

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

U.S. Patent No. 8,304,193

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Attorney Docket No.: P50031US00

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For: METHOD FOR CONDUCTING AN
AUTOCATALYTIC REACTION IN
PLUGS IN A MICROFLUIDIC SYSTEM

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REQUEST FOR *EX PARTE* REEXAMINATION OF
U.S. PATENT NO. 8,304,193

Mail Stop *Ex Parte* Reexam
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

Pursuant to the provisions of 35 U.S.C. § 302 and 37 C.F.R. § 1.510 et seq., the undersigned, on behalf of 10X Genomics, Inc., requests *ex parte* patent reexamination of claims 1-14 of U.S. Patent No. 8,304,193 (“the ‘193 patent,” Exhibit 1001).

***Ex Parte* Patent Reexamination Filing Requirements**

Pursuant to 37 C.F.R. § 1.510(b)(1), statements pointing out at least one substantial new question of patentability based on material, non-cumulative prior art patents for claims 1-14 of the ‘193 patent are provided in Section VI of this Request. Although some of these prior art references were previously cited in the record during the *ex parte* prosecution of the ‘193 patent, these references have not been considered in the new light demonstrated by the proposed substantial new questions of patentability.

Pursuant to 37 C.F.R. § 1.510(b)(2), reexamination of claims 1-14 of the '193 patent is requested, and a detailed explanation of the pertinence and manner of applying the cited prior art to claims 1-14 is provided in Section VII of this Request.

Pursuant to 37 C.F.R. § 1.510(b)(3), copies of every patent relied upon or referred to in the statement pointing out each substantial new question of patentability or in the detailed explanation of the pertinence and manner of applying the cited prior art are provided as Exhibits 1002-1110 of this Request.

Pursuant to 37 C.F.R. § 1.510(b)(4), a copy of the '193 patent is provided as Exhibit 1001 of this Request.

Pursuant to 37 C.F.R. § 1.510(b)(5), the attached Certificate of Service indicates that a copy of this Request, in its entirety, has been served on Patent Owner at the following address of record for Patent Owner, in accordance with 37 C.F.R. § 1.33(c):

Brown Rudnick LLP
One Financial Center
Boston, MA 02111

Also submitted herewith is the fee set forth in 37 C.F.R. § 1.20(c)(1).

Pursuant to 37 C.F.R. § 1.510(b)(6), Requester hereby certifies that neither the statutory estoppel provisions of 35 U.S.C. § 315(e)(1) nor 35 U.S.C. § 325(e)(1) prohibit Requester from filing this *ex parte* patent reexamination request.

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1002	Prosecution history of U.S. Patent No. 8,304,193 (U.S. Patent Application No. 13/024,155)
1003	Provisional application serial no. 60/379,927 by Ismagilov (priority document)
1004	Declaration of Dr. Eric Shaqfeh
1005	Curriculum Vitae of Dr. Eric Shaqfeh
1006	Brody, J. P. et al., <i>Biotechnology at Low Reynolds Numbers</i> , Biophysical Journal, vo. 71, December 1996, pp. 3430-3441. (“Brody”)
1007	Burns, J. R. et al., <i>The Intensification of Rapid Reactions in Multiphase Systems Using Slug Flow in Capillaries</i> , Lab on a Chip, vol. 1, 2001, pp. 10-15 (“Burns I”)
1008	Burns, M.A. et al., <i>Microfabricated structures for integrated DNA analysis</i> , Proc. Natl. Acad. Sci. USA, May 1996, vol. 93, pp. 5556-5561. (“Burns II”)
1009	Chiem, N. H. et al., <i>Microchip systems for immunoassay: an integrated immunoreactor with electrophoretic separation for serum theophylline determination</i> , Clin. Chem., 1998, vol. 44, no. 3, pp. 591-598 (“Chiem”)
1010	Corbett et al., U.S. Patent No. 5,270,183, assigned to Beckman Research Institute of the City of Hope (“Corbett”)
1011	Dolnik, V. et al., <i>Capillary Electrophoresis on a microchip</i> , Electrophoresis, 2000, vol. 21, pp. 41-54 (“Dolnik”)
1012	Erbacher, C. et al., <i>Towards Integrated Continuous-Flow Chemical Reactors</i> , <i>Mikrochim. Acta</i> , 1999, vol. 131, pp. 19-24 (“Erbacher”)
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- 1022 Johnson, T.J. et al., *Rapid Microfluidic Mixing*, Anal. Chem., 2002 (published on Web 12/06/2001), vol. 74, pp. 45-51 (“Johnson”)
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- 1025 Knapp et al., U.S. Patent No. 6,444,461, assigned to Caliper Technologies Corp. (“Knapp I”)
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- 1027 Kopp, M.U. et al., *Chemical Amplification: Continuous-Flow PCR on a Chip*, Science, 1998, vol. 280, pp. 1046-48 (“Kopp”)
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- 1036 Ramsey, U.S. Patent No. 6,001,229, assigned to Martin Marietta Energy Systems, Inc. (“Ramsey I”)
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- 1049 Final Written Decision cancelling the claims of U.S. Patent No. 8,658,430, IPR2015-01558, Paper No. 46
- 1050 Petition for *Inter Partes* Review of U.S. Patent No. 8,304,193, IPR2015-01163
- 1051 Patent Owner’s Preliminary Response, *Inter Partes* Review of U.S. Patent No. 8,304,193, IPR2015-01163

- 1052 Decision Denying Institution of *Inter Partes* Review of U.S. Patent No. 8,304,193, IPR2015-01163, Paper No. 14
- 1053 Amended Complaint in *RainDance Technologies, Inc. et al. v. 10X Genomics, Inc.*, Case No. 1-15-cv-00152 (D.Del.)
- 1054 Claim construction memorandum opinion, DED 1-15-CV-00152, Docket No. 116
- 1055 Claim construction order, DED 1-15-CV-00152, Docket No. 121
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- 1058 Portions of file history of U.S. 7,294,503
- 1059 Petition for *Inter Partes* Review of U.S. 7,294,503, IPR2015-00009
- 1060 Declaration of Prof. Shelly Anna Ph.D., submitted as Ex. 1002 in IPR2015-00009 (*Inter Partes* Review of U.S. 7,294,503).
- 1061 Plaintiffs' Amended Fed. R. Civ. P. 7.1 Statement in *RainDance Technologies, Inc. et al v. 10X Genomics, Inc.*, Case No. 1-15-cv-00152 (D.Del.)
- 1062 Claim chart showing the correspondence between the claims of the '193 patent and the combination of the Quake, Corbett and Lagally references.
- 1063 Claim chart showing the correspondence between the claims of the '193 patent and the combination of the Quake, Corbett and Burns II references.
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- 1079 Claim chart showing the correspondence between the claims of the '193 patent and the combination of the Burns I, Kopp, and Vogelstein references.
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- 1081 Belgrader et al., "A Reusable Flow-Through Polymerase Chain Reaction Instrument for the Continuous Monitoring of Infectious Biological Agents," Anal. Chem. 2003, 75, 3446-3450 ("Belgrader")
- 1082 Burns MA, et al., "An integrated nanoliter DNA analysis device," Science, 1998, 282(5388), 484-487 ("Burns II 1998")
- 1083 U.S. Pat. No. 60/283527 ("Knapp II Provisional")
- 1084 De Mello A. J., Manz A., "Chip technology for micro-separation," Microsystem Technology, BioMethods, Köhler J.M., Mejevaia T., Saluz H.P. (eds), (1999), Birkhäuser, Basel ("de Mello and Manz 1999")

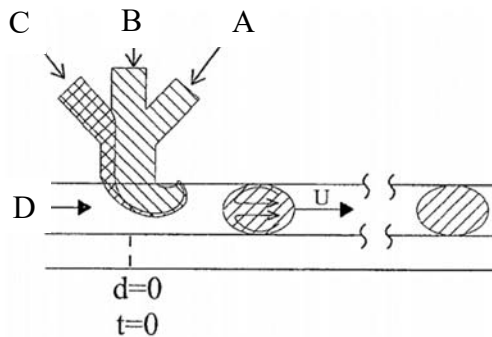
- 1085 De Mello, A. J., “DNA amplification: does ‘small’ really mean ‘efficient’?,” *Lab on a Chip*, 2001, 1, 24N–29N (“de Mello 2001”)
- 1086 Wilding, P., et al., “PCR in a silicon microstructure,” *Clin Chem* 1994; 40:1815–8 (“Wilding”)
- 1087 Harrison DJ, et al., “Micromachining a miniaturized capillary electrophoresis based chemical analysis system on a chip,” *Science* 1993; 261:895–7 (“Harrison”)
- 1088 Jakeway S.C. et al, “Miniaturized total analysis systems for biological analysis,” *Fesenius J. Anal. Chem.*, 2000, 366:525–539 (“Jakeway”)
- 1089 Lagally E.T., et al., “Monolithic integrated microfluidic DNA amplification and capillary electrophoresis analysis system,” *Sens Actuators B Chem* 2000;63:138– 46. (“Lagally 2000”)
- 1090 LoNostro P et al., *J. Phys. Chem. B* 1999, 103, 5347-5352 (1999) (“LoNostro”)
- 1091 Lazar JG, “Advanced methods in PCR product detection,” *PCR Methods Appl.*, 4, 1-14 (1994) (“Lazar”)
- 1092 Manz A, et al., “Planar chips technology for miniaturization and integration of separation techniques into monitoring systems: capillary electrophoresis on a chip,” *J Chromatogr* 1992; 593:253– 8. (“Manz 1992”)
- 1093 Manz, et al., “Miniaturized Total Chemical Analysis Systems: a Novel Concept for Chemical Sensing,” *Sensors and Actuators, BI* (1990) 244-248, (“Manz 1990”)
- 1094 Matsubara Y, et al., “Microchamber array based DNA quantification and specific sequence detection from a single copy via PCR in nanoliter volumes,” *Biosens Bioelectron*, 2005;20:1482–90. (“Matsubara”)
- 1095 Nagai H, et al., “Development of a microchamber array for picoliter PCR,” *Anal Chem* 2001; 73(5):1043–7 (“Nagai”)
- 1096 Nisasako et al., “Formation of Droplets Using Branch Channels in a Microfluidic Circuit,” *SICE 2002, Osaka, Japan, SICE02-0475*, (“Nisasako”)
- 1097 Northrup MA, et al., “A miniature analytical instrument for nucleic acids based on micromachined silicon reaction chambers,” *Anal Chem* 1998; 70(5):918– 22. (“Northrup 1998”)
- 1098 Northrup MA et al., “A Mems-based Miniature DNA Analysis System (“Northrup 1995”)

- 1099 Saiki R.K., et al., “Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase,” *Science*, 1988, Vol. 239, No. 4839, 487-491 (“Saiki”)
- 1100 Thorsen, T, et al., “Dynamic pattern formation in a vesicle-generating microfluidic device,” *Phys. Rev. Lett.*, 2001, 86, 4163-4166 (“Thorsen”)
- 1101 U.S. Patent No. 5,739,036 to Parris titled “Method for analysis” (“Parris”)
- 1102 U.S. Patent No. 5,980,936 to Krafft et al., titled “Multiple emulsions comprising a hydrophobic continuous phase” (“Krafft”)
- 1103 U.S. Patent No. 4,683,202 to Mullis et al. titled "Process for amplifying nucleic acid sequences." (“Mullis”)
- 1104 Shaw Stewart, P., “The development and applications of a new liquid handling device,” Thesis, (1988), Imperial College of London (“Shaw Stewart Thesis”)
- 1105 Cold Spring Harbor 51st Symposium on Quantitative Biology in 1986
- 1106 National Center for Biotechnology Information, “Polymerase Chain Reaction (PCR),” Retrieved October 1, 2017, from <https://www.ncbi.nlm.nih.gov/probe/docs/techpcr/>
- 1107 Jungkind D, “Automation of laboratory testing for infectious diseases using the polymerase chain reaction-- our past, our present, our future.” *J Clin Virol.* 2001 Jan;20(1-2):1-6.
- 1108 U.S. Patent No. 6,485,705 to Schneider et al. titled “Mixable combination for generating a suspension of stable microbubbles for ultrasonic imaging” (“Schneider”)
- 1109 Prosecution history of U.S. Patent No. 8,329,407 (U.S. Patent Application No. 13/024,145)
- 1110 “Autocatalysis.” ScienceDaily, www.sciencedaily.com/terms/autocatalysis.htm. Accessed 27 Oct. 2017.

I. INTRODUCTION

This request for reexamination and the proposed grounds of rejection raised herein are supported by a declaration from Dr. Eric Shaqfeh, Chair of Stanford's Department of Chemical Engineering and author of one hundred journal articles relating to fluid mechanics. *See* Ex. 1004. Dr. Shaqfeh's declaration summarizes and reflects his knowledge, technical expertise, and understanding of the scope and content of the prior art applied in this request for reexamination. *Id.* at ¶¶ 3-12, 31-56, 106-109, MPEP § 2258. The state of the art at the time of the filing of the '193 patent is presented by Dr. Shaqfeh in this request for reexamination.

The '193 patent is generally directed to microfabricated substrates and methods of conducting reactions within these substrates. Ex. 1001 at Abstract. As shown in Fig. 2A of the '193 patent, reproduced at right, the reactions occur in plugs transported in the flow of a carrier-fluid *Id.* at 11:64-65, 17:37-44, Fig. 2A. In a preferred embodiment, two aqueous reagents form laminar streams that are separated by a "divider" aqueous stream. *Id.* ¶17:37-41. "The three streams enter a channel with flowing oil D, at which point plugs form and plug fluids mix." *Id.* ¶17:41-43.



As discussed in Section IV.C, *infra*, the prosecution history taken as a whole indicates that the '193 patent was allowed primarily because the Examiner believed that the prior art failed to teach conducting reactions in droplets formed from two continuously flowing immiscible fluids. Significantly, the Examiner was not made aware that '193 patent's disclosure concerning forming plugs from continuous streams of immiscible fluids was **copied almost verbatim from one of the prior art references of record**. See Sections III.A and IV.C, *infra*.

The PTAB denied institution of an *inter partes* review of U.S. Patent No. 8,304,193 because the petition failed to explain why a skilled artisan would have believed that the PCR technique of Haff could have been conducted on a microfluidic scale as taught in Quake. *See* Section IV.D, *infra*. Accordingly, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for conducting reactions in droplets formed from two continuously flowing immiscible fluids that could be readily modified to perform the reaction in question.**¹

As discussed in Section VI, *infra*, the prior art submitted herewith (much of which was not previously presented to the Office) shows that i) droplet micro reactors that formed plugs from continuous flowing immiscible fluids were well known, ii) microfluidic PCR devices were well known, and iii) there were compelling reasons to modify the known microfluidic droplet reactors to conduct the known continuous flow PCR reactions.

The Quake, Burns I and Shaw Stewart references show that droplet microreactors which formed plugs from continuous flowing immiscible fluids were well known. As noted by the Patent Trial and Appeal Board (PTAB), Quake (Ex. 1033) discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids. Indeed, **Patent Owner copied this subject matter almost word-for-word from the Quake reference**, a fact about which the Office has not yet been made aware. Burns et al. also shows that plugs in microchannels also could be readily formed by continuous flow of fluids at an intersecting channel. Ex. 1007 (Burns I). The Shaw Stewart British Application (Ex. 1040) also teaches conducting reactions in droplets formed from two continuously flowing immiscible fluids. Significantly, the exclusive licensee of the '193 patent affirmatively (and correctly) took the position that Shaw Stewart

¹ Requester notes that independent claim generically recites a reaction involving a biological molecule whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the '193 patent, Ex. 1061, Ex. 1053 ¶43) explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams. Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

As of the earliest claimed priority date, many groups had demonstrated that PCR could be successfully performed in microfluidic plugs, as demonstrated by Corbett, Burns II, Kopp, Lagally and Vogelstein. In 1991, eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Ex. 1010 (Corbett). In 1996, Mark A. Burns from the University of Michigan reported the use of microfluidic devices to perform PCR. Ex. 1008 (Burns II). By 1998 Kopp *et al.* had successfully implemented continuous flow PCR on a chip. Ex. 1027 (Kopp). By 1999, Vogelstein *et al.* had reported various applications for single-molecule microfluidic PCR. Ex. 1044 (Vogelstein). By 2001, Lagally reported using microfluidic PCR to amplify single-molecule of DNA template to, among other things, detect rare genetic mutations. Ex. 1028 (Lagally). Corbett, Kopp, Burns II, and Vogelstein show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the combinations proposed herein, the microfluidic droplet reactors of Quake, Burns I and Shaw Stewart are relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).² Ex. 1001 at 78:12-15. In the proffered combinations, this limitation is met by using the microfluidic droplet reactors to perform PCR reactions, wherein each droplet (slug or plug) is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the substrate molecule) and reagents, *e.g.*, polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, Burns I or Shaw Stewart to conduct microfluidic PCR as taught by Corbett, Burns II, Kopp, Lagally or Vogelstein. Ex. 1004 ¶¶72-82. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic reactor of Quake, Burns I or Shaw Stewart to perform continuous flow droplet formation. *Id.* Moreover, as evidenced by Kopp, Burns II, Lagally and Vogelstein’s contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing, one would have a high expectation of success for the performance of PCR using the droplet reactors of Quake, Burns I or Shaw Stewart. *Id.*

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactors of Quake, Burns I or Shaw Stewart because doing so have provided the substantial benefits known to be associated with such microfluidic reactors. *Id.* ¶106. As noted

² Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565 [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. “In addition, decreasing the scale of PCR allows the reaction to be carried out **more efficiently**, producing more product in **less time** with **less side reactions.**” Ex. 1013 at 192, [emphasis added]. It was also well known that miniaturization of PCR reactions to the microfluidic level provided the **substantial advantage of making PCR reactors highly portable** (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Skilled artisans would also have recognized that performing Corbett’s PCR reaction using two continuous immiscible fluids in the microfluidic reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer** relative to then-traditional approaches. Ex. 1024 at 841. Moreover, using the microfluidic reactors for PCR reactions to create many droplets would have **substantially increased the tolerance of PCR reactions to primer non-specificity.** Ex. 1044 at 9236, 9239; Ex.1004 ¶80. This reduction in signal-to-noise ratio **enabled detection of rare mutations, dislocations and allelic imbalances.** Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86.

Moreover, a skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp, Burns II, Lagally or Vogelstein) to incorporate a droplet reactor (Quake, Burns I or Shaw Stewart). Ex. 1004 ¶107. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught

in Kopp, Burns II, Lagally or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II or Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also **decrease contamination** and **increase the number of reactors** that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Further, a skilled artisan would have **fully expected these combinations to be successful**. Ex. 1004 ¶109. In 1998, four years prior to the earliest claimed priority date, Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, and continuous separations) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” Ex. 1027 at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article, Lagally et al. provided an overview of that evolution and further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR. Ex. 1028. Accordingly, the prior art shows that **many forms of PCR had been successfully achieved in microfluidic plugs** in the years leading up to the earliest claimed priority date.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Kopp or Lagally to work in the microfluidic reactor of Quake, Burns I or Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo

developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.

Section VI, *infra*, establishes that a reasonable Examiner would consider the foregoing teachings relevant in deciding whether claims 1-14 of the ‘193 patent are patentable.

Section VII, *infra*, provides a detailed explanation of how the teachings apply to the claims of the ‘193 patent and demonstrates that the claims of the ‘193 patent should be rejected as anticipated and obvious.

II. RELATED AND CO-PENDING PROCEEDINGS; ESTOPPEL

Neither 35 U.S.C. § 315(e)(1) nor 35 U.S.C. § 325(e)(1) prohibit Requester from filing this *ex parte* patent reexamination request. Only the former is relevant here, as the latter applies to Covered Business Method Review proceedings, which have not occurred relative to the ‘193 patent.

The ‘193 patent is currently the subject of *Bio-Rad Laboratories, Inc. et al v. 10X Genomics, Inc.*, Case No. 1-15-cv-00152 (D.Del.). Ex. 1053. RainDance Technologies and the University of Chicago brought suit against 10X Genomics, Inc. for infringement of the ‘193 patent in the District Court for the District of Delaware. *Id.* RainDance is the exclusive licensee under the ‘193 patent. Ex. 1053 at ¶44. RainDance was subsequently acquired by Bio-Rad Laboratories (Ex. 1061), and Bio-Rad Laboratories was officially substituted as the Plaintiff on

May 25, 2017. The District Court has interpreted various claim terms appearing the ‘193 patent. Exhibits 1054-57. Dispositive motions are due by November 20, 2017. Trial is scheduled for April 16, 2018.

The ‘193 patent was the subject of IPR2015-01163 in which the Board declined to institute an *inter partes* review trial. Ex. 1052. Because the Board did not issue a final written decision, no estoppel can arise under 35 U.S.C. § 315(e)(1).

III. CITATION OF PRIOR ART PATENTS AND PRINTED PUBLICATIONS RELIED UPON IN REQUEST FOR REEXAMINATION

Reexamination of claims 1-14 of the ‘193 patent is requested in view of the following prior art patents and printed publications:

A. Quake (from which Key Passages Were Copied Almost Verbatim by Patent Owner)

WO 02/23163 A1 to Quake et al. (“Quake PCT”) is attached as Exhibit 1034. Quake was filed on September 14, 2001, the WIPO publication was in the English language, and the PCT application designated the United States. Accordingly, the Quake PCT has a § 102(e) date of September 14, 2001 and qualifies as prior art under that section.

The Quake PCT corresponds to U.S. Published Application 2002/0058332 to Quake et al., which is attached as Exhibit 1033 (“Quake”).

The Quake PCT is relied upon herein in lieu of the published U.S. application because the Quake PCT demonstrates that key portions of the ‘193 patent specification were copied almost verbatim from the Quake PCT. As shown in the table below, the supposed point of novelty of the ‘193 patent (i.e., forming droplets from two continuous streams of immiscible fluids) was copied almost verbatim from the Quake PCT. The Patent Owner copied verbatim the underlined portions from the Quake PCT.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and '193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	Provisional at 22:12-19, '193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

Attached as Ex. 1035 is a table showing the numerous passages which Ismagilov copied key disclosures verbatim or nearly verbatim from the Quake PCT.

As discussed in sections VI and VII, below, notwithstanding statements to the contrary during *ex parte* prosecution of the '193 patent, it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids. Indeed, the Patent Trial and Appeal Board expressly found that Quake disclosed such a technique. Ex. 1052 at 11-14. However, neither the examiner nor the PTAB appear to have been made aware that Ismagilov's disclosure concerning forming plugs from continuous streams of immiscible fluids was copied almost word-for-word from Quake.

1. Potential applicability of Dynamic Drinkware

According to the Federal Circuit, claims of an issued U.S. patent must be supported by the disclosure of the application to which it claims priority for the patent to qualify as prior art

under 35 U.S.C. §102(e) as of the priority date. *Dynamic Drinkware, LLC v. National Graphics, Inc.*, 800 F.3d 1375 (Fed. Cir. 2015). The court cited *In re Wertheim*, 646 F.2d 527, 537 (CCPA 1981), and found that because Petitioner failed to compare the claims of the issued prior art patent to the disclosures of its provisional, Petitioner could not establish an earlier prior art date for the patent. *Id.* However, it appears that this holding has not been extended to U.S. published applications or WIPO publications by any court. It should not be so extended, as explained below.

The rationale of *In re Wertheim* and *Dynamic Drinkware* applies at most to alleged prior art issued patents under 35 U.S.C. § 102(e)(2) and not to prior art publications under 35 U.S.C. § 102(e)(1). *Dynamic Drinkware* referred to the status of an “issued patent” as prior art under Section 102(e)(2). *Dynamic Drinkware*, Slip Op. at 11, 1. Indeed, the rationale of *In re Wertheim* was that “in a situation where there are continuation-in-part applications,” the “type of new matter added must be inquired into” because “if a patent could not theoretically have issued the day the application was filed, it is not entitled to be used against another” *In re Wertheim*, 646 F.2d 527, 536-37 (CCPA 1981). By contrast, as the BPAI has explained, “[w]hen a patent application is published . . . the PTO has yet to have made a final determination as to whether the claimed subject matter conforms to the patentability requirements of Chapter 35 Yet, the published application makes its disclosure, and that of any parent applications, available to the public. Such disclosure . . . is explicit evidence of the activity of another as of the earliest claimed filing date. . . . [Thus] a published patent application . . . constitutes prior art for all that it discloses on its earliest filing date.” *Ex parte Jo Anne Robbins*, No. 2009-001866, 2009 WL 3490271, *4 (BPAI Oct. 27, 2009); *see also Ex parte Yamaguchi*, 88 U.S.P.Q.2d 1606 (BPAI 2008) (declining to apply *Wertheim* to, *inter alia*, publications and holding “a provisional

application is considered prior art for all that it teaches”). As noted in the legislative history of the AIA: “Wertheim...was almost completely overruled by the American Inventors Protection Act of 1999. . . which, by making any published application prior art [under §102(e)(1)], effectively displaced Wertheim's requirement that the application have been capable of becoming a patent on the day that it was filed.” *See* 157 Cong. Rec. S1360-02, 2011 WL 797877 (Mar. 8, 2011). In short, *In re Wertheim* and *Dynamic Drinkware* have no application to published applications under Section 102(e)(1).

Moreover, the provisional application at issue in *Dynamic Drinkware* was not incorporated by reference into the alleged prior art patent (as is the case with the Quake PCT). The rationale of *In re Wertheim* and *Dynamic Drinkware* applies when the content of a provisional application is not published, but is simply referred to by a purported prior art patent. In such circumstances, courts have held that the disclosure of the provisional must provide written description for the later issued patent. However, where, as here, the entire disclosure of the provisional is incorporated by reference, and thereby effectively published on the publication date, this rationale does not apply. *See Telemac Cellular Corp. v. Topp Telecom, Inc.*, 247 F.3d 1316, 1329 (Fed. Cir. 2001) (document “‘incorporated by reference’ . . . becomes effectively part of the host document”); MPEP § 2127(I) (subject matter of application may be relied on under Section 102(e) if it is “actually included or incorporated by reference”).

Accordingly, the *Dynamic Drinkware* rule should not apply to the WIPO publications such as the Quake PCT.

2. Chart Showing Compliance with *Dynamic Drinkware*

However, in the event *Dynamic Drinkware* is deemed to apply to WIPO publications, the chart below shows that the claims of the WIPO publication are indeed supported by the priority document.

WO 02/23163 A1	U.S. Pat. App. No. 60/233,037
1. A microfluidic device comprising	This invention relates to microfluidic devices and methods, including microfabricated, multi-layered elastomeric devices with active pumps and valves. P. 1, lines 11-14.
a main channel, and	The devices and methods of the invention comprise a main channel , through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. p.1, lines 14-17.
at least one inlet region in communication with the main channel at a droplet extrusion region.	A junction or " droplet extrusion region " joins the sample inlet channel to the main channel such that the aqueous solution can be introduced to the main channel, <i>e.g.</i> , at an angle that is perpendicular to the stream of oil. p. 1, lines 17-19.
28. A device for sorting biological material comprising:	The invention also provides a device for sorting biological material comprising: p.8, line 6.
(a) a microfabricated substrate having at least one main channel, an inlet region which meets the main channel at a droplet extrusion region, and at least two branch channels meeting at a junction downstream from the droplet extrusion region;	The invention also provides a device for sorting biological material comprising: a microfabricated substrate...In more detail, the microfabricated substrate has at least one main channel, an inlet which meets the main channel at a droplet extrusion region, and at least two branch channels meeting at a junction downstream from the droplet extrusion region. p. 8, lines 6-10.
(b) a detection region within or coincident with at least a portion of the main channel and associated with a detector; and	The detection region of the device is within or coincident with at least a portion of the main channel, and is also associated with a detector. p. 8, lines 10-12.
38. A method for sorting biological material, said method comprising:	The invention also provides a method for sorting biological material. p. 9, line 3.
(a) furnishing an extrusion fluid to a main channel of a microfabricated substrate;	The method, which is preferably implemented using a microfabricated device of the invention...p. 9. lines 7-9 An extrusion fluid , which is incompatible with the sample fluid, flows through the main channel so that the droplets of the sample

	fluid are within the flow of the extrusion fluid in the main channel. p. 9, lines 14-16.
(b) providing droplets of a sample fluid containing the biological material to the main channel, wherein the sample fluid is incompatible with the extrusion fluid;	(a) providing droplets of a sample fluid containing the biological material to the main channel of a microfabricated substrate; p. 9. lines 9-10 An extrusion fluid, which is incompatible with the sample fluid, flows through the main channel so that the droplets of the sample fluid are within the flow of the extrusion fluid in the main channel. p. 9, lines 14-16.
(c) interrogating the biological material in each droplet for a predetermined characteristic as it passes through a detection region associated with the main channel; and	(b) interrogating each droplet (or the biological material within each droplet) for a predetermined characteristic as it passes through a detection region associated with the main channel; p. 9. lines 10-12.
(d) directing the flow of each droplet into a selected branch channel according to the results of the interrogation.	(c) directing the flow of each droplet into a selected branch channel according to the results of the interrogation. p. 12-14,

B. Burns I (Cited but Not Discussed or Applied by the Examiner)

Burns, J. R. et al., *The Intensification of Rapid Reactions in Multiphase Systems Using Slug Flow in Capillaries*, Lab on a Chip, vol. 1, 2001, pp. 10-15 (“Burns I”) is attached as Exhibit 1007. Burns I indicates on its face that it was published on the web August 9, 2001. Burns I is thus available as prior art under 35 U.S.C. § 102(a). Burns I was among the hundreds of references cited but was not applied or discussed by the Examiner during *ex parte* prosecution of the ‘193 patent. Requester notes that another reference by a *different* Burns (*i.e.*, Mark A. Burns) was cited by the Examiner in an office action for a related application. (Ex. 1109 at July 11, 2011 rejection p 7.) That article is referenced herein as Exhibit 1008 “Burns II.”

C. Burns II (Cited but Not Previously Applied to the Independent Claim)

Burns, M.A. et al., *Microfabricated structures for integrated DNA analysis*, Proc. Natl. Acad. Sci. USA, May 1996, vol. 93, pp. 5556-5561 (Burns II) is attached as Exhibit 1008. Burns II was published in 1996 and is available as prior art under 35 U.S.C. § 102(b). Burns II was cited by the Examiner only against dependent claims in a related application reciting autocatalytic reactions, enzymes or DNA. Ex. 1002 at June 21, 2011 rejection p. 7. Burns II was not applied against any independent claim.

D. Shaw Stewart British Application (Cited but Not Previously Applied to the Independent Claim)

UK Patent Application GB 2,097,692A to Shaw Stewart (the “Shaw Stewart British Application”) is attached as Exhibit 1040. The Shaw Stewart British Application published in 1982 is available as prior art under 35 U.S.C. § 102(b). The British Application was specifically considered only for teachings concerning the surfactants recited in dependent claims 10 and 11. Ex. 1002 at July 11, 2011 rejection p. 6-7.

E. Corbett (Not Previously Considered)

U.S. Patent No. 5,270,183 to Corbett et al. (“Corbett”) is attached as Exhibit 1010. Corbett issued in 1993 and is available as prior art under 35 U.S.C. § 102(b). Corbett was not previously disclosed or considered in connection with *ex parte* prosecution or any post-grant proceeding involving the ‘193 patent.

F. Kopp (Not Previously Considered)

Kopp, M.U. et al., Chemical Amplification: Continuous-Flow PCR on a Chip, Science, 1998, vol. 280, pp. 1046-48 (“Kopp”) is attached as Exhibit 1027. Kopp published in 1998 and is available as prior art under 35 U.S.C. § 102(b). Kopp was not previously disclosed or considered in connection with *ex parte* prosecution or any post-grant proceeding involving the ‘193 patent.

G. Lagally (Not Previously Considered)

Lagally, E.T. et al., Single-Molecule DNA Amplification and Analysis in an Integrated Microfluidic Device, *Anal. Chem.*, 2001, vol. 73, pp. 565-570 (“Lagally”) is attached as Exhibit 1028. Lagally indicates on its face that it was published on the web on January 3, 2001. Lagally is available as prior art under 35 U.S.C. § 102(b). Lagally was not previously disclosed or considered in connection with *ex parte* prosecution or any post-grant proceeding involving the ‘193 patent.

H. Vogelstein (Cited but Not Discussed or Applied by the Examiner)

Vogelstein, B. et al., *Digital PCR*, *Proc. Natl. Acad. Sci.*, August 1999, Vol. 96, pp. 9236–9241 (“Vogelstein”) is attached as Exhibit 1044. Vogelstein was published in August 1999 and is thus available as prior art under 35 U.S.C. § 102(b). Vogelstein was among the hundreds of references cited but was not applied or discussed by the Examiner during *ex parte* prosecution of the ‘193 patent.

I. Additional References

Requester further relies on various of the other references cited in the Table of Exhibits to support the proposed grounds of rejection, as explained below, in the claim charts appended hereto, and in the declaration of Dr. Shaqfeh (Ex. 1004). For example, various additional references are relied upon to establish the state of the art and the level of skill in the art (Section IV.A) and motivations to combine the references as set forth in the proposed grounds set forth in Sections VI and VII.

IV. OVERVIEW OF THE ‘193 PATENT

A. State of the Art and Level of Skill in the Art³

In the years leading up to the earliest claimed priority date (May 9, 2002), there was a substantial body of literature describing performing continuous flow biochemical assays, such as polymerase chain reaction, and other reactions on a microfabricated chip.

In 1996, Brody, J. P. et al. (“Brody”) (not previously presented to the USPTO) published an article, *Biotechnology at Low Reynolds Numbers*, *Biophysical Journal*, vol. 71, December 1996, pp. 3430-3441 (Ex. 1006). Brody, in summarizing the existing state of the art in microfluidics, stated

“[t]here has been a **surge of interest in the ‘lab-on-a-chip’ concept**, which involves the miniaturization of many chemical processes onto a single silicon chip. [] Because these systems allow one to manipulate single cells, and even single macromolecules, there is great interest in the biotechnology community in using microfluid systems for analytical tests. For example, in the **polymerase chain reaction, amplification of DNA** in a microenvironment is attractive both because the temperature can be rapidly cycled and because the sample volume is extremely small.”

Ex. 1006 at 3430 [emphasis added].

Brody explained that “[a]t [micrometer] scales, viscous forces dominate over inertial forces, turbulence is nonexistent, surface tension can be a powerful force, diffusion becomes the basic method for mixing, and evaporation acts quickly on exposed liquid surfaces.” Ex. 1006 at 3430. Brody noted that in microfluidic devices “[m]ixing must be chiefly done by diffusion, and in fact diffusion cannot be neglected in the design of these devices.” *Id.* at 3440.

³ See Ex. 1004 (*Shaqfeh Decl.*) ¶¶26-56 for full Background discussion, including evidentiary support.

In 1996, Mark A. Burns from the University of Michigan reported the use of microfluidic devices to perform PCR. *Microfabricated structures for integrated DNA analysis*, Proc. Natl. Acad. Sci. USA, May 1996, vol. 93, pp. 5556-5561. (Ex. 1008, Burns II).⁴ Burns II reported that “[n]o new biochemistries or DNA detection methods are required for their use; the components simply reproduce current laboratory procedures in a micron-sized environment.” *Id.* at 5560. In other words, the PCR chemistry did not need to be modified – it worked without modification on the microfluidic scale. In the method of Burns II, a sample containing a DNA plasmid was loaded into one of the reaction wells (A) and a mixture of Taq enzyme and buffer was loaded into the other. *Id.* at 5558. Each formed droplets of about 60 nl in the parallel 500 x 25 μm channels (B) which came together at the Y junction to form a single larger droplet (about 120 nl). *Id.* at 5558-59; Ex.1004 ¶40.

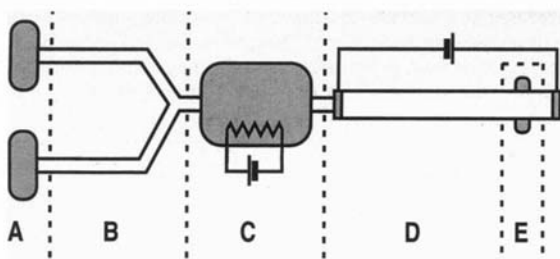


FIG. 1. Example of an integrated DNA analysis system, represented schematically. The individual components of the system are injection entry ports (A), liquid pumping channels (B), thermally controlled reaction chamber (C), electrophoresis channel (D), and DNA band migration detector (E). Each component would have associated sensors, control circuitry, and external connections.

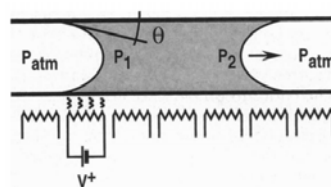


FIG. 2. Schematic drawing showing the principle of thermally induced drop motion in a closed channel. The case of a single aqueous drop in a hydrophilic channel is presented, where V is an applied voltage, P_{atm} is atmospheric pressure, P_1 is the receding-edge internal pressure, P_2 is the advancing-edge internal pressure, and θ is the contact angle of the liquid-gas-solid interface. The contact angle will depend on the surface characteristics of the channel and the constituents of the drop, with a hydrophilic interaction giving θ between 0 and 90 degrees, and a hydrophobic surface giving θ between 90 and 180 degrees. Surface treatments can also reduce contact angle hysteresis and, therefore, reduce the temperature difference necessary for drop motion.

The droplet then passed through the resistive heater portion (C) in which PCR amplification occurred. *Id.* at 5556. Afterwards the amplification results were optically detected in zone D via gel electrophoresis. *Id.* at 5559. Burns II noted that “[t]he use of common fabrication methods

⁴ This author (**Mark** Burns) reference should not be confused with **J.R.** Burns of the University of Newcastle. The latter published a different article relevant to this reexamination request: Burns, J. R. et al., *The Intensification of Rapid Reactions in Multiphase Systems Using Slug Flow in Capillaries*, Lab on a Chip, vol. 1, 2001, pp. 10-15. (“Burns I”, Ex. 1007)

allows the assembly of increasingly complex, multicomponent, integrated systems from a small, defined set of standardized elements” and that “[f]uture design efforts have a strong impetus to reduce component size and improve efficiency.” *Id.* at 5560-61.

In 1997, Knapp *et al.* described microchannel reactors in which multiple reactants were continuously or intermittently flowed into and reacted within a microchannel. In U.S. Patent No. 6,444,461 to Knapp (Ex. 1025, priority 1997, not presented to the USPTO), Knapp described the system as follows:

The use of an integrated microfluidic system as herein before described, wherein said biochemical system flows through one of said channels substantially **continuously**, enabling sequential testing of said plurality of test compounds. . . .

Finally, in a screening run, streams of antibody, antigen and sample are **flowed continuously** into the reaction channel 404a and into waste well 428. A slug of this mixture is then injected into the separation channel 404b. . . .

Modulating voltages are then concomitantly applied to the various reservoirs to affect a **desired fluid flow characteristic, e.g., continuous or discontinuous** (e.g., a regularly pulsed field causing the flow to oscillate direction of travel) flow of receptor/enzyme, ligand/substrate toward the waste reservoir with the periodic introduction of test compounds.

Ex. 1025 at 59:21-25, 29:43-46, 51:64-52:2 [emphasis added].

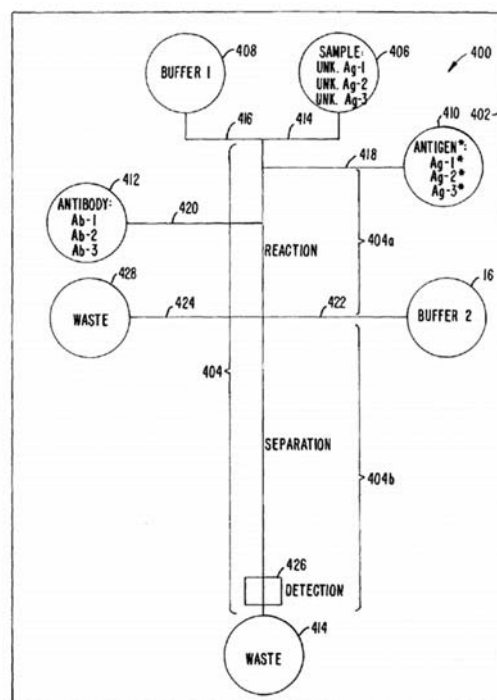


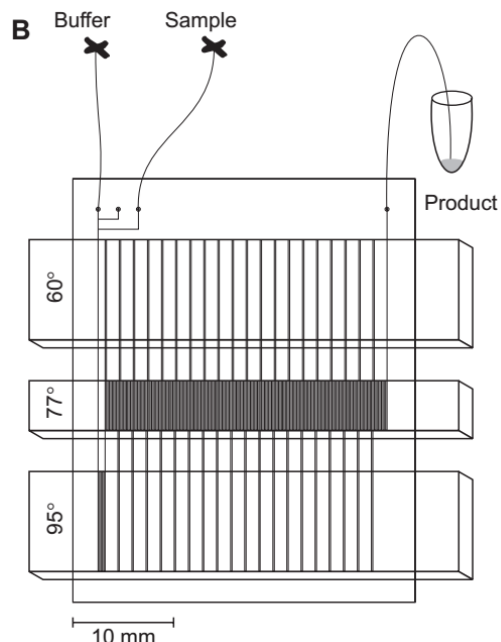
FIG. 4A.

By 1998, Kopp *et al.* had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 [emphasis added].

Kopp *et al.* also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

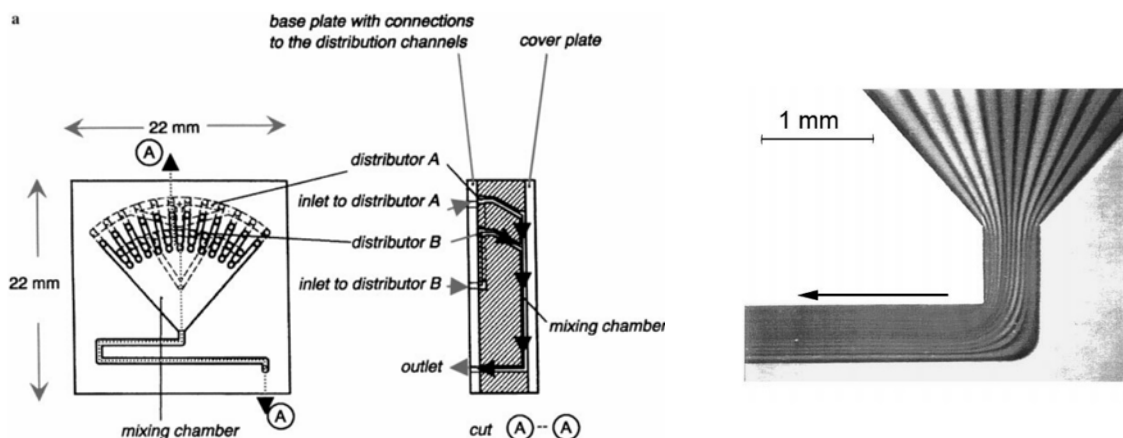
To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene

could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 [emphasis added].

Starting in 1999, consistent with Kopp's prediction, other groups reported conducting various other types of reactions in continuous flow microchannel devices. Also in 1999, Erbacher et al., reported in Erbacher *et al.*, *Towards Integrated Continuous-Flow Chemical Reactors*, *Mikrochim. Acta*, 1999, vol. 131, pp. 19-24, Ex. 1012, ("Erbacher") (another article not previously presented to the USPTO) on their development of a continuous flow microchannel reactor in which complete mixing of the reactants occurred within just a few seconds. Ex. 1012 at 19. The figures of Erbacher's article, reproduced below, illustrate the mixing manifold and the manner in which the fluids mix after they are introduced into the same conduit.



In 2000, Kenis *et al.* reported (in another article not previously disclosed to the USPTO) that a similar continuous flow microchannel reactor could be used for various fabrication reactions. Ex. 1024 at 841; Ex. 1004 ¶46. Kenis *et al.* explained that the laminar flow which predominates in microchannel devices makes them well suited as microreactors:

Channels that are 0.02-2 mm wide -- channels which, in this Account, we call almost interchangeably “microchannels” or “capillaries” – can be considered as reaction vessels with two unusual characteristics. First, their interior volumes are small, but readily and rapidly accessible from the outside by pumping fluids or gases into them. Second, fluids moving in them at low to moderate velocities flow laminarly, that is, without turbulence. This laminar flow can be used to deliver reagents spatially inside capillaries with **remarkable precision**. These characteristics, combined with the ease with which microchannels can be assembled and disassembled using soft lithography and other techniques, suggest them as a new system of reactors with which to carry out microfabrication.

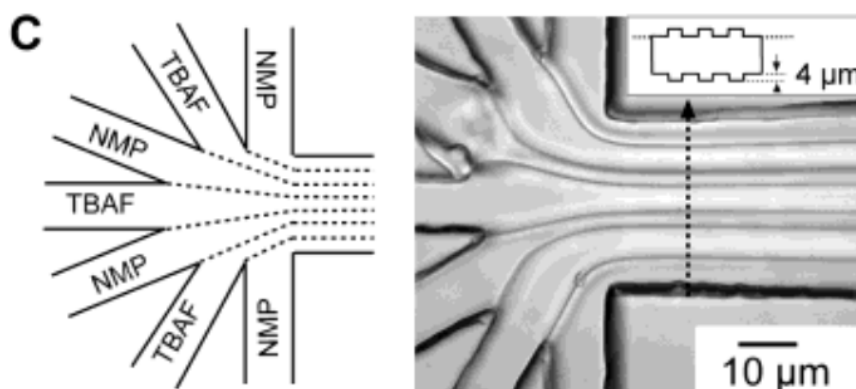
Id. at 841 [emphasis added]. Kenis *et al.* explained that a wide range of technologies had already been used to accomplish chemical reactions in microchannels or capillaries, including deposition of proteins and cells.

Table 1. Techniques Used for Microfabrication inside Microchannels

<i>Strategy for Microfabrication</i> Process or Technology	<i>Reference</i>
<i>Multilayer Photolithography</i>	2
<i>Single-Stream Liquid Flow</i>	
Chemical Vapor Deposition	3
Light Tweezers assisted Deposition	4
Polymer Deposition	8
Electroless Deposition	9
Electrochemical Fiber Growth	10
Etching of Optical Fibers	11,12
Micromolding in capillaries (MIMIC)	13-17
Deposition of Proteins	18
<i>Multiple-Stream Liquid Flow</i>	
Electroless Deposition	20
Etching	20
Nucleation and Crystallization (CaCO ₃)	20
Deposition of Proteins	24
Deposition of Cells	24

Id. at 842, 846.

Kenis *et al.* reported that “[t]he occurrence of laminar flow on the microscopic scale allows liquid streams to be maintained as entities that are separate and capable of delivering different reagents” and that “[f]luid flows have clear potential to pattern delicate biological structures” such as those shown in Fig. 4C, reproduced below. *Id.* at 845.



Also in the year 2000, Floyd, *et al.*, “*Novel Liquid Phase Microreactors for Safe Production of Hazardous Specialty Chemicals*,” *Microreaction Technology: Industrial Prospects*, 2000 (“Floyd”), reported the development of “liquid-phase microreactor with thin film temperature sensing, good thermal management, and fast mixing has been fabricated for the production of hazardous specialty chemicals, specifically for organic peroxides.” Ex. 1014 at 171 (also not previously presented to the USPTO). In Floyd’s microreactor, multiple two different reactants are **continuously** injected in a fan of converging channels. *Id.* at 177, Fig. 1 (reproduced in part below). The convergence and fast mixing of acid and base reactants is shown in Floyd’s Fig. 9 (reproduced in part below). *Id.* at 173, 178.

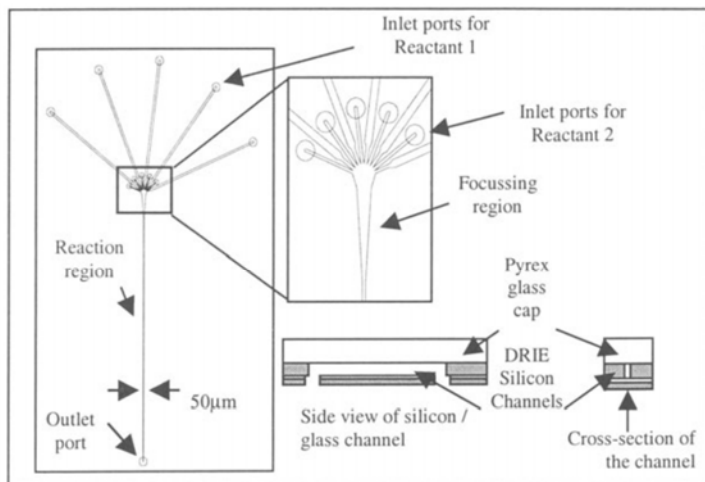


Fig. 1. First generation reactor design

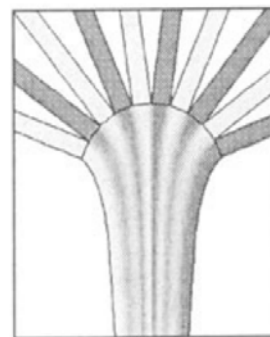


Fig. 9. Laminating

Also in 2000, Ferrance, *et al.* published a book chapter, Ferrance, J. P. et al., "*Toward effective PCR-based amplification of DNA on microfabricated chips*," Methods in molecular biology (Clifton, NJ), 2000, vol. 163, pp. 191-204 ("Ferrance"), (not previously considered by the Office) which provided an overview of known techniques and apparatus for performing continuous flow **PCR in microfabricated chips**. Ex. 1013.

To utilize this sequence most efficiently there **should be a continuous flow** from sample collection to diagnosis. . . .

The **same advantages of reduced time, sample, and reagents** brought to the separations field by miniaturization also apply to low volume PCR in capillaries. Microchip formats have also been developed for PCR where the reactions are carried out in reservoirs or microreaction chambers formed in glass, silicon, or plastic microchips. In addition, decreasing the scale of PCR allows the reaction to be carried out **more efficiently**, producing more product in **less time** with **less side reactions**. Both capillaries and microchip devices have reduced the time needed for PCR but cycle times concomitant with the fast separations now possible are still being developed. . .

Although capillaries have proven useful for small scale PCR, true **integration of the PCR and fast separation steps** will require a

microchip device where continuous flow of the PCR products to the separation channels is achievable on a single coordinated platform (see Note 2). With this type of platform, integration not only of the last two steps shown in Fig. 1 is possible, but total integration of the complete process. . . .

Ex. 1013 at 191, 192 [emphasis added]. Ferrance noted that several groups had reported successful PCR reactions with sample volumes on the nano-liter scale.

Kalinina et al. (12) have **reduced the volume of reaction by using capillaries as small as 20 pm** in diameter to hold a total volume of 10 nL, allowing single copies of template DNA to be amplified and detected after 30 cycles using fluorescent energy transfer (FET). . . .

The system of Oda et al. (8) has been further modified by Huhmer and Landers (13) to carry out reactions in 150-pm id capillaries, which hold volumes as low as 100 nL. . . . The submicroliter volumes and noncontact, direct heating of the PCR solution by the IR radiation **allowed cycle times to be reduced to less than 3 s** in this system.

Id. at 196 [emphasis added].

In 2001, in another article not previously presented to the USPTO, Lagally *et al.*, reported using a microfluidic reactor to conduct single-molecule DNA amplification by PCR. Lagally summarized the state of the art as of February 2001, explaining **continuous flow PCR microreactors had already undergone substantial evolution:**

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template

concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

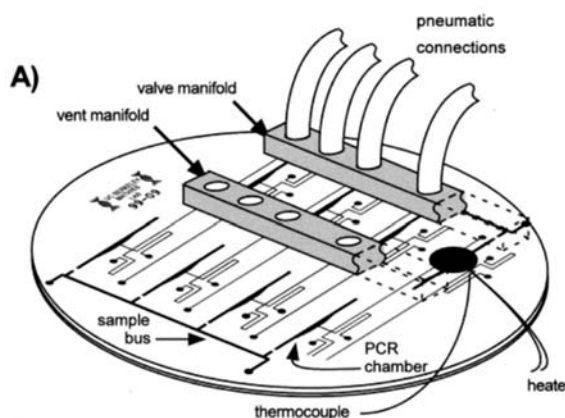
Ex. 1028 at 565-566.

Lagally *et al.* developed a further improvement – a device and method for achieving single molecule amplification in a microchannel environment.

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials

are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

Microfluidic PCR-CE device. The PCR chambers are connected to a common sample bus through a set of valves. Hydrophobic vents at the other end of the PCR chambers are used to locate the sample and to eliminate gas. The PCR chambers are directly connected to the cross channel of the CE system for product injection and analysis. Two aluminum manifolds, one each for the vents and valves, are placed onto the respective ports and clamped in place using vacuum. The manifolds connect to external solenoid valves for pressure and vacuum actuation. Thermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.



Ex. 1028 at 566 (see figure legend).

In the same year, Whitesides published an article (also not previously considered by the USPTO) which surveyed the state of the art and known applications for microchannel reactors. Whitesides explained that “[f]or the relatively large feature sizes used in biology ($>50\text{ }\mu\text{m}$), production of prototype patterns and structures is convenient, inexpensive, and rapid.” Ex.1047

at 335. The table of contents in Whitesides shows that microfabricated reactors were widely used in PCR, DNA detection and analysis, biochemical analysis, and other applications.

APPLICATIONS OF SOFT LITHOGRAPHY IN MICROSCOPIC	
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Id. at 336. Whitesides explained that as of 2001 “[a] variety of companies and academic research groups have developed systems for nucleic acid amplification and/or detection.” *Id.* at 353.

Among the work specifically cited by Whitesides was Kopp (affiliated with Zeneca/SmithKline Beecham), Lagally (University of California Berkeley), and Burns II (University of Michigan).

Also in 2001, the University of Newcastle in the United Kingdom published its findings concerning the suitability of microchannel slug flow reactors for industrial, high-throughput chemical reactions like nitrations, hydrogenations, sulfonations and oxidations. Ex. 1007. In the article *The intensification of rapid reactions in multiphase systems using slug flow in capillaries*, Lab on a Chip, 2001, vol. 1, pp. 10-15

(Burns I), Burns reported that circulation within the plugs caused by shear forces against the wall may greatly enhance the mass transfer or mixing and hence the rate

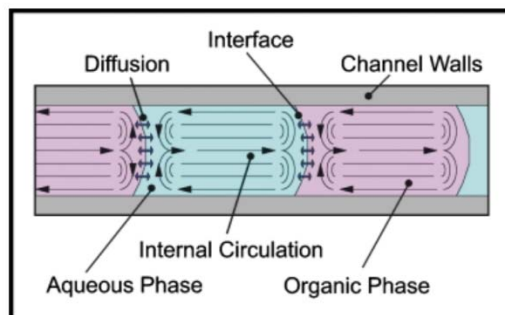


Fig. 1 Illustration of internal circulation generated within immiscible slug flow.

of reaction. *Id.* Burns taught that “[i]n practice, as shown in Fig. 1, a **circulation is generated within the slugs which is stimulated by the shear** between the stationary fluid at the capillary wall and the slug axis. This convects fluid from the slug ends and establishes a concentration gradient in the radial direction. The resulting mass transfer between the wall flow and that along the axis is enhanced because: (i) the path length for diffusion is approximately equal to the tube radius—generally much shorter than the slug half length; (ii) diffusion occurs across an area which approaches the cylindrical slug area—generally larger than the capillary cross-section area. **Thus circulation can provide a powerful boost to the inter-phase mass transfer.**” *Id.* at 10 [emphasis added].

Burns I also taught that the slugs may be formed from the continuous flow of fluids at an intersecting channel. Burns explained that “various methods may be used to generate slugs of liquid within a microreactor.” *Id.* at 10. Burns I selected a method in which “the **continuous flow** of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel **whilst the other phase**

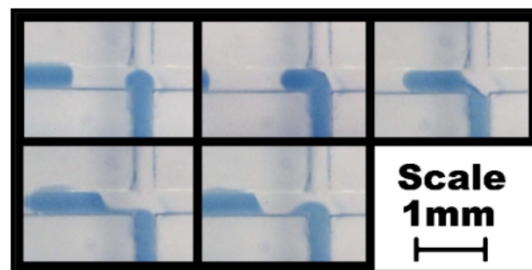
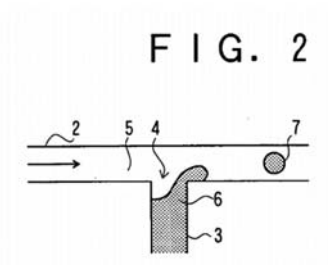


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 11 [emphasis added]. “Fig. 3 shows the typical pattern of slug generation within this device when an organic phase, with a room temperature viscosity of 27 cP, composed of 67% silicone oil (1000 cP) and 33% kerosene (dyed blue) was used in conjunction with water. The slug lengths produced were around 1.5 mm long with a flow velocity of 5.6 mm s^{-1} .” *Id.* at 11.

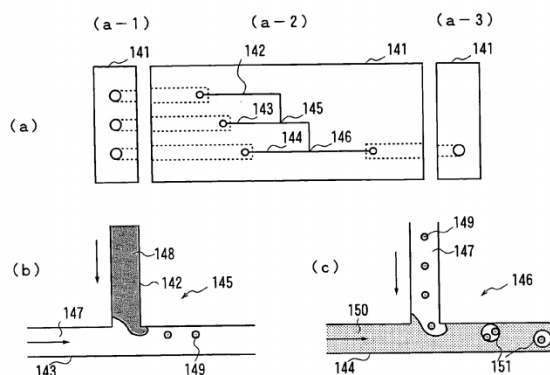
In early 2001, a group from the University of Tokyo developed a droplet reactor that appears to fall within the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are relevant to the state of the art at the time of filing.⁵

Higuchi I discloses a microreactor in which a dispersion phase (6) is ejected from a dispersion phase feeding port (4) toward a continuous phase (5) flowing in a microchannel (2) in such a manner that flows of the dispersion phase (6) and the continuous phase (5) cross each other, thereby obtaining microdroplets (7), formed by the shear force of the continuous phase (5), having a size smaller than the width of the channel for feeding the dispersion phase (6). Ex. 1018 at Fig. 2 (reproduced at right). Higuchi teaches many variations of the embodiment of Figure 2 and one representative variation is shown in Fig. 19.



⁵ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

FIG. 19



In [Figures 19a-c], reference numeral 141 represents a main body of the microcapsule-forming apparatus, reference numeral 142 represents a microchannel in which a dispersion phase (for example, water) flows, reference numeral 143 represents a microchannel in which a first continuous phase (for example, oil) flows, reference numeral 144 represents a microchannel in which a second continuous phase (for example, water) flows, reference numeral 145 represents the first junction at which flows of the dispersion phase and the first continuous phase are joined together, reference numeral 146 represents the second junction at which flows of the dispersion phase, the first continuous phase, and the second continuous phase are joined together, reference numeral 147 represents the first continuous phase, reference numeral 148 represents the dispersion phase, reference numeral 149 represents an emulsion (for example, water), reference numeral 150 represents the second continuous phase, and reference numeral 151 represents formed microcapsules. The microcapsules 151 can contain one or more emulsions 149. Numeral 143 represents a microchannel in which a first continuous phase (for example, oil) flows, reference numeral 144 represents a microchannel in which a second continuous phase (for example, water) flows, reference numeral 145 represents the first junction at which flows of the dispersion phase and the first continuous phase are joined together, reference numeral 146 represents the second junction at which flows of the dispersion phase, the first continuous phase, and the second continuous phase are joined together, reference numeral 147 represents the first continuous phase, reference numeral 148 represents the dispersion phase, reference numeral 149 represents an emulsion (for example, water), reference numeral 150 represents the second continuous phase, and reference numeral 151 represents formed microcapsules. The microcapsules 151 can contain one or more emulsions 149.

Ex. 1018 ¶100.

The microchannel device of Higuchi I-III was specifically intended to be used to perform chemical reactions; indeed, that is typically why emulsions are created. Ex. 1004 ¶56; Ex. 1043 (A contemporaneous article by Higuchi entitled *Chemical reactions in microdroplets by electrostatic manipulation of droplets in liquid media*). Higuchi I-III militate in favor of a finding that show that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was actually within the level of ordinary skill in the art at the time of filing.

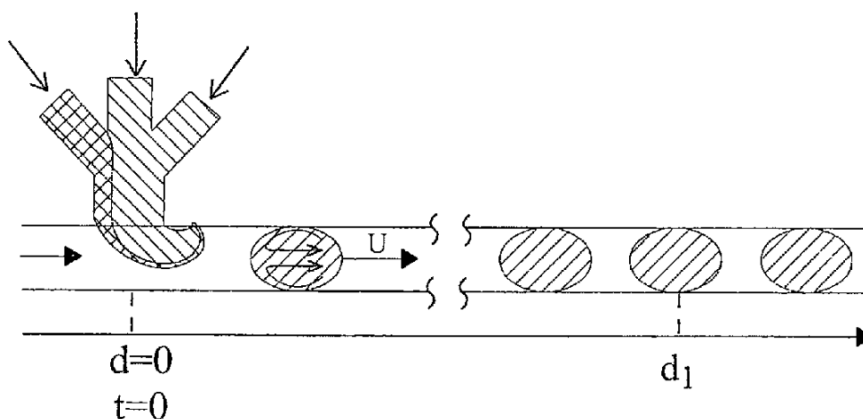
In light of the foregoing references, which demonstrate the state of the art, a person having ordinary skill in the art would have had knowledge of the scientific literature concerning microfluidic devices and the methods of using such devices before May 9, 2002. A skilled artisan would have had a **Ph.D. in chemistry, biochemistry, mechanical engineering, or a related discipline, with two years of experience in using, designing or creating microfluidic devices**. A person of ordinary skill in the art would have known how to research the scientific literature in fields relating to microfluidics, including fluid dynamics, microscale reactions, chemistry, biochemistry, and mechanical engineering, and to consult with team members having specialized skills in these fields. Ex. 1004 at ¶29. The Patent Trial and Appeal Board has adopted this definition in an *inter partes* review of another microfluidic device patent owned assigned to RainDance Technologies with a priority date of 2011. Ex. 1049 (Final written decision in IPR2015-01558 cancelling the claims of U.S. Patent No. 8,658,430) at p. 7.

B. Summary of the ‘193 Patent

Against this backdrop, Ismagilov *et al.* filed the patent application that issued as the ‘193 patent. The ‘193 patent claims priority to May 9, 2002, and is assigned to the University of Chicago (“Patent Owner”), according to the Office’s electronic-assignment records. The ‘193

patent claims methods of performing a reaction in plugs of an aqueous fluid flowing in an oil in a microfluidic device. Ex. 1001 at 78:8-26. The methods claimed in the '193 patent recite flowing an aqueous fluid through a first channel into a junction with at least one other channel. The aqueous fluid comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction between the substrate molecule and the reagents. And upon introduction of the aqueous fluid from the first channel into the junction, the oil partitions the aqueous fluid into a plug that is substantially surrounded by the oil and that then flows through the channel. *Id.*

Figure 2A-2 of the '193 patent illustrates this process:



Independent claim 1 of the '193 patent reads as follows:

1. A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:
 - providing the microfluidic system comprising at least two channels having at least one junction;
 - flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;
 - flowing an oil through the second channel of the at least two channels;
 - forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents

for conducting an autocatalytic reaction with the at least one substrate molecule; and
providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Ex. 1001 at 78:8-29

C. Prosecution History of the '193 Patent

In U.S. Patent Application Serial No. 13/024,155 Patent Owner presented a single independent claim:

1. A method comprising the steps of:
providing a microfluidic system comprising one or more channels;
providing within the one or more channels a carrier fluid comprising an oil and at least one plug substantially surrounded by the carrier fluid, wherein the plug comprises reagents sufficient for an autocatalytic reaction including a first species of molecule in a concentration such that the plug contains no more than a single molecule of the first species; and
conducting an autocatalytic reaction such that the single molecule is amplified.

Ex. 1002 at February 9, 2011 application as filed, p. 116.

Examiner Gakh rejected the pending claims as indefinite and either anticipated or rendered obvious by Beer et al. (Anal. Chem., 2007). Ex. 1002 at July 11, 2011 Non-final Office Action, 3-7. Examiner Gakh made the following findings:

Beer teaches “On-Chip, Real-Time, Single-Copy Polymerase Chain Reaction in Picoliter Droplets” (Title) as the following:

“The first lab-on-chip system for picoliter droplet generation and PCR amplification with real-time fluorescence detection has performed PCR in isolated droplets at volumes 10^6 smaller than commercial real-time PCR instruments. The system utilized a shearing T-junction in a silicon device to generate a stream of monodisperse picoliter droplets that were isolated from the microfluidic channel walls and each other by the oil-phase carrier. An off-chip valving system stopped the droplets on-chip, allowing them to be thermally cycled through the PCR protocol without droplet motion. With this system, a 10-pL droplet, encapsulating less than one copy of viral genomic DNA through Poisson statistics, showed realtime PCR amplification curves with a cycle threshold of ~18, 20 cycles earlier than commercial instruments. This combination of the established real-time PCR assay with digital microfluidics is ideal for isolating single-copy nucleic acids in a complex environment.” (Abstract)

The method comprises heating (see page 8473).

Ex. 1002 at July 11, 2011 Non-final Office Action, p. 5.

In response, the Applicants amended independent claim 1 as follows:

1. (Currently amended) A method comprising the steps of:
 - providing a microfluidic system comprising one or more channels;
 - ~~providing within the one or more channels~~ forming a plug of aqueous fluid by partitioning the aqueous fluid as it continuously flows through a first of the one or more channels, the a carrier fluid comprising an oil and at least one plug being substantially surrounded by ~~the carrier fluid~~ an oil, wherein the plug comprises a single substrate molecule and reagents sufficient for conducting an autocatalytic reaction with the single substrate molecule including a first species of molecule in a concentration such that the plug contains no more than a single molecule of the first species; and
 - conducting an autocatalytic reaction in the plug such that the single substrate molecule is amplified.

Ex. 1002 at December 16, 2011 Amendment/Req. Reconsideration-After Non-Final Reject, p. 2.

The Applicants argued that the pending claims were allowable for because Beer was not available as prior art:

Beer is not prior art and cannot be used to reject the present claims. Beer is a journal article that published in 2007, and thus the date that Beer is available as a prior art reference under 35 U.S.C. §102 and 35 U.S.C. §103(a) is 2007.

Applicants have shown above that the subject matter of the pending claims is adequately described in each of U.S. patent application serial number 12/777,099 and U.S. patent application serial number 10/765,718. Since claims of the present application are adequately described and enabled in each of 12/777,099 and U.S. patent application serial number 10/765,718, the present claims are entitled to at least the priority date of January 26, 2004.

Ex. 1002 at December 16, 2011 Applicant Arguments/Remarks Made in an amendment p. 78.

Examiner Gakh found that the new claims were not supported in the priority documents because the latter did not describe single molecule reactions:

Even for this reaction and even for femtoliter volumes of the plugs the concentration of the molecules statistically is almost two molecules (1.8) per plug. Nowhere is it shown that single molecules were obtained in the plugs. PCR is mentioned only as another example of possible autocatalytic reaction. However, the Applicants did not show the possession of the claimed invention at the time of filing the priority document. Therefore, the instant case does not obtain the priority date of the parent application.

Id. at January 31, 2012 Final Rejection, pg. 4 [emphasis added].

The Examiner also rejected the claims as being indefinite. For instance, the Examiner found that the claims did not clearly set forth how the partitioning occurs:

Claim 1 recites providing a microfluidic system comprising one or more channels and forming a plug of aqueous fluid by partitioning the aqueous fluids as it continuously flows through a first of one of the one or more channels. First of all, it is not clear, how the plugs can be formed just within one channel with continuously flowing water. It requires at least two channels – for flowing water and for flowing the carrier fluid. Also, it is not clear, how the plug is formed with only a single molecule of the substrate and reagents? How is it possible? The recitation of the claims differs from the disclosure of the specification.

Id. at 2012-01-31 Final Rejection p. 6 [emphasis added]. Lastly, the Examiner maintained that the claims were anticipated by or rendered obvious by Beer et al. (Anal. Chem., 2007). *Id.* at 2012-01-31 Final Rejection p. 7-8.

The Applicants next conducted an in-person interview with Examiner Gakh and tentatively agreed on amended claim language that would address the Examiner's enablement, written description and art-based rejections. *Id.* at July 27, 2012 Interview Summary. The Applicants also persuaded the Examiner that the amended claims were entitled to the asserted priority date. *Id.*

The Applicants filed the amended claims without a further explanation of the substantive reasons underlying the Examiner's change in position. Independent claim 1 was amended as follows:

1. (Currently amended) A method for conducting an autocatalytic reaction, comprising the steps of:
 - providing a microfluidic system comprising one or more channels;
 - forming a at least one plug of aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction by partitioning the aqueous fluid as it continuously flows through a first of the one or more channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises a single at least one substrate molecule and reagents for conducting an autocatalytic reaction with the single at least one substrate molecule; and
 - conducting providing conditions suitable for an autocatalytic reaction in the at least one plug such that the single at least one substrate molecule is amplified.

Id. at 2012- 07- 27 Response After Final Action, p. 3. In their remarks, the Applicants took the position that the foregoing amendments did not narrow the scope of the claims but provided no disclosure of the reasons the Examiner agreed to withdraw her rejections:

Applicants and their counsel thank Examiner Gakh for comments the Examiner made on claims submitted in a facsimile from Applicant's counsel dated June 28, 2012. The amendment submitted herewith is to clarify the claims and is not intended to, and does not, change the scope of the claims. The amended claims in this Amendment and Response retain the amendments previously made in the facsimile response submitted on June 28, 2012, as that response has not officially been entered at this time. As agreed with the Examiner, these claims are in condition for allowance pending a supplemental prior art search by the Examiner. If there are any further issues for discussion, the Examiner is kindly invited to contact the undersigned.

Id. at 2012- 07- 27 Response After Final Action p. 5. [emphasis added]

After the Examiner's supplemental search, the Examiner required additional amendments to the claims. The Examiner made the following amendment to independent claim 1 with the Applicants' permission:

1. (Currently amended) A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:
providing a the microfluidic system comprising ~~one or more~~ at least two channels having at least one junction;
flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;
flowing an oil through the second channel of the at least two channels;
forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents ~~for conducting an autocatalytic reaction~~ by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels as it continuously flows through a first of the one or more channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule; and
providing conditions suitable for ~~an~~ the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.
Claim 16 (New). The method of claim 1, further comprising a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction.

Id. at 2012-08-07 Notice of Allowance and Fees Due (PTOL-85), pp. 2-3. Examiner Gakh stated that she was allowing the further amended claims because the Thorson and Quake prior art did

not appear to disclose performing chemical reactions in droplets formed at the intersection of two channels.

The following is an examiner's statement of reasons for allowance: the claims recite the subject matter which was disclosed in the parent case having priority over all relevant art found by the examiner. In particular, *Takahashi and Inoue* disclose analogous reaction in US 2006/0257893; however this is not the prior art, as its filing date is February 17, 2006.

The review by *Gu et al.* "*Droplets Formation and Merging in Two-Phase Flow Microfluidics*" (*Int. J. Mol. Sci.*, 2011) refers to the formation of the droplets from continuous flows of two liquids with the first publication of such method by *Thorsen et al.* "*Dynamic Pattern Formation in a Vesicle-Generating Microfluidic Device*" (*Phys. Rev. Lett.* 2001). The publication by Thorsen et al. is devoted to the study of the physical-chemical processes of droplet formation in microfluidic systems and the effects of different conditions and channel designs for such formation. The paper does not mention any application of the systems for the chemical reactions in droplets formed at the junction of two channels by two constantly flowing immiscible fluids. The review of *Atencia and Beebe* "*Controlled microfluidic interfaces*"

(*Nature*, 2005) refers to the microfluidic platform for performing a two-step reaction, which is disclosed in the publication from Ismagilov's group, *Shestopalov, I., Tice, J. D. and Ismagilov, R. F.* "*Multi-step synthesis of nanoparticles performed on millisecond time scale in a microfluidic droplet-based system* (*Lab Chip*, 2004, IDS).

Quake and Thorsen in US 2002/0058332 (IDS) disclose a system with two channels and the method of forming plugs (droplets) with reagents in immiscible oil; however, the droplets in the oil are not formed from two continuously flowing immiscible fluids, but rather by a discrete dispensing the solution with biological material into the carrier fluid. This is a different approach from the claimed invention.

Id. at 2012-08-07 Notice of Allowance and Fees Due (PTOL-85), pp. 3-4 [emphasis added]. The '155 application issued as U.S. Patent No. 8,304,193 (Exhibit 1001), the subject of the instant Request.

Significantly, the examiner was not made aware that '193 patent's disclosure concerning forming plugs from continuous streams of immiscible fluids was plagiarized from Quake. As shown in the table below, the supposed point of novelty of the '193 patent (i.e., forming droplets from two continuous streams of immiscible fluids) was copied almost verbatim from the Quake PCT.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and '193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	Provisional at 22:12-19, '193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

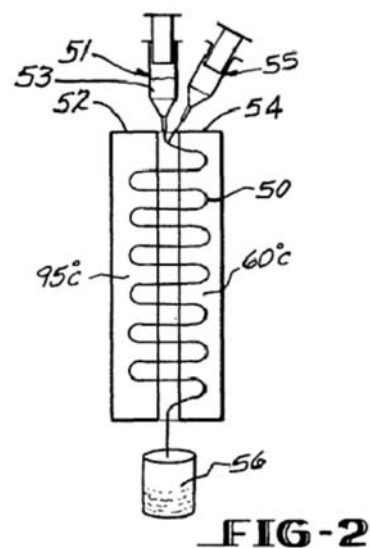
Attached as Ex. 1035 is a table showing the numerous passages which Ismagilov copied key disclosures verbatim or nearly verbatim from the Quake PCT.

In sum, the prosecution history taken as a whole indicates that the '193 patent was allowed primarily because the Examiner believed that the prior art failed to teach **conducting reactions in droplets formed from two flowing immiscible fluids, despite the fact that Patent Owner copied this subject matter almost verbatim from Quake.**

D. The *Inter Partes* Review Proceedings

Requester filed an *inter partes* review petition (Ex. 1050) asserting that claims 1-5, 7-9, and 11 of the '193 patent were rendered obvious by US 6,033,880, issued Mar. 7, 2000 to Haff (Ex. 1017) in view of Quake (Ex. 1033, "Quake '332"); and claim 6 was rendered obvious by Haff in view of Quake and further in view of US 6,524,456 B1, issued Feb. 25, 2003 to Ramsey (Ex. 1037, "Ramsey II").

Haff discloses an automated machine for performing polymerase chain reaction (“PCR”). Ex. 1017, 1:12–15. Haff describes capillary tube PCR instruments use various mechanisms to heat and cool the reaction mixture and various fluid handling mechanisms to move the reaction mixture. As shown in Figure 2, reproduced at right, capillary tubing 50 defines a fluid path that alternately passes through hot and cold zones 52, 54. *Id.* at 9:58–65. Syringe 51 is used to dispense the PCR reaction mixture 53. Syringe 55 optionally contains an immiscible carrier fluid. *Id.* at 10:20–32. Syringe 55 is used to intermittently inject the immiscible fluid into the flow stream of reaction mixture 53. *Id.* at 10:32–35. This creates “slugs” of PCR reaction mixture separated by the immiscible carrier fluid. *Id.* at 9:50–52. The PCR amplification is controlled by the size of the tube, the flow rate and the temperatures of the hot and cold zones. *Id.* at 10:15–19.



Turning to Quake, the petition relied primarily on the embodiment shown in Figure 16A, reproduced below. *See, e.g.*, Ex. 1050 at pp. 22, 23, 27. Quake teaches that a “junction or ‘droplet extrusion region’ joins the sample inlet channel to the main channel such that the aqueous solution can be introduced to the main channel, e.g., at an angle that is perpendicular to the stream of oil. By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established between the two channels such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets.” Ex. 1033 ¶ 3.

The droplets of the sample fluid “contain [] the biological material for analysis, reaction or sorting” *Id.* ¶ 20. Optionally, the droplets of the sample fluid each “contain, on average, no more than one particle of the biological material.” *Id.*

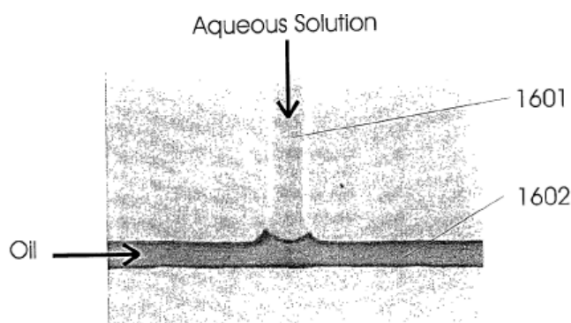


FIG. 16A

In Figure 16A, channel 1601 contains the aqueous solution and intersects with main channel 1602, which contains the oil. *Id.* ¶ 292.

The Board denied institution on the basis that the petition did not sufficiently explain why a person of skill in the art would combine Haff and Quake. Ex. 1052. In particular, the Board held that

[W]e determine that Petitioner has not explained why a person of ordinary skill in the art would have had a reasonable expectation of success combining Haff’s capillary tube PCR system with Quake’s microfluidic system to amplify a substrate in droplets. Simply stating that it would have been “routine” is insufficient. To the extent Petitioner relies on the background information to support a reasonable expectation of success, Petitioner fails to explain any connection between the two. For example, if conducting PCR in a stream of reaction mixture in a microfluidic device was known, Petitioner has not explained why, with that knowledge, it would have been routine to conduct PCR in droplets in a microfluidic device. Moreover, even though Haff describes conducting PCR in a capillary tube device, neither Petitioner nor its declarant explains whether the conditions for conducting PCR in microfluidic droplets would differ,

or why a person of ordinary skill in the art would consider conducting PCR in microfluidic droplets to be “routine.”

Ex. 1052 at 16-17.

The Board was not presented, and the decision did not consider, the teachings of Burns I, Burns II, Shaw Stewart, Corbett, Kopp, Lagally or Vogelstein. Nor did the Board consider any testimony that is comparable to that of Dr. Shaqfeh. Ex. 1004.

V. CLAIM CONSTRUCTION

Claim terms are generally given their ordinary and customary meaning as would be understood by one of ordinary skill in the art. *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312-13 (Fed. Cir. 2005) (*en banc*). In an *ex parte* reexamination, a claim in an unexpired patent is “given the broadest reasonable interpretation consistent with the specification.” MPEP § 2258(I)(G).

A. “Microfluidic system” (Claim 1)

Claim 1 recites “providing the microfluidic system comprising at least two channels having at least one junction.” The Board did not find it necessary to interpret this term for purposes of its decision. Ex. 1052 at 9-11. The District Court adopted the following definition under *Phillips*:

The term "microfluidic system" (as used in the '193, '148, '083, and '407 patents) means a "system comprised of at least one substrate having a network of channels of micrometer dimension through which fluid may be transported." A "microfluidic system" is not limited to or the equivalent of a "substrate."

Ex. 1055 at 1.

Consistent with this definition, the expert retained by the exclusive licensee of the ‘193 patent (Bio-Rad), averred in a declaration that the term “[m]icrofluidics” is “understood by those of ordinary skill in the art, like myself, to encompass devices and methods for control and manipulation of fluids that contain geometric features that are on the sub-millimeter scale (i.e., less than 1000 microns). Ex. 1060 at 19.

B. “Plug of Aqueous Fluid” (Claim 1)

Independent claim 1 recites the step of “forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents” Ex. 1001 at 78:18-19. The ‘193 patent includes two paragraphs specifically addressing the meaning of the term “plug”:

“Plugs” in accordance with the present invention are formed in a substrate when a stream of at least one plug-fluid is introduced into the flow of a carrier-fluid in which it is substantially immiscible. The flow of the fluids in the device is induced by a driving force or stimulus that arises, directly or indirectly, from the presence or application of, for example, pressure, radiation, heat, vibration, sound waves, an electric field, or a magnetic field. Plugs in accordance with the present invention may vary in size but when formed, their cross-section **should be** substantially similar to the cross-section of the channels in which they are formed. When plugs merge or get trapped inside plug traps, the cross-section of the plugs may change. For example, when a plug enters a wider channel, its cross-section typically increases.

Further, plugs in accordance with the present invention may vary in shape, and for example **may be** spherical or non-spherical. The shape of the plug may be independent of the shape of the channel (e.g., a plug may be a deformed sphere traveling in a rectangular channel). The plugs **may be** in the form of plugs comprising an aqueous plug-fluid containing one or more reagents and/or one or more products formed from a reaction of

the reagents, wherein the aqueous plug-fluid is surrounded by a non-polar or hydrophobic fluid such as an oil. The plugs **may also be** in the form of plugs comprising mainly a non-polar or hydrophobic fluid which is surrounded by an aqueous fluid. The plugs may be encased by one or more layers of molecules that comprise both hydrophobic and hydrophilic groups or moieties. The term “plugs” also includes plugs comprising one or more smaller plugs, that is, plugs-within-plugs. The relative amounts of reagents and reaction products contained in the plugs at any given time depend on factors such as the extent of a reaction occurring within the plugs. **Preferably**, plugs contain a mixture of at least two plug fluids.

Ex. 1001 at 9:27-60. [emphasis added].

Consistent with the foregoing, the Board broadly interpreted the term “plug” as to mean “volumes of aqueous fluid formed when a stream of aqueous fluid is introduced into the flow of a substantially immiscible carrier-fluid.” Ex. 1052 at 7-8. The Board noted that this did not incorporate various features of the preferred embodiments such as plug size and its separation from the channel wall. *Id.*

The District Court did not specifically construe the term “plug” but noted that preamble of claim 1 (“A method for conducting an autocatalytic reaction in plugs in a microfluidic system”) was nonlimiting. Ex. 1054 at 12.

C. “Providing Conditions Suitable for the Autocatalytic Reaction” (Claim 1)

Independent claim 1 recites the step of “providing conditions suitable for the autocatalytic reaction in the at least one plug.” The Board interpreted this term to refer to “a global set of physical and chemical conditions that allows one to conduct an autocatalytic reaction (including PCR) in droplets.” Ex. 1052 at 8-9.

The District Court, applying the different claim construction standard set forth in *Phillips v. A WH Corp.*, 415 F.3d 1303, 1312 (Fed. Cir. 2005), interpreted the term “providing conditions

suitable for the reaction” (Ex. 1054 at 4) as “providing a set of physical and chemical conditions that allow the autocatalytic reaction to occur.” Ex. 1055 at 1. The District Court similarly found that the term “reaction” was not limited by the specification to any particular type of reaction. Ex. 1054 at 10-11.

D. “Polymerase Chain Reaction” (Claim 3)

Claim 4 recites that “the autocatalytic reaction is polymerase chain reaction.” The Board did not find it necessary to interpret this term for purposes of its decision. Ex. 1052 at 8-11. The District Court adopted the following definition under *Phillips*:

The Court has determined that the term "polymerase chain reaction" shall be given the following meaning:

“Method of amplifying a target sequence of nucleic acids that involves repeated cycles of DNA replication, wherein 1) strands of DNA are denatured to form single-strand templates; 2) the templates are treated with oligonucleotide primers and a polymerase enzyme is used to extend the primers to produce replicated double-stranded DNA; 3) the replicated DNA then serves as a template for additional replication. The target sequence need not be specifically identified as a target in advance of the reaction.”

Ex. 1057.

VI. STATEMENT POINTING OUT EACH SUBSTANTIAL NEW QUESTION OF PATENTABILITY FOR THE CHALLENGED CLAIMS

A. Summary of the Significant New Questions of Patentability

As discussed in Section IV.C, above, the prosecution history taken as a whole indicates that the ‘193 patent was allowed primarily because the Examiner believed that the prior art failed

to teach conducting reactions in droplets formed from two continuously flowing immiscible fluids.

As discussed in Section IV.D, above, the PTAB denied institution of an *inter partes* review of U.S. Patent No. 8,304,193 because the petition failed to explain why a skilled artisan would have believed that the PCR technique of Haff could have been conducted on a microfluidic scale as taught in Quake.

Accordingly, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for conducting reactions in droplets formed from two continuously flowing immiscible fluids that could be readily modified to perform the reaction in question.**⁶

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuous flowing immiscible fluids were well known, ii) microfluidic PCR reactions were well known, and iii) there were compelling reasons to modify the microfluidic droplet reactors to conduct continuous flow PCR.

1. Quake, Burns I and Shaw Stewart Show that Droplet Microreactors which Formed Plugs from Continuous Flowing Immiscible Fluids Were Well known

As noted by the Patent Trial and Appeal Board (PTAB), Quake (Ex. 1033) discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids. “Quake’s devices are designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil.” [Ex. 1052 at 11, citing Quake (Ex. 1033) ¶ 3]. “The devices comprise a main channel, through which a pressurized stream of oil is passed, and at

⁶ Requester notes that independent claim generically recites an autocatalytic reaction whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

least one sample inlet channel, through which a pressurized stream of aqueous solution is passed.” *Id.* “A junction joins the main channel with the sample inlet channel.” *Id.* “By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets.” *Id.* Figure 16A of Quake is reproduced below:

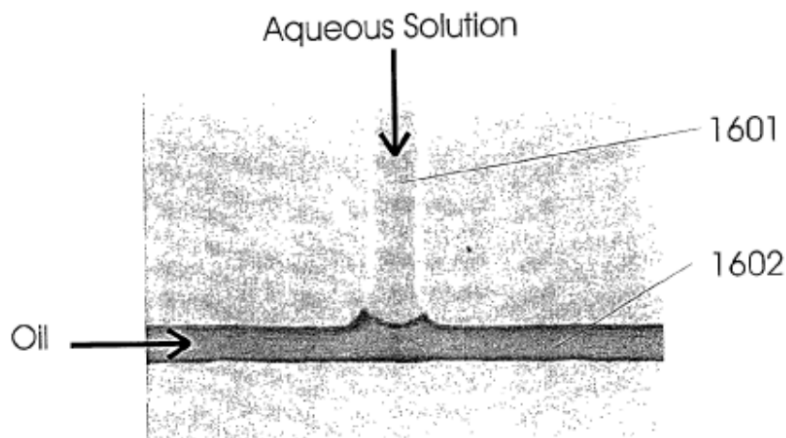


FIG. 16A

“Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil.” *Id.*, citing Quake (Ex. 1033) ¶ 292. The Petitioner asserted that this disclosure in Quake met the plug-formed-by-streams limitations of the ‘193 patent and neither the Patent Owner nor the Board contested that premise. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous streams. When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, p. 15. Accordingly,

notwithstanding statements to the contrary during *ex parte* prosecution of the '193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids.

Another body of work from the University of Newcastle shows that plugs in microchannels also could be readily formed by continuous flow of fluids at an intersecting channel. Burns explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10 (“Burns I”). Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of **one phase flowing into the channel whilst the other phase moves into the intersection**, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 11. [emphasis added]. Fig. 3 shows oil-in-water slug generation and

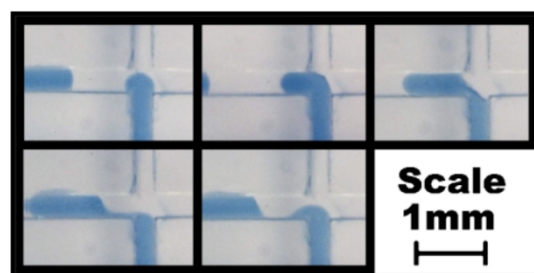


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns was a well-known alternative to periodically injecting a measured slug of reactant into the main channel. See Ex. 1010; 1027; Ex. 1025 at 23:2-8. Burns I demonstrates that making slugs or plugs with continuous flows of immiscible fluids was a mere design choice as of 2001.

The Shaw Stewart British Application also teaches conducting reactions in droplets formed from two continuously flowing immiscible fluids. The Shaw Stewart British Application discloses a “droplet reactor” and illustrates at Figure 1 a carrier phase 1 flowing along a conduit into which reagent 2 is injected, thereby forming droplet 4. Ex. 1040 at 1:1-20, 70-90. “[I]f large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” *Id.* at 1:83-90.

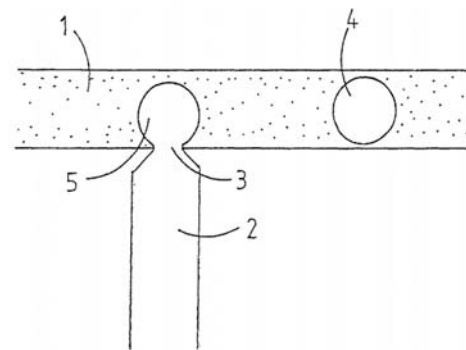


Figure 1.

Significantly, the exclusive licensee of the ‘193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the ‘193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO’s primary arguments was that Stewart I

only disclosed introducing fluid in a “stepwise fashion,” whereas in the ’503 Patent droplets are formed by “combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.” *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

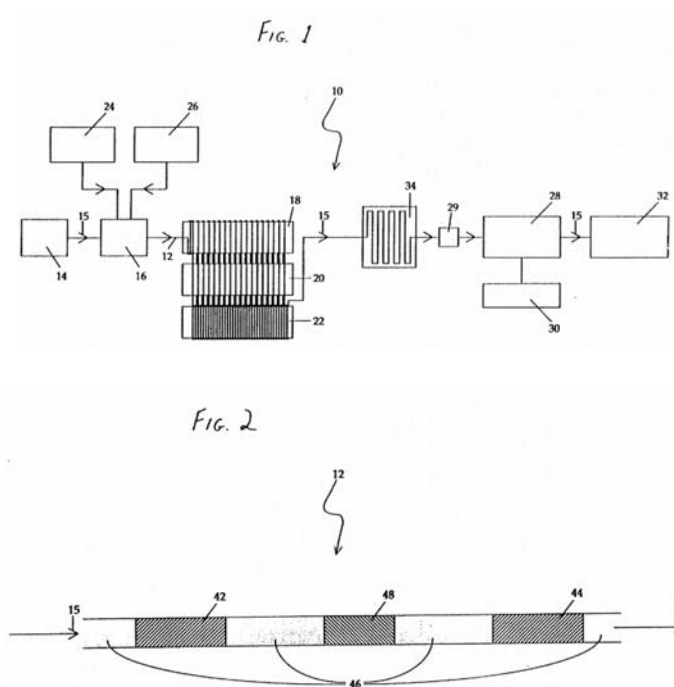
To summarize on the first point, the Shaw Stewart British Application, (Ex. 1040), Quake (Ex. 1034) and Burns I (Ex. 1007) each provide the teaching that the *ex parte* Examiner believed was missing from the prior art: conducting reactions in droplets formed from two continuously flowing immiscible fluids.

2. Corbett, Kopp, Burns II, Lagally and Vogelstein Show that Microfluidic PCR Devices Were Well Known

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett, Kopp, Burns II, Lagally and Vogelstein. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical

laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12.

“Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.



“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

In 1996, Mark A. Burns from the University of Michigan reported the use of microfluidic devices to perform PCR. *Microfabricated structures for integrated DNA analysis*, Proc. Natl. Acad. Sci. USA, May 1996, vol. 93, pp. 5556-5561. (Ex. 1008, Burns II).⁷ Burns II reported that “[n]o new biochemistries or DNA detection methods are required for their use; the components simply reproduce current laboratory procedures in a micron-sized environment.” *Id.* at 5560. In other words, the PCR chemistry did not need to be modified – it worked without modification on the microfluidic scale. In the method of Burns II, a sample containing a DNA plasmid was loaded into one of the reaction wells (A) and a mixture of Taq enzyme and buffer was loaded into the other. *Id.* at 5558. Each formed droplets of about 60 nl in the parallel 500 x 25 μm channels (B) which came together at the Y junction to form a single larger droplet (about 120 nl). *Id.* at 5558-59; Ex. 1004 ¶40.

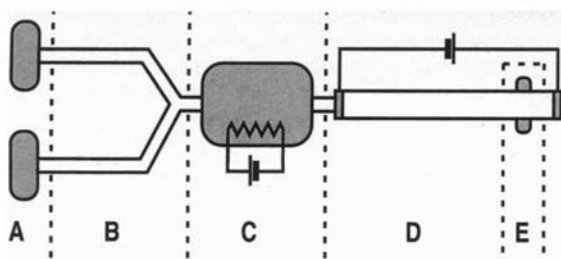


FIG. 1. Example of an integrated DNA analysis system, represented schematically. The individual components of the system are injection entry ports (A), liquid pumping channels (B), thermally controlled reaction chamber (C), electrophoresis channel (D), and DNA band migration detector (E). Each component would have associated sensors, control circuitry, and external connections.

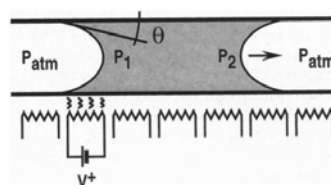


FIG. 2. Schematic drawing showing the principle of thermally induced drop motion in a closed channel. The case of a single aqueous drop in a hydrophilic channel is presented, where V is an applied voltage, P_{atm} is atmospheric pressure, P_1 is the receding-edge internal pressure, P_2 is the advancing-edge internal pressure, and θ is the contact angle of the liquid-gas-solid interface. The contact angle will depend on the surface characteristics of the channel and the constituents of the drop, with a hydrophilic interaction giving θ between 0 and 90 degrees, and a hydrophobic surface giving θ between 90 and 180 degrees. Surface treatments can also reduce contact angle hysteresis and, therefore, reduce the temperature difference necessary for drop motion.

The droplet then passed through the resistive heater portion (C) in which PCR amplification occurred. Ex. 1008 at 5556. Afterwards the amplification results were optically detected in zone D via gel electrophoresis. *Id.* at 5559. Burns II noted that “[t]he use of common fabrication

⁷ This author (Mark Burns) reference should not be confused with J.R. Burns of the University of Newcastle. The latter published a different article relevant to this reexamination request: Burns, J. R. et al., *The Intensification of Rapid Reactions in Multiphase Systems Using Slug Flow in Capillaries*, Lab on a Chip, vol. 1, 2001, pp. 10-15. (“Burns I”, Ex. 1007)

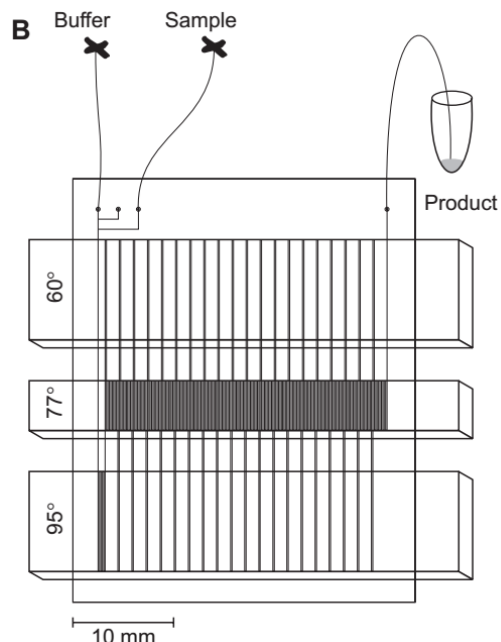
methods allows the assembly of increasingly complex, multicomponent, integrated systems from a small, defined set of standardized elements” and that “[f]uture design efforts have a strong impetus to reduce component size and improve efficiency.” *Id.* at 5560-61.

By 1998 Kopp et al. had successfully implemented continuous flow PCR on a chip. In his 1998 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 [emphasis added].

Kopp et al. also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene

could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 [emphasis added].

In 2001, in another article not previously considered by the Office, Lagally reported using a microfluidic reactor to conduct single-molecule DNA amplification by PCR. Lagally summarized the state of the art as of February 2001, explaining continuous flow PCR microreactors had recently undergone substantial evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-

volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

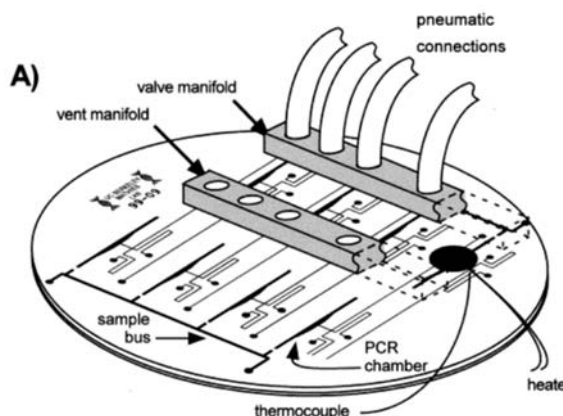
Ex. 1028 at 565-566.

Lagally *et al.* developed a further improvement – a device and method for achieving single molecule amplification in a microchannel environment.

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

Microfluidic PCR-CE device. The PCR chambers are connected to a common sample bus through a set of valves. Hydrophobic

vents at the other end of the PCR chambers are used to locate the sample and to eliminate gas. The PCR chambers are directly connected to the cross channel of the CE system for product injection and analysis. Two aluminum manifolds, one each for the vents and valves, are placed onto the respective ports and clamped in place using vacuum. The manifolds connect to external solenoid valves for pressure and vacuum actuation. Thermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.



Ex. 1028 at 566 (see figure legend).

By 1999, Vogelstein had reported **various applications for single-molecule microfluidic PCR**. Ex. 1044 at 9241. In a seminal article which has been cited over by over 1,000 other publications, Vogelstein explained that PCR amplification of small aliquots or droplets containing single molecules was effective for “detection of a small number of mutant-containing cells among a large excess of normal cells.” *Id.* at 9236. In Vogelstein’s method, the DNA was diluted into 7 μ l volumes such that, on average, there was one template molecule for every two wells (i.e., approximately half of the wells contained no DNA template molecule and approximately half contained a single DNA template molecule). *Id.* at 9236, 9237. This avoided the problem of background noise impairing the determination of whether each volume contained the mutant sequence. *Id.* By counting the number of volumes which indicated wild type or mutant templates, the presence or absence of the mutation (e.g., a sequence associated with colorectal cancer) can be determined. *Id.* at 9238. The accuracy of the test is a function of the number of discrete volumes into which the sample is diluted. *Id.* at 9239. Vogelstein taught that

diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) The article explained that the same technique would be useful for **detecting a wide array of mutations, dislocations and allelic imbalances**, as summarized in Table 1. *Id.* at 9241. Vogelstein thus taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby overcoming the signal-to-noise limitations associated with certain other PCR techniques and enabling the detection of relatively rare mutations, dislocations and allelic imbalances. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86.

In sum, Corbett, Kopp, Burns II, Lagally and Vogelstein show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

3. There Were Strong Motivations to Modify the Microfluidic Droplet Reactors of Quake, Burns I or Shaw Stewart to Conduct Microfluidic PCR as Taught by Corbett, Kopp, Burns II, Lagally or Vogelstein

In the combinations proffered herein, the microfluidic droplet reactors of Quake, Burns I and Shaw Stewart are relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two

channels”).⁸ Ex. 1001 at 78:12-15 and, in the cases of Burns I and Shaw Stewart, the reaction is autocatalytic. In the proffered combinations, these limitations are met by using the microfluidic droplet reactors that utilize two continuously flowing immiscible fluids to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, Burns I or Shaw Stewart to conduct microfluidic PCR as taught by Corbett, Kopp, Burns II, Lagally or Vogelstein. *Id.* ¶¶72-82. Stated a different way, a skilled artisan would have considered it obvious to miniaturize and modify the PCR apparatus of Corbett by modifying it to incorporate the microfluidic reactor using two continuously flowing immiscible fluids of Quake, Burns or Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein’s contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Quake **specifically suggests that his microfluidic device could be used to perform PCR**. Quake explained that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR...” Ex. 1034 at 23:26-30. Quake noted that “PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen.” *Id.* at 2:31-3:2.

Further, it was **well known that PCR could be performed in plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported

⁸ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”)

More generally, a skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactors of Quake, Burns I or Shaw Stewart because doing so would have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed**.” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost**.” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions, and using two continuous streams as opposed to injecting the

aqueous fluid into the immiscible oil, would have reduced the amounts of reagents used, which would in turn **decrease operating costs**. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall process. Ex. 1004 ¶47; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the **substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing)**. Ex. 1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Quake, Burns or Shaw Stewart to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing a PCR reaction in the microfluidic droplet reactors of Quake, Burns I or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the

thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241;

Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. “It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.” Ex. 1044 at 9239. The Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Corbett, Kopp, Burns II, Lagally or Vogelstein) to incorporate a droplet reactor (Quake, Burns I or Shaw Stewart). Ex. 1004 ¶107. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the microfluidic PCR devices taught in Kopp, Burns II, Lagally or Vogelstein. The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II or Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease

contamination and increase the number of reactors that can be created on a given chip.

Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk

heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-566.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with

microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching**

whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

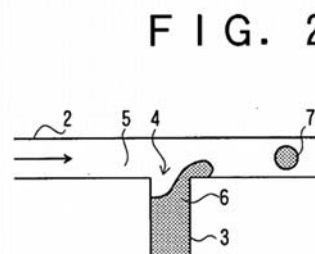
Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Kopp or Lagally to work in the microfluidic droplet reactor of Quake, Burns or Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation limitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁹

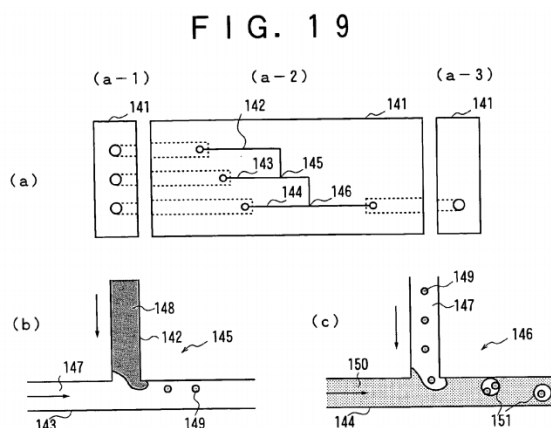
⁹ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so

Higuchi I discloses a microreactor in which a dispersion phase (6) is ejected from a dispersion phase feeding port (4) toward a continuous phase (5) flowing in a microchannel (2) in such a manner that flows of the dispersion phase (6) and the continuous phase (5) cross each other, thereby obtaining microdroplets (7), formed by the

shear force of the continuous phase (5), having a size smaller than the width of the channel for feeding the dispersion phase (6). Ex. 1018 at Fig. 2 (reproduced at right). Higuchi teaches



many variations of the embodiment of Figure 2 and one representative variation is shown in Fig. 19.



In [Figures 19a-c], reference numeral 141 represents a main body of the microcapsule-forming apparatus, reference numeral 142 represents a microchannel in which a dispersion phase (for example, water) flows, reference numeral 143 represents a microchannel in which a first continuous phase (for example, oil) flows, reference numeral 144 represents a microchannel in which a second continuous phase (for example, water) flows, reference numeral 145 represents the first junction at which flows of the dispersion phase and the first continuous phase are joined together,

ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

reference numeral 146 represents the second junction at which flows of the dispersion phase, the first continuous phase, and the second continuous phase are joined together, reference numeral 147 represents the first continuous phase, reference numeral 148 represents the dispersion phase, reference numeral 149 represents an emulsion (for example, water), reference numeral 150 represents the second continuous phase, and reference numeral 151 represents formed microcapsules. The microcapsules 151 can contain one or more emulsions 149. Numeral 143 represents a microchannel in which a first continuous phase (for example, oil) flows, reference numeral 144 represents a microchannel in which a second continuous phase (for example, water) flows, reference numeral 145 represents the first junction at which flows of the dispersion phase and the first continuous phase are joined together, reference numeral 146 represents the second junction at which flows of the dispersion phase, the first continuous phase, and the second continuous phase are joined together, reference numeral 147 represents the first continuous phase, reference numeral 148 represents the dispersion phase, reference numeral 149 represents an emulsion (for example, water), reference numeral 150 represents the second continuous phase, and reference numeral 151 represents formed microcapsules. The microcapsules 151 can contain one or more emulsions 149.

Ex. 1018 ¶100.

The microchannel device of Higuchi I-III was specifically intended to be used to perform emulsion-based chemical reactions. Ex. 1004 ¶56; Ex. 1043 (contemporaneous article by Higuchi entitled *Chemical reactions in microdroplets by electrostatic manipulation of droplets in liquid media*). Higuchi I-III thus demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, Burns I or Shaw Stewart to conduct microfluidic PCR as taught by Corbett, Kopp, Burns II or Lagally. Ex. 1004 ¶¶72-82.

Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR

apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Quake, Burns or Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.*

B. The Combination of Quake, Corbett and Lagally Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Section IV.C, above, the prosecution history taken as a whole indicates that the '193 patent was allowed primarily because the Examiner believed that the prior art failed to teach conducting reactions in droplets formed from two continuously flowing immiscible fluids.

As discussed in Section IV.D, above, the PTAB denied institution of an *inter partes* review of U.S. Patent No. 8,304,193 because the petition failed to explain why a skilled artisan would have believed that the PCR technique of Haff could have been conducted on a microfluidic scale as taught in Quake.

Accordingly, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for conducting autocatalytic reactions in droplets formed from two continuously flowing immiscible fluids that could be readily modified to perform the reaction in question.**¹⁰

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Quake), ii) microfluidic PCR devices were well known (Corbett and Lagally), and iii) there were compelling reasons to use the

¹⁰ Requester notes that independent claim generically recites a reaction involving a biological molecule whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

microfluidic droplet reactors to conduct microfluidic PCR (Lagally, Kopp, Burns II and Vogelstein).

As to the first feature – forming plugs from continuously flowing immiscible fluids – the ‘193 patent’s teachings in this regard were copied almost verbatim from the Quake PCT (Ex. 1034). The table below compares the Quake PCT publication with the Ismagilov specification, which was filed about two months later.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and ‘193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	Provisional at 22:12-19, ‘193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

Attached as Ex. 1035 is a table showing the numerous passages which Ismagilov copied key disclosures verbatim or nearly verbatim from the Quake PCT. These were not merely background or “state of the art” descriptions; rather, they go to the heart of the claimed subject matter.

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake (Ex. 1033) discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

Quake's devices are designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [Ex. 1052 at 11, citing Quake Ex. 1033 at ¶ 3]....

The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* A junction joins the main channel with the sample inlet channel. *Id.* By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

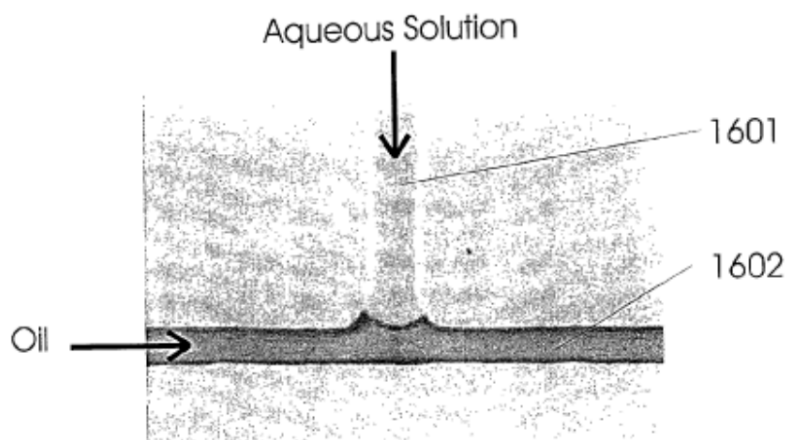


FIG. 16A

Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil.

In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR. *Id.* ¶ 80.

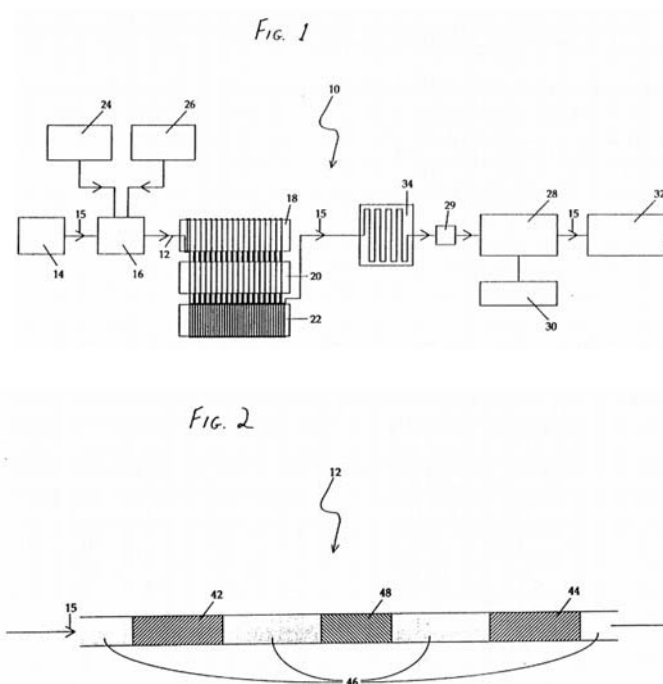
Ex. 1052 at 11-14.

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by-streams limitations of the '193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous streams.¹¹ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the '193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids.

Turning the second issue, as of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Lagally. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream

¹¹ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.

includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.



“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

In 2001, in another article not previously considered by the Office, Lagally reported using a single fluid microfluidic reactor to conduct single-molecule DNA amplification by PCR. Lagally summarized the state of the art as of February 2001, explaining continuous flow PCR microreactors had recently undergone substantial evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or **continuous flow** through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with

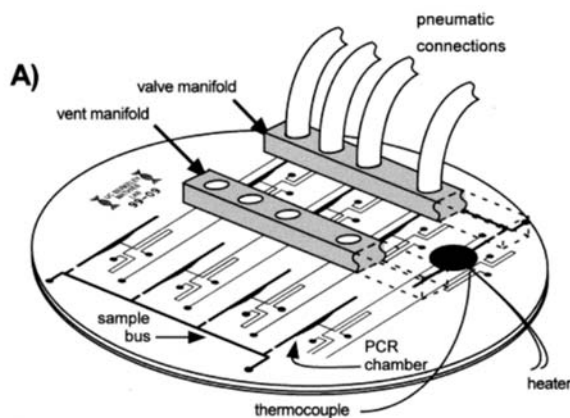
sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-566, [emphasis added].

Lagally *et al.* developed a further improvement – a device and method for achieving single molecule amplification in a microchannel environment.

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

Microfluidic PCR-CE device. The PCR chambers are connected to a common sample bus through a set of valves. Hydrophobic vents at the other end of the PCR chambers are used to locate the sample and to eliminate gas. The PCR chambers are directly connected to the cross channel of the CE system for product injection and analysis. Two aluminum manifolds, one each for the vents and valves, are placed onto the respective ports and clamped in place using vacuum. The manifolds connect to external solenoid valves for pressure and vacuum



actuation. Thermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.

Ex. 1028 at 566 (see figure legend).

In sum, Corbett and Lagally show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitation reciting that the flowing aqueous fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).¹² Ex. 1001 at 78:12-15. In the instant combination, this limitation is met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR in droplets as taught by Corbett, or to modify the reactions of Lagally to be performed in microfluidic droplets instead of continuous flow of a single fluid in a microfluidic channel. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to use two continuous streams of immiscible fluid as used in a microfluidic droplet reactor of Quake, as

¹² Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

evidenced by Lagally's contemporaneous report that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.*

Quake **specifically suggests that his microfluidic device could be used to perform PCR.** Quake explained that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR...” Ex. 1034 at 23:26-27. Quake noted that “PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen.” *Id.* at 2:31-3:2.

More generally, a skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactors of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565 [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556 [emphasis added]. The modification of Corbett to microfluidic dimensions and the use of two continuous streams of immiscible fluids would have reduced the amounts of reagents used, which would in turn **decrease operating costs.** Ex. 1004 ¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Quake to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve

product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86.

Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article

explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Lagally) to incorporate a droplet reactor (Quake) *Id.* Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Lagally. The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow

PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides

an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an

autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. Ex. 1004 ¶91.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Lagally to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹³ As discussed in Section

¹³ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985)

VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Corbett, Kopp, Burns II or Lagally. Ex. 1004 ¶¶72-82. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Quake, as evidenced by Lagally's contemporaneous report that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶48-49.

Not only would a reasonable Examiner consider these teachings relevant in deciding whether the claims of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. See MPEP § 2242. The correspondence between claims 1-14 of the '193 patent and the combined teachings of the Quake, Corbett and Lagally references are set forth in Section VII.A, below.

C. The Combination of Quake, Corbett and Burns II Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Section IV.C and IV.D above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for**

(‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

conducting reactions in droplets formed from two continuously flowing immiscible fluids that could be readily modified to perform the reaction in question.¹⁴

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Quake), ii) microfluidic PCR devices were well known (Corbett and Burns II), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Lagally, Kopp, Burns II and Vogelstein).

As to the first feature – forming plugs from continuously flowing immiscible fluids – the ‘193 patent’s teachings in this regard were copied almost verbatim from the Quake PCT (Ex. 1034). The table below compares the Quake PCT publication with the Ismagilov specification, which was filed about two months later.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and ‘193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	Provisional at 22:12-19, ‘193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

¹⁴ Requester notes that independent claim generically recites a reaction involving a biological molecule whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

Attached as Ex. 1035 is a table showing the numerous passages which Ismagilov copied key disclosures verbatim or nearly verbatim from the Quake PCT. These were not merely background or “state of the art” descriptions; rather, they go to the heart of the claimed subject matter.

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake (Ex. 1033) discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

Quake relates to microfluidic “devices designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [citing Quake ‘332 Ex. 1033 at ¶ 3]. The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* A junction joins the main channel with the sample inlet channel. *Id.* By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

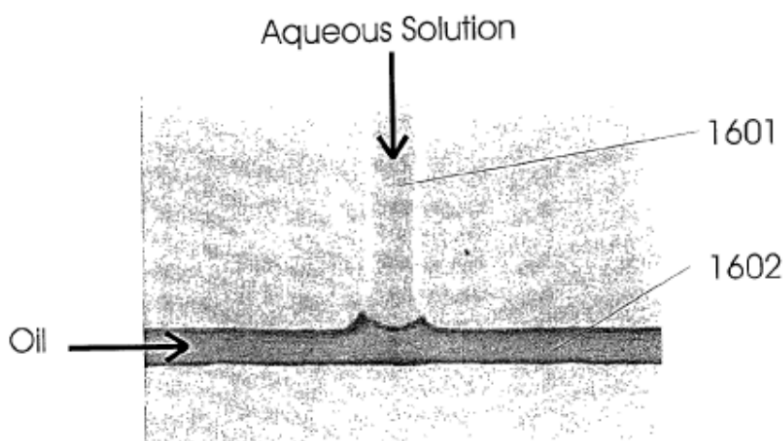


FIG. 16A

Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil. [Ex. 1052 at 12 citing Quake (Ex. 1033) ¶ 292]

In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* ¶ 80.

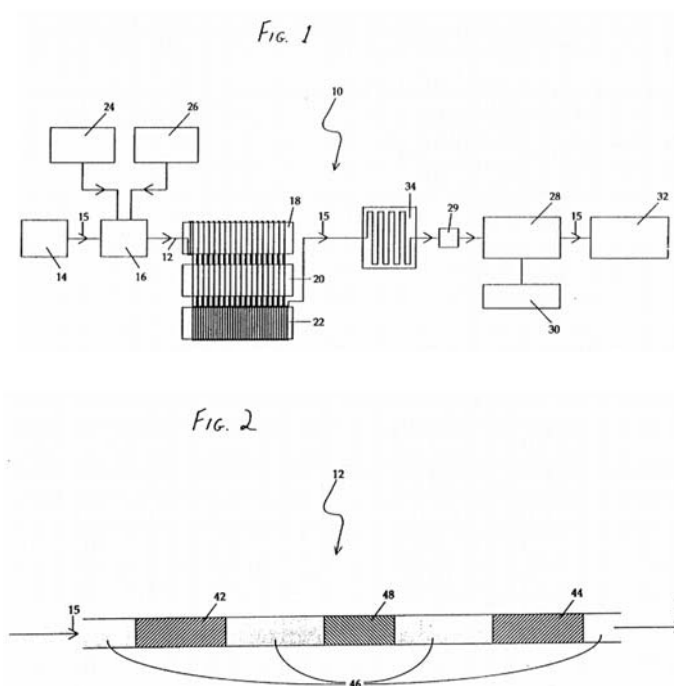
Ex. 1052 at 11-14.

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by-streams limitations of the ‘193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous streams.¹⁵ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the ‘193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids.

Turning the second issue, as of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Burns II. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences

¹⁵ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.

capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.



“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

In 1996, Burns II reported the use of microfluidic devices to perform PCR. Ex. 1008 at 5556. Burns II explained that “[n]o new biochemistries or DNA detection methods are required for their use; the components simply reproduce current laboratory procedures in a micron-sized environment.” *Id.* at 5560. In other words, the PCR chemistry did not need to be modified – it worked without modification on the microfluidic scale. In the method of Burns II, a sample containing a DNA plasmid was loaded into one of the reaction wells (A) and a mixture of Taq enzyme and buffer was loaded into the other. Ex. 1008 at 5558. Each formed droplets of about 60 nl in the parallel 500 x 25 μm channels (B) which came together at the Y junction to form a single larger droplet (about 120 nl). *Id.* at 5558-59; Ex. 1004 ¶40.

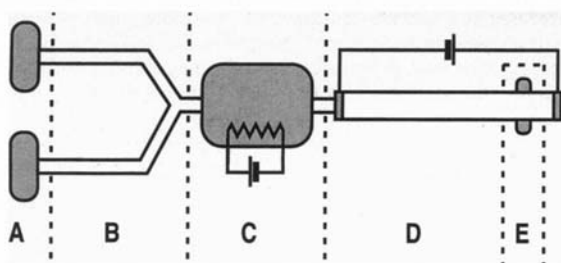


FIG. 1. Example of an integrated DNA analysis system, represented schematically. The individual components of the system are injection entry ports (A), liquid pumping channels (B), thermally controlled reaction chamber (C), electrophoresis channel (D), and DNA band migration detector (E). Each component would have associated sensors, control circuitry, and external connections.

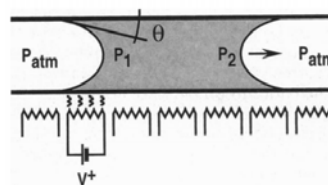


FIG. 2. Schematic drawing showing the principle of thermally induced drop motion in a closed channel. The case of a single aqueous drop in a hydrophilic channel is presented, where V is an applied voltage, P_{atm} is atmospheric pressure, P_1 is the receding-edge internal pressure, P_2 is the advancing-edge internal pressure, and θ is the contact angle of the liquid-gas-solid interface. The contact angle will depend on the surface characteristics of the channel and the constituents of the drop, with a hydrophilic interaction giving θ between 0 and 90 degrees, and a hydrophobic surface giving θ between 90 and 180 degrees. Surface treatments can also reduce contact angle hysteresis and, therefore, reduce the temperature difference necessary for drop motion.

The droplet then passed through the resistive heater portion (C) in which PCR amplification occurred. *Id.* at 5556-5557. Afterwards the amplification results were optically detected in zone D via gel electrophoresis. *Id.* at 5557-5561. Burns II noted that “[t]he use of common fabrication methods allows the assembly of increasingly complex, multicomponent, integrated systems from a small, defined set of standardized elements” and that “[f]uture design efforts have a strong impetus to reduce component size and improve efficiency.” *Id.* at 5560-61.

In sum, Corbett and Burns II show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").¹⁶ Ex. 1001 at 78:12-15. In the instant combination, this limitation is met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Corbett and Burns II. *Id.* Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Quake, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.*

Quake **specifically suggests that his microfluidic device could be used to perform PCR.** Quake explained that "[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR..." Ex. 1034 at 23:26-30. Quake noted that "PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen." *Id.* at 2:31-3:2.

¹⁶ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

More generally, a skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactor of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Quake to perform

PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett's PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample

leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239 (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* 9239. Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Burns II) to incorporate a droplet reactor (Quake). Ex. 1004 ¶¶92-109. Using two flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Burns II. *Id.* The use of two flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents

needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In

1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Ex. 1004 ¶¶39-57. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. *Id.*

As further evidence that a skilled artisan would have expected to be successful in modifying Burns II to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation limitation

of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹⁷ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Corbett and Burns II. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Quake, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

¹⁷ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See id. at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. *See* MPEP § 2242. The correspondence between the claims of the '193 patent and the combined teachings of the Quake, Corbett and Burns II are set forth in Section VII.B, below.

D. The Combination of Quake, Corbett and Vogelstein Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Section IV.C and IV.D above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for conducting reactions in droplets formed from two flowing immiscible fluids that could be readily modified to perform the reaction in question.**¹⁸

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from flowing immiscible fluids were well known (Quake), ii) microfluidic PCR devices were well known (Corbett and Vogelstein), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Lagally, Kopp and Vogelstein).

As to the first feature – forming plugs from continuously flowing immiscible fluids – the '193 patent's teachings in this regard were copied almost verbatim from the Quake PCT (Ex. 1034). The table below compares the Quake PCT publication with the Ismagilov specification, which was filed about two months later.

Quake PCT (published March 21, 2002 (Ex. 1034)	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and '193 patent (Ex. 1001)
34:32-35:7	Provisional at 22:12-19, '193 patent at Ex. 1001 at 21:56-65

¹⁸ Requester notes that independent claim generically recites a reaction involving a biological molecule whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

<u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	<u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

Attached as Ex. 1035 is a table showing the numerous passages which Ismagilov copied key disclosures verbatim or nearly verbatim from the Quake PCT. These were not merely background or “state of the art” descriptions; rather, they go to the heart of the claimed subject matter.

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake (Ex. 1033) discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

Quake’s devices are designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [Ex. 1052 at 11, citing Quake Ex. 1033 at ¶ 3]. The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* A junction joins the main channel with the sample inlet channel. *Id.* By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

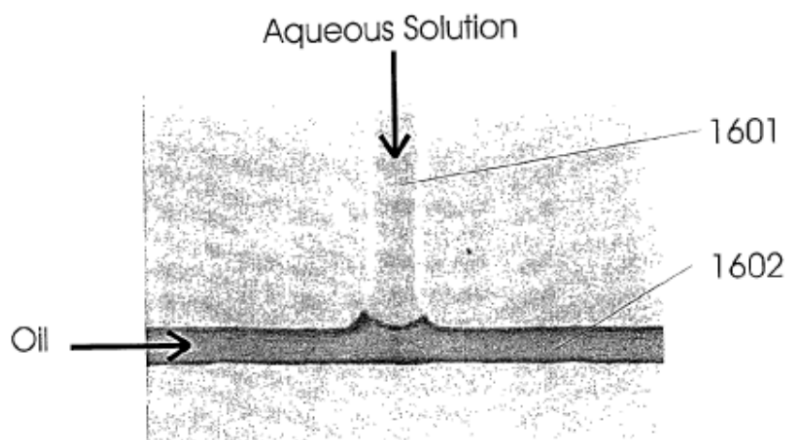


FIG. 16A

Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil. *Id.* [citing Quake (Ex. 1033) ¶ 292].

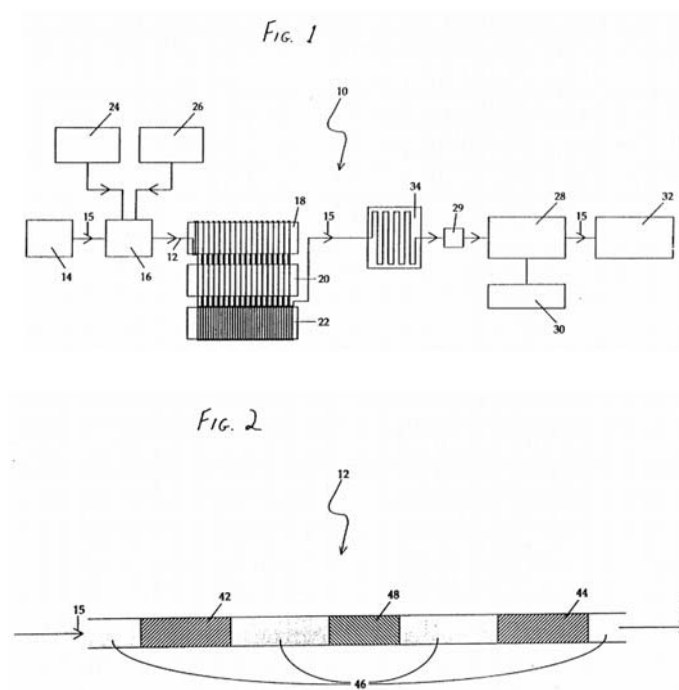
In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* ¶ 80.

Ex. 1052 at 11-14.

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by-streams limitations of the ‘193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous streams.¹⁹ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the ‘193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids.

¹⁹ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.

Turning the second issue, as of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Vogelstein. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.



“The pump is actuated and the reaction mixture of 20 µl or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

By 1999, Vogelstein had reported **various applications for single-molecule microfluidic PCR**. Ex. 1044 at 9241. In a seminal article which has been cited over by over 1,000 other publications, Vogelstein explained that PCR amplification of small aliquots or droplets containing single molecules was effective for “detection of a small number of mutant-containing cells among a large excess of normal cells.” *Id.* at 9236. In Vogelstein’s method, the DNA was diluted into 7 µl volumes such that, on average, there was one template molecule for every two wells (i.e., approximately half of the wells contained no DNA template molecule and approximately half contained a single DNA template molecule). *Id.* at 9236, 9237. This avoided the problem of background noise impairing the determination of whether each volume contained the mutant sequence. *Id.* By counting the number of volumes which indicated wild type or mutant templates, the presence or absence of the mutation (e.g., a sequence associated with colorectal cancer) can be determined. *Id.* at 9238. The accuracy of the test is a function of the number of discrete volumes into which the sample is diluted. *Id.* at 9239. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the

sensitivity by another order of magnitude.”) The article explained that the same technique would be useful for **detecting a wide array of mutations, dislocations and allelic imbalances**, as summarized in Table 1. *Id.* at 9241. Vogelstein thus taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby overcoming the signal-to-noise limitations associated with certain other PCR techniques and enabling the detection of relatively rare mutations, dislocations and allelic imbalances. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86.

In sum, Corbett and Vogelstein show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing fluid with the reagent also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).²⁰ Ex. 1001 at 78:12-15. In the instant combination, this limitation is met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Corbett and Vogelstein. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR

²⁰ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Quake, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.*

¶¶92-109.

Quake **specifically suggests that his microfluidic device could be used to perform PCR.** Quake explained that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR...” Ex. 1034 at 23:26-30. Quake noted that “PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen.” *Id.* at 2:31-3:2.

More generally, a skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactor of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, emphasis added. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex. 1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Quake to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve

product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.*

Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Vogelstein) to incorporate a droplet reactor (Quake). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to the droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to

perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-

volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the

development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with

the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Vogelstein to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.²¹ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

²¹ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Corbett and Vogelstein. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Quake, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. See MPEP § 2242. The correspondence between the claims of the '193 patent and the combined teachings of the Quake, Corbett and Vogelstein are set forth in Section VII.C, below.

E. The Combination of Quake, Kopp and Lagally Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Sections IV.C and IV.D, above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for conducting reactions in droplets formed from two flowing immiscible fluids that could be readily modified to perform the reaction in question.**²²

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Quake), ii) microfluidic PCR devices were well known (Kopp and Lagally), and iii) there were compelling reasons to use the

²² Requester notes that independent claim generically recites a reaction involving a biological molecule whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

As to the first feature – forming plugs from continuously flowing immiscible fluids – the ‘193 patent’s teachings in this regard were copied almost verbatim from the Quake PCT (Ex. 1034). The table below compares the Quake PCT publication with the Ismagilov specification, which was filed about two months later.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and ‘193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	Provisional at 22:12-19, ‘193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

Attached as Ex. 1035 is a table showing the numerous passages which Ismagilov copied key disclosures verbatim or nearly verbatim from the Quake PCT. These were not merely background or “state of the art” descriptions; rather, they go to the heart of the claimed subject matter.

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake (Ex. 1033) discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

“Quake’s devices are designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [Ex. 1052 at 11, citing Quake (Ex. 1033) ¶ 3]. The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* A junction joins the main channel with the sample inlet channel. *Id.* By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

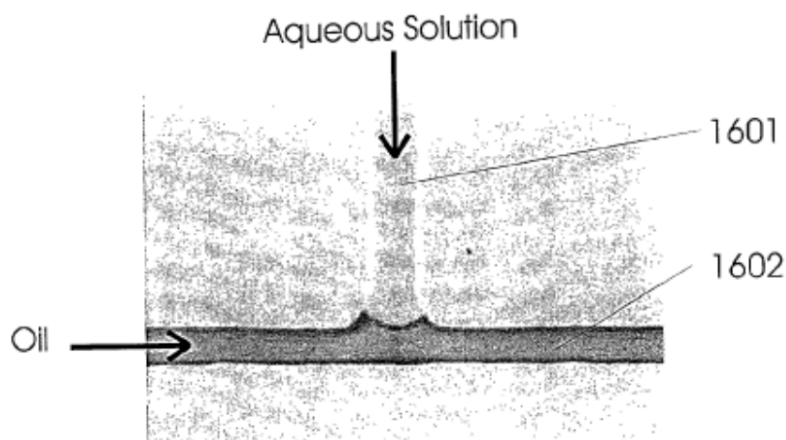


FIG. 16A

Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil. [Ex.1052 at 12 citing Quake (Ex. 1033) ¶ 292]. In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* ¶ 80.

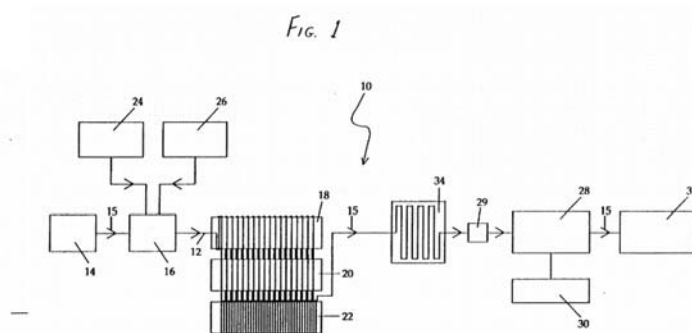
Ex. 1052 at 11-14.

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by-streams limitations of the '193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous streams.²³ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the '193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids.

Turning the second issue, as of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Lagally. By 1998 Kopp *et al.* had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed.

The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input

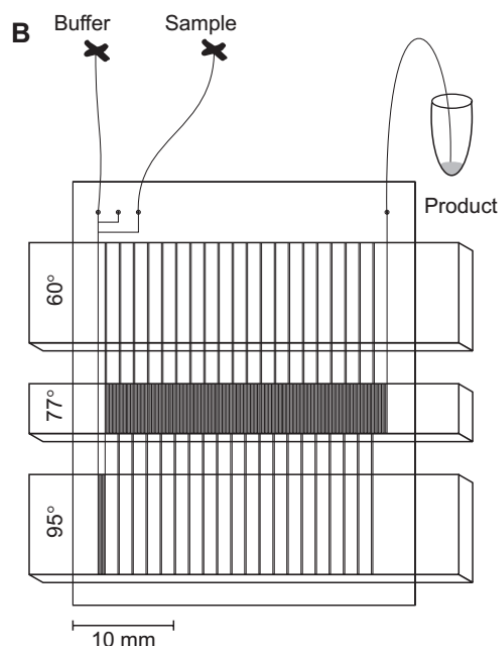


²³ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.

and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 [emphasis added].

Kopp *et al.* also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis

systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 [emphasis added].

In 2001, in another article not previously considered by the Office, Lagally reported using a microfluidic reactor to conduct single-molecule DNA amplification by PCR. Lagally summarized the state of the art as of February 2001, explaining continuous flow PCR microreactors had recently undergone substantial evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize

noncontact methods¹⁶ or **continuous flow** through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

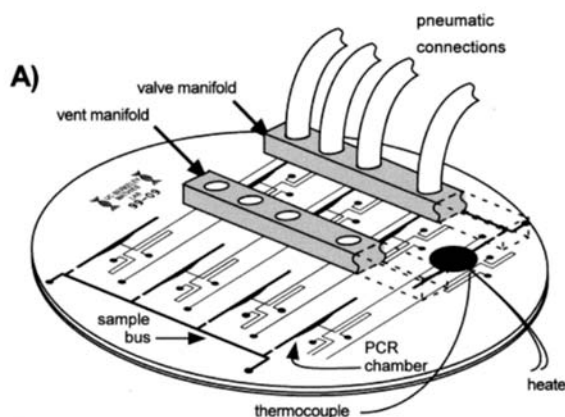
Ex. 1028 at 565-566, [emphasis added].

Lagally *et al.* developed a further improvement – a device and method for achieving single molecule amplification in a microchannel environment.

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson

distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

Microfluidic PCR-CE device. The PCR chambers are connected to a common sample bus through a set of valves. Hydrophobic vents at the other end of the PCR chambers are used to locate the sample and to eliminate gas. The PCR chambers are directly connected to the cross channel of the CE system for product injection and analysis. Two aluminum manifolds, one each for the vents and valves, are placed onto the respective ports and clamped in place using vacuum. The manifolds connect to external solenoid valves for pressure and vacuum actuation. Thermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.



Ex. 1028 at 565-66 (see figure legend).

In sum, Kopp and Lagally show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an

autocatalytic reaction through a first channel of the at least two channels”).²⁴ Ex. 1001 at 78:12-15. In the instant combination, this limitation is met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

Quake **specifically suggests that his microfluidic device could be used to perform PCR.** Quake explained that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR...” Ex. 1034 at 23:26-30. Quake noted that “PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen.” *Id.* at 2:31-3:2.

More generally, a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Kopp and Lagally. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions

²⁴ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047 .It was thus known that using the droplet reactor of Quake to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler

is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed

sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp, Burns II, Lagally or Vogelstein) to incorporate a droplet reactor (Quake, Burns I or Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Lagally. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful.**

In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” Ex. 1027 at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually

transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an

autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp or Lagally to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.²⁵ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that

²⁵ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985)

the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Kopp and Lagally.

Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. See MPEP § 2242. The correspondence between the claims of the '193 patent and the combined teachings of the Quake, Kopp and Lagally are set forth in Section VII.D, below.

F. The Combination of Quake, Kopp and Burns II Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Sections IV.C and IV.D, above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for conducting reactions in droplets formed from two flowing immiscible fluids that could be readily modified to perform the reaction in question.**²⁶

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Quake), ii) microfluidic PCR devices were well known (Kopp and Burns II), and iii) there were compelling reasons to use the

(‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

²⁶ Requester notes that independent claim generically recites a reaction involving a biological molecule whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

As to the first feature – forming plugs from continuously flowing immiscible fluids – the ‘193 patent’s teachings in this regard were copied almost verbatim from the Quake PCT (Ex. 1034). The table below compares the Quake PCT publication with the Ismagilov specification, which was filed about two months later.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and ‘193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	Provisional at 22:12-19, ‘193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

Attached as Ex. 1035 is a table showing the numerous passages which Ismagilov copied key disclosures verbatim or nearly verbatim from the Quake PCT. These were not merely background or “state of the art” descriptions; rather, they go to the heart of the claimed subject matter.

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake (Ex. 1033) discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

“Quake’s devices are designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [Ex. 1052 at 11, citing Quake Ex. 1033 at ¶ 3].

The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* A junction joins the main channel with the sample inlet channel. *Id.* By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

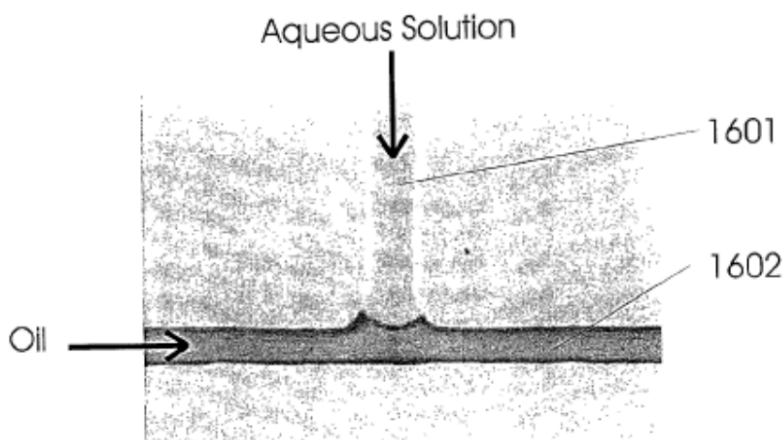


FIG. 16A

Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil. [Ex. 1052 at 12 citing Quake (Ex. 1033) ¶ 292].

In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* ¶ 80.

Ex. 1052 at 11-14.

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by-streams limitations of the '193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous streams.²⁷ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the '193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids. Turning the second issue, as of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Burns II.

In 1996, Burns II reported the use of microfluidic devices to perform PCR. Ex. 1008 at 5556. Burns II explained that “[n]o new biochemistries or DNA detection methods are required for their use; the components simply reproduce current laboratory procedures in a micron-sized environment.” *Id.* at 5560. In other words, the PCR chemistry did not need to be modified – it worked without modification on the microfluidic scale. In the method of Burns II, a sample containing a DNA plasmid was loaded into one of the reaction wells (A) and a mixture of Taq enzyme and buffer was loaded into the other. Ex. 1008 at 5558. Each formed droplets of about 60 nl in the parallel 500 x 25 μm channels (B) which came together at the Y junction to form a single larger droplet (about 120 nl). *Id.* at 5558-59; Ex. 1004 ¶40.

²⁷ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.

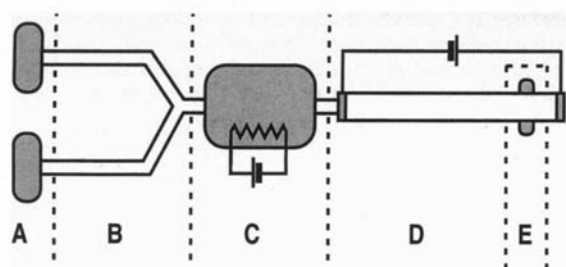


FIG. 1. Example of an integrated DNA analysis system, represented schematically. The individual components of the system are injection entry ports (A), liquid pumping channels (B), thermally controlled reaction chamber (C), electrophoresis channel (D), and DNA band migration detector (E). Each component would have associated sensors, control circuitry, and external connections.

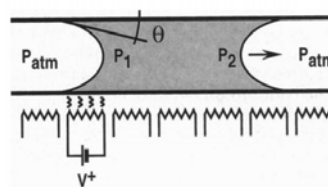


FIG. 2. Schematic drawing showing the principle of thermally induced drop motion in a closed channel. The case of a single aqueous drop in a hydrophilic channel is presented, where V is an applied voltage, P_{atm} is atmospheric pressure, P_1 is the receding-edge internal pressure, P_2 is the advancing-edge internal pressure, and θ is the contact angle of the liquid-gas-solid interface. The contact angle will depend on the surface characteristics of the channel and the constituents of the drop, with a hydrophilic interaction giving θ between 0 and 90 degrees, and a hydrophobic surface giving θ between 90 and 180 degrees. Surface treatments can also reduce contact angle hysteresis and, therefore, reduce the temperature difference necessary for drop motion.

The droplet then passed through the resistive heater portion (C) in which PCR amplification occurred. *Id.* at 5556-5557. Afterwards the amplification results were optically detected in zone D via gel electrophoresis. *Id.* at 5557-5561. Burns II noted that “[t]he use of common fabrication methods allows the assembly of increasingly complex, multicomponent, integrated systems from a small, defined set of standardized elements” and that “[f]uture design efforts have a strong impetus to reduce component size and improve efficiency.” *Id.* at 5560-61.

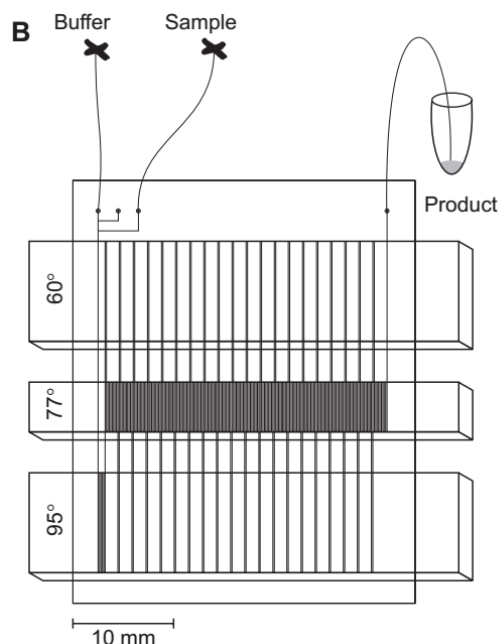
By the late 1990s Kopp *et al.* had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product

quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 [emphasis added].

Kopp *et al.* also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been

presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 [emphasis added].

In sum, Kopp and Burns II show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").²⁸ Ex. 1001 at 78:12-15. In the instant combination, this limitation is met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

Quake **specifically suggests that his microfluidic device could be used to perform PCR**. Quake explained that "[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR..." Ex. 1034 at 23:26-30. Quake noted that "PCR is [] generally the method of choice to detect viral DNA or RNA directly in

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clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen.” *Id.* at 2:31-3:2.

More generally, a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, to conduct microfluidic PCR as taught by Kopp and Burns II. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactor of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶¶92-100; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in

development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047 .It was thus known that using the droplet reactor of Quake to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Burns II Lagally or Vogelstein) to incorporate a droplet reactor (Quake, Burns I or Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Burns II. Id. The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers,

continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” Ex. 1027 at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with

polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic

diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with

the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp or Lagally to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.²⁹ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

²⁹ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Kopp and Burns II.

Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. See MPEP § 2242. The correspondence between the claims of the '193 patent and the combined teachings of the Quake, Kopp and Burns II are set forth in Section VII.E, below.

G. The Combination of Quake, Kopp and Vogelstein Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Sections IV.C and IV.D, above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for conducting reactions in droplets formed from two continuously flowing immiscible fluids that could be readily modified to perform the reaction in question.**³⁰

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Quake), ii) microfluidic PCR devices were well known (Kopp and Vogelstein), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

³⁰ Requester notes that independent claim generically recites a reaction involving a biological molecule whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

As to the first feature – forming plugs from continuously flowing immiscible fluids – the ‘193 patent’s teachings in this regard were copied almost verbatim from the Quake PCT (Ex. 1034). The table below compares the Quake PCT publication with the Ismagilov specification, which was filed about two months later.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and ‘193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	Provisional at 22:12-19, ‘193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

Attached as Ex. 1035 is a table showing the numerous passages which Ismagilov copied key disclosures verbatim or nearly verbatim from the Quake PCT. These were not merely background or “state of the art” descriptions; rather, they go to the heart of the claimed subject matter.

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

“Quake’s devices are designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil [Ex. 1052 at 11, citing Quake

(Ex. 1033) ¶ 3]. The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* A junction joins the main channel with the sample inlet channel. *Id.* By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

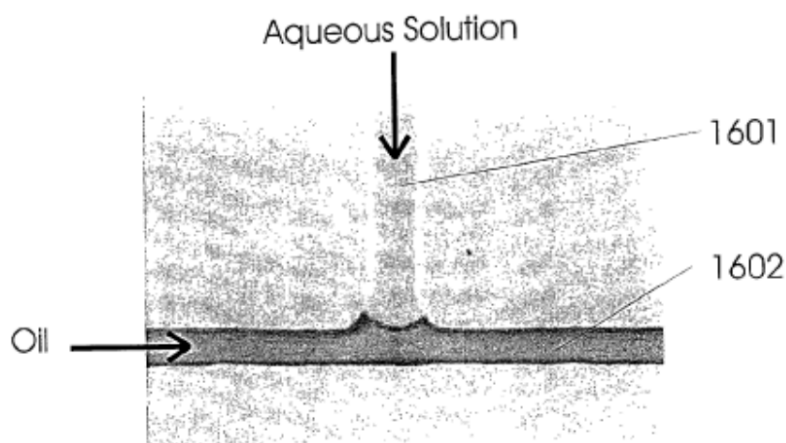


FIG. 16A

Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil. [Ex. 1052 at 12 citing Quake ¶ 292].

In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* ¶ 80.

Ex. 1052 at 11-14.

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by-streams limitations of the ‘193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous

streams.³¹ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the '193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids.

Turning the second issue, as of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Vogelstein. By the late 1990s Kopp *et al.* had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

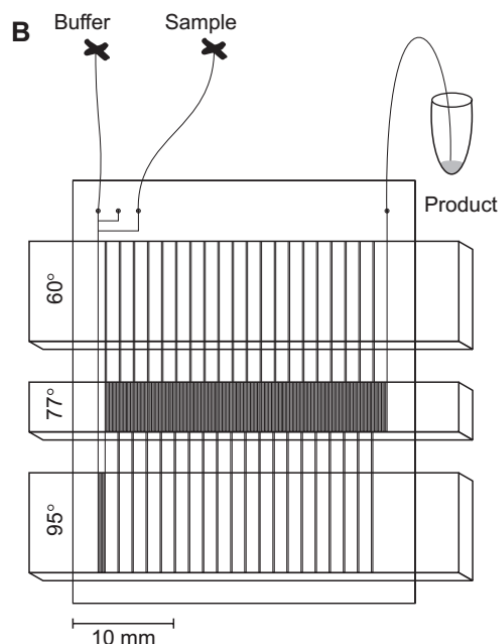
[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without

³¹ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.

major cleaning. . . .



Ex. 1027 at 1046-1047 [emphasis added].

Kopp *et al.* also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene

could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 [emphasis added].

By 1999, Vogelstein had reported **various applications for single-molecule microfluidic PCR**. Ex. 1044 at 9241. In a seminal article which has been cited over by over 1,000 other publications, Vogelstein explained that PCR amplification of small aliquots or droplets containing single molecules was effective for “detection of a small number of mutant-containing cells among a large excess of normal cells.” *Id.* at 9236. In Vogelstein's method, the DNA was diluted into 7 μ l volumes such that, on average, there was one template molecule for every two wells (i.e., approximately half of the wells contained no DNA template molecule and approximately half contained a single DNA template molecule). *Id.* at 9236, 9237. This avoided the problem of background noise impairing the determination of whether each volume contained the mutant sequence. *Id.* By counting the number of volumes which indicated wild type or mutant templates, the presence or absence of the mutation (e.g., a sequence associated with colorectal cancer) can be determined. *Id.* at 9238. The accuracy of the test is a function of the number of discrete volumes into which the sample is diluted. *Id.* at 9239. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) The article explained that the same technique would be useful for **detecting a wide array of mutations, dislocations and allelic imbalances**, as summarized in Table 1. *Id.* at 9241. Vogelstein thus taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby

overcoming the signal-to-noise limitations associated with certain other PCR techniques and enabling the detection of relatively rare mutations, dislocations and allelic imbalances. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86.

In sum, Kopp and Vogelstein show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").³² Ex. 1001 at 78:12-15. In the instant combination, this limitation is met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

Quake **specifically suggests that his microfluidic device could be used to perform PCR.** Quake explained that "[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR..." Ex. 1034 at 23:26-30. Quake noted that "PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen." *Id.* at 2:31-3:2.

More generally, a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, to conduct microfluidic PCR as taught by Kopp and

³² Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

Vogelstein. Ex. 1004 ¶¶88-109. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactor of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. *Id.* As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Quake to perform

PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett's PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample

leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044. at 9239 (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Vogelstein) to incorporate a droplet reactor (Quake). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and

sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In

1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp or Vogelstein to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation

of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.³³ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Kopp and Vogelstein.

Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. See MPEP § 2242. The correspondence between the claims of the '193 patent and the combined teachings of the Quake, Kopp and Vogelstein are set forth in Section VII.F, below.

³³ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

H. The Combination of Shaw Stewart, Corbett and Lagally Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Sections IV.C and IV.D, above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for conducting reactions in droplets formed from two flowing immiscible fluids that could be readily modified to perform the reaction in question.**³⁴

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Shaw Stewart), ii) microfluidic PCR devices were well known (Corbett and Lagally), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

The Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. The Shaw Stewart British Application discloses a “droplet reactor” and illustrates at Figure 1 a carrier phase 1 flowing along a conduit into which reagent 2 is injected, thereby forming droplet 4. Ex. 1040 at 1:1-20, 70-90. “[I]f large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” *Id.* at 1:83-90.

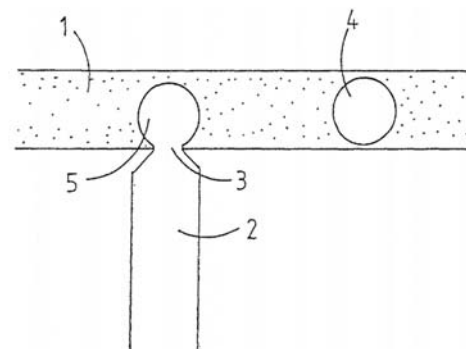


Figure 1.

³⁴ Requester notes that independent claim generically recites a reaction involving a biological molecule whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

Significantly, the exclusive licensee of the '193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the '193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner ("PO") mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO's arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO's primary arguments was that Stewart I only disclosed introducing fluid in a "stepwise fashion," whereas in the '503 Patent droplets are formed by "combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid." *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

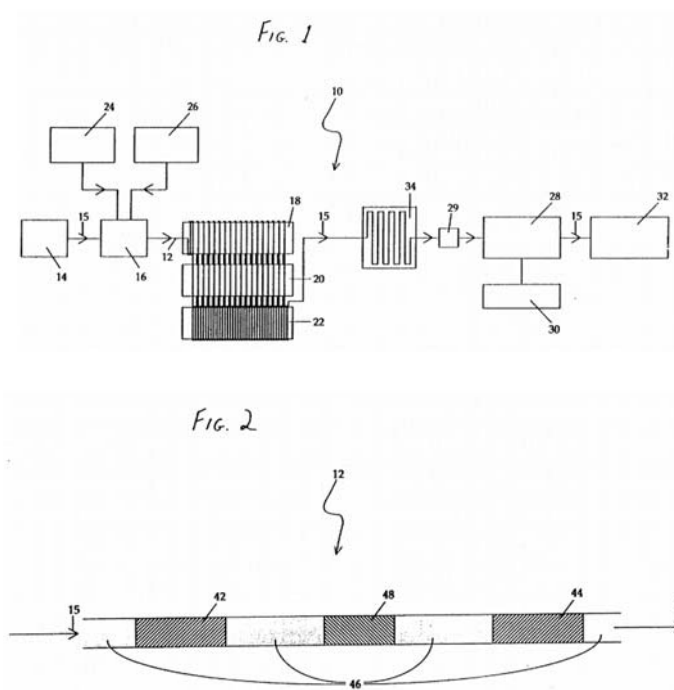
If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Lagally. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.



“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77. In 2001, in another article not previously considered by the Office, Lagally reported using a microfluidic droplet reactor to conduct single-molecule DNA amplification by PCR. Lagally summarized the state of the art as of February 2001, explaining continuous flow PCR microreactors had recently undergone substantial evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or **continuous flow** through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually

transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

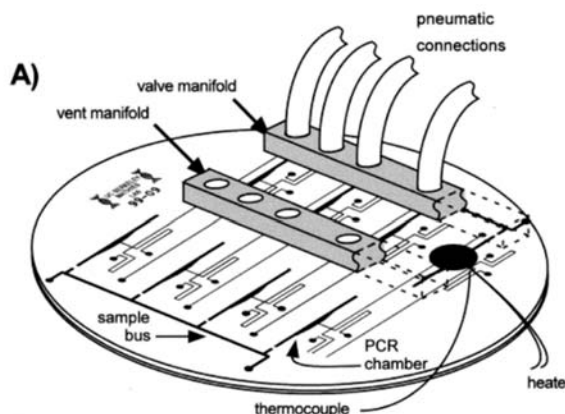
Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-566.

Lagally *et al.* developed a further improvement – a device and method for achieving single molecule amplification in a microchannel environment.

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

Microfluidic PCR-CE device. The PCR chambers are connected to a common sample bus through a set of valves. Hydrophobic vents at the other end of the PCR chambers are used to locate the sample and to eliminate gas. The PCR chambers are directly connected to the cross channel of the CE system for product injection and analysis. Two aluminum manifolds, one each for the vents and valves, are placed onto the respective ports and clamped in place using vacuum. The manifolds connect to external solenoid valves for pressure and vacuum actuation. Thermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.



Ex. 1028 at 565-66 (see figure legend).

In sum, Corbett and Lagally show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactors of Shaw Stewart are relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").³⁵ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination,

³⁵ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Corbett and Lagally. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. *Id.* ¶¶92-96. As noted by Lagally, "[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**" Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that "[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**" Ex. 1008 at 5556,, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have

increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Shaw Stewart to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of

off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity Ex. 1044 at 9239 (“It is also possible that Dig-PCR can be performed in

microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* at 9239 Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Lagally) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Lagally. The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful.**

In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually

transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-566.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an

autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactors Shaw Stewart could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Lagally to work in the microfluidic droplet reactor of Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.³⁶ As discussed in

³⁶ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985)

Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Corbett and Lagally. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. See MPEP § 2242. The correspondence between the claims of the '193 patent and the combined teachings of the Shaw Stewart, Corbett and Lagally are set forth in Section VII.G, below.

I. The Combination of Shaw Stewart, Corbett and Burns II Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Sections IV.C and IV.D above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for**

(‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

conducting reactions in droplets formed from two flowing immiscible fluids that could be readily modified to perform the reaction in question.³⁷

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Shaw Stewart), ii) microfluidic PCR devices were well known (Corbett and Burns II), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Lagally, Kopp, Burns II and Vogelstein).

The Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. The Shaw Stewart British Application discloses a “droplet reactor” and illustrates at Figure 1 a carrier phase 1 flowing along a conduit into which reagent 2 is injected, thereby forming droplet 4. Ex. 1040 at 1:1-20, 70-90. “[I]f large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” *Id.* at 1:83-90.

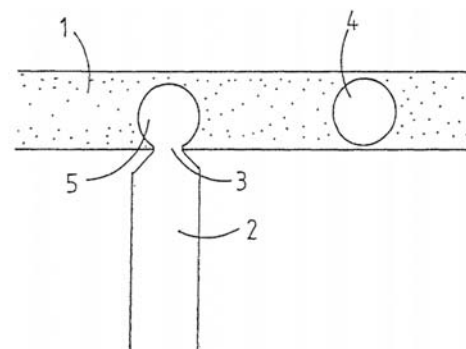


Figure 1.

Significantly, the exclusive licensee of the ‘193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the ‘193 patent, Ex. 1061,

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Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a “stepwise fashion,” whereas in the ’503 Patent droplets are formed by “combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.” *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

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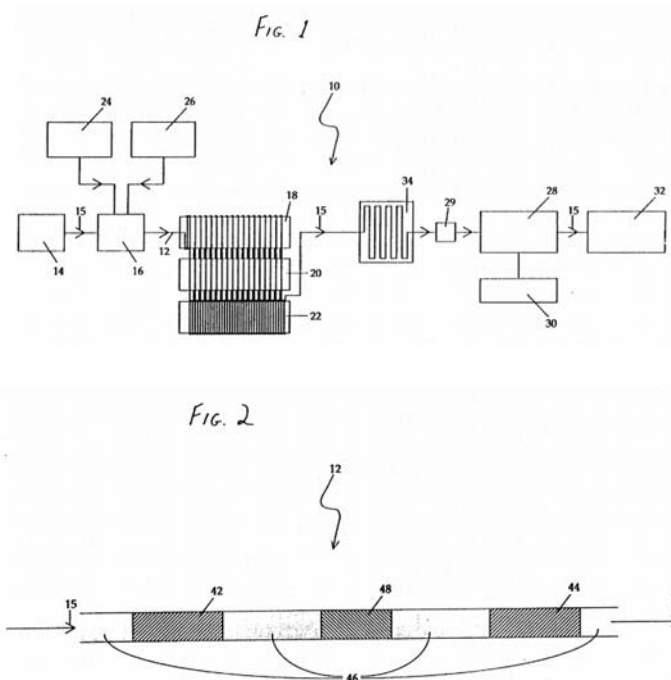
Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Burns II. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of

reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.



“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as

of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

In 1996, Burns II reported the use of microfluidic devices to perform PCR. Ex. 1008 at 5556. Burns II explained that “[n]o new biochemistries or DNA detection methods are required for their use; the components simply reproduce current laboratory procedures in a micron-sized environment.” *Id.* at 5560. In other words, the PCR chemistry did not need to be modified – it worked without modification on the microfluidic scale. In the method of Burns II, a sample containing a DNA plasmid was loaded into one of the reaction wells (A) and a mixture of Taq enzyme and buffer was loaded into the other. Ex. 1008 at 5558. Each formed droplets of about 60 nl in the parallel 500 x 25 μm channels (B) which came together at the Y junction to form a single larger droplet (about 120 nl). *Id.* at 5558-59; Ex. 1004 ¶40.

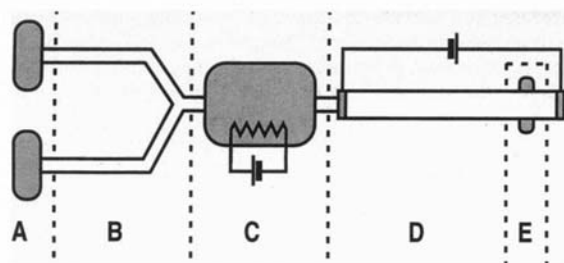


FIG. 1. Example of an integrated DNA analysis system, represented schematically. The individual components of the system are injection entry ports (A), liquid pumping channels (B), thermally controlled reaction chamber (C), electrophoresis channel (D), and DNA band migration detector (E). Each component would have associated sensors, control circuitry, and external connections.

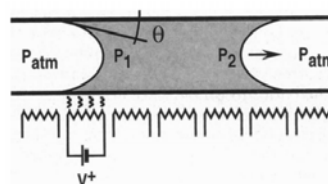


FIG. 2. Schematic drawing showing the principle of thermally induced drop motion in a closed channel. The case of a single aqueous drop in a hydrophilic channel is presented, where V is an applied voltage, P_{atm} is atmospheric pressure, P_1 is the receding-edge internal pressure, P_2 is the advancing-edge internal pressure, and θ is the contact angle of the liquid-gas-solid interface. The contact angle will depend on the surface characteristics of the channel and the constituents of the drop, with a hydrophilic interaction giving θ between 0 and 90 degrees, and a hydrophobic surface giving θ between 90 and 180 degrees. Surface treatments can also reduce contact angle hysteresis and, therefore, reduce the temperature difference necessary for drop motion.

The droplet then passed through the resistive heater portion (C) in which PCR amplification occurred. *Id.* at 5556-5557. Afterwards the amplification results were optically detected in zone D via gel electrophoresis. *Id.* at 5557-5561. Burns II noted that “[t]he use of common fabrication methods allows the assembly of increasingly complex, multicomponent, integrated systems from

a small, defined set of standardized elements” and that “[f]uture design efforts have a strong impetus to reduce component size and improve efficiency.” *Id.* at 5560-61.

In sum, Corbett and Burns II show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Shaw Stewart is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).³⁸ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct PCR as taught by Corbett and Burns II. *Id.* ¶¶88-109. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein’s contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

³⁸ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactor of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. *Id.* ¶¶92-96. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Shaw Stewart to

perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett's PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample

leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) Ex.1044 at 9239. Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Burns II) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶92-109. Using two flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Burns II. *Id.* The use of two flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents

needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-566.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In

1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Shaw Stewart could be successfully modified to perform PCR reactions.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Burns II to work in the microfluidic droplet reactor of Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as

early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.³⁹ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Corbett and Burns II. Ex. 1004 ¶106. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.*

³⁹ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. See MPEP § 2242. The correspondence between the claims of the '193 patent and the combined teachings of the Shaw Stewart, Corbett and Burns II are set forth in Section VII.H, below.

J. The Combination of Shaw Stewart, Corbett and Vogelstein Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Sections IV.C and IV.D above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for conducting reactions in droplets formed from two flowing immiscible fluids that could be readily modified to perform the reaction in question.**⁴⁰

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Shaw Stewart), ii) microfluidic PCR devices were well known (Corbett and Vogelstein), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Lagally, Kopp, Burns II and Vogelstein).

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The Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. The Shaw Stewart British Application discloses a “droplet reactor” and illustrates at Figure 1 a carrier phase 1 flowing along a conduit into which reagent 2 is injected, thereby forming droplet 4. Ex. 1040 at 1:1-20, 70-90. “[I]f large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” *Id.* at 1:83-90.

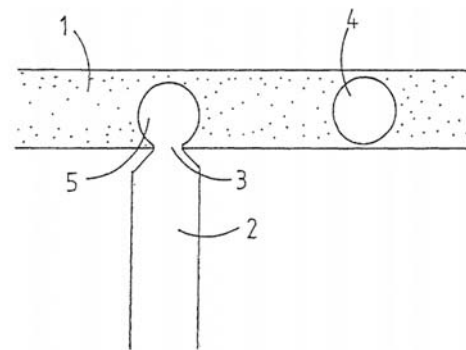


Figure 1.

Significantly, the exclusive licensee of the ‘193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the ‘193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

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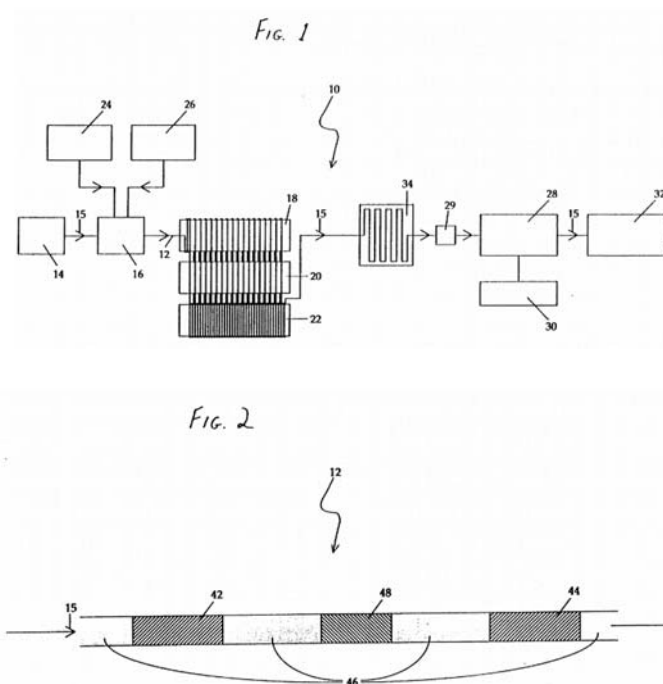
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Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Vogelstein. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream

includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.



“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

By 1999, Vogelstein had reported **various applications for single-molecule microfluidic PCR**. Ex. 1044 at 9241. In a seminal article which has been cited over by over 1,000 other publications, Vogelstein explained that PCR amplification of small aliquots or droplets containing single molecules was effective for “detection of a small number of mutant-containing cells among a large excess of normal cells.” *Id.* at 9236. In Vogelstein’s method, the

DNA was diluted into 7 μ l volumes such that, on average, there was one template molecule for every two wells (i.e., approximately half of the wells contained no DNA template molecule and approximately half contained a single DNA template molecule). *Id.* at 9236, 9237. This avoided the problem of background noise impairing the determination of whether each volume contained the mutant sequence. *Id.* By counting the number of volumes which indicated wild type or mutant templates, the presence or absence of the mutation (e.g., a sequence associated with colorectal cancer) can be determined. *Id.* at 9238. The accuracy of the test is a function of the number of discrete volumes into which the sample is diluted. *Id.* at 9239. Vogelstein taught that diluting the sample into $\sim$ 1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) The article explained that the same technique would be useful for **detecting a wide array of mutations, dislocations and allelic imbalances**, as summarized in Table 1. *Id.* at 9241. Vogelstein thus taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby overcoming the signal-to-noise limitations associated with certain other PCR techniques and enabling the detection of relatively rare mutations, dislocations and allelic imbalances. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86.

In sum, Corbett and Vogelstein show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Shaw Stewart is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the

limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).⁴¹ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Corbett and Vogelstein. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein’s contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactor of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized

⁴¹ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

devices may provide significant improvements in DNA analysis **speed, portability, and cost.”**

Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Shaw Stewart to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In

larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of**

relatively rare mutations, dislocations and allelic imbalances. Ex. 1044 at 9236, 9239, 9241 Ex. 1004 ¶¶81. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* at 9239. Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I, and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Vogelstein) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the

number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful.**

In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling

profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced

microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic**

droplet (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Shaw Stewart could be successfully modified to perform PCR reactions.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Vogelstein to work in the microfluidic droplet reactor of Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁴² As discussed in

⁴² “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985)

Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Corbett and Vogelstein. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. See MPEP § 2242. The correspondence between the claims of the '193 patent and the combined teachings of the Shaw Stewart, Corbett and Vogelstein are set forth in Section VII.I, below.

K. The Combination of Shaw Stewart, Kopp and Lagally Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Sections IV.C and IV.D, above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for**

(‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

conducting reactions in droplets formed from two flowing immiscible fluids that could be readily modified to perform the reaction in question.⁴³

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Shaw Stewart), ii) microfluidic PCR devices were well known (Kopp and Lagally), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

The Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. The Shaw Stewart British Application discloses a “droplet reactor” and illustrates at Figure 1 a carrier phase 1 flowing along a conduit into which reagent 2 is injected, thereby forming droplet 4. Ex. 1040 at 1:1-20, 70-90. “[I]f large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” *Id.* at 1:83-90

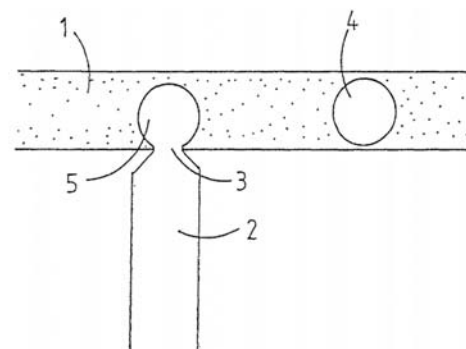


Figure 1.

Significantly, the exclusive licensee of the ‘193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the ‘193 patent, Ex. 1061,

⁴³ Requester notes that independent claim generically recites a reaction involving a biological molecule whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a “stepwise fashion,” whereas in the ’503 Patent droplets are formed by “combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.” *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

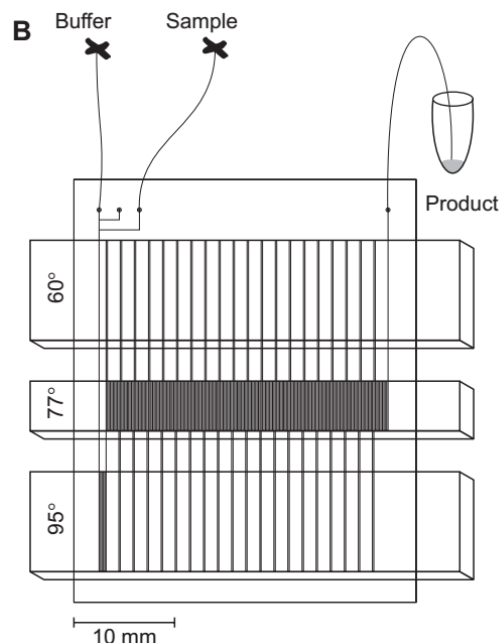
As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Lagally. By 1998 Kopp et al. had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary

electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 [emphasis added].

Kopp et al. also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1025 at 1047 [emphasis added].

In 2001, in another article not previously considered by the Office, Lagally reported using a microfluidic reactor to conduct single-molecule DNA amplification by PCR. Lagally summarized the state of the art as of February 2001, explaining continuous flow PCR microreactors had recently undergone substantial evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis

microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or **continuous flow** through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

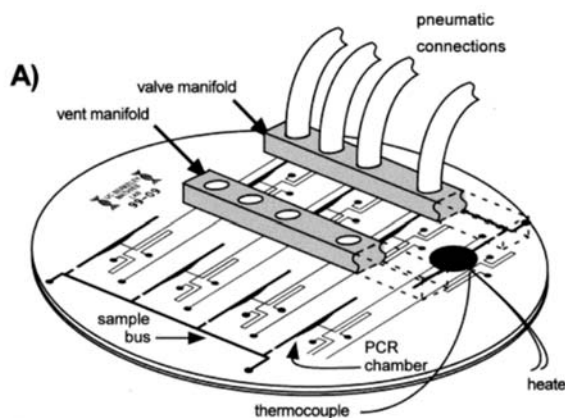
Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

Lagally et al. developed a further improvement – a device and method for achieving single molecule amplification in a microchannel environment.

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

Microfluidic PCR-CE device. The PCR chambers are connected to a common sample bus through a set of valves. Hydrophobic vents at the other end of the PCR chambers are used to locate the sample and to eliminate gas. The PCR chambers are directly connected to the cross channel of the CE system for product injection and analysis. Two aluminum manifolds, one each for the vents and valves, are placed onto the respective ports and clamped in place using vacuum. The manifolds connect to external solenoid valves for pressure and vacuum actuation. Thermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.



Ex. 1028 at 565-66 (see figure legend).

In sum, Kopp and Lagally show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Shaw Stewart is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).⁴⁴ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Kopp and Lagally. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactors of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556,

⁴⁴ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

[emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶¶92-100; Ex. 1008 at Abstract; Ex. 1027. It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex. 1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Shaw Stewart to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production of non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler

is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed

sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239 (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Lagally) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Lagally. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can

be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful.**

In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” Ex. 1027 at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling

profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced

microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic**

droplet (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Shaw Stewart could be successfully modified to perform PCR reactions.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp or Lagally to work in the microfluidic reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁴⁵ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that

⁴⁵ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985)

the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Kopp and Lagally.

Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. See MPEP § 2242. The correspondence between the claims of the '193 patent and the combined teachings of the Shaw Stewart, Kopp and Lagally are set forth in Section VII.J, below.

L. The Combination of Shaw Stewart, Kopp and Burns II Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Sections IV.C and IV.D, above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for conducting reactions in droplets formed from two flowing immiscible fluids that could be readily modified to perform the reaction in question.**⁴⁶

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Shaw Stewart), ii) microfluidic PCR devices were well known (Kopp and Burns II), and iii) there were compelling reasons to

(‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

⁴⁶ Requester notes that independent claim generically recites a reaction involving a biological molecule whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

The Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. The Shaw Stewart British Application discloses a “droplet reactor” and illustrates at Figure 1 a

carrier phase 1 flowing along a conduit into which reagent 2 is injected, thereby forming droplet 4. Ex. 1040 at 1:1-20, 70-90. “[I]f large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet

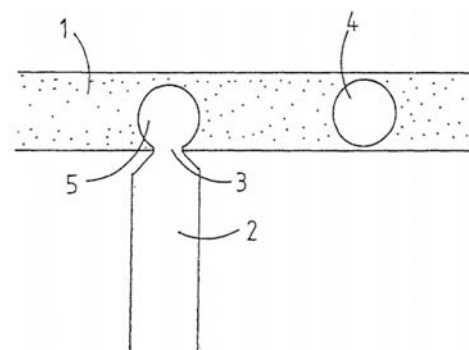


Figure 1.

almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” *Id.* at 1:83-90

Significantly, the exclusive licensee of the ‘193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the ‘193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a “stepwise fashion,” whereas in the ’503 Patent droplets are formed by “combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.” *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids. As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Burns II.

In 1996, Burns II reported the use of microfluidic devices to perform PCR. Ex. 1008 at 5556. Burns II explained that “[n]o new biochemistries or DNA detection methods are required for their use; the components simply reproduce current laboratory procedures in a micron-sized environment.” *Id.* at 5560. In other words, the PCR chemistry did not need to be modified – it worked without modification on the microfluidic scale. In the method of Burns II, a sample containing a DNA plasmid was loaded into one of the reaction wells (A) and a mixture of Taq

enzyme and buffer was loaded into the other. Ex. 1008 at 5558. Each formed droplets of about 60 nl in the parallel 500 x 25 μm channels (B) which came together at the Y junction to form a single larger droplet (about 120 nl). *Id.* at 5558-59; Ex. 1004 ¶40.

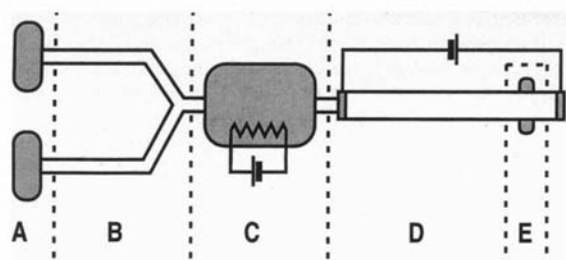


FIG. 1. Example of an integrated DNA analysis system, represented schematically. The individual components of the system are injection entry ports (A), liquid pumping channels (B), thermally controlled reaction chamber (C), electrophoresis channel (D), and DNA band migration detector (E). Each component would have associated sensors, control circuitry, and external connections.

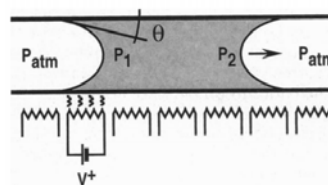


FIG. 2. Schematic drawing showing the principle of thermally induced drop motion in a closed channel. The case of a single aqueous drop in a hydrophilic channel is presented, where V is an applied voltage, P_{atm} is atmospheric pressure, P_1 is the receding-edge internal pressure, P_2 is the advancing-edge internal pressure, and θ is the contact angle of the liquid-gas-solid interface. The contact angle will depend on the surface characteristics of the channel and the constituents of the drop, with a hydrophilic interaction giving θ between 0 and 90 degrees, and a hydrophobic surface giving θ between 90 and 180 degrees. Surface treatments can also reduce contact angle hysteresis and, therefore, reduce the temperature difference necessary for drop motion.

The droplet then passed through the resistive heater portion (C) in which PCR amplification occurred. *Id.* at 5556-5557. Afterwards the amplification results were optically detected in zone D via gel electrophoresis. *Id.* at 5557-5561. Burns II noted that “[t]he use of common fabrication methods allows the assembly of increasingly complex, multicomponent, integrated systems from a small, defined set of standardized elements” and that “[f]uture design efforts have a strong impetus to reduce component size and improve efficiency.” *Id.* at 5560-61.

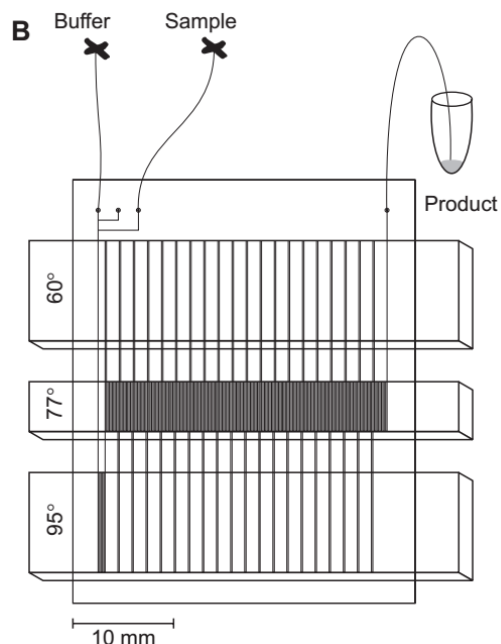
By the late 1990s Kopp et al. had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass

microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 [emphasis added].

Kopp et al. also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis

systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 [emphasis added].

In sum, Kopp and Burns II show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Shaw Stewart is relied upon to meet all of the limitations of the independent claim of the '193 patent except the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").⁴⁷ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

⁴⁷ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart, to conduct microfluidic PCR as taught by Kopp and Burns II. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactor of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶¶92-100; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of

therapy.” Ex. 1027 at 1047 It was thus known that using the droplet reactor of Shaw Stewart to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely

reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044. at 9239 (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby

enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Burns II) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Burns II. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with

sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

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In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

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Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Shaw Stewart could be successfully modified to perform PCR reactions.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp or Lagally to work in the microfluidic droplet reactor of Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same

invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁴⁸ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Kopp and Burns II.

Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the

⁴⁸ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

‘193 patent in view of these teachings. See MPEP § 2242. The correspondence between the claims of the ‘193 patent and the combined teachings of the Shaw Stewart, Kopp and Burns II are set forth in Section VII.K, below.

M. The Combination of Shaw Stewart, Kopp and Vogelstein Presents a Substantial New Question of Patentability for Claims 1-14 of the ‘193 Patent

As discussed in Sections IV.C and IV.D, above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for conducting reactions in droplets formed from two flowing immiscible fluids that could be readily modified to perform the reaction in question.**⁴⁹

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Shaw Stewart), ii) microfluidic PCR devices were well known (Kopp and Vogelstein), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

The Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. The Shaw Stewart British Application discloses a “droplet reactor” and illustrates at Figure 1 a carrier phase 1 flowing along a conduit into which reagent 2 is injected, thereby forming droplet 4. Ex. 1040 at 1:1-20, 70-90. “[I]f large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of

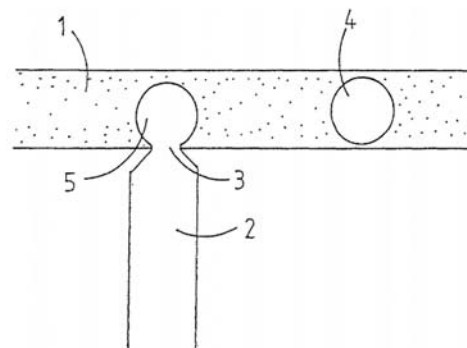


Figure 1.

⁴⁹ Requester notes that independent claim generically recites a reaction involving a biological molecule whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” *Id.* at 1:83-90

Significantly, the exclusive licensee of the ‘193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the ‘193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a “stepwise fashion,” whereas in the ’503 Patent droplets are formed by “combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.” *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

Ex. 1008, pg. 1, col. 2, ll. 83-88.

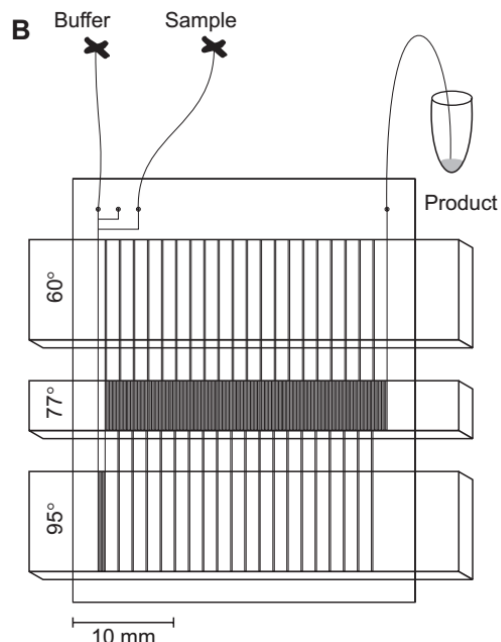
Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids. As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Vogelstein. By the late 1990s Kopp et al. had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 [emphasis added].

Kopp et al. also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene

could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 [emphasis added].

By 1999, Vogelstein had reported **various applications for single-molecule microfluidic PCR**. Ex. 1044 at 9241. In a seminal article which has been cited over by over 1,000 other publications, Vogelstein explained that PCR amplification of small aliquots or droplets containing single molecules was effective for “detection of a small number of mutant-containing cells among a large excess of normal cells.” *Id.* at 9236. In Vogelstein's method, the DNA was diluted into 7 μ l volumes such that, on average, there was one template molecule for every two wells (i.e., approximately half of the wells contained no DNA template molecule and approximately half contained a single DNA template molecule). *Id.* at 9236, 9237. This avoided the problem of background noise impairing the determination of whether each volume contained the mutant sequence. *Id.* By counting the number of volumes which indicated wild type or mutant templates, the presence or absence of the mutation (e.g., a sequence associated with colorectal cancer) can be determined. *Id.* at 9238. The accuracy of the test is a function of the number of discrete volumes into which the sample is diluted. *Id.* at 9239. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) The article explained that the same technique would be useful for **detecting a wide array of mutations, dislocations and allelic imbalances**, as summarized in Table 1. *Id.* at 9241. Vogelstein thus taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby

overcoming the signal-to-noise limitations associated with certain other PCR techniques and enabling the detection of relatively rare mutations, dislocations and allelic imbalances. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86.

In sum, Kopp and Vogelstein show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Shaw Stewart is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").⁵⁰ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart, to conduct microfluidic PCR as taught by Kopp and Vogelstein. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactor of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, "[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of**

⁵⁰ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

material and reagents needed.” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.”** Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047 It was thus known that using the droplet reactor of Shaw Stewart to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions

suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each

contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239 (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Vogelstein) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp, Burns II, Lagally or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces**

the volumes of reagent compared droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize

noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson

distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at

1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Shaw Stewart could be successfully modified to perform PCR reactions.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp or Lagally to work in the microfluidic droplet reactor of Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁵¹ As discussed in Section VI.A.3, above, Higuchi I-III

⁵¹ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent

demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date. As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Kopp and Vogelstein.

Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. See MPEP § 2242. The correspondence between the claims of the '193 patent and the combined teachings of the Shaw Stewart, Kopp and Vogelstein are set forth in Section VII.L, below.

N. The Combination of Burns I, Corbett and Lagally Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Sections IV.C and IV.D, above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for conducting reactions in droplets formed from two flowing immiscible fluids that could be readily modified to perform the reaction in question.**⁵²

development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

⁵² Requester notes that independent claim generically recites a reaction involving a biological molecule whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Burns I), ii) microfluidic PCR devices were well known (Corbett and Lagally), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10. Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment,

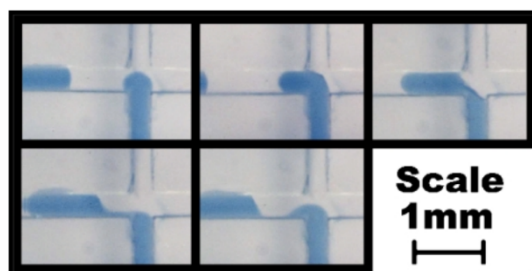
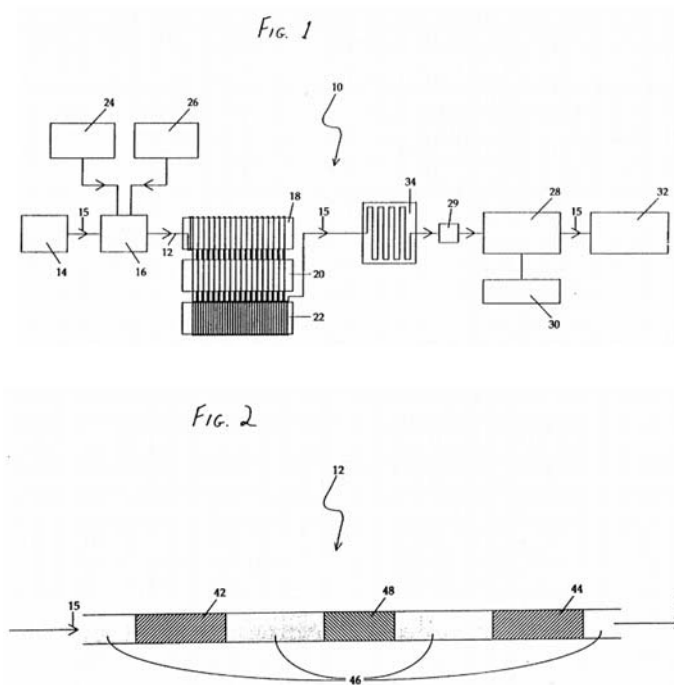


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel. See Ex. See Ex. 1010; 1027; Ex. 1025 at 23:2-8 When read in combination with Higuchi I-III, Quake and Shaw Stewart, Burns I demonstrates that making reactant slugs or plugs with continuous flows of immiscible fluids was a mere design choice as of 2001.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Lagally. In 1991, about eleven

years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.



“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by

the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

In 2001, in another article not previously considered by the Office, Lagally reported using a microfluidic reactor to conduct single-molecule DNA amplification by PCR. Lagally summarized the state of the art as of February 2001, explaining continuous flow PCR microreactors had recently undergone substantial evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or **continuous flow** through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

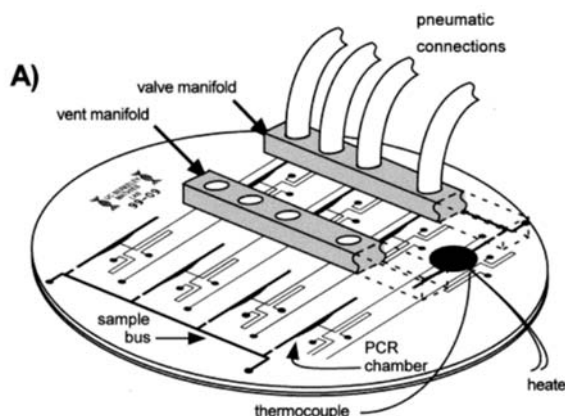
Ex. 1028 at 565-556.

Lagally *et al.* developed a further improvement – a device and method for achieving single molecule amplification in a microchannel environment.

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

Microfluidic PCR-CE device. The PCR chambers are connected to a common sample bus through a set of valves. Hydrophobic

vents at the other end of the PCR chambers are used to locate the sample and to eliminate gas. The PCR chambers are directly connected to the cross channel of the CE system for product injection and analysis. Two aluminum manifolds, one each for the vents and valves, are placed onto the respective ports and clamped in place using vacuum. The manifolds connect to external solenoid valves for pressure and vacuum actuation. Thermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.



Ex. 1028 at 565-66 (see figure legend).

In sum, Corbett and Lagally show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactors of Burns I are relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).⁵³ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug

⁵³ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Lagally. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic reactor of Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactors of Burns I because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. *Id.* ¶¶92-96. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶¶92-109; Ex. 1008 at Abstract; Ex. 1027

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047 It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactor of Burns I would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve

product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239 (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.*

Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Burns I to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Lagally) to incorporate a droplet reactor (Burns I). Ex. 1004 ¶¶92-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Lagally. The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow

PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides

an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an

autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Lagally to work in the microfluidic reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁵⁴ As discussed in Section

⁵⁴ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985)

VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Lagally. Ex. 1004 ¶¶88-109. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic reactor of Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.*

Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. See MPEP § 2242. The correspondence between the claims of the '193 patent and the combined teachings of the Burns I, Corbett and Lagally are set forth in Section VII.M, below.

O. The Combination of Burns I, Corbett and Burns II Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Section IV.C and IV.D above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for**

(‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

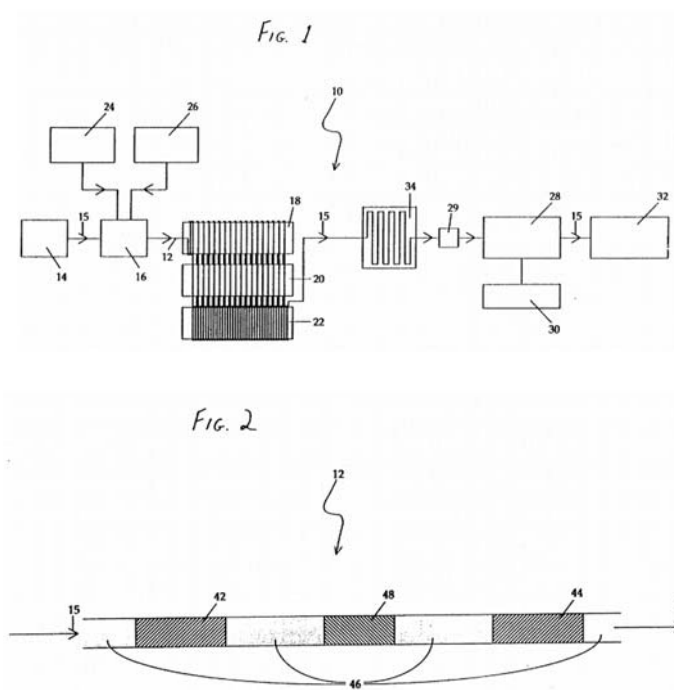
conducting reactions in droplets formed from two flowing immiscible fluids that could be readily modified to perform the reaction in question.⁵⁵

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Burns I), ii) microfluidic PCR devices were well known (Corbett and Burns II), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Lagally, Kopp, Burns II and Vogelstein).

Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10. Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. [emphasis added]. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel. See Ex. 1010; Ex. 1027; Ex. 1025 at 23:2-8. When read in combination with Higuchi I-III, Quake and Shaw Stewart, Burns I demonstrates that making reactant slugs or plugs with continuous flows of immiscible fluids was a mere design choice as of 2001.

⁵⁵ Requester notes that independent claim generically recites a reaction involving a biological molecule whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Burns II. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.



“The pump is actuated and the reaction mixture of 20 μl or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

In 1996, Burns II reported the use of microfluidic devices to perform PCR. Ex. 1008 at 5556. Burns II explained that “[n]o new biochemistries or DNA detection methods are required for their use; the components simply reproduce current laboratory procedures in a micron-sized environment.” *Id.* at 5560. In other words, the PCR chemistry did not need to be modified – it worked without modification on the microfluidic scale. In the method of Burns II, a sample containing a DNA plasmid was loaded into one of the reaction wells (A) and a mixture of Taq enzyme and buffer was loaded into the other. Ex. 1008 at 5558. Each formed droplets of about 60 nl in the parallel 500 x 25 μm channels (B) which came together at the Y junction to form a single larger droplet (about 120 nl). *Id.* at 5558-59; Ex. 1004 ¶40.

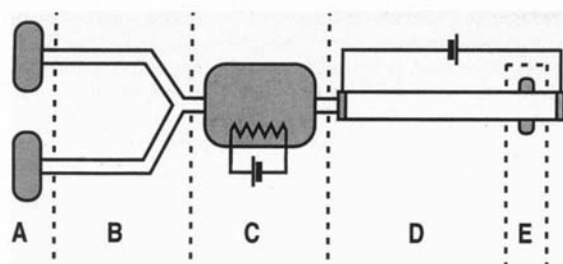


FIG. 1. Example of an integrated DNA analysis system, represented schematically. The individual components of the system are injection entry ports (A), liquid pumping channels (B), thermally controlled reaction chamber (C), electrophoresis channel (D), and DNA band migration detector (E). Each component would have associated sensors, control circuitry, and external connections.

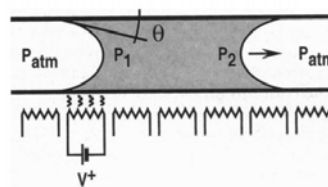


FIG. 2. Schematic drawing showing the principle of thermally induced drop motion in a closed channel. The case of a single aqueous drop in a hydrophilic channel is presented, where V is an applied voltage, P_{atm} is atmospheric pressure, P_1 is the receding-edge internal pressure, P_2 is the advancing-edge internal pressure, and θ is the contact angle of the liquid-gas-solid interface. The contact angle will depend on the surface characteristics of the channel and the constituents of the drop, with a hydrophilic interaction giving θ between 0 and 90 degrees, and a hydrophobic surface giving θ between 90 and 180 degrees. Surface treatments can also reduce contact angle hysteresis and, therefore, reduce the temperature difference necessary for drop motion.

The droplet then passed through the resistive heater portion (C) in which PCR amplification occurred. *Id.* at 5556-5557. Afterwards the amplification results were optically detected in zone D via gel electrophoresis. *Id.* at 5557-5561. Burns II noted that “[t]he use of common fabrication methods allows the assembly of increasingly complex, multicomponent, integrated systems from a small, defined set of standardized elements” and that “[f]uture design efforts have a strong impetus to reduce component size and improve efficiency.” *Id.* at 5560-61.

In sum, Corbett and Burns II show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Burns I is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).⁵⁶ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Burns II. Ex. 1004 ¶106. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic reactor of Burns I, as

⁵⁶ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.*

¶¶92-109.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactor of Burns I because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶¶92-109; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene

could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy." Ex. 1027 at 1047 It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett's PCR reaction in the microfluidic droplet reactor of Burns I would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a

sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby

enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Burns II) to incorporate a droplet reactor (Burns I). Ex. 1004 ¶¶92-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp, Burns II, Lagally or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with

sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Burns II to work in the microfluidic reactor of

Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁵⁷ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Burns II. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic reactor of

⁵⁷ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. See MPEP § 2242. The correspondence between the claims of the '193 patent and the combined teachings of the Burns I, Corbett and Burns II are set forth in Section VII.N, below.

P. The Combination of Burns I, Corbett and Vogelstein Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Section IV.C and IV.D above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for conducting reactions in droplets formed from two continuously flowing immiscible fluids that could be readily modified to perform the reaction in question.**⁵⁸

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Burns I), ii) microfluidic PCR devices were well known (Corbett and Vogelstein), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Lagally, Kopp, Burns II and Vogelstein).

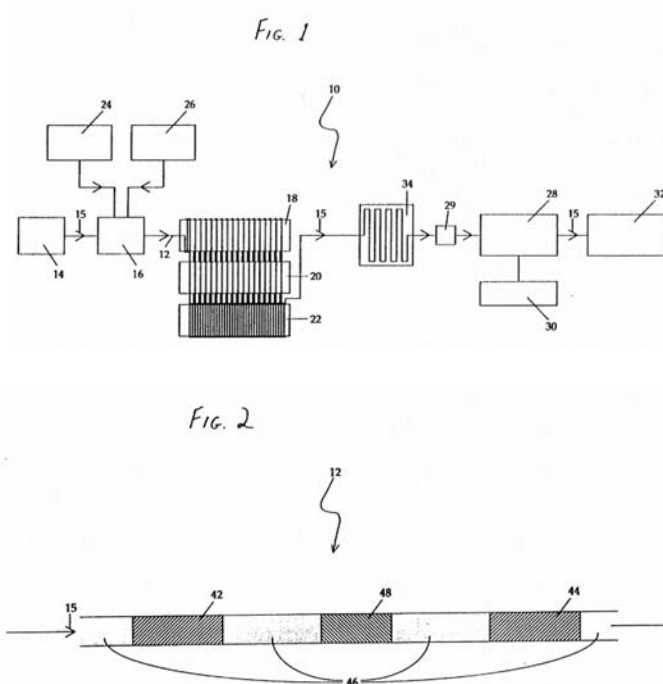
Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that "various methods may be used to generate slugs of liquid within a microreactor." Ex. 1007 at 10. Burns I chose a method in which

⁵⁸ Requester notes that independent claim generically recites a reaction involving a biological molecule whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

“the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel. See Ex. See Ex. 1010; Ex. 1027; Ex. 1025 at 23:2-8. When read in combination with Higuchi I-III, Quake and Shaw Stewart, Burns I demonstrates that making reactant slugs or plugs with continuous flows of immiscible fluids was a mere design choice as of 2001.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Vogelstein. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream

includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.



“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

By 1999, Vogelstein had reported **various applications for single-molecule microfluidic PCR**. Ex. 1044 at 9241. In a seminal article which has been cited over by over 1,000 other publications, Vogelstein explained that PCR amplification of small aliquots or droplets containing single molecules was effective for “detection of a small number of mutant-containing cells among a large excess of normal cells.” *Id.* at 9236. In Vogelstein’s method, the

DNA was diluted into 7 μ l volumes such that, on average, there was one template molecule for every two wells (i.e., approximately half of the wells contained no DNA template molecule and approximately half contained a single DNA template molecule). *Id.* at 9236, 9237. This avoided the problem of background noise impairing the determination of whether each volume contained the mutant sequence. *Id.* By counting the number of volumes which indicated wild type or mutant templates, the presence or absence of the mutation (e.g., a sequence associated with colorectal cancer) can be determined. *Id.* at 9238. The accuracy of the test is a function of the number of discrete volumes into which the sample is diluted. *Id.* at 9239. Vogelstein taught that diluting the sample into $\sim$ 1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) The article explained that the same technique would be useful for **detecting a wide array of mutations, dislocations and allelic imbalances**, as summarized in Table 1. *Id.* at 9241. Vogelstein thus taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby overcoming the signal-to-noise limitations associated with certain other PCR techniques and enabling the detection of relatively rare mutations, dislocations and allelic imbalances. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86.

In sum, Corbett and Vogelstein show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Burns I is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations

reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).⁵⁹ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Vogelstein. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic reactor of Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein’s contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactor of Burns I because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may

⁵⁹ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶¶92-100; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047 It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactor of Burns I would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger

volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

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A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp, Burns II, Lagally Vogelstein) to incorporate a droplet reactor (Quake, Burns I or Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease

contamination and increase the number of reactors that can be created on a given chip.

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Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

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In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with

microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching**

whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Vogelstein to work in the microfluidic reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁶⁰ As discussed in Section

⁶⁰ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so

VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Vogelstein. Ex. 1004 ¶¶88-109. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic reactor of Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.*

Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. See MPEP § 2242. The correspondence between the claims of the '193 patent and the combined teachings of the Burns I, Corbett and Vogelstein are set forth in Section VII.O, below.

ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

Q. The Combination of Burns I, Kopp and Lagally Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Sections IV.C and IV.D, above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for conducting reactions in droplets formed from two flowing immiscible fluids that could be readily modified to perform the reaction in question.**⁶¹

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Burns I), ii) microfluidic PCR devices were well known (Kopp and Lagally), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10. Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11, [emphasis added]. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel. See Ex. 1010; Ex. 1027; Ex. 1025 at 23:2-8. When read in

⁶¹ Requester notes that independent claim generically recites a reaction involving a biological molecule whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

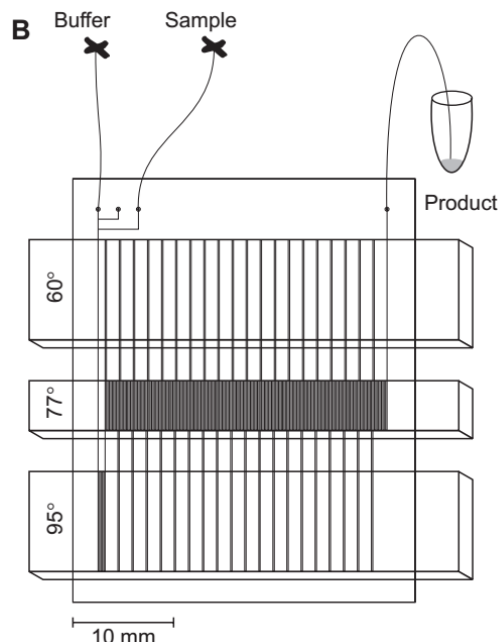
combination with Higuchi I-III, Quake and Shaw Stewart, Burns I demonstrates that making reactant slugs or plugs with continuous flows of immiscible fluids was a mere design choice as of 2001.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Lagally. By 1998 Kopp *et al.* had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 [emphasis added].

Kopp *et al.* also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene

could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 [emphasis added].

In 2001, in another article not previously considered by the Office, Lagally reported using a microfluidic reactor to conduct single-molecule DNA amplification by PCR. Lagally summarized the state of the art as of February 2001, explaining continuous flow PCR microreactors had recently undergone substantial evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or **continuous flow** through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-

volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

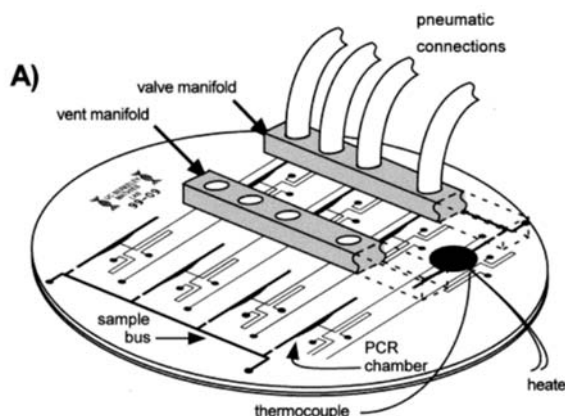
Ex. 1028 at 565-556.

Lagally *et al.* developed a further improvement – a device and method for achieving single molecule amplification in a microchannel environment.

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

Microfluidic PCR-CE device. The PCR chambers are connected to a common sample bus through a set of valves. Hydrophobic

vents at the other end of the PCR chambers are used to locate the sample and to eliminate gas. The PCR chambers are directly connected to the cross channel of the CE system for product injection and analysis. Two aluminum manifolds, one each for the vents and valves, are placed onto the respective ports and clamped in place using vacuum. The manifolds connect to external solenoid valves for pressure and vacuum actuation. Thermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.



Ex. 1028 at 565-66 (see figure legend).

In sum, Kopp and Lagally show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Burns I is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").⁶² Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug

⁶² Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Kopp and Lagally. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactors of Burns I because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶¶92-109; See Ex. 1010; Ex. 1027; Ex. 1025 at 23:2-8.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in

development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047 It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactor of Burns I would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Burns I to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Lagally) to incorporate a droplet reactor (Burns I). Ex. 1004 ¶¶92-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Lagally. *Id.* The use of two flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous

flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

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time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp or Lagally to work in the microfluidic reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁶³ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Kopp and Lagally.

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Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. See MPEP § 2242. The correspondence between the claims of the '193 patent and the combined teachings of the Burns I, Kopp and Lagally references are set forth in Section VII.P, below.

R. The Combination of Burns I, Kopp and Burns II Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Sections IV.C and IV.D, above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for conducting reactions in droplets formed from two flowing immiscible fluids that could be readily modified to perform the reaction in question.**⁶⁴

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Burns I), ii) microfluidic PCR devices were well known (Kopp and Burns II), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10. Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing

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the process.” Ex. 1007 at 10-11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel. See Ex. 1010; 1027; Ex. 1025 at 23:2-8. When read in combination with Higuchi I-III, Quake and Shaw Stewart, Burns I demonstrates that making reactant slugs or plugs with continuous flows of immiscible fluids was a mere design choice as of 2001.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Burns II. In 1996, Burns II reported the use of microfluidic devices to perform PCR. Ex. 1008 at 5556. Burns II explained that “[n]o new biochemistries or DNA detection methods are required for their use; the components simply reproduce current laboratory procedures in a micron-sized environment.” *Id.* at 5560. In other words, the PCR chemistry did not need to be modified – it worked without modification on the microfluidic scale. In the method of Burns II, a sample containing a DNA plasmid was loaded into one of the reaction wells (A) and a mixture of Taq enzyme and buffer was loaded into the other. Ex. 1008 at 5558. Each formed droplets of about 60 nl in the parallel 500 x 25 μm channels (B) which came together at the Y junction to form a single larger droplet (about 120 nl). *Id.* at 5558-59; Ex. 1004 ¶40.

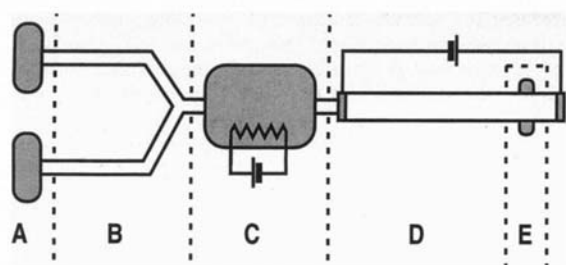


FIG. 1. Example of an integrated DNA analysis system, represented schematically. The individual components of the system are injection entry ports (A), liquid pumping channels (B), thermally controlled reaction chamber (C), electrophoresis channel (D), and DNA band migration detector (E). Each component would have associated sensors, control circuitry, and external connections.

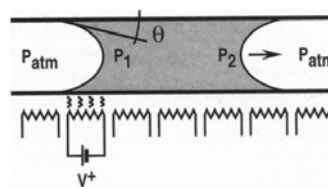


FIG. 2. Schematic drawing showing the principle of thermally induced drop motion in a closed channel. The case of a single aqueous drop in a hydrophilic channel is presented, where V is an applied voltage, P_{atm} is atmospheric pressure, P_1 is the receding-edge internal pressure, P_2 is the advancing-edge internal pressure, and θ is the contact angle of the liquid-gas-solid interface. The contact angle will depend on the surface characteristics of the channel and the constituents of the drop, with a hydrophilic interaction giving θ between 0 and 90 degrees, and a hydrophobic surface giving θ between 90 and 180 degrees. Surface treatments can also reduce contact angle hysteresis and, therefore, reduce the temperature difference necessary for drop motion.

The droplet then passed through the resistive heater portion (C) in which PCR amplification occurred. *Id.* at 5556-5557. Afterwards the amplification results were optically detected in zone D via gel electrophoresis. *Id.* at 5557-5561. Burns II noted that “[t]he use of common fabrication methods allows the assembly of increasingly complex, multicomponent, integrated systems from a small, defined set of standardized elements” and that “[f]uture design efforts have a strong impetus to reduce component size and improve efficiency.” *Id.* at 5560-61.

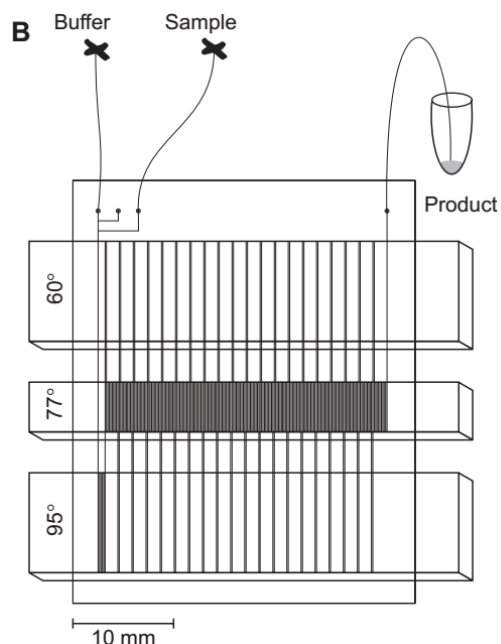
By the late 1990s Kopp *et al.* had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product

quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 [emphasis added].

Kopp *et al.* also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been

presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 [emphasis added].

In sum, Kopp and Burns II show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Burns I is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").⁶⁵ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I, to conduct microfluidic PCR as taught by Kopp and Burns II. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactor of

⁶⁵ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

Burns I because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp, Burns II, Lagally or Vogelstein) to incorporate a droplet reactor (Quake, Burns I or Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp, Burns II, Lagally or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents

needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In

1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp to work in the microfluidic reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation

of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁶⁶ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Kopp and Burns II.

Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. See MPEP § 2242. The correspondence between the claims of the '193 patent and the combined teachings of the Burns I, Kopp and Burns II are set forth in Section VII.Q, below.

⁶⁶ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

S. The Combination of Burns I, Kopp and Vogelstein Presents a Substantial New Question of Patentability for Claims 1-14 of the '193 Patent

As discussed in Sections IV.C and IV.D, above, the claims have been considered nonobvious by the Office because the **prior art was not believed to teach a technique for conducting reactions in droplets formed from two flowing immiscible fluids that could be readily modified to perform the reaction in question.**⁶⁷

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Burns I), ii) microfluidic PCR devices were well known (Kopp and Vogelstein), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10. Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel. See Ex. 1010; 1027; Ex. 1025 at 23:2-8. When read in

⁶⁷ Requester notes that independent claim generically recites a reaction involving a biological molecule whereas dependent claim 3 and the Haff reference asserted in the *inter partes* review relate specifically to PCR.

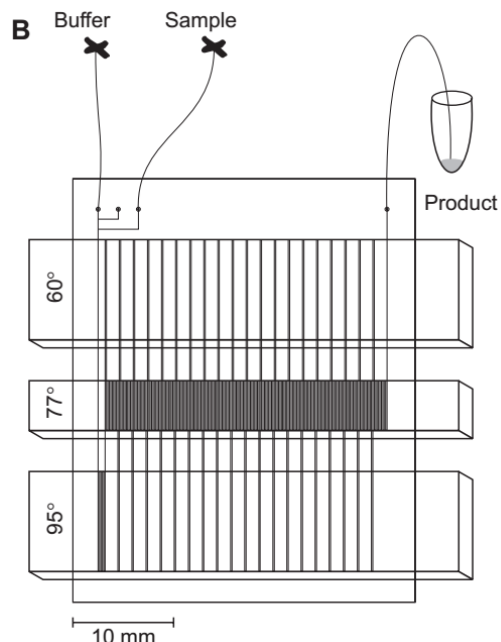
combination with Higuchi I-III, Quake and Shaw Stewart, Burns I demonstrates that making reactant slugs or plugs with continuous flows of immiscible fluids was a mere design choice as of 2001.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Vogelstein. By the late 1990s Kopp *et al.* had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 [emphasis added].

Kopp *et al.* also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene

could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 [emphasis added].

By 1999, Vogelstein had reported **various applications for single-molecule microfluidic PCR**. Ex. 1044 at 9241. In a seminal article which has been cited over by over 1,000 other publications, Vogelstein explained that PCR amplification of small aliquots or droplets containing single molecules was effective for “detection of a small number of mutant-containing cells among a large excess of normal cells.” *Id.* at 9236. In Vogelstein's method, the DNA was diluted into 7 μ l volumes such that, on average, there was one template molecule for every two wells (i.e., approximately half of the wells contained no DNA template molecule and approximately half contained a single DNA template molecule). *Id.* at 9236, 9237. This avoided the problem of background noise impairing the determination of whether each volume contained the mutant sequence. *Id.* By counting the number of volumes which indicated wild type or mutant templates, the presence or absence of the mutation (e.g., a sequence associated with colorectal cancer) can be determined. *Id.* at 9238. The accuracy of the test is a function of the number of discrete volumes into which the sample is diluted. *Id.* at 9239. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) The article explained that the same technique would be useful for **detecting a wide array of mutations, dislocations and allelic imbalances**, as summarized in Table 1. *Id.* at 9241. Vogelstein thus taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby

overcoming the signal-to-noise limitations associated with certain other PCR techniques and enabling the detection of relatively rare mutations, dislocations and allelic imbalances. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86.

In sum, Kopp and Vogelstein show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Burns I is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).⁶⁸ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I, to conduct microfluidic PCR as taught by Kopp and Vogelstein. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactor of Burns I because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and**

⁶⁸ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

reagents needed.” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added].

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactor of Burns I would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is

too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up

to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity.

Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Vogelstein) to incorporate a droplet reactor (Burns I). Ex. 1004 ¶¶92-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful.**

In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

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transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

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In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an

autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp to work in the microfluidic reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁶⁹ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that

⁶⁹ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985)

the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Kopp and Vogelstein.

Not only would a reasonable Examiner consider these teachings relevant in deciding whether claims 1-14 of the '193 patent are patentable, but would have rejected the claims of the '193 patent in view of these teachings. See MPEP § 2242. The correspondence between the claims of the '193 patent and the combined teachings of the Burns I, Kopp and Vogelstein are set forth in Section VII.L, below.

VII. DETAILED EXPLANATION OF THE PERTINENCE AND MANNER OF APPLYING THE PRIOR ART REFERENCES TO EVERY CLAIM FOR WHICH REEXAMINATION IS REQUESTED

In accordance with 37 C.F.R. § 1.510(b)(2), Requester provides the following detailed explanation of the pertinence and manner of applying the prior art to claims 1-14 of the '193 patent, for which reexamination is requested.

A. The Combination of Quake, Corbett and Lagally Renders Obvious Claims 1-14 of the '193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Quake), ii) microfluidic PCR

(‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

devices were well known (Corbett and Lagally), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Lagally and Vogelstein).

As to the first feature – forming plugs from continuously flowing immiscible fluids – the ‘193 patent’s teachings in this regard were copied almost verbatim from the Quake PCT (Ex. 1034). The table below compares the Quake PCT publication with the Ismagilov specification, which was filed about two months later.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and ‘193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	Provisional at 22:12-19, ‘193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

Attached as Ex. 1035 is a table showing the numerous passages which Ismagilov copied key disclosures verbatim or nearly verbatim from the Quake PCT. These were not merely background or “state of the art” descriptions; rather, they go to the heart of the claimed subject matter.

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

Quake relates to microfluidic devices designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [citing Quake '332 publication ¶ 3, Ex. 1033 ¶ 3]. The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. A junction joins the main channel with the sample inlet channel. By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. Figure 16A of Quake is reproduced below:

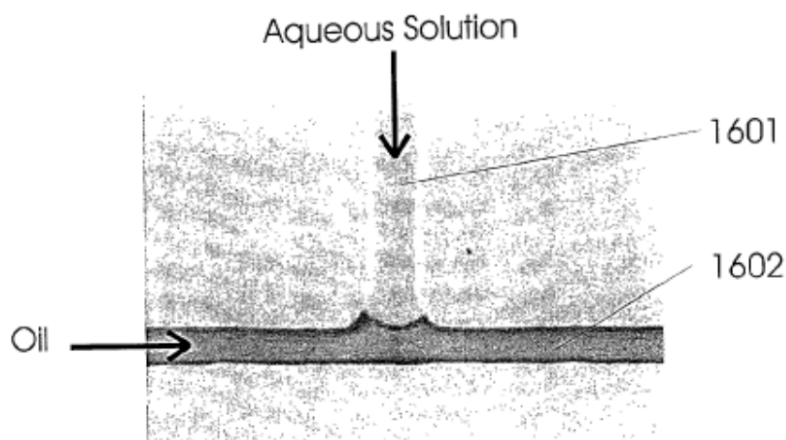


FIG. 16A

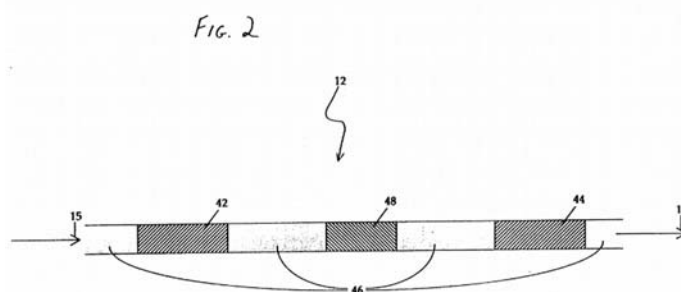
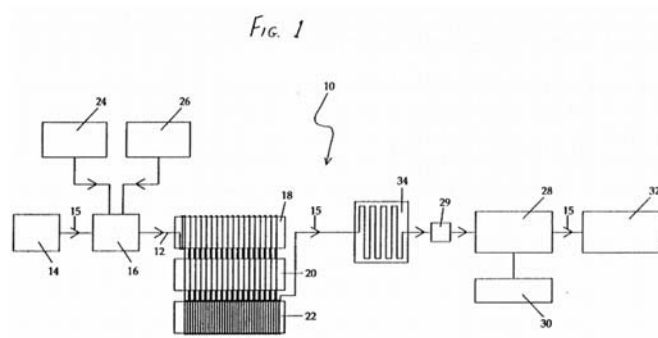
Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil. [Ex. 1052 at 12 citing Quake (Ex. 1033) ¶ 292]. In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* ¶ 80.

Ex. 1052 at 11-14.

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by-streams limitations of the '193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous streams.⁷⁰ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the '193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids.

Turning to the second issue, as of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Lagally. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.

⁷⁰ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.



“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

In 2001, in another article not previously considered by the Office, Lagally reported using a single fluid microfluidic reactor to conduct single-molecule DNA amplification by PCR. Lagally summarized the state of the art as of February 2001, explaining continuous flow PCR microreactors had recently undergone substantial evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents

needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or **continuous flow** through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

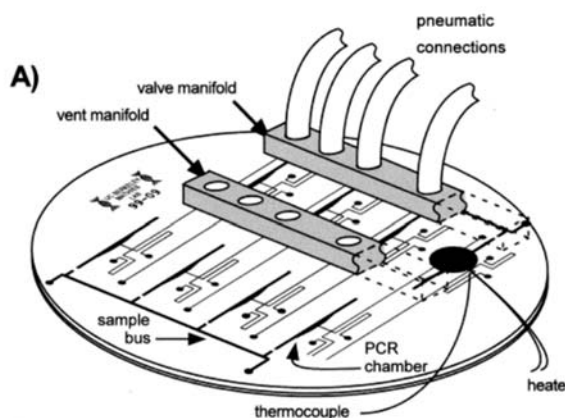
Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

Lagally *et al.* developed a further improvement – a device and method for achieving single molecule amplification in a microchannel environment.

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

Microfluidic PCR-CE device. The PCR chambers are connected to a common sample bus through a set of valves. Hydrophobic vents at the other end of the PCR chambers are used to locate the sample and to eliminate gas. The PCR chambers are directly connected to the cross channel of the CE system for product injection and analysis. Two aluminum manifolds, one each for the vents and valves, are placed onto the respective ports and clamped in place using vacuum. The manifolds connect to external solenoid valves for pressure and vacuum actuation. Thermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.



Ex. 1028 at 565-66 (see figure legend).

In sum, Corbett and Lagally show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").⁷¹ Ex. 1001 at 78:12-15. In the instant combination, this limitation is met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR in droplets as taught by Corbett, or to modify the reactions of Lagally to be performed in microfluidic droplets instead of continuous flow of a single fluid in a microfluidic channel. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to use two continuous streams of immiscible fluid as used in a microfluidic droplet reactor of Quake, as evidenced by Lagally's contemporaneous report that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.*

Quake **specifically suggests that his microfluidic device could be used to perform PCR.** Quake explained that "[m]icrofabrication permits other technologies to be integrated or

⁷¹ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

combined with flow cytometry on a single chip, such as PCR...” Ex. 1034 at 23:26-30. Quake noted that “PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen.” *Id.* at 2:31-3:2.

More generally, a skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions and the use of two continuous streams of immiscible fluids would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. Likewise, the reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶¶92-100; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047.

Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047 .It was thus known that using the droplet reactor of Quake to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id* 9239. Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Lagally) to incorporate a droplet reactor (Quake). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Lagally. The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at

Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The

first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic

diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Ex. 1004 ¶¶39-

57. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. *Id.*

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Lagally to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. A group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20(Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁷² As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

⁷² "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

As explained in more detail below, in the accompanying claim chart (Ex. 1062), and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Corbett and Lagally. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Quake, as evidenced by Lagally's contemporaneous report that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. Ex. 1004 ¶¶39-57.

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Quake discloses “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided. . . . “For instance, **in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials**, a water soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Ex. 1034 at 28:20-23.

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Quake may be used to perform PCR reactions which are autocatalytic. Ex. 1034 pp. 2, 3, 23.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1062.

Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

Quake's device comprises a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed: "The channel architecture for the droplet extrusion region of the first device is shown in FIG. 16A. In this device, the inlet channel 1601 (inner diameter 30 μm) intersects the main channel 1602 (inner diameter 30 μm) at a T-intersection (i.e., an angle perpendicular to the main channel). Other intersections and angles may be used. The walls of the inlet and main channels were not tapered in this device." Ex. 1034 at 79:23-28.

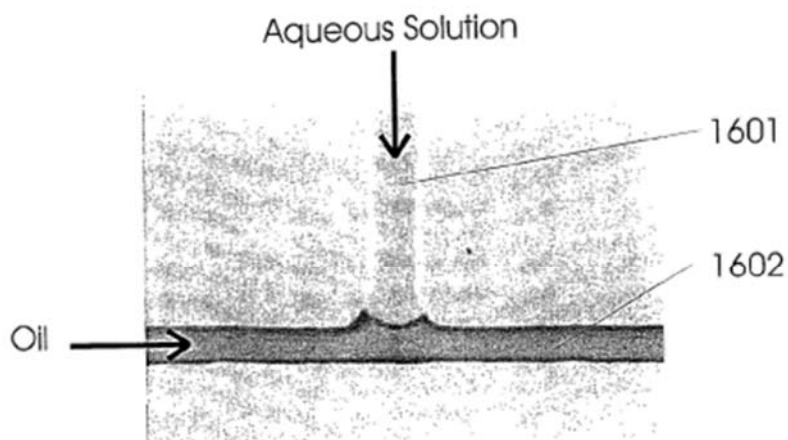


FIG. 16A

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1062.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

Quake teaches that “[a] first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the second fluid, passes through the inlet region so that droplets of the second fluid are sheared into the main channel. ... The second phase or fluid which passes through the inlet region can be an **aqueous solution**, for example ultra pure water, TE buffer, phosphate buffer saline and acetate buffer. The second fluid may also contain a **biological sample** (e.g., molecules of an enzyme or a **substrate**, or one or more cells, or one or more viral particles) for analysis or sorting in the device. In preferred embodiments the second fluid includes a biological sample that comprises one or more **molecules**, cells, virions or particles.” Ex. 1034 at 6:6-16. Quake further teaches that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a “sample” or “droplet” fluid, passes or flows through the inlet region. ... Thus, droplets of the sample fluid containing the biological material for analysis, reaction or sorting are sheared at the droplet extrusion region into the flow of the extrusion fluid in the main channel.” *Id.* at 7:30-8:5.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing aqueous fluid with the reagents also includes at least one substrate molecule. This limitation is met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR in droplets as taught by Corbett, or to modify the reactions of Lagally to be performed in microfluidic droplets instead of continuous flow of a single fluid in a microfluidic channel. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to use two continuous streams of immiscible fluid as used in a microfluidic droplet reactor of Quake, as evidenced by Lagally's contemporaneous report that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.*

Quake **specifically suggests that his microfluidic device could be used to perform PCR**. Quake explained that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR...” Ex. 1034 at 23:26-30. Quake noted that “PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen.” *Id.* at 2:31-3:2.

More generally, a skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. Ex. 1004 ¶¶88-109. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed**.” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost**.” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett

to microfluidic dimensions and the use of two streams of immiscible fluids would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. Likewise, the reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48. Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Quake to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as

the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241;

Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Lagally) to incorporate a droplet reactor (Quake). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Lagally. The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller

reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling

profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced

microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic**

droplet (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Ex. 1004 ¶¶39-57. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. *Id.*

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Lagally to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁷³ As discussed in Section

⁷³ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the

VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained further in the accompanying claim chart (Ex. 1062) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Corbett and Lagally. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Quake, as evidenced by Corbett and Lagally's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1062.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

Quake discloses that “[p]referably, the fluid introduced to the main channel through the extrusion region is ‘incompatible’ (i.e., **immiscible**) with the fluid in the main channel so that droplets of the fluid introduced through the extrusion region are sheared off into the stream of fluid in the main channel. For example, the first phase or fluid which flows through the main channel can be a non-polar solvent, such as decane (e.g., tetradecane or hexadecane) or another **oil** (for example, mineral oil).” Ex. 1034 at 19:29-32, [emphasis added].

art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) (‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1062.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

As discussed above, the written descriptive support in the '193 patent for this claim element (which was the supposed point of novelty) was copied almost verbatim from Quake. The table below shows the correspondence between the relevant disclosures in the Quake PCT (Ex. 1034) and the Ismagilov provisional application (Ex. 1003) and '193 patent.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and '193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	Provisional at 22:12-19, '193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

Quake relates to microfluidic devices designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [citing Quake '332 publication ¶ 3, Ex. 1033 ¶ 3]. The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. A junction joins the main channel with the sample inlet channel. By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. Figure 16A of Quake is reproduced below:

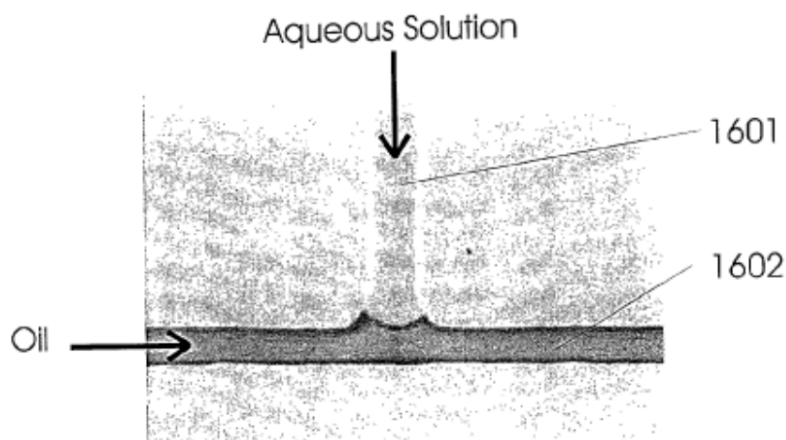


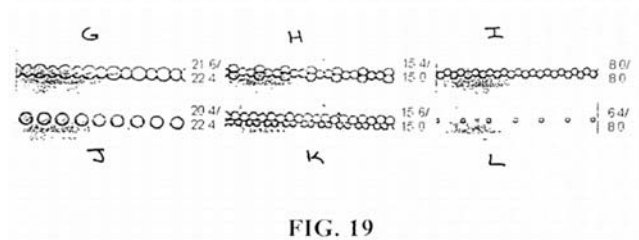
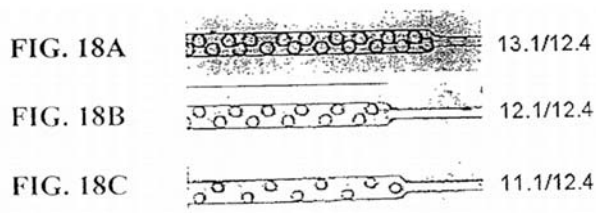
FIG. 16A

Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil. [Ex. 1052 at 12 citing Quake (Ex. 1033) ¶ 292]. In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* ¶ 80.

Ex. 1052 at 11-14.

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by-streams limitations of the '193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous streams.⁷⁴ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the '193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids.

The recitation that the plug is “substantially surrounded by the oil flowing through the channel” is met by Figures 18 and 19 of Quake. “FIGS. 18A-C are photomicrographs showing droplets of aqueous solution in a flow of oil (hexadecane with 2% Span 80 surfactant) in a microfluidic device with rectangular channels.” Ex. 1034 at 11:24-27. “FIG. 19 provides photomicrographs (Frames A-L) showing droplets of aqueous solution in a flow of oil (hexadecane with 2% Span 80 surfactant) in a microfluidic device with rounded channels.” *Id.* at 11:29-31. Figures 18B, 18C, 19J and 19L (reproduced above) each depict droplets being completely surrounded by the carrier fluid.



⁷⁴ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1062.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Quake teaches that “droplets of the sample fluid containing the biological material for analysis, **reaction** or sorting are sheared at the droplet extrusion region into the flow of the extrusion fluid in the main channel.” Ex. 1034 at 8. “Microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as **PCR** [i.e., a reaction].” *Id.* at 23.

As discussed above in connection with element 1[b], in the instant combination Quake is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing aqueous fluid with the reagents also includes a substrate molecule which reacts with the reagents. Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. This limitation is met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71. The DNA is amplified through the PCR reaction. *Id.*

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1062.

Thus, claim 1 is shown to be rendered obvious by the combination of Quake, Corbett and Lagally.

Dependent Claim 2

Claim 2 of the '193 patent recites "wherein the at least one substrate molecule is a single biological molecule."

Quake discloses that "[t]he invention also provides a method for sorting biological material. In various embodiments of the method, the biological material may be, e.g., molecules (for example, polynucleotides, polypeptides, enzymes, substrates or mixtures thereof), cells or viral particles, or mixtures thereof." Ex. 1034 at 8:19-22. "The term 'molecule' means any distinct or distinguishable structural unit of matter comprising one or more atoms, and includes for example polypeptides and polynucleotides." *Id.* at 13:10-12. "The term 'polynucleotide' as used herein refers to a polymeric molecule having a backbone that supports bases capable of hydrogen bonding to typical polynucleotides, ... Polymeric molecules include double and single stranded RNA and DNA, and backbone modifications thereof, for example, methylphosphonate linkages." *Id.* at 13:16-23.

Quake further specifies that, "[p]referably, each droplet of this multi-phase mixture encapsulates a single particle." *Id.* at 5. "In particular, the sample concentration should be dilute enough that most of the droplets contain no more than a single molecule, cell or virion, with only a small statistical chance that a droplet will contain two or more molecules, cells or virions." *Id.* at 36-7.

In the combined method of Quake, Corbett and Lagally, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1062.

Thus, claim 2 is shown to be rendered obvious by the combination of Quake, Corbett and Lagally.

Dependent Claims 3 and 4

Claim 3 of the '193 patent recites "wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase chain reaction." Claim 4 of the '193 patent recites "wherein the providing step includes heating." PCR reactions involve thermocycling. Ex. 1004 ¶¶32-38.

Quake discloses that "microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR." Ex. 1034 at 23:26-30. In the combined method of Quake, Corbett and Lagally, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of an aqueous fluid and an oil and includes a mixture of DNA (the substrate molecule) and reagents, e.g., polymerase, primers, buffers and the like. To affect the PCR reaction, the carrier fluid is heated. ("The carrier fluid then passes through a plurality of temperature zones in which the polymerase chain reactions take place") Ex. 1010 (Corbett), ("The PCR chambers were thermally cycled.") Ex. 1028 at 567 (Lagally).

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1062.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Quake, Corbett and Lagally.

Dependent Claim 5

Claim 5 of the '193 patent further recites "providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred."

Quake discloses that “[t]he device of the invention may also comprise a **detection region** which is within or coincident with at least a portion of the main channel at or downstream of the droplet extrusion region. The device may also have a detector, preferably an **optical detector** such as a microscope, associated with the detection region.” Ex. 1034 at 6, [emphasis added].

Corbett similarly teaches that “[d]ownstream of temperature zone 34 is in-line analysis means 29 and recovery means 28. Connected to recovery means 28 is **detection means 30**. Detection means 30 determines when a reaction mixture in the carrier fluid reaches recovery means 28. This may be done by measuring the conductivity or **optical density** of the stream of carrier fluid.” Ex. 1010 at 8:12-18, [emphasis added].

Lagally teaches “[s]tochastic PCR amplification of single DNA template molecules followed by **capillary electrophoresis** (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Ex. 1028 at 565. The results of capillary electrophoresis were generally detected optically at the time of filing. Ex. 1011 (Dolnik) at 46.

In the combined method of Quake, Corbett and Lagally, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶68-69.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1062.

Thus, claim 5 is shown to be rendered obvious by the combination of Quake, Corbett and Lagally.

Dependent Claims 6-8

Claim 6 of the ‘193 patent recites “wherein the oil is fluorinated oil.” Claim 7 of the ‘193 patent recites “wherein the carrier fluid further comprises a surfactant.” Claim 8 of the ‘193 patent recites “wherein the surfactant is fluorinated surfactant.”

Quake discloses that “[t]he fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). **Exemplary surfactants** include Tween, Span, **fluorinated oils**, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel.” Ex. 1034 at 35, [emphasis added].

The phrase “carrier fluid” in claim 7, having no antecedent basis, is construed to mean “a fluid that is immiscible with a plug fluid,” as this is the way the term is defined in the specification. See Ex. 1001 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1062.

Thus, claims 6-8 are shown to be rendered obvious by the combination of Quake, Corbett and Lagally.

Dependent Claim 9

Claim 9 of the ‘193 patent recites “wherein the at least one plug is a merged plug.”

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1062.

Thus, claim 9 is shown to be rendered obvious by the combination of Quake, Corbett and Lagally.

Dependent Claim 10

Claim 10 of the '193 patent recites “trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels.”

Quake describes a “detection region” which “should generally be at least about twice, preferably at least about five times as large per side or in diameter as the diameter of the largest molecule, cell or droplet that will be passing through it.” Ex. 1034 at 26. This may be contrasted to Quake’s generally recommended dimensions of the fluid channels, which are shaped to cause the droplets to “conform to the size and shape of the channels” by “becom[ing] longer and thinner” as channels narrow. *Id.* at 27. Quake teaches that “an optical trap, or laser tweezers, may be used to sort or direct cells.... The optical trap remains in an ‘off’ mode until a desired particle is detected in the detection region. After detection of a desired characteristic, the particle or cell is ‘trapped’, and thereby directed or moved into the predetermined branch channel for collection.” *Id.* at 67, *see also* FIGs. 14A & B (Input). Thus, Quake’s detection region is an “expansion portion” where the at least one plug would be “trapp[ed]... for a period of time... after the reaction” as recited in dependent claim 10.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1062.

Thus, claim 10 is shown to be rendered obvious by the combination of Quake, Corbett and Lagally.

Dependent Claim 11

Claim 11 of the '193 patent recites “wherein the at least one plug is substantially spherical in shape.”

As discussed by Quake, “the microfabricated devices of this invention generate round, monodisperse droplets (such as those illustrated in Frames J and L of FIG. 19” (reproduced below). Ex. 1034 at 27, FIG. 19.

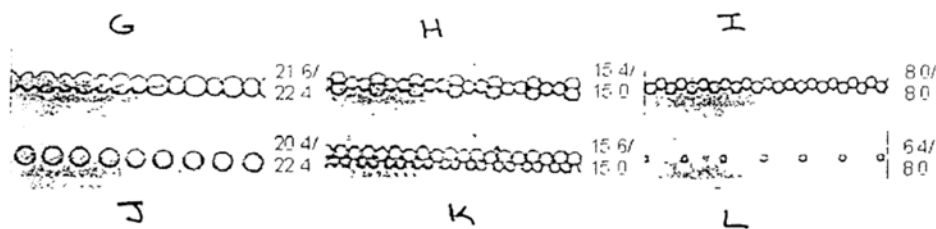


FIG. 19

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1062.

Thus, claim 11 is shown to be rendered obvious by the combination of Quake, Corbett and Lagally.

Dependent Claim 12

Claim 12 of the '193 patent recites “a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction.”

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1062.

Thus, claim 12 is shown to be rendered obvious by the combination of Quake, Corbett and Lagally.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '193 patent recites "wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters." Claim 14 of the '193 patent recites "wherein the relevant parameters are selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns."

Corbett teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Corbett meets both claims 13 and 14 under the broadest reasonable interpretation of the term "special design," which in and of itself imparts no meaningful structural limitation. Ex. 1010 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1062.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Quake, Corbett, and Lagally.

B. The Combination of Quake, Corbett and Burns II Renders Obvious Claims 1-14 of the '193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Quake), ii) microfluidic PCR devices were well known (Corbett and Burns II), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Lagally, Kopp, Burns II and Vogelstein).

As to the first feature – forming plugs from continuously flowing immiscible fluids – the '193 patent's teachings in this regard were copied almost verbatim from the Quake PCT (Ex.

1034). The table below compares the Quake PCT publication with the Ismagilov specification, which was filed about two months later.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and ‘193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	Provisional at 22:12-19, ‘193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

Attached as Ex. 1035 is a table showing the numerous passages which Ismagilov copied key disclosures verbatim or nearly verbatim from the Quake PCT. These were not merely background or “state of the art” descriptions; rather, they go to the heart of the claimed subject matter.

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

Quake relates to microfluidic devices designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [citing Quake ‘332 publication ¶ 3, Ex. 1033 ¶ 3]. The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet

channel, through which a pressurized stream of aqueous solution is passed. A junction joins the main channel with the sample inlet channel. By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. Figure 16A of Quake is reproduced below:

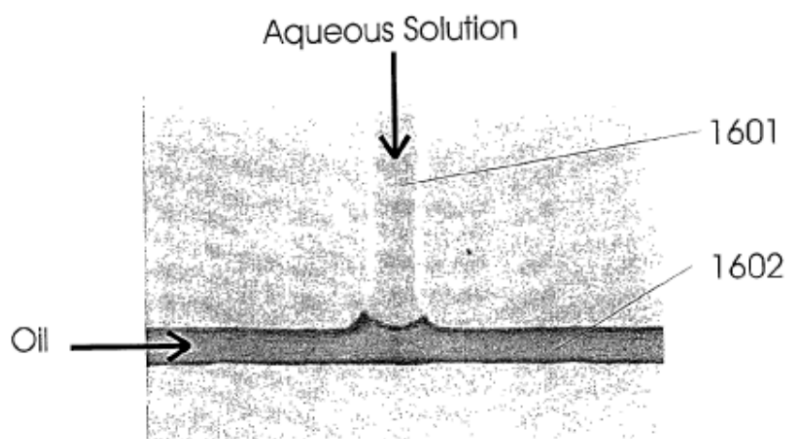


FIG. 16A

Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil. [Ex. 1052 at 12 citing Quake (Ex. 1033) ¶ 292].

In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* ¶ 80.

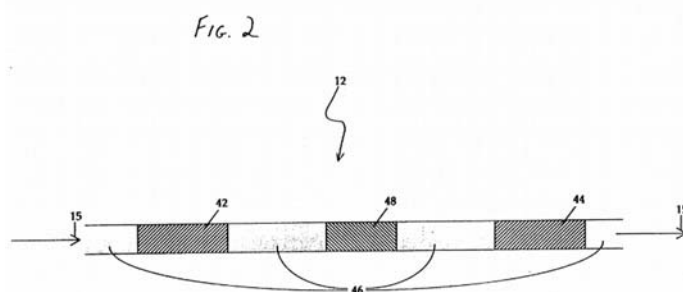
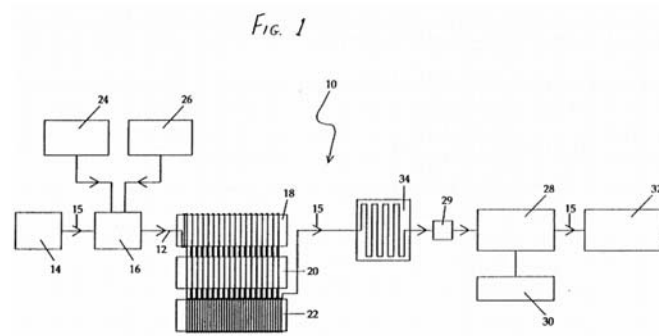
Ex. 1052 at 11-14.

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by-streams limitations of the ‘193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous

streams.⁷⁵ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the '193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from streams of immiscible fluids.

Turning the second issue, as of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Burns II. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.

⁷⁵ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.



“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

In 1996, Burns II reported the use of microfluidic devices to perform PCR. Ex. 1008 at 5556. Burns II explained that “[n]o new biochemistries or DNA detection methods are required for their use; the components simply reproduce current laboratory procedures in a micron-sized environment.” *Id.* at 5560. In other words, the PCR chemistry did not need to be modified – it worked without modification on the microfluidic scale. In the method of Burns II, a sample containing a DNA plasmid was loaded into one of the reaction wells (A) and a mixture of Taq enzyme and buffer was loaded into the other. Ex. 1008 at 5558. Each formed droplets of about

60 nl in the parallel 500 x 25 μm channels (B) which came together at the Y junction to form a single larger droplet (about 120 nl). *Id.* at 5558-59; Ex. 1004 ¶40.

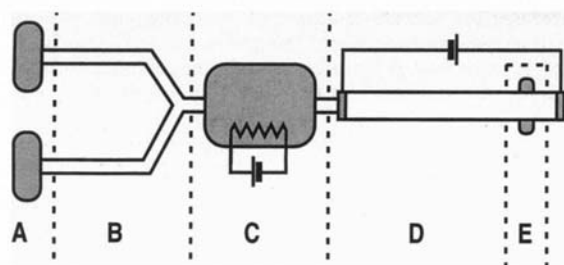


FIG. 1. Example of an integrated DNA analysis system, represented schematically. The individual components of the system are injection entry ports (A), liquid pumping channels (B), thermally controlled reaction chamber (C), electrophoresis channel (D), and DNA band migration detector (E). Each component would have associated sensors, control circuitry, and external connections.

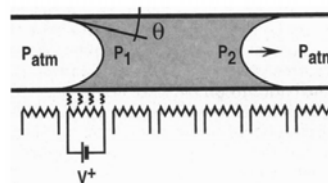


FIG. 2. Schematic drawing showing the principle of thermally induced drop motion in a closed channel. The case of a single aqueous drop in a hydrophilic channel is presented, where V is an applied voltage, P_{atm} is atmospheric pressure, P_1 is the receding-edge internal pressure, P_2 is the advancing-edge internal pressure, and θ is the contact angle of the liquid-gas-solid interface. The contact angle will depend on the surface characteristics of the channel and the constituents of the drop, with a hydrophilic interaction giving θ between 0 and 90 degrees, and a hydrophobic surface giving θ between 90 and 180 degrees. Surface treatments can also reduce contact angle hysteresis and, therefore, reduce the temperature difference necessary for drop motion.

The droplet then passed through the resistive heater portion (C) in which PCR amplification occurred. *Id.* at 5556-5557. Afterwards the amplification results were optically detected in zone D via gel electrophoresis. *Id.* at 5557-5561. Burns II noted that “[t]he use of common fabrication methods allows the assembly of increasingly complex, multicomponent, integrated systems from a small, defined set of standardized elements” and that “[f]uture design efforts have a strong impetus to reduce component size and improve efficiency.” *Id.* at 5560-61.

In sum, Corbett and Burns II show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).⁷⁶ Ex. 1001 at 78:12-

⁷⁶ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

15. In the instant combination, this limitation is met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents.

Ex. 1004 ¶¶32-38.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Corbett and Burns II. *Id.* Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Quake, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.*

Quake **specifically suggests that his microfluidic device could be used to perform PCR.** Quake explained that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR...” Ex. 1034 at 23:26-30. Quake noted that “PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen.” *Id.* at 2:31-3:2.

More generally, a skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactor of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-

contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶¶92-100; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Quake to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In

larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of**

relatively rare mutations, dislocations and allelic imbalances. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239 (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* 9239. Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Burns II) to incorporate a droplet reactor (Quake). Ex. 1004 ¶¶88-109. Using two flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Burns II. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination

and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk

heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with

microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching**

whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Ex. 1004 ¶¶39-57. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. *Id.*

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Burns II to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁷⁷ As discussed in Section

⁷⁷ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so

VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Corbett and Burns II. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Quake, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

As explained in more detail below, in the accompanying claim chart (Ex. 1063), and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Corbett and Burns II. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Quake, as evidenced by Burns II's contemporaneous report that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Quake discloses “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided. . . . For instance, **in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials**, a water soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Ex. 1034 at 28:20-23 , [emphasis added].

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Quake may be used to perform PCR reactions which are autocatalytic. Ex. 1034 pp. 2, 3, 23.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1063.

Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

Quake’s device comprises a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed: “The channel architecture for the droplet extrusion region of the first device is shown in FIG. 16A. In this device, the inlet channel 1601 (inner diameter 30 μm) intersects the main channel 1602 (inner diameter 30 μm) at a T-intersection (i.e., an angle perpendicular to the

main channel). Other intersections and angles may be used. The walls of the inlet and main channels were not tapered in this device.” Ex. 1034 at 79:23-28.

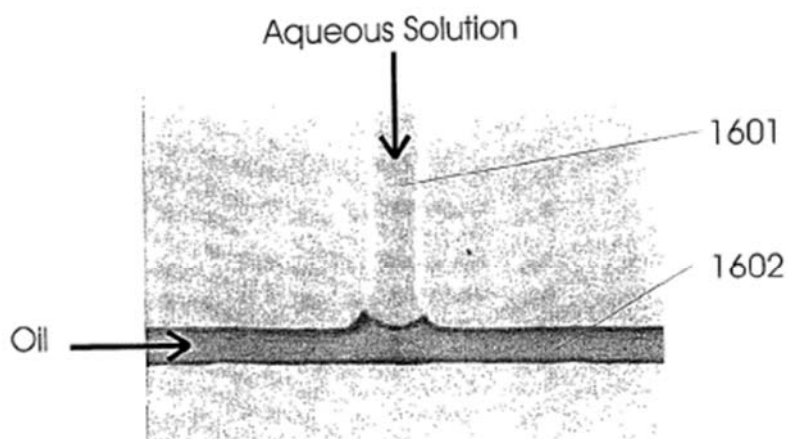


FIG. 16A

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1063.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

Quake teaches that “[a] first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the second fluid, passes through the inlet region so that droplets of the second fluid are sheared into the main channel. ... The second phase or fluid which passes through the inlet region can be an **aqueous solution**, for example ultra pure water, TE buffer, phosphate buffer saline and acetate buffer. The second fluid may also contain a **biological sample** (e.g., molecules of an enzyme or a substrate, or one or more cells, or one or more viral particles) for analysis or sorting in the device. In preferred embodiments the second

fluid includes a biological sample that comprises one or more **molecules**, cells, virions or particles.” Ex. 1034 at 6:6-16, [emphasis added]. Quake further teaches that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a “sample” or “droplet” fluid, passes or flows through the inlet region. ... Thus, droplets of the sample fluid containing the biological material for analysis, reaction or sorting are sheared at the droplet extrusion region into the flow of the extrusion fluid in the main channel.” *Id.* at 7:30-8:5.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).⁷⁸ Ex. 1001 at 78:12-15. In the instant combination, this limitation is met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-38.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Corbett and Burns II. Ex. 1004 ¶¶88-109. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Quake, as evidenced by Kopp, Burns II, Lagally and Vogelstein’s contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.*

⁷⁸ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

Quake **specifically suggests that his microfluidic device could be used to perform PCR.** Quake explained that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR...” Ex. 1034 at 23:26-30. Quake noted that “PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen.” *Id.* at 2:31-3:2.

More generally, a skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. Ex. 1004 ¶¶88-109. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions and the use of two streams of immiscible fluids would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. Likewise, the reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶¶92-100; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR

microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Quake to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex.

1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”). *Id.* 9239. Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Burns II) to incorporate a droplet reactor (Quake). Ex. 1004 ¶¶88-109. Using two flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Burns II. *Id.* The use of two flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at

Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The

first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic

diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Ex. 1004 ¶¶39-

57. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. *Id.*

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Burns II to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁷⁹ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

⁷⁹ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

As explained further in the accompanying claim chart (Ex. 1063) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Burns II. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Quake, as evidenced by contemporaneous reports by Corbett, Kopp, Burns II and Lagally that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1063.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

Quake discloses that “[p]referably, the fluid introduced to the main channel through the extrusion region is ‘incompatible’ (i.e., immiscible) with the fluid in the main channel so that droplets of the fluid introduced through the extrusion region are sheared off into the stream of fluid in the main channel. For example, the first phase or fluid which flows through the main channel can be a non-polar solvent, such as **decane (e.g., tetradecane or hexadecane) or another oil (for example, mineral oil).**” Ex. 1034 at 19, 87, [emphasis added].

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1063.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially

surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

As discussed above, the written descriptive support in the '193 patent for this claim element (which was the supposed point of novelty) was copied almost verbatim from Quake. The table below shows the correspondence between the relevant disclosures in the Quake PCT (Ex. 1034) and the Ismagilov provisional application (Ex. 1003) and '193 patent.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and '193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	Provisional at 22:12-19, '193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

Quake relates to microfluidic devices designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [citing Quake '332 publication ¶ 3, Ex. 1033 ¶ 3]. The devices comprise a main channel,

through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* A junction joins the main channel with the sample inlet channel. *Id.* By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

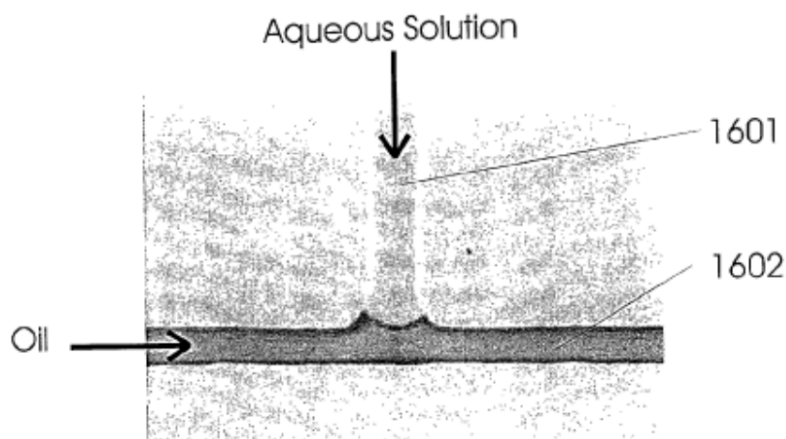


FIG. 16A

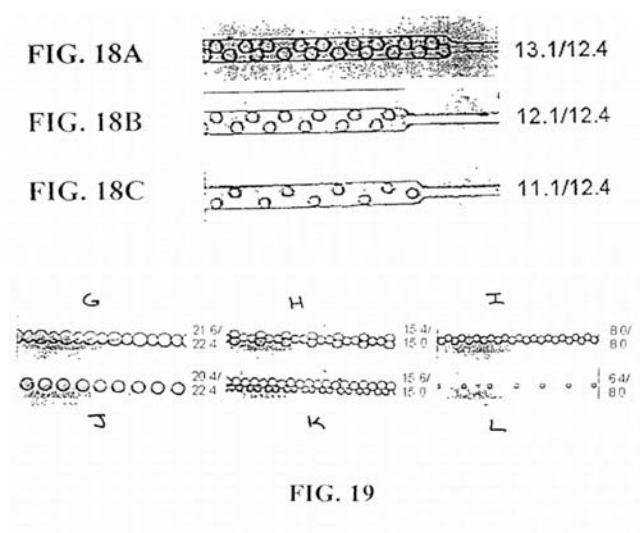
Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil.

In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* at 12 [which corresponds to Ex. 1033 at ¶ 80].

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by -streams limitations of the ‘193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his

invention as involving continuous streams.⁸⁰ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the '193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from streams of immiscible fluids.

The recitation that the plug is “substantially surrounded by an oil flowing through the channel” is met by Figures 18 and 19 of Quake. “FIGS. 18A-C are photomicrographs showing droplets of aqueous solution in a flow of oil (hexadecane with 2% Span 80 surfactant) in a microfluidic device with rectangular channels.” Ex. 1034 at 11. “FIG. 19 provides photomicrographs (Frames A-L) showing droplets of aqueous solution in a flow of oil (hexadecane with 2% Span 80 surfactant) in a microfluidic device with rounded channels.” *Id.* at 11. Figures 18B, 18C, 19J and 19L each depict droplets being completely surrounded by the carrier fluid.



Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1063.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Quake teaches that “droplets of the sample fluid containing the biological material for analysis, **reaction** or sorting are sheared at the droplet extrusion region into the flow of the

⁸⁰ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.

extrusion fluid in the main channel.” Ex. 1034 at 8, [emphasis added]. “Microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR [i.e., a reaction].” *Id.* at 23.

As discussed above in connection with element 1[b], in the instant combination Quake is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule. Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. This limitation is met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-38. The DNA is amplified through the PCR reaction. *Id.*

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1063.

Thus, claim 1 is shown to be rendered obvious by the combination of Quake, Corbett and Burns II.

Dependent Claim 2

Claim 2 of the ‘193 patent recites “wherein the at least one substrate molecule is a single biological molecule.”

Quake discloses that “[t]he invention also provides a method for sorting biological material. In various embodiments of the method, the biological material may be, e.g., molecules (for example, polynucleotides, polypeptides, enzymes, substrates or mixtures thereof), cells or viral particles, or mixtures thereof.” Ex. 1034 at 8:19-22. “The term ‘molecule’ means any distinct or distinguishable structural unit of matter comprising one or more atoms, and includes for example polypeptides and polynucleotides.” *Id.* at 13:10-12. “The term ‘polynucleotide’ as

used herein refers to a polymeric molecule having a backbone that supports bases capable of hydrogen bonding to typical polynucleotides, ... Polymeric molecules include double and single stranded RNA and DNA, and backbone modifications thereof, for example, methylphosphonate linkages.” *Id.* at 13:16-23.

Quake further specifies that, “[p]referably, each droplet of this multi-phase mixture encapsulates a single particle.” *Id.* at 5. “In particular, the sample concentration should be dilute enough that most of the droplets contain no more than a single molecule, cell or virion, with only a small statistical chance that a droplet will contain two or more molecules, cells or virions.” *Id.* at 36-7.

In the combined method of Quake, Corbett and Burns II, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using flows of two immiscible fluids and includes a mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1063.

Thus, claim 2 is shown to be rendered obvious by the combination of Quake, Corbett and Burns II.

Dependent Claims 3 and 4

Claim 3 of the ‘193 patent recites “wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.” Claim 4 of the ‘193 patent recites “wherein the providing step includes heating.” PCR reactions involve thermocycling. Ex. 1004 ¶¶32-38.

Quake discloses that “microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” Ex. 1034 at 23:26-30. In the

combined method of Quake, Corbett and Burns II, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using flows of an aqueous fluid and an oil and includes a mixture of DNA (the substrate molecule) and reagents, e.g., polymerase, primers, buffers and the like. To affect the PCR reaction, the carrier fluid is heated. (“The carrier fluid then passes through a plurality of temperature zones in which the polymerase chain reactions take place”) Ex. 1010 (Corbett), (“The enzymatic reaction occurred by moving two drops (Taq I restriction enzyme and supercoiled plasmid) down separate channels using the thermocapillary technique, combining the drops, and heating the merged sample to 65°C in the channel.”). Ex. 1008 at 5559 (Burns II)

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1063.

Thus, claims 3-4 are shown to be rendered obvious by the combination of Quake, Corbett and Burns II.

Dependent Claim 5

Claim 5 of the ‘193 patent further recites “providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.”

Quake discloses that “[t]he device of the invention may also comprise a **detection region** which is within or coincident with at least a portion of the main channel at or downstream of the droplet extrusion region. The device may also have a detector, preferably an **optical detector** such as a microscope, associated with the detection region.” Ex. 1034 at 6:28-31, [emphasis added].

Corbett similarly teaches that “[d]ownstream of temperature zone 34 is in-line analysis means 29 and recovery means 28. Connected to recovery means 28 is **detection means 30**.

Detection means 30 determines when a reaction mixture in the carrier fluid reaches recovery means 28. This may be done by measuring the conductivity or **optical density** of the stream of carrier fluid.” Ex. 1010 at 8:12-18, [emphasis added].

Burns II teaches that “[c]apillary gel electrophoresis of DNA samples was performed using a Beckman P/ACE instrument with a laser-induced fluorescence detector and 37 cm length, 100 μ m diameter, linear polymerase gel capillary according to manufacturer’s recommendation.” Ex. 1008 at 5558. The results of capillary electrophoresis were generally detected optically at the time of filing. Ex. 1011 (Dolnik) at 46.

In the combined method of Quake, Corbett and Burns II, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶68-69.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1-63

Thus, claim 5 is shown to be rendered obvious by the combination of Quake, Corbett and Burns II.

Dependent Claims 6-8

Claim 6 of the ‘193 patent recites “wherein the oil is fluorinated oil.” Claim 7 of the ‘193 patent recites “wherein the carrier fluid further comprises a surfactant.” Claim 8 of the ‘193 patent recites “wherein the surfactant is fluorinated surfactant.”

Quake discloses that “[t]he fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). **Exemplary surfactants** include Tween, Span, **fluorinated oils**, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the

shear force needed to extrude or inject droplets into an intersecting channel.” Ex. 1034 at 35:18-22 , [emphasis added].

The phrase “carrier fluid” in claim 7, having no antecedent basis, is construed to mean “a fluid that is immiscible with a plug fluid,” as this is the way the term is defined in the specification. See Ex. 1001 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1063.

Thus, claims 6-8 are shown to be rendered obvious by the combination of Quake, Corbett and Burns II.

Dependent Claim 9

Claim 9 of the ‘193 patent recites “wherein the at least one plug is a merged plug.”

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1063.

Thus, claim 9 is shown to be rendered obvious by the combination of Quake, Corbett and Burns II.

Dependent Claim 10

Claim 10 of the ‘193 patent recites “trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels.”

Quake describes a “detection region” which “should generally be at least about twice, preferably at least about five times as large per side or in diameter as the diameter of the largest

molecule, cell or droplet that will be passing through it.” Ex. 1034 at 26. This may be contrasted to Quake’s generally recommended dimensions of the fluid channels, which are shaped to cause the droplets to “conform to the size and shape of the channels” by “becom[ing] longer and thinner” as channels narrow. *Id.* at 27. Quake teaches that “an optical trap, or laser tweezers, may be used to sort or direct cells.... The optical trap remains in an ‘off’ mode until a desired particle is detected in the detection region. After detection of a desired characteristic, the particle or cell is ‘trapped’, and thereby directed or moved into the predetermined branch channel for collection.” *Id.* at 67, *see also* FIGs. 14A & B (Input). Thus, Quake’s detection region is an “expansion portion” where the at least one plug would be “trapp[ed]... for a period of time... after the reaction” as recited in dependent claim 10.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1063.

Thus, claim 10 is shown to be rendered obvious by the combination of Quake, Corbett and Burns II.

Dependent Claim 11

Claim 11 of the ‘193 patent recites “wherein the at least one plug is substantially spherical in shape.”

As discussed by Quake, “the microfabricated devices of this invention generate round, monodisperse droplets (such as those illustrated in Frames J and L of FIG. 19” (reproduced below). Ex. 1034 at 27, FIG. 19.

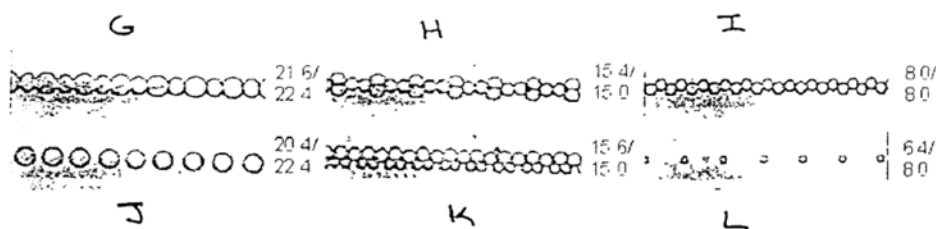


FIG. 19

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1063.

Thus, claim 11 is shown to be rendered obvious by the combination of Quake, Corbett and Burns II.

Dependent Claim 12

Claim 12 of the '193 patent recites "a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction."

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1063.

Thus, claim 12 is shown to be rendered obvious by the combination of Quake, Corbett and Burns II.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '193 patent recites "wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters." Claim 14 of the '193 patent recites "wherein the relevant parameters are

selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns.”

Corbett teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Corbett meets both claims 13 and 14 under the broadest reasonable interpretation of the term “special design,” which in an of itself imparts no meaningful structural limitation. Ex. 1010 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1063.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Quake, Corbett, and Burns II.

C. The Combination of Quake, Corbett and Vogelstein Renders Obvious Claims 1-14 of the ‘193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Quake), ii) microfluidic PCR devices were well known (Corbett and Vogelstein), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Lagally, Kopp, Burns II and Vogelstein).

As to the first feature – forming plugs from continuously flowing immiscible fluids – the ‘193 patent’s teachings in this regard were copied almost verbatim from the Quake PCT (Ex. 1034). The table below compares the Quake PCT publication with the Ismagilov specification, which was filed about two months later.

Quake PCT (published March 21, 2002 (Ex. 1034)	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and ‘193 patent (Ex. 1001)

34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	Provisional at 22:12-19, '193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

Attached as Ex. 1035 is a table showing the numerous passages which Ismagilov copied key disclosures verbatim or nearly verbatim from the Quake PCT. These were not merely background or “state of the art” descriptions; rather, they go to the heart of the claimed subject matter.

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

Quake relates to microfluidic devices designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [citing Quake '332 publication Ex. 1033 at ¶ 3]. The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* A junction joins the main channel with the sample inlet channel. *Id.* By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular

frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

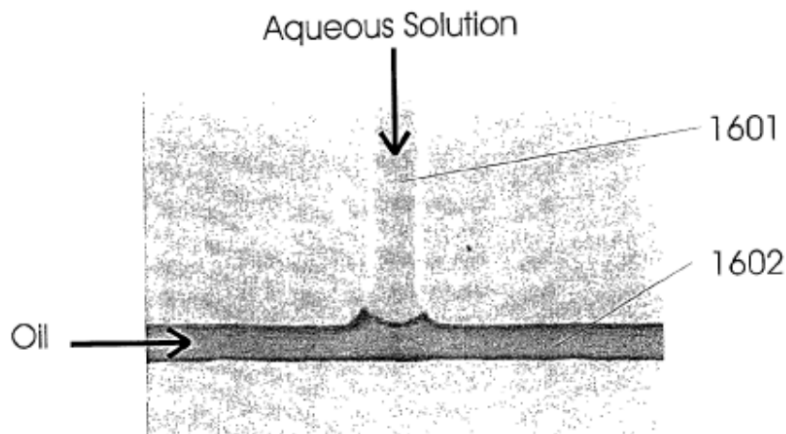


FIG. 16A

Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil. [Ex. 1052 at 12 citing Quake ¶ 292].

In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* ¶ 80.

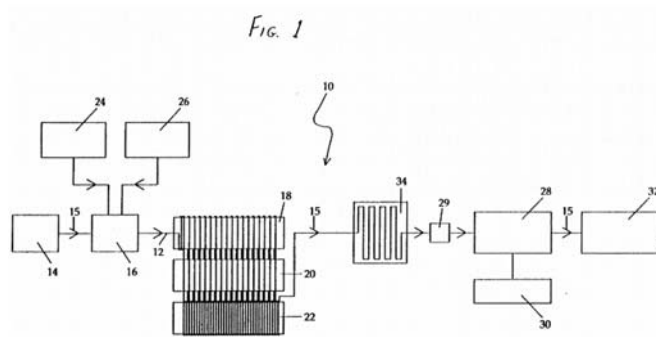
Ex. 1052 at 11-14.

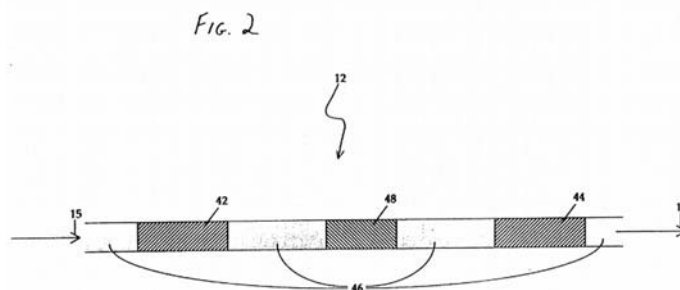
The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by -streams limitations of the ‘193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous streams.⁸¹ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution

⁸¹ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.

of the '193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from streams of immiscible fluids.

Turning the second issue, as of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Vogelstein. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.





“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

By 1999, Vogelstein had reported **various applications for single-molecule microfluidic PCR**. Ex. 1044 at 9241. In a seminal article which has been cited over by over 1,000 other publications, Vogelstein explained that PCR amplification of small aliquots or droplets containing single molecules was effective for “detection of a small number of mutant-containing cells among a large excess of normal cells.” *Id.* at 9236. In Vogelstein’s method, the DNA was diluted into 7 μ l volumes such that, on average, there was one template molecule for every two wells (i.e., approximately half of the wells contained no DNA template molecule and approximately half contained a single DNA template molecule). *Id.* at 9236, 9237. This avoided the problem of background noise impairing the determination of whether each volume contained the mutant sequence. *Id.* By counting the number of volumes which indicated wild type or mutant templates, the presence or absence of the mutation (e.g., a sequence associated with colorectal cancer) can be determined. *Id.* at 9238. The accuracy of the test is a function of the

number of discrete volumes into which the sample is diluted. *Id.* at 9239. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) The article explained that the same technique would be useful for **detecting a wide array of mutations, dislocations and allelic imbalances**, as summarized in Table 1. *Id.* at 9241. Vogelstein thus taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby overcoming the signal-to-noise limitations associated with certain other PCR techniques and enabling the detection of relatively rare mutations, dislocations and allelic imbalances. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86.

In sum, Corbett and Vogelstein show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date. *Id.* ¶¶39-57.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).⁸² Ex. 1001 at 78:12-15. In the instant combination, this limitation is met by using the microfluidic droplet reactors

⁸² Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

with two flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents.

Ex. 1004 ¶¶32-38.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Corbett and Vogelstein. *Id.* ¶¶92-109. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Quake, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.*

Quake **specifically suggests that his microfluidic device could be used to perform PCR.** Quake explained that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR...” Ex. 1034 at 23:26-30. Quake noted that “PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen.” *Id.* at 2:31-3:2.

More generally, a skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactor of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed,**

portability, and cost.” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶¶92-100; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Quake to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as

the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241;

Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp, Burns II, Lagally or Vogelstein) to incorporate a droplet reactor (Quake, Burns I or Shaw Stewart). Ex. 1004 ¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip.

Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful.**

In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or **continuous flow** through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling

profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced

microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic**

droplet (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Vogelstein to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁸³ As discussed in Section

⁸³ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the

VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail below, in the accompanying claim chart (Ex. 1064), and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Corbett and Vogelstein. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Quake, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Quake discloses “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided. . . . For instance, **in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials**, a water

art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) (‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Ex. 1034 at 28..

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Quake may be used to perform PCR reactions which are autocatalytic. Ex. 1034 ¶ 6.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1064.

Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

Quake’s device comprises a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed: “The channel architecture for the droplet extrusion region of the first device is shown in FIG. 16A. In this device, the inlet channel 1601 (inner diameter 30 μm) intersects the main channel 1602 (inner diameter 30 μm) at a T-intersection (i.e., an angle perpendicular to the main channel). Other intersections and angles may be used. The walls of the inlet and main channels were not tapered in this device.” Ex. 1034 at 79:23-28.

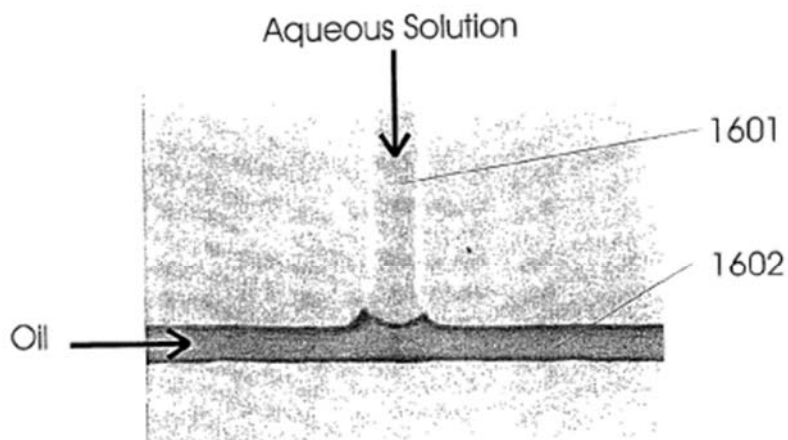


FIG. 16A

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit .

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

Quake teaches that “[a] first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the second fluid, passes through the inlet region so that droplets of the second fluid are sheared into the main channel. ... The second phase or fluid which passes through the inlet region can be an **aqueous solution**, for example ultra pure water, TE buffer, phosphate buffer saline and acetate buffer. The second fluid may also contain a **biological sample** (e.g., molecules of an enzyme or a substrate, or one or more cells, or one or more viral particles) for analysis or sorting in the device. In preferred embodiments the second fluid includes a biological sample that comprises one or more **molecules**, cells, virions or particles.” Ex. 1034 at 6-16. Quake further teaches that “[i]n preferred embodiments, a first

fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a "sample" or "droplet" fluid, passes or flows through the inlet region. ... Thus, droplets of the sample fluid containing the biological material for analysis, reaction or sorting are sheared at the droplet extrusion region into the flow of the extrusion fluid in the main channel.” *Id.* at 7:30-8:5.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing aqueous fluid with the reagents also includes at least one substrate molecule. This limitation is met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-38.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR in droplets as taught by Corbett, or to modify the reactions of Vogelstein to be performed in microfluidic droplets instead of continuous flow of a single fluid in a microfluidic channel. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to use two continuous streams of immiscible fluid as used in a microfluidic droplet reactor of Quake, as evidenced by contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

Quake **specifically suggests that his microfluidic device could be used to perform PCR.** Quake explained that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR...” Ex. 1034 at 23:26-30. Quake

noted that “PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen.” *Id.* at 2:31-3:2.

More generally, a skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions and the use of two continuous streams of immiscible fluids would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. Likewise, the reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶¶92-100; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in

development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047 .It was thus known that using the droplet reactor of Quake to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Vogelstein) to incorporate a droplet reactor (Quake). Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Vogelstein) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous

flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant

time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁸⁴ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained further in the accompanying claim chart (Ex. 1064) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Corbett and Vogelstein. Alternatively, a skilled artisan

⁸⁴ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Quake, as evidenced by contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1064.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

Quake discloses that “[p]referably, the fluid introduced to the main channel through the extrusion region is ‘incompatible’ (i.e., **immiscible**) with the fluid in the main channel so that droplets of the fluid introduced through the extrusion region are sheared off into the stream of fluid in the main channel. For example, the first phase or fluid which flows through the main channel can be a non-polar solvent, such as decane (e.g., tetradecane or hexadecane) or another oil (for example, mineral oil).” Ex. 1034 at 19, 87, [emphasis added].

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1064.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

As discussed above, the written descriptive support in the ‘193 patent for this claim element (which was the supposed point of novelty) was copied almost verbatim from Quake. The

table below shows the correspondence between the relevant disclosures in the Quake PCT (Ex. 1034) and the Ismagilov provisional application (Ex. 1003) and ‘193 patent.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and ‘193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	Provisional at 22:12-19, ‘193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

Quake relates to microfluidic devices designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [citing Quake ‘332 publication Ex. 1033 at ¶ 3]. The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* A junction joins the main channel with the sample inlet channel. *Id.* By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

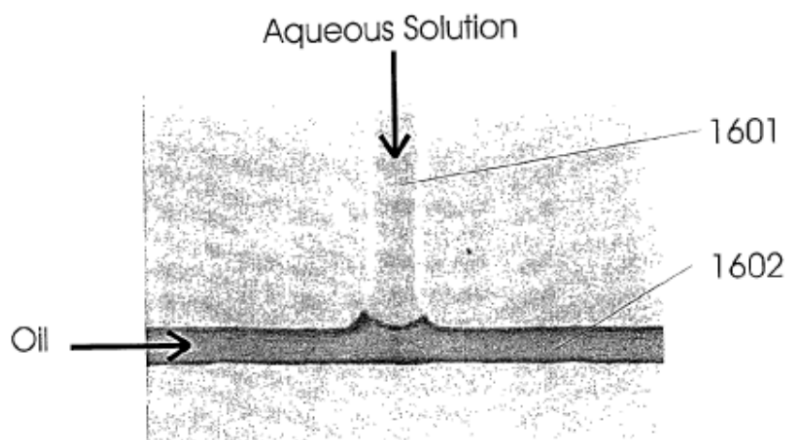


FIG. 16A

Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil. [Ex. 1052 at 12 citing Quake ¶ 292].

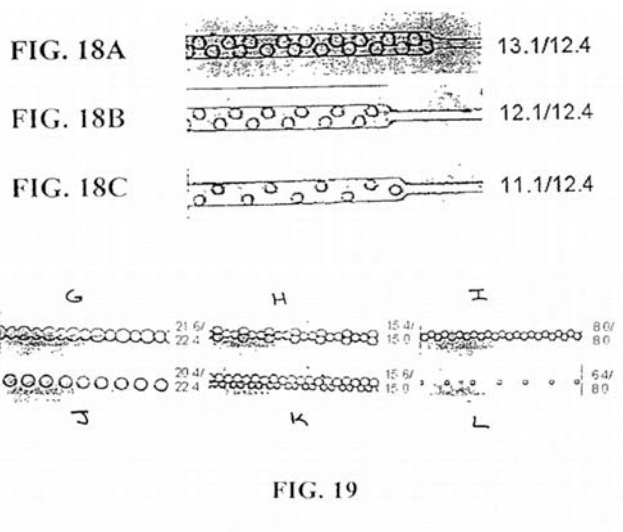
In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* ¶ 80.

Ex. 1052 at 11-14.

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by -streams limitations of the ‘193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous streams.⁸⁵ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the ‘193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids.

⁸⁵ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.

The recitation that the plug is “substantially surrounded by the oil flowing through the channel” is met by Figures 18 and 19 of Quake. “FIGS. 18A-C are photomicrographs showing droplets of aqueous solution in a flow of oil (hexadecane with 2% Span 80 surfactant) in a microfluidic device with rectangular channels.” Ex. 1034 at 11: 24-27.. “FIG. 19 provides photomicrographs (Frames A-L) showing droplets of aqueous solution in a flow of oil (hexadecane with 2% Span 80 surfactant) in a microfluidic device with rounded channels.” *Id.* at 11:29-31. Figures 18B, 18C, 19J and 19L each depict droplets being completely surrounded by the carrier fluid.



Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1062.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Quake teaches that “droplets of the sample fluid containing the biological material for analysis, **reaction** or sorting are sheared at the droplet extrusion region into the flow of the extrusion fluid in the main channel.” Ex. 1034 at 8, [emphasis added]. “Microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as **PCR** [i.e., a reaction].” *Id.* at 23. [emphasis added].

As discussed above in connection with element 1[b], in the instant combination Quake is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule.

Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. This limitation is met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71. The DNA is amplified through the PCR reaction. *Id.*

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1064

Thus, claim 1 is shown to be rendered obvious by the combination of Quake, Corbett and Vogelstein.

Dependent Claim 2

Claim 2 of the '193 patent recites "wherein the at least one substrate molecule is a single biological molecule."

Quake discloses that "[t]he invention also provides a method for sorting biological material. In various embodiments of the method, the biological material may be, e.g., molecules (for example, polynucleotides, polypeptides, enzymes, substrates or mixtures thereof), cells or viral particles, or mixtures thereof." Ex. 1034 at 8: 19-22. "The term 'molecule' means any distinct or distinguishable structural unit of matter comprising one or more atoms, and includes for example polypeptides and polynucleotides." *Id.* at 13: 10-12. "The term 'polynucleotide' as used herein refers to a polymeric molecule having a backbone that supports bases capable of hydrogen bonding to typical polynucleotides, ... Polymeric molecules include double and single stranded **RNA and DNA**, and backbone modifications thereof, for example, methylphosphonate linkages." *Id.* at 13: 16-23.

Quake further specifies that, "[p]referably, each droplet of this multi-phase mixture encapsulates a single particle." *Id.* at 5. "In particular, the sample concentration should be dilute

enough that most of the droplets contain no more than a single molecule, cell or virion, with only a small statistical chance that a droplet will contain two or more molecules, cells or virions.” *Id.* at 36-7.

In the combined method of Quake, Corbett and Lagally, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1064.

Thus, claim 2 is shown to be rendered obvious by the combination of Quake, Corbett and Vogelstein.

Dependent Claims 3 and 4

Claim 3 of the ‘193 patent recites “wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.” Claim 4 of the ‘193 patent recites “wherein the providing step includes heating.” PCR reactions involve thermocycling. Ex. 1004 ¶¶33-34.

Quake discloses that “microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” Ex. 1034 at 2:26-303. In the combined method of Quake, Corbett and Lagally, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. To affect the PCR reaction, the carrier fluid is heated. (“The carrier fluid then passes through a plurality of temperature zones in which the polymerase chain reactions take place”) Ex. 1010 (Corbett), (“If

a PCR product whose sequence is complementary to the loop sequence is present during the heating/cooling cycle, hybridization of the MB to one strand of the PCR product will increase the distance between the Dabcyl and the dye, resulting in increased fluorescence.”) Ex. 1044 at 9237(Vogelstein).

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1064.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Quake, Corbett and Vogelstein.

Dependent Claim 5

Claim 5 of the ‘193 patent further recites “providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.”

Quake discloses that “[t]he device of the invention may also comprise a **detection region** which is within or coincident with at least a portion of the main channel at or downstream of the droplet extrusion region. The device may also have a detector, preferably an **optical detector** such as a microscope, associated with the detection region.” Ex. 1034 at 6:28-31, [emphasis added].

Corbett similarly teaches that “[d]ownstream of temperature zone 34 is in-line analysis means 29 and recovery means 28. Connected to recovery means 28 is **detection means 30**. Detection means 30 determines when a reaction mixture in the carrier fluid reaches recovery means 28. This may be done by measuring the conductivity or **optical density** of the stream of carrier fluid.” Ex. 1010 at 8:12-18, [emphasis added].

Vogelstein teaches that the “[r]eactions were analyzed immediately or stored at room temperature for up to 36 h before fluorescence analysis.” Ex. 1044 at 9236.

In the combined method of Quake, Corbett and Lagally, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶102, 108.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1064.

Thus, claim 5 is shown to be rendered obvious by the combination of Quake, Corbett and Vogelstein.

Dependent Claims 6-8

Claim 6 of the '193 patent recites "wherein the oil is fluorinated oil." Claim 7 of the '193 patent recites "wherein the carrier fluid further comprises a surfactant." Claim 8 of the '193 patent recites "wherein the surfactant is fluorinated surfactant."

Quake discloses that "the first phase or fluid which flows through the main channel can be a non-polar solvent, such as decane (e.g., tetradecane or hexadecane) or another oil (for example, **mineral oil**). Ex. 1034 at 6:8-10, [emphasis added]. "The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). **Exemplary surfactants** include Tween, Span, **fluorinated oils**, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel." Ex. 1034 at 35:18-22, [emphasis added].

The phrase "carrier fluid" in claim 7, having no antecedent basis, is construed to mean "a fluid that is immiscible with a plug fluid," as this is the way the term is defined in the specification. Ex. 1001 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1064.

Thus, claims 6-8 are shown to be rendered obvious by the combination of Quake, Corbett and Vogelstein.

Dependent Claim 9

Claim 9 of the '193 patent recites "wherein the at least one plug is a merged plug."

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1064.

Thus, claim 9 is shown to be rendered obvious by the combination of Quake, Corbett and Vogelstein.

Dependent Claim 10

Claim 10 of the '193 patent recites "trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels."

Quake describes a "detection region" which "should generally be at least about twice, preferably at least about five times as large per side or in diameter as the diameter of the largest molecule, cell or droplet that will be passing through it." Ex. 1034 at 26. This may be contrasted to Quake's generally recommended dimensions of the fluid channels, which are shaped to cause the droplets to "conform to the size and shape of the channels" by "becom[ing] longer and thinner" as channels narrow. *Id.* at 27. Quake teaches that "an optical trap, or laser tweezers, may be used to sort or direct cells.... The optical trap remains in an 'off' mode until a desired particle is detected in the detection region. After detection of a desired characteristic, the particle or cell is 'trapped', and thereby directed or moved into the predetermined branch channel for

collection.” *Id.* at 67, *see also* FIGs. 14A & B (Input). Thus, Quake’s detection region is an “expansion portion” where the at least one plug would be “trapp[ed]... for a period of time... after the reaction” as recited in dependent claim 10.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1064.

Thus, claim 10 is shown to be rendered obvious by the combination of Quake, Corbett and Vogelstein.

Dependent Claim 11

Claim 11 of the ‘193 patent recites “wherein the at least one plug is substantially spherical in shape.”

As discussed by Quake, “the microfabricated devices of this invention generate round, monodisperse droplets (such as those illustrated in Frames J and L of FIG. 19” (reproduced below). Ex. 1034 at 27, FIG. 19.

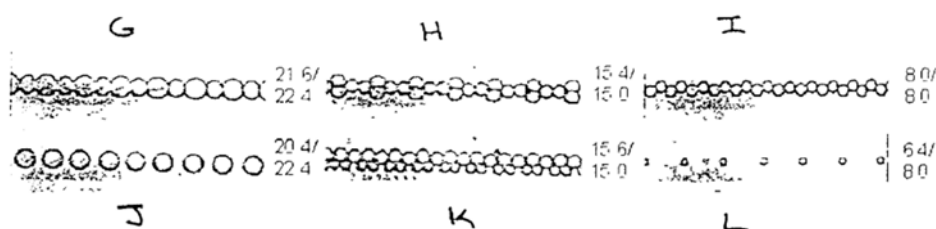


FIG. 19

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1064.

Thus, claim 11 is shown to be rendered obvious by the combination of Quake, Corbett and Vogelstein.

Dependent Claim 12

Claim 12 of the '193 patent recites “a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction.”

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1064.

Thus, claim 12 is shown to be rendered obvious by the combination of Quake, Corbett and Vogelstein.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '193 patent recites “wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters.” Claim 14 of the '193 patent recites “wherein the relevant parameters are selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns.”

Corbett teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Corbett meets both claims 13 and 14 under the broadest reasonable interpretation of the term “special design,” which in an of itself imparts no meaningful structural limitation. Ex. 1010 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1064.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Quake, Corbett, and Vogelstein.

D. The Combination of Quake, Kopp and Lagally Renders Obvious Claims 1-14 of the '193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Quake), ii) microfluidic PCR devices were well known (Kopp and Lagally), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

As to the first feature – forming plugs from flowing immiscible fluids – the '193 patent's teachings in this regard were copied almost verbatim from the Quake PCT (Ex. 1034). The table below compares the Quake PCT publication with the Ismagilov specification, which was filed about three months later.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and '193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby</u>	Provisional at 22:12-19, '193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-</u>

<u>controlling the size and periodicity of the droplets.</u>	<u>forming region, thereby controlling the size and periodicity of the plugs.</u>

Attached as Ex. 1035 is a table showing the numerous passages which Ismagilov copied key disclosures verbatim or nearly verbatim from the Quake PCT. These were not merely background or “state of the art” descriptions; rather, they go to the heart of the claimed subject matter.

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

Quake relates to microfluidic devices designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [citing Quake ‘332 publication Ex. 1033 at ¶ 3]. The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* A junction joins the main channel with the sample inlet channel. *Id.* By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

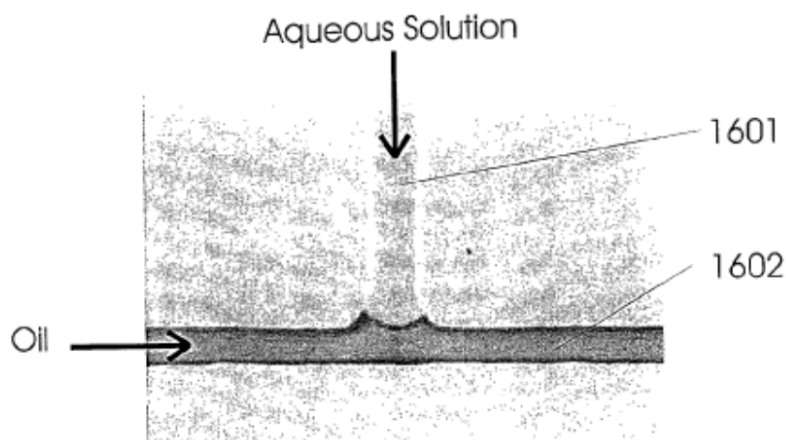


FIG. 16A

Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil. [Ex. 1052 at 12 citing Quake ¶ 292].

In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* ¶ 80.

Ex. 1052 at 11-14.

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by -streams limitations of the ‘193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous streams.⁸⁶ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the ‘193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids.

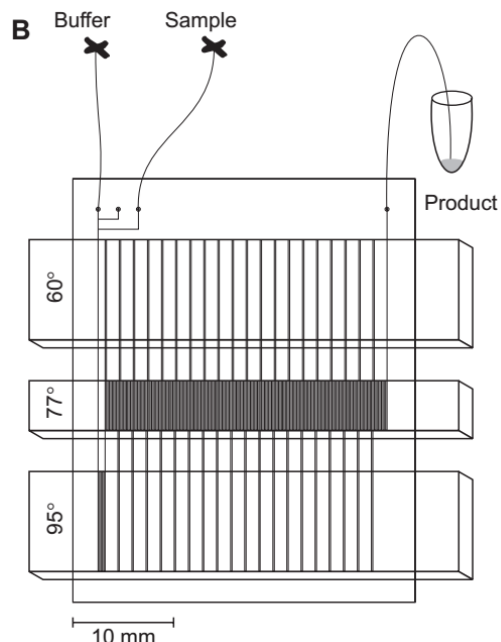
⁸⁶ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.

Turning the second issue, as of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Lagally. By 1998 Kopp *et al.* had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 [emphasis added].

Kopp *et al.* also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene

could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 [emphasis added].

In 2001, in another article not previously considered by the Office, Lagally reported using a microfluidic reactor to conduct single-molecule DNA amplification by PCR. Lagally summarized the state of the art as of February 2001, explaining continuous flow PCR microreactors had recently undergone substantial evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or **continuous flow** through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-

volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

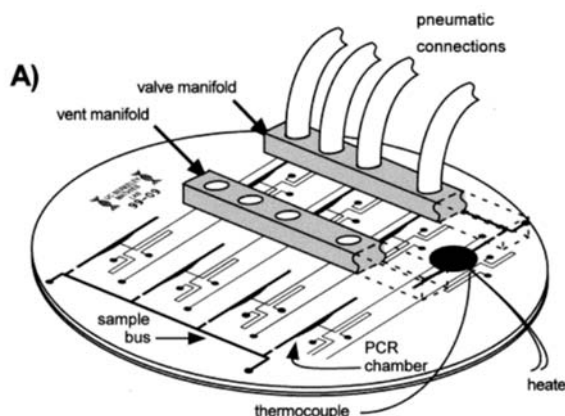
Ex. 1028 at 565-556 [emphasis added].

Lagally *et al.* developed a further improvement – a device and method for achieving single molecule amplification in a microchannel environment.

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

Microfluidic PCR-CE device. The PCR chambers are connected to a common sample bus through a set of valves. Hydrophobic

vents at the other end of the PCR chambers are used to locate the sample and to eliminate gas. The PCR chambers are directly connected to the cross channel of the CE system for product injection and analysis. Two aluminum manifolds, one each for the vents and valves, are placed onto the respective ports and clamped in place using vacuum. The manifolds connect to external solenoid valves for pressure and vacuum actuation. Thermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.



Ex. 1028 at 565-66 (see figure legend).

In sum, Kopp and Lagally show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").⁸⁷ Ex. 1001 at 78:12-15. In the instant combination, this limitation is met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions,

⁸⁷ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-38.

Quake **specifically suggests that his microfluidic device could be used to perform PCR.** Quake explained that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR...” Ex. 1034 at 23:26-30. Quake noted that “PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen.” *Id.* at 2:31-3:2.

More generally, a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Kopp and Lagally. Ex. 1004 ¶¶88-109. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. *Id.* As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶¶88-109; Ex. 1008 at Abstract; Ex. 1027

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide

point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Quake to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling

times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Lagally) to incorporate a droplet reactor (Quake). Ex. 1004 ¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Lagally. The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Lagally). Ex. 1004 ¶88-90. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at

Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The

first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic

diagnostic devices even if they do not operate at the single-molecule limit.

On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with

the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp or Lagally to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the '93 patent at least as early as February 23, 2001, more than a year prior to the '93 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '93 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁸⁸ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail below, in the accompanying claim chart (Ex. 1065) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith

⁸⁸ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Kopp and Lagally.

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Quake discloses “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided. . . . For instance, **in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials**, a water soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Ex. 1034 at 28., [emphasis added].

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Quake may be used to perform PCR reactions which are autocatalytic. Ex. 1034 ¶ 6.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1065.

Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

Quake’s device comprises a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed: “The channel architecture for the droplet extrusion region of the first device is shown in FIG. 16A. In this device, the inlet channel 1601 (inner diameter 30 μm) intersects the

main channel 1602 (inner diameter 30 μm) at a T-intersection (i.e., an angle perpendicular to the main channel). Other intersections and angles may be used. The walls of the inlet and main channels were not tapered in this device.” Ex. 1034 at 79-23-28.

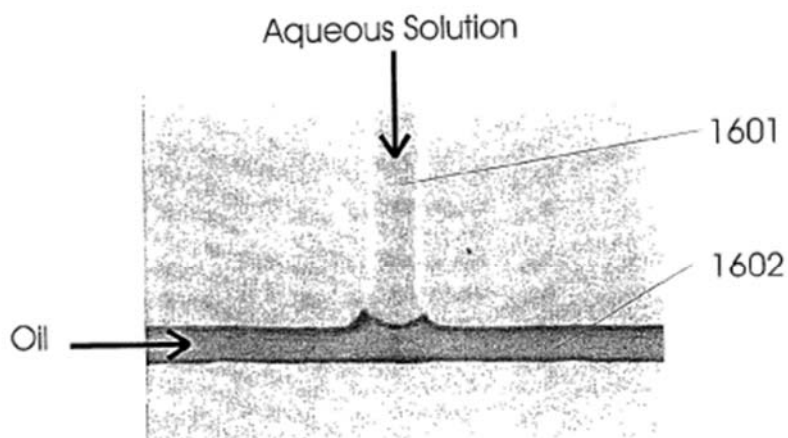


FIG. 16A

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1065.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

Quake teaches that “[a] first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the second fluid, passes through the inlet region so that droplets of the second fluid are sheared into the main channel. ... The second phase or fluid which passes through the inlet region can be an **aqueous solution**, for example ultra pure water, TE buffer, phosphate buffer saline and acetate buffer. The second fluid may also contain a **biological sample** (e.g., molecules of an enzyme or a substrate, or one or more cells, or one or

more viral particles) for analysis or sorting in the device. In preferred embodiments the second fluid includes a biological sample that comprises one or more **molecules**, cells, virions or particles.” Ex. 1034 at 6:6-16, [emphasis added]. Quake further teaches that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a “sample” or “droplet” fluid, passes or flows through the inlet region. ... Thus, droplets of the sample fluid containing the biological material for analysis, reaction or sorting are sheared at the droplet extrusion region into the flow of the extrusion fluid in the main channel.” *Id.* at 7:30-8:5.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).⁸⁹ Ex. 1001 at 78:12-15. In the instant combination, this limitation is met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions (as taught in Kopp and Lagally), wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents.

Quake **specifically suggests that his microfluidic device could be used to perform PCR**. Quake explained that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR...” Ex. 1034 at 23:26-30. Quake noted that “PCR is [] generally the method of choice to detect viral DNA or RNA directly in

⁸⁹ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen.” *Id.* at 2:31-3:2.

More generally, a skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. Likewise, the reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Quake to perform

PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett's PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample

leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* 9239. Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Kopp and Lagally. Ex. 1004 ¶37, 85.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Lagally) to incorporate a droplet reactor (Quake). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Lagally. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents

needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In

1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in modifying Lagally or Kopp to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet

formation recitation of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁹⁰ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained further in the accompanying claim chart (Ex. 1065) and the declaration of Dr. Shafqeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Kopp and Lagally.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1065.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

Quake discloses that “[p]referably, the fluid introduced to the main channel through the extrusion region is ‘incompatible’ (i.e., **immiscible**) with the fluid in the main channel so that

⁹⁰ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) (‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

droplets of the fluid introduced through the extrusion region are sheared off into the stream of fluid in the main channel. For example, the first phase or fluid which flows through the main channel can be a non-polar solvent, such as **decane (e.g., tetradecane or hexadecane) or another oil** (for example, mineral oil).” Ex. 1034 at 6:8-10. , [emphasis added].

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1065.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

As discussed above, the written descriptive support in the ‘193 patent for this claim element (which was the supposed point of novelty) was copied almost verbatim from Quake. The table below shows the correspondence between the relevant disclosures in the Quake PCT (Ex. 1034) and the Ismagilov provisional application (Ex. 1003) and ‘193 patent.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and ‘193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated.</u>	Provisional at 22:12-19, ‘193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated.</u>

Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.

Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

Quake relates to microfluidic devices designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [citing Quake '332 publication Ex. 1033 at ¶ 3]. The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* A junction joins the main channel with the sample inlet channel. *Id.* By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

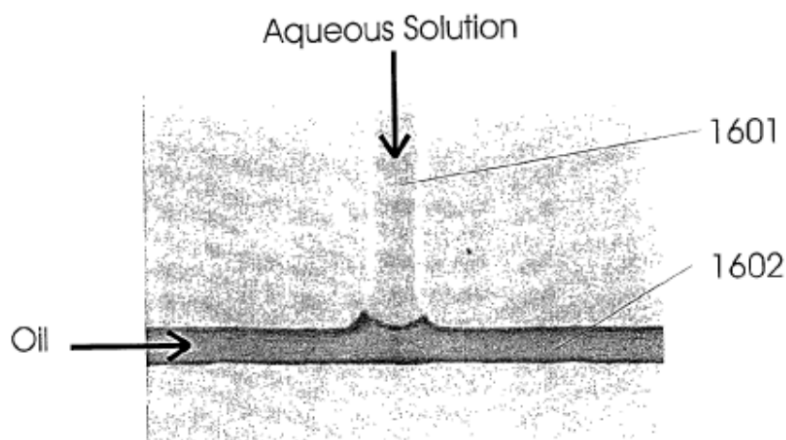


FIG. 16A

Figure 16A depicts the channel architecture for the droplet extrusion region.

Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil. [Ex. 1052 at 12 citing Quake ¶ 292].

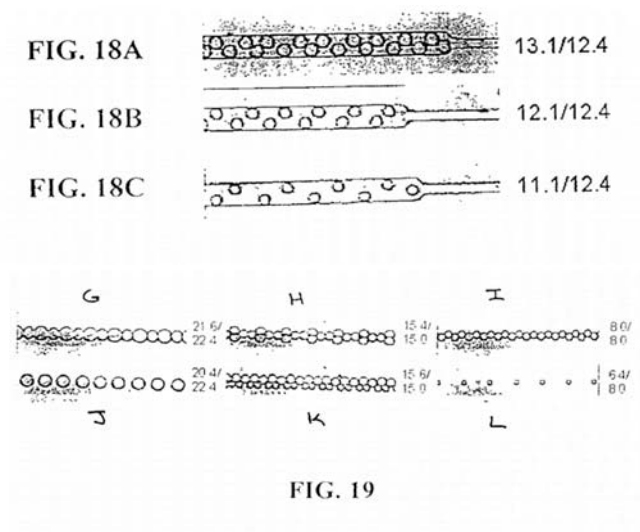
In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* ¶ 80.

Ex. 1052 at 11-14.

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by-streams limitations of the ‘193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous streams.⁹¹ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the ‘193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids.

⁹¹ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.

The recitation that the plug is “substantially surrounded by the oil flowing through the channel” is met by Figures 18 and 19 of Quake. “FIGS. 18A-C are photomicrographs showing droplets of aqueous solution in a flow of oil (hexadecane with 2% Span 80 surfactant) in a microfluidic device with rectangular channels.” Ex. 1034 at 11:24-27. “FIG. 19 provides photomicrographs (Frames A-L) showing droplets of aqueous solution in a flow of oil (hexadecane with 2% Span 80 surfactant) in a microfluidic device with rounded channels.” *Id.* at 11:29-31. Figures 18B, 18C, 19J and 19L each depict droplets being completely surrounded by the carrier fluid.



Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1065.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Quake teaches that “droplets of the sample fluid containing the biological material for analysis, **reaction** or sorting are sheared at the droplet extrusion region into the flow of the extrusion fluid in the main channel.” Ex. 1034 at 7:30-8:58, [emphasis added]. “Microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as **PCR** [i.e., a reaction].” *Id.* at 23:26-30. [emphasis added].

As discussed above in connection with element 1[b], in the instant combination Quake is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule.

Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. This limitation is met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71. The DNA is amplified through the PCR reaction. *Id.*

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1065

Thus, claim 1 is shown to be rendered obvious by the combination of Quake, Kopp and Lagally.

Dependent Claim 2

Claim 2 of the '193 patent recites "wherein the at least one substrate molecule is a single biological molecule."

Quake discloses that "[t]he invention also provides a method for sorting biological material. In various embodiments of the method, the biological material may be, e.g., molecules (for example, polynucleotides, polypeptides, enzymes, substrates or mixtures thereof), cells or viral particles, or mixtures thereof." Ex. 1034 at 8:19-22. "The term 'molecule' means any distinct or distinguishable structural unit of matter comprising one or more atoms, and includes for example polypeptides and polynucleotides." *Id.* at 13:10-12 "The term 'polynucleotide' as used herein refers to a polymeric molecule having a backbone that supports bases capable of hydrogen bonding to typical polynucleotides, ... Polymeric molecules include double and single stranded **RNA and DNA**, and backbone modifications thereof, for example, methylphosphonate linkages." *Id.* at 13:16-23.

Quake further specifies that, "[p]referably, each droplet of this multi-phase mixture encapsulates a single particle." *Id.* at 5. "In particular, the sample concentration should be dilute

enough that most of the droplets contain no more than a single molecule, cell or virion, with only a small statistical chance that a droplet will contain two or more molecules, cells or virions.” *Id.* at 36-7.

In the combined method of Quake, Kopp and Lagally, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1065.

Thus, claim 2 is shown to be rendered obvious by the combination of Quake, Kopp and Lagally.

Dependent Claims 3 and 4

Claim 3 of the ‘193 patent recites “wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.” Claim 4 of the ‘193 patent recites “wherein the providing step includes heating.” PCR reactions involve thermocycling. Ex. 1004 ¶¶33-34.

Quake discloses that “microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” Ex. 1034 at 2:26-30. In the combined method of Quake, Kopp and Lagally, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. To affect the PCR reaction, the carrier fluid is heated. (“The individual steps are simply performed by heating or cooling the sample to characteristic temperatures: 95°C for dsDNA melting, 50° to 65°C for primer

annealing, and 72° to 77°C for primer extension at the optimum enzyme temperature.”) Ex. 1027 at 1046 (Kopp), (“The PCR chambers were thermally cycled.”) Ex. 1028 at 567.

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1065.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Quake, Kopp and Lagally.

Dependent Claim 5

Claim 5 of the ‘193 patent further recites “providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.”

Quake discloses that “[t]he device of the invention may also comprise a **detection region** which is within or coincident with at least a portion of the main channel at or downstream of the droplet extrusion region. The device may also have a detector, preferably an **optical detector** such as a microscope, associated with the detection region.” Ex. 1034 at 6:23-31, [emphasis added].

Lagally teaches “[s]tochastic PCR amplification of single DNA template molecules followed by **capillary electrophoresis** (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Ex. 1028 at 565, [emphasis added]. The results of capillary electrophoresis were generally detected optically at the time of filing. Ex. 1011 (Dolnik) at 46; Ex. 1004 ¶¶68-69.

In the combined method of Quake, Kopp and Lagally, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶102, 108.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1065.

Thus, claim 5 is shown to be rendered obvious by the combination of Quake, Kopp and Lagally.

Dependent Claims 6-8

Claim 6 of the '193 patent recites "wherein the oil is fluorinated oil." Claim 7 of the '193 patent recites "wherein the carrier fluid further comprises a surfactant." Claim 8 of the '193 patent recites "wherein the surfactant is fluorinated surfactant."

Quake discloses that "the first phase or fluid which flows through the main channel can be a non-polar solvent, such as decane (e.g., tetradecane or hexadecane) or another oil (for example, **mineral oil**). Ex. 1034 at 6:8-10, [emphasis added]. "The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). **Exemplary surfactants** include Tween, Span, **fluorinated oils**, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel." Ex. 1034 at 35:18-22, [emphasis added].

The phrase "carrier fluid" in claim 7, having no antecedent basis, is construed to mean "a fluid that is immiscible with a plug fluid," as this is the way the term is defined in the specification. See Ex. 1001 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1065.

Thus, claims 6-8 are shown to be rendered obvious by the combination of Quake, Kopp and Lagally.

Dependent Claim 9

Claim 9 of the '193 patent recites "wherein the at least one plug is a merged plug."

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1065.

Thus, claim 9 is shown to be rendered obvious by the combination of Quake, Kopp and Lagally.

Dependent Claim 10

Claim 10 of the '193 patent recites "trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels."

Quake describes a "detection region" which "should generally be at least about twice, preferably at least about five times as large per side or in diameter as the diameter of the largest molecule, cell or droplet that will be passing through it." Ex. 1034 at 26. This may be contrasted to Quake's generally recommended dimensions of the fluid channels, which are shaped to cause the droplets to "conform to the size and shape of the channels" by "becom[ing] longer and thinner" as channels narrow. *Id.* at 27. Quake teaches that "an optical trap, or laser tweezers, may be used to sort or direct cells.... The optical trap remains in an 'off' mode until a desired particle is detected in the detection region. After detection of a desired characteristic, the particle or cell is 'trapped', and thereby directed or moved into the predetermined branch channel for collection." *Id.* at 67, *see also* FIGs. 14A & B (Input). Thus, Quake's detection region is an

“expansion portion” where the at least one plug would be “trapp[ed]... for a period of time... after the reaction” as recited in dependent claim 10.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1065.

Thus, claim 10 is shown to be rendered obvious by the combination of Quake, Kopp and Lagally.

Dependent Claim 11

Claim 11 of the ‘193 patent recites “wherein the at least one plug is substantially spherical in shape.”

As discussed by Quake, “the microfabricated devices of this invention generate round, monodisperse droplets (such as those illustrated in Frames J and L of FIG. 19” (reproduced below). Ex. 1034 at 27, FIG. 19.

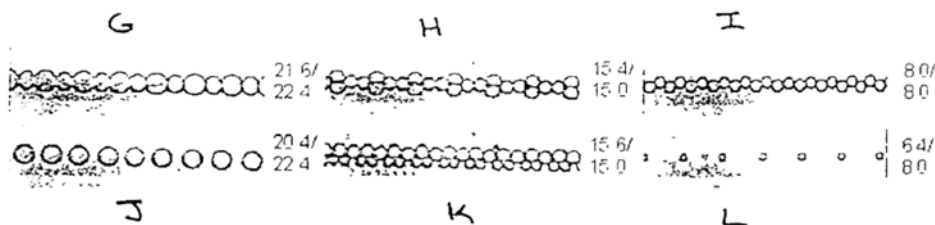


FIG. 19

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1065.

Thus, claim 11 is shown to be rendered obvious by the combination of Quake, Kopp and Lagally.

Dependent Claim 12

Claim 12 of the ‘193 patent recites “a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction.”

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1065.

Thus, claim 12 is shown to be rendered obvious by the combination of Quake, Kopp and Lagally.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '193 patent recites "wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters." Claim 14 of the '193 patent recites "wherein the relevant parameters are selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns."

Kopp teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Kopp meets both claims 13 and 14 under the broadest reasonable interpretation of the term "special design," which in an of itself imparts no meaningful structural limitation. Ex. 1027 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1065.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Quake, Kopp, and Lagally.

E. The Combination of Quake, Kopp and Burns II Renders Obvious Claims 1-14 of the '193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Quake), ii) microfluidic PCR devices were well known (Kopp and Burns II), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

As to the first feature – forming plugs from continuously flowing immiscible fluids – the '193 patent's teachings in this regard were copied almost verbatim from the Quake PCT (Ex. 1034). The table below compares the Quake PCT publication with the Ismagilov specification, which was filed about three months later.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and '193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	Provisional at 22:12-19, '193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

Attached as Ex. 1035 is a table showing the numerous passages which Ismagilov copied key disclosures verbatim or nearly verbatim from the Quake PCT. These were not merely background or “state of the art” descriptions; rather, they go to the heart of the claimed subject matter.

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

Quake relates to microfluidic devices designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [citing Quake ‘332 publication Ex. 1033 at ¶ 3]. The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* A junction joins the main channel with the sample inlet channel. *Id.* By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

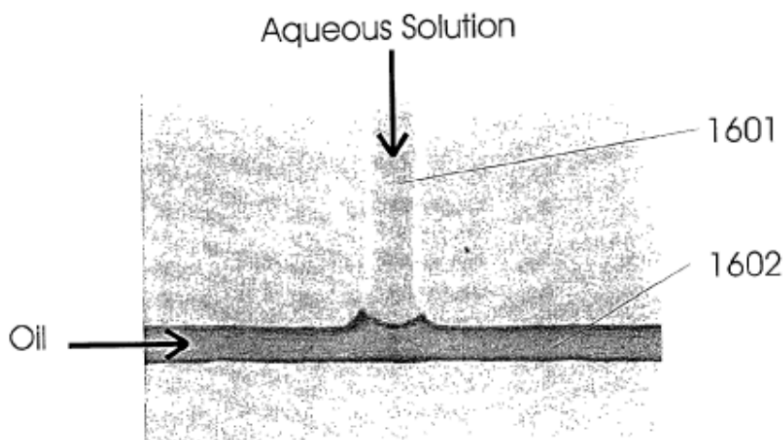


FIG. 16A

Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil. [Ex. 1052 at 12 citing Quake ¶ 292].

In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* ¶ 80.

Ex. 1052 at 11-14.

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by-streams limitations of the ‘193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous streams.⁹² Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the ‘193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids.

Turning the second issue, as of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Burns II. In 1996, Burns II reported the use of microfluidic devices to perform PCR. Ex. 1008 at 5556. Burns II explained that “[n]o new biochemistries or DNA detection methods are required for their use; the components simply reproduce current laboratory procedures in a micron-sized environment.” *Id.* at 5560. In other words, the PCR chemistry did not need to be modified – it worked without modification on the microfluidic scale. In the method of Burns II, a sample containing a DNA plasmid was loaded into one of the reaction wells (A) and a mixture of Taq

⁹² When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.

enzyme and buffer was loaded into the other. Ex. 1008 at 5558. Each formed droplets of about 60 nl in the parallel 500 x 25 μm channels (B) which came together at the Y junction to form a single larger droplet (about 120 nl). *Id.* at 5558-59; Ex. 1004 ¶40.

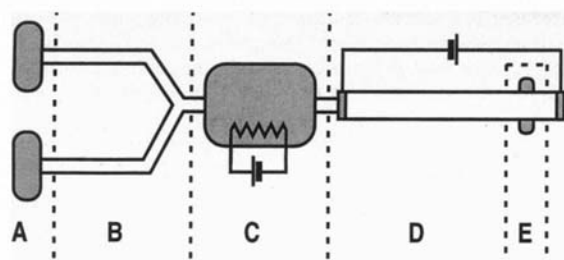


FIG. 1. Example of an integrated DNA analysis system, represented schematically. The individual components of the system are injection entry ports (A), liquid pumping channels (B), thermally controlled reaction chamber (C), electrophoresis channel (D), and DNA band migration detector (E). Each component would have associated sensors, control circuitry, and external connections.

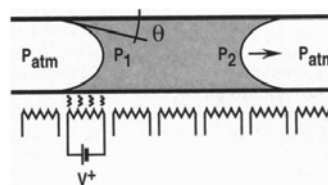


FIG. 2. Schematic drawing showing the principle of thermally induced drop motion in a closed channel. The case of a single aqueous drop in a hydrophilic channel is presented, where V is an applied voltage, P_{atm} is atmospheric pressure, P_1 is the receding-edge internal pressure, P_2 is the advancing-edge internal pressure, and θ is the contact angle of the liquid-gas-solid interface. The contact angle will depend on the surface characteristics of the channel and the constituents of the drop, with a hydrophilic interaction giving θ between 0 and 90 degrees, and a hydrophobic surface giving θ between 90 and 180 degrees. Surface treatments can also reduce contact angle hysteresis and, therefore, reduce the temperature difference necessary for drop motion.

The droplet then passed through the resistive heater portion (C) in which PCR amplification occurred. *Id.* at 5556-5557. Afterwards the amplification results were optically detected in zone D via gel electrophoresis. *Id.* at 5557-5561. Burns II noted that “[t]he use of common fabrication methods allows the assembly of increasingly complex, multicomponent, integrated systems from a small, defined set of standardized elements” and that “[f]uture design efforts have a strong impetus to reduce component size and improve efficiency.” *Id.* at 5560-61.

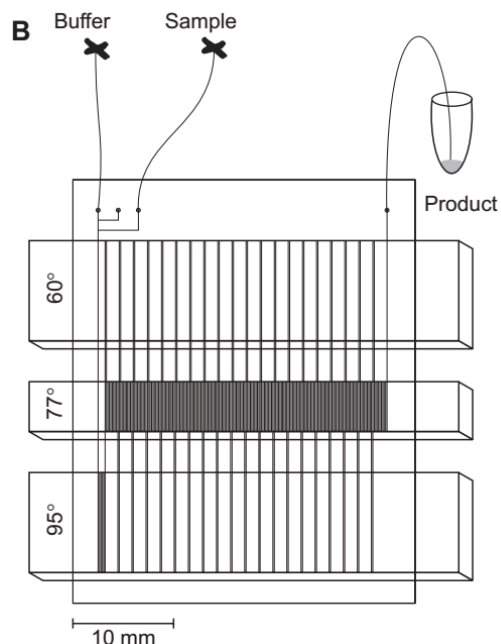
By the late 1990s Kopp *et al.* had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass

microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 [emphasis added].

Kopp *et al.* also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis

systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 [emphasis added].

In sum, Kopp and Burns II show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").⁹³ Ex. 1001 at 78:12-15. In the instant combination, this limitation is met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents.

⁹³ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

Quake **specifically suggests that his microfluidic device could be used to perform PCR.** Quake explained that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR...” Ex. 1034 at 23:26-30. Quake noted that “PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen.” *Id.* at 2:31-3:2.

More generally, a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, to conduct microfluidic PCR as taught by Kopp and Burns II. Ex. 1004 ¶¶88-109. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactor of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. *Id.* As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the

presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047.

Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Quake to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex.

1004 ¶¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Burns II) to incorporate a droplet reactor (Quake). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Burns II. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also **decrease contamination** and **increase the number of reactors** that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow

PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides

an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an

autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp or Burns II to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁹⁴ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that

⁹⁴ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985)

the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail below, in the accompanying claim chart (Ex. 1066) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Kopp and Burns II.

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Quake discloses “[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and enzymes; viruses and cells) and methods for its use are provided. . . . For instance, **in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials**, a water soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Ex. 1034 at 28., [emphasis added].

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Quake may be used to perform PCR reactions which are autocatalytic. Ex. 1034 ¶ 6.

(‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1066.

Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

Quake's device comprises a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed: "The channel architecture for the droplet extrusion region of the first device is shown in FIG. 16A. In this device, the inlet channel 1601 (inner diameter 30 μm) intersects the main channel 1602 (inner diameter 30 μm) at a T-intersection (i.e., an angle perpendicular to the main channel). Other intersections and angles may be used. The walls of the inlet and main channels were not tapered in this device." Ex. 1034 at 79:23-28.

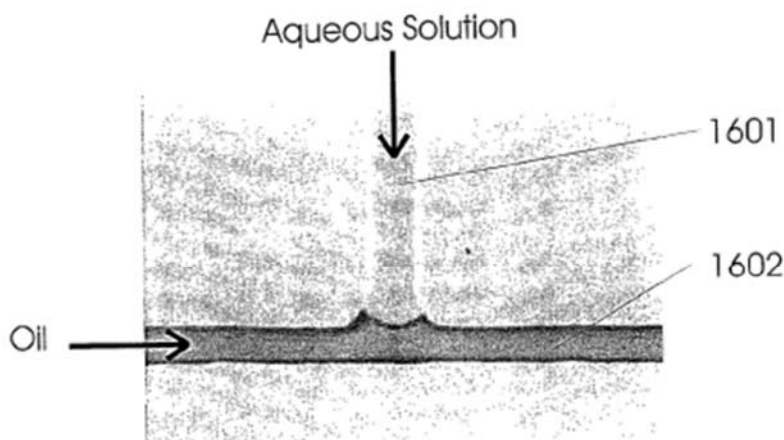


FIG. 16A

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1066.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

Quake teaches that “[a] first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the second fluid, passes through the inlet region so that droplets of the second fluid are sheared into the main channel. ... The second phase or fluid which passes through the inlet region can be an **aqueous solution**, for example ultra pure water, TE buffer, phosphate buffer saline and acetate buffer. The second fluid may also contain a **biological sample** (e.g., molecules of an enzyme or a substrate, or one or more cells, or one or more viral particles) for analysis or sorting in the device. In preferred embodiments the second fluid includes a biological sample that comprises one or more **molecules**, cells, virions or particles.” Ex. 1034 at 6:6-16. Quake further teaches that “[i]n preferred embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a “sample” or “droplet” fluid, passes or flows through the inlet region. ... Thus, droplets of the sample fluid containing the biological material for analysis, reaction or sorting are sheared at the droplet extrusion region into the flow of the extrusion fluid in the main channel.” *Id.* at 8.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).⁹⁵ Ex. 1001 at 78:12-15. In the instant combination, this limitation is met by using the microfluidic droplet reactors

⁹⁵ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions (as taught in Kopp and Burns II), wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents.

Quake **specifically suggests that his microfluidic device could be used to perform PCR**. Quake explained that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR...” Ex. 1034 at 23:26-30. Quake noted that “PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen.” *Id.* at 2:31-3:2.

More generally, a skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed**.” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost**.” Ex. 1008 at 5556, [emphasis added]. Likewise, the reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶92-100; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide

point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Quake to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling

times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Burns II) to incorporate a droplet reactor (Quake). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Burns II. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful.**

In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually

transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an

autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Burns II, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in modifying Kopp to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.⁹⁶ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that

⁹⁶ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985)

the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained further in the accompanying claim chart (Ex. 1066) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Kopp and Burns II.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1066.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

Quake discloses that “[p]referably, the fluid introduced to the main channel through the extrusion region is ‘incompatible’ (i.e., **immiscible**) with the fluid in the main channel so that droplets of the fluid introduced through the extrusion region are sheared off into the stream of fluid in the main channel. For example, the first phase or fluid which flows through the main channel can be a non-polar solvent, such as **decane (e.g., tetradecane or hexadecane) or another oil** (for example, mineral oil Ex. 1034 at 19:29-32, [emphasis added]).

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1066.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug

(“Development by others may also be pertinent to a determination of the obviousness of an invention”). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

As discussed above, the written descriptive support in the '193 patent for this claim element (which was the supposed point of novelty) was copied almost verbatim from Quake. The table below shows the correspondence between the relevant disclosures in the Quake PCT (Ex. 1034) and the Ismagilov provisional application (Ex. 1003) and '193 patent.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and '193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	Provisional at 22:12-19, '193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

Quake relates to microfluidic devices designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [citing Quake '332 publication Ex. 1033 at ¶ 3]. The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* A

junction joins the main channel with the sample inlet channel. *Id.* By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

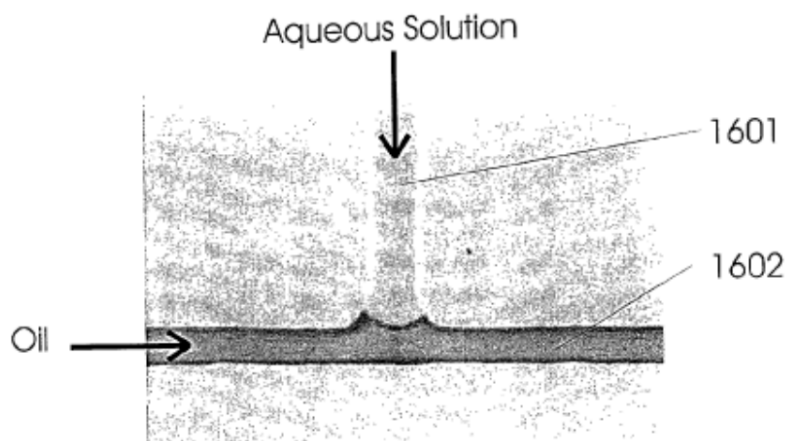


FIG. 16A

Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil. [Ex. 1052 at 12 citing Quake ¶ 292].

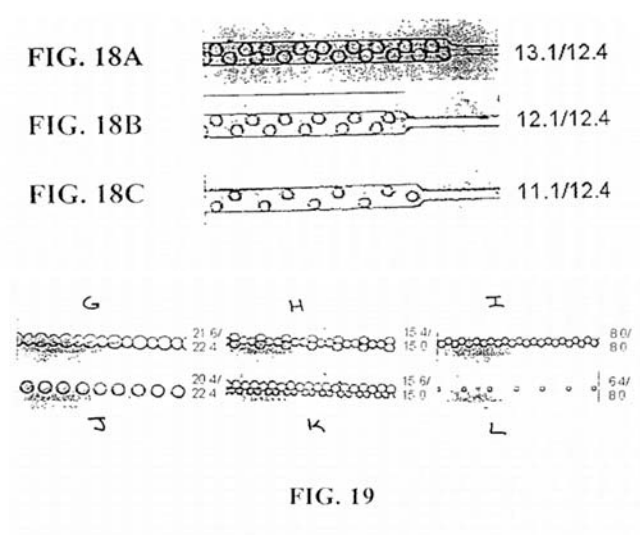
In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* ¶ 80.

Ex. 1052 at 11-14.

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by-streams limitations of the ‘193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous

streams.⁹⁷ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the '193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids.

The recitation that the plug is “substantially surrounded by the oil flowing through the channel” is met by Figures 18 and 19 of Quake. “FIGS. 18A-C are photomicrographs showing droplets of aqueous solution in a flow of oil (hexadecane with 2% Span 80 surfactant) in a microfluidic device with rectangular channels.” Ex. 1034 at 11. “FIG. 19 provides photomicrographs (Frames A-L) showing droplets of aqueous solution in a flow of oil (hexadecane with 2% Span 80 surfactant) in a microfluidic device with rounded channels.” *Id.* at 11. Figures 18B, 18C, 19J and 19L each depict droplets being completely surrounded by the carrier fluid.



Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1066.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Quake teaches that “droplets of the sample fluid containing the biological material for analysis, **reaction** or sorting are sheared at the droplet extrusion region into the flow of the

⁹⁷ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.

extrusion fluid in the main channel.” Ex. 1034 at 7:30-8:58. “Microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as **PCR** [i.e., a reaction].” *Id.* at 23:26-30.

As discussed above in connection with element 1[b], in the instant combination Quake is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule. Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. This limitation is met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71. The DNA is amplified through the PCR reaction. *Id.*

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1066.

Thus, claim 1 is shown to be rendered obvious by the combination of Quake, Kopp and Burns II.

Dependent Claim 2

Claim 2 of the ‘193 patent recites “wherein the at least one substrate molecule is a single biological molecule.”

Quake discloses that “[t]he invention also provides a method for sorting biological material. In various embodiments of the method, the biological material may be, e.g., molecules (for example, polynucleotides, polypeptides, enzymes, substrates or mixtures thereof), cells or viral particles, or mixtures thereof.” Ex. 1034 at 8:19-22. “The term ‘molecule’ means any distinct or distinguishable structural unit of matter comprising one or more atoms, and includes for example polypeptides and polynucleotides.” *Id.* at 13:10-12. “The term ‘polynucleotide’ as

used herein refers to a polymeric molecule having a backbone that supports bases capable of hydrogen bonding to typical polynucleotides, ... Polymeric molecules include double and single stranded **RNA and DNA**, and backbone modifications thereof, for example, methylphosphonate linkages.” *Id.* at 13:16-23.

Quake further specifies that, “[p]referably, each droplet of this multi-phase mixture encapsulates a single particle.” *Id.* at 5. “In particular, the sample concentration should be dilute enough that most of the droplets contain no more than a single molecule, cell or virion, with only a small statistical chance that a droplet will contain two or more molecules, cells or virions.” *Id.* at 36-7. In the combined method of Quake, Kopp and Burns II, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1066.

Thus, claim 2 is shown to be rendered obvious by the combination of Quake, Kopp and Burns II.

Dependent Claims 3 and 4

Claim 3 of the ‘193 patent recites “wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.” Claim 4 of the ‘193 patent recites “wherein the providing step includes heating.” PCR reactions involve thermocycling. Ex. 1004 ¶¶33-34.

Quake discloses that “microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” Ex. 1034 at 23:26-30. In the combined method of Quake, Kopp and Burns II, microfluidic droplet reactors are used to

perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. To affect the PCR reaction, the carrier fluid is heated. (“The individual steps are simply performed by heating or cooling the sample to characteristic temperatures: 95°C for dsDNA melting, 50° to 65°C for primer annealing, and 72° to 77°C for primer extension at the optimum enzyme temperature.”) Ex. 1027 at 1046 (Kopp), (“The enzymatic reaction occurred by moving two drops (Taq I restriction enzyme and supercoiled plasmid) down separate channels using the thermocapillary technique, combining the drops, and heating the merged sample to 65°C in the channel.”) Ex. 1008 at 5559 (Burns II).

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1066.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Quake, Kopp and Burns II.

Dependent Claim 5

Claim 5 of the ‘193 patent further recites “providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.”

Quake discloses that “[t]he device of the invention may also comprise a **detection region** which is within or coincident with at least a portion of the main channel at or downstream of the droplet extrusion region. The device may also have a detector, preferably an **optical detector** such as a microscope, associated with the detection region.” Ex. 1034 at 6:28-31, [emphasis added].

Burns II teaches that “[c]apillary gel electrophoresis of DNA samples was performed using a Beckman P/ACE instrument with a laser-induced fluorescence detector and 37 cm length, 100 μ m diameter, linear polymerase gel capillary according to manufacturer’s recommendation.” Ex. 1008 at 5558. The results of capillary electrophoresis were generally detected optically at the time of filing. Ex. 1011 (Dolnik) at 46.

In the combined method of Quake, Kopp and Burns II, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶68-69.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1066.

Thus, claim 5 is shown to be rendered obvious by the combination of Quake, Kopp and Burns II.

Dependent Claims 6-8

Claim 6 of the ‘193 patent recites “wherein the oil is fluorinated oil.” Claim 7 of the ‘193 patent recites “wherein the carrier fluid further comprises a surfactant.” Claim 8 of the ‘193 patent recites “wherein the surfactant is fluorinated surfactant.”

Quake discloses that “the first phase or fluid which flows through the main channel can be a non-polar solvent, such as decane (e.g., tetradecane or hexadecane) or another oil (for example, **mineral oil**).” Ex. 1034 at 6:8-10, [emphasis added]. “The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). **Exemplary surfactants** include Tween, Span, **fluorinated oils**, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel.” Ex. 1034 at 35:18-33, [emphasis added].

The phrase “carrier fluid” in claim 7, having no antecedent basis, is construed to mean “a fluid that is immiscible with a plug fluid,” as this is the way the term is defined in the specification. See Ex. 1001 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1066.

Thus, claims 6-8 are shown to be rendered obvious by the combination of Quake, Kopp and Burns II.

Dependent Claim 9

Claim 9 of the ‘193 patent recites “wherein the at least one plug is a merged plug.”

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1066.

Thus, claim 9 is shown to be rendered obvious by the combination of Quake, Kopp and Burns II.

Dependent Claim 10

Claim 10 of the ‘193 patent recites “trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels.”

Quake describes a “detection region” which “should generally be at least about twice, preferably at least about five times as large per side or in diameter as the diameter of the largest molecule, cell or droplet that will be passing through it.” Ex. 1034 at 26. This may be contrasted to Quake’s generally recommended dimensions of the fluid channels, which are shaped to cause

the droplets to “conform to the size and shape of the channels” by “becom[ing] longer and thinner” as channels narrow. *Id.* at 27. Quake teaches that “an optical trap, or laser tweezers, may be used to sort or direct cells.... The optical trap remains in an ‘off’ mode until a desired particle is detected in the detection region. After detection of a desired characteristic, the particle or cell is ‘trapped’, and thereby directed or moved into the predetermined branch channel for collection.” *Id.* at 67, *see also* FIGs. 14A & B (Input). Thus, Quake’s detection region is an “expansion portion” where the at least one plug would be “trapp[ed]... for a period of time... after the reaction” as recited in dependent claim 10.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1066.

Thus, claim 10 is shown to be rendered obvious by the combination of Quake, Kopp and Burns II.

Dependent Claim 11

Claim 11 of the ‘193 patent recites “wherein the at least one plug is substantially spherical in shape.”

As discussed by Quake, “the microfabricated devices of this invention generate round, monodisperse droplets (such as those illustrated in Frames J and L of FIG. 19” (reproduced below). Ex. 1034 at 27, FIG. 19.

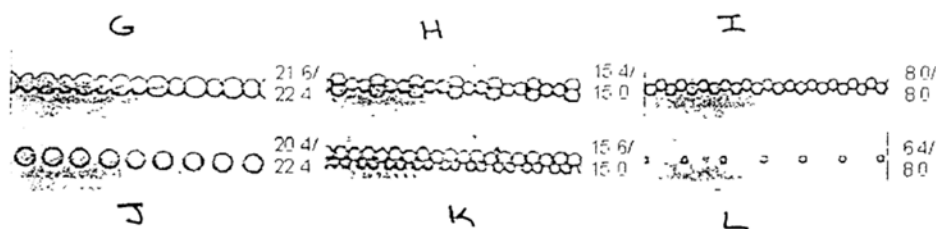


FIG. 19

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1066.

Thus, claim 11 is shown to be rendered obvious by the combination of Quake, Kopp and Burns II.

Dependent Claim 12

Claim 12 of the '193 patent recites "a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction."

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1066.

Thus, claim 12 is shown to be rendered obvious by the combination of Quake, Kopp and Burns II.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '193 patent recites "wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters." Claim 14 of the '193 patent recites "wherein the relevant parameters are selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns."

Kopp teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Kopp meets both claims 13 and 14 under the broadest reasonable

interpretation of the term “special design,” which in an of itself imparts no meaningful structural limitation. Ex. 1027 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1066.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Quake, Kopp, and Burns II.

F. The Combination of Quake, Kopp and Vogelstein Renders Obvious Claims 1-14 of the ‘193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Quake), ii) microfluidic PCR devices were well known (Kopp and Vogelstein), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

As to the first feature – forming plugs from continuously flowing immiscible fluids – the ‘193 patent’s teachings in this regard were copied almost verbatim from the Quake PCT (Ex. 1034). The table below compares the Quake PCT publication with the Ismagilov specification, which was filed about three months later.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and ‘193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the</u>	Provisional at 22:12-19, ‘193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of</u>

<u>droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	<u>the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

Attached as Ex. 1035 is a table showing the numerous passages which Ismagilov copied key disclosures verbatim or nearly verbatim from the Quake PCT. These were not merely background or “state of the art” descriptions; rather, they go to the heart of the claimed subject matter.

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

Quake relates to microfluidic devices designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [citing Quake ‘332 publication Ex. 1033 at ¶ 3]. The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* A junction joins the main channel with the sample inlet channel. *Id.* By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

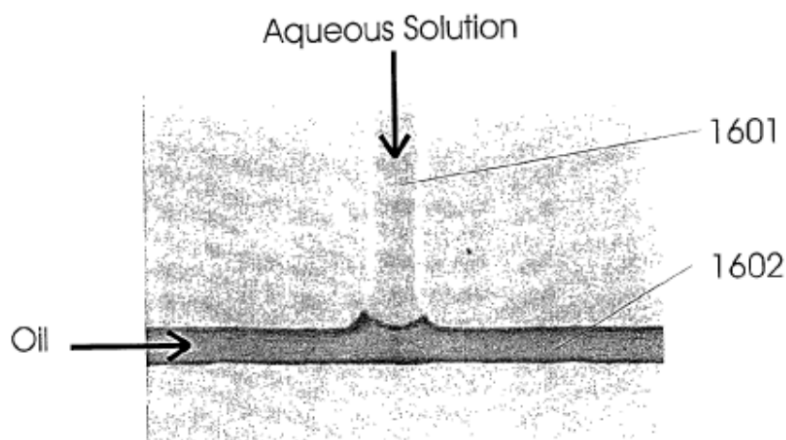


FIG. 16A

Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil. [Ex. 1052 at 12 citing Quake ¶ 292].

In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* ¶ 80.

Ex. 1052 at 11-14.

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by-streams limitations of the ‘193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous streams.⁹⁸ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the ‘193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids. Turning the second issue, as of the

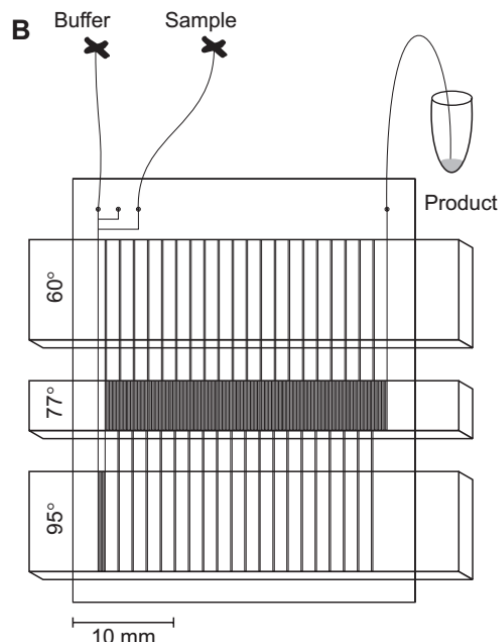
⁹⁸ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.

earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Vogelstein. By the late 1990s Kopp *et al.* had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 [emphasis added].

Kopp *et al.* also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene

could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 [emphasis added].

By 1999, Vogelstein had reported **various applications for single-molecule microfluidic PCR**. Ex. 1044 at 9241. In a seminal article which has been cited over by over 1,000 other publications, Vogelstein explained that PCR amplification of small aliquots or droplets containing single molecules was effective for “detection of a small number of mutant-containing cells among a large excess of normal cells.” *Id.* at 9236. In Vogelstein's method, the DNA was diluted into 7 μ l volumes such that, on average, there was one template molecule for every two wells (i.e., approximately half of the wells contained no DNA template molecule and approximately half contained a single DNA template molecule). *Id.* at 9236, 9237. This avoided the problem of background noise impairing the determination of whether each volume contained the mutant sequence. *Id.* By counting the number of volumes which indicated wild type or mutant templates, the presence or absence of the mutation (e.g., a sequence associated with colorectal cancer) can be determined. *Id.* at 9238. The accuracy of the test is a function of the number of discrete volumes into which the sample is diluted. *Id.* at 9239. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) The article explained that the same technique would be useful for **detecting a wide array of mutations, dislocations and allelic imbalances**, as summarized in Table 1. *Id.* at 9241. Vogelstein thus taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby

overcoming the signal-to-noise limitations associated with certain other PCR techniques and enabling the detection of relatively rare mutations, dislocations and allelic imbalances. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86.

In sum, Kopp and Vogelstein show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").⁹⁹ Ex. 1001 at 78:12-15. In the instant combination, this limitation is met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents.

Quake **specifically suggests that his microfluidic device could be used to perform PCR.** Quake explained that "[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR..." Ex. 1034 at 23:26-30. Quake noted that "PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen." *Id.* at 2:31-3:2.

More generally, a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake, to conduct microfluidic PCR as taught by Kopp and

⁹⁹ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

Vogelstein. Ex. 1004 ¶¶88-109. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactor of Quake because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. *Id.* As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Kopp to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Quake to perform

PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett's PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample

leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Vogelstein) to incorporate a droplet reactor (Quake). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and

sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In

1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp or Lagally to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation

of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹⁰⁰ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail below, in the accompanying claim chart (Ex. 1067) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Kopp and Vogelstein.

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Quake discloses "[a] microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides, including proteins and

¹⁰⁰ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

enzymes; viruses and cells) and methods for its use are provided. . . . For instance, **in those embodiments where aqueous droplets are used as microreactors for chemical reactions (including biochemical reactions) or are used to analyze and/or sort biomaterials**, a water soluble surfactant such as SDS may denature or inactivate the contents of the droplet.” Ex. 1034 at 28, [emphasis added].

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Quake may be used to perform PCR reactions which are autocatalytic. Ex. 1034 pp. 2-3, 23.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1067.

Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

Quake’s device comprises a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed: “The channel architecture for the droplet extrusion region of the first device is shown in FIG. 16A. In this device, the inlet channel 1601 (inner diameter 30 μm) intersects the main channel 1602 (inner diameter 30 μm) at a T-intersection (i.e., an angle perpendicular to the main channel). Other intersections and angles may be used. The walls of the inlet and main channels were not tapered in this device.” Ex. 1034 at 79:23-28.

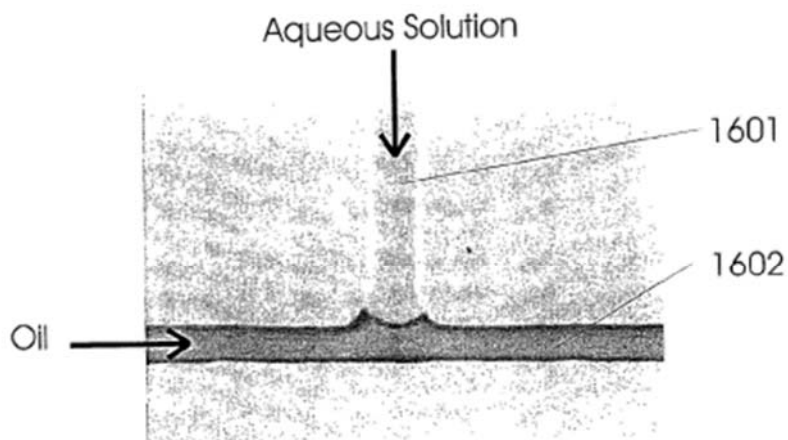


FIG. 16A

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1067.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

Quake teaches that “[a] first fluid flows through the main channel, and a second fluid, which is incompatible or immiscible with the second fluid, passes through the inlet region so that droplets of the second fluid are sheared into the main channel. ... The second phase or fluid which passes through the inlet region can be an **aqueous solution**, for example ultra pure water, TE buffer, phosphate buffer saline and acetate buffer. The second fluid may also contain a **biological sample** (e.g., molecules of an enzyme or a substrate, or one or more cells, or one or more viral particles) for analysis or sorting in the device. In preferred embodiments the second fluid includes a biological sample that comprises one or more **molecules**, cells, virions or particles.” Ex. 1034 at 6:6-16, [emphasis added]. Quake further teaches that “[i]n preferred

embodiments, a first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device and a second fluid, referred to as a "sample" or "droplet" fluid, passes or flows through the inlet region. ... Thus, droplets of the sample fluid containing the biological material for analysis, reaction or sorting are sheared at the droplet extrusion region into the flow of the extrusion fluid in the main channel.” *Id.* at 7:30-8:5.

In the instant combination, the microfluidic droplet reactor of Quake is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).¹⁰¹ Ex. 1001 at 78:12-15. In the instant combination, this limitation is met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Quake) to perform PCR reactions (as taught in Kopp and Vogelstein), wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents.

Quake **specifically suggests that his microfluidic device could be used to perform PCR.** Quake explained that “[m]icrofabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR...” Ex. 1034 at 23:26-30. Quake noted that “PCR is [] generally the method of choice to detect viral DNA or RNA directly in clinical specimens. The advantage of PCR for viral diagnostics is its high sensitivity; PCR can detect very low numbers of viruses in a small clinical specimen.” *Id.* at 2:31-3:2.

More generally, a skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Quake because doing so have provided the

¹⁰¹ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. Likewise, the reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Quake to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the**

PCR reaction with a given primer and reduce the production of non-specific products

relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* 9239. Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Vogelstein) to incorporate a droplet reactor (Quake). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the

fluid microfluidic PCR devices taught in Kopp or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/μL, in volumes down to 1 μL.¹¹ Since then, stand-alone PCR reactors have been constructed in

silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control.

The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-

chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Burns II, a skilled artisan would have expected that the microfluidic droplet reactor of Quake could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in modifying Kopp or Vogelstein to work in the microfluidic droplet reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered

as evidence of the level of skill in the art.¹⁰² As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained further in the accompanying claim chart (Ex. 1067) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Quake to conduct microfluidic PCR as taught by Kopp and Vogelstein.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1067.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

Quake discloses that “[p]referably, the fluid introduced to the main channel through the extrusion region is ‘incompatible’ (i.e., **immiscible**) with the fluid in the main channel so that droplets of the fluid introduced through the extrusion region are sheared off into the stream of fluid in the main channel. For example, the first phase or fluid which flows through the main channel can be a non-polar solvent, such as **decane (e.g., tetradecane or hexadecane) or another oil** (for example, mineral oil).” Ex. 1034 at 19:29-32, [emphasis added].

During the *inter partes* review proceeding, Patent Owner did not contest that Quake met

¹⁰² “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) (‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

this limitation. Ex. 1051, *passim*. The PTAB appears to have concluded that this limitation was met by Quake. Ex. 1052, *passim*.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1067.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

As discussed above, the written descriptive support in the ‘193 patent for this claim element (which was the supposed point of novelty) was copied almost verbatim from Quake. The table below shows the correspondence between the relevant disclosures in the Quake PCT (Ex. 1034) and the Ismagilov provisional application (Ex. 1003) and ‘193 patent.

Quake PCT (published March 21, 2002 (Ex. 1034))	Ismagilov provisional application (filed May 9, 2002) (Ex. 1003) and ‘193 patent (Ex. 1001)
34:32-35:7 <u>The pressure at the droplet extrusion region can also be regulated by adjusting the pressure on the main and sample inlets, for example, with pressurized syringes feeding into those inlets. By controlling the pressure difference between the oil and water sources at the droplet extrusion region, the size and periodicity of the droplets generated may be regulated. Alternatively, a valve may be placed at or coincident to either the droplet extrusion region or the sample inlet connected thereto to control the flow of solution into the droplet extrusion region, thereby controlling the size and periodicity of the droplets.</u>	Provisional at 22:12-19, ‘193 patent at Ex. 1001 at 21:56-65 <u>The pressure at the plug-forming region can also be regulated by adjusting the pressure on the main and sample inlets, for example with pressurized syringes feeding into those inlets. By controlling the difference between the oil and water flow rates at the plug-forming region, the size and periodicity of the plugs generated may be regulated. Alternatively, a valve may be placed at or coincident to either the plug forming region or the sample inlet connected thereto to control the flow of solution into the plug-forming region, thereby controlling the size and periodicity of the plugs.</u>

The disclosure of Quake was summarized by the Patent Trial and Appeal Board in its November 15, 2015 decision. Therein the Board found that Quake discloses a droplet reactor in which droplets are formed by continuous streams of immiscible fluids:

Quake relates to microfluidic devices designed to compartmentalize small droplets of aqueous solution within microfluidic channels filled with oil. [citing Quake '332 publication Ex. 1033 at ¶ 3]. The devices comprise a main channel, through which a pressurized stream of oil is passed, and at least one sample inlet channel, through which a pressurized stream of aqueous solution is passed. *Id.* A junction joins the main channel with the sample inlet channel. *Id.* By adjusting the pressure of the oil and/or the aqueous solution, a pressure difference can be established such that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream, thereby forming droplets. *Id.* Figure 16A of Quake is reproduced below:

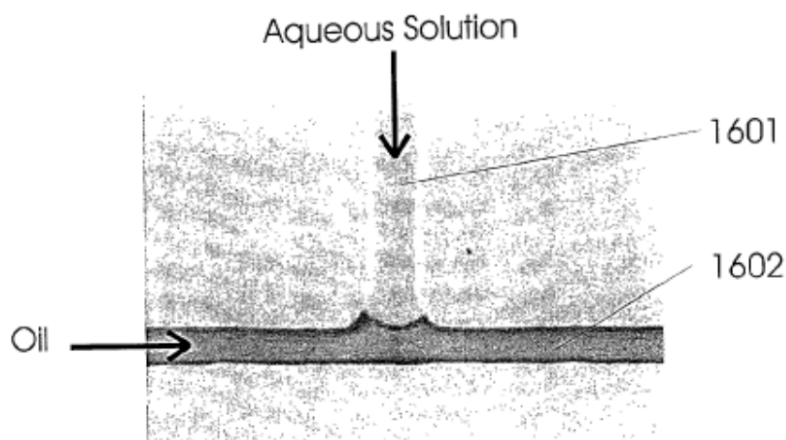


FIG. 16A

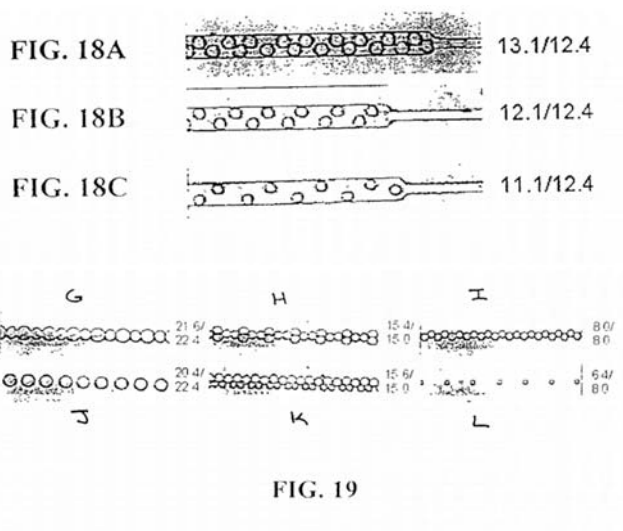
Figure 16A depicts the channel architecture for the droplet extrusion region. Channel 1601 containing an aqueous solution intersects with main channel 1602 containing oil. [Ex. 1052 at 12 citing Quake ¶ 292].

In preferred embodiments, the droplets have a volume of approximately 0.1 to 100 picoliters. *Id.* According to Quake, microfabrication of the device “permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” *Id.* ¶ 80.

Ex. 1052 at 11-14.

The Patent Owner did not contest in the *inter partes* review proceeding that Quake met the plug-formed-by-streams limitations of the '193 patent. Ex. 1051 *passim*, Ex. 1052, *passim*. Moreover, Quake himself during prosecution characterized his invention as involving continuous streams.¹⁰³ Accordingly, notwithstanding statements to the contrary during *ex parte* prosecution of the '193 patent (discussed in Section IV.C above) it is clear that Quake does in fact teach forming plugs from continuous streams of immiscible fluids.

The recitation that the plug is “substantially surrounded by the oil flowing through the channel” is met by Figures 18 and 19 of Quake. “FIGS. 18A-C are photomicrographs showing droplets of aqueous solution in a flow of oil (hexadecane with 2% Span 80 surfactant) in a microfluidic device with rectangular channels.” Ex. 1034 at 11: 24-27. “FIG. 19 provides photomicrographs (Frames A-L) showing droplets of aqueous solution in a flow of oil (hexadecane with 2% Span 80 surfactant) in a microfluidic device with rounded channels.” *Id.* at 1:29-31. Figures 18B, 18C, 19J and 19L each depict droplets being completely surrounded by the carrier fluid.



Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1067.

¹⁰³ When distinguishing his invention over one of the embodiments of Shaw Stewart in which reagent is periodically injected, Quake noted that his “invention relates, in part, to the discovery that combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid (e.g., decane) it is possible to produce small, discrete, monodisperse droplets of the aqueous solution flowing in the second fluid.” Ex. 1058 at September 25, 2006 response, page 15.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Quake teaches that “droplets of the sample fluid containing the biological material for analysis, **reaction** or sorting are sheared at the droplet extrusion region into the flow of the extrusion fluid in the main channel.” Ex. 1034 at 7:30-8:5. [emphasis added]. “Microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as **PCR** [i.e., a reaction].” Ex. 1034 at 23:26-30. [emphasis added].

As discussed above in connection with element 1[b], in the instant combination Quake is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule. Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. This limitation is met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Quake) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71. The DNA is amplified through the PCR reaction. *Id.*

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1067.

Thus, claim 1 is shown to be rendered obvious by the combination of Quake, Kopp and Vogelstein.

Dependent Claim 2

Claim 2 of the ‘193 patent recites “wherein the at least one substrate molecule is a single biological molecule.”

Quake discloses that “[t]he invention also provides a method for sorting biological material. In various embodiments of the method, the biological material may be, e.g., molecules

(for example, polynucleotides, polypeptides, enzymes, substrates or mixtures thereof), cells or viral particles, or mixtures thereof.” Ex. 1034 at 8:19-22. “The term ‘molecule’ means any distinct or distinguishable structural unit of matter comprising one or more atoms, and includes for example polypeptides and polynucleotides.” *Id.* at 13:10-12. “The term ‘polynucleotide’ as used herein refers to a polymeric molecule having a backbone that supports bases capable of hydrogen bonding to typical polynucleotides, ... Polymeric molecules include double and single stranded **RNA and DNA**, and backbone modifications thereof, for example, methylphosphonate linkages.” *Id.* at 13:16-23.

Quake further specifies that, “[p]referably, each droplet of this multi-phase mixture encapsulates a single particle.” *Id.* at 5. “In particular, the sample concentration should be dilute enough that most of the droplets contain no more than a single molecule, cell or virion, with only a small statistical chance that a droplet will contain two or more molecules, cells or virions.” *Id.* at 36-7.

In the combined method of Quake, Kopp and Vogelstein, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1067.

Thus, claim 2 is shown to be rendered obvious by the combination of Quake, Kopp and Vogelstein.

Dependent Claims 3 and 4

Claim 3 of the ‘193 patent recites “wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.” Claim 4 of the ‘193 patent recites

“wherein the providing step includes heating.” PCR reactions involve thermocycling. Ex. 1004 ¶¶33-34.

Quake discloses that “microfabrication permits other technologies to be integrated or combined with flow cytometry on a single chip, such as PCR.” Ex. 1034 at 23:26-30. In the combined method of Quake, Kopp and Vogelstein, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. To affect the PCR reaction, the carrier fluid is heated. (“The individual steps are simply performed by heating or cooling the sample to characteristic temperatures: 95°C for dsDNA melting, 50° to 65°C for primer annealing, and 72° to 77°C for primer extension at the optimum enzyme temperature.”) Ex. 1027 at 1046 (Kopp), (“If a PCR product whose sequence is complementary to the loop sequence is present during the heating/cooling cycle, hybridization of the MB to one strand of the PCR product will increase the distance between the Dabcyl and the dye, resulting in increased fluorescence.”) Ex. 1044 at 9237 (Vogelstein).

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1067.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Quake, Kopp and Vogelstein.

Dependent Claim 5

Claim 5 of the ‘193 patent further recites “providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.”

Quake discloses that “[t]he device of the invention may also comprise a **detection region** which is within or coincident with at least a portion of the main channel at or downstream of the droplet extrusion region. The device may also have a detector, preferably an **optical detector** such as a microscope, associated with the detection region.” Ex. 1034 at 6, [emphasis added].

Vogelstein teaches that the “[r]eactions were analyzed immediately or stored at room temperature for up to 36 h before fluorescence analysis.” Ex. 1044 at 9236.

In the combined method of Quake, Kopp and Vogelstein, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶68-69.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1067.

Thus, claim 5 is shown to be rendered obvious by the combination of Quake, Kopp and Vogelstein.

Dependent Claims 6-8

Claim 6 of the ‘193 patent recites “wherein the oil is fluorinated oil.” Claim 7 of the ‘193 patent recites “wherein the carrier fluid further comprises a surfactant.” Claim 8 of the ‘193 patent recites “wherein the surfactant is fluorinated surfactant.”

Quake discloses that “the first phase or fluid which flows through the main channel can be a non-polar solvent, such as decane (e.g., tetradecane or hexadecane) or another oil (for example, **mineral oil**).” Ex. 1034 at 19, 87, [emphasis added]. “The fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). **Exemplary surfactants** include Tween, Span, **fluorinated oils**, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and

uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel.” Ex. 1034 at 35:18-22, [emphasis added].

The phrase “carrier fluid” in claim 7, having no antecedent basis, is construed to mean “a fluid that is immiscible with a plug fluid,” as this is the way the term is defined in the specification. Ex. 1001 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1067.

Thus, claims 6-8 are shown to be rendered obvious by the combination of Quake, Kopp and Vogelstein.

Dependent Claim 9

Claim 9 of the ‘193 patent recites “wherein the at least one plug is a merged plug.”

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1067.

Thus, claim 9 is shown to be rendered obvious by the combination of Quake, Kopp and Vogelstein.

Dependent Claim 10

Claim 10 of the ‘193 patent recites “trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels.”

Quake describes a “detection region” which “should generally be at least about twice, preferably at least about five times as large per side or in diameter as the diameter of the largest

molecule, cell or droplet that will be passing through it.” Ex. 1034 at 26. This may be contrasted to Quake’s generally recommended dimensions of the fluid channels, which are shaped to cause the droplets to “conform to the size and shape of the channels” by “becom[ing] longer and thinner” as channels narrow. *Id.* at 27. Quake teaches that “an optical trap, or laser tweezers, may be used to sort or direct cells.... The optical trap remains in an ‘off’ mode until a desired particle is detected in the detection region. After detection of a desired characteristic, the particle or cell is ‘trapped’, and thereby directed or moved into the predetermined branch channel for collection.” *Id.* at 67, *see also* FIGs. 14A & B (Input). Thus, Quake’s detection region is an “expansion portion” where the at least one plug would be “trapp[ed]... for a period of time... after the reaction” as recited in dependent claim 10.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1067.

Thus, claim 10 is shown to be rendered obvious by the combination of Quake, Kopp and Vogelstein.

Dependent Claim 11

Claim 11 of the ‘193 patent recites “wherein the at least one plug is substantially spherical in shape.”

As discussed by Quake, “the microfabricated devices of this invention generate round, monodisperse droplets (such as those illustrated in Frames J and L of FIG. 19” (reproduced below). Ex. 1034 at 27, FIG. 19.

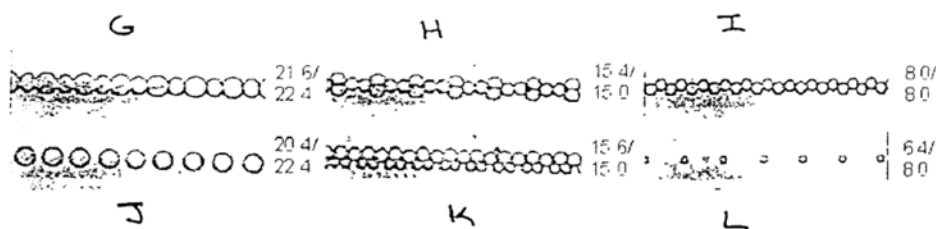


FIG. 19

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1067.

Thus, claim 11 is shown to be rendered obvious by the combination of Quake, Kopp and Vogelstein.

Dependent Claim 12

Claim 12 of the '193 patent recites "a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction."

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1067.

Thus, claim 12 is shown to be rendered obvious by the combination of Quake, Kopp and Vogelstein.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '193 patent recites "wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters." Claim 14 of the '193 patent recites "wherein the relevant parameters are

selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns.”

Kopp teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Kopp meets both claims 13 and 14 under the broadest reasonable interpretation of the term “special design,” which in and of itself imparts no meaningful structural limitation. Ex. 1027 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1067.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Quake, Kopp, and Vogelstein.

G. The Combination of Shaw Stewart, Corbett and Lagally Renders Obvious Claims 1-14 of the ‘193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Shaw Stewart), ii) microfluidic PCR devices were well known (Corbett and Lagally), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

The Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. The Shaw Stewart British Application discloses a “droplet reactor” and illustrates at Figure 1 a carrier phase 1 flowing along a conduit into which reagent 2 is injected, thereby forming droplet 4. Ex. 1040 at 1:1-20, 70-90. “[I]f large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” *Id.* at 1:83-90.

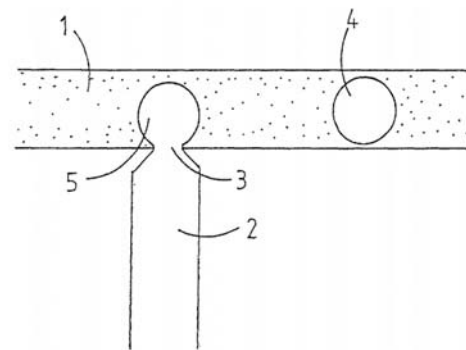


Figure 1.

Significantly, the exclusive licensee of the ‘193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the ‘193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO’s primary arguments was that Stewart I

only disclosed introducing fluid in a “stepwise fashion,” whereas in the ’503 Patent droplets are formed by “combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.” *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

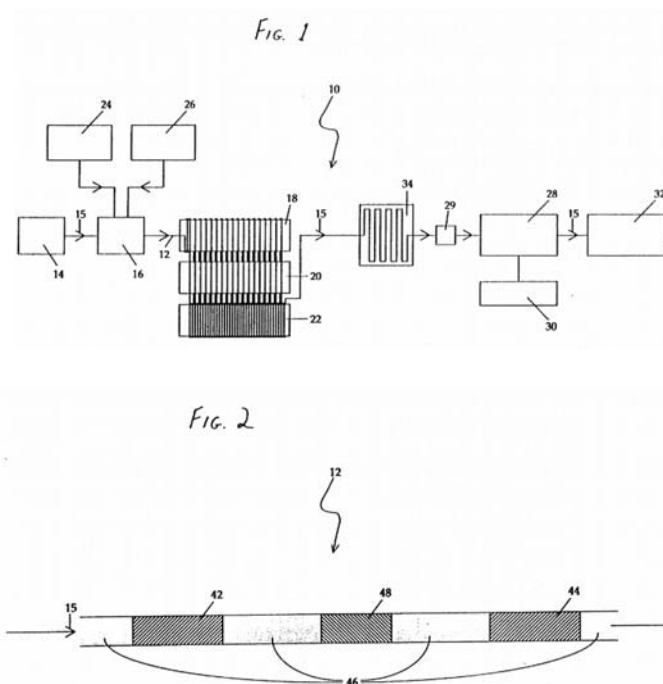
Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Lagally. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream

includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.



“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

In 2001, in another article not previously considered by the Office, Lagally reported using a microfluidic droplet reactor to conduct single-molecule DNA amplification by PCR. Lagally summarized the state of the art as of February 2001, explaining continuous flow PCR microreactors had recently undergone substantial evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or **continuous flow** through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with

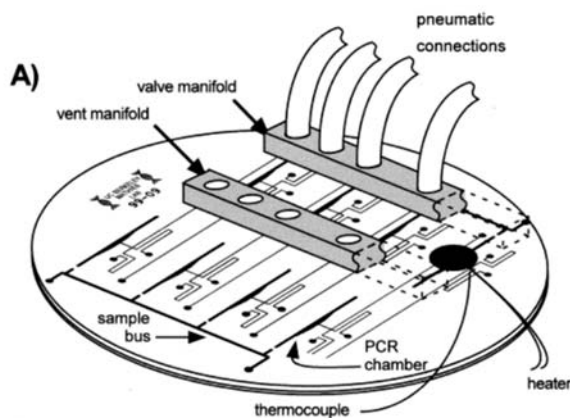
sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-566, [emphasis added].

Lagally *et al.* developed a further improvement – a device and method for achieving single molecule amplification in a microchannel environment.

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

Microfluidic PCR-CE device. The PCR chambers are connected to a common sample bus through a set of valves. Hydrophobic vents at the other end of the PCR chambers are used to locate the sample and to eliminate gas. The PCR chambers are directly connected to the cross channel of the CE system for product injection and analysis. Two aluminum manifolds, one each for the vents and valves, are placed onto the respective ports and clamped in place using vacuum. The manifolds connect to external solenoid valves for pressure and vacuum



actuation. Thermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.

Ex. 1028 at 566 (see figure legend).

In sum, Corbett and Lagally show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactors of Shaw Stewart are relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").¹⁰⁴ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶31-39.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Corbett and Lagally. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that

¹⁰⁴ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶¶92-100; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of

therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Shaw Stewart to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely

reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) . at 9239. Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Shaw Stewart to dilute the samples and thereby enable the detection of

mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Lagally) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Lagally. The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with

sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactors Shaw Stewart could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Lagally to work in the microfluidic droplet

reactor of Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹⁰⁵ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail below, in the accompanying claim chart (Ex. 1068), and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Corbett and Lagally. Alternatively, a skilled artisan would have considered it obvious to miniaturize the

¹⁰⁵ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Shaw Stewart discloses that "[t]he method of the invention may be used for initiating and controlling a chemical reaction or preparing mixtures of reagents..." Ex. 1040 at 1:7-9.

"The system is particularly suited **to the manipulation of microscopic quantities of reagents, with volumes of less than one microliter,...**" *Id.* at 1:20-22.

In the instant combination, microfluidic droplet reactors (as taught in Shaw Stewart) are used to perform PCR reactions (as taught in Corbett and Lagally), wherein each plug includes a mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-38. For example, as noted by Lagally, "[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**" Ex. 1028 at 565, PCR reactions are autocatalytic. Ex. 1004 ¶32; Ex. 1001 44:58-61.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1068.

Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

As shown in Shaw Stewart's Figure 1, "[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3).

When the correct volume has passed into the tube, it is separated and carried away by a current

of carrier phase, down the tube.” Ex. 1040 at 1:70-5.

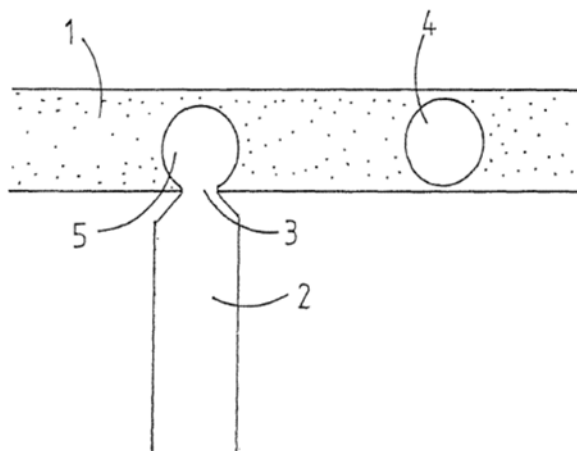


Figure 1.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1068.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

As shown in Shaw Stewart’s Figure 1, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Ex. 1040 at 1:70-5. Shaw Stewart teaches that “[f]or **aqueous reagents**, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Ex. 1040 at 1:62-66, [emphasis added]. “[I]f large numbers of droplets are required, a **continuous flow of reagent** through the opening will be produced, while a **continuous current of carrier phase flows** down the tube.” Ex. 1040 at 1:83-86, [emphasis added].

In the instant combination, the microfluidic droplet reactors of Shaw Stewart are relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").¹⁰⁶ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-39.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Corbett and Lagally. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modifying it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, "[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of**

¹⁰⁶ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

material and reagents needed.” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Shaw Stewart to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions

suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each

contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Lagally) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp, Burns II, Lagally or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the**

volumes of reagent compared to the single fluid in microfluidic chip reactor (e.g., Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/μL, in volumes down to 1 μL.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use

resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1

template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at

1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactors Shaw Stewart could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Lagally to work in the microfluidic droplet reactor of Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly,

the Higuchi references are offered as evidence of the level of skill in the art.¹⁰⁷ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained further in the accompanying claim chart (Ex. 1068) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Corbett and Lagally. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modifying it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1068.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

As shown in Shaw Stewart's Figure 1, "[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3).

When the correct volume has passed into the tube, it is separated and carried away by a current

¹⁰⁷ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

of carrier phase, down the tube.” Ex. 1040 at 1:70-5. Shaw Stewart teaches that “[f]or **aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable**, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Ex. 1040 at 1:62-66, [emphasis added]. Shaw Stewart notes this can be **silicon oil** or, alternatively, light mineral oil. *Id.* at Abstract, [emphasis added]. “[I]f large numbers of droplets are required, **a continuous flow of reagent** through the opening will be produced, while **a continuous current of carrier phase flows** down the tube.” Ex. 1040 at 1:83-86, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1068.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

As discussed above, the Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. Significantly, the exclusive licensee of the ‘193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the ‘193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a “stepwise fashion,” whereas in the ’503 Patent droplets are formed by “combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.” *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

With regard to the recitation that the plug is “substantially surrounded by the oil flowing through the channel,” this is shown in Shaw Stewart’s Figure 1. Shaw Stewart’s specification also explains that the droplets are smaller than the conduits (“A method as claimed in claim (1) in which the discrete volumes of chemical reagents are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.”) . Ex. 1040 at 3:101-104.

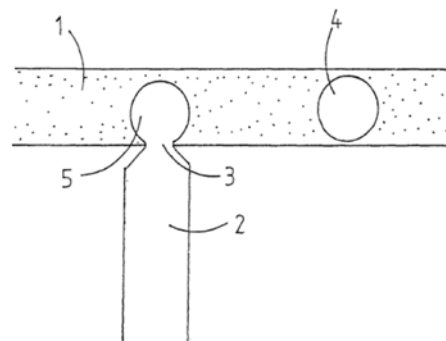


Figure 1.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1068.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Shaw Stewart discloses that “[r]egions of the device can be heated by electric circuits or coils or by electromagnetic radiation to allow the merged droplets to be incubated at the required temperature.” Ex. 1040 at 2:44-47. “If a colour change reaction is involved, such a colour change can be recorded immediately or after incubation, using thermostatically controlled heating coil (27).” *Id.* at 3:57-60

As discussed above in connection with element 1[b], in the instant combination Shaw Stewart is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule and the autocatalytic reaction. Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. These limitations are met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1040 at 1. The DNA is amplified through the PCR reaction. *Id.*

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1068.

Thus, claim 1 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Lagally.

Dependent Claim 2

Claim 2 of the ‘193 patent recites “wherein the at least one substrate molecule is a single biological molecule.”

Shaw Stewart discloses that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.”¹⁰⁸ Ex. 1040 at 3:82-86.

In the combined method of Shaw Stewart, Corbett and Lagally, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90.

As discussed by Corbett, “a duplex DNA segment of up to 5,000 base pairs in length may be amplified many million-fold by means of the polymerase chain reaction (PCR;[1]), starting from as little as **a single copy**.” Ex. 1010 at 1:17-20

As discussed by Lagally, “[s]tochastic PCR amplification of **single DNA template molecules** followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Ex. 1028 at 565, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1068.

Thus, claim 2 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Lagally.

Dependent Claims 3 and 4

Claim 3 of the ‘193 patent recites “wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.” Claim 4 of the ‘193 patent recites

¹⁰⁸ Requester notes that Shaw does disclose use of a biological molecule (Ex. 1040 4:30-33), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

“wherein the providing step includes heating.” PCR reactions involve thermocycling. Ex. 1004 ¶¶33-34.

Shaw Stewart discloses that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.”¹⁰⁹ Ex. 1040 at 3:82-86. In the combined method of Shaw Stewart, Corbett and Lagally, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. To affect the PCR reaction, the carrier fluid is heated. (“The carrier fluid then passes through a plurality of temperature zones in which the polymerase chain reactions take place”) Ex. 1010 (Corbett); (“The PCR chambers were thermally cycled.”) Ex. 1028 at 567.

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1068.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Lagally.

Dependent Claim 5

Claim 5 of the ‘193 patent further recites “providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.”

Shaw Stewart discloses that “parts of the device itself can be adapted to form the sample chambers of the standard instruments of chemical or biochemical analysis.” Ex. 1040 at 2:62-64.

¹⁰⁹ Requester notes that Shaw does disclose use of a biological molecule (Ex. 1040 4:30-33), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

“For example ducts can be formed with two plain transparent walls to form the sample chambers of spectrophotometers.” Ex. 1040 at 2:64-66.

Corbett similarly teaches that “[d]ownstream of temperature zone 34 is in-line analysis means 29 and recovery means 28. Connected to recovery means 28 is **detection means 30**. Detection means 30 determines when a reaction mixture in the carrier fluid reaches recovery means 28. This may be done by measuring the conductivity or **optical density** of the stream of carrier fluid.” Ex. 1010 at 8:12-18, [emphasis added].

Lagally teaches “[s]tochastic PCR amplification of single DNA template molecules followed by **capillary electrophoresis** (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Ex. 1028 at 565, [emphasis added]. The results of capillary electrophoresis were generally detected optically at the time of filing. Ex. 1011 (Dolnik) at 46.

In the combined method of Shaw Stewart, Corbett and Lagally, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶68-69.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1068.

Thus, claim 5 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Lagally.

Dependent Claims 6-8

Claim 6 of the ‘193 patent recites “wherein the oil is fluorinated oil.” Claim 7 of the ‘193 patent recites “wherein the carrier fluid further comprises a surfactant.” Claim 8 of the ‘193 patent recites “wherein the surfactant is fluorinated surfactant.”

Shaw Stewart discloses that “[s]uitable carrier phases include **mineral oils**, water, light silicones, or **Freons**.” Ex. 1040 at 1:39-41; (Freons are comprised of chlorofluorocarbons. Ex.

1108). “**Surface acting agents** may also be included in the carrier and/or reagents phases to produce suitable surface properties, for example to allow efficient merging.” Ex. 1040 at 1:44-47, [emphasis added].

The phrase “carrier fluid” in claim 7, having no antecedent basis, is construed to mean “a fluid that is immiscible with a plug fluid,” as this is the way the term is defined in the specification. *See, e.g.*, Ex. 1001 at 7:42-43.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1068.

To the extent the Office determines that any element of claims 6-8 is not disclosed by Shaw Stewart, it would have been obvious to use the recited fluorinated surfactants and oils in light of Quake’s disclosure that fluorinated oils serve as surfactants which “aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel.” Ex. 1034 at 35.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claims 6-8 are shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Lagally and further in view of Quake.

Dependent Claim 9

Claim 9 of the ‘193 patent recites “wherein the at least one plug is a merged plug.”

Shaw Stewart describe merging droplets of reactants with droplets of reagents. Ex. 1040 at 1:49-53 (“The liquid components will be contained in a system of tubes and conduits allowing the droplets of reactant to be moved by the carrier phase so as to be merged with the droplets containing other reagents”), *see also* 1:114-121.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1068.

Thus, claim 9 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Lagally.

Dependent Claim 10

Claim 10 of the '193 patent recites "trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels."

Quake describes a "detection region" which "should generally be at least about twice, preferably at least about five times as large per side or in diameter as the diameter of the largest molecule, cell or droplet that will be passing through it." Ex. 1034 at 26. This may be contrasted to Quake's generally recommended dimensions of the fluid channels, which are shaped to cause the droplets to "conform to the size and shape of the channels" by "becom[ing] longer and thinner" as channels narrow. *Id.* at 27. Quake teaches that "an optical trap, or laser tweezers, may be used to sort or direct cells.... The optical trap remains in an 'off' mode until a desired particle is detected in the detection region. After detection of a desired characteristic, the particle or cell is 'trapped', and thereby directed or moved into the predetermined branch channel for collection." *Id.* at 67, *see also* FIGs. 14A & B (Input). Thus, Quake's detection region is an "expansion portion" where the at least one plug would be "trapp[ed]... for a period of time... after the reaction" as recited in dependent claim 10.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1068.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claim 10 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett, Lagally, and Quake.

Dependent Claim 11

Claim 11 of the '193 patent recites “wherein the at least one plug is substantially spherical in shape.”

Shaw Stewart’s droplets are described as being “sufficiently small to form substantially **spherical droplets** with diameters less than the diameters of the conduits.” Ex. 1040 at 3:102-104, [emphasis added]. Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1068.

Thus, claim 11 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Lagally.

Dependent Claim 12

Claim 12 of the '193 patent recites “a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction.”

Shaw Stewart describes mixing by “introduce[ing] two reagents, which are separated into two smaller volumes at the T junctions then these smaller volumes are merged at the 'Y' junction by withdrawing oil from the center conduit.” Ex. 1040 at Abstract. The merging causing mixing to occur. Further, Shaw Stewart states “[a]fter coalescence the constituents of two droplets can be mixed by . . . passing it through a constriction once or several times.” Ex. 1040 at 2:53-56.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1068.

Thus, claim 12 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Lagally.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '193 patent recites “wherein the special design of the at least one channel comprises periodic or aperiodic turns and

relevant parameters.” Claim 14 of the ‘193 patent recites “wherein the relevant parameters are selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns.”

Corbett teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Corbett meets both claims 13 and 14 under the broadest reasonable interpretation of the term “special design,” which in and of itself imparts no meaningful structural limitation. Ex. 1010 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1068.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Lagally.

H. The Combination of Shaw Stewart, Corbett and Burns II Renders Obvious Claims 1-14 of the ‘193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Shaw Stewart), ii) microfluidic PCR devices were well known (Corbett and Burns II), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Lagally, Kopp, Burns II and Vogelstein).

The Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. The Shaw Stewart British Application discloses a “droplet reactor” and illustrates at Figure 1 a carrier phase 1 flowing along a conduit into which reagent 2 is injected, thereby forming droplet 4. Ex. 1040 at 1:1-20, 70-90. “[I]f large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” *Id.* at 1:83-90

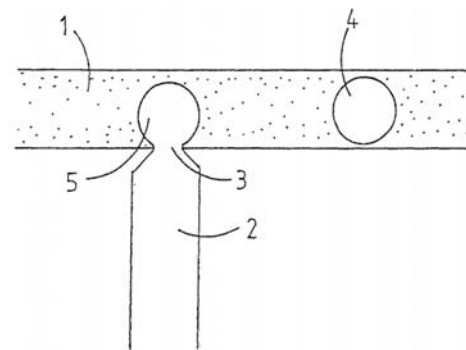


Figure 1.

Significantly, the exclusive licensee of the ‘193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the ‘193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO’s primary arguments was that Stewart I

only disclosed introducing fluid in a “stepwise fashion,” whereas in the ’503 Patent droplets are formed by “combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.” *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

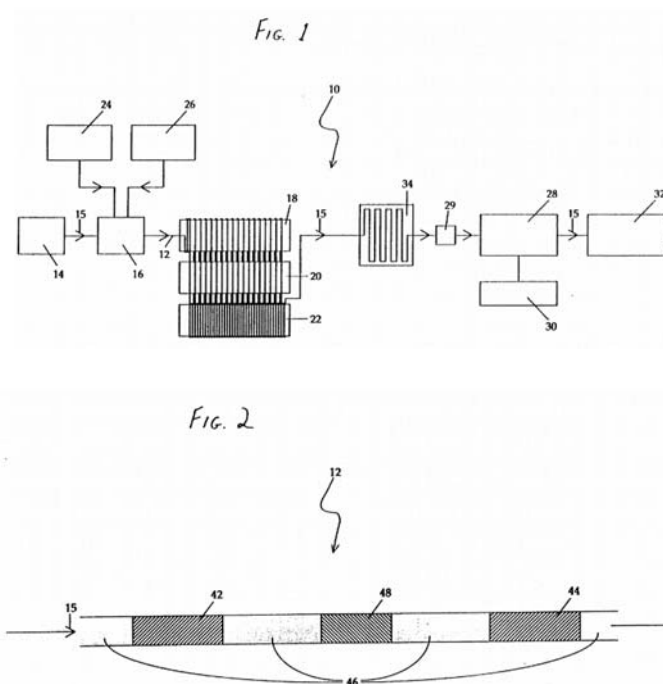
Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Burns II. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream

includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.



“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

In 1996, Burns II reported the use of microfluidic devices to perform PCR. Ex. 1008 at 5556. Burns II explained that “[n]o new biochemistries or DNA detection methods are required for their use; the components simply reproduce current laboratory procedures in a micron-sized environment.” *Id.* at 5560. In other words, the PCR chemistry did not need to be modified – it worked without modification on the microfluidic scale. In the method of Burns II, a sample

containing a DNA plasmid was loaded into one of the reaction wells (A) and a mixture of Taq enzyme and buffer was loaded into the other. Ex. 1008 at 5558. Each formed droplets of about 60 nl in the parallel 500 x 25 μm channels (B) which came together at the Y junction to form a single larger droplet (about 120 nl). *Id.* at 5558-59; Ex. 1004 ¶40.

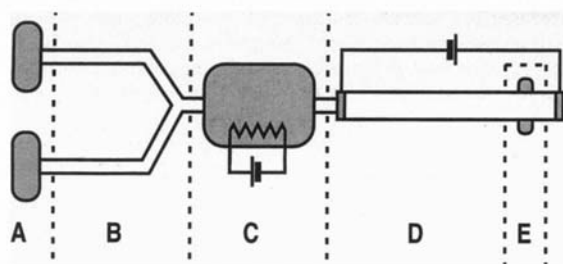


FIG. 1. Example of an integrated DNA analysis system, represented schematically. The individual components of the system are injection entry ports (A), liquid pumping channels (B), thermally controlled reaction chamber (C), electrophoresis channel (D), and DNA band migration detector (E). Each component would have associated sensors, control circuitry, and external connections.

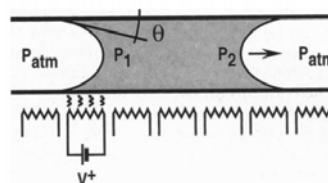


FIG. 2. Schematic drawing showing the principle of thermally induced drop motion in a closed channel. The case of a single aqueous drop in a hydrophilic channel is presented, where V is an applied voltage, P_{atm} is atmospheric pressure, P_1 is the receding-edge internal pressure, P_2 is the advancing-edge internal pressure, and θ is the contact angle of the liquid-gas-solid interface. The contact angle will depend on the surface characteristics of the channel and the constituents of the drop, with a hydrophilic interaction giving θ between 0 and 90 degrees, and a hydrophobic surface giving θ between 90 and 180 degrees. Surface treatments can also reduce contact angle hysteresis and, therefore, reduce the temperature difference necessary for drop motion.

The droplet then passed through the resistive heater portion (C) in which PCR amplification occurred. *Id.* at 5556-5557. Afterwards the amplification results were optically detected in zone D via gel electrophoresis. *Id.* at 5557-5561. Burns II noted that “[t]he use of common fabrication methods allows the assembly of increasingly complex, multicomponent, integrated systems from a small, defined set of standardized elements” and that “[f]uture design efforts have a strong impetus to reduce component size and improve efficiency.” *Id.* at 5560-61.

In sum, Corbett and Burns II show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Shaw Stewart is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting

an autocatalytic reaction through a first channel of the at least two channels”).¹¹⁰ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct PCR as taught by Corbett and Burns II. *Id.* ¶¶88-109. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein’s contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactor of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions

¹¹⁰ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Shaw Stewart to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler

is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed

sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Burns II) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶88-109. Using two flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Burns II. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller

reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96. Moreover, a skilled artisan would have **fully expected the combination to be successful**.

In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample

volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced

microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic**

droplet (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Shaw Stewart could be successfully modified to perform PCR reactions.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Burns II to work in the microfluidic droplet reactor of Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹¹¹ As discussed in

¹¹¹ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570

Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail below, in the accompanying claim chart (Ex. 1069) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Corbett and Burns II. Ex. 1004 ¶106. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Shaw Stewart discloses that “[t]he method of the invention may be used for initiating and controlling a chemical reaction or preparing mixtures of reagents...” Ex. 1040 at 1:7-9.

“The system is particularly suited **to the manipulation of microscopic quantities of reagents, with volumes of less than one microliter,**...” *Id.* at 1:20-22.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1069.

(Fed.Cir.1985) (‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

As shown in Shaw Stewart's Figure 1, "[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube." Ex. 1040 at 1:70-5 .

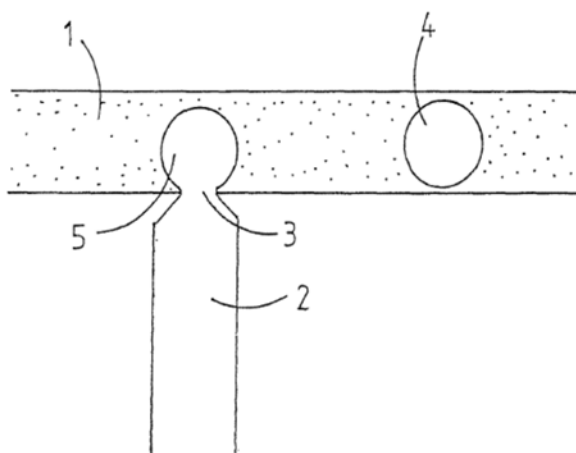


Figure 1.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1069.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

As shown in Shaw Stewart's Figure 1, "[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube." Ex. 1040 at 1:70-75. Shaw Stewart teaches that "[f]or **aqueous reagents**, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon

interface.” Ex. 1040 at 1:62-66, [emphasis added]. “[I]f large numbers of droplets are required, a **continuous flow of reagent** through the opening will be produced, while a **continuous current of carrier phase flows** down the tube.” Ex. 1040 at 1:83-86, [emphasis added].

In the instant combination, the microfluidic droplet reactors of Shaw Stewart are relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).¹¹² Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-39.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Corbett and Burns II. *Id.* ¶106. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modifying it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein’s contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Shaw Stewart because doing so have provided the substantial

¹¹² Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Shaw Stewart to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett's PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026

(Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp, Burns II, Lagally

or Vogelstein) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp, Burns II, Lagally or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II or Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and

sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In

1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactors Shaw Stewart could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Burns II to work in the microfluidic droplet reactor of Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet

reactor that falls within the droplet formation recitation of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹¹³ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained further in the accompanying claim chart (Ex. 1069) and the declaration of Dr. Shafqeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Corbett and Burns II. Ex. 1004 ¶106. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modifying it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that

¹¹³ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1069.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

As shown in Shaw Stewart's Figure 1, "[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube." Ex. 1040 at 1:70-5. Shaw Stewart teaches that "[f]or **aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable**, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface." Ex. 1040 at 1:62-66, [emphasis added]. "[I]f large numbers of droplets are required, **a continuous flow of reagent** through the opening will be produced, while a **continuous current of carrier phase flows** down the tube." Ex. 1040 at 1:83-86, emphasis added.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1069.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

As discussed above, the Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. Significantly, the exclusive licensee of the '193 patent affirmatively (and correctly) took the position that Shaw

Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the '193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner ("PO") mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO's arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO's primary arguments was that Stewart I only disclosed introducing fluid in a "stepwise fashion," whereas in the '503 Patent droplets are formed by "combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid." *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

With regard to the recitation that the plug is “substantially surrounded by the oil flowing through the channel,” this is shown in Shaw Stewart’s Figure 1. Shaw Stewart’s specification also explains that the droplets are smaller than the conduits. (“A method as claimed in claim (1) in which the discrete volumes of chemical reagents are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.”). Ex. 1040 at 3:101-104.

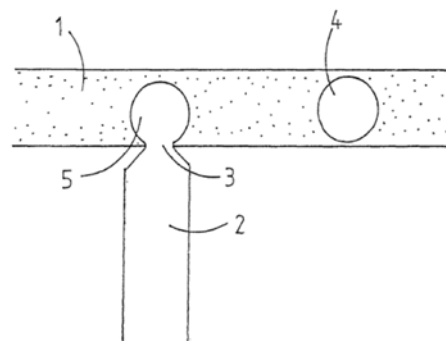


Figure 1.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1069.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Shaw Stewart discloses that “[r]egions of the device can be heated by electric circuits or coils or by electromagnetic radiation to allow the merged droplets to be incubated at the required temperature.” Ex. 1040 at 2:44-47. “If a colour change reaction is involved, such a colour change can be recorded immediately or after incubation, using thermostatically controlled heating coil (27).” *Id.* at 3:58-60.

As discussed above in connection with element 1[b], in the instant combination Shaw Stewart is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule and the autocatalytic reaction. Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. These limitations are met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase,

primers, and other reagents. Ex. 1004 ¶¶58-71. The DNA is amplified through the PCR reaction. Id.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1069.

Thus, claim 1 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Burns II.

Dependent Claim 2

Claim 2 of the '193 patent recites "wherein the at least one substrate molecule is a single biological molecule."

Shaw Stewart discloses that "[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work."¹¹⁴ Ex. 1040 at 3:82-86.

As discussed by Corbett, "a duplex DNA segment of up to 5,000 base pairs in length may be amplified many million-fold by means of the polymerase chain reaction (PCR:[1]), starting from as little as **a single copy**." Ex. 1010 at 1:17-20

In the combined method of Shaw Stewart, Corbett and Burns II, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1069.

¹¹⁴ Requester notes that Shaw does disclose use of a biological molecule (Ex. 1040 4:30-33), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

Thus, claim 2 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Burns II.

Dependent Claims 3 and 4

Claim 3 of the '193 patent recites "wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction." Claim 4 of the '193 patent recites "wherein the providing step includes heating." PCR reactions involve thermocycling. Ex. 1004 ¶¶33-34.

Shaw Stewart discloses that "[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work."¹¹⁵ Ex. 1040 at 3:82-86. In the combined method of Shaw Stewart, Corbett and Burns II, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. To affect the PCR reaction, the carrier fluid is heated. ("The carrier fluid then passes through a plurality of temperature zones in which the polymerase chain reactions take place") Ex. 1010 (Corbett), ("The enzymatic reaction occurred by moving two drops (Taq I restriction enzyme and supercoiled plasmid) down separate channels using the thermocapillary technique, combining the drops, and heating the merged sample to 65°C in the channel.") Ex. 1008 at 5559 (Burns II) Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1069.

¹¹⁵ Requester notes that Shaw does disclose use of a biological molecule (Ex. 1040 4:30-33), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Burns II.

Dependent Claim 5

Claim 5 of the '193 patent further recites “providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.”

Shaw Stewart discloses that “parts of the device itself can be adapted to form the sample chambers of the standard instruments of chemical or biochemical analysis.” Ex. 1040 at 2:62-64. “For example ducts can be formed with two plain transparent walls to form the sample chambers of spectrophotometers.” Ex. 1040 at 2:64-66.

Corbett similarly teaches that “[d]ownstream of temperature zone 34 is in-line analysis means 29 and recovery means 28. Connected to recovery means 28 is **detection means 30**. Detection means 30 determines when a reaction mixture in the carrier fluid reaches recovery means 28. This may be done by measuring the conductivity or **optical density** of the stream of carrier fluid.” Ex. 1010 at 8:12-18, [emphasis added].

Burns II teaches that “[c]apillary gel electrophoresis of DNA samples was performed using a Beckman P/ACE instrument with a laser-induced fluorescence detector and 37 cm length, 100 μ m diameter, linear polymerase gel capillary according to manufacturer’s recommendation.” Ex. 1008 at 5558. The results of capillary electrophoresis were generally detected optically at the time of filing. Ex. 1011 (Dolnik) at 46.

In the combined method of Shaw Stewart, Corbett and Burns II, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶102, 108.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1069.

Thus, claim 5 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Burns II.

Dependent Claims 6-8

Claim 6 of the '193 patent recites "wherein the oil is fluorinated oil." Claim 7 of the '193 patent recites "wherein the carrier fluid further comprises a surfactant." Claim 8 of the '193 patent recites "wherein the surfactant is fluorinated surfactant."

Shaw Stewart discloses that "[s]uitable carrier phases include **mineral oils**, water, light silicones, or **Freons**." Ex. 1040 at 1:39-41, [emphasis added]; (Freons are comprised of chlorofluorocarbons. Ex. 1108). "**Surface acting agents** may also be included in the carrier and/or reagents phases to produce suitable surface properties, for example to allow efficient merging." Ex. 1040 at 1:44-47, [emphasis added].

The phrase "carrier fluid" in claim 7, having no antecedent basis, is construed to mean "a fluid that is immiscible with a plug fluid," as this is the way the term is defined in the specification. See Ex. 1001 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1069.

To the extent the Office determines that any element of claims 6-8 is not disclosed by Shaw Stewart, it would have been obvious to use the recited fluorinated surfactants and oils in light of Quake's disclosure that fluorinated oils serve as surfactants which "aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel." Ex. 1034 at 35.

Thus, claims 6-8 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Burns II and further in view of Quake.

Dependent Claim 9

Claim 9 of the '193 patent recites "wherein the at least one plug is a merged plug."

Shaw Stewart describe merging droplets of reactants with droplets of reagents. Ex. 1040 at 1:49-53 ("The liquid components will be contained in a system of tubes and conduits allowing the droplets of reactant to be moved by the carrier phase so as to be merged with the droplets containing other reagents"), *see also* 1:114-121.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1069.

Thus, claim 9 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Burns II.

Dependent Claim 10

Claim 10 of the '193 patent recites "trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels."

Quake describes a "detection region" which "should generally be at least about twice, preferably at least about five times as large per side or in diameter as the diameter of the largest molecule, cell or droplet that will be passing through it." Ex. 1034 at 26. This may be contrasted to Quake's generally recommended dimensions of the fluid channels, which are shaped to cause the droplets to "conform to the size and shape of the channels" by "becom[ing] longer and thinner" as channels narrow. *Id.* at 27. Quake teaches that "an optical trap, or laser tweezers, may be used to sort or direct cells.... The optical trap remains in an 'off' mode until a desired particle is detected in the detection region. After detection of a desired characteristic, the particle or cell is 'trapped', and thereby directed or moved into the predetermined branch channel for

collection.” *Id.* at 67, *see also* FIGs. 14A & B (Input). Thus, Quake’s detection region is an “expansion portion” where the at least one plug would be “trapp[ed]... for a period of time... after the reaction” as recited in dependent claim 10.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1069.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claim 10 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett, Burns II and Quake.

Dependent Claim 11

Claim 11 of the ‘193 patent recites “wherein the at least one plug is substantially spherical in shape.”

Shaw Stewart’s droplets are described as being “sufficiently small to form substantially **spherical droplets** with diameters less than the diameters of the conduits.” Ex. 1040 at 3:102-104, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1069.

Thus, claim 11 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Burns II.

Dependent Claim 12

Claim 12 of the ‘193 patent recites “a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction.”

Shaw Stewart describes mixing by “introduce[ing] two reagents, which are separated into two smaller volumes at the T junctions then these smaller volumes are merged at the 'Y' junction by withdrawing oil from the center conduit.” Ex. 1040 at Abstract. The merging causing mixing

to occur. Further, Shaw Stewart states “[a]fter coalescence the constituents of two droplets can be mixed by . . . passing it through a constriction once or several times.” Ex. 1040 at 2:53-56.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1069.

Thus, claim 12 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Burns II.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the ‘193 patent recites “wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters.” Claim 14 of the ‘193 patent recites “wherein the relevant parameters are selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns.”

Corbett teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Corbett meets both claims 13 and 14 under the broadest reasonable interpretation of the term “special design,” which in and of itself imparts no meaningful structural limitation. Ex. 1010 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1069.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Burns II.

I. The Combination of Shaw Stewart, Corbett and Vogelstein Renders Obvious Claims 1-14 of the ‘193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Shaw Stewart), ii) microfluidic

PCR devices were well known (Corbett and Vogelstein), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Lagally, Kopp, Burns II and Vogelstein).

The Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. The Shaw Stewart British Application discloses a “droplet reactor” and illustrates at Figure 1 a carrier phase 1 flowing along a conduit into which reagent 2 is injected, thereby forming droplet 4. Ex. 1040 at 1:1-20, 70-90. “[I]f large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” *Id.* at 1:83-90

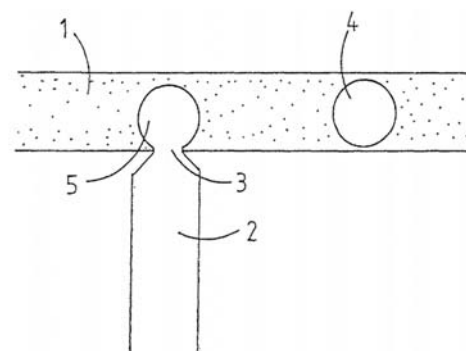


Figure 1.

Significantly, the exclusive licensee of the ‘193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the ‘193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. *See, e.g., Ex. 1003 at 174-180.* For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a “stepwise fashion,” whereas in the ’503 Patent droplets are formed by “combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.” *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

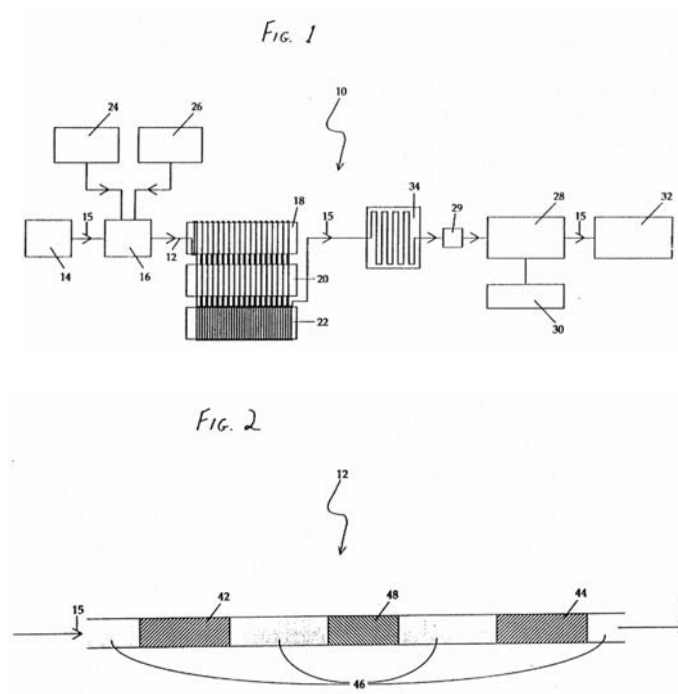
Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Vogelstein. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being

amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.



“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

By 1999, Vogelstein had reported **various applications for single-molecule microfluidic PCR**. Ex. 1044 at 9241. In a seminal article which has been cited over by over 1,000 other publications, Vogelstein explained that PCR amplification of small aliquots or droplets containing single molecules was effective for “detection of a small number of mutant-containing cells among a large excess of normal cells.” *Id.* at 9236. In Vogelstein’s method, the DNA was diluted into 7 µl volumes such that, on average, there was one template molecule for every two wells (i.e., approximately half of the wells contained no DNA template molecule and approximately half contained a single DNA template molecule). *Id.* at 9236, 9237. This avoided the problem of background noise impairing the determination of whether each volume contained the mutant sequence. *Id.* By counting the number of volumes which indicated wild type or mutant templates, the presence or absence of the mutation (e.g., a sequence associated with colorectal cancer) can be determined. *Id.* at 9238. The accuracy of the test is a function of the number of discrete volumes into which the sample is diluted. *Id.* at 9239. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) The article explained that the same technique would be useful for **detecting a wide array of mutations, dislocations and allelic imbalances**, as summarized in Table 1. *Id.* at 9241. Vogelstein thus taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby overcoming the signal-to-noise limitations associated with certain other PCR techniques and

enabling the detection of relatively rare mutations, dislocations and allelic imbalances. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86.

In sum, Corbett and Vogelstein show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Shaw Stewart is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").¹¹⁶ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-74.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Corbett and Vogelstein. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

¹¹⁶ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactor of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047 . It was thus known that using the droplet reactor of Shaw Stewart to

perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett's PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample

leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239 (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein Ex. 1044 at 9236, 9241.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp, Burns II, Lagally or Vogelstein) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp, Burns II, Lagally or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II or Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the

speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In

1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Shaw Stewart could be successfully modified to perform PCR reactions.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Vogelstein to work in the microfluidic droplet reactor of Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as

early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹¹⁷ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail below, in the accompanying claim chart (Ex. 1070) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Corbett and Vogelstein. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

¹¹⁷ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Shaw Stewart discloses that “[t]he method of the invention may be used for initiating and controlling a chemical reaction or preparing mixtures of reagents...” Ex. 1040 at 1:7-9.

“The system is particularly suited **to the manipulation of microscopic quantities of reagents, with volumes of less than one microliter,**...” *Id.* at 1:20-22.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1070.

Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

As shown in Shaw Stewart’s Figure 1, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Ex. 1040 at 1:70-5.

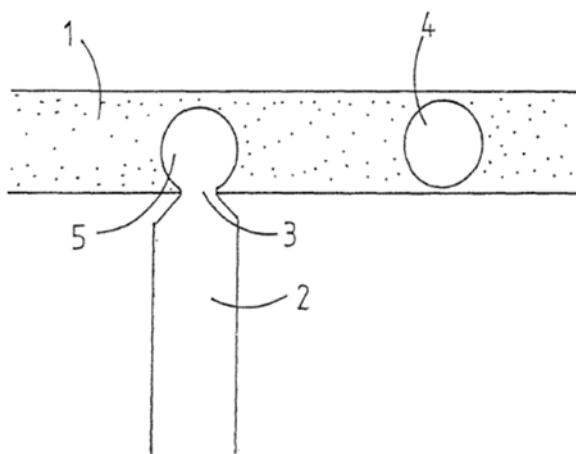


Figure 1.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

As shown in Shaw Stewart's Figure 1, "[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube." Ex. 1040 at 1:70-5. Shaw Stewart teaches that "[f]or **aqueous reagents**, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface." Ex. 1040 at 1:62-66, [emphasis added]. "[I]f large numbers of droplets are required, a **continuous flow of reagent** through the opening will be produced, while a **continuous current of carrier phase flows** down the tube." Ex. 1040 at 1:83-86, [emphasis added].

In the instant combination, the microfluidic droplet reactors of Shaw Stewart are relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").¹¹⁸ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-39.

¹¹⁸ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Corbett and Vogelstein. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modifying it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. *Id.* ¶¶92-96. As noted by Lagally, "[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**" Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that "[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**" Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR

microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Shaw Stewart to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex.

1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances** Ex. 1044 at 9236, 9239, 9241. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp, Burns II, Lagally or Vogelstein) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp, Burns II, Lagally or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II or Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to

perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-

volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the

development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with

the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactors Shaw Stewart could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Vogelstein to work in the microfluidic droplet reactor of Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹¹⁹ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

¹¹⁹ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

As explained further in the accompanying claim chart (Ex. 1070) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Corbett and Vogelstein. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modifying it to incorporate the microfluidic droplet reactor of Shaw Stewart, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1070.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

As shown in Shaw Stewart's Figure 1, "[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube." Ex. 1040 at 1:70-5. Shaw Stewart teaches that "[f]or **aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable**, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface." Ex. 1040 at 1:62-66, [emphasis added]. "[I]f large numbers of droplets are required, **a continuous flow of reagent** through the opening will be produced, while a **continuous current of carrier phase flows** down the tube." Ex. 1040 at 1:83-86, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1070.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

As discussed above, the Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. Significantly, the exclusive licensee of the '193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the '193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner ("PO") mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO's arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO's primary arguments was that Stewart I

only disclosed introducing fluid in a “stepwise fashion,” whereas in the ’503 Patent droplets are formed by “combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.” *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

With regard to the recitation that the plug is “substantially surrounded by the oil flowing through the channel,” this is shown in Shaw Stewart’s Figure 1. Shaw Stewart’s specification also explains that the droplets are smaller than the conduits. (“A method as claimed in claim (1) in which the discrete volumes of chemical reagents are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.”) . Ex. 1040 at 3:101-104

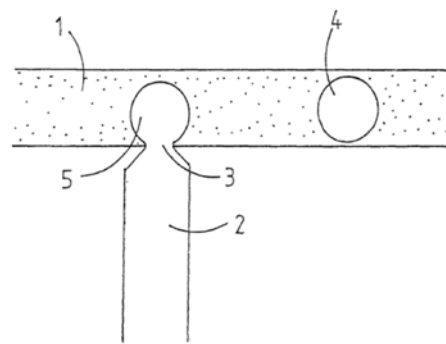


Figure 1.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1070.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Shaw Stewart discloses that “[r]egions of the device can be heated by electric circuits or coils or by electromagnetic radiation to allow the merged droplets to be incubated at the required temperature.” Ex. 1040 at 2:44-47. “If a colour change reaction is involved, such a colour change can be recorded immediately or after incubation, using thermostatically controlled heating coil (27).” *Id.* at 3:58-60.

As discussed above in connection with element 1[b], in the instant combination Shaw Stewart is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule and the autocatalytic reaction. Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. These limitations are met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. The DNA is amplified through the PCR reaction. *Id.*

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1070.

Thus, claim 1 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Vogelstein.

Dependent Claim 2

Claim 2 of the ‘193 patent recites “wherein the at least one substrate molecule is a single biological molecule.”

Shaw Stewart discloses that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.”¹²⁰ Ex. 1040 at 3:82-86.

As discussed by Corbett, “a duplex DNA segment of up to 5,000 base pairs in length may be amplified many million-fold by means of the polymerase chain reaction (PCR;[1]), starting from as little as **a single copy**.” Ex. 1010 at 1:17-20

In the combined method of Shaw Stewart, Corbett and Vogelstein, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1070.

Thus, claim 2 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Vogelstein.

Dependent Claims 3 and 4

Claim 3 of the ‘193 patent recites “wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.” Claim 4 of the ‘193 patent recites “wherein the providing step includes heating.” PCR reactions involve thermocycling. Ex. 1004 ¶¶33-34.

¹²⁰ Requester notes that Shaw does disclose use of a biological molecule (Ex. 1040 4:30-33), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

Shaw Stewart discloses that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.”¹²¹ Ex. 1040 at 3:82-86. In the combined method of Shaw Stewart, Corbett and Vogelstein, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. To affect the PCR reaction, the carrier fluid is heated. (“The carrier fluid then passes through a plurality of temperature zones in which the polymerase chain reactions take place”) Ex. 1010 at Abstract (Corbett), (“If a PCR product whose sequence is complementary to the loop sequence is present during the heating/cooling cycle, hybridization of the MB to one strand of the PCR product will increase the distance between the Dabcyl and the dye, resulting in increased fluorescence.”) Ex. 1044 at 9237(Vogelstein).

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1070.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Vogelstein.

Dependent Claim 5

Claim 5 of the ‘193 patent further recites “providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.”

¹²¹ Requester notes that Shaw does disclose use of a biological molecule (Ex. 1040 4:30-33), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

Shaw Stewart discloses that “parts of the device itself can be adapted to form the sample chambers of the standard instruments of chemical or biochemical analysis.” Ex. 1040 at 2:62-64. “For example ducts can be formed with two plain transparent walls to form the sample chambers of spectrophotometers.” Ex. 1040 at 2:64-66.

Corbett similarly teaches that “[d]ownstream of temperature zone 34 is in-line analysis means 29 and recovery means 28. Connected to recovery means 28 is **detection means 30**. Detection means 30 determines when a reaction mixture in the carrier fluid reaches recovery means 28. This may be done by measuring the conductivity or **optical density** of the stream of carrier fluid.” Ex. 1010 at 8:12-18, [emphasis added].

Vogelstein teaches that the “[r]eactions were analyzed immediately or stored at room temperature for up to 36 h before **fluorescence analysis**.” Ex. 1044 at 9236, [emphasis added].

In the combined method of Shaw Stewart, Corbett and Vogelstein, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶102, 108.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1070.

Thus, claim 5 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Vogelstein.

Dependent Claims 6-8

Claim 6 of the ‘193 patent recites “wherein the oil is fluorinated oil.” Claim 7 of the ‘193 patent recites “wherein the carrier fluid further comprises a surfactant.” Claim 8 of the ‘193 patent recites “wherein the surfactant is fluorinated surfactant.”

Shaw Stewart discloses that “[s]uitable carrier phases include **mineral oils**, water, light silicones, or **Freons**.” Ex. 1040 at 1:39-41, [emphasis added]; (Freons are comprised of

chorofluorocarbons. Ex. 1108). “**Surface acting agents** may also be included in the carrier and/or reagents phases to produce suitable surface properties, for example to allow efficient merging.” Ex. 1040 at 1:44-47, [emphasis added].

The phrase “carrier fluid” in claim 7, having no antecedent basis, is construed to mean “a fluid that is immiscible with a plug fluid,” as this is the way the term is defined in the specification. See Ex. 1001 at 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1070.

To the extent the Office determines that any element of claims 6-8 is not disclosed by Shaw Stewart, it would have been obvious to use the recited fluorinated surfactants and oils in light of Quake’s disclosure that fluorinated oils serve as surfactants which “aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel.” Ex. 1034 at 35.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claims 6-8 are shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Vogelstein and further in view of Quake.

Dependent Claim 9

Claim 9 of the ‘193 patent recites “wherein the at least one plug is a merged plug.”

Shaw Stewart describe merging droplets of reactants with droplets of reagents. Ex. 1040 at 1:49-53 (“The liquid components will be contained in a system of tubes and conduits allowing the droplets of reactant to be moved by the carrier phase so as to be merged with the droplets containing other reagents”), *see also* 1:114-121.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1070.

Thus, claim 9 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Vogelstein.

Dependent Claim 10

Claim 10 of the '193 patent recites "trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels."

Quake describes a "detection region" which "should generally be at least about twice, preferably at least about five times as large per side or in diameter as the diameter of the largest molecule, cell or droplet that will be passing through it." Ex. 1034 at 26. This may be contrasted to Quake's generally recommended dimensions of the fluid channels, which are shaped to cause the droplets to "conform to the size and shape of the channels" by "becom[ing] longer and thinner" as channels narrow. *Id.* at 27. Quake teaches that "an optical trap, or laser tweezers, may be used to sort or direct cells.... The optical trap remains in an 'off' mode until a desired particle is detected in the detection region. After detection of a desired characteristic, the particle or cell is 'trapped', and thereby directed or moved into the predetermined branch channel for collection." *Id.* at 67, *see also* FIGs. 14A & B (Input). Thus, Quake's detection region is an "expansion portion" where the at least one plug would be "trapp[ed]... for a period of time... after the reaction" as recited in dependent claim 10.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1070.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claim 10 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett, Vogelstein and Quake.

Dependent Claim 11

Claim 11 of the '193 patent recites “wherein the at least one plug is substantially spherical in shape.”

Shaw Stewart's droplets are described as being “sufficiently small to form substantially **spherical droplets** with diameters less than the diameters of the conduits.” Ex. 1040 at 3:102-104, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1070.

Thus, claim 11 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Vogelstein.

Dependent Claim 12

Claim 12 of the '193 patent recites “a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction.”

Shaw Stewart describes mixing by “introduc[ing] two reagents, which are separated into two smaller volumes at the T junctions then these smaller volumes are merged at the 'Y' junction by withdrawing oil from the center conduit.” Ex. 1040 at Abstract. The merging causing mixing to occur. Further, Shaw Stewart states “[a]fter coalescence the constituents of two droplets can be mixed by . . . passing it through a constriction once or several times.” Ex. 1040 at 2:53-56.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1070.

Thus, claim 12 is shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Vogelstein.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '193 patent recites "wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters." Claim 14 of the '193 patent recites "wherein the relevant parameters are selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns."

Corbett teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Corbett meets both claims 13 and 14 under the broadest reasonable interpretation of the term "special design," which in and of itself imparts no meaningful structural limitation. Ex. 1010 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1070.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Shaw Stewart, Corbett and Vogelstein.

J. The Combination of Shaw Stewart, Kopp and Lagally Renders Obvious Claims 1-14 of the '193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Shaw Stewart), ii) microfluidic PCR devices were well known (Kopp and Lagally), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

The Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. The Shaw Stewart British Application discloses a “droplet reactor” and illustrates at Figure 1 a carrier phase 1 flowing along a conduit into which reagent 2 is injected, thereby forming droplet 4. Ex. 1040 at 1:1-20, 70-90. “[I]f large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” *Id.* at 1:83-90

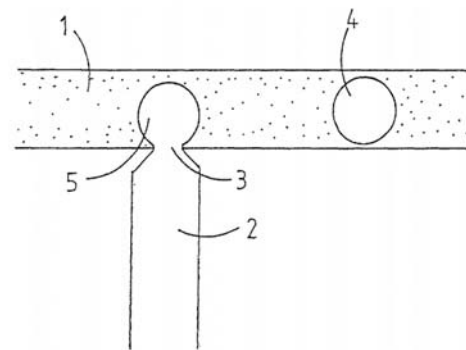


Figure 1.

Significantly, the exclusive licensee of the ‘193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the ‘193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO’s primary arguments was that Stewart I

only disclosed introducing fluid in a “stepwise fashion,” whereas in the ’503 Patent droplets are formed by “combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.” *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

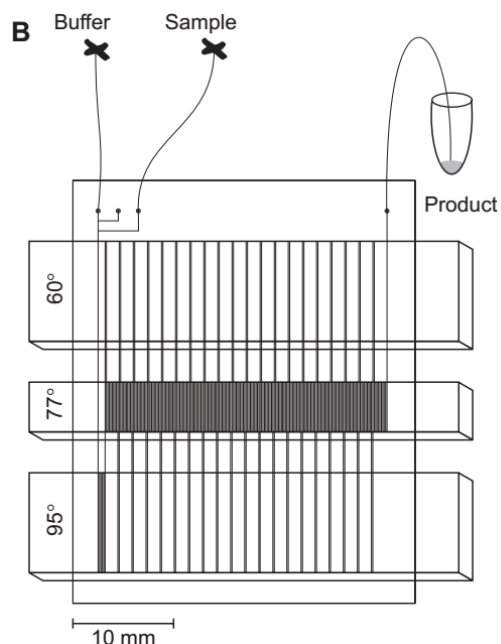
As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Lagally. By 1998 Kopp et al. had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product

quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 [emphasis added].

Kopp et al. also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been

presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 emphasis added.

In 2001, in another article not previously considered by the Office, Lagally reported using a microfluidic reactor to conduct single-molecule DNA amplification by PCR. Lagally summarized the state of the art as of February 2001, explaining continuous flow PCR microreactors had recently undergone substantial evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or **continuous flow** through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample

volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

Lagally *et al.* developed a further improvement – a device and method for achieving single molecule amplification in a microchannel environment.

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced

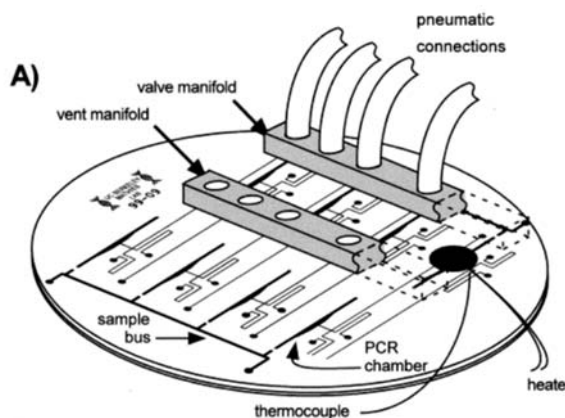
microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

Microfluidic PCR-CE device. The PCR chambers are connected to a common sample bus through a set of valves. Hydrophobic vents at the other end of the PCR chambers are used to locate the sample and to eliminate gas. The PCR chambers are directly

connected to

the cross channel of the CE system for product injection and analysis. Two aluminum manifolds, one each for the vents and valves, are placed

onto the respective ports and clamped in place using vacuum. The manifolds connect to external solenoid valves for pressure and vacuum actuation. Thermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.



Ex. 1028 at 565-66 (see figure legend).

In sum, Kopp and Lagally show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Shaw Stewart is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").¹²² Ex.

¹²² Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Kopp and Lagally. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactors of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶¶92-100; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047.

Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Shaw Stewart to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp, Burns II, Lagally or Vogelstein) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp, Burns II, Lagally or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II or Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation

of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for

thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask

important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Shaw Stewart could be successfully modified to perform PCR reactions.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp or Lagally to work in the microfluidic reactor of Quake, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹²³ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail below, in the accompanying claim chart (Ex. 1071), and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the

¹²³ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Kopp and Lagally.

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Shaw Stewart discloses that “[t]he method of the invention may be used for initiating and controlling a chemical reaction or preparing mixtures of reagents...” Ex. 1040 at 1:7-9.

“The system is particularly suited **to the manipulation of microscopic quantities of reagents, with volumes of less than one microliter,**...” *Id.* at 1:20-22.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1071.

Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

As shown in Shaw Stewart’s Figure 1, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Ex. 1040 at 1:70-5.

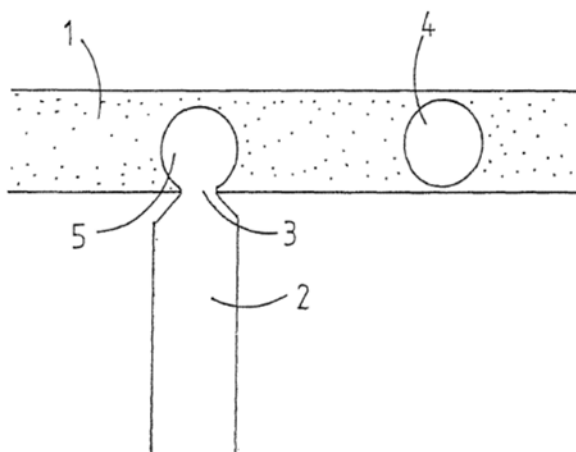


Figure 1.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1071.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

As shown in Shaw Stewart's Figure 1, "[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube." Ex. 1040 at 1:70-5. Shaw Stewart teaches that "[f]or **aqueous reagents**, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface." Ex. 1040 at 1:62-66, [emphasis added]. "[I]f large numbers of droplets are required, a **continuous flow of reagent** through the opening will be produced, while a **continuous current of carrier phase flows** down the tube." Ex. 1040 at 1:83-86, [emphasis added].

In the instant combination, the microfluidic droplet reactors of Shaw Stewart are relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").¹²⁴ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents.

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A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Kopp and Lagally. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactors of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Shaw Stewart to

perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett's PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample

leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

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In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In

1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactors Shaw Stewart could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in modifying Kopp or Lagally to work in the microfluidic droplet reactor of Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet

formation recitation of the claims of the '193 patent at least as early as February 23, 2001, more than a year prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹²⁵ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained further in the accompanying claim chart (Ex. 1071) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Kopp and Lagally.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1071.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

As shown in Shaw Stewart's Figure 1, "[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3).

¹²⁵ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Ex. 1040 at 1:70-5. Shaw Stewart teaches that “[f]or **aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable**, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Ex. 1040 at 1:62-66, [emphasis added]. “[I]f large numbers of droplets are required, **a continuous flow of reagent** through the opening will be produced, while a **continuous current of carrier phase flows** down the tube.” Ex. 1040 at 1:83-86, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1071.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

As discussed above, the Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. Significantly, the exclusive licensee of the ‘193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the ‘193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO’s primary arguments was that Stewart I only disclosed introducing fluid in a “stepwise fashion,” whereas in the ’503 Patent droplets are formed by “combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.” *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

With regard to the recitation that the plug is “substantially surrounded by the oil flowing through the channel,” this is shown in Shaw Stewart’s Figure 1. Shaw Stewart’s specification also explains that the droplets are smaller than the conduits. E (“A method as claimed in claim (1) in which the discrete volumes of chemical reagents are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.”) . Ex. 1040 at 3:101-104

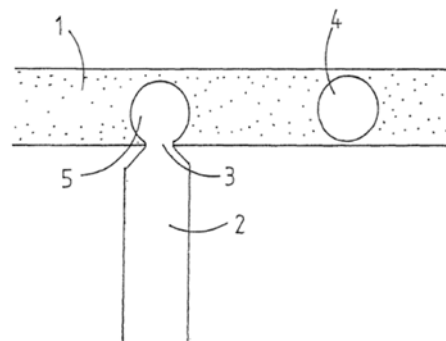


Figure 1.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1071.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Shaw Stewart discloses that “[r]egions of the device can be heated by electric circuits or coils or by electromagnetic radiation to allow the merged droplets to be incubated at the required temperature.” Ex. 1040 at 2:44-47. “If a colour change reaction is involved, such a colour change can be recorded immediately or after incubation, using thermostatically controlled heating coil (27).” *Id.* at 3:58-60.

As discussed above in connection with element 1[b], in the instant combination Shaw Stewart is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule and the autocatalytic reaction. Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. These limitations are met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. The DNA is amplified through the PCR reaction. *Id.*

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1071.

Thus, claim 1 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Lagally.

Dependent Claim 2

Claim 2 of the ‘193 patent recites “wherein the at least one substrate molecule is a single biological molecule.”

Shaw Stewart discloses that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.”¹²⁶ Ex. 1040 at 3:82-86.

In the combined method of Shaw Stewart, Kopp and Lagally, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90.

As discussed by Lagally, “[s]tochastic PCR amplification of **single DNA template molecules** followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Ex. 1028 at 565, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1071.

Thus, claim 2 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Lagally.

Dependent Claims 3 and 4

Claim 3 of the ‘193 patent recites “wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.” Claim 4 of the ‘193 patent recites “wherein the providing step includes heating.” PCR reactions involve thermocycling. Ex. 1004 ¶¶33-34.

¹²⁶ Requester notes that Shaw does disclose use of a biological molecule (Ex. 1040 4:30-33), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

Shaw Stewart discloses that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.”¹²⁷ Ex. 1040 at 3:82-86. In the combined method of Shaw Stewart, Kopp and Lagally, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. To affect the PCR reaction, the carrier fluid is heated. (“The individual steps are simply performed by heating or cooling the sample to characteristic temperatures: 95°C for dsDNA melting, 50° to 65°C for primer annealing, and 72° to 77°C for primer extension at the optimum enzyme temperature.”) Ex. 1027 at 1046 (Kopp), (“The PCR chambers were thermally cycled.”) Ex. 1028 (Lagally) at 567.

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1071.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Lagally.

Dependent Claim 5

Claim 5 of the ‘193 patent further recites “providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.”

Shaw Stewart discloses that “parts of the device itself can be adapted to form the sample chambers of the standard instruments of chemical or biochemical analysis.” Ex. 1040 at 2:62-64.

¹²⁷ Requester notes that Shaw does disclose use of a biological molecule (Ex. 1040 4:30-33), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

“For example ducts can be formed with two plain transparent walls to form the sample chambers of spectrophotometers.” Ex. 1040 at 2:64-66.

Lagally teaches “[s]tochastic PCR amplification of single DNA template molecules followed by **capillary electrophoresis** (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Ex. 1028 at 565, [emphasis added]. The results of capillary electrophoresis were generally detected optically at the time of filing. Ex. 1011 (Dolnik) at 46; Ex. 1004 ¶¶68-69.

In the combined method of Shaw Stewart, Kopp and Lagally, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶102, 108.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1071.

Thus, claim 5 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Lagally.

Dependent Claims 6-8

Claim 6 of the ‘193 patent recites “wherein the oil is fluorinated oil.” Claim 7 of the ‘193 patent recites “wherein the carrier fluid further comprises a surfactant.” Claim 8 of the ‘193 patent recites “wherein the surfactant is fluorinated surfactant.”

Shaw Stewart discloses that “[s]uitable carrier phases include **mineral oils**, water, light silicones, or **Freons**.” Ex. 1040 at 1:39-41, [emphasis added]; (Freons are comprised of chorofluorocarbons. Ex. 1108). “**Surface acting agents** may also be included in the carrier and/or reagents phases to produce suitable surface properties, for example to allow efficient merging.” Ex. 1040 at 1:44-47, [emphasis added].

The phrase “carrier fluid” in claim 7, having no antecedent basis, is construed to mean “a fluid that is immiscible with a plug fluid,” as this is the way the term is defined in the specification. See Ex. 1001 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1071.

To the extent the Office determines that any element of claims 6-8 is not disclosed by Shaw Stewart, it would have been obvious to use the recited fluorinated surfactants and oils in light of Quake’s disclosure that fluorinated oils serve as surfactants which “aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel.” Ex. 1034 at 35.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claims 6-8 are shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Lagally and further in view of Quake.

Dependent Claim 9

Claim 9 of the ‘193 patent recites “wherein the at least one plug is a merged plug.”

Shaw Stewart describe merging droplets of reactants with droplets of reagents. Ex. 1040 at 1:49-53 (“The liquid components will be contained in a system of tubes and conduits allowing the droplets of reactant to be moved by the carrier phase so as to be merged with the droplets containing other reagents”), *see also* 1:114-121.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1071.

Thus, claim 9 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Lagally.

Dependent Claim 10

Claim 10 of the '193 patent recites “trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels.”

Quake describes a “detection region” which “should generally be at least about twice, preferably at least about five times as large per side or in diameter as the diameter of the largest molecule, cell or droplet that will be passing through it.” Ex. 1034 at 26. This may be contrasted to Quake’s generally recommended dimensions of the fluid channels, which are shaped to cause the droplets to “conform to the size and shape of the channels” by “becom[ing] longer and thinner” as channels narrow. *Id.* at 27. Quake teaches that “an optical trap, or laser tweezers, may be used to sort or direct cells.... The optical trap remains in an ‘off’ mode until a desired particle is detected in the detection region. After detection of a desired characteristic, the particle or cell is ‘trapped’, and thereby directed or moved into the predetermined branch channel for collection.” *Id.* at 67, *see also* FIGs. 14A & B (Input). Thus, Quake’s detection region is an “expansion portion” where the at least one plug would be “trapp[ed]... for a period of time... after the reaction” as recited in dependent claim 10.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1071.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claim 10 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Lagally and Quake.

Dependent Claim 11

Claim 11 of the '193 patent recites “wherein the at least one plug is substantially spherical in shape.”

Shaw Stewart's droplets are described as being "sufficiently small to form substantially **spherical droplets** with diameters less than the diameters of the conduits." Ex. 1040 at 3:102-104, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1071.

Thus, claim 11 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Lagally.

Dependent Claim 12

Claim 12 of the '193 patent recites "a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction."

Shaw Stewart describes mixing by "introduc[ing] two reagents, which are separated into two smaller volumes at the T junctions then these smaller volumes are merged at the 'Y' junction by withdrawing oil from the center conduit." Ex. 1040 at Abstract. The merging causing mixing to occur. Further, Shaw Stewart states "[a]fter coalescence the constituents of two droplets can be mixed by . . . passing it through a constriction once or several times." Ex. 1040 at 2:53-56.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1071.

Thus, claim 12 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Lagally.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '193 patent recites "wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters." Claim 14 of the '193 patent recites "wherein the relevant parameters are

selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns.”

Kopp teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Kopp meets both claims 13 and 14 under the broadest reasonable interpretation of the term “special design,” which in and of itself imparts no meaningful structural limitation. Ex. 1027 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1071.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Shaw Stewart, Kopp, and Lagally.

K. The Combination of Shaw Stewart, Kopp and Burns II Renders Obvious Claims 1-14 of the ‘193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Shaw Stewart), ii) microfluidic PCR devices were well known (Kopp and Burns II), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

The Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. The Shaw Stewart British Application discloses a “droplet reactor” and illustrates at Figure 1 a carrier phase 1 flowing along a conduit into which reagent 2 is injected, thereby forming droplet 4. Ex. 1040 at 1:1-20, 70-90. “[I]f large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” *Id.* at 1:83-90

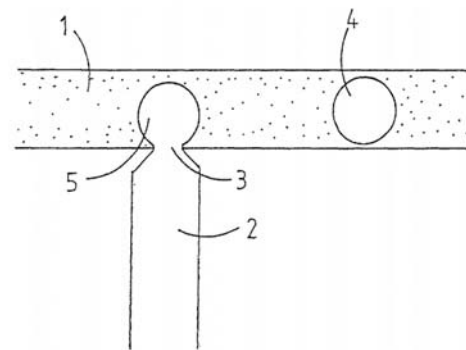


Figure 1.

Significantly, the exclusive licensee of the ‘193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the ‘193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO’s primary arguments was that Stewart I

only disclosed introducing fluid in a “stepwise fashion,” whereas in the ’503 Patent droplets are formed by “combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.” *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Burns II. In 1996, Burns II reported the use of microfluidic devices to perform PCR. Ex. 1008 at 5556. Burns II explained that “[n]o new biochemistries or DNA detection methods are required for their use; the components simply reproduce current laboratory procedures in a micron-sized environment.” *Id.* at 5560. In other words, the PCR chemistry did not need to be modified – it worked without modification on the microfluidic scale. In the method of Burns II, a sample containing a DNA plasmid was loaded into one of the reaction wells (A) and a mixture of Taq enzyme and buffer was loaded into the other. Ex. 1008 at 5558. Each formed droplets of about 60 nl in the parallel 500 x 25 μm channels (B) which came together at the Y junction to form a single larger droplet (about 120 nl). *Id.* at 5558-59; Ex. 1004 ¶40.

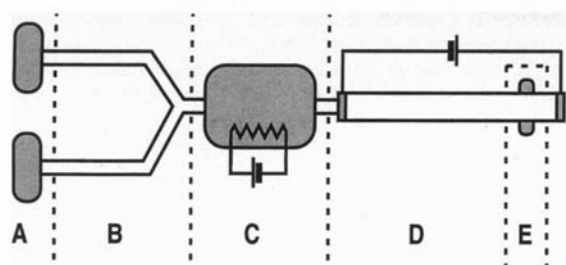


FIG. 1. Example of an integrated DNA analysis system, represented schematically. The individual components of the system are injection entry ports (A), liquid pumping channels (B), thermally controlled reaction chamber (C), electrophoresis channel (D), and DNA band migration detector (E). Each component would have associated sensors, control circuitry, and external connections.

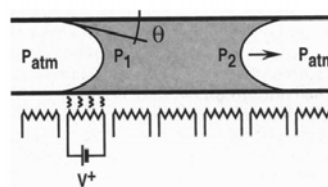


FIG. 2. Schematic drawing showing the principle of thermally induced drop motion in a closed channel. The case of a single aqueous drop in a hydrophilic channel is presented, where V is an applied voltage, P_{atm} is atmospheric pressure, P_1 is the receding-edge internal pressure, P_2 is the advancing-edge internal pressure, and θ is the contact angle of the liquid-gas-solid interface. The contact angle will depend on the surface characteristics of the channel and the constituents of the drop, with a hydrophilic interaction giving θ between 0 and 90 degrees, and a hydrophobic surface giving θ between 90 and 180 degrees. Surface treatments can also reduce contact angle hysteresis and, therefore, reduce the temperature difference necessary for drop motion.

The droplet then passed through the resistive heater portion (C) in which PCR amplification occurred. *Id.* at 5556-5557. Afterwards the amplification results were optically detected in zone D via gel electrophoresis. *Id.* at 5557-5561. Burns II noted that “[t]he use of common fabrication methods allows the assembly of increasingly complex, multicomponent, integrated systems from a small, defined set of standardized elements” and that “[f]uture design efforts have a strong impetus to reduce component size and improve efficiency.” *Id.* at 5560-61.

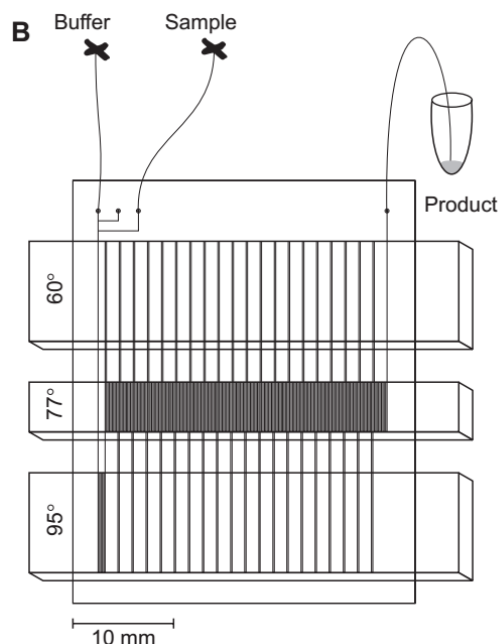
By the late 1990s Kopp et al. had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product

quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 (emphasis added).

Kopp et al. also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been

presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 (emphasis added).

In sum, Kopp and Burns II show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Shaw Stewart is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").¹²⁸ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart, to conduct microfluidic PCR as taught by Kopp and Burns II. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic

¹²⁸ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

droplet reactor of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Shaw Stewart to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett's PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026

(Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp, Burns II, Lagally

or Vogelstein) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp, Burns II, Lagally or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II or Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and

sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In

1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Shaw Stewart could be successfully modified to perform PCR reactions.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp or Lagally to work in the microfluidic droplet reactor of Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year

prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹²⁹ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Kopp and Burns II

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Shaw Stewart discloses that "[t]he method of the invention may be used for initiating and controlling a chemical reaction or preparing mixtures of reagents...." Ex. 1040 at 1:7-9.

¹²⁹ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

“The system is particularly suited **to the manipulation of microscopic quantities of reagents, with volumes of less than one microliter,...**” *Id.* at 1:20-22.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1072.

Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

As shown in Shaw Stewart’s Figure 1, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Ex. 1040 at 1:70-5.

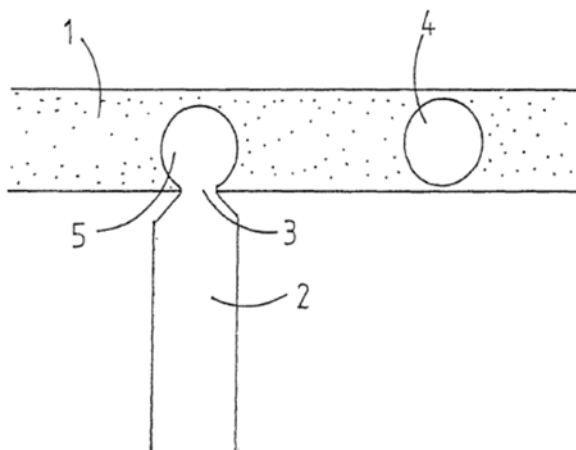


Figure 1.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1072.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

As shown in Shaw Stewart’s Figure 1, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3).

When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Ex. 1040 at 1:70-5. Shaw Stewart teaches that “[f]or **aqueous reagents**, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Ex. 1040 at 1:62-66, [emphasis added]. “[I]f large numbers of droplets are required, a **continuous flow of reagent** through the opening will be produced, while a **continuous current of carrier phase flows** down the tube.” Ex. 1040 at 1:83-86, [emphasis added].

In the instant combination, the microfluidic droplet reactors of Shaw Stewart are relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).¹³⁰ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Kopp and Burns II. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactors of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small

¹³⁰ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Shaw Stewart to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each

other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise**

limitations associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

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Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp, Burns II) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp, Burns II, Lagally or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II)

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Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

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noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson

distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at

1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactors Shaw Stewart could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in modifying Kopp or Burns II to work in the microfluidic droplet reactor of Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered

as evidence of the level of skill in the art.¹³¹ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained further in the accompanying claim chart (Ex. 1072) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Kopp and Burns II.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1072.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

As shown in Shaw Stewart's Figure 1, "[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube." Ex. 1040 at 1:70-5. Shaw Stewart teaches that "[f]or **aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable**, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface." Ex. 1040 at 1:62-66, [emphasis added]. "[I]f large numbers of droplets are

¹³¹ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

required, **a continuous flow of reagent** through the opening will be produced, while a **continuous current of carrier phase flows** down the tube.” Ex. 1040 at 1:83-86, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1072.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

As discussed above, the Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. Significantly, the exclusive licensee of the ‘193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the ‘193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO’s primary arguments was that Stewart I

only disclosed introducing fluid in a “stepwise fashion,” whereas in the ’503 Patent droplets are formed by “combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.” *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

With regard to the recitation that the plug is “substantially surrounded by the oil flowing through the channel,” this is shown in Shaw Stewart’s Figure 1. Shaw Stewart’s specification also explains that the droplets are smaller than the conduits. (“A method as claimed in claim (1) in which the discrete volumes of chemical reagents are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.”) . Ex. 1040 at 3:101-104

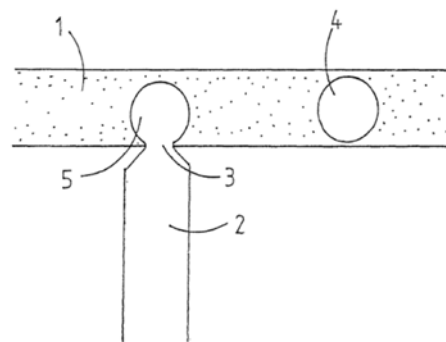


Figure 1.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1072.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Shaw Stewart discloses that “[r]egions of the device can be heated by electric circuits or coils or by electromagnetic radiation to allow the merged droplets to be incubated at the required temperature.” Ex. 1040 at 2:44-47. “If a colour change reaction is involved, such a colour change can be recorded immediately or after incubation, using thermostatically controlled heating coil (27).”

As discussed above in connection with element 1[b], in the instant combination Shaw Stewart is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule and the autocatalytic reaction. Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. These limitations are met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. The DNA is amplified through the PCR reaction. *Id.*

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1072.

Thus, claim 1 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Burns II.

Dependent Claim 2

Claim 2 of the ‘193 patent recites “wherein the at least one substrate molecule is a single biological molecule.”

Shaw Stewart discloses that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very

small quantities, such as forensic and recombinant DNA work.”¹³² Ex. 1040 at 3:82-86. As evidenced by Lagally, in microfluidic reactors, a single DNA molecule may be involved in a reaction. Ex. 1028 p. 564 (“[s]tochastic PCR amplification of **single DNA template molecules** followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device.”); Ex. 1028 at 565.

In the combined method of Shaw Stewart, Kopp and Burns II, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1072.

Thus, claim 2 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Burns II.

Dependent Claims 3 and 4

Claim 3 of the ‘193 patent recites “wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.” Claim 4 of the ‘193 patent recites “wherein the providing step includes heating.” PCR reactions involve thermocycling. Ex. 1004 ¶¶33-34.

Shaw Stewart discloses that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very

¹³² Requester notes that Shaw does disclose use of a biological molecule (Ex. 1040 4:30-33), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

small quantities, such as forensic and recombinant DNA work.”¹³³ Ex. 1040 at 3:82-86. In the combined method of Shaw Stewart, Kopp and Burns II, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. To affect the PCR reaction, the carrier fluid is heated. (“The individual steps are simply performed by heating or cooling the sample to characteristic temperatures: 95°C for dsDNA melting, 50° to 65°C for primer annealing, and 72° to 77°C for primer extension at the optimum enzyme temperature.”) Ex. 1027 at 1046 (Kopp), (“The enzymatic reaction occurred by moving two drops (Taq I restriction enzyme and supercoiled plasmid) down separate channels using the thermocapillary technique, combining the drops, and heating the merged sample to 65°C in the channel.”) Ex. 1008 at 5559 (Burns II).

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1072.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Burns II.

Dependent Claim 5

Claim 5 of the ‘193 patent further recites “providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.”

Shaw Stewart discloses that “parts of the device itself can be adapted to form the sample chambers of the standard instruments of chemical or biochemical analysis.” Ex. 1040 at 2:62-64.

¹³³ Requester notes that Shaw does disclose use of a biological molecule (Ex. 1040 4:30-33), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

“For example ducts can be formed with two plain transparent walls to form the sample chambers of spectrophotometers.” Ex. 1040 at 2:64-66.

Burns II teaches that “[c]apillary gel electrophoresis of DNA samples was performed using a Beckman P/ACE instrument with a laser-induced fluorescence detector and 37 cm length, 100 μ m diameter, linear polymerase gel capillary according to manufacturer’s recommendation.” Ex. 1008 at 5558. The results of capillary electrophoresis were generally detected optically at the time of filing. Ex. 1011 (Dolnik) at 46; Ex. 1004 ¶¶68-69.

In the combined method of Shaw Stewart, Kopp and Burns II, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶102, 108.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1072.

Thus, claim 5 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Burns II.

Dependent Claims 6-8

Claim 6 of the ‘193 patent recites “wherein the oil is fluorinated oil.” Claim 7 of the ‘193 patent recites “wherein the carrier fluid further comprises a surfactant.” Claim 8 of the ‘193 patent recites “wherein the surfactant is fluorinated surfactant.”

Shaw Stewart discloses that “[s]uitable carrier phases include **mineral oils**, water, light silicones, or **Freons**.” Ex. 1040 at 1:39-41, [emphasis added]; (Freons are comprised of chorofluorocarbons. Ex. 1108). “**Surface acting agents** may also be included in the carrier and/or reagents phases to produce suitable surface properties, for example to allow efficient merging.” Ex. 1040 at 1:44-47, [emphasis added].

The phrase “carrier fluid” in claim 7, having no antecedent basis, is construed to mean “a fluid that is immiscible with a plug fluid,” as this is the way the term is defined in the specification. See Ex. 1001 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1072.

To the extent the Office determines that any element of claims 6-8 is not disclosed by Shaw Stewart, it would have been obvious to use the recited fluorinated surfactants and oils in light of Quake’s disclosure that fluorinated oils serve as surfactants which “aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel.” Ex. 1034 at 35.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claims 6-8 are shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Burns II and further in view of Quake.

Dependent Claim 10

Claim 10 of the ‘193 patent recites “trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels.”

Quake describes a “detection region” which “should generally be at least about twice, preferably at least about five times as large per side or in diameter as the diameter of the largest molecule, cell or droplet that will be passing through it.” Ex. 1034 at 26. This may be contrasted to Quake’s generally recommended dimensions of the fluid channels, which are shaped to cause the droplets to “conform to the size and shape of the channels” by “becom[ing] longer and thinner” as channels narrow. *Id.* at 27. Quake teaches that “an optical trap, or laser tweezers, may be used to sort or direct cells.... The optical trap remains in an ‘off’ mode until a desired particle is detected in the detection region. After detection of a desired characteristic, the particle or cell

is ‘trapped’, and thereby directed or moved into the predetermined branch channel for collection.” *Id.* at 67, *see also* FIGs. 14A & B (Input). Thus, Quake’s detection region is an “expansion portion” where the at least one plug would be “trapp[ed]... for a period of time... after the reaction” as recited in dependent claim 10.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1072.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claim 10 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp, Burns II and Quake.

Dependent Claim 9

Claim 9 of the ‘193 patent recites “wherein the at least one plug is a merged plug.”

Shaw Stewart describe merging droplets of reactants with droplets of reagents. Ex. 1040 at 1:49-53 (“The liquid components will be contained in a system of tubes and conduits allowing the droplets of reactant to be moved by the carrier phase so as to be merged with the droplets containing other reagents”), *see also* 1:114-121.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1072.

Thus, claim 9 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Burns II.

Dependent Claim 11

Claim 11 of the ‘193 patent recites “wherein the at least one plug is substantially spherical in shape.”

Shaw Stewart's droplets are described as being "sufficiently small to form substantially **spherical droplets** with diameters less than the diameters of the conduits." Ex. 1040 at 3:102-104, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1072.

Thus, claim 11 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Burns II.

Dependent Claim 12

Claim 12 of the '193 patent recites "a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction."

Shaw Stewart describes mixing by "introduc[ing] two reagents, which are separated into two smaller volumes at the T junctions then these smaller volumes are merged at the 'Y' junction by withdrawing oil from the center conduit." Ex. 1040 at Abstract. The merging causing mixing to occur. Further, Shaw Stewart states "[a]fter coalescence the constituents of two droplets can be mixed by . . . passing it through a constriction once or several times." Ex. 1040 at 2:53-56.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1072.

Thus, claim 12 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Burns II.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '193 patent recites "wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters." Claim 14 of the '193 patent recites "wherein the relevant parameters are

selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns.”

Kopp teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Kopp meets both claims 13 and 14 under the broadest reasonable interpretation of the term “special design,” which in an of itself imparts no meaningful structural limitation. Ex. 1027 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1072.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Burns II.

L. The Combination of Shaw Stewart, Kopp and Vogelstein Renders Obvious Claims 1-14 of the ‘193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Shaw Stewart), ii) microfluidic PCR devices were well known (Kopp and Vogelstein), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

The Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. The Shaw Stewart British Application discloses a “droplet reactor” and illustrates at Figure 1 a carrier phase 1 flowing along a conduit into which reagent 2 is injected, thereby forming droplet 4. Ex. 1040 at 1:1-20, 70-90. “[I]f large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off. The exact size of the droplets produced will depend on the relative and absolute magnitude of the two currents.” *Id.* at 1:83-9

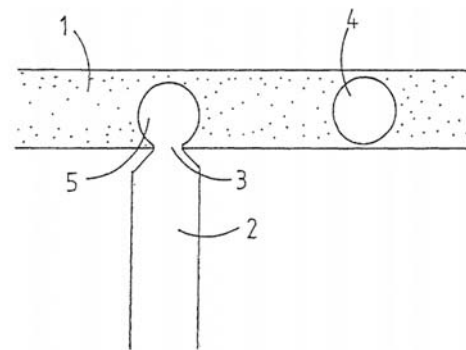


Figure 1.

Significantly, the exclusive licensee of the ‘193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the ‘193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner (“PO”) mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO’s arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO’s primary arguments was that Stewart I

only disclosed introducing fluid in a “stepwise fashion,” whereas in the ’503 Patent droplets are formed by “combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.” *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

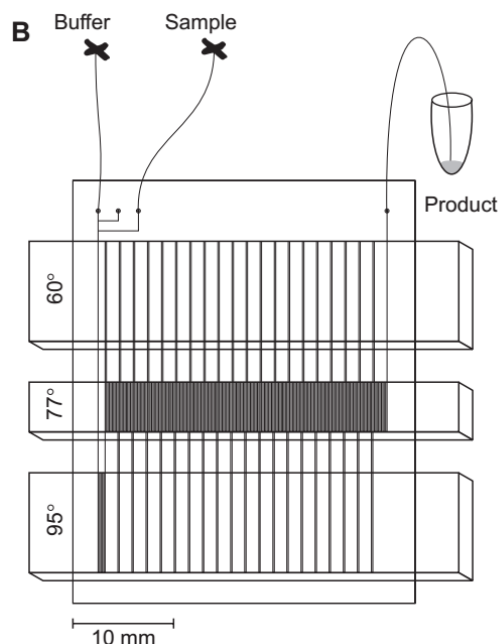
As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Vogelstein. By the late 1990s Kopp et al. had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product

quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 [emphasis added].

Kopp et al. also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been

presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 emphasis added.

By 1999, Vogelstein had reported **various applications for single-molecule microfluidic PCR**. Ex. 1044 at 9241. In a seminal article which has been cited over by over 1,000 other publications, Vogelstein explained that PCR amplification of small aliquots or droplets containing single molecules was effective for “detection of a small number of mutant-containing cells among a large excess of normal cells.” *Id.* at 9236. In Vogelstein's method, the DNA was diluted into 7 μ l volumes such that, on average, there was one template molecule for every two wells (i.e., approximately half of the wells contained no DNA template molecule and approximately half contained a single DNA template molecule). *Id.* at 9236, 9237. This avoided the problem of background noise impairing the determination of whether each volume contained the mutant sequence. *Id.* By counting the number of volumes which indicated wild type or mutant templates, the presence or absence of the mutation (e.g., a sequence associated with colorectal cancer) can be determined. *Id.* at 9238. The accuracy of the test is a function of the number of discrete volumes into which the sample is diluted. *Id.* at 9239. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the

sensitivity by another order of magnitude.”) The article explained that the same technique would be useful for **detecting a wide array of mutations, dislocations and allelic imbalances**, as summarized in Table 1. *Id.* at 9241. Vogelstein thus taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby overcoming the signal-to-noise limitations associated with certain other PCR techniques and enabling the detection of relatively rare mutations, dislocations and allelic imbalances. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86.

In sum, Kopp and Vogelstein show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Shaw Stewart is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).¹³⁴ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart, to conduct microfluidic PCR as taught by Kopp and Vogelstein. A

¹³⁴ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactor of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, emphasis added. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Shaw Stewart to

perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett's PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample

leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Vogelstein) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and

sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In

1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Shaw Stewart could be successfully modified to perform PCR reactions.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp or Lagally to work in the microfluidic droplet reactor of Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year

prior to the '193 patent's earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹³⁵ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail below, in the accompanying claim chart (Ex. 1073), and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Kopp and Vogelstein.

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Shaw Stewart discloses that “[t]he method of the invention may be used for initiating and controlling a chemical reaction or preparing mixtures of reagents....” Ex. 1040 at 1:7-9..

¹³⁵ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) (‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

“The system is particularly suited **to the manipulation of microscopic quantities of reagents, with volumes of less than one microliter,...**” *Id.* at 1:20-22.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1073.

Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

As shown in Shaw Stewart’s Figure 1, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Ex. 1040 at 1:70-5 .

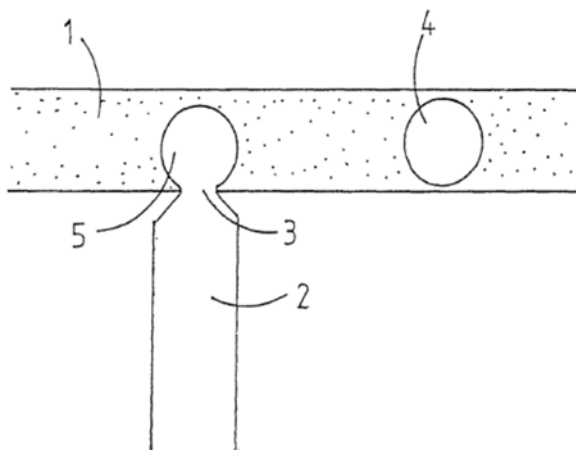


Figure 1.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1073.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

As shown in Shaw Stewart’s Figure 1, “[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3).

When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube.” Ex. 1040 at 1:70-5 . Shaw Stewart teaches that “[f]or **aqueous reagents**, glass tubing and a light silicon carrier phase would be particularly suitable, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface.” Ex. 1040 at 1:62-66, [emphasis added]. “[I]f large numbers of droplets are required, a **continuous flow of reagent** through the opening will be produced, while a **continuous current of carrier phase flows** down the tube.” Ex. 1040 at 1:83-86, [emphasis added].

In the instant combination, the microfluidic droplet reactors of Shaw Stewart are relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).¹³⁶ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Kopp and Vogelstein. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactors of Shaw Stewart because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at

¹³⁶ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Shaw Stewart to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each

other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise**

limitations associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Vogelstein) to incorporate a droplet reactor (Shaw Stewart). Ex. 1004 ¶¶88-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Vogelstein. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn further **reduces the volumes of reagent** compared to droplets produced using injection of an aqueous fluid into an

immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk

heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with

microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching**

whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactors Shaw Stewart could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in modifying Kopp or Vogelstein to work in the microfluidic droplet reactor of Shaw Stewart, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹³⁷ As discussed in Section

¹³⁷ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at

VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained further in the accompanying claim chart (Ex. 1073) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Shaw Stewart to conduct microfluidic PCR as taught by Kopp and Vogelstein.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1073.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

As shown in Shaw Stewart's Figure 1, "[t]he reagent is introduced into a tube containing carrier phase (1) from a side arm (2) by being sucked or pushed through a small opening (3). When the correct volume has passed into the tube, it is separated and carried away by a current of carrier phase, down the tube." Ex. 1040 at 1:70-5. Shaw Stewart teaches that "[f]or **aqueous reagents, glass tubing and a light silicon carrier phase would be particularly suitable**, as this combination would prevent wetting of the glass, due to a strong interaction at the glass-silicon interface." Ex. 1040 at 1:62-66, [emphasis added]. "[I]f large numbers of droplets are required, **a continuous flow of reagent** through the opening will be produced, while a **continuous current of carrier phase flows** down the tube." Ex. 1040 at 1:83-86, [emphasis

884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1073.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

As discussed above, the Shaw Stewart British Application teaches conducting reactions in microdroplets formed from two continuously flowing immiscible fluids. Significantly, the exclusive licensee of the '193 patent affirmatively (and correctly) took the position that Shaw Stewart teaches plug or droplet formation combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid. More particularly, Bio-Rad (who recently acquired RainDance, the original exclusive licensee of the '193 patent, Ex. 1061, Ex. 1053 ¶43), explained that another party had mischaracterized the Shaw Stewart reference by failing to point out the above-quoted portion of Shaw Stewart, in which Shaw Stewart specifically teaches that in certain embodiments droplets are formed from intersecting and continuously flowing streams.

To overcome Stewart I and secure allowance of their patent, the Patent Owner ("PO") mischaracterized the device disclosed in Stewart I (and hence Stewart II), misdirecting the Examiner to irrelevant portions of Stewart I while ignoring other portions that directly contradicted PO's arguments. *See, e.g.*, Ex. 1003 at 174-180. For example, one of PO's primary arguments was that Stewart I

only disclosed introducing fluid in a “stepwise fashion,” whereas in the ’503 Patent droplets are formed by “combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid.” *Id.* at 174. In truth, however, Stewart I also discloses combining a flowing stream of an aqueous solution and a flowing stream of an immiscible fluid:

If large numbers of droplets are required, a continuous flow of reagent through the opening will be produced, while a continuous current of carrier phase flows down the tube. When each droplet almost spans the tube, it will be broken off.

Ex. 1008, pg. 1, col. 2, ll. 83-88.

Ex. 1059 at 2-3.

Thus, the exclusive licensee (Bio-Rad) expressly agreed that Shaw Stewart discloses forming plugs from intersecting streams of continuously flowing immiscible fluids.

With regard to the recitation that the plug is “substantially surrounded by the oil flowing through the channel,” this is shown in Shaw Stewart’s Figure 1. Shaw Stewart’s specification also explains that the droplets are smaller than the conduits. (“A method as claimed in claim (1) in which the discrete volumes of chemical reagents are sufficiently small to form substantially spherical droplets with diameters less than the diameters of the conduits.”) . Ex. 1040 at 3:101-104

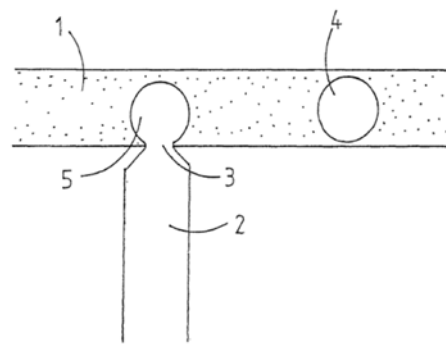


Figure 1.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1073.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Shaw Stewart discloses that “[r]egions of the device can be heated by electric circuits or coils or by electromagnetic radiation to allow the merged droplets to be incubated at the required temperature.” Ex. 1040 at 2:44-47. “If a colour change reaction is involved, such a colour change can be recorded immediately or after incubation, using thermostatically controlled heating coil (27).” *Id.* at 3:58-60.

As discussed above in connection with element 1[b], in the instant combination Shaw Stewart is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule and the autocatalytic reaction. Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. These limitations are met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Shaw Stewart) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71. The DNA is amplified through the PCR reaction. *Id.*

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1073.

Thus, claim 1 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Vogelstein.

Dependent Claim 2

Claim 2 of the ‘193 patent recites “wherein the at least one substrate molecule is a single biological molecule.”

In the combined method of Shaw Stewart, Kopp and Vogelstein, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the

biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90.

Shaw Stewart discloses that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.”¹³⁸ Ex. 1040 at 3:82-86.

Vogelstein explains “PCR-amplification from single cells isolated by physical separation or dilution has been used to address a variety of interesting biologic questions.” Ex. 1044 p. 9239. Thus, a skilled artisan would be motivated to isolate a single molecule for PCR reaction. Ex. 1004 ¶¶81-87.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1073.

Thus, claim 2 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Vogelstein.

Dependent Claims 3 and 4

Claim 3 of the ‘193 patent recites “wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.” Claim 4 of the ‘193 patent recites “wherein the providing step includes heating.” PCR reactions involve thermocycling. Ex. 1004 ¶¶33-34.

Shaw Stewart discloses that “[t]his invention may have applications in many branches of medicine, chemistry, biochemistry, geology, etc., especially in procedures which utilize very small quantities, such as forensic and recombinant DNA work.”¹³⁹ Ex. 1040 at 3:82-86. In the

¹³⁸ Requester notes that Shaw does disclose use of a biological molecule (Ex. 1040 4:30-33), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

¹³⁹ Requester notes that Shaw does disclose use of a biological molecule (Ex. 1040 4:30-33), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

combined method of Shaw Stewart, Kopp and Vogelstein, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. To affect the PCR reaction, the carrier fluid is heated. (“The individual steps are simply performed by heating or cooling the sample to characteristic temperatures: 95°C for dsDNA melting, 50° to 65°C for primer annealing, and 72° to 77°C for primer extension at the optimum enzyme temperature.”) Ex. 1027 at 1046 (Kopp), (“If a PCR product whose sequence is complementary to the loop sequence is present during the heating/cooling cycle, hybridization of the MB to one strand of the PCR product will increase the distance between the Dabcyl and the dye, resulting in increased fluorescence.”) Ex. 1044 at 9237(Vogelstein)..

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1073.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Vogelstein.

Dependent Claim 5

Claim 5 of the ‘193 patent further recites “providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.”

Shaw Stewart discloses that “parts of the device itself can be adapted to form the sample chambers of the standard instruments of chemical or biochemical analysis.” Ex. 1040 at 2:62-64. “For example ducts can be formed with two plain transparent walls to form the sample chambers of spectrophotometers.” Ex. 1040 at 2:64-66.

Vogelstein teaches that the “[r]eactions were analyzed immediately or stored at room temperature for up to 36 h before **fluorescence analysis**.” Ex. 1044 at 9236. , [emphasis added].

In the combined method of Shaw Stewart, Kopp and Vogelstein, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶102, 108.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1073.

Thus, claim 5 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Vogelstein.

Dependent Claims 6-8

Claim 6 of the ‘193 patent recites “wherein the oil is fluorinated oil.” Claim 7 of the ‘193 patent recites “wherein the carrier fluid further comprises a surfactant.” Claim 8 of the ‘193 patent recites “wherein the surfactant is fluorinated surfactant.”

Shaw Stewart discloses that “[s]uitable carrier phases include **mineral oils**, water, light silicones, or **Freons**.” Ex. 1040 at 1:39-41, [emphasis added]; (Freons are comprised of chlorofluorocarbons. Ex. 1108). “**Surface acting agents** may also be included in the carrier and/or reagents phases to produce suitable surface properties, for example to allow efficient merging.” Ex. 1040 at 1:44-47, [emphasis added].

The phrase “carrier fluid” in claim 7, having no antecedent basis, is construed to mean “a fluid that is immiscible with a plug fluid,” as this is the way the term is defined in the specification. See Ex. 1001 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1073.

To the extent the Office determines that any element of claims 6-8 is not disclosed by Shaw Stewart, it would have been obvious to use the recited fluorinated surfactants and oils in light of Quake's disclosure that fluorinated oils serve as surfactants which "aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel." Ex. 1034 at 35.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claims 6-8 are shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Vogelstein and further in view of Quake.

Dependent Claim 9

Claim 9 of the '193 patent recites "wherein the at least one plug is a merged plug."

Shaw Stewart describe merging droplets of reactants with droplets of reagents. Ex. 1040 at 1:49-53 ("The liquid components will be contained in a system of tubes and conduits allowing the droplets of reactant to be moved by the carrier phase so as to be merged with the droplets containing other reagents"), *see also* 1:114-121.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1073.

Thus, claim 9 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Vogelstein.

Dependent Claim 10

Claim 10 of the '193 patent recites "trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels."

Quake describes a "detection region" which "should generally be at least about twice, preferably at least about five times as large per side or in diameter as the diameter of the largest molecule, cell or droplet that will be passing through it." Ex. 1034 at 26. This may be contrasted

to Quake's generally recommended dimensions of the fluid channels, which are shaped to cause the droplets to "conform to the size and shape of the channels" by "becom[ing] longer and thinner" as channels narrow. *Id.* at 27. Quake teaches that "an optical trap, or laser tweezers, may be used to sort or direct cells.... The optical trap remains in an 'off' mode until a desired particle is detected in the detection region. After detection of a desired characteristic, the particle or cell is 'trapped', and thereby directed or moved into the predetermined branch channel for collection." *Id.* at 67, *see also* FIGs. 14A & B (Input). Thus, Quake's detection region is an "expansion portion" where the at least one plug would be "trapp[ed]... for a period of time... after the reaction" as recited in dependent claim 10.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1073.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claim 10 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Vogelstein, and Quake.

Dependent Claim 11

Claim 11 of the '193 patent recites "wherein the at least one plug is substantially spherical in shape."

Shaw Stewart's droplets are described as being "sufficiently small to form substantially **spherical droplets** with diameters less than the diameters of the conduits." Ex. 1040 at 3:102-104, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1073.

Thus, claim 11 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Vogelstein.

Dependent Claim 12

Claim 12 of the '193 patent recites “a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction.”

Shaw Stewart describes mixing by “introduc[ing] two reagents, which are separated into two smaller volumes at the T junctions then these smaller volumes are merged at the 'Y' junction by withdrawing oil from the center conduit.” Ex. 1040 at Abstract. The merging causing mixing to occur. Further, Shaw Stewart states “[a]fter coalescence the constituents of two droplets can be mixed by . . . passing it through a constriction once or several times.” Ex. 1040 at 2:53-56.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1073.

Thus, claim 12 is shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Vogelstein.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '193 patent recites “wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters.” Claim 14 of the '193 patent recites “wherein the relevant parameters are selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns.”

Kopp teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Kopp meets both claims 13 and 14 under the broadest reasonable interpretation of the term “special design,” which in and of itself imparts no meaningful structural limitation. Ex. 1027 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1073.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Shaw Stewart, Kopp and Vogelstein.

M. The Combination of Burns I, Corbett and Lagally Renders Obvious Claims 1-14 of the '193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Burns I), ii) microfluidic PCR devices were well known (Corbett and Lagally), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10. Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.”

Ex. 1007 at 10-11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug

generation. *Id.* at 11. In Burns' Fig. 4 embodiment,

an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel. See Ex. 1010; 1027; Ex. 1025 at 23:2-8.

When read in combination with Higuchi I-III, Quake and Shaw Stewart, Burns I demonstrates

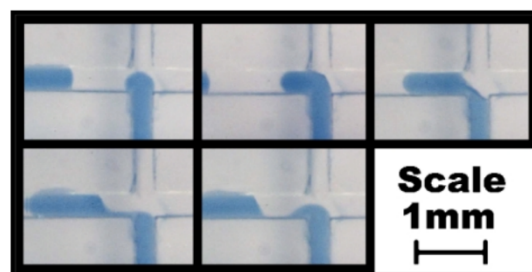
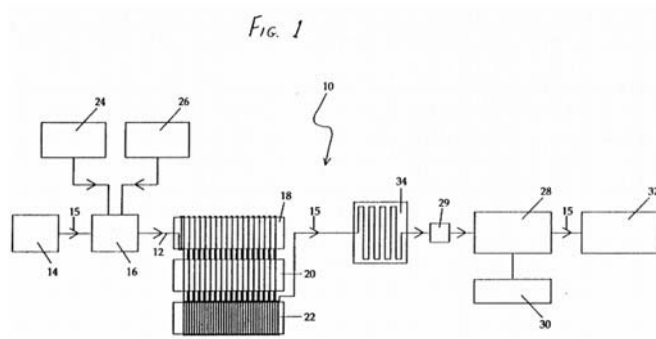
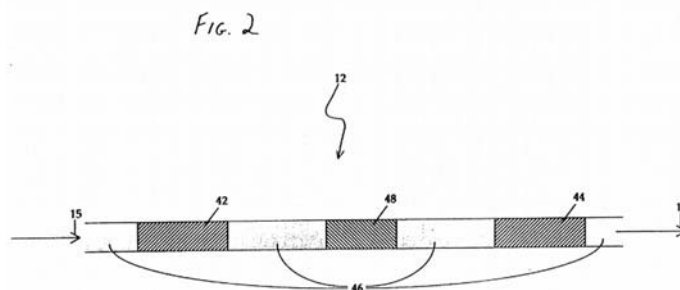


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

that making reactant slugs or plugs with continuous flows of immiscible fluids was a mere design choice as of 2001.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Lagally. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.





“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77. In 2001, in another article not previously considered by the Office, Lagally reported using a microfluidic reactor to conduct single-molecule DNA amplification by PCR. Lagally summarized the state of the art as of February 2001, explaining continuous flow PCR microreactors had recently undergone substantial evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize

noncontact methods¹⁶ or **continuous flow** through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

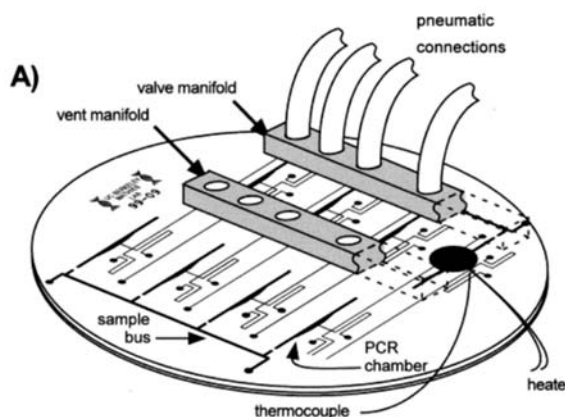
Ex. 1028 at 565-556.

Lagally *et al.* developed a further improvement – a device and method for achieving single molecule amplification in a microchannel environment.

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson

distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

Microfluidic PCR-CE device. The PCR chambers are connected to a common sample bus through a set of valves. Hydrophobic vents at the other end of the PCR chambers are used to locate the sample and to eliminate gas. The PCR chambers are directly connected to the cross channel of the CE system for product injection and analysis. Two aluminum manifolds, one each for the vents and valves, are placed onto the respective ports and clamped in place using vacuum. The manifolds connect to external solenoid valves for pressure and vacuum actuation. Thermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.



Ex. 1028 at 565-66 (see figure legend).

In sum, Corbett and Lagally show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactors of Burns I are relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an

autocatalytic reaction through a first channel of the at least two channels”).¹⁴⁰ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Lagally. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic reactor of Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein’s contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactors of Burns I because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. *Id.* ¶¶92-96. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic

¹⁴⁰ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler

is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Lagally) to incorporate a droplet reactor (Burns I). Ex. 1004 ¶¶92-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Lagally. The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the

single fluid in microfluidic chip reactor (e.g., Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use

resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1

template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at

1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Lagally to work in the microfluidic reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi

references are offered as evidence of the level of skill in the art.¹⁴¹ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail below, in the accompanying claim chart (Ex. 1074) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Lagally. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic reactor of Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Burns I discloses “[a] multiphase microreactor based upon the use of slug flow through a narrow channel....” Ex. 1007 at 10. “The experimental work discussed here is based on the use

¹⁴¹ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) (‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

of alternating slugs of two liquid phases to provide the environment for mass transfer and reaction within the microreactor.” *Id.* at 10.

In the instant combination, microfluidic droplet reactors (as taught in Burns I) are used to perform PCR reactions (as taught in Corbett and Lagally), wherein each plug includes a mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-38. For example, as noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565. PCR reactions are autocatalytic. Ex. 1004 ¶32; Ex. 1001 44:58-61.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1074.

Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

Burns I explains that “[t]he method discussed in this work **uses the continuous flow of both phases through T or cross-shaped intersections.** Slugs are generated by the action of **one phase flowing into the channel whilst the other phase moves into the intersection,** eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1074.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

As noted above, Burns I teaches that “[t]he method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Figure 1 shows that the reactant slug is composed of an “**aqueous phase**.” *Id.* at 11, [emphasis added].

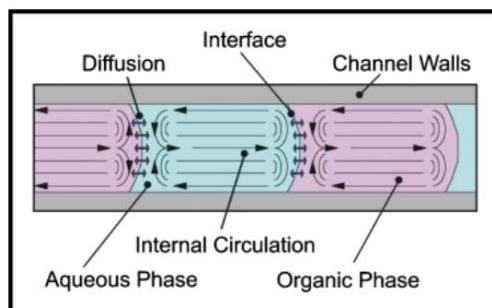


Fig. 1 Illustration of internal circulation generated within immiscible slug flow.

Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase.

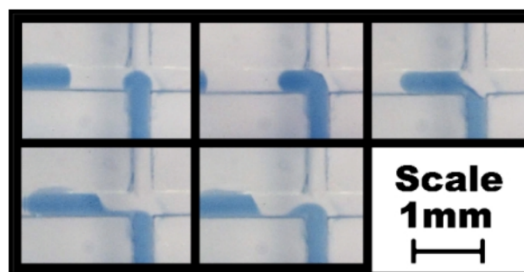


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

In the instant combination, the microfluidic droplet reactors of Burns I are relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).¹⁴² Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug

¹⁴² Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-39.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Lagally. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modifying it to incorporate the microfluidic droplet reactor of Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Burns I because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. *Id.* ¶¶92-96. As noted by Lagally, "[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**" Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that "[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**" Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve

product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Lagally) to incorporate a droplet reactor (Burns I). Ex. 1004 ¶¶92-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Lagally. The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip.

Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful.**

In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling

profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced

microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic**

droplet (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactors Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Lagally to work in the microfluidic droplet reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹⁴³ As discussed in

¹⁴³ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary

Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained further in the accompanying claim chart (Ex. 1074) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Lagally. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modifying it to incorporate the microfluidic droplet reactor of Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1074.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

As noted above, Burns I teaches that "[t]he method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the

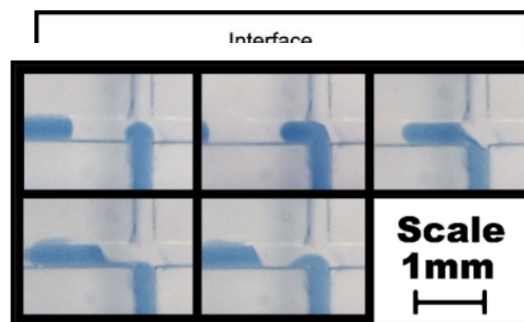


Fig. 3 Typical slug generation in glass device with 380 μm wide flow channels.

knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Figure 1 shows that the reactant slug is composed of an “organic phase.” *Id.* at 11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1074.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of oil at an intersecting channel. Burns I explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10. Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.”

Ex. 1007 at 10-11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug

generation. *Id.* at 11. In Burns’ Fig. 4 embodiment,

an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel See Ex. 1010; 1027; Ex. 1025 at 23:2-8. When

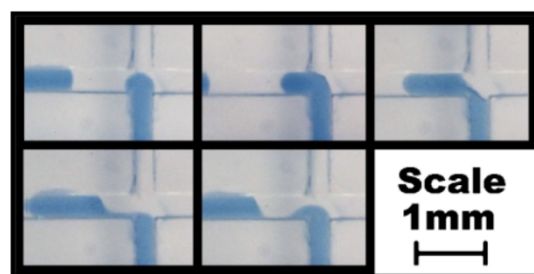


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

read in combination with Higuchi I-III, Quake and Shaw Stewart, Burns I demonstrates that making reactant slugs or plugs with continuous flows of oil was a mere design choice as of 2001.

In the present combination, the slugs or plugs contain a substrate molecule and reagents for performing a PCR reaction. Ex. 1028 at 565, (Lagally) (“The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.”).

With regard to the recitation that “the plug [is] substantially surrounded by the immiscible carrier fluid,” Requester submits that this would necessarily occur for any slug or droplet having a volume small enough to not bridge the entire channel width. Indeed, such scenarios are illustrated in Quake (Ex. 1033 Fig. 18) and Shaw Stewart (Ex. 1040 Fig. 1). Accordingly, it would have been obvious to utilize the Burns I droplet generator in a manner that meets the “substantially surrounded” limitation.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1074.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Burns I discloses that “[t]he high heat and mass transfer capability offered by capillary microreactors allows high intensity reactions to be precisely controlled for optimum yield and selectivity.” Ex. 1007 at 10. “The internal circulation, which is stimulated within the slugs by their passage along the channel, is responsible for a **large enhancement** in the interfacial mass transfer and the **reaction rate**.” Ex. 1007 at 10, [emphasis added].

As discussed above in connection with element 1[b], in the instant combination Burns I is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the

limitations reciting that the flowing fluid with the reagents also includes a substrate molecule and the autocatalytic reaction. Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. These limitations are met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. The DNA is amplified through the PCR reaction. Ex. 1004 ¶¶33-34.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1074.

Thus, claim 1 is shown to be rendered obvious by the combination of Burns I, Corbett and Lagally.

Dependent Claim 2

Claim 2 of the '193 patent recites "wherein the at least one substrate molecule is a single biological molecule."

In the combined method of Burns I, Corbett and Lagally, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90.

As discussed by Lagally, "[s]tochastic PCR amplification of **single DNA template molecules** followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device." Ex. 1028 at 565, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1074.

Thus, claim 2 is shown to be rendered obvious by the combination of Burns I, Corbett and Lagally.

Dependent Claims 3 and 4

Claim 3 of the '193 patent recites "wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction." Claim 4 of the '193 patent recites "wherein the providing step includes heating." PCR reactions involve thermocycling. Ex. 1004 ¶¶33-34.

In the combined method of Burns I, Corbett and Lagally, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. To affect the PCR reaction, the carrier fluid is heated. ("The carrier fluid then passes through a plurality of temperature zones in which the polymerase chain reactions take place") Ex. 1010 (Corbett),, ("The PCR chambers were thermally cycled.") Ex. 1028 (Lagally) at 567. Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1074.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Burns I, Corbett and Lagally.

Dependent Claim 5

Claim 5 of the '193 patent further recites "providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred."

Corbett similarly teaches that "[d]ownstream of temperature zone 34 is in-line analysis means 29 and recovery means 28. Connected to recovery means 28 is **detection means 30**. Detection means 30 determines when a reaction mixture in the carrier fluid reaches recovery

means 28. This may be done by measuring the conductivity or **optical density** of the stream of carrier fluid.” Ex. 1010 at 8:12-18, [emphasis added].

Lagally teaches “[s]tochastic PCR amplification of single DNA template molecules followed by **capillary electrophoresis** (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Ex. 1028 at 565, [emphasis added]. The results of capillary electrophoresis were generally detected optically at the time of filing. Ex. 1011 (Dolnik) at 46; Ex. 1004 ¶¶68-69.

In the combined method of Burns I, Corbett and Lagally, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶102, 108.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1074.

Thus, claim 5 is shown to be rendered obvious by the combination of Burns I, Corbett and Lagally.

Dependent Claims 6-8

Claim 6 of the ‘193 patent recites “wherein the oil is fluorinated oil.” Claim 7 of the ‘193 patent recites “wherein the carrier fluid further comprises a surfactant.” Claim 8 of the ‘193 patent recites “wherein the surfactant is fluorinated surfactant.”

Quake discloses that “[t]he fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). **Exemplary surfactants** include Tween, Span, **fluorinated oils**, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel.” Ex. 1034 at 35, [emphasis added].

The phrase “carrier fluid” in claim 7, having no antecedent basis, is construed to mean “a fluid that is immiscible with a plug fluid,” as this is the way the term is defined in the specification. See Ex. 1001 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1074.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claims 6-8 are shown to be rendered obvious by the combination of Burns I, Corbett, Lagally, and Quake.

Dependent Claim 9

Claim 9 of the ‘193 patent recites “wherein the at least one plug is a merged plug.”

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and finally mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1074.

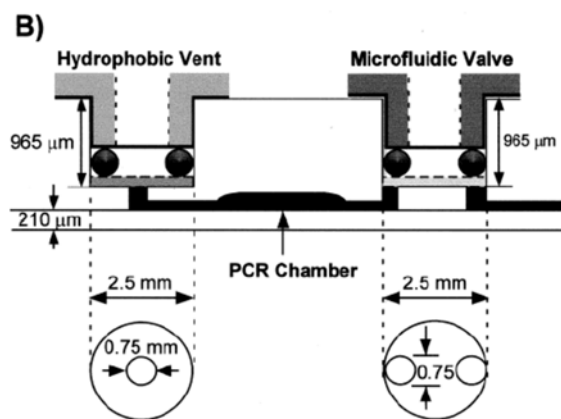
A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claim 9 is shown to be rendered obvious by the combination of Burns I, Corbett, Lagally and Quake.

Dependent Claim 10

Claim 10 of the '193 patent recites “trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels.”

Lagally designed “PCR chambers etched into a glass substrate, connected to microfluidic valves and hydrophobic vents for sample introduction and **immobilization** during thermal cycling.” Ex. 1028 at 566. Lagally’s system vacuum traps the sample during a heating period of time. *Id.* at 567. As illustrated in Figure 1, reproduced to the right, Lagally’s PCR chamber, used to trap the sample, is expanded in relation to the sample bus connected thereto. Ex. 1028 at 566; Ex. 1004

¶49.



Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1074.

Thus, claim 10 is shown to be rendered obvious by the combination of Burns I, Corbett, and Lagally.

Dependent Claim 11

Claim 11 of the '193 patent recites “wherein the at least one plug is substantially spherical in shape.”

As discussed by Quake, “the microfabricated devices of this invention generate round, monodisperse droplets (such as those illustrated in Frames J and L of FIG. 19” (reproduced below). Ex. 1034 at 27, FIG. 19.

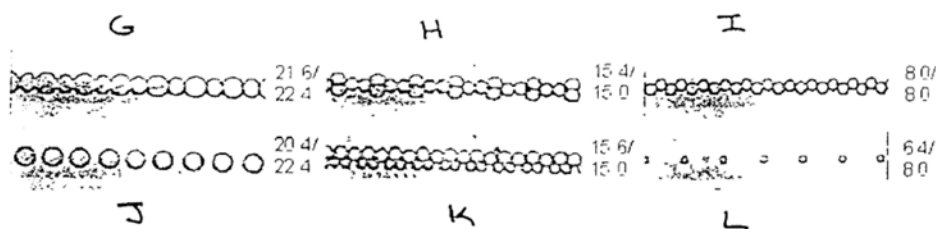


FIG. 19

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1074.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claim 11 is shown to be rendered obvious by the combination of Burns I, Corbett, Lagally, and Quake.

Dependent Claim 12

Claim 12 of the '193 patent recites "a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction."

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1074.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claim 12 is shown to be rendered obvious by the combination of Burns I, Corbett, Lagally, and Quake.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '193 patent recites "wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters." Claim 14 of the '193 patent recites "wherein the relevant parameters are selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns."

Corbett teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Corbett meets both claims 13 and 14 under the broadest reasonable interpretation of the term "special design," which in and of itself imparts no meaningful structural limitation. Ex. 1010 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1074.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Burns I, Corbett and Lagally.

N. The Combination of Burns I, Corbett and Burns II Renders Obvious Claims 1-14 of the '193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

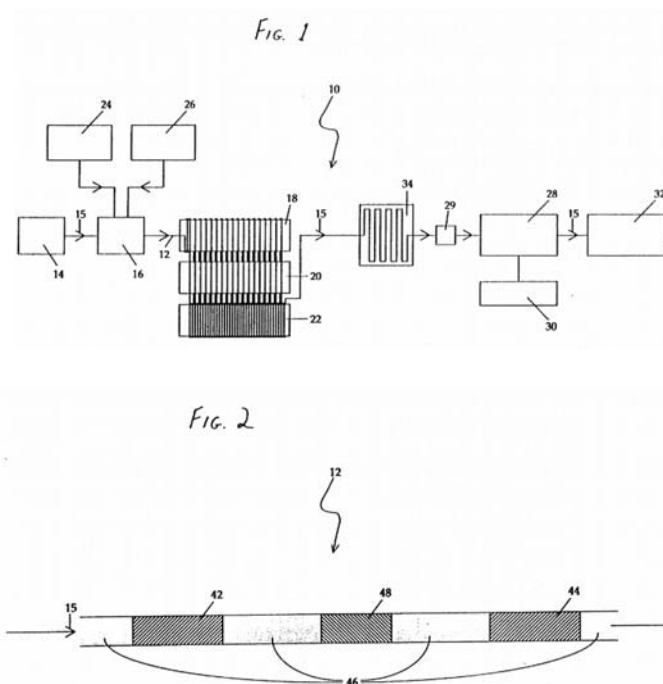
The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Burns I), ii) microfluidic PCR devices were well known (Corbett and Burns II), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Lagally, Kopp, Burns II and Vogelstein).

Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that "various methods may be used to generate slugs of liquid within a microreactor." Ex. 1007 at 10. Burns I chose a method in which

“the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel. See Ex. 1010; 1027; Ex. 1025 at 23:2-8. Burns I demonstrates that making reactant slugs or plugs with continuous flows of immiscible fluids was a mere design choice as of 2001.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Burns II. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream

includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.



“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

In 1996, Burns II reported the use of microfluidic devices to perform PCR. Ex. 1008 at 5556. Burns II explained that “[n]o new biochemistries or DNA detection methods are required for their use; the components simply reproduce current laboratory procedures in a micron-sized environment.” *Id.* at 5560. In other words, the PCR chemistry did not need to be modified – it worked without modification on the microfluidic scale. In the method of Burns II, a sample

containing a DNA plasmid was loaded into one of the reaction wells (A) and a mixture of Taq enzyme and buffer was loaded into the other. Ex. 1008 at 5558. Each formed droplets of about 60 nl in the parallel 500 x 25 μm channels (B) which came together at the Y junction to form a single larger droplet (about 120 nl). *Id.* at 5558-59; Ex. 1004 ¶40.

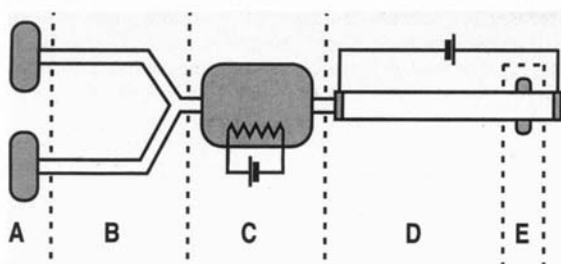


FIG. 1. Example of an integrated DNA analysis system, represented schematically. The individual components of the system are injection entry ports (A), liquid pumping channels (B), thermally controlled reaction chamber (C), electrophoresis channel (D), and DNA band migration detector (E). Each component would have associated sensors, control circuitry, and external connections.

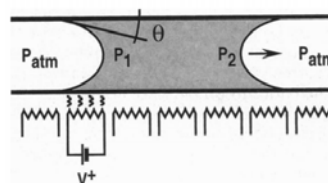


FIG. 2. Schematic drawing showing the principle of thermally induced drop motion in a closed channel. The case of a single aqueous drop in a hydrophilic channel is presented, where V is an applied voltage, P_{atm} is atmospheric pressure, P_1 is the receding-edge internal pressure, P_2 is the advancing-edge internal pressure, and θ is the contact angle of the liquid-gas-solid interface. The contact angle will depend on the surface characteristics of the channel and the constituents of the drop, with a hydrophilic interaction giving θ between 0 and 90 degrees, and a hydrophobic surface giving θ between 90 and 180 degrees. Surface treatments can also reduce contact angle hysteresis and, therefore, reduce the temperature difference necessary for drop motion.

The droplet then passed through the resistive heater portion (C) in which PCR amplification occurred. *Id.* at 5556-5557. Afterwards the amplification results were optically detected in zone D via gel electrophoresis. *Id.* at 5557-5561. Burns II noted that “[t]he use of common fabrication methods allows the assembly of increasingly complex, multicomponent, integrated systems from a small, defined set of standardized elements” and that “[f]uture design efforts have a strong impetus to reduce component size and improve efficiency.” *Id.* at 5560-61.

In sum, Corbett and Burns II show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Burns I is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an

autocatalytic reaction through a first channel of the at least two channels”).¹⁴⁴ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Burns II. Ex. 1004 ¶106. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic reactor of Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein’s contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactor of Burns I because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. *Id.* ¶¶92-96. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic

¹⁴⁴ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactor of Burns I would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is

too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed

sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Burns I to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Burns II) to incorporate a droplet reactor (Burns I). Ex. 1004 ¶¶92-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Burns II. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a

given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful.**

In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/μL, in volumes down to 1 μL.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling

profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced

microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic**

droplet (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Burns II to work in the microfluidic reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹⁴⁵ As discussed in Section

¹⁴⁵ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary

VI.A.3, above, Higuchi I-III thus demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail below, in the accompanying claim chart (Ex. 1075) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Burns II. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic reactor of Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Burns I discloses "[a] multiphase microreactor based upon the use of slug flow through a narrow channel...." Ex. 1007 at 10. "The experimental work discussed here is based on the use of alternating slugs of two liquid phases to provide the environment for mass transfer and reaction within the microreactor." *Id.* at 10.

In the instant combination, microfluidic droplet reactors (as taught in Burns I) are used to perform PCR reactions (as taught in Corbett and Burns II), wherein each plug includes a mixture

knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-38. PCR reactions are autocatalytic. Ex. 1004 ¶32; Ex. 1001 44:58-61.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1075.

Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

Burns I explains that “[t]he method discussed in this work **uses the continuous flow of both phases through T or cross-shaped intersections**. Slugs are generated by the action of **one phase flowing into the channel whilst the other phase moves into the intersection**, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1075.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

As noted above, Burns I teaches that “[t]he method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Figure 1 shows that

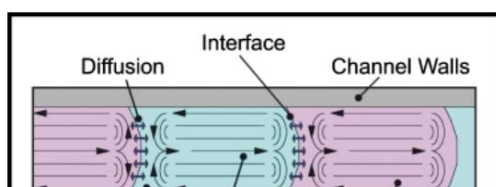
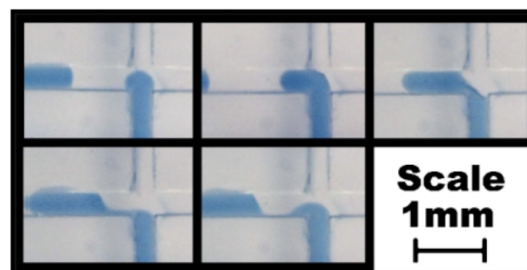


Fig. 1
flow.



slug

Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

the reactant slug is composed of an “**aqueous phase.**” *Id.* at 11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase.

In the instant combination, the microfluidic droplet reactors of Burns I are relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).¹⁴⁶ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-39.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Burns II. Ex. 1004 ¶106. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modifying it to incorporate the microfluidic droplet reactor of Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein’s contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Burns I because doing so have provided the substantial

¹⁴⁶ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

benefits known to be associated with microfluidic droplet reactors. *Id.* ¶¶92-96. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶¶92-100; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett's PCR reaction in the microfluidic droplet reactors of Quake or Shaw Stewart would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026

(Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity Ex. 1044 at 9239 (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Burns II) to incorporate

a droplet reactor (Burns I). Ex. 1004 ¶¶92-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Burns II. *Id.* The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid

chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that

“the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactors Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Burns II to work in the microfluidic droplet reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority

date. Ex. 1018-20 (Higuchi I, II and III). The group's work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the '193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹⁴⁷ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained further in the accompanying claim chart (Ex. 1075) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Burns II. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modifying it to incorporate the microfluidic droplet reactor of Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1075.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

¹⁴⁷ "In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references 'do not qualify as 'prior art' under [§ 102 or § 103(A)],' the references were nonetheless 'relevant to obviousness as a secondary consideration.' See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 ('Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration') (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) ('Development by others may also be pertinent to a determination of the obviousness of an invention'). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

As noted above, Burns I teaches that “[t]he method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Figure 1 shows that the reactant slug is composed of an “**organic phase.**” *Id.* at 11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase.

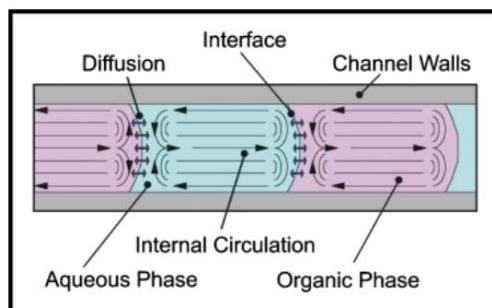


Fig. 1 Illustration of internal circulation generated within immiscible slug flow.

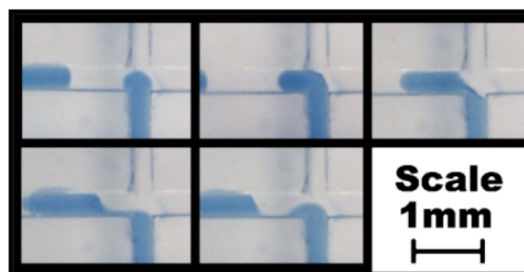


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1075.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10. Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Fig. 3 shows oil-in-

water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug

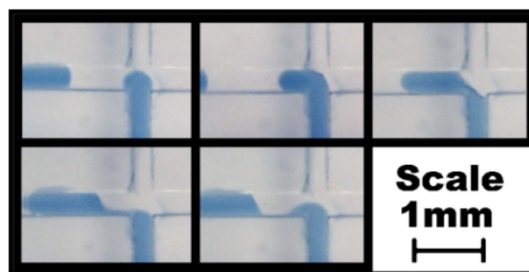


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel See Ex. 1010; 1027; Ex. 1025 at 23:2-8. When read in combination with Higuchi I-III, Quake and Shaw Stewart, Burns I demonstrates that making reactant slugs or plugs with continuous flows of oil was a mere design choice as of 2001.

With regard to the recitation that “the plug [is] substantially surrounded by the immiscible carrier fluid,” Requester submits that this would necessarily occur for any slug or droplet having a volume small enough to not bridge the entire channel width. Indeed, such scenarios are illustrated in Quake (Ex. 1033 Fig. 18) and Shaw Stewart (Ex. 1040 Fig. 1). Accordingly, it would have been obvious to utilize the Burns I droplet generator in a manner that meets the “substantially surrounded” limitation.

In the present combination, the slugs or plugs contain a substrate molecule and reagents for performing a PCR reaction. Ex. 1008 p. 5556 (“In the proposed format, sample and reagent

are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C).”).

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1075.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Burns I discloses that “[t]he high heat and mass transfer capability offered by capillary microreactors allows high intensity reactions to be precisely controlled for optimum yield and selectivity.” Ex. 1007 at 10. “The internal circulation, which is stimulated within the slugs by their passage along the channel, is responsible for a **large enhancement** in the interfacial mass transfer and the **reaction rate**.” Ex. 1007 at 10, [emphasis added].

As discussed above in connection with element 1[b], in the instant combination Burns I is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule and the autocatalytic reaction and the autocatalytic reaction. Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. These limitations are met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-39. The DNA is amplified through the PCR reaction. Ex. 1004 ¶¶33-34.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1075.

Thus, claim 1 is shown to be rendered obvious by the combination of Burns I, Corbett and Burns II.

Dependent Claim 2

Claim 2 of the '193 patent recites "wherein the at least one substrate molecule is a single biological molecule."

In the combined method of Burns I, Corbett and Burns II, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. As evidenced by Lagally, in microfluidic reactors, a single DNA molecule may be involved in a reaction. Ex. 1028 p. 564 ("[s]tochastic PCR amplification of **single DNA template molecules** followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device."); Ex. 1028 at 565.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1075.

Thus, claim 2 is shown to be rendered obvious by the combination of Burns I, Corbett and Burns II.

Dependent Claims 3 and 4

Claim 3 of the '193 patent recites "wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction." Claim 4 of the '193 patent recites "wherein the providing step includes heating." PCR reactions involve thermocycling. Ex. 1004 ¶¶33-34.

In the combined method of Burns I, Corbett and Burns II, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow

of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. To affect the PCR reaction, the carrier fluid is heated. (“The carrier fluid then passes through a plurality of temperature zones in which the polymerase chain reactions take place”) Ex. 1010 (Corbett), (“The enzymatic reaction occurred by moving two drops (Taq I restriction enzyme and supercoiled plasmid) down separate channels using the thermocapillary technique, combining the drops, and heating the merged sample to 65°C in the channel.”) Ex. 1008 at 5559 (Burns II).

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1075.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Burns I, Corbett and Burns II.

Dependent Claim 5

Claim 5 of the ‘193 patent further recites “providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.”

Corbett similarly teaches that “[d]ownstream of temperature zone 34 is in-line analysis means 29 and recovery means 28. Connected to recovery means 28 is **detection means 30**. Detection means 30 determines when a reaction mixture in the carrier fluid reaches recovery means 28. This may be done by measuring the conductivity or **optical density** of the stream of carrier fluid.” Ex. 1010 at 8:12-18, [emphasis added].

Burns II teaches that “[c]apillary gel electrophoresis of DNA samples was performed using a Beckman P/ACE instrument with a laser-induced fluorescence detector and 37 cm length, 100 µm diameter, linear polymerase gel capillary according to manufacturer’s

recommendation.” Ex. 1008 at 5558. The results of capillary electrophoresis were generally detected optically at the time of filing. Ex. 1011 (Dolnik) at 46; Ex. 1004 ¶¶68-69.

In the combined method of Burns I, Corbett and Burns II, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶102, 108.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1075.

Thus, claim 5 is shown to be rendered obvious by the combination of Burns I, Corbett and Burns II.

Dependent Claims 6-8

Claim 6 of the ‘193 patent recites “wherein the oil is fluorinated oil.” Claim 7 of the ‘193 patent recites “wherein the carrier fluid further comprises a surfactant.” Claim 8 of the ‘193 patent recites “wherein the surfactant is fluorinated surfactant.”

Quake discloses that “[t]he fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). **Exemplary surfactants** include Tween, Span, **fluorinated oils**, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel.” Ex. 1034 at 35, [emphasis added].

The phrase “carrier fluid” in claim 7, having no antecedent basis, is construed to mean “a fluid that is immiscible with a plug fluid,” as this is the way the term is defined in the specification. See Ex. 1001 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1075.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claims 6-8 are shown to be rendered obvious by the combination of Burns I, Corbett, Burns II, and Quake.

Dependent Claim 9

Claim 9 of the '193 patent recites "wherein the at least one plug is a merged plug."

Burns II describes mixing two biological materials within a plug by first creating a first droplet with Taq I restriction enzyme, then creating a second droplet with plasmid DNA, and finally mixing the biological materials by merging the first droplet and the second droplet. Ex. 1008 pp. 5558-60.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1075.

Thus, claim 9 is shown to be rendered obvious by the combination of Burns I, Corbett and Burns II.

Dependent Claim 10

Claim 10 of the '193 patent recites "trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels."

Quake describes a "detection region" which "should generally be at least about twice, preferably at least about five times as large per side or in diameter as the diameter of the largest molecule, cell or droplet that will be passing through it." Ex. 1034 at 26. This may be contrasted to Quake's generally recommended dimensions of the fluid channels, which are shaped to cause the droplets to "conform to the size and shape of the channels" by "becom[ing] longer and thinner" as channels narrow. *Id.* at 27. Quake teaches that "an optical trap, or laser tweezers, may be used to sort or direct cells.... The optical trap remains in an 'off' mode until a desired particle is detected in the detection region. After detection of a desired characteristic, the particle or cell

is ‘trapped’, and thereby directed or moved into the predetermined branch channel for collection.” *Id.* at 67, *see also* FIGs. 14A & B (Input). Thus, Quake’s detection region is an “expansion portion” where the at least one plug would be “trapp[ed]... for a period of time... after the reaction” as recited in dependent claim 10.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1075.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claim 10 is shown to be rendered obvious by the combination of Burns I, Corbett, Burns II, and Quake

Dependent Claim 11

Claim 11 of the ‘193 patent recites “wherein the at least one plug is substantially spherical in shape.”

As discussed by Quake, “the microfabricated devices of this invention generate round, monodisperse droplets (such as those illustrated in Frames J and L of FIG. 19” (reproduced below). Ex. 1034 at 27, FIG. 19.

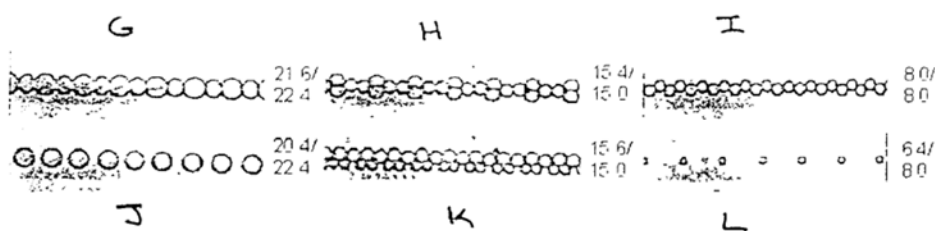


FIG. 19

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1075.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claim 11 is shown to be rendered obvious by the combination of Burns I, Corbett, Burns II, and Quake.

Dependent Claim 12

Claim 12 of the '193 patent recites "a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction."

As Burns II discusses, "[a]ctivating the heaters under the left interfaces propels the drops forward to the channel intersection where they meet and join to form a single larger drop (Fig. 4 B and C). The combined drop is stopped by turning off all heating elements (Fig. 4D) and can be reversed by heating the right interface. Additionally, circulation patterns generated in the drop during motion aid in mixing the liquid sample." Ex. 1008 at 5558.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1075.

Thus, claim 12 is shown to be rendered obvious by the combination of Burns I, Corbett and Burns II.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '193 patent recites "wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters." Claim 14 of the '193 patent recites "wherein the relevant parameters are selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns."

Corbett teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Corbett meets both claims 13 and 14 under the broadest reasonable

interpretation of the term “special design,” which in an of itself imparts no meaningful structural limitation. Ex. 1010 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1075.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Burns I, Corbett, and Burns II.

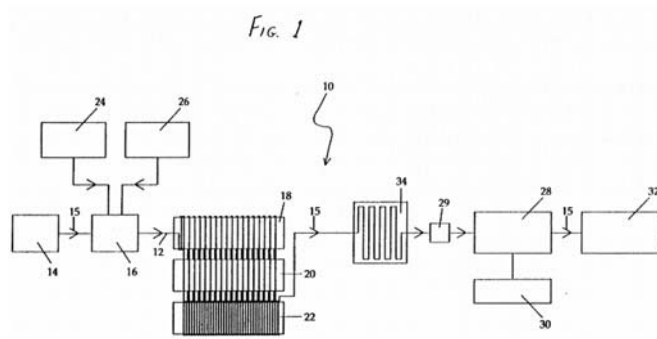
**O. The Combination of Burns I, Corbett and Vogelstein Renders Obvious
Claims 1-14 of the ‘193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)**

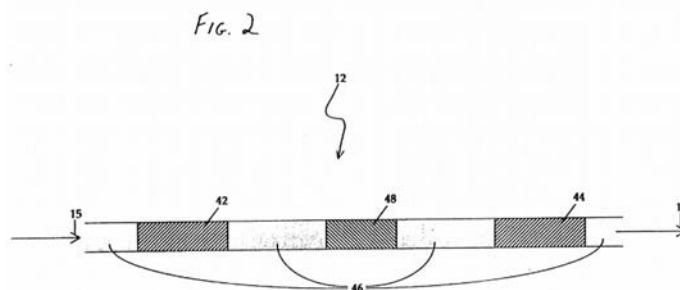
The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Burns I), ii) microfluidic PCR devices were well known (Corbett and Vogelstein), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Lagally, Kopp, Burns II and Vogelstein).

Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10. Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel. See Ex. 1010; 1027; Ex. 1025 at 23:2-8. Burns I demonstrates

that making reactant slugs or plugs with continuous flows of immiscible fluids was a mere design choice as of 2001.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Corbett and Vogelstein. In 1991, about eleven years prior to the earliest effective filing date, Corbett disclosed using a microchannel or capillary device to conduct DNA amplification reactions such as PCR by injecting plugs of reactants into a carrier stream that is passed through different temperature zones. Corbett explained that as of 1991 “[t]he most critical problem in applying nucleic acid amplification procedures, particularly in repetitive diagnostic assays in a clinical laboratory, is contamination of the reaction mixture by small amounts of DNA which contain sequences capable of being amplified under the conditions of assay.” Ex. 1010 at 3:7-12. “Automation of procedures minimize[s] the opportunity for such contamination and allows the implementation of stringent quality control.” *Id.* 3:15-19 “As is shown in FIG. 1, the apparatus 10 comprises tube 12 through which a fluid stream passes. As is more clearly shown in FIG. 2, the fluid stream includes pockets of reaction mixtures 42 and 44 and purging solution 48 separated by carrier fluid 46.” *Id.* at 7:58-62.





“The pump is actuated and the reaction mixture of 20 μ l or less is injected into the mineral oil or silicone oil carrier fluid and specific DNA sequences (whose limits are defined by the oligonucleotide primers) present in the sample is amplified as it passes cyclically through the temperature zones.” Ex. 1010 at 9:35-40. Corbett thus demonstrates that it was known at least as of 1991 that reduction of contamination risk in PCR could be achieved by performing the PCR on microliter sized plugs of reactant in capillary tube which passes through various temperature zones. Ex. 1004 ¶77.

By 1999, Vogelstein had reported **various applications for single-molecule microfluidic PCR**. Ex. 1044 at 9241. In a seminal article which has been cited over by over 1,000 other publications, Vogelstein explained that PCR amplification of small aliquots or droplets containing single molecules was effective for “detection of a small number of mutant-containing cells among a large excess of normal cells.” *Id.* at 9236. In Vogelstein’s method, the DNA was diluted into 7 μ l volumes such that, on average, there was one template molecule for every two wells (i.e., approximately half of the wells contained no DNA template molecule and approximately half contained a single DNA template molecule). *Id.* at 9236, 9237. This avoided the problem of background noise impairing the determination of whether each volume contained the mutant sequence. *Id.* By counting the number of volumes which indicated wild type or mutant templates, the presence or absence of the mutation (e.g., a sequence associated with colorectal cancer) can be determined. *Id.* at 9238. The accuracy of the test is a function of the

number of discrete volumes into which the sample is diluted. *Id.* at 9239. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) The article explained that the same technique would be useful for **detecting a wide array of mutations, dislocations and allelic imbalances**, as summarized in Table 1. *Id.* at 9241. Vogelstein thus taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby overcoming the signal-to-noise limitations associated with certain other PCR techniques and enabling the detection of relatively rare mutations, dislocations and allelic imbalances. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86.

In sum, Corbett and Vogelstein show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Burns I is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitation reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).¹⁴⁸ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug

¹⁴⁸ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Vogelstein. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic reactor of Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactor of Burns I because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. *Id.* ¶¶92-96. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactor of Burns I would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production of non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve

product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity Ex. 1044 at 9239 (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.*

Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Quake, Burns I and Shaw Stewart to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp, Burns II, Lagally or Vogelstein) to incorporate a droplet reactor (Quake, Burns I or Shaw Stewart). Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp, Burns II, Lagally or Vogelstein. The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II or Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would **enhance temperature control and reaction specificity**. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful.**

In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually

transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an

autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Vogelstein to work in the microfluidic reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹⁴⁹ As discussed in Section

¹⁴⁹ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570

VI.A.3, above, Higuchi I-III thus demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail below, in the accompanying claim chart (Ex. 1076) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Vogelstein. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modify it to incorporate the microfluidic reactor of Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Burns I discloses “[a] multiphase microreactor based upon the use of slug flow through a narrow channel....” Ex. 1007 at 10. “The experimental work discussed here is based on the use of alternating slugs of two liquid phases to provide the environment for mass transfer and reaction within the microreactor.” *Id.* at 10.

In the instant combination, microfluidic droplet reactors (as taught in Burns I) are used to perform PCR reactions (as taught in Corbett and Vogelstein), wherein each plug includes a mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-38. PCR reactions are autocatalytic. Ex. 1004 ¶32; Ex. 1001 44:58-61.

(Fed.Cir.1985) (‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1076.

Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

Burns I explains that “[t]he method discussed in this work **uses the continuous flow of both phases through T or cross-shaped intersections**. Slugs are generated by the action of **one phase flowing into the channel whilst the other phase moves into the intersection**, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1076.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

As noted above, Burns I teaches that “[t]he method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Figure 1 shows that the reactant slug is composed of an “**aqueous phase**.” *Id.* at 11.

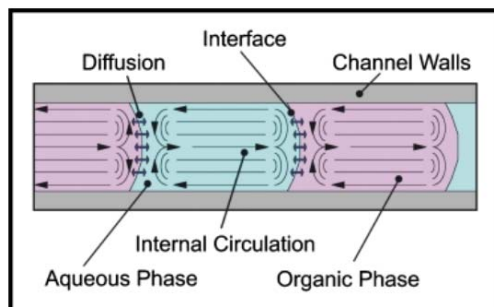


Fig. 1 Illustration of internal circulation generated within immiscible slug flow.

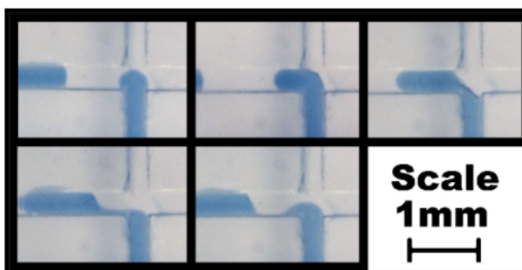


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns' Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase.

In the instant combination, the microfluidic droplet reactors of Burns I are relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").¹⁵⁰ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-39.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Vogelstein. Stated a different way, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modifying it to incorporate the microfluidic droplet reactor of Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing. *Id.* ¶¶92-109.

A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Burns I because doing so have provided the substantial

¹⁵⁰ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Corbett to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett's PCR reaction in the microfluidic droplet reactor of Burns I would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026

(Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

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incorporate a droplet reactor (Burns I). Ex. 1004 ¶¶92-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Burns II or Vogelstein. The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

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Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

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In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of

single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

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Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority

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Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactors Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in miniaturizing and modifying Corbett or modifying Vogelstein to work in the microfluidic droplet reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the

PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹⁵¹ As discussed in Section VI.A.3, above, Higuchi I-III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained further in the accompanying claim chart (Ex. 1076) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Corbett and Vogelstein. Alternatively, a skilled artisan would have considered it obvious to miniaturize the PCR apparatus of Corbett and modifying it to incorporate the microfluidic droplet reactor of Burns I, as evidenced by Kopp, Burns II, Lagally and Vogelstein's contemporaneous reports that PCR had been widely and successfully implemented at the microfluidic scale at the time of filing.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1076.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

¹⁵¹ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) (‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

As noted above, Burns I teaches that “[t]he method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Figure 1 shows that the reactant slug is composed of an “**organic phase**.” *Id.* at 11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase.

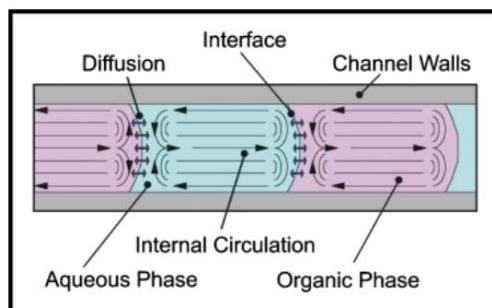


Fig. 1 Illustration of internal circulation generated within immiscible slug flow.

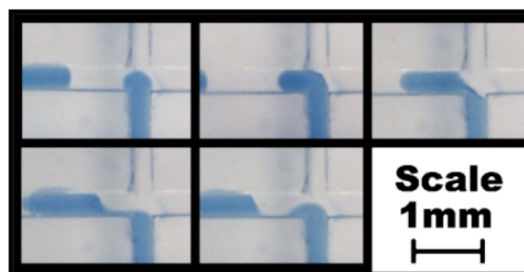


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1076.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10. Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.”

Ex. 1007 at 10-11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug

generation. *Id.* at 11. In Burns’ Fig. 4 embodiment,

an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel. See Ex. 1010; 1027; Ex. 1025 at 23:2-8.

When read in combination with Higuchi I-III, Quake and Shaw Stewart, Burns I demonstrates that making reactant slugs or plugs with continuous flows of oil was a mere design choice as of 2001.

With regard to the recitation that “the plug [is] substantially surrounded by the immiscible carrier fluid,” Requester submits that this would necessarily occur for any slug or droplet having a volume small enough to not bridge the entire channel width. Indeed, such scenarios are illustrated in Quake (Ex. 1033 Fig. 18) and Shaw Stewart (Ex. 1040 Fig. 1). Accordingly, it would have been obvious to utilize the Burns I droplet generator in a manner that meets the “substantially surrounded” limitation.

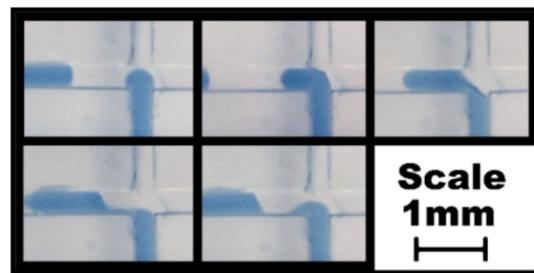


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

In the present combination, the slugs or plugs contain a substrate molecule and reagents for performing a PCR reaction. Ex. 1044 p. 9238.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1076.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Burns I discloses that “[t]he high heat and mass transfer capability offered by capillary microreactors allows high intensity reactions to be precisely controlled for optimum yield and selectivity.” Ex. 1007 at 10. “The internal circulation, which is stimulated within the slugs by their passage along the channel, is responsible for a **large enhancement** in the interfacial mass transfer and the **reaction rate**.” Ex. 1007 at 10, [emphasis added].

As discussed above in connection with element 1[b], in the instant combination Burns I is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule and the autocatalytic reaction and the autocatalytic reaction. Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. These limitations are met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. The DNA is amplified through the PCR reaction. Ex. 1004 ¶¶31-38; Ex. 1044 p. 9238.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1076.

Thus, claim 1 is shown to be rendered obvious by the combination of Burns I, Corbett and Vogelstein.

Dependent Claim 2

Claim 2 of the '193 patent recites “wherein the at least one substrate molecule is a single biological molecule.”

In the combined method of Burns I, Corbett and Vogelstein, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. Vogelstein explains “PCR-amplification from single cells isolated by physical separation or dilution has been used to address a variety of interesting biologic questions.” Ex. 1044 p. 9239. Thus a skilled artisan would be motivated to isolate a single molecule for PCR reaction. Ex. 1004 ¶¶81-87.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1076.

Thus, claim 2 is shown to be rendered obvious by the combination of Burns I, Corbett and Vogelstein.

Dependent Claims 3 and 4

Claim 3 of the '193 patent recites “wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.” Claim 4 of the '193 patent recites “wherein the providing step includes heating.” PCR reactions involve thermocycling. Ex. 1004 ¶¶33-34.

In the combined method of Burns I, Corbett and Vogelstein, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. To affect the PCR reaction, the carrier fluid is heated. (“The carrier fluid then passes through a plurality of

temperature zones in which the polymerase chain reactions take place”) Ex. 1010 (Corbett),, (“If a PCR product whose sequence is complementary to the loop sequence is present during the heating/cooling cycle, hybridization of the MB to one strand of the PCR product will increase the distance between the Dabcyl and the dye, resulting in increased fluorescence.”) Ex. 1044 at 9237(Vogelstein)..

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1076.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Burns I, Corbett and Vogelstein.

Dependent Claim 5

Claim 5 of the ‘193 patent further recites “providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.”

Corbett similarly teaches that “[d]ownstream of temperature zone 34 is in-line analysis means 29 and recovery means 28. Connected to recovery means 28 is **detection means 30**. Detection means 30 determines when a reaction mixture in the carrier fluid reaches recovery means 28. This may be done by measuring the conductivity or **optical density** of the stream of carrier fluid.” Ex. 1010 at 8:12-18, [emphasis added].

Vogelstein teaches that the “[r]eactions were analyzed immediately or stored at room temperature for up to 36 h before **fluorescence analysis**.” Ex. 1044 at 9236. , [emphasis added].

In the combined method of Burns I, Corbett and Vogelstein, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶102, 108.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1076.

Thus, claim 5 is shown to be rendered obvious by the combination of Burns I, Corbett and Vogelstein.

Dependent Claims 6-8

Claim 6 of the '193 patent recites "wherein the oil is fluorinated oil." Claim 7 of the '193 patent recites "wherein the carrier fluid further comprises a surfactant." Claim 8 of the '193 patent recites "wherein the surfactant is fluorinated surfactant."

Quake discloses that "[t]he fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). **Exemplary surfactants** include Tween, Span, **fluorinated oils**, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel." Ex. 1034 at 35, [emphasis added].

The phrase "carrier fluid" in claim 7, having no antecedent basis, is construed to mean "a fluid that is immiscible with a plug fluid," as this is the way the term is defined in the specification. See Ex. 1001 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1076.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claims 6-8 are shown to be rendered obvious by the combination of Burns I, Corbett, Vogelstein, and Quake.

Dependent Claim 9

Claim 9 of the '193 patent recites "wherein the at least one plug is a merged plug."

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1076.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claim 9 is shown to be rendered obvious by the combination of Burns I, Corbett, Vogelstein, and Quake.

Dependent Claim 10

Claim 10 of the '193 patent recites "trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels."

Quake describes a "detection region" which "should generally be at least about twice, preferably at least about five times as large per side or in diameter as the diameter of the largest molecule, cell or droplet that will be passing through it." Ex. 1034 at 26. This may be contrasted to Quake's generally recommended dimensions of the fluid channels, which are shaped to cause the droplets to "conform to the size and shape of the channels" by "becom[ing] longer and thinner" as channels narrow. *Id.* at 27. Quake teaches that "an optical trap, or laser tweezers, may be used to sort or direct cells.... The optical trap remains in an 'off' mode until a desired particle is detected in the detection region. After detection of a desired characteristic, the particle or cell is 'trapped', and thereby directed or moved into the predetermined branch channel for collection." *Id.* at 67, *see also* FIGs. 14A & B (Input). Thus, Quake's detection region is an "expansion portion" where the at least one plug would be "trapp[ed]... for a period of time... after the reaction" as recited in dependent claim 10.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1076.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claim 10 is shown to be rendered obvious by the combination of Burns I, Corbett, Vogelstein, and Quake

Dependent Claim 11

Claim 11 of the '193 patent recites "wherein the at least one plug is substantially spherical in shape."

As discussed by Quake, "the microfabricated devices of this invention generate round, monodisperse droplets (such as those illustrated in Frames J and L of FIG. 19" (reproduced below). Ex. 1034 at 27, FIG. 19.

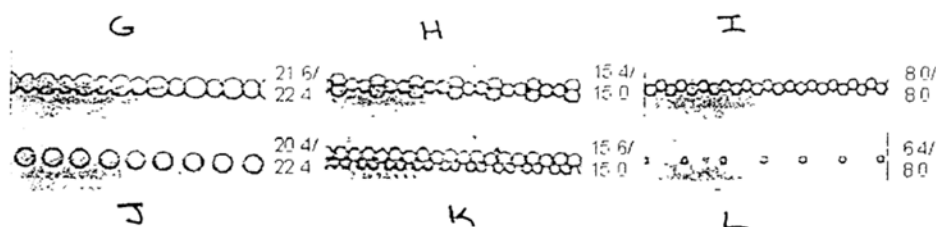


FIG. 19

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1076.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claim 11 is shown to be rendered obvious by the combination of Burns I, Corbett, Vogelstein, and Quake.

Dependent Claim 12

Claim 12 of the '193 patent recites "a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction."

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1076.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claim 12 is shown to be rendered obvious by the combination of Burns I, Corbett, Vogelstein, and Quake.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '93 patent recites "wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters." Claim 14 of the '93 patent recites "wherein the relevant parameters are selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns."

Corbett teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Corbett meets both claims 13 and 14 under the broadest reasonable interpretation of the term "special design," which in and of itself imparts no meaningful structural limitation. Ex. 1010 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1076.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Burns I, Corbett, and Vogelstein.

P. The Combination of Burns I, Kopp and Lagally Renders Obvious Claims 1-14 of the '193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Burns I), ii) microfluidic PCR devices were well known (Kopp and Lagally), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10. Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel See Ex. 1010; 1027; Ex. 1025 at 23:2-8. Burns I demonstrates that making reactant slugs or plugs with continuous flows of immiscible fluids was a mere design choice as of 2001.

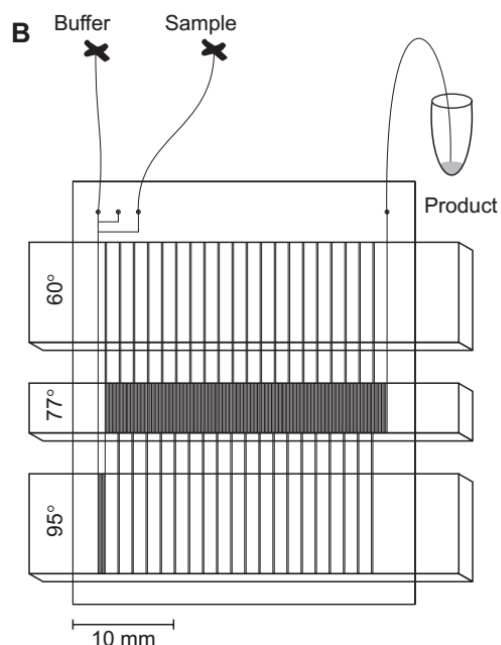
As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Lagally. By 1998 Kopp *et al.* had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary

electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 (emphasis added).

Kopp *et al.* also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 (emphasis added).

In 2001, in another article not previously considered by the Office, Lagally reported using a microfluidic reactor to conduct single-molecule DNA amplification by PCR. Lagally summarized the state of the art as of February 2001, explaining continuous flow PCR microreactors had recently undergone substantial evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis

microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or **continuous flow** through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

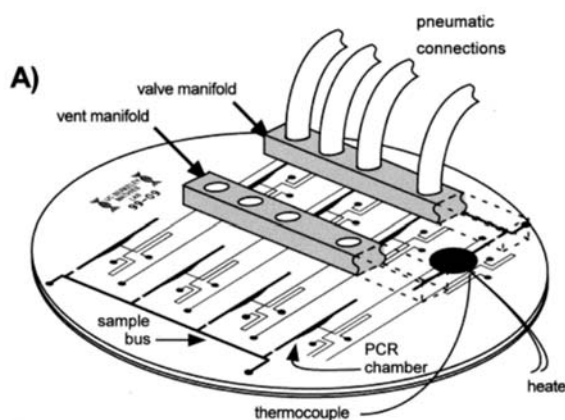
Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556 [emphasis added].

Lagally *et al.* developed a further improvement – a device and method for achieving single molecule amplification in a microchannel environment.

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

Microfluidic PCR-CE device. The PCR chambers are connected to a common sample bus through a set of valves. Hydrophobic vents at the other end of the PCR chambers are used to locate the sample and to eliminate gas. The PCR chambers are directly connected to the cross channel of the CE system for product injection and analysis. Two aluminum manifolds, one each for the vents and valves, are placed onto the respective ports and clamped in place using vacuum. The manifolds connect to external solenoid valves for pressure and vacuum actuation. Thermal cycling is accomplished using a resistive heater and a miniature thermocouple below the 280-nL chamber.



Ex. 1028 at 565-66 (see figure legend).

In sum, Kopp and Lagally show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Burns I is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).¹⁵² Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Kopp and Lagally. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactors of Burns I because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556,

¹⁵² Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

[emphasis added]. The modification of Kopp to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Lagally) to incorporate a droplet reactor (Burns I). Ex. 1004 ¶¶92-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Lagally. The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Lagally) or droplets produced

using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful.**

In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally et al. provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize

noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson

distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at

1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp or Lagally to work in the microfluidic reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the

level of skill in the art.¹⁵³ As discussed in Section VI.A.3, above, Higuchi I-III thus demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail below, in the accompanying claim chart (Ex. 1077), and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Kopp and Lagally.

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Burns I discloses “[a] multiphase microreactor based upon the use of slug flow through a narrow channel....” Ex. 1007 at 10. “The experimental work discussed here is based on the use of alternating slugs of two liquid phases to provide the environment for mass transfer and reaction within the microreactor.” *Id.* at 10.

In the instant combination, microfluidic droplet reactors (as taught in Burns I) are used to perform PCR reactions (as taught in Kopp and Lagally), wherein each plug includes a mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-38. For example, as noted by

¹⁵³ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) (‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565. PCR reactions are autocatalytic. Ex. 1004 ¶32; Ex. 1001 44:58-61.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1077.

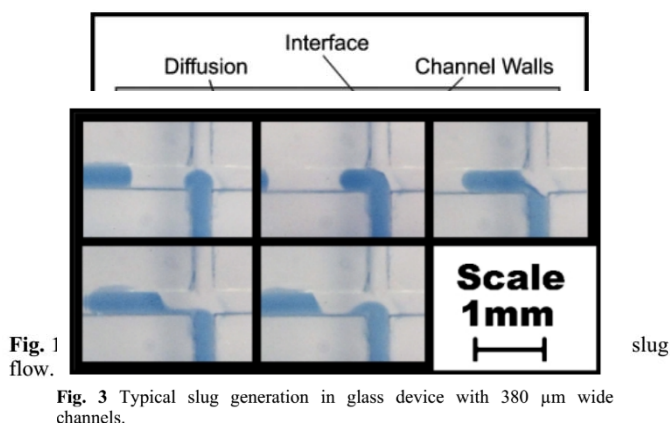
Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

Burns I explains that “[t]he method discussed in this work **uses the continuous flow of both phases through T or cross-shaped intersections.** Slugs are generated by the action of **one phase flowing into the channel whilst the other phase moves into the intersection,** eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1077.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

As noted above, Burns I teaches that “[t]he method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the



flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Figure 1 shows that the reactant slug is composed of an “**aqueous phase**.” *Id.* at 11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase.

In the instant combination, the microfluidic droplet reactors of Burns I are relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).¹⁵⁴ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Kopp and Lagally. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Burns I because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed**.” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide

¹⁵⁴ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

Skilled artisans would also have recognized that performing Corbett’s PCR reaction in the microfluidic droplet reactor of Burns I would **enhance the precision of the PCR reaction with a given primer and reduce the production of non-specific products** relative to then-traditional approaches. As of the filing date it was well known that PCR reactions suffered from the limitation that the primers could bind to non-specific sequences, including each other, leading to production on non-specific products. Ex. 1004 ¶35; Ex. 1032 at 106-07. In larger volume PCR reactions, the temperature of the reactants ramps up and down gradually as the reaction mixture is thermocycled. Ex. 1004 ¶¶92, 93. If the ramp speed of the thermocycler is

too slow, spurious annealing may occur due to lower temperature and sufficient time for nonspecific binding. Ex. 1004 ¶93. These long ramping periods thus increase the likelihood of off-target effects, such binding to non-target sequences and the production of primer dimers. *Id.* Rapid ramp times of the temperature-dependent reactions in PCR amplification can improve product specificity significantly while decreasing the cycle time by an order of magnitude. Ex. 1045 at Abstract (Wittwer). It is therefore highly desirable to have short heating and cooling times to achieve more precise and efficient reactions with fewer off-target reaction products. Ex. 1004 ¶93. Accordingly, at the time of filing it was well-known that, for a given primer, the PCR reaction is more efficient and precise where the temperature ramping time is minimized. *Id.*

Moreover, using the microfluidic droplet reactors for PCR reactions would have **substantially increased the ability to successfully utilize degraded or low-level DNA** in a sample. For example, the fragmentation of DNA derived from aged or degraded tissue severely reduces the efficiency of the PCR. Using conventional PCR on such a degraded or aged sample leads to competition for enzyme due to template reconstruction and resulting depletion of dNTPs, both of which hinder the efficient amplification of a target product. Ex. 1015 at 5026 (Golenberg). Thus, at the time of filing, skilled artisans understood that using a microfluidic droplet system would prevent such template reconstruction and dNTP depletion.

Vogelstein taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby **overcoming the signal-to-noise limitations** associated with certain other PCR techniques and **enabling the detection of relatively rare mutations, dislocations and allelic imbalances**. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86. Vogelstein taught that diluting the sample into ~1,500 discrete volumes allowed

sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. Ex. 1044 at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the sensitivity by another order of magnitude.”) *Id.* Vogelstein article explained this technique was useful for detecting an array of mutations, translocations and other rare genetic conditions:

Table 1. Potential applications of Dig-PCR

Application	Example	Probe 1 detects	Probe 2 detects
Base substitution mutations	Cancer gene mutations in stool, blood, and lymph nodes	Mutant or WT alleles	WT PCR products
Chromosomal translocations	Residual leukemia cells after therapy (DNA or RNA)	Normal or translocated alleles	Translocated allele
Gene amplifications	Determine presence or extent of amplification	Sequence within amplicon	Sequence from another part of same chromosome arm
Alternatively spliced products	Determine fraction of alternatively spliced transcripts from same gene (RNA)	Minor exons	Common exons
Changes in gene expression	Determine relative levels of expression of two genes (RNA)	First transcript	Reference transcript
Allelic discrimination	Two different alleles mutated vs. one mutation in each of two alleles	First mutation	Second mutation
Allelic imbalance	Quantitative analysis with nonpolymorphic markers	Marker from test chromosome	Marker from reference chromosome

Id. at 9241. A skilled artisan would thus have been strongly motivated to use droplet reactors such as those taught in Burns I to dilute the samples and thereby enable the detection of mutations, translocations and other rare genetic conditions by PCR as taught in Vogelstein. Ex. 1004 ¶¶85,86.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Lagally) to incorporate a droplet reactor (Burns I). Ex. 1004 ¶¶92-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Lagally. The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Lagally) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a

given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful.**

In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μ L, in volumes down to 1 μ L.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling

profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced

microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic**

droplet (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactors Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in modifying Lagally or Kopp to work in the microfluidic droplet reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹⁵⁵ As discussed in Section VI.A.3, above, Higuchi I-

¹⁵⁵ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary

III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained further in the accompanying claim chart (Ex. 1077) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Kopp and Lagally.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1077.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

As noted above, Burns I teaches that “[t]he method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11.

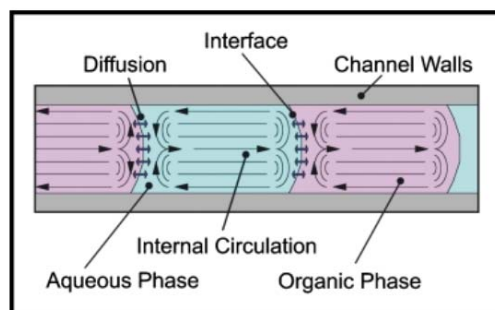


Fig. 1 Illustration of internal circulation generated within immiscible slug flow.

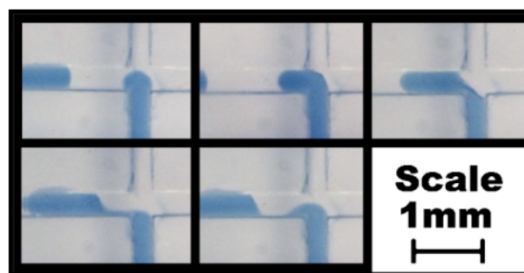


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) (‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

Figure 1 shows that the reactant slug is composed of an “**organic phase.**” *Id.* at 11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1077.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10. Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.”

Ex. 1007 at 10-11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug

generation. *Id.* at 11. In Burns’ Fig. 4 embodiment,

an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel. See Ex. 1010; 1027; Ex. 1025 at 23:2-8.

When read in combination with Higuchi I-III, Quake and Shaw Stewart, Burns I demonstrates

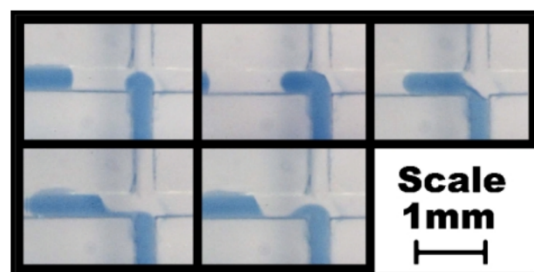


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

that making reactant slugs or plugs with continuous flows of oil was a mere design choice as of 2001.

With regard to the recitation that “the plug [is] substantially surrounded by the immiscible carrier fluid,” Requester submits that this would necessarily occur for any slug or droplet having a volume small enough to not bridge the entire channel width. Indeed, such scenarios are illustrated in Quake (Ex. 1033 Fig. 18) and Shaw Stewart (Ex. 1040 Fig. 1). Accordingly, it would have been obvious to utilize the Burns I droplet generator in a manner that meets the “substantially surrounded” limitation.

In the present combination, the slugs or plugs contain a substrate molecule and reagents for performing a PCR reaction. Ex. 1028 at 565(Lagally) (“The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed.”).

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1077.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Burns I discloses that “[t]he high heat and mass transfer capability offered by capillary microreactors allows high intensity reactions to be precisely controlled for optimum yield and selectivity.” Ex. 1007 at 10. “The internal circulation, which is stimulated within the slugs by their passage along the channel, is responsible for a **large enhancement** in the interfacial mass transfer and the **reaction rate**.” Ex. 1007 at 10, [emphasis added].

As discussed above in connection with element 1[b], in the instant combination Burns I is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the

limitations reciting that the flowing fluid with the reagents also includes a substrate molecule and the autocatalytic reaction and the autocatalytic reaction. Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. These limitations are met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-39. The DNA is amplified through the PCR reaction. Ex. 1004 ¶¶33-34.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1077.

Thus, claim 1 is shown to be rendered obvious by the combination of Burns I, Kopp and Lagally.

Dependent Claim 2

Claim 2 of the '193 patent recites "wherein the at least one substrate molecule is a single biological molecule."

In the combined method of Burns I, Kopp and Lagally, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90.

As discussed by Lagally, "[s]tochastic PCR amplification of **single DNA template molecules** followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device." Ex. 1028 at 565, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1077.

Thus, claim 2 is shown to be rendered obvious by the combination of Burns I, Kopp and Lagally.

Dependent Claims 3 and 4

Claim 3 of the '193 patent recites "wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction." Claim 4 of the '193 patent recites "wherein the providing step includes heating." PCR reactions involve thermocycling. Ex. 1004 ¶¶33-34.

In the combined method of Burns I, Kopp and Lagally, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. To affect the PCR reaction, the carrier fluid is heated. ("The individual steps are simply performed by heating or cooling the sample to characteristic temperatures: 95°C for dsDNA melting, 50° to 65°C for primer annealing, and 72° to 77°C for primer extension at the optimum enzyme temperature.") Ex. 1027 at 1046 (Kopp), ("The PCR chambers were thermally cycled.") Ex. 1028 (Lagally) at 567.

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1077.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Burns I, Kopp and Lagally.

Dependent Claim 5

Claim 5 of the '193 patent further recites "providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred."

Lagally teaches “[s]tochastic PCR amplification of single DNA template molecules followed by **capillary electrophoresis** (CE) analysis of the products is demonstrated in an integrated microfluidic device.” Ex. 1028 at 565, [emphasis added]. The results of capillary electrophoresis were generally detected optically at the time of filing. Ex. 1011 (Dolnik) at 46; Ex. 1004 ¶¶68-69.

In the combined method of Burns I, Kopp and Lagally, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶102, 108.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1077.

Thus, claim 5 is shown to be rendered obvious by the combination of Burns I, Kopp and Lagally.

Dependent Claims 6-8

Claim 6 of the ‘193 patent recites “wherein the oil is fluorinated oil.” Claim 7 of the ‘193 patent recites “wherein the carrier fluid further comprises a surfactant.” Claim 8 of the ‘193 patent recites “wherein the surfactant is fluorinated surfactant.”

Quake discloses that “[t]he fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). **Exemplary surfactants** include Tween, Span, **fluorinated oils**, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel.” Ex. 1034 at 35, [emphasis added].

The phrase “carrier fluid” in claim 7, having no antecedent basis, is construed to mean “a fluid that is immiscible with a plug fluid,” as this is the way the term is defined in the specification. See Ex. 1001 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1077.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claims 6-8 are shown to be rendered obvious by the combination of Burns I, Kopp, Lagally, and Quake

Dependent Claim 9

Claim 9 of the ‘193 patent recites “wherein the at least one plug is a merged plug.”

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1077.

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claim 9 is shown to be rendered obvious by the combination of Burns I, Kopp, Lagally, and Quake

Dependent Claim 10

Claim 10 of the ‘193 patent recites “trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels.”

Lagally designed “PCR chambers etched into a glass substrate, connected to microfluidic valves and hydrophobic vents for sample introduction and **immobilization** during thermal

cycling.” Ex. 1028 at 566. Lagally’s system vacuum traps the sample during a heating period of time. *Id.* at 567. As illustrated in Figure 1, reproduced to the right, Lagally’s PCR chamber, used to trap the sample, is expanded in relation to the sample bus connected thereto. Ex. 1028 at 566; Ex. 1004 ¶49.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1077.

Thus, claim 10 is shown to be rendered obvious by the combination of Burns I, Kopp, and Lagally.

Dependent Claim 11

Claim 11 of the ‘193 patent recites “wherein the at least one plug is substantially spherical in shape.”

As discussed by Quake, “the microfabricated devices of this invention generate round, monodisperse droplets (such as those illustrated in Frames J and L of FIG. 19” (reproduced below). Ex. 1034 at 27, FIG. 19.

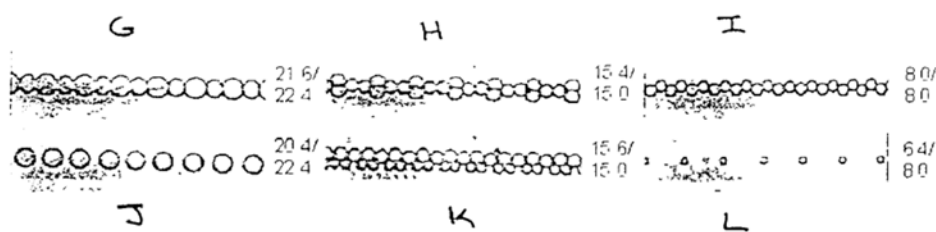


FIG. 19

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1077.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claim 11 is shown to be rendered obvious by the combination of Burns I, Kopp, Lagally, and Quake.

Dependent Claim 12

Claim 12 of the '193 patent recites "a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction."

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and finally mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17, .

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1077.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claim 12 is shown to be rendered obvious by the combination of Burns I, Kopp, Lagally, and Quake.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '193 patent recites "wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters." Claim 14 of the '193 patent recites "wherein the relevant parameters are selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns."

Kopp teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Kopp meets both claims 13 and 14 under the broadest reasonable

interpretation of the term “special design,” which in an of itself imparts no meaningful structural limitation. Ex. 1027 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1077.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Burns I, Kopp, and Lagally.

Q. The Combination of Burns I, Kopp and Burns II Renders Obvious Claims 1-14 of the ‘193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Burns I), ii) microfluidic PCR devices were well known (Kopp and Burns II), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10. Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel. See Ex. 1010; 1027; Ex. 1025 at 23:2-8. Burns I demonstrates

that making reactant slugs or plugs with continuous flows of immiscible fluids was a mere design choice as of 2001.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Burns II. In 1996, Burns II reported the use of microfluidic devices to perform PCR. Ex. 1008 at 5556. Burns II explained that “[n]o new biochemistries or DNA detection methods are required for their use; the components simply reproduce current laboratory procedures in a micron-sized environment.” *Id.* at 5560. In other words, the PCR chemistry did not need to be modified – it worked without modification on the microfluidic scale. In the method of Burns II, a sample containing a DNA plasmid was loaded into one of the reaction wells (A) and a mixture of Taq enzyme and buffer was loaded into the other. Ex. 1008 at 5558. Each formed droplets of about 60 nl in the parallel 500 x 25 μm channels (B) which came together at the Y junction to form a single larger droplet (about 120 nl). *Id.* at 5558-59; Ex. 1004 ¶40.

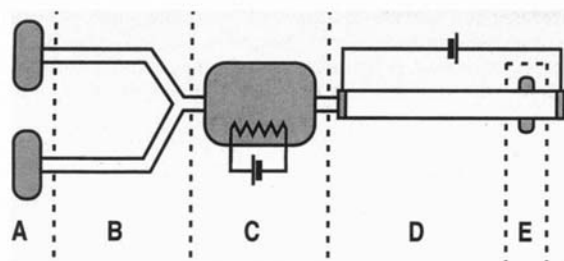


FIG. 1. Example of an integrated DNA analysis system, represented schematically. The individual components of the system are injection entry ports (A), liquid pumping channels (B), thermally controlled reaction chamber (C), electrophoresis channel (D), and DNA band migration detector (E). Each component would have associated sensors, control circuitry, and external connections.

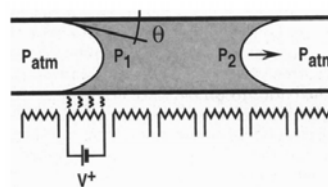


FIG. 2. Schematic drawing showing the principle of thermally induced drop motion in a closed channel. The case of a single aqueous drop in a hydrophilic channel is presented, where V is an applied voltage, P_{atm} is atmospheric pressure, P_1 is the receding-edge internal pressure, P_2 is the advancing-edge internal pressure, and θ is the contact angle of the liquid-gas-solid interface. The contact angle will depend on the surface characteristics of the channel and the constituents of the drop, with a hydrophilic interaction giving θ between 0 and 90 degrees, and a hydrophobic surface giving θ between 90 and 180 degrees. Surface treatments can also reduce contact angle hysteresis and, therefore, reduce the temperature difference necessary for drop motion.

The droplet then passed through the resistive heater portion (C) in which PCR amplification occurred. *Id.* at 5556-5557. Afterwards the amplification results were optically detected in zone D via gel electrophoresis. *Id.* at 5557-5561. Burns II noted that “[t]he use of common fabrication methods allows the assembly of increasingly complex, multicomponent, integrated systems from

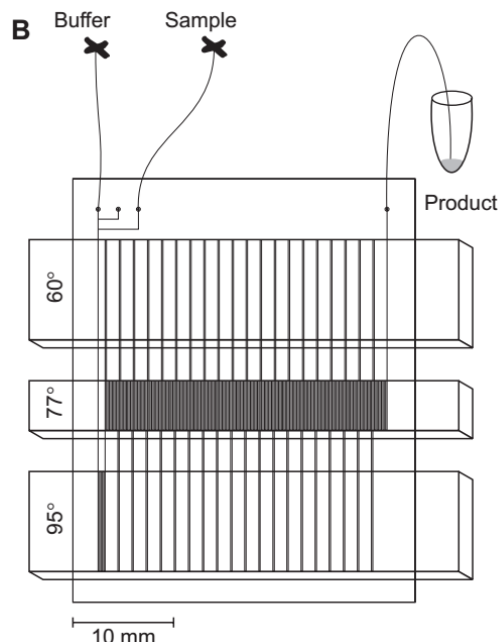
a small, defined set of standardized elements” and that “[f]uture design efforts have a strong impetus to reduce component size and improve efficiency.” *Id.* at 5560-61.

By the late 1990s Kopp *et al.* had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 (emphasis added).

Kopp *et al.* also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary gel electrophoresis (as a conventional batch process), have already been presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene

could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 (emphasis added).

In sum, Kopp and Burns II show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Burns I is relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").¹⁵⁶ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I, to conduct microfluidic PCR as taught by Kopp and Burns II. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactor of Burns I because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, "[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents**

¹⁵⁶ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

needed.” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added]. The modification of Kopp to microfluidic dimensions would have reduced the amounts of reagents used, which would in turn decrease operating costs. Ex. 1004 ¶¶106. The reduction of fluid plug size would also have increased the rate at which the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction. Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Burns II) to incorporate a droplet reactor (Burns I). Ex. 1004 ¶¶92-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the

fluid microfluidic PCR devices taught in Kopp or Burns II. The use of two flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template

concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials

are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority

date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp or Burns II to work in the microfluidic reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English.

Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹⁵⁷ As discussed in Section VI.A.3, above, Higuchi I-III thus demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail below, in the accompanying claim chart (Ex. 1078) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Kopp and Burns II.

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Burns I discloses “[a] multiphase microreactor based upon the use of slug flow through a narrow channel....” Ex. 1007 at 10. “The experimental work discussed here is based on the use of alternating slugs of two liquid phases to provide the environment for mass transfer and reaction within the microreactor.” *Id.* at 10.

In the instant combination, microfluidic droplet reactors (as taught in Burns I) are used to perform PCR reactions (as taught in Kopp and Burns II), wherein each plug includes a mixture

¹⁵⁷ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) (‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-38. PCR reactions are autocatalytic. Ex. 1004 ¶32; Ex. 1001 44:58-61.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1078.

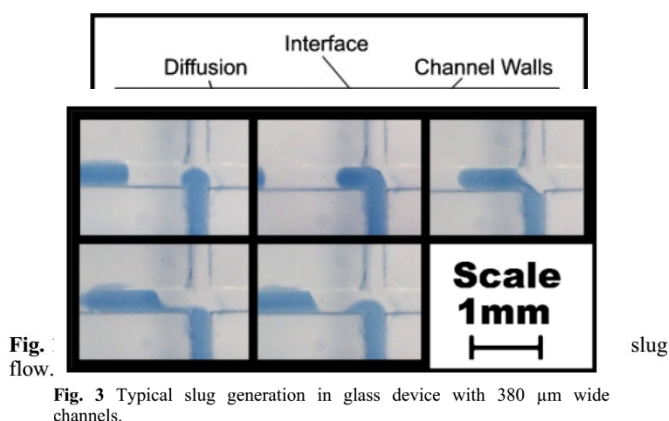
Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

Burns I explains that “[t]he method discussed in this work **uses the continuous flow of both phases through T or cross-shaped intersections**. Slugs are generated by the action of **one phase flowing into the channel whilst the other phase moves into the intersection**, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1078.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

As noted above, Burns I teaches that “[t]he method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Figure 1 shows that the reactant slug is composed of an “**aqueous phase**.” *Id.* at 11. Fig. 3 shows oil-in-



water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns' Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase.

In the instant combination, the microfluidic droplet reactors of Burns I are relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").¹⁵⁸ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Kopp and Burns II. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Burns I because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, "[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**" Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that "[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**" Ex. 1008 at 5556, [emphasis added]. The reduction of fluid plug size would also have increased the rate at which

¹⁵⁸ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction.

Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Burns II) to incorporate a droplet reactor (Burns I). Ex. 1004 ¶¶92-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Burns II. The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to the single fluid in microfluidic chip reactor (e.g., Burns II) or droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a

given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful.**

In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/μL, in volumes down to 1 μL.¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling

profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced

microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic**

droplet (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactors Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in modifying Burns II or Kopp to work in the microfluidic droplet reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation recitation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹⁵⁹ As discussed in Section VI.A.3, above, Higuchi I-

¹⁵⁹ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary

III demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained further in the accompanying claim chart (Ex. 1078) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Kopp and Burns II.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1078.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

As noted above, Burns I teaches that “[t]he method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Figure 1 shows that the

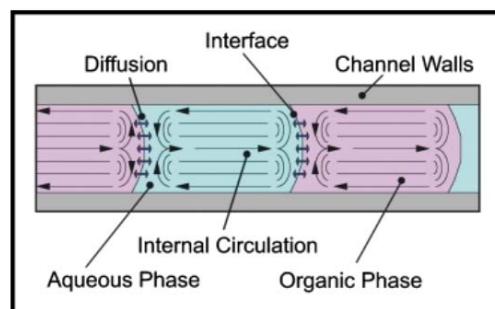


Fig. 1 Illustration of internal circulation generated within immiscible slug flow.

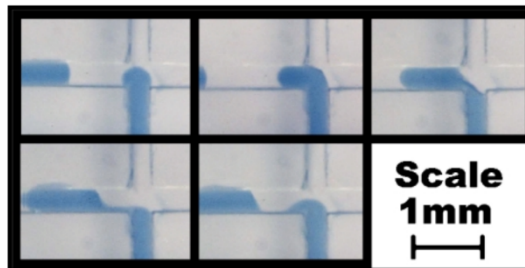


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) (‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

reactant slug is composed of an “**organic phase.**” *Id.* at 11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1078.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10. Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.”

Ex. 1007 at 10-11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug

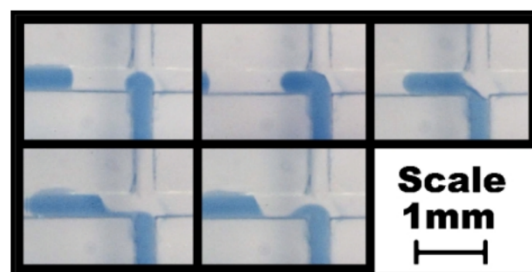


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

generation. *Id.* at 11. In Burns’ Fig. 4 embodiment,

an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel. See Ex. 1010; 1027; Ex. 1025 at 23:2-8.

When read in combination with Higuchi I-III, Quake and Shaw Stewart, Burns I demonstrates

that making reactant slugs or plugs with continuous flows of oil was a mere design choice as of 2001.

With regard to the recitation that “the plug [is] substantially surrounded by the immiscible carrier fluid,” Requester submits that this would necessarily occur for any slug or droplet having a volume small enough to not bridge the entire channel width. Indeed, such scenarios are illustrated in Quake (Ex. 1033 Fig. 18) and Shaw Stewart (Ex. 1040 Fig. 1). Accordingly, it would have been obvious to utilize the Burns I droplet generator in a manner that meets the “substantially surrounded” limitation.

In the present combination, the slugs or plugs contain a substrate molecule and reagents for performing a PCR reaction. Ex. 1008 (Burns II) p. 5556 (“In the proposed format, sample and reagent are injected into the device through entry ports (Fig. 1A) and the solutions pumped through channels (Fig. 1B) to a thermally controlled reactor where mixing and restriction enzyme digestion or PCR amplification occurs (Fig. 1C).”).

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1078.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Burns I discloses that “[t]he high heat and mass transfer capability offered by capillary microreactors allows high intensity reactions to be precisely controlled for optimum yield and selectivity.” Ex. 1007 at 10. “The internal circulation, which is stimulated within the slugs by their passage along the channel, is responsible for a **large enhancement** in the interfacial mass transfer and the **reaction rate**.” Ex. 1007 at 10, [emphasis added].

As discussed above in connection with element 1[b], in the instant combination Burns I is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the

limitations reciting that the flowing fluid with the reagents also includes a substrate molecule and the autocatalytic reaction. Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. These limitations are met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. The DNA is amplified through the PCR reaction. Ex. 1004 ¶¶33-34.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1078.

Thus, claim 1 is shown to be rendered obvious by the combination of Burns I, Kopp and Burns II.

Dependent Claim 2

Claim 2 of the '193 patent recites "wherein the at least one substrate molecule is a single biological molecule."

In the combined method of Burns I, Kopp and Burns II, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. As evidenced by Lagally, in microfluidic reactors, a single DNA molecule may be involved in a reaction. Ex. 1028 p. 564 ("[s]tochastic PCR amplification of **single DNA template molecules** followed by capillary electrophoretic (CE) analysis of the products is demonstrated in an integrated microfluidic device."); Ex. 1028 at 565.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1078.

Thus, claim 2 is shown to be rendered obvious by the combination of Burns I, Kopp and Burns II.

Dependent Claims 3 and 4

Claim 3 of the '193 patent recites "wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction." Claim 4 of the '193 patent recites "wherein the providing step includes heating." PCR reactions involve thermocycling. Ex. 1004 ¶¶33-34.

In the combined method of Burns I, Kopp and Burns II, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. To affect the PCR reaction, the carrier fluid is heated. ("The individual steps are simply performed by heating or cooling the sample to characteristic temperatures: 95°C for dsDNA melting, 50° to 65°C for primer annealing, and 72° to 77°C for primer extension at the optimum enzyme temperature.") Ex. 1027 at 1046 (Kopp), ("The enzymatic reaction occurred by moving two drops (Taq I restriction enzyme and supercoiled plasmid) down separate channels using the thermocapillary technique, combining the drops, and heating the merged sample to 65°C in the channel.") Ex. 1008 at 5559 (Burns II)

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1078.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Burns I, Kopp and Burns II.

Dependent Claim 5

Claim 5 of the '193 patent further recites “providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.”

Burns II teaches that “[c]apillary gel electrophoresis of DNA samples was performed using a Beckman P/ACE instrument with a laser-induced fluorescence detector and 37 cm length, 100 μ m diameter, linear polymerase gel capillary according to manufacturer’s recommendation.” Ex. 1008 at 5558. The results of capillary electrophoresis were generally detected optically at the time of filing. Ex. 1011 (Dolnik) at 46; Ex. 1004 ¶¶68-69.

In the combined method of Burns I, Kopp and Burns II, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶102, 108.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1078.

Thus, claim 5 is shown to be rendered obvious by the combination of Burns I, Kopp and Burns II.

Dependent Claims 6-8

Claim 6 of the '193 patent recites “wherein the oil is fluorinated oil.” Claim 7 of the '193 patent recites “wherein the carrier fluid further comprises a surfactant.” Claim 8 of the '193 patent recites “wherein the surfactant is fluorinated surfactant.”

Kopp teaches using a “non-ionic surfactant ... as the PCR buffer”. Ex. 1027 p. 1047. In the present combination, the non-ionic surfactant would be provided by the silicone oil of Burns I. Ex. 1007 at 11.

Quake discloses that “[t]he fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). **Exemplary surfactants** include Tween, Span, **fluorinated oils**, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel.” Ex. 1034 at 35, [emphasis added].

The phrase “carrier fluid” in claim 7, having no antecedent basis, is construed to mean “a fluid that is immiscible with a plug fluid,” as this is the way the term is defined in the specification. See Ex. 1001 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1078.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claims 6-8 are shown to be rendered obvious by the combination of Burns I, Kopp Burns II, and Quake.

Dependent Claim 9

Claim 9 of the ‘193 patent recites “wherein the at least one plug is a merged plug.”

Burns II describes mixing two biological materials within a plug by first creating a first droplet with Taq I restriction enzyme, then creating a second droplet with plasmid DNA, and final mixing the biological materials by merging the first droplet and the second droplet. Ex. 1008 pp. 5558-60.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1078.

Thus, claim 9 is shown to be rendered obvious by the combination of Burns I, Kopp and Burns II.

Dependent Claim 10

Claim 10 of the '193 patent recites “trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels.”

Quake describes a “detection region” which “should generally be at least about twice, preferably at least about five times as large per side or in diameter as the diameter of the largest molecule, cell or droplet that will be passing through it.” Ex. 1034 at 26. This may be contrasted to Quake’s generally recommended dimensions of the fluid channels, which are shaped to cause the droplets to “conform to the size and shape of the channels” by “becom[ing] longer and thinner” as channels narrow. *Id.* at 27. Quake teaches that “an optical trap, or laser tweezers, may be used to sort or direct cells.... The optical trap remains in an ‘off’ mode until a desired particle is detected in the detection region. After detection of a desired characteristic, the particle or cell is ‘trapped’, and thereby directed or moved into the predetermined branch channel for collection.” *Id.* at 67, *see also* FIGs. 14A & B (Input). Thus, Quake’s detection region is an “expansion portion” where the at least one plug would be “trapp[ed]... for a period of time... after the reaction” as recited in dependent claim 10.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1078.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claim 10 is shown to be rendered obvious by the combination of Burns I, Kopp, Burns II, and Quake.

Dependent Claim 11

Claim 11 of the '193 patent recites “wherein the at least one plug is substantially spherical in shape.”

As discussed by Quake, “the microfabricated devices of this invention generate round, monodisperse droplets (such as those illustrated in Frames J and L of FIG. 19” (reproduced below). Ex. 1034 at 27, FIG. 19.

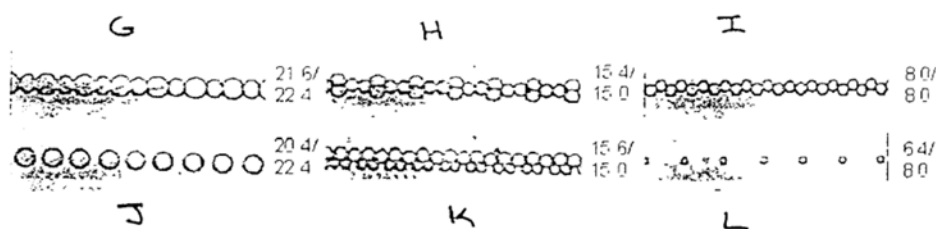


FIG. 19

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1078.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claim 11 is shown to be rendered obvious by the combination of Burns I, Kopp, Burns II, and Quake.

Dependent Claim 12

Claim 12 of the ‘193 patent recites “a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction.”

As Burns II discusses, “[a]ctivating the heaters under the left interfaces propels the drops forward to the channel intersection where they meet and join to form a single larger drop (Fig. 4 B and C). The combined drop is stopped by turning off all heating elements (Fig. 4D) and can be reversed by heating the right interface. Additionally, circulation patterns generated in the drop during motion aid in mixing the liquid sample.” Ex. 1008 at 5558.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1078.

Thus, claim 12 is shown to be rendered obvious by the combination of Burns I, Kopp and Burns II.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '193 patent recites "wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters." Claim 14 of the '193 patent recites "wherein the relevant parameters are selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns."

Kopp teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Kopp meets both claims 13 and 14 under the broadest reasonable interpretation of the term "special design," which in and of itself imparts no meaningful structural limitation. Ex. 1027 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1078.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Burns I, Kopp, and Burns II.

R. The Combination of Burns I, Kopp and Vogelstein Renders Obvious Claims 1-14 of the '193 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The prior art submitted herewith shows that i) droplet micro reactors that formed plugs from continuously flowing immiscible fluids were well known (Burns I), ii) microfluidic PCR devices were well known (Kopp and Vogelstein), and iii) there were compelling reasons to use the microfluidic droplet reactors to conduct microfluidic PCR (Kopp, Burns II, Lagally and Vogelstein).

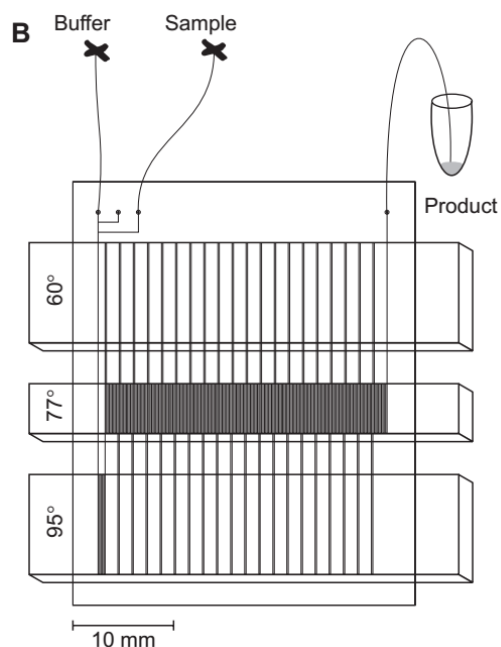
Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10. Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns’ Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel See Ex. 1010; 1027; Ex. 1025 at 23:2-8. Burns I demonstrates that making reactant slugs or plugs with continuous flows of immiscible fluids was a mere design choice as of 2001.

As of the earliest claimed priority date microfluidic devices were routinely being used to perform high-throughput PCR, as demonstrated by Kopp and Vogelstein. By the late 1990s Kopp *et al.* had successfully implemented continuous flow PCR on a chip. In his 1988 article (not previously presented to the USPTO), Kopp explained that “[a] wide range of microreactors, microcapillary electrophoresis devices, and microcell manipulation devices have been described in recent years.” Ex. 1027 at 1046. Building on that body of work, Kopp’s group developed

[a] micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in **continuous flow** at high speed. The device is analogous to an electronic amplifier and relies on the movement of sample through thermostated temperature zones on a glass microchip. Input and output of material (DNA) is **continuous**, and amplification is independent of input concentration. . . .

The results demonstrate that with a very simple device and virtually no optimization, PCR can be performed in **continuous flow**, yielding product quality and quantity comparable to standard thermal cycling methods (Fig. 2B). . . .

We suggest that cross-contamination in a **continuous-flow** format may be much less of a problem than in a stationary-tube PCR format. Thus, a flow-through PCR chip can be reused for extended periods without major cleaning. . . .



Ex. 1027 at 1046-1047 (emphasis added).

Kopp *et al.* also explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications:

To build a complex system analogous to an electronic integrated circuit, it is necessary to integrate a variety of other continuously working parts. A cursory glance at developments in miniaturized total analysis systems (uTAS) during the past 5 years reveals that most of the relevant components, such as **continuous-flow mixers** (8), **continuous-flow microreactors** (9), **continuous separations** (10), and high-speed capillary

gel electrophoresis (as a conventional batch process), have already been presented (11). Accordingly, the **continuous-flow microamplifier** should allow the creation of highly elaborate analysis and synthesis systems.

Although chemical amplification reactors will obviously not be applicable to all reactions, neither will they be limited to PCR. Devices of this kind open up whole new areas of application in medical diagnostics. For example, the online amplification and monitoring of a specific gene could show the patient's ability to metabolize a given drug so as to determine the ideal course of therapy.

Ex. 1027 at 1047 (emphasis added).

By 1999, Vogelstein had reported **various applications for single-molecule microfluidic PCR**. Ex. 1044 at 9241. In a seminal article which has been cited over by over 1,000 other publications, Vogelstein explained that PCR amplification of small aliquots or droplets containing single molecules was effective for “detection of a small number of mutant-containing cells among a large excess of normal cells.” *Id.* at 9236. In Vogelstein's method, the DNA was diluted into 7 μ l volumes such that, on average, there was one template molecule for every two wells (i.e., approximately half of the wells contained no DNA template molecule and approximately half contained a single DNA template molecule). *Id.* at 9236, 9237. This avoided the problem of background noise impairing the determination of whether each volume contained the mutant sequence. *Id.* By counting the number of volumes which indicated wild type or mutant templates, the presence or absence of the mutation (e.g., a sequence associated with colorectal cancer) can be determined. *Id.* at 9238. The accuracy of the test is a function of the number of discrete volumes into which the sample is diluted. *Id.* at 9239. Vogelstein taught that diluting the sample into $\sim$ 1,500 discrete volumes allowed sensitivities up to about 0.1% and that further miniaturization would dramatically increase the assay sensitivity. *Id.* at 9239. (“It is also possible that Dig-PCR can be performed in microarray format, potentially increasing the

sensitivity by another order of magnitude.”) The article explained that the same technique would be useful for **detecting a wide array of mutations, dislocations and allelic imbalances**, as summarized in Table 1. *Id.* at 9241. Vogelstein thus taught that microfluidic PCR had the substantial advantage that it enabled a sample to be diluted into thousands or tens of thousands of discrete reaction volumes that each contained either zero or one DNA template molecule, thereby overcoming the signal-to-noise limitations associated with certain other PCR techniques and enabling the detection of relatively rare mutations, dislocations and allelic imbalances. Ex. 1044 at 9236, 9239, 9241; Ex. 1004 ¶86.

In sum, Kopp and Vogelstein show that microfluidic PCR devices had reached a state of scientific maturity and were in widespread use as of the earliest claimed priority date.

In the instant combination, the microfluidic droplet reactor of Burns I is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., “flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels”).¹⁶⁰ Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶58-71.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I, to conduct microfluidic PCR as taught by Kopp and Vogelstein. A skilled

¹⁶⁰ Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

artisan would have been strongly motivated to perform such PCR reactions in the microfluidic reactor of Burns I because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, “[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**” Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that “[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**” Ex. 1008 at 5556, [emphasis added].

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Vogelstein) to incorporate a droplet reactor (Burns I). Ex. 1004 ¶¶92-109. Using two continuously flowing

immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Vogelstein. . The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful**. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template

concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μL silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μL in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials

are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority

date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactor of Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful modifying Kopp or Vogelstein to work in the microfluidic reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35

U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹⁶¹ As discussed in Section VI.A.3, above, Higuchi I-III thus demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained in more detail in the following sections, the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Kopp and Vogelstein.

Independent Claim 1

Claim 1 [preamble]: A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:

Burns I discloses “[a] multiphase microreactor based upon the use of slug flow through a narrow channel....” Ex. 1007 at 10. “The experimental work discussed here is based on the use of alternating slugs of two liquid phases to provide the environment for mass transfer and reaction within the microreactor.” *Id.* at 10.

In the instant combination, microfluidic droplet reactors (as taught in Burns I) are used to perform PCR reactions (as taught in Kopp and Vogelstein), wherein each plug includes a mixture

¹⁶¹ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570 (Fed.Cir.1985) (‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

of DNA, polymerase, primers, and other reagents. Ex. 1004 ¶¶32-38. PCR reactions are autocatalytic. Ex. 1004 ¶32; Ex. 1001 44:58-61.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1079.

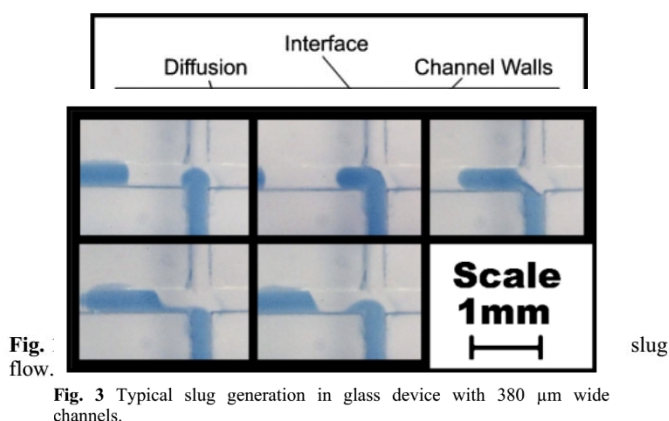
Claim 1[a]: providing the microfluidic system comprising at least two channels having at least one junction;

Burns I explains that “[t]he method discussed in this work **uses the continuous flow of both phases through T or cross-shaped intersections**. Slugs are generated by the action of **one phase flowing into the channel whilst the other phase moves into the intersection**, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11, [emphasis added].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1079.

Claim 1[b]: flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels;

As noted above, Burns I teaches that “[t]he method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Figure 1 shows that the reactant slug is composed of an “**aqueous phase**.” *Id.* at 11. Fig. 3 shows oil-in-



water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns' Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase.

In the instant combination, the microfluidic droplet reactors of Burns I are relied upon to meet all of the limitations of the independent claim of the '193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule (i.e., "flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel of the at least two channels").¹⁶² Ex. 1001 at 78:12-15 and the autocatalytic reaction. Ex. 1001 at 78:8-26. In the instant combination, these limitations are met by using the microfluidic droplet reactors with two continuously flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and other reagents.

A skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Kopp and Vogelstein. A skilled artisan would have been strongly motivated to perform such PCR reactions in the microfluidic droplet reactors of Burns I because doing so have provided the substantial benefits known to be associated with microfluidic droplet reactors. As noted by Lagally, "[t]he advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to **increase the speed** of these assays and to **reduce the amount of material and reagents needed.**" Ex. 1028 at 565, [emphasis added]. Burns II similarly explained that "[t]he combination of pump and components into self-contained miniaturized devices may provide significant improvements in DNA analysis **speed, portability, and cost.**" Ex. 1008 at 5556, [emphasis added]. The reduction of fluid plug size would also have increased the rate at which

¹⁶² Requestor notes that Quake does disclose use of PCR (Ex. 1033 ¶¶6, 80), although that disclosure is not expressly relied upon herein as satisfying any PCR-related limitations.

the droplets or slugs could be thermocycled, thereby increasing the speed of the overall reaction.

Ex. 1004 ¶48; Ex. 1008 at Abstract; Ex. 1027.

It was also well known that miniaturization of PCR reactions to the microfluidic level provided the substantial advantage of making PCR reactors highly portable (sufficient to provide point of care testing). Ex.1027 at 1047; Ex. 1004 ¶100. Kopp explained that such portable PCR microreactors could enable “on-site analysis of patient samples [which] could demonstrate the presence of bacterial DNA and any susceptibility to antibiotic treatment.” Ex. 1027 at 1047. Moreover generally, it was known that portable PCR reactors were useful to aid physicians in development of treatment of various conditions. For instance, identification of a “specific gene could show the patient’s ability to metabolize a given drug so as to determine the ideal course of therapy.” Ex. 1027 at 1047. It was thus known that using the droplet reactor of Burns I to perform PCR advantageously would have allowed PCR reactions to be performed in point of care diagnostic applications.

A skilled artisan would also have been strongly motivated to combine the references in the “opposite” direction, i.e., modifying the microfluidic PCR apparatus (Kopp or Vogelstein) to incorporate a droplet reactor (Burns I). Ex. 1004 ¶¶92-109. Using two continuously flowing immiscible fluids to create droplets would have further enhanced the benefits provided by the fluid microfluidic PCR devices taught in Kopp or Vogelstein. . The use of two continuously flowing, immiscible fluids creates droplets which in turn **further reduces the volumes of reagent** compared to droplets produced using injection of an aqueous fluid into an immiscible fluid (e.g., Corbett or Kopp). Droplet reactors also decrease contamination and increase the number of reactors that can be created on a given chip. Moreover, the smaller reaction volumes would enhance temperature control and reaction specificity. Ex. 1004 ¶¶95, 96.

Moreover, a skilled artisan would have **fully expected the combination to be successful.**

In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR on a microfluidic chip. (“A micromachined chemical amplifier was successfully used to perform the polymerase chain reaction (PCR) in continuous flow at high speed.” Ex. 1027 at Abstract). Kopp noted that all of the underlying components (continuous-flow mixers, continuous-flow microreactors, continuous separations and high speed gel electrophoresis) were already known and predicted that “the continuous-flow microamplifier should allow the creation of highly elaborate analysis and synthesis systems.” *Id.* at 1047. In 1999 and 2000, continuous flow PCR microreactors underwent substantial evolution. In a 2001 article Lagally *et al.* provided an overview of that evolution:

The advent of biological microdevices allows one to consider conducting bioanalytical assays such as PCR at very small volumes to increase the speed of these assays and to reduce the amount of material and reagents needed. Our own work in this area has included DNA fragment sizing and sequencing on capillary and capillary array electrophoresis microdevices,^{7,8} integrated electrochemical detection,⁹ and amino acid chirality analysis.¹⁰ The first microfabricated PCR reactors were constructed from silicon and glass and amplified DNA from template concentrations down to roughly 2000 copies/ μL , in volumes down to 1 μL .¹¹ Since then, stand-alone PCR reactors have been constructed in silicon,¹²⁻¹⁵ glass,¹⁶⁻¹⁸ and fused-silica capillaries.¹⁹ Most designs use resistive heaters surrounding the chambers, but some designs utilize noncontact methods¹⁶ or continuous flow through three differentially heated regions.¹⁸ Unfortunately, most of these designs either require bulk heating,^{16,17} making parallel analyses with different thermal cycling profiles difficult, or require large starting concentrations or large sample volumes.¹⁸ Furthermore, typically the sample must be manually

transferred to an analysis device, introducing external contamination and increasing the time needed for the assay.

Integrated systems combining rapid thermal cycling PCR amplification with capillary electrophoretic (CE) analysis address most of the problems outlined above. In previous work, we developed two generations of small-volume PCR chambers integrated with glass CE microdevices.^{20,21} The first integrated PCRCE device used 10- μ L silicon chambers with polysilicon heaters that were attached to a glass CE microchannel.²¹ This device was capable of amplifying 4×10^5 template copies/ μ L in a time of 15 min. Later work from other laboratories utilizing Peltier elements for thermal cycling have been successful, but have not shown a significant time or sensitivity advantage over conventional thermal cycling systems. An integrated microdevice utilizing submicroliter sample volumes with sensitivity to the single-molecule limit would avoid these shortcomings and could serve as a platform for high-throughput parallelization.

Ex. 1028 at 565-556.

In 2001, Lagally further improved on the state of the art by describing a microfluidic reactor that was successfully used to conduct **single-molecule** DNA amplification by PCR:

Here we present an extensive series of PCR-CE chip experiments at very low concentrations of target to explore the stochastic amplification of single DNA molecule templates. A statistically significant number of trials are performed and analyzed with calculations of normalized peak areas between the single-molecule product and an internal amplification control. The event amplitudes and frequencies at a mean occupancy of ~ 1 template molecule in the reactor are found to conform to a Poisson distribution, demonstrating that we are operating at the single-molecule level. The capability of amplifying single-copy templates combined with microfluidic integration will facilitate the development of enhanced microfluidic diagnostic devices as well as studies of single-cell genetic phenomena and expression variation. . . .

The ability of our device to amplify single DNA molecules provides an unprecedented molecular limit of detection using microfabricated PCR reactors, and this coupled with the integrated capillary electrophoretic analysis forms the basis of a powerful device for high-sensitivity detection of DNA targets. On one hand, this accomplishment will lead to the development of more complex high-density, low-volume microfluidic diagnostic devices even if they do not operate at the single-molecule limit. On the other hand, single-molecule nucleic acid analyses should facilitate studies of expression from individual cells in a population as well as genetic heterogeneity in cases where population averages mask important biological complexity and variation.

Ex. 1028 at 566, 570.

Further, at the time of filing it was **well-known that PCR could be performed in microfluidic plugs or droplets**. More than ten years before the earliest claimed priority date, Corbett reported that PCR was successfully performed in aqueous slugs. Ex. 1010 6:60-62. In 1996, Burns II likewise reported that PCR was successfully performed in aqueous droplets and further explained that “[n]o new biochemistries or DNA detection methods are required” and that “the components simply reproduce current laboratory procedures in a micron-sized environment.” Ex. 1008 pp. 5558-59. In 1998, four years prior to the earliest claimed priority date, Kopp reported continuous flow PCR in microfluidic plugs and explained that because all the relevant underlying components and technologies had already been developed, the lab-on-a-chip concept was applicable to a wide range of diagnostic and medical applications. Ex. 1027 at 1047. As of 2002, there was nothing new or unpredictable about performing PCR in microfluidic plugs or droplets. Consistent with this fact, the **‘193 patent provides no teaching whatsoever as to how to conduct the claimed method of performing PCR in a microfluidic droplet** (claim 3). Ex. 1001 at 44:58-59 (simply stating that “[a]nother example of an

autocatalytic reaction is the polymerase-chain reaction (PCR), which is a very effective amplification method that has been widely used in the biological sciences.”).

Accordingly, the prior art shows that many forms of PCR had been successfully achieved in microfluidic plugs in the years leading up to the earliest claimed priority date. Consistent with the observations of Kopp and Lagally, a skilled artisan would have expected that the microfluidic droplet reactors Burns I could be successfully modified to perform PCR reactions. Ex. 1004 ¶¶92-109.

As further evidence that a skilled artisan would have expected to be successful in modifying Vogelstein or Kopp to work in the microfluidic droplet reactor of Burns I, the new teachings set forth above must be considered in light of the previously undisclosed fact that the same invention was contemporaneously and independently developed by others. In early 2001 a group from the University of Tokyo developed a droplet reactor that falls within the droplet formation of the claims of the ‘193 patent at least as early as February 23, 2001, more than a year prior to the ‘193 patent’s earliest claimed priority date. Ex. 1018-20 (Higuchi I, II and III). The group’s work was published in a patent application (Higuchi I, Ex. 1018) that would qualify as prior art to the ‘193 patent but for the fact that the PCT was published in Japanese rather than English. Pre-AIA 35 U.S.C. §102(e). Accordingly, the Higuchi references are offered as evidence of the level of skill in the art.¹⁶³ As discussed in Section VI.A.3, above, Higuchi I-III

¹⁶³ “In *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH*, 139 F.3d 877 (Fed. Cir.1998), the Federal Circuit considered two particular references before it that were submitted as evidence of contemporaneous independent development, and determined that while the references ‘do not qualify as ‘prior art’ under [§ 102 or § 103(A)],’ the references were nonetheless ‘relevant to obviousness as a secondary consideration.’ See 139 F.3d at 884. In so ruling, the Federal Circuit also noted that evidence of contemporaneous independent invention is also relevant to the level of ordinary skill in the art insofar as the obviousness inquiry is concerned. See *id.* at 883 (‘Although this court has noted the relevance of contemporaneous independent invention to the level of ordinary knowledge or skill in the art, it has also acknowledged the view that this evidence is relevant as a secondary consideration’) (citations omitted); see also *Stewart-Warner Corp. v. City of Pontiac, Mich.*, 767 F.2d 1563, 1570

demonstrate that the use of microdroplet reactors to create droplets from continuously flowing streams of water and oil was within the level of skill in the art as of the earliest effective priority date.

As explained further in the accompanying claim chart (Ex. 1079) and the declaration of Dr. Shaqfeh (Ex. 1004), the prior art and evidence submitted herewith demonstrates that a skilled artisan would have seen compelling reasons to modify the microfluidic droplet reactor of Burns I to conduct microfluidic PCR as taught by Kopp and Vogelstein.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1079.

Claim 1[c]: flowing an oil through the second channel of the at least two channels;

As noted above, Burns I teaches that “[t]he method discussed in this work uses the continuous flow of both phases through T or cross-shaped intersections. Slugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Figure 1 shows that the reactant slug is composed of an “**organic phase**.” *Id.* at 11. Fig. 3 shows oil-in-water slug

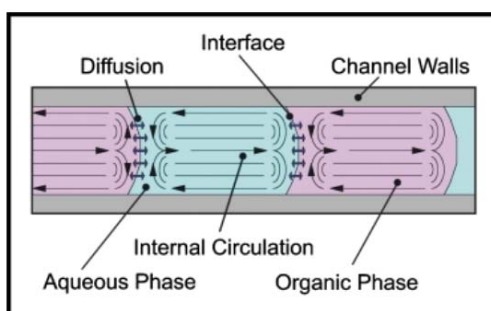


Fig. 1 Illustration of internal circulation generated within immiscible slug flow.

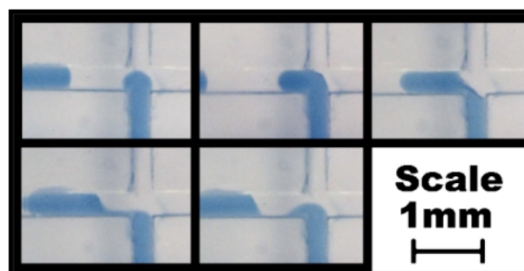


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

(Fed.Cir.1985) (‘Development by others may also be pertinent to a determination of the obviousness of an invention’). *Stewart-Warner Corp. v. City of Pontiac, Michigan*, 767 F. 2d 1563 (Fed.Cir. 1985)

generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns' Fig. 4 embodiment, an aqueous plug of reagents is formed in a continuous oil phase.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1079.

Claim 1[d]: forming at least one plug of the aqueous fluid containing the at least one substrate molecule and reagents by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels, the plug being substantially surrounded by an oil flowing through the channel, wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule;

Burns I teaches forming plugs in the channels of a microreactor by the continuous flow of fluids at an intersecting channel. Burns I explained that “various methods may be used to generate slugs of liquid within a microreactor.” Ex. 1007 at 10. Burns I chose a method in which “the continuous flow of both phases through T or cross-shaped intersections” and “[s]lugs are generated by the action of one phase flowing into the channel whilst the other phase moves into the intersection, eventually cutting off the flow of the first phase into the channel and reversing the process.” Ex. 1007 at 10-11. Fig. 3 shows oil-in-water slug generation and Fig. 4 shows water-in-oil slug generation. *Id.* at 11. In Burns' Fig. 4 embodiment,

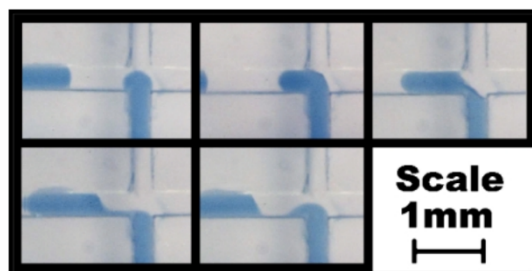


Fig. 3 Typical slug generation in glass device with 380 μm wide channels.

an aqueous plug of reagents is formed in a continuous oil phase. The continuous flow plug formation used by Burns I was a well-known alternative to the approach of periodically injecting a measured slug of reactant into the main channel. See Ex. 1010; 1027; Ex. 1025 at 23:2-8. When read in combination with Higuchi I-III, Quake and Shaw Stewart, Burns I demonstrates that making reactant slugs or plugs with continuous flows of oil was a mere design choice as of 2001.

With regard to the recitation that “the plug [is] substantially surrounded by the immiscible carrier fluid,” Requester submits that this would necessarily occur for any slug or droplet having a volume small enough to not bridge the entire channel width. Indeed, such scenarios are illustrated in Quake (Ex. 1033 Fig. 18) and Shaw Stewart (Ex. 1040 Fig. 1). Accordingly, it would have been obvious to utilize the Burns I droplet generator in a manner that meets the “substantially surrounded” limitation.

In the present combination, the slugs or plugs contain a substrate molecule and reagents for performing a PCR reaction. Ex. 1044 (Vogelstein) p. 9238

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1079.

Claim 1[e]: providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

Burns I discloses that “[t]he high heat and mass transfer capability offered by capillary microreactors allows high intensity reactions to be precisely controlled for optimum yield and selectivity.” Ex. 1007 at 10. “The internal circulation, which is stimulated within the slugs by their passage along the channel, is responsible for a **large enhancement** in the interfacial mass transfer and the **reaction rate**.” Ex. 1007 at 10, [emphasis added].

As discussed above in connection with element 1[b], in the instant combination Burns I is relied upon to meet all of the limitations of the independent claim of the ‘193 patent except the limitations reciting that the flowing fluid with the reagents also includes a substrate molecule and the autocatalytic reaction. Further, as claimed above, this reaction causes the at least one substrate molecule to be amplified. These limitations are met by using the microfluidic droplet reactors with two flowing immiscible fluids (as taught in Burns I) to perform PCR reactions, wherein each droplet or slug includes an aqueous mixture of DNA, polymerase, primers, and

other reagents. Ex. 1004 ¶¶32-39. The DNA is amplified through the PCR reaction. Ex. 1004 ¶¶33-34.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1079.

Thus, claim 1 is shown to be rendered obvious by the combination of Burns I, Kopp and Vogelstein.

Dependent Claim 2

Claim 2 of the '193 patent recites "wherein the at least one substrate molecule is a single biological molecule."

In the combined method of Burns I, Kopp and Vogelstein, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes an aqueous mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. Ex. 1004 ¶¶75, 90. Vogelstein explains "PCR-amplification from single cells isolated by physical separation or dilution has been used to address a variety of interesting biologic questions." Ex. 1044 p. 9239. Thus a skilled artisan would be motivated to isolate a single molecule for PCR reaction. Ex. 1004 ¶¶81-87.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1079.

Thus, claim 2 is shown to be rendered obvious by the combination of Burns I, Kopp and Vogelstein.

Dependent Claims 3 and 4

Claim 3 of the '193 patent recites "wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction." Claim 4 of the '193 patent recites

“wherein the providing step includes heating.” PCR reactions involve thermocycling. Ex. 1004 ¶¶33-34.

In the combined method of Burns I, Kopp and Vogelstein, microfluidic droplet reactors are used to perform PCR reactions wherein each droplet or slug is created using continuous flow of two immiscible fluids and includes a mixture of DNA (the biological molecule) and reagents, e.g., polymerase, primers, buffers and the like. To affect the PCR reaction, the carrier fluid is heated. (“The individual steps are simply performed by heating or cooling the sample to characteristic temperatures: 95°C for dsDNA melting, 50° to 65°C for primer annealing, and 72° to 77°C for primer extension at the optimum enzyme temperature.”) Ex. 1027 at 1046 (Kopp), (“If a PCR product whose sequence is complementary to the loop sequence is present during the heating/cooling cycle, hybridization of the MB to one strand of the PCR product will increase the distance between the Dabcyl and the dye, resulting in increased fluorescence.”) Ex. 1044 at 9237(Vogelstein).

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit 1079.

Thus, claims 3 and 4 are shown to be rendered obvious by the combination of Burns I, Kopp and Vogelstein.

Dependent Claim 5

Claim 5 of the ‘193 patent further recites “providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.”

Vogelstein teaches that the “[r]eactions were analyzed immediately or stored at room temperature for up to 36 h before fluorescence analysis.” Ex. 1044 at 9236. .

In the combined method of Burns I, Kopp and Vogelstein, microfluidic droplet reactors are used to perform PCR reactions after which the reaction products are detected optically, for instance, by electrophoresis. Ex. 1004 ¶¶102, 108.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1079.

Thus, claim 5 is shown to be rendered obvious by the combination of Burns I, Kopp and Vogelstein.

Dependent Claims 6-8

Claim 6 of the '193 patent recites "wherein the oil is fluorinated oil." Claim 7 of the '193 patent recites "wherein the carrier fluid further comprises a surfactant." Claim 8 of the '193 patent recites "wherein the surfactant is fluorinated surfactant."

Quake discloses that "[t]he fluids used in the invention may contain additives, such as agents which reduce surface tensions (surfactants). **Exemplary surfactants** include Tween, Span, **fluorinated oils**, and other agents that are soluble in oil relative to water. Surfactants may aid in controlling or optimizing droplet size, flow and uniformity, for example by reducing the shear force needed to extrude or inject droplets into an intersecting channel." Ex. 1034 at 35, [emphasis added].

The phrase "carrier fluid" in claim 7, having no antecedent basis, is construed to mean "a fluid that is immiscible with a plug fluid," as this is the way the term is defined in the specification. See Ex. 1001 at 7:42-43. Thus, the above quote applies to claims 7 and 8 as well.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1079.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claims 6-8 are shown to be rendered obvious by the combination of Burns I, Kopp, Vogelstein, and Quake.

Dependent Claim 9

Claim 9 of the '193 patent recites "wherein the at least one plug is a merged plug."

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and finally mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1079.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claim 9 is shown to be rendered obvious by the combination of Burns I, Kopp, Vogelstein, and Quake.

Dependent Claim 10

Claim 10 of the '193 patent recites "trapping the at least one plug for a period of time during or after the reaction in an expansion portion in the one or more channels."

Quake describes a "detection region" which "should generally be at least about twice, preferably at least about five times as large per side or in diameter as the diameter of the largest molecule, cell or droplet that will be passing through it." Ex. 1034 at 26. This may be contrasted to Quake's generally recommended dimensions of the fluid channels, which are shaped to cause the droplets to "conform to the size and shape of the channels" by "becom[ing] longer and thinner" as channels narrow. *Id.* at 27. Quake teaches that "an optical trap, or laser tweezers, may be used to sort or direct cells.... The optical trap remains in an 'off' mode until a desired particle

is detected in the detection region. After detection of a desired characteristic, the particle or cell is ‘trapped’, and thereby directed or moved into the predetermined branch channel for collection.” *Id.* at 67, *see also* FIGs. 14A & B (Input). Thus, Quake’s detection region is an “expansion portion” where the at least one plug would be “trapp[ed]... for a period of time... after the reaction” as recited in dependent claim 10.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1079.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Seciton VI.A.3. Thus, claim 10 is shown to be rendered obvious by the combination of Burns I, Kopp, Vogelstein, and Quake.

Dependent Claim 11

Claim 11 of the ‘193 patent recites “wherein the at least one plug is substantially spherical in shape.”

As discussed by Quake, “the microfabricated devices of this invention generate round, monodisperse droplets (such as those illustrated in Frames J and L of FIG. 19” (reproduced below). Ex. 1034 at 27, FIG. 19.

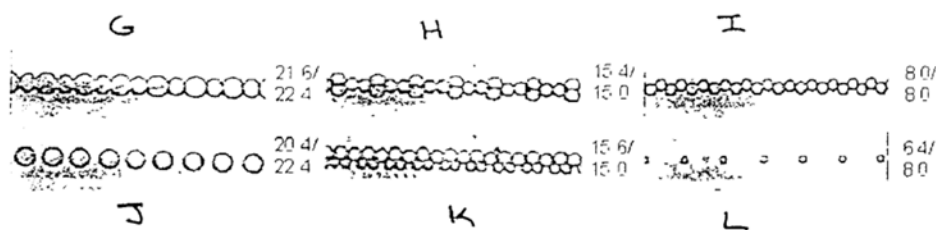


FIG. 19

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit 1079.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claim 11 is shown to be rendered obvious by the combination of Burns I, Kopp, Vogelstein, and Quake.

Dependent Claim 12

Claim 12 of the '193 patent recites "a mixing step, wherein the mixing step occurs via a special design of the at least one channel of the at least two channels below the junction."

Quake describes mixing two biological materials within a plug by first creating a first droplet with the first biological material, then creating a second droplet with the second biological material, and finally mixing the biological materials by merging the first droplet and the second droplet. Ex. 1034 7:10-17.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1079.

A skilled artisan would be motivated to combine the references for the reasons set forth above in Section VI.A.3. Thus, claim 12 is shown to be rendered obvious by the combination of Burns I, Kopp, Vogelstein, and Quake.

Dependent Claims 13-14

Claims 13 and 14 depend from dependent claim 12. Claim 13 of the '193 patent recites "wherein the special design of the at least one channel comprises periodic or aperiodic turns and relevant parameters." Claim 14 of the '193 patent recites "wherein the relevant parameters are selected from the group comprising channel width, period, radius of curvature, and sequence of turns based on the direction of the turns."

Kopp teaches in Fig. 1 that the channel comprises periodic turns. The turns have a periodicity, and thus Kopp meets both claims 13 and 14 under the broadest reasonable

interpretation of the term “special design,” which in an of itself imparts no meaningful structural limitation. Ex. 1027 at Fig. 1.

Additional correspondence between these claim elements and the cited references is shown in the claim chart submitted herewith as Exhibit 1079.

Thus, claims 13 and 14 are shown to be rendered obvious by the combination of Burns I, Kopp, and Vogelstein.

VIII. CONCLUSION

Based on the above, the accompanying references show that there exists at least one substantial new question of patentability as to claims 1-14 of the ‘193 patent. For the reasons set forth in this Request, it is respectfully requested that the *ex parte* reexamination of the ‘193 patent be ordered.

Respectfully submitted,

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