

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

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DROPWORKS, INC.,  
Petitioner,

v.

THE UNIVERSITY OF CHICAGO,  
Patent Owner.

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Patent No. 8,304,193

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**DECLARATION OF CARL D. MEINHART, PH.D.**

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I, Carl D. Meinhart, declare as follows:

**I. ENGAGEMENT**

1. I have been retained by counsel for Dropworks, Inc. to provide my opinion about the patentability of claims 1-9 and 11 of U.S. Patent No. 8,304,193 to Ismagilov, *et al.* (“the ’193 patent,” EX1001). I am being compensated for my time at a rate of \$600 per hour and understand that I will be reimbursed for any reasonable expenses I incur while performing this work. I have no financial or other personal interest in the ’193 patent and my compensation is not contingent on the outcome of this matter or the specifics of my testimony.

**II. QUALIFICATIONS**

2. I am currently a Professor of Engineering at the University of California – Santa Barbara and have been employed there as a professor, associate professor, or assistant professor since 1996. Among other things, I have been teaching undergraduate courses on fluid mechanics and thermodynamics as well as graduate courses on microscale flow transport phenomena. I have served as the advisor dozens of graduate and post-doctoral students from the late-1990s until today.

3. In addition to my academic appointments, I served as a Summer Faculty Fellow for NASA Johnson Space Center and previously was a research scientist at Ford Motor Company. I served as the Chief Technology Officer at

SpectraFluidics Inc., and was a Co-Founder of Pi-MEMS, Inc. and a Founder of Ti-MEMS, Inc. I currently serve as the Chief Executive Officer of Numerical Design, Inc. I have also previously served as an industry consultant and expert witness, including on the subjects of fluid diagnostic instrumentation, heat transfer, polymeric solutions for biomedical applications, microfluidic designs, DNA sequencing, medical devices, and MEMS-based Oscillators.

4. My current research interests include micro-resolution particle image velocimetry for micro-scale flow, enhancing immunoassays and stem cell culturing techniques, Ti-based thermal ground planes for cooling IC chips, and developing free surface fluidics for trace chemical detection.

5. I received my B.S. in Agricultural Engineering, M.S. in Theoretical and Applied Mechanics, and Ph.D. in Theoretical and Applied Mechanics from the University of Illinois at Urbana-Champaign in 1989, 1991, and 1994.

6. Over the years I have been an invited or keynote speaker or seminar presenter at many national and international conferences, including on the topics of microfluidic devices, flow, imaging, velocimetry, BioMEMS & biomedical Nanotech, Nanomedicine, Biological Diagnostics, Micro and NanoTechnology, miniaturization of analytical instruments and micro-systems, Lab on a Chip

7. I am listed as an inventor or co-inventor on more than 15 issued U.S. patents and I am the author or co-author of well over 150 scientific publications

and conference papers. My work has been published in many journals, including *The Proceedings of the National Academy of Sciences; The Journal of Biomedical Microdevices; Biomicrofluidics; Lab on a Chip; Micro and Nanofluidics; The Journal of Physical Chemistry, Analytical Chemistry; Nanomedicine: Nanotechnology, Biology and Medicine; Nanoscale; Journal of Colloid and Interfacial Science; Analyst; Electrochimica Acta; ACS Applied Material Interfaces; Material Research Letters; Physics Review of Fluids; Journal of Fluid Mechanics; Journal of Fluids Engineering; ACS Nano; Physics of Fluids; Journal of Chromatography A; Langmuir; Journal of Nanophotonics; Journal of Heat Transfer; Journal of Physics; Annual Review of Fluid Mechanics; and Experiments in Fluids.*

8. I have served as a reviewer and/or referee for many publications, including The Proceedings of the National Academy of Science, The Journal of Fluid Mechanics, Experiments in Fluids, ASME Journal of Fluids Engineering, among others.

9. I have closely followed the field of microfluidics over the past 25 years, including through research and publications. My experience extends not only to the fluid mechanics involved in such systems, but in the engineering involved in using microfluidic systems for chemical reactions, including enzymatic and other biological reactions.

10. Please see my *curriculum vitae*, which is submitted as EX1003, for a more detailed listing of my credentials and publications.

### **III. LEGAL PRINCIPLES**

11. I have been advised that a claimed invention is not patentable under 35 U.S.C. §103 if the claimed invention as a whole would have been obvious to a person of ordinary skill in the art (“POSA”) at the time the claimed invention was made. I understand that the obviousness analysis does not require that all elements of the challenged claim be disclosed in a single prior art reference.

12. I further have been advised that the ultimate conclusion of whether a claim is obvious should be based upon several factual determinations. That is, a determination of obviousness requires inquiries into: (1) the level of ordinary skill in the field; (2) the scope and content of the prior art; (3) what difference, if any, existed between the claimed invention and the prior art; and (4) any secondary evidence bearing on obviousness.

13. I have been advised that, in determining the level of ordinary skill in the field that someone would have had at the time the claimed invention was made, I should consider: (1) the levels of education and experience of persons working in the field; (2) the types of problems encountered in the field; and (3) the sophistication of the technology.

14. I have also been advised that, in determining the scope and content of the prior art, a reference may be relevant if it is in the same field as the claimed invention or is from another field to which a person of ordinary skill in the field would have looked to solve a known problem.

15. I have been advised that, in considering the obviousness of a patent claim composed of several elements, I should consider whether there was a reason that would have prompted a person of ordinary skill in the field to combine the elements or concepts from the prior art in the same way as in the claimed invention.

16. I have been further advised that obviousness may be demonstrated by showing the claim was merely the result of applying common sense and ordinary skill to solve a known problem. I have been advised that market forces or other design incentives may be what produced a change, rather than true inventiveness. I understand that I may consider whether the claimed subject matter was merely the predictable result of using prior art elements according to their known functions and whether the claimed innovation applies a known technique that had been used to improve a similar device or method in a similar way. I may also consider whether there is some teaching or suggestion in the prior art to make the modification or combination of elements claimed in the patent. I may also consider whether the claimed invention would have been obvious to try, meaning



that the claimed innovation was one of a relatively small number of possible approaches to the problem with a reasonable expectation of success by those skilled in the art.

17. I have also been advised, however, that I must be careful not to determine obviousness using the benefit of hindsight; many true inventions could seem obvious after the fact, in view of what we know today. Thus, in my analysis below, I put myself in the position of a person of ordinary skill in the field at the time the claimed invention was made and did not consider what is known today or what is learned from the teaching of the patent in reaching my conclusions.

18. Finally, I have been advised that any obviousness rationale for modifying or combining prior art should include a showing that a person of ordinary skill would have had a reasonable expectation of success.

19. With regard to secondary considerations of nonobviousness, I have been advised that objective evidence may be considered for an indication that the claimed invention would not have been obvious at the time the claimed invention was made. I understand that the purpose of secondary considerations is to prevent a hindsight analysis of the obviousness of the claims.

20. I have been advised that there are several factors that may be contemplated as a secondary consideration. These factors include the commercial success of the invention, industry praise for the invention, skepticism of those in

the field regarding the invention, licensing of the invention, copying of the invention, a long-felt need that the invention solved, failure of others to achieve the invention, and unexpected results of the invention.

21. I have been further advised that in order for evidence of secondary considerations to be significant, there must be a sufficient nexus between the claimed invention and the evidence of secondary considerations. I understand that this nexus must provide a link between the merits of the claimed invention and the evidence of secondary considerations provided.

#### **IV. SUMMARY OF OPINIONS**

22. In my opinion, each of claims 1-9 and 11 the '193 patent (EX1001) is obvious. Each of these claims recites a method for conducting an autocatalytic reaction in aqueous plugs in a microfluidic system. At a high level, the claims require, among other things, forming a plug at the junction of two microfluidic channels by continuously flowing an aqueous fluid from one channel into the oil continuously flowing in the second channel. The plug is substantially surrounded by the oil flowing through the channel. What the patent's claims call a plug is a water-in-oil (w/o) droplet.

23. The claims require the aqueous fluid flowing through the first channel to contain a substrate molecule and reagents for conducting an autocatalytic reaction. The claims also require providing conditions suitable for the

autocatalytic reaction in the plug such that the substrate molecule is amplified.

The patent specifically identifies polymerase chain reaction of DNA as a qualifying autocatalytic reaction. As I set forth in more detail below, including by analyzing each element of the claims on an individual basis and also as a whole, each of claims 1-9 and 11 as a whole would have been obvious to a person of ordinary skill in the art before the '193 patent. My analysis is based on printed publications that published before the earliest identified priority date of the '193 patent.

24. For example, International Publication No. WO 02/23163 to Quake and Thorsen ("Quake, EX1005) discloses the same steps for forming the aqueous plugs in a microfluidic system as claimed in the '193 patent. Indeed, portions of the '193 patent appear to be identical to what was published earlier in Quake.

25. Quake discloses that its w/o droplets should be used for micro-reactions, including reactions using DNA and other biological materials, and reactions using enzyme catalysts. Quake specifically calls out the enzymatic DNA reaction called polymerase chain reaction ("PCR") for application in its microfluidic system. Quake cites and incorporates by reference Kopp (EX1011) for teaching the fundamentals of microfluidic PCR for amplification of DNA. By disclosing its droplets as enzymatic bioreactors for biological molecules including DNA and specifically calling out PCR for performance on its

microfluidic device, it is my opinion that Quake suggested to a person of ordinary skill in the art to perform PCR in its droplets.

26. It is my opinion that a person of ordinary skill in the art would have had a reasonable expectation for using Quake's system to form droplets in which PCR successfully could be performed before the '193 patent. A person of ordinary skill in the art already would have known that PCR amplification of DNA routinely was performed by combining DNA template with PCR reagents (*e.g.*, primers, deoxynucleotide triphosphate (dNTP) monomers, and polymerase enzyme in aqueous buffer) and then repeatedly cycling the mixture through three temperature stages to initiate and complete the amplification reaction. Quake disclosed all the necessary steps for performing PCR in its droplets through its incorporation of Kopp.

27. Because POSAs knew full-well that PCR does not occur spontaneously upon polymerase contact with DNA substrate, but instead requires thermocycling to perform the amplification reaction, my opinion is that it would have been obvious before the '193 patent to form Quake's droplets using an aqueous fluid that already contained the DNA template and PCR reagents. In fact, a POSA would have recognized that this would have been the simplest and most straightforward way to form Quake's droplets for a PCR reaction and a most suitable and simpler alternative to Quake's proposal to initiate enzymatic

reactions by merging droplets after formation. Claim 1 as a whole thus would have been obvious.

28. Prior art printed publications from before the '193 patent already disclosed combining the DNA template with PCR reagents before droplet formation and subsequently amplifying the DNA template successfully in the droplet using PCR. In view of these references, discussed below, a POSA would have had good reason to include the DNA template and PCR reagents in Quake's aqueous fluid before droplet formation at the junction of the first and second microfluidic channels and it would have been obvious to do so. These references also confirm a POSA had a reasonable expectation of success for achieving what is claimed, including for providing conditions suitable for the autocatalytic reaction such that the DNA substrate is amplified.

29. For example, International Publication No. WO 02/022869 to Holliger and Gahdessy ("Holliger," EX1006), teaches successful PCR amplification of DNA in sub-microliter (*e.g.*, picoliter) microscopic w/o droplets formed in mineral oil in the presence of standard surfactants, such as Span, Tween, and Triton X100. Holliger combined the DNA template and PCR reagents in the aqueous fluid before droplet formation, formed the droplets by adding the aqueous PCR mixture to mineral oil and applying mechanical energy (in this case stirring), and conducted PCR in the droplets by thermocycling. Holliger

demonstrated successful microfluidic w/o droplet PCR and that neither the tiny size of the droplets, any oil-interface interactions, or any surfactant interactions precluded successful PCR amplification with the droplets.

30. As another example, “Synthesis and Analysis of Chemical Components in Nanoscale” by Litborn *et al.* (“Litborn,” EX1007), teaches successful PCR amplification of DNA in sub-microliter (*e.g.*, nanoliter) microscopic w/o droplets in flowing octane oil. Litborn also combined the DNA template and PCR reagents in the aqueous fluid before droplet formation. Litborn formed its w/o droplets in flowing oil and also successfully performed PCR amplification of the DNA template in flowing oil. Litborn demonstrated successful microfluidic w/o droplet PCR in a microfluidic device and that neither the tiny size of the droplets, any wall interactions, or any oil-interface interactions precluded successful PCR amplification with the droplets.

31. As another example, “Flow-through polymerase chain reactions in chip thermocyclers” by Schneegaß and Köhler (“Schneegaß,” EX1009), teaches successful PCR amplification of DNA in sub-microliter microscopic w/o droplets in flowing mineral oil. Schneegaß also combined the DNA template and PCR reagents in the aqueous fluid before droplet formation. Like Litborn, Schneegaß formed its w/o droplets in flowing oil and also successfully performed PCR amplification of the DNA template in flowing oil. Schneegaß demonstrated

successful microfluidic w/o droplet PCR in a microfluidic chip. Schneegaß demonstrated successful microfluidic w/o droplet PCR in a microfluidic device and that neither the tiny size of the droplets, any channel wall interactions, any oil-interface interactions, or any surfactant interactions precluded successful PCR amplification with the droplets.

32. As discussed in detail below, the remaining elements recited in the challenged dependent claims were also disclosed in the prior art and were obvious before the '193 patent.

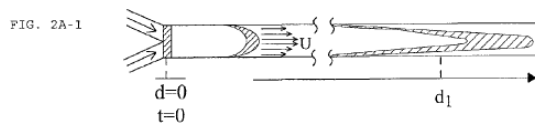
33. Below are exemplary grounds upon which I believe claims 1-9 and 11 of the '193 patent to be unpatentable.

Ground	Claims	References
1	1-9, 11	Obvious over Quake in view of Holliger
2	1-9, 11	Obvious over Quake and Holliger in view of Shaw Stewart (EX1008)
3	1-9, 11	Obvious over Quake in view of Litborn
4	1-9, 11	Obvious over Quake and Litborn in view of Shaw Stewart

## V. SUMMARY OF THE '193 PATENT

34. The '193 patent is entitled “Method for Conducting an Autocatalytic Reaction in Plugs in a Microfluidic System” and is assigned to The University of Chicago (“Patent Owner”).

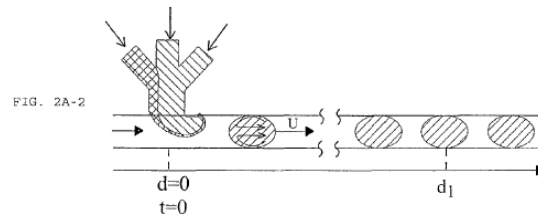
35. According to the '193 patent, “[m]icrofluidics deal with the transport of fluids through networks of channels, typically having micrometer dimensions.” EX1001, 1:20-22. The '193 patent states that fluids are most commonly pumped through microfluidic channels using pressure or electroosmotic flow, that pressure-driven flows are typically significantly less sensitive to surface chemistry than electroosmotic flow, but that pressure-driven flow is prone to Taylor dispersion. EX1001, 1:56-2:2. As illustrated in Figure 2A-1 of the '193 patent, reproduced below, Taylor dispersion can broaden and dilute sample plugs, leaving part of the sample behind the plug as a tail, and resulting in cross-contamination of samples in different plugs. EX1001, 2:3-6, 3:39-40, 17:37-38.



36. The '193 patent states that one way this cross-contamination problem was addressed in the prior art was by introducing samples into the channels individually, separated by buffer or by washing the system with buffer between samples, but that this reduces throughput of the system. EX1001, 2:6-10. In



contrast, the '193 patent employs at least one plug (illustrated in Figure 2A-2 below) that is substantially surrounded by an oil carrier fluid. EX1001, 2:54-58.

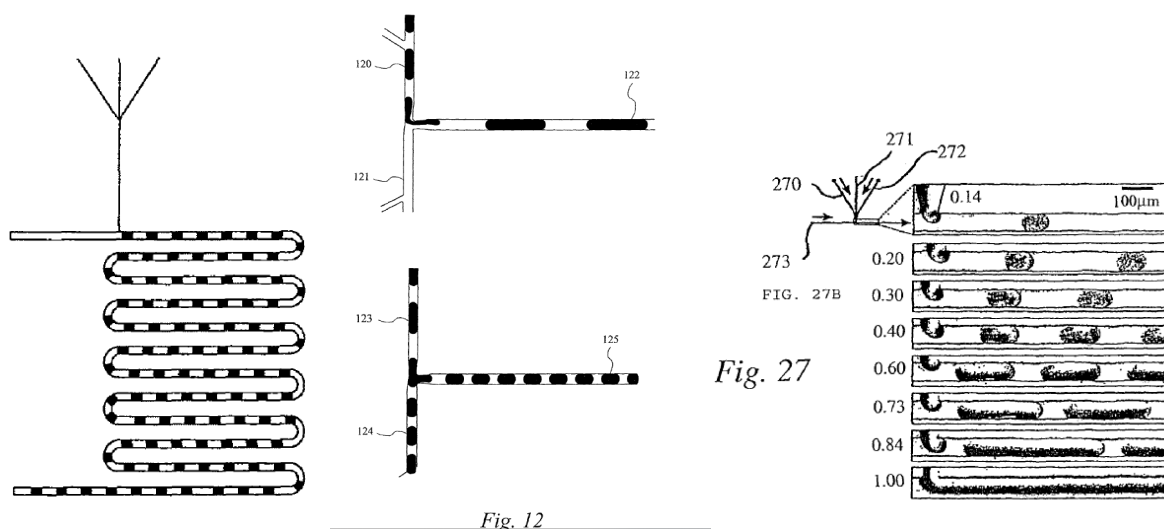


37. The '193 patent teaches that these plugs are formed by injecting at least one aqueous solution into a flowing oil carrier fluid. EX1001, 3:50-56, 18:38-46 (Figs. 4-5 illustrate plug formation through the flow of plug fluid into flowing oil), 20:37-39 (shear force used to “extrude or inject plugs into an intersecting channel”), 21:33-38 (“In the invention, plugs are formed by introducing the plug-fluid, at the plug-forming region, into the flow of carrier-fluid passing through the first channel.”), 71:53-59 (syringe pumps were used to inject aqueous solution and carrier fluid into the microchannels); *see also id.*, 1:25-32, 14:38-41 (using syringe pump to introduce plug and carrier fluid into the channels); 19:45-57 (“The force and direction of flow can be controlled by any desired method for controlling flow, for example, by a pressure differential, or by valve action.”).

38. As illustrated in Figures 3, 12, and 27 (reproduced below), the '193 patent teaches that a stream of plugs may flow down the channel, that the plugs

may be short or elongated, that multiple plugs may be merged together, and that an elongated plug may be separated into smaller plugs after plug formation.

EX1001, 3:46-49, 4:23-25, 5:25-32, 18:14-21, 27:25-27, 28:25-27, 64:20-23.



39. Although the '193 patent forms the plug by injecting a flow of aqueous fluid into a flowing stream of oil, the patent discloses the fluid flow may cease after plug formation. "The flow stream in the first channel is typically, but not necessarily continuous and may be stopped and started, reversed or changed in speed." EX1001, 21:45-47. For a crystallization amplification reaction, the '193 patent states that "the flow is preferably stopped once the desired number of plugs are formed." EX1001, 55:7-8; *see also id.*, 55:17-20 ("the plugs are being formed in the presence of flow"). The '193 patent also contemplates significant spacing between individual plugs. For example, the '193 patent states that "the spacing between plugs can be increased or the oil composition changed to reduce

plug-plug diffusion” and that “spacing of about 2.5 mm in paraffin oil” was used for crystallization trials. EX1001, 56:37-41. The ’193 patent also states that “a sample plug could be split into smaller plugs and forwarded to individual amplification cascades.” EX1001, 12:60-64-10.

40. The ’193 patent states that plugs corresponding to the dimensions of the channel “can be formed in channels having cross sectional areas of, for example, from 20x20 to 200x200  $\mu\text{m}^2$ , which correspond to plug volumes of between about 16 picoliters (pL) to 16 nanoliters (nL), and that the “size of channels may be increased to about 500  $\mu\text{m}$  (corresponding to a volume of about 250 nL) or more.” EX1001, 19:45-57; *see also id.*, 11:24-53 (invention can be used to manipulate and monitor solutions or reactions for microseconds’ to weeks’ in time and at volumes of femtoliters to hundreds of nanoliters).

41. The ’193 patent states that the carrier fluid, the plug fluid, or both may contain additives, such as surfactants to reduce surface tensions and reduce the shear force needed “to extrude or inject plugs into an intersecting channel” or “to control the wetting of the channel walls by fluids,” and that preferred surfactants that may be used that are compatible with the carrier and plug-fluids include Tween<sup>TM</sup>, Span<sup>TM</sup>, and fluorinated surfactants such as Zonyl<sup>TM</sup>, that fluorinated surfactants with a hydrophilic head group are preferred when the carrier fluid is a fluorinated fluid and the plug fluid is an aqueous solution, and that water soluble

surfactants such as SDS may denature or inactivate the contents of the plug.

EX1001, 20:31-52.

42. The '193 patent states that the various devices and methods according to the invention are used to (1) localize reagents inside plugs that are encapsulated by an immiscible carrier-fluid to reduce reagent consumption resulting from substantial dispersion of solutes in microfluidic channels; (2) mix reagent inside the plugs based on chaotic advection to accelerate the mixing process; and (3) carefully control the surface chemistry to which solutions are exposed through a careful selection of surfactants that assemble at the interface between the plugs and the immiscible fluid that surrounds them. EX1001, 11:54-12:17. The '193 patent states that polyethylene glycols (PEGs) and oligoethylene glycols (OEGs) are known to reduce non-specific adsorption of proteins on surfaces and were useful in the claimed invention to do the same thing. EX1001, 35:63-65. A POSA would have understood that the generally-understood difference between OEGs and PEGs is that OEGs have six or fewer carbon atoms in each chain and PEGs have more than 6 carbon atoms in each chain. This distinction is noted, for example, in Sigma Aldrich's product materials.

43. The '193 patent states that the plug can include reagents sufficient for an autocatalytic reaction that amplifies a molecule, that the autocatalytic reaction may be polymerase chain reaction, and that the amplified molecule may be DNA

or RNA. EX1001, 2:58-67. The '193 patent includes no examples where DNA was amplified through an autocatalytic reaction, including PCR. EX1001, 44:58-61, 78:32-35. The provisional applications of the '193 patent do not reference PCR but only "polymerization" and disparate and unconnected reference to manipulating "DNA" in completely different portions of the specification. EX1021, 6:24-25, 8:3-4, 9:12-21, 13:13-20, 22:9-12, 43:4-13, 46:9-10; EX1022, 3:24-27, 5:3-4, 6:13-22, 11:7-16, 31:26-31, 33:11-15, 46:28-47:5, 49:9-10.

44. The '193 patent has 14 claims. Only claim 1 is independent. I have reproduced the text of claim 1 below, but have added notations in brackets to identify individual elements of the claim.

1.[preamble] A method for conducting an autocatalytic reaction in plugs in a micro fluidic system, comprising the steps of:

[a] providing the microfluidic system comprising at least two channels having at least one junction;

[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;

[c] flowing an oil through the second channel of the at least two channels;

[d] forming at least one plug of the aqueous fluid containing 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, [d.1] the plug being substantially surrounded by an oil flowing through the channel, [d.2] 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

[e] providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified.

EX1001, 78:8-29.

45. Claims 2-9 and 11 depend directly or indirectly from claim 1.

EX1001, 78:30-52. Claim 2 recites the at least one substrate molecule is a single biological molecule. Claim 3 depends from claim 2 and recites the substrate molecule is DNA and the autocatalytic reaction is polymerase-chain reaction.

Claim 4 recites the providing step includes heating. Claim 5 recites the method comprises providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during or after it has occurred.

Claim 6 recites the oil is fluorinated oil. Claim 7 recites the carrier fluid comprises a surfactant. Claim 8 depends from claim 7 and recites the surfactant is fluorinated surfactant. Claim 9 recites the at least one plug is a merged plug.

Claim 11 recites the plug is substantially spherical in shape.

### **A. Priority Date for the '193 patent Claims**

46. The '193 patent states on its front page that it was filed on February 9, 2011 as U.S. Patent Application No. 13/024,155. The patent states that this application is a continuation of application No. 12/777,099, filed on May 10, 2010, which is a continuation of application No. 10/765,718, filed on Jan. 26, 2004, which is a continuation-in-part of application No. 10/434,970, filed on May 9, 2003. The '193 patent also claims entitlement to Provisional application No. 60/394,544, filed on July 8, 2002, and Provisional application No. 60/379,927, filed on May 9, 2002. As such, I understand the earliest claimed priority date of the '193 patent (and a relevant date for evaluating whether the claims are obvious) to be May 9, 2002, the filing date of the '927 Provisional application

### **B. File History of the '193 patent**

47. Before issuance, I understand that the patent examiner rejected certain claims as anticipated by a 2007 publication because the examiner believed the claims were not entitled to a priority date before 2007. EX1004, 0496-0501. The examiner also rejected the claims because the examiner believed the '155 application failed to provide written description support for performing an autocatalytic reaction for a plug containing only a single first species of molecule. EX1004, 497.

48. In response, Patent Owner stated that the claims were entitled to the May 9, 2002, priority date but only cited examples as far back as the '718 application (filed Jan. 26, 2004 to disqualify the 2007 publication as prior art. *Id.*, 0331, 0404-0405. Patent Owner argued that there was adequate support for the pending claims of the '155 application in the '099 application, including identifying Example 8 as an example of an autocatalytic amplification reaction, Example 9 as amplification for single molecule detection, and visual detection of a single molecule of DNA labeled with nanoparticles via an autocatalytic pathway. EX1004, 0331-0359. Patent Owner similarly argued there was support in the 10/765,718 application to provide a priority date of January 26, 2004. EX1004, 0359-0386.

49. To address the written description rejection, Patent Owner argued that Examples 8-9 of the '155 application describe conducting an autocatalytic reaction on a single substrate molecule within a plug of fluid to amplify the substrate molecule. EX1004, 0387-0401. Patent Owner argued that there was support for the PCR dependent claim because that specification identifies PCR as an autocatalytic reaction that may be conducted with the methods of the invention, because Examples 8-9 report conducting autocatalytic reactions on single substrate molecules in plug fluids to amplify the substrate molecule, and



because Example 10 shows conducting enzyme based reactions in a plug fluid.

EX1004, 0402.

50. The Examiner responded that Examples 8-9 disclose an autocatalytic reaction exclusively for inorganic complex of  $\text{Co}^{2+}$  but that the concentration of substrate molecules was almost two per plug. EX1004, 0316-0320, 0324-0325.

Patent Owner responded by further amending the claims, including to replace the claim element of “conducting an autocatalytic reaction” such that at least one substrate molecule is amplified to “providing conditions suitable for” an autocatalytic reaction such that at least one substrate molecule is amplified.

EX1004, 0298.

51. The Examiner issued a Notice of Allowance saying that the claimed subject matter “was disclosed in the parent case having priority over all relevant art found by the examiner” and that the pending claims were also supported by the disclosure of the instant application, as originally filed. EX1004, 0282, 0274.

52. The originally-filed claims did not specify how the at least one plug was formed and required that the plug contain no more than a single molecule “of the first species.” EX1004, 0679. In response to indefiniteness rejections, Patent Owner amended the claim to specify that the plug of aqueous fluid is formed “by partitioning the aqueous fluid as it continuously flows through a first of the one or more channels.” *Id.*, 0329. Patent Owner subsequently amended the claims to

specify that the plug is substantially surrounded by an oil “flowing through the channel” and asserted that the amendment “is not intended to, and does not, change the scope of the claims.” *Id.*, 0298, 0300. The Examiner issued an Examiner’s Amendment specifying that the aqueous fluid flows through a first channel, the oil flows through a second channel, and the at least one plug is formed by partitioning the aqueous fluid with the flowing oil “at the junction” of the two channels. *Id.*, 0281.

53. In the Notice of Allowance, the Examiner stated that US 2006/0257893 discloses an “analogous reaction” but is not prior art because it was filed on February 17, 2006. *Id.*, 0282. The examiner stated that a 2001 article by Thorsen refers to the formation of droplets by continuous flows of two liquids but “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.” *Id.* The examiner stated that Quake and Thorsen in US 2002/0058332 “disclose a system with two channels and the method of forming plugs (droplets) with reagents in immiscible oil” but that “the droplets in 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,” which the Examiner stated “is a different approach from the claimed invention.” EX1004, 0283.

### **C. Patent Office Proceedings After Issuance**

54. I understand that a different company than Dropworks twice asked the Patent Office to review the '193 patent after the patent issued. The company, 10X Genomics, Inc., first asked the Patent Trial and Appeal Board to review the patent based on Haff (EX1010) and US 2002/0058332 to Quake and Thorsen (EX1029), with Ramsey (EX1037) being asserted against claim 6. EX1025, i, 1-2

55. 10X Genomics asserted that Haff demonstrated that microfluidic PCR had been successfully performed by May 2002 and that Quake demonstrated generating plugs of an aqueous fluid encapsulated by an immiscible carrier fluid before May 2002. *Id.*, 1. 10X argued that a POSA would have been motivated to perform PCR in Quake's microscopic droplets to reduce the amount of reagents used and that combining the two references would require "no more than routine skill." *Id.*, 1-2.

56. In response, Patent Owner argued that 10X "fails to identify a single reference that teaches chemical reactions in microfluidic droplets," and had effectively conceded that "no one had ever discovered the inventions protected by the claims of the '193 patent." EX1026, 1. Patent Owner argued that the "use of microfluidic droplets as chemical microreactors was at most an aspiration until Ismagilov and co-workers made this goal a reality." *Id.* Patent Owner conceded that "microfluidic systems and microfluidic droplets had long been known," but

argued that “researchers had not successfully used droplets as ‘microreactors’ for chemical reactions.” *Id.*, 7.

57. Patent Owner argued that the “tiny size” of “microfluidic droplets” ranging from femtoliter to nanoliter range presented “a host of unique problems inapplicable to bulk scale chemical experiments” and “prevented researchers from using microfluidic droplets as chemical reactors.” *Id.* Among other things, Patent Owner argued that difficulties with mixing reagents, adherence of droplet material to channel walls and the droplet/carrier-fluid interface, and “environments incompatible with biological molecules” prevented researchers from using microfluidic droplets as chemical reactors. *Id.*, 7-8. Patent Owner thus argued that PCR and other autocatalytic reactions “could, for the first time, be carried out using microfluidic droplets” only with the benefit of the “Ismagilov Patents.” *Id.*, 8-9. Patent Owner argued that the use of polyethylene glycols (PEGs) or oligoethylene glycols (OEGs) to reduce non-specific adsorption of proteins on the droplet-carrier-fluid interface “must be addressed in seeking to conduct autocatalytic reactions (such as PCR) in droplets.” *Id.*, 20.

58. With reference to the references 10X asserted, Patent Owner stated that US 2002/0058332 to Quake and Thorsen (EX1029) forms droplets “by introducing a flow of a fluid that may ‘contain a biological sample (e.g., molecules of an enzyme or a substrate, or one or more cells, or one or more viral

particles)’ into a main channel through which a fluid is flowing that is immiscible with the first fluid.” *Id.*, 13-14. Patent Owner also stated that “the defining attribute of plugs” as claimed is that they “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.” *Id.*, 18-19.

59. Patent Owner also agreed that US 2002/0058332 is “in the microfluidics field” and “teaches the use of microfluidic droplets” but argued that Quake uses microfluidic droplets only for particle sorting and that “Ismagilov and colleagues had ‘pioneered’ PCR reactions in droplets.” *Id.*, 2, 12. Patent Owner argued that US 2002/0058332 “teaches forming droplets containing only a single biological molecule, without a reagent,” and merging this droplet with another droplet containing reagents, instead of forming the droplet with the biological molecule and the reagent both inside. *Id.*, 25-26.

60. Patent Owner argued against combining Haff and US 2002/0058332, saying that there cannot be a thermal insulator between different portions of a channel as disclosed in Haff when the channel is integrated into a wafer substrate, as in US 2002/0058332. *Id.*, 39. Patent Owner also argued that US 2002/0058332 provides no teaching how to maintain a thermal gradient over the smaller dimensions of Quake. *Id.*, 40. As discussed in detail below, it was well within the skill of the art to thermocycle PCR reactions in microscopic w/o

droplets in a microfluidic device, as evidenced by the prior art Litborn and Schneegaß references.

61. Patent Owner argued that it would not have been straightforward to package PCR reagents into microfluidic droplets at the right concentration. *Id.*, 40-41. As discussed in detail below, it was well within the skill of the art to package PCR reagents into microfluidic droplets at the right concentration in microscopic w/o droplets, as evidenced by the prior art Holliger, Litborn and Schneegaß references.

62. Patent Owner also argued that the carrier fluid flow rate for forming droplets in US 2002/0058332 was likely 6.4 cm/second, and that performing PCR at this flow rate would require channels 21 meters long. *Id.*, 41. As discussed in detail below, it was well within the skill of the art to have adequate time to complete thermocycling of PCR reactions in microscopic w/o droplets in microfluidic devices, as evidenced by the prior art Litborn and Schneegaß references, among others.

63. Patent Owner argued that the tiny volumes of microfluidic droplets, relatively large surface-to-volume ratios, and chemical interactions with the carrier fluid would lead to adsorption of proteins. *Id.*, 43. As discussed in detail below, it was well within the skill of the art to successfully amplify DNA in PCR reactions in microscopic w/o droplets despite the large surface-to-volume ratio

and potential interactions with (including protein absorption by) the oil carrier fluid, as evidenced by the prior art Holliger, Litborn and Schneegaß references.

64. Patent Owner also argued that there was a “long felt need for methods of conducting biological reactions in [microfluidic] droplets” that was solved by “Ismagilov and co-workers” in 2003. *Id.*, 48-49. Patent Owner argued that there was a long-felt need for creating the conditions suitable for an autocatalytic reaction in a plug that Ismagilov and co-workers filled when they allegedly “unlocked microfluidic droplets as systems for studying autocatalytic biological reactions” by “the use of specific fluorinated surfactants including a hydrophilic oligoethylene glycol head group (Rf-OEG surfactants) to provide an inert, biocompatible interface.” *Id.*, 55-56. As discussed in detail below, it was well within the skill of the art to successfully amplify DNA in PCR reactions in microscopic w/o droplets without the ’193 patent and without the use of Rf-OEG surfactants (which are not claimed in the ’193 patent), as evidenced by the prior art Holliger, Litborn, and Schneegaß references.

65. Although Patent Owner conceded that Haff teaches successful PCR amplification, Patent Owner argued that “Haff operates on a size-scale” of 10 microliters to 1.5 milliliters using capillary tubes with inner diameters of 1-2 mm, “orders of magnitude greater than expected in a microfluidic system” and is “expressly direct to *increasing* the volume of a PCR reaction, the exact opposite

of what one hopes to achieve by using droplets[.]” *Id.*, 3, 11-12 (emphasis in original). In contrast, Patent Owner argued that channels in the ‘193 patent invention typically have micrometer dimensions. *Id.*, 6. Moreover, Patent Owner argued that Haff does not teach flowing an aqueous fluid containing substrate molecule and reagents for an autocatalytic reaction through one channel and flowing an oil through another channel, but instead teaches “‘*alternatively* injecting’ aqueous fluid containing reagents and then immiscible fluid.” *Id.*, 24-25. In contrast, none of Holliger, Litborn or Schneegaß are directed to increasing the volume of the PCR reaction, but are instead directed to performing PCR in microscopic (e.g., picoliter- or nanoliter-sized w/o droplets) formed in an immiscible oil.

66. The PTAB concluded that “Haff’s disclosure of PCR in a larger-volume capillary tube devices logically would have commended itself to the inventors’ attention when considering methods of conducting PCR in smaller microfluidic devices.” EX1027, 15. The PTAB stated that “we found that the inventors logically would have considered how PCR is conducted in capillary tube devices to figure out how to scale down the reaction for microfluidic devices.” *Id.*

67. The PTAB denied 10X’s petition. EX1027, 15-18. The PTAB said that 10X cited insufficient evidence to support its statement that it “would have



required no more than routine skill and knowledge in the art to adapt Haff's capillary PCR device to use Quake's method of creating encapsulated plugs...to perform PCR in a microfluidic device." *Id.*, 16. The PTAB stated that 10X "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." *Id.*, 16-17. The Board concluded that "[s]imply stating it would have been 'routine' is insufficient." *Id.*, 17. The Board noted that 10X had "not explained why" conducting PCR in a *stream* of reaction mixture in a microfluidic device would make it routine to conduct PCR in *droplets* in a microfluidic device. *Id.*, 17. The Board also noted that 10X did not explain "whether the conditions for conducting PCR in microfluidic droplets would differ[.]" *Id.*, 17. The Board found that 10X's expert testimony was an "unexplained opinion" that did not disclose the underlying facts or data on which it was based. *Id.*, 17-18. In contrast, the prior art Holliger, Litborn and Schneegaß references asserted here demonstrate POSAs already had successfully conducted PCR in microfluidic w/o droplets, including in microfluidic devices.

68. 10X subsequently asked the Patent Office to have another examiner review the '193 patent. EX1028, 4399. That Examiner rejected claims 1-14 as obvious over WO 2002/023163 ("Quake," EX1005) in view of Kopp (EX1011)

and GB 2,097,692 (EX1020). *Id.*, 1899-1900. The Examiner found that Quake teaches microfluidic devices that may be used to conduct PCR, an autocatalytic reaction. *Id.*, 1899. The Examiner found Quake's microfluidic device forms the claimed plugs of aqueous fluid at the junction of two channels by flowing the aqueous fluid containing the sample into a flowing oil so that the plugs are substantially surrounded by the flowing oil. *Id.*, 1899-1900. The Examiner found that Quake teaches continuous flow droplet formation as claimed. *Id.*, 1915-16.

69. The Examiner found that Quake does not specify that the aqueous fluid contains at least one substrate molecule and reagents for conducting the autocatalytic PCR reaction and does not discuss what conditions are suitable for an autocatalytic reaction. *Id.*, 1900. The Examiner found that Kopp discloses performing PCR in a microfluidic device and that the sample fluid contains PCR reagents. *Id.*, 1900. The examiner found that GB 2,097,692 teaches a microfluidic apparatus much like that disclosed by Quake for initiating and controlling chemical reactions. *Id.*, 1900-01. The examiner found that it would have been obvious to provide the substrate molecule and reagents specific to PCR and to provide the necessary conditions (temperature cycling) for autocatalytic reaction disclosed by Kopp in Quake's droplets because Quake specifically points to the PCR method of Kopp for practicing PCR in the microfluidic device. *Id.*, 1901.

70. Regarding claim 2, the Examiner found that Quake discloses that each droplet contains on average no more than one particle of biological material, which may be a polynucleotide. *Id.*, 1901. For claim 5, the Examiner found that Quake discloses a detection region downstream of the droplet extrusion region and that Kopp discloses fluorescence analysis. *Id.*, 1902. For claims 6-8, the Examiner found Quake teaches the oil may comprise fluorinated oils and other surfactant additives to aid in controlling droplet size, flow, and uniformity. *Id.*, 1902. For claim 9, the Examiner found that Quake teaches forming merged plugs and that Kopp teaches the addition of intermixed reactants (Taq polymerase, primers, DNA templates, and nucleotides). *Id.*, 1902. For claim 10, the Examiner found that Fig. 6 of GB 2,097,692 discloses trapping the plug within an expansion portion during or after the reaction. *Id.*, 1903. For claims 12-13, the Examiner found that Quake teaches routing the droplets through different channel architectures such as an S-shaped channel. *Id.*, 1903. For claim 14, the Examiner found that Quake discloses more curvature is beneficial where less resistance to flow is desired. *Id.*

71. During an examiner interview, Patent Owner argued microdroplets have “unique surface chemistry” and that a “unique set of conditions” is “necessary to achieve autocatalytic reaction within a microdroplet,” including “a specific surfactant to prevent denaturation of proteins within the microdroplet

and that not all surfactants will achieve the necessary conditions.” *Id.*, 1882. Patent Owner argued that the decane oil and mineral oil disclosed in Quake “would not be effective in creating the conditions suitable for autocatalytic reaction,” including Quake’s fluorinated oil additive. *Id.*, 1882. Patent Owner argued that Kopp pertains to “slug flow,” which slugs it said do not require the same reaction conditions as droplets, and that Quake does not suggest performing PCR in droplets, but merely that it can be performed on the chip. *Id.*, 1882. Patent Owner argued that GB 2,097,692 does not disclose using its droplets for any reaction. *Id.*, 1882.

72. Patent Owner repeated these arguments in its Office Action Response. *Id.*, 244-277. Patent Owner argued that there were no droplets in prior art continuous flow systems such as Kopp. *Id.*, 249-50. Patent Owner argued that Kopp is not a microfluidic droplet reference and instead injects discrete 10 µl slugs into the channels one-by-one, does not use a carrier fluid that is immiscible with a reagent fluid, and does not teach conditions suitable for carrying out PCR in droplets. *Id.* 264-65. Patent Owner argued that combining Quake’s speed with Kopp would require a channel length of 11.5 m to complete PCR. *Id.*, 275.

73. For water-in-oil droplets, in contrast, Patent Owner argued that the droplet-carrier interface had to be “inert to the adsorption of proteins” and that using “an incorrect oil/surfactant systems leads to the destruction of proteins and

thus does not provide suitable conditions for PCR in droplets.” *Id.*, 254-56. In particular, Patent Owner argued that using fluorinated oils as the carrier fluid integrated with fluorinated surfactant provides “an inert surface that prevents destruction of proteins” and “was not disclosed in the prior art.” *Id.*, 257-58. In contrast, Patent Owner argued that “traditional hydrocarbons, such as decane, tetradecane, hexadecane, and mineral oil” used as a carrier fluid in Quake “are not remotely bioincompatible and will destroy proteins.” *Id.*, 261. Patent Owner argued that the fluorinated oil surfactant additives Quake teaches would coat the channel walls would be insufficient to achieve biocompatible conditions for PCR in a droplet. *Id.*, 261. Patent Owner also argued that scientists praised Ismagilov for identifying fluorinated oils that were nearly impermeable to water, oligoethylene glycol-capped perfluorinated surfactants to prevent protein adsorption at the interface of the droplets, and perfluoramines to provide high surface tension. *Id.*, 277. As discussed above, Patent Owner’s assertions that traditional hydrocarbons such as decane and mineral oil would preclude PCR amplification and that fluorinated oils, OEG-capped perfluorinated surfactants and perfluoroamines were necessary for performing droplet PCR are simply incorrect, as demonstrated as least by Holliger, Litborn, and Schneegaß.

74. Accepting Patent Owner’s representations, the Examiner found the claims allowable. The Examiner concluded that Kopp does not teach a droplet

using oil as a carrier fluid. *Id.*, 9. The Examiner found that the providing step of the claims includes preventing enough unwanted protein adsorption on the droplet surface to conduct PCR in the droplet, such as by using a perfluorocarbon carrier fluid. *Id.*, 7-8. The examiner stated: “Instead of using biocompatible fluorinated oils and fluorosurfactants, Quake teaches traditional hydrocarbons which are not biocompatible and will destroy proteins.” *Id.*, 8-9. As discussed above, however, Patent Owner’s assertions that traditional hydrocarbons such as decane and mineral oil would preclude PCR amplification and that fluorinated oils, OEG-capped perfluorinated surfactants and perfluoroamines were necessary for performing droplet PCR are simply incorrect, as demonstrated as least by Holliger, Litborn, and Schneegaß.

#### **D. Claim Terms**

75. I have been advised that the claims terms of the ’193 patent are to be given their plain and ordinary meaning, *i.e.*, the meaning that the terms would have to a person of ordinary skill in the art at the time of the invention.

76. I understand that this analysis focuses on intrinsic evidence, including how the patentee used the claim term in the claims, specification, and prosecution history. I also understand that dictionaries or other extrinsic sources may assist in determining the plain and ordinary meaning but cannot override a meaning that is

unambiguous from the intrinsic evidence. I have followed these principles in my analysis throughout this declaration.

77. The '193 patent defines certain claims terms. For example, the patent defines the term "channel" to refer "to a conduit that is typically enclosed, although it may be at least partially open, and that allows the passage through it of one or more types of substances or mixtures" and can "assume any form or shape such as tubular or cylindrical," and may have one or more cross sectional shapes such as rectangular, circular, triangular. EX1001, 7:45-55. A channel "may be connected to at least one other channel through another type of conduit such as a valve." EX1001, 7:61-63.

78. As another example, the '193 patent defines the term "flow" to mean "any movement of a solid or a fluid such as a liquid" and specifies that the force "used to provide a flow" includes "without limitation: pressure, capillary action, electro-osmosis, electrophoresis, dielectrophoresis, optical tweezers, and combinations thereof[.]" EX1001, 8:30-41.

79. I am informed that certain claim terms of the '193 patent have been interpreted by the courts. For example, I understand that a federal trial judge interpreted the term "microfluidic system" to mean "system comprised of at least one substrate having a network of channels of micrometer dimension through which fluid may be transported." EX1031, 6-7.

80. I understand that the trial judge interpreted the term “reagent” to mean “component of a plug-fluid that undergoes or participates in at least one type of reaction to produce one or more reaction products or intermediates which may undergo a further reaction or series of reactions.” *Id.*, 7-9. The judge interpreted the term “reaction” to mean “physical, chemical, biochemical, or biological transformation.” *Id.*, 9-10. The judge determined that the term “providing conditions suitable for” the autocatalytic reaction means “providing a set of physical and chemical conditions that allow” the reaction to occur. *Id.*, 10-11.

81. I understand that a federal appellate court determined the preamble to claim 1 limits the claims and requires that the reaction take place in the droplets while the droplets are in the microfluidic system. EX1035, 20-24 & n.3-4. I understand that the federal trial judge found that the claims do not require the plug to continue flowing through the channel while the autocatalytic reaction takes place. EX1031, 6-7; *see also* EX1032, 3-5.

82. I understand that this same federal trial judge interpreted the term “polymerase chain reaction” to mean a method of amplifying target sequence of nucleic acid 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. EX1033.

83. I understand that the federal trial judge interpreted the term “autocatalytic reaction” to mean a reaction in which a product of a reaction is also a reagent for the same reaction. EX1034, 2-3. The judge interpreted the term “fluorinated oil” to mean an oil that includes one or more fluorine atoms.” *Id.* The judge interpreted the term “fluorinated surfactant” to mean an agent that reduces the surface tension between two liquids or a liquid and a solid, which includes one or more fluorine atoms. *Id.* The judge interpreted the term “plug” to mean a volume of liquid formed in a substrate when a stream of at least one plug-fluid is introduced into a flow of a carrier-fluid in which it is substantially immiscible.” *Id.*, 3. The judge interpreted the term “surfactant” to mean an agent that reduces the surface tension between two liquids or a liquid and a solid. *Id.*

84. I have applied the courts’ interpretations of the claims in my analysis below.

85. As discussed above, the prosecution Examiner distinguished the prior art then of record on the basis that the claims require that the plugs be formed “from two continuously flowing immiscible fluids,” as opposed to being formed “by a discrete dispensing the solution with biological material into the carrier fluid,” which the Examiner stated “is a different approach from the claimed

invention.” EX1004, 0282-0283. As discussed in detail below, Quake discloses the claimed droplet formation steps, including the requirement that the aqueous fluid continuously flows into the flowing oil. EX1001, 78:8-26.

## **VI. PERSON OF ORDINARY SKILL IN THE ART**

86. I have been asked to consider the level of ordinary skill in the art as of May 2002 and May 2003. As I noted earlier in Section III, I have been advised that, in determining the level of ordinary skill in the field that someone would have had at the time the claimed invention was made, I should consider: (1) the levels of education and experience of persons working in the field; (2) the types of problems encountered in the field; and (3) the sophistication of the technology.

87. The '193 patent relates to the field of microfluidic systems and conducting reactions in microfluidic systems. EX1001, 1:15-2:50. I am very familiar with each of these fields. I have worked with microfluidic systems and conducting reactions in microfluidic systems since the mid 1990s. I have followed the state of the art throughout the ensuing decades.

88. In my opinion, a person of ordinary skill in the art at the relative time would have been a researcher interested in microfluidics with at least a bachelor's degree in a science such as bioengineering, chemical engineering, chemistry, physics, mechanical or aeronautical engineering, or biochemistry; or a bachelor's or master's degree in a relevant discipline and three to five years of

relevant experience. A POSA may have worked as part of team, which may have included a biochemist, biologist, or a PCR specialist. Those of ordinary skill in the art would have been familiar with the state of the art as described below in Section VII.

## **VII. STATE OF THE ART**

89. The discussion below summarizes the state of the art as of May 2002 and May 2003. Based on my experience, those of ordinary skill in the art were familiar with the state of the art as outlined below and were capable of implementing the features discussed below.

90. Polymerase chain reaction, PCR for short, is a reaction routinely carried out using a polymerase enzyme such as *Taq* as the catalyst for making a new polynucleotide polymer molecule, normally DNA, by interacting with an existing polynucleotide (DNA) template polymer molecule. PCR is thus a species of autocatalytic amplification reaction as that term is used in the claims of the '193 patent. During the PCR reaction, deoxynucleotide triphosphate monomers (dNTPs) are polymerized together into the new DNA strand polymer. The PCR reaction was published in the mid-1980s and was extremely well known, routine, and conventional, and was used widely by 2002 and 2003. U.S. Patent No. 8,033,800 to Haff et al., issued in March 2000, provides a description of PCR as

a DNA amplification reaction that falls within the meaning of autocatalytic reaction as used in the '193 patent. EX1010, 1:16-45.

91. As explained in Haff and countless other publications available before 2002 and 2003, one routinely performs PCR by first creating an aqueous PCR mixture containing template DNA, polymerase enzyme, polymerase primers, dNTPs, and buffer. EX1010, 1:16-45; EX1009, 103. These reagents were all well-known well before the claimed priority date. EX1010, 1:16-45; EX1011, 1046-47; EX1009, 103.

92. After the PCR mixture is combined, the PCR reaction is routinely initiated and completed by repeatedly cycling the mixture between three temperature stages. EX1010, 1:16-45; EX1009, 103. This is called thermocycling. In the first stage, the temperature is high enough to separate the two strands of the double helix DNA molecule from one another. This is often referred to as denaturation, unzipping, or melting the DNA. 94-95°C was a most common temperature for melting DNA for PCR. The second temperature stage is lower than the first. At this temperature stage, the primers, which are short polynucleotide molecules, bind to their DNA targets. This is called annealing. Annealing temperature depended on the specific primers used but 50-60°C was a most common temperature for annealing. The third temperature stage is when the polymerase enzyme adds dNTPs to the primers. This is called extension. 72°C

was a most common temperature for extension. During the extension phase, the nucleotide monomers are added to the ends of the primers to extend the strand and create a second strand of DNA that is complementary to the DNA strand used by the polymerase as a template.

93. Each cycle of three temperature stages doubles the amount of template DNA in the mixture, thereby exponentially amplifying the DNA. EX1010, 1:16-45; EX1009, 103. In each cycle, the DNA templates created as the product of the last polymerase reaction cycle serves as a reagent (the polynucleotide template) for the next polymerase reaction cycle. PCR is thus a species of autocatalytic reaction as that term is used in the '193 patent. As discussed above, PCR was so well-characterized that practitioners knew how to perform it and which variables could impact its success. Persons of ordinary skill were well-versed in successfully performing PCR and well-equipped to successfully perform PCR.

94. The first PCR thermocyclers operated on reaction tubes and later in mm-scale capillary tubes, as described in Haff. EX1010, 1:50-2:6. PCR reaction mixture volumes were thus on the scale of tens of microliters and generally were no smaller than 10  $\mu$ l. EX1010, 2:23-27; EX1016, 484; EX1012, 138; EX1013, 271). Haff, for example, disclosed an apparatus for performing PCR amplification in a capillary tube with an inner diameter of 1-3 mm. EX1010,

Cover, Abstract, 3:6-9, 8:28-37. Haff thus did not perform PCR in a microfluidic system.

95. Whereas PCR reaction tubes are thermocycled by increasing and decreasing the temperature of essentially stationary vials (sometimes called reaction tubes), flow-through PCR thermocycles the PCR mixture by flowing the mixture through a tube or channel that proceeds through temperature zones to perform the melting, annealing, and extension phases of PCR. EX1010, Abstract, 3:6-9, 8:28-37. Because different PCR reactions would flow serially through the same tube, Haff discloses injecting an air bubble or immiscible fluid bubbles such as from an oil (including mineral oil) into the aqueous flow stream in the tubing to provide a discontinuity to demark the ends of the reaction mixture sample volume and to drive the reaction mix predictably through the temperature zones. *Id.*, 8:37-42, 10:29-34.

96. Haff discloses that the time required to change the temperature of a PCR mixture is greater when there is a small surface/volume ratio, such as when the reaction mixture volume is large, than with a large surface/volume ratio, such as when the similarly shaped mixture has a smaller volume. *Id.*, 2:27-49. The reason behind this observation is straightforward. The more surface area available for heat exchange for a given volume, the faster that heat exchange will occur and the faster the volume will equilibrate at the temperature that surrounds

it. Haff thus teaches that spreading out its large samples in the mm-scale capillary tube effectively increased the surface/volume ratio of the PCR reaction mixture, enabling faster and easier thermocycling for large volumes. *Id.*, 7:9-11, 7:27-31. Haff thus taught that capillary tube PCR enabled PCR of larger sample sizes by providing more rapid thermal incubation transitions than a comparable reaction volume in a stationary reaction chamber. *Id.*, 2:50-53, 5:11-40.

97. A person of ordinary skill readily would have recognized that reducing the volume of the PCR reaction mixture similarly would have increased the surface-to-volume ratio of the mixture as compared to a similarly-shaped, larger reaction volume. Once again, there would be more surface area available for heat exchange, resulting in faster heat exchange and faster temperature equilibration. Thermocycling thus can be completed faster for smaller reaction volumes.

98. The Patent Owner's assumptions about channel length for flow-through PCR discussed above in Section V.C vastly overstate the channel length required for performing PCR because, in part, they fail to take into account the smaller droplet volume and resulting increased surface/volume ratio of the microreactors. A POSA would have recognized that the smaller inherent length scale of microfluidic droplets would have aided both kinetics and heat transfer rates associated with microfluidic droplets. Patent Owner's arguments also

assumed a carrier fluid flow much faster than required to generate a microfluidic droplet, and ignored the option of forming droplets in a flowing stream and subsequently thermocycling the droplets on the chip in a stationary microreaction chamber. It was also known at the time that channel length could be increased by using a larger chip or stacked chips. EX1007, 451. In my opinion, it would have been well-within the skill of the ordinary artisan in 2002 and 2003 to design a microfluidic device (including a microfluidic chip) with channels long enough to successfully complete PCR of a microfluidic (*e.g.*, nanoliter-, picoliter-, or femtoliter-sized) droplet.

99. Recognizing that reducing reaction volume permits faster thermocycling using less energy and otherwise saves time and expensive reagents, microfluidic practitioners began creating microfluidic PCR devices in the 1990s. Printed publications describe such devices. For example, Lagally *et al.*, published the article “Monolithic integrated microfluidic DNA amplification and capillary electrophoresis analysis system” in the year 2000 in Volume 63 of the journal *Sensors and Actuators B*. EX1012, 138. Lagally states that their lab “produced one of the first microdevices to both amplify and detect PCR products without intermediate steps, which used a silicon reaction chamber, a 10- $\mu$ L reaction volume, and performed PCR amplifications in 15 minutes. *Id.*, 128, 145 (citing 1996 publication in n.3).



100. Microfluidic PCR devices performed PCR amplification by thermocycling the PCR reaction either in stationary micro reaction chambers or using continuous flow. EX1012, 138; EX1009, 104-05, 108. Lagally notes that others successfully fabricated microfluidic PCR devices in the 1990s in silicon, glass, hybrids, and in fused silica capillaries. EX1012, 138, 145 (citing n.4-11). Others successfully designed microfluidic continuous-flow devices in which the same is repeatedly flowed through three differently heated regions. *Id.*, 138, 145 (citing n.12-13). In both types of microfluidic PCR systems, the aqueous PCR mixture must cycle through the melting, annealing, and extension phases common to all PCR reactions to initiate and complete the PCR reaction. EX1012, 142, 144 (Fig. 5 caption); EX1013, 274, 277; EX1011, 1046; EX1014, 11:1-24 & Fig. 4. In other words, unlike some reactions, PCR does not spontaneously occur upon mixing enzyme with reagents. The timing of the reaction initiation thus depends on introducing thermocycling, not combining the DNA template with the PCR reagents.

101. Early PCR microreactors had reaction volumes on the order of 1  $\mu$ L, with reaction volumes between 250-280 nL also reported by 2001. EX1012, Abstract, 144 (microchamber); EX1013, 280 (microfluidic capillary). For example, Lagally notes that microfluidic PCR chamber volumes had been reduced to a 1- $\mu$ L reaction volume with roughly 2000 starting copies. EX1012,

138. Lagally states that these devices should be further improved by amplifying small amounts of template, meaning fewer starting copies of DNA. *Id.*, 138-39. Lagally thus developed a sub-microliter PCR chamber for amplification of “submicroliter volumes of DNA.” *Id.*, 139 (0.28- $\mu$ l PCR chamber).

102. Persons of ordinary skill knew that using sub-microliter PCR reaction volumes conserves time. For example, Lagally expressly teaches that “[s]mall operating volumes...make the device well-suited to rapid cycling.” EX1012, 144. Lagally successfully performed on-chip microfluidic PCR amplification using 20 cycles of 95°C for 5 seconds, 53°C for 15 seconds, and 72°C for 10 seconds, for a total run time of 10 minutes. *Id.*, 141-42. In contrast, conventional Peltier thermocycling required 12-times as much time for melting, 4-times as much time for annealing, and 6-times as much time for extension. *Id.*, 142. In Lagally’s system with a stationary sub-microliter reaction chamber, the “rate-limiting step” was thus the “thermal transition time, rather than energy transfer from the heater to the sample.” *Id.*, 144. Lagally thus provides an example of how decreasing reaction volume size decreases the time required for thermocycling, as discussed above. In a flow-through system, the thermal transition time is decreased further as compared to a stationary microreaction chamber because the heating and cooling zone temperatures essentially remain constant. Persons of ordinary skill

thus recognized that sub-microliter reaction volumes in flow-through devices provided means for even further reduced thermocycling times.

103. Lagally also teaches that using sub-microliter PCR reaction volumes makes possible using less DNA template, thereby enabling quantitative PCR methods. For example, Lagally used a template concentration of 20 copies per microliter, which amounts to approximately 5 copies for a 0.28- $\mu$ l (280 nanoliter) volume. *Id.*, 143. Lagally states that adding an additional 5-10 cycles would enable amplification using only a single starting copy in the chamber. *Id.*, 143. Lagally teaches it was possible to perform quantitative studies of trace target sequences potentially to a single molecule level because of the improve molecular limit of detection. *Id.*, 143-44.

104. Such improvements were not limited to stationary PCR microreactors. For example, He *et al.*, published the article “Capillary-based fully integrated and automated system for nanoliter polymerase chain reaction analysis directly from cheek cells” in the year 2001 in Volume 924 of the Journal of Chromatography A. EX1013, 271. He *et al.* successfully performed PCR amplification of DNA in a 250 nL microreaction volume. *Id.*, 284. He *et al.* notes that PCR reactions “at the nanoliter scale will provide significant advantages to the biochemist in terms of speed, cost and automation.” *Id.*, 272.

105. He *et al.* notes that reducing the scale of the reaction such that there is a “large surface-to-volume ratio” may increase the amount of interference between the surface of the channel (e.g., capillary inner diameter). *Id.*, 273. Surface adhesion of polymerase protein in microreactors, and solutions for it, were “well known.” *Id.*, 280 (reducing surface inhibition by adding BSA to the PCR mixture and/or by increasing enzyme concentration); *see also* EX1009, 110 (using BSA, glycerol and PEG to prevent polymerase protein adsorption).

106. As with non-microfluidic flow-through PCR devices, POSAs recognized that it was important to isolate individual PCR reaction mixtures from subsequent samples in microfluidic flow-through PCR devices. One approach was to inject small bubbles of air or immiscible fluid between PCR reaction mixtures. EX1010, 10:20-37; EX1015, [0095]-[0116].

107. Another approach, similar to the approach used with stationary PCR microreaction chambers, was to wash the internal surfaces with aqueous fluid between sample runs. In 1998, for example, Kopp and co-workers published “Chemical Amplification: Continuous-Flow PCR on a Chip” in Volume 280 of the journal *Science*. EX1011, 1046. Kopp reported successful continuous-flow PCR amplification in a microfluidic channel on a chip. EX1011, 1046. Kopp used precision syringe pumps to alternately inject the PCR reaction mixture or just the aqueous buffer into the channels on the chip at a ratio of 1:0.8. *Id.*, 1047.

The DNA-negative buffer thus served to separate samples and wash the channel walls to prevent cross-over.

108. Before 2002, however, it was known that using air or oil bubbles between samples may leave some cross-contamination between samples, and that using an aqueous DNA-blank wash between samples as Kopp did reduced through-put. *See, e.g.*, EX1009, 108 (interspacing serial samples in a flow by air or gas is not preferred). Practitioners thus turned to performing PCR in water-in-oil (w/o) droplets, sometimes also referred to as emulsion PCR (ePCR), to prevent cross-over between samples. Before 2002, practitioners had successfully used such (w/o) droplets formed in standard hydrocarbons such as octane and mineral oil to reduce cross-talk between PCR samples. These practitioners successfully performed microfluidic PCR in sub-microliter w/o droplets. *See, e.g.*, EX1008; EX1009; see also EX1006.

109. In addition to standard hydrocarbons such as octane, decane, and mineral oil, POSAs were well aware that fluorinated oils could be advantageously used as a carrier fluid for aqueous droplet microreactors. Forming droplets in fluorinated oils using fluorinated surfactants to safely handle biological materials was well known before 2002.

110. For example, Patrick Shaw Stewart published his Master's Thesis in 1988 at the University of London and the Imperial College of Science and

Technology in the Centre for Biotechnology. EX1023, 1. The Stewart Thesis described a new device for carrying out liquid chemical reactions on a very small scale and its application to a number of problems in the biological sciences including clinical biochemistry, cell manipulation, and crystallography. *Id.*, 2 (Abstract).

111. The device operated by containing all reactants as droplets in an inert, immiscible liquid within a system of conduits. EX1023, 2 (Abstract). The device disperses, subdivides, stores, or coalesces the droplets by moving the immiscible liquid. *Id.* The Stewart Thesis created reaction droplets as small as 10 nL. *Id.*, 21. The Stewart Thesis specifically discusses including DNA samples and enzymes together in the droplets, including to perform DNA sequencing. *Id.*, 147-48.

112. If aqueous reactants are to be used, the Stewart Thesis discloses that the carrier liquid should be a hydrocarbon, an organic fluorocarbon (oil), or a silicon oil. EX1023, 18. The Stewart Thesis notes that fluorocarbon liquid previously has been used in continuous flow analyzers as a separating and encapsulating fluid. *Id.* The Stewart Thesis evaluated the sensitivity of reagents used in biochemical assays, especially enzymes, to certain carrier oils and found that silicone oil, fluorocarbon and washed white spirit were acceptable carrier oils for biochemical enzymatic reactions in microfluidic water-in-oil droplets. *Id.*, 89-92. The Stewart Thesis found there was no significant reduction in activity of

the enzyme when exposed to any of these carriers and concluded that the droplet reaction “is suitable for carrying out this and perhaps other enzyme assays.” *Id.*, 91-92. The Stewart Thesis advised testing compatibility of future reagents with the carrier fluid, to test for enzyme inactivation without excess enzyme. *Id.*, 91-92.

113. In contrast to simple reaction droplets, the Stewart Thesis noted that it was much more difficult to perform reactions in entire *cells* encapsulated in the droplets because the bacterial cells tended to contaminate the walls of the conduits. *Id.*, 36-37.

114. A practitioner of ordinary skill would have found the Stewart Thesis’s teaching to use fluorinated oils as a carrier fluid for enzymatic reactions in water-in-oil droplets to be extremely persuasive. For example, such a practitioner have known that perfluorinated oils are inert, including for use with biological materials, extremely immiscible with aqueous fluids, but generally chemically compatible with (and hold an affinity for) other fluorinated compounds. Indeed, these principles of fluorine chemistry are regularly taught to undergraduate students. A practitioner of ordinary skill would have recognized that these properties were favorable for promoting enzymatic reactions in droplets while minimizing both cross-over between droplets and deactivation of enzyme through

affinity or interaction with the carrier fluid. Indeed, publications available before 2002 teach fluorinated oils were desirable because of these known properties.

115. For example, Rüdiger et al. published “Perfluorocarbons—Useful Tools for Medicine” in volume 5 of the European Journal of Medical Research in the year 2000. EX1017. Rüdiger teaches that perfluorocarbons “combine rather unique chemical and physical properties together with physiological inertness.” *Id.*, Abstract. Rüdiger teaches that these properties made perfluorocarbons attractive to scientists all over the world because of their “unique suitability for a variety of biological-medical applications.” *Id.*, 209 (Introduction). Rüdiger states that there were many existing publications and patents “covering all aspects of synthesis of fluorocarbons and their use in biology and medicine[.]” *Id.*, 215. Rüdiger teaches several well-known perfluorocarbons, including specifically perfluorodecalin. *Id.*, 209.

116. Rüdiger teaches that the unique properties of perfluorocarbons are a result of the specific properties of fluorine and the C-F bond. Rüdiger teaches that the fluorine atoms in perfluorocarbons shield its carbon-carbon bonds from chemical attack, giving them “extraordinary” resistance to chemical and thermal attack, making them “chemically highly inert” and “as a consequence, they are physiologically acceptable, too.” *Id.*, 212. Rüdiger teaches that they also have a high ability to “wet any solid surface,” “strongly hydrophobic, but also



oleophobic” such that they are “immiscible with water and very limited miscible with oleophilic liquids. *Id.*, 212; *see also id.*, 213 (they are “practically insoluble in water,” are “very limited miscible with or soluble in lipophilic liquids, too”). Indeed, Rüdiger teaches that perfluorocarbons “are very poor solvents for all but fluorophilic solid substances.” *Id.*, 212. In other words, perfluorinated oils such as perfluorodecalin not only will not mix with water, but they barely mix with other oils and oil-like substances (*e.g.*, non-fluorinated surfactants).

117. In contrast, it was well known that one could find compounds with a good affinity or compatibility for fluorinated oils by looking to other fluorinated compounds. Rüdiger, for example, teaches that forming a stable microemulsion using water in a perfluorinated oil requires the use of fluorosurfactants. *Id.*, 213. Rüdiger explains that fluorosurfactants are necessary to stabilize the emulsion and that they “become enriched at the liquid-liquid interface.” *Id.*, 213. The fluorosurfactants arrange themselves at the water-oil interface with the hydrophilic head facing the aqueous droplet and the fluorophilic end facing the fluorinated oil. *Id.*, 213.

118. Rüdiger was not unique in discussing the benefits of using fluorinated surfactants together with fluorinated oils. For example, Krafft and Riess published “Highly fluorinated amphiphiles and colloidal systems, and their applications in the biomedical field. A contribution” in Volume 80 of the journal

Biochimie in 1998. EX1018. Krafft similarly lauded the biological inertness, low surface tension, and low water solubility properties of fluorocarbons. EX1018, Summary. Krafft explains that perfluoroalkyl chains are “more hydrophobic” than non-fluorinated alkanes and are also “lipophobic as well.” *Id.* Krafft explains that “[f]luorinated chains confer to surfactants a powerful driving force for collecting and organizing at interfaces” and have “a much stronger capacity to self-aggregate into discrete molecular assemblies when dispersed in water and other solvents” as compared to non-fluorinated analogs.” *Id.* Krafft discloses that fluorinated amphiphiles (fluorinated surfactants) are desirable for forming colloidal or microemulsion systems for biological applications. *Id.* Krafft reports that a large variety of well-defined fluorinated surfactants were known whose “polar head groups consist of polyols” or other polar moieties. *Id.* Polyethylene glycol (PEG) is a typical example of a polyol. *Id.*, 507.

119. Krafft disclosed colloidal systems that were water-in-fluorinated oil emulsions created using fluorinated surfactants and explained that using these fluorinated surfactants to form the water-in-fluorinated oil emulsions was beneficial for biological application because they were chemically and biologically inert and because these surfactants organize at the oil/water interface of the droplets in the emulsion. EX1018, Summary, 489-90, 492, 496, 510, Fig. 6. As discussed in detail below, workers in the field described

successfully performing PCR amplification reactions in microscopic water-in-oil droplets, which emulsion they referred to as a colloidal mixture. *See, e.g.*, EX1006, 47:7-8.

## **VIII. BRIEF OVERVIEW OF PRIOR ART**

### **A. Quake (EX1005)**

120. International PCT Publication No. WO 02/23163 to Stephen Quake and Todd Thorsen, entitled “Microfabricated Crossflow Devices and Methods,” states on its cover that it published on March 21, 2002. Accordingly, I understand that Quake is prior art to the ’193 patent.

121. Quake provides a microfluidic device for analyzing and/or sorting biological materials. EX1005, Abstract. The device comprises a main channel into which droplets of solution containing the biological material are deposited through a droplet extrusion region. *Id.* A fluid different from and incompatible (*i.e.*, immiscible) with the solution containing the biological material flows through the main channel so that the droplets containing the biological material do not diffuse or mix. *Id.*, Abstract, 19:30. Biological materials within the droplets can be analyzed by detecting a predetermined characteristic of the biological sample in each droplet and the droplet can be sorted accordingly. *Id.*

122. Quake’s microfluidic device compartmentalizes the small droplets of aqueous solution within pressurized oil continuously flowing in the main

microfluidic channel by continuously flowing a pressurized stream of aqueous solution from a sample inlet channel into the main channel via a junction in a droplet extrusion region. EX1005, 1:15-28, 5:21-23, 6:4-8, 7:30-8:5, 11:20-12:12, 22:6-16. The pressures of the oil and aqueous solutions are adjusted so that the stream of aqueous solution is sheared off at a regular frequency as it enters the oil stream to form the droplets. *Id.*, 1:15-28, 19:26-32, 78:17-79:31, 81:31-82:8, Figs. 16-22. Quake teaches that the droplets are trapped by the immiscible carrier fluid so that separate droplets do not diffuse or mix, such that individual particles can be compartmentalized inside individual droplets and be analyzed or reacted as desired. *Id.*, 5:1-12; *Id.*, 5:29-32 (“there is no problem with the material diffusing or exchanging positions” between droplets). The continuously flowing oil separates the aqueous droplets from one another and the channel wall. *Id.*, 29:33-30:2. Each droplet preferably encapsulates only a single particle. *Id.*, 5:7-8, 6:16-21, 8:5-8, 9:5-9, 36:29-37:28.

123. The size and periodicity of the droplets may be regulated by adjusting the pressures in the two channels from the pressurized syringes feeding those channels. *Id.*, 34:32-35:8, 80:31-81:2, 82:25-29, 83:4-7, 83:22-31, 84:4-16 & Fig. 19. Droplet size is also dependent on the channel radius. *Id.*, 84:4-16. The droplets slow down significantly after being partitioned into the oil stream. *Id.*,

83:30-31. Quake teaches that the droplets have a preferred volume of 0.1-100 picoliters. *Id.*, 1:15-28.

124. In preferred embodiments, the droplet extrusion region comprises a T-shaped junction between the two channels. *Id.*, 6:23-27, 24:18-27:3, Figs. 17A-C. Channels are preferably rounded and of micron dimensions, droplets conform to the size and shape of the channels and are also of micron dimensions, and are preferably round and monodisperse. *Id.*, 26:30-29:32. Quake also discloses special channel architectures below the junction in which extruded droplets are routed through periodic turns (*e.g.*, S-shaped channel segments and rectilinear U-shaped channel segments). *Id.*, 79:32-80:16, Figs. 17A-C.

125. Quake discloses using the microfluidic device to identify and/or sort virions or virus particles and states PCR is “generally the method of choice to detect viral DNA or RNA directly in clinical specimens.” *Id.*, 1:29-2:9, 2:29-32. Quake teaches benefits of PCR include high sensitivity but that it is vulnerable to cross-contamination between samples. *Id.*, 2:32-3:6. Quake proposes its droplets as a solution for cross-contamination because forming the w/o droplets at the junction of the two channels, as discussed above, reduces the risk of cross contamination between droplets. *Id.*, 5:1-12; 5:25-27 (invention obviates the need for cleaning and prevents cross-contamination). Quake states that PCR can be

integrated with flow cytometry on the chip, citing M.U. Kopp et al., Science, 280:1046 (1998), a reference that teaches flow-through PCR. *Id.*, 23:26-30.

126. Quake also discloses the channel walls may be coated to minimize adhesion, including coating with a surfactant or with the fluorinated coating TEFLON. *Id.*, 28:1-6. Quake discloses exemplary surfactants, including Span and others, and teaches that the oil carrier fluid may contain a surfactant as an additive to coat the channel walls as the oil flows through the channel. *Id.*, 28:7-29, 82:2-3. In contrast, Quake teaches to avoid water soluble surfactants such as SDS for biochemical reactions because the SDS may denature or inactive the contents of the droplets. *Id.*, 28:18-23. Quake discloses that the oil carrier fluid can be a non-polar solvent, and gives decane, tetradecane, hexadecane, and mineral oil as examples. *Id.*, 6:8-10, 8:12-13, 9:2-4, 21:26-22:5, 35:14-17, 82:1-2. Quake discloses that the oil carrier fluid preferably contains at least one surfactant or other additives. *Id.*, 8:15-18, 9:4-5; 35:18-24

127. Quake discloses that the aqueous fluids used for forming droplets may comprise a biological sample, a substrate, an enzyme, buffer (e.g., phosphate buffer saline), and reaction reagents. *Id.*, 6:10-16, 8:14-15, 35:9-14. Quake discloses that an enzyme and a biological material substrate of the enzyme may react with one another within a droplet and the reaction may produce a signal that is detected as the droplet passes through a detection region. *Id.*, 7:15-21, 22:27-

23:3 (discussing detection region and detection of, for example, “a chemical reaction of a substrate catalyzed by an enzyme”), 32:1-34:11 (discussing detection, including using fluorescent reporters), 17:3-25 (discussing fluorescent labels and other reporters), 51:13-21 (same), 52:6-21 (same); *id.*, 15:2-6 (defining enzyme as a catalyst and substrate as any substance on which an enzyme acts), 49:13-25 (discussing using reporters to quantitate nucleic acid content for cellular DNA/RNA analysis), 50:7-23 (discussing fluorescent reporters activated by interaction of biological material with one or more reagents added to the fluid medium—any reporter or combination of substrate, enzyme and product can be used for detection). Quake discloses that the biological materials may thus be “analyzed and/or identified” but also may be sorted. *Id.*, Abstract, 1:29-2:9, 9:10-13. Quake discloses that the biological materials include polynucleotides, substrates, and mixtures thereof or viral particles. *Id.*, 8:19-23; *id.*, 15:18-21; *id.*, 13:16-26 (specifying polynucleotides include DNA); *id.*, 16:16-17 (“Viruses typically comprise a nucleic acid core (comprising DNA or RNA)”)

128. Quake discloses the device may comprise a detector, preferably an optical detector downstream of the droplet extrusion region. *Id.*, 6:28-31. Quake discloses that droplets can be sorted and/or enriched according to their contents but that sorting is not necessary to practice the invention. *Id.*, 6:22-23, 7:22-29,

8:8-12, 9:10-13. Quake also discloses that separate droplets may be controllably merged together. *Id.*, 7:6-15, 36:9-28, 85:9-86:6 & Fig. 22.

**B. Holliger (EX1006)**

129. International Publication No. WO 02/22869 to Holliger and Gahdessy, entitled “Directed Evolution Method,” states on its cover that it published on March 21, 2002. Accordingly, I understand that Holliger is prior art.

130. Holliger explains that polymerases are at the heart of modern biology and enable core technologies such as polymerase chain reaction (PCR). EX1006, 11:6-8. Holliger teaches its invention can be used to select polymerases that are highly active, including under various reaction conditions (*e.g.*, thermostable polymerases). *Id.*, 14:12-17. Holliger thus discloses directed evolution of polymerases used for PCR amplification and other nucleic acid-processing enzymes by compartmentalizing the nucleic acids encoding the enzymes together with the enzyme in water-in-oil (w/o) droplets, allowing the processing (*e.g.*, PCR amplification) to occur in the droplets, and detecting the processing (*e.g.*, PCR amplification) of the nucleic acid member by the enzyme. *Id.*, Abstract, 2:4-11, 3:14-20, 19:3, 19:22-20:12, 21:8-9, 21:23-22, 27:19-20).

131. Holliger teaches how to perform PCR amplification using thermocycling. *Id.*, 21:23-22:20, 25:24-26:22. Holliger explains that the polymerase enzyme amplifies nucleic acid from a nucleic acid template and that



the polymerase chain reaction preferably uses DNA *Taq* polymerase, *Id.*, 5:7-10, 5:19-23, 6:20-21, 10:20-25. Holliger discloses that the composition of the compartments must not abolish the function of the enzymes in amplifying the DNA in the compartment and discloses that the w/o emulsion may be used for this purpose. *Id.*, 44:19-21. 47:7-48:5, 48:22-26.

132. Holliger discloses forming the compartments as aqueous droplets in an emulsion (heterogeneous systems of two immiscible liquid phase with one of the phases dispersed in the other as droplets of microscopic or colloidal size). *Id.*, 47:9-11. Holliger discloses that the compartments are preferably produced by adding the aqueous phase to the oil phase in the presence of a surfactant comprising 4.5% v/v/ Span 80, 0.4% v/v Tween 80 and 0.05-0.1% v/v Triton X100. *Id.*, 6:11-15, 7:16-20, 52:7-15. Holliger teaches that light white mineral oil was a suitable oil. *Id.*, 47:16-48:5. Holliger discloses that the preferred water:oil phase ratio of 1:2 “leads to adequate droplet size.” *Id.*, 6:15-17. Holliger teaches that creation of the emulsion generally requires the application of mechanical energy to force the phases together and that this can be accomplished in a variety of ways. *Id.*, 48:14-21. Holliger discloses that the w/o droplets “are generally stable with little if any exchange of polypeptides or nucleic acids between compartments.” *Id.* 48:22-26, 53:1-6. Holliger reports that its droplets did not change size or coalesce even after 20 cycles of PCR. *Id.*, 52:25-53:2.

133. Holliger discloses combining the nucleic acid substrate and PCR reagents in aqueous solution before forming the droplets in oil. For example, Holliger discloses a general scheme of first suspending the polymerase and the encoding gene substrate in reaction buffer containing flanking primers and nucleotide triphosphates (dNTPs) and then segregating this PCR solution into aqueous compartments. *Id.*, 8:5-13 & Fig. 1B. Holliger teaches the aqueous phase preferably comprises a PCR reaction mix as well as nucleic acid and polymerase. *Id.*, 50:21-22. Regarding Example 3, Holliger describes combining the DNA template, primers, dNTPs, and *Taq* polymerase enzyme into a PCR reaction mix before the PCR mix was compartmentalized into droplets in mineral oil with surfactant additives. *Id.*, 59:10-16. In some embodiments, Holliger used *E. coli* bacteria overexpressing *Taq* polymerases as delivery vehicles placed together with “sufficient amounts of PCR reagents” NTPs and primers into aqueous compartments in the w/o emulsion. *Id.*, 49:20-27.

134. Holliger called the droplet PCR emulsion PCR (ePCR). *Id.*, 12:21-26. Holliger said that its created  $> 10^9$  droplets per milliliter, which equates to picoliter-scale or smaller droplets. *Id.*, 12:21-26. Holliger states that its successful performance of ePCR at this scale “offers the possibility of parallel PCR multiplexing on a[n] unprecedented scale” and “may also have applications for large-scale diagnostic PCR applications like “Digital PCR.” *Id.*, 12:21-26. In

preferred embodiments, each water-in-oil compartment contains substantially one nucleic acid member, the polypeptide encoded by that nucleic acid member, and there is minimal or substantially no interchange of macromolecules (such as nucleic acids and polypeptides) between different compartments. *Id.*, 13:30-14:8, 43:11-44:18, 53:1-6. Holliger discloses that diffusion of dNTPs between droplets occurs at a 1:2 water:oil ratio but that such diffusion can be controlled by increasing the water:oil phase ratio to 1:4. *Id.*, 7:20-23.

135. Holliger reports that aqueous compartment dimensions averaged 15 micron in size. *Id.*, 53:1-6 & Figs. 2-3. Holliger teaches that a preferred upper and lower limits for microcapsule diameter when also performing transcription and translation in the droplet is approximately 1 micron and 100 nm. *Id.*, 49:10-16, 51:5-6. Holliger teaches that the size of the compartments may be varied simply by tailoring the emulsion conditions. *Id.*, 51:21-26.

136. Holliger reported successfully amplifying DNA templates in the droplets. *Id.*, 8:21-9:6, 9:12, 18:3-7, 49:20-27, 59:4-23 & Figs. 3-4, and 7. Holliger states that “polymerase chain reaction proceeded efficiently within the compartments of this water-in-oil composition, to approach the rates observed in solution PCR.” *Id.*, 52:25-53:2. Holliger determined that conditions in the aqueous compartments in the emulsion were suitable for the autocatalytic amplification reaction called PCR by emulsifying a standard reaction mix and

successfully carrying out PCR, which led to amplification of the template gene and had sufficient yield to allow visualization using standard agarose gel electrophoresis. *Id.*, 63:9-13 (Example 9). Holliger also successfully performed PCR in emulsified compartments using *E. coli* cells to deliver the *Taq* Polymerase. *Id.*, 63:14-23. Figs. 3, 4, 8.

### **C. Litborn (EX1007)**

137. Litborn and co-workers published “Synthesis and Analysis of Chemical Components in Nanoscale” in 2000 in the Journal Micro Total Analysis Systems. Micro Total Analysis Systems is a periodical publication that was publicly available to persons of ordinary skill in the art before 2003. I understand that Litborn is prior art to the ’193 patent.

138. Litborn states that the interest in chemistry performed in nanoscale was growing rapidly in 2000 and for biochemistry in particular because of the need for enhanced throughput for biochemical reactions. EX1007, 447 (Abstract). Litborn thus discusses a number of strategies for miniaturized chemistry and analysis using microfluidic devices for applications ranging from nanotitration to PCR amplification. *Id.*

139. Litborn explains that the 1990s represented a drastic change for opening “the use of microchips” to the majority of chemists. EX1007, 447. Litborn explains that microchips had become “a major part of analytical

conferences” by 2000 and that “the number of people active in this area is growing tremendously.” Litborn noted that chip-based separation systems often sacrificed resolution for speed of analysis and that “more attention should be given to scale-related contributions of forces in selective mass transport.” *Id.* Litborn notes, however, that there are “many obvious advantages” to using microchips for chemical reactions, including the fact that “miniaturized chip based systems allow massive parallel chemistry to be performed, at comparatively low cost,” and permit high sample concentration to be maintained due to reduced volumes. *Id.* These benefits open the door to accelerated development of new materials, designing high-throughput platforms for applications including drug screening, medical diagnostics, and proteomics, and improves reaction kinetics and detectability. *Id.* Litborn thus predicted that “[c]hemical reactions in miniaturized formats will become more and more important.” *Id.*

140. Litborn explains that miniaturized reactors either keep sample and reagents in a static environment, such as a nanovial or a capillary tube, or in a dynamic environment, such as by transporting the sample through a capillary tube or a channel. *Id.*, 448. Litborn explains that reagents can be added in advance or downstream depending on desired reaction conditions. *Id.* For example, Litborn discloses PCR amplification performed in microvials and

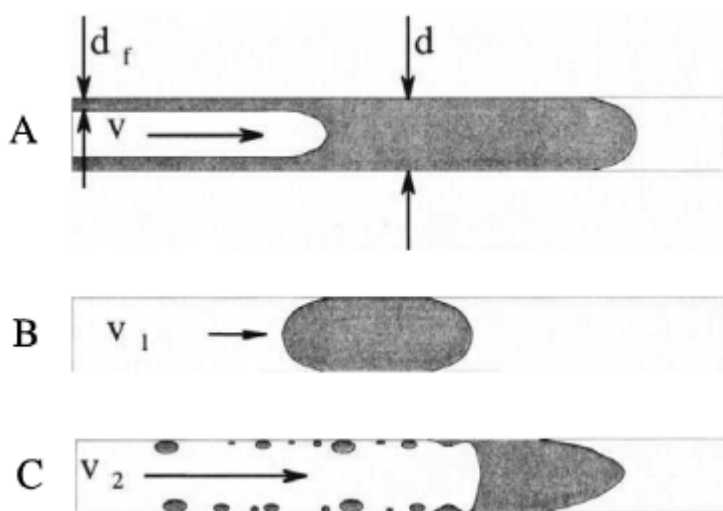
teaches that it was useful to cover nanoliter-sized PCR reaction mixtures in the microvial (*e.g.*, volumes down to 50 nL) with hexane or octane to prevent evaporation. *Id.*, 450-51. In contrast to the stationary microvial reaction, Section 4 of Litborn teaches successful PCR amplification of DNA in so-called dynamic, flow-based reactors in microfluidic systems. *Id.*, 451-53. Litborn specifically discloses that this successful PCR amplification occurs in water-in-oil droplets flowing in a microfluidic stream.

141. Litborn states that “[m]icrofluidic systems also offer exciting possibilities for nanoscale reactions,” and that “the simplest version of a fluidic reactors is the transport of a pre-mixed sample through a channel, which has the desired reaction conditions *e.g.* an elevated temperature zone and/or zones[.]” EX1007, 451. Litborn discloses that “[s]uch microreactors can be stacked, and utilized in a continuous mode[.]” *Id.* Litborn discloses that a single channel or an array of channels with different samples may be employed for analytical purposes and that practitioners had already presented “[a]n elegant example of a microflow reactor” and successfully “realized flow PCR in a chip-based channel.” *Id.* In contrast to the simplest reaction where a pre-mixed sample travels through the channel, Litborn discloses that different or additional reactions may be conducted along the flow trajectory by adding reagents via downstream side channels. *Id.*

142. Litborn discloses that an attractive feature of flow reactors is the ability to create a serial sequence of reaction mixtures separated by smaller zones to enhance the “component number capacity,” or in other words, the number of samples that can be reacted in a given flow. *Id.*, 451. Litborn teaches that obtaining this benefit requires accounting for cross-contamination between samples, such as is caused by the “well-known Taylor dispersion in a pressure driven fluid flow” where the sample lags on the channel walls and the sample becomes diluted. *Id.* Litborn teaches that Taylor dispersion is particularly problematic for “small sample zones. *Id.* Litborn notes that electrically-driven flows do not suffer from Taylor dispersion but come with their own set of issues, including electrophoretic separation of reagents and a limited degree of system freedom from electrostatic and ionic condition requirements. *Id.*, 451-52. Litborn thus points out that “[r]educing carry-over is of major importance to enhance the sample number capacity of flow reactors.” *Id.*, 452.

143. Litborn discloses that one way to attempt to reduce carry-over is to create sample plugs interspaced by air or gas, but that this approach remains unsatisfactory because “a sample plug, passing a wetted surface, will leave a thin film behind the beside-meniscus of the plug” which will be “picked up by the next plug,” resulting in “a severe cross contamination.” *Id.*, 452. Litborn states that the thickness of the sample residue on the channel depends on the internal

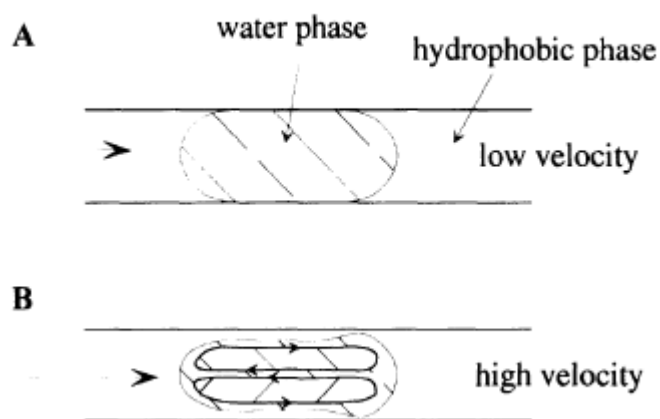
friction within the liquid, the velocity and the wetting angle. *Id.* Litborn states that non-wetted surfaces theoretically will not lead to cross-contamination, but that cross-contamination remains a problem because of contact angle hysteresis, micro roughness of the channel wall, and chemical affinity of the channel wall. *Id.* Litborn also notes that cross-contamination results when the cohesive forces within the fluid are offset by friction with the channel surface, causing the backside meniscus of the water in air droplet to disintegrate, leaving very small droplets behind. *Id.* Litborn illustrates the problem of cross-contamination using a water-in-air droplet in Figure 6, reproduced below:



144. As a solution for these cross-contamination problems, Litborn proposes forming water in oil droplets for microfluidic reactions. Litborn states that “the use of systems with two immiscible fluids” provides “one of the best



potentials for high throughput sequential flow reactions.” *Id.*, 452. Litborn teaches that “a droplet of water in a continuous flow of a hydrocarbon in a hydrophobized channel will keep well separated from an adjacent water droplet, even at high velocities.” *Id.* Litborn states: “Instead of disintegrating into smaller droplets, the water droplet detaches from the surface while the hydrocarbon creates a layer between the channel surface and the droplet (Figure 7).” *Id.* For these water droplets flowing in oil in a microfluidic channel, Litborn discloses that “there is no more surface contact of the water (and no more risk of surface interactions).” *Id.*, 453. Litborn depicts the microfluidic droplet flowing through the microfluidic channel and being substantially surrounded by the hydrophobic carrier oil in Figure 7, reproduced below:



145. Litborn states: “An additional benefit of this situation is that the friction between the hydrocarbon and the water leads to an intrasegmental movement within the droplet, which means that an effective mixing action is obtained.” *Id.*, 453.

146. Litborn reports successfully having performed PCR amplification of DNA in nanoliter-sized water-in-oil droplets flowing through a hydrocarbon. Litborn states:

We have carried out preliminary experiments with a flow based PCR reactions, where the reaction mixture is interspaced with a hydrocarbon, as described above. Amplification was successful, and the results were fully comparable with the results of the commercial standard procedure. It seems that this mode of operation could hold the potential to carry out a massive number of PCR reactions in nanoliter or even sub-nanoliter plugs of solution. This would have fundamental impacts on throughput bottle-necks in molecular biology.

*Id.*, 453.

**D. Shaw Stewart (EX1008)**

147. International Patent Publication No. WO 84/02000 to Patrick Shaw Stewart, entitled “Chemical Droplet Reactor,” published in 1984. Accordingly, I understand that Shaw Stewart is prior art.

148. Shaw Stewart discloses a microfluidic system designed to reduce or eliminate contamination by performing chemical reactions, including biological

assays, in nanoliter-sized droplets. EX1009, Abstract, 2 (objects 1 and 5), 8.

Shaw Stewart discloses the conduit (channel) size is “of the same order of magnitude as the cross-section of the droplets.” *Id.*, 4. Shaw Stewart discloses injecting a small volume or droplet of reactant through a constriction and into a conduit at a right angle. *Id.*, Abstract, 3. The discrete reactant droplets are thus separated from the parent volume by passing carrier phase along the conduit such that the droplets are separated from each other by an inert, immiscible liquid. *Id.*, Abstract, 3. Shaw Stewart discloses mineral oil or a fluorinated hydrocarbon may be used as the carrier. *Id.*, Abstract, 4.

#### **E. Schneegaß (EX1009)**

149. In December 2001, Schneegaß and Köhler published “Flow-through polymerase chain reactions in chip thermocyclers” in volume 82 of Reviews in Molecular Biotechnology, a periodical publication that was publicly available to persons of ordinary skill in the art before 2003. EX1009. I understand that Schneegaß is prior art to the ’193 patent.

150. Schneegaß teaches microfluidic devices for “rapid DNA amplification” and detection of amplified DNA EX1009, Abstract; *id.*, 115 (devices fabricated using standard microlithographic techniques and channels were of microfluidic dimensions), 118 (discussing laser-induced fluorescence as “the preferred detection method of DNA in microchannels”). Schneegaß provides

an overview of the development of miniaturization of PCR in micro total analysis systems because of the desire for high sample-throughput with low reagent consumptions.. *Id.*, 102-03. Schneegaß explains that PCR is a temperature-controlled and enzyme catalyzed process that consists of the periodic repetition of three different temperatures, and explains how this is performed. *Id.*, 103. Schneegaß discloses that miniaturized PCR in capillaries solves the problems of slow heating and cooling rates and that shrinking the reaction volumes to the microliter range decreases the consumption of expensive reaction materials. *Id.*, 104.

151. Schneegaß discloses performing polymerase chain reaction (PCR) in microreactors after paying special attention to the condition of internal surfaces to address the pronounced surface effects from increased surface to volume ratio upon miniaturization. *Id.*, Abstract, 118. Schneegaß discloses that its flow-through thermocyclers transported “pre-mixed samples” through a single channel and subjected them to thermal cycling” and described how to accomplish thermocycling. *Id.*, 111-13.

152. Schneegaß identified “cross-talk between samples” as one of two main concerns for “serial processing of samples in microfluidic systems.” *Id.*, 102. Schneegaß explains that this cross-talk can be addressed by using microheterogeneous phase systems, as the liquid/liquid interface reduces the

dispersion of the samples drastically because of the interface tension between the liquids and because the embedding liquid (*e.g.*, oil) can insulate the reacting liquid volume fractions from the wall surfaces. *Id.*, 102. Schneegaß also teaches that the embedding liquid prevents “chemical gettering of templates and enzymes by reaction sites at the wall surfaces.” *Id.*, 102. Schneegaß states that these points are confirmed by “results from the flow-through thermocycler devices in performing DNA amplification.” *Id.*

153. Schneegaß uses the “Two-phase system for flow segmentation in the serial flow mode” described in Section 4.5 because “a system with two immiscible fluids, a liquid/liquid two-phase system,” has the “best potential for generating serial sample sequence reducing surface contacts.” *Id.*, 109-09. But Schneegaß addressed not only interaction with the channel wall, but also potential interactions at the liquid-liquid interface. *Id.*, 108 (noting that “interface tensions and ***interface interaction*** play an important role in fluid control”). Schneegaß discloses that they “supplemented the PCR mixture” with Tween 20, a non-ionic surfactant, to reduce the surface tension and stabilize the sample droplets in the oil flow and to stabilize the polymerase during PCR. *Id.*, 110. Schneegaß noted that the addition of polyethylene glycol (PEG) for preventing protein (polymerase) adsorption was known. *Id.*, 110.

154. Schneegaß reported that using “dodecane as carrier flow medium did interfere with the PCR and resulted in low product amounts,” but that “non volatile mineral oil...led to the ideal sample transport and showed the expected amplification results.” *Id.*, 109. Schneegaß also states that “mineral oil does not interfere with the analysis method of gel slab electrophoresis we used for visualization of amplification results.” *Id.*, 109.

155. Schneegaß discloses that its aqueous microreactors were w/o droplets that are substantially surrounded by the oil carrier fluid. For example, Schneegaß states: “Using mineral oil for generation of a two-phase system for sample transfer, we could demonstrate, that an *aqueous sample droplet in a continuous carrier flow of the oil* in a hydrophobized channel moves well separated from the subsequent sample droplet....” *Id.*, 109. Schneegaß reported that “[t]he *aqueous sample droplet detaches from the channel surface* because the oil forms a thin layer on the channel surface and reduces the surface contact of the sample..., which leads to a lower risk of cross-contamination of samples.” *Id.*, 109.

156. Schneegaß reported PCR products of three different systems amplified in the droplets using the flow-through PCR chip. *Id.*, 110, Fig. 6 & caption. Schneegaß discloses that, after applying its described modifications to address surface effects, they “demonstrated amplification results in [their] miniaturized devices with an adequate product yield and specificity comparable to the results

achieved in a conventional thermocycler. *Id.*, 110 (citing Fig. 6). In addition to reporting their own successful PCR amplification in microfluidic droplets formed in flowing mineral oil, Schneegaß also noted that Litborn (EX1007) previously reported successful DNA amplification in w/o droplets using octane for generating carrier flow. *Id.*, 109.

157. Schneegaß discloses that it created its serial flow mode by “periodically inject[ing] small sample volumes into the continuous carrier flow, which was generated by a syringe pump[.]” *Id.*, 109. Schneegaß discloses that the sample injection was performed automatically by a precision syringe drive module. *Id.*, 109. Schneegaß proposed using an adapted T-shaped injector channel “[f]or a higher sample throughput.” *Id.*, 109.

158. Schneegaß describes a continuous sample application (*i.e.*, flow of pre-mixed aqueous fluid containing DNA template and PCR reagent) from the injector channel into oil flowing in the main microchannel to form multiple small droplets within the microchannel. *Id.*, 108-113, Fig. 5. Schneegaß validated its continuous sample application droplet PCR thermocycling chips using a variety of template lengths and cycling parameters, all in w/o droplets that a person of ordinary skill in the art would understand, based on the size of the cross-sectional area of the microchannels, to have volumes in the nanoliter range. *Id.* & Table 1. A person of ordinary skill in the art would have recognized from Schneegaß’s

disclosure that it performed droplet PCR in microchannels that fit on a standardized microfluidic chip. *Id.*, 112-13 & Figs. 9-11, Table 1.

159. Schneegaß concluded that miniaturized continuous flow and serial flow DNA amplification “are both very promising techniques for nucleic acid analysis” and that PCR results “equivalent in yield and specificity to a conventional thermocycler” can be achieved for the flow-through thermocycler chip by optimizing the thermal and fluidic design, surface treatments, and PCR processing, as discussed in Schneegaß. *Id.*, 118. Schneegaß concluded that “the serial flow mode with a liquid/liquid two-phase system enables a high-throughput of small samples without cross-contaminations and establishes the feasibility of performing regular screening assays for clinically relevant sequences. *Id.*, 118. In contrast, Schneegaß reported that “continuous flow amplification was especially effective using larger samples volume of a few microliters” but “could not be proved to be consistently free of cross-contamination, a problem which was solved by the introduction of the two-phase system.” *Id.*, 118. Schneegaß concluded that “[t]he reliable operation of the flow-through chip device in amplifying DNA was verified by various amplification systems, and it was proven that flow-through thermocycler chips are suited for rapid  $\mu$ -PCR and are applicable to very different templates,” with the amplification being “efficient and highly selective for all templates.” *Id.*, 118.



**IX. GROUND 1: CLAIMS 1-9 AND 11 ARE OBVIOUS OVER QUAKE IN VIEW OF HOLLIGER**

160. It is my opinion that each of claims 1-9 and 11 would have been obvious before May 9, 2002, based on the disclosures of Quake and Holliger. It is my opinion that Quake and Holliger together teach each element of each of these claims and render each claim obvious as a whole. It is my opinion that a POSA would have had strong motivation to apply the below-cited teachings of Holliger in microfluidic droplets such as those described by Quake and would have, in the process, obtained exactly what is claimed in claims 1-9 and 11. It is my opinion that a POSA not only would have had motivation to do so, but would have had a reasonable expectation of success in obtaining exactly what is claimed.

161. A POSA would have looked to combine the teachings of Quake and Holliger to perform PCR in water-in-oil (w/o) droplets in a microfluidic device. Indeed, both references create microscopic w/o droplets for performing enzymatic reactions and teach they were useful for performing PCR analyses. Quake teaches a microfluidic device for making microfluidic water-in-oil (w/o) droplets, including picoliter-sized w/o droplets, for analysis, reaction or sorting. Quake's aqueous droplets are made in standard hydrocarbons such as decane or mineral oil at the junction of two microfluidic channels by continuously flowing the aqueous solution from the injection channel into the oil continuously flowing

in the main flow channel. EX1005, 1:18-24, 5:6-7, 5:21-23, 6:4-8, 6:23-27, 7:30-8:5, 11:20-12:12, 22:6-16, 30:1-2.

162. Quake teaches its microfluidic w/o droplets are useful for microanalysis and as enzyme bioreactors. EX1005, 6:10-16, 8:14-15, 35:9-14 (aqueous fluid contains biological sample, substrate, and enzyme); *id.*, 7:15-21, 15:2-6, 22:27-23:3 (discussing enzymatic reaction of biological substrate within the droplets). Quake specifically teaches these droplet bioreactions include reactions using DNA polynucleotides as the biological material. *Id.*, 8:19-23, 13:16-26, 15:18-21. Quake also specifically teaches its microfluidic devices should be used to conduct on-chip PCR, which is an autocatalytic reaction, such as for identification of viral DNA. *Id.* 2:25-3:6, 23:26-30.

163. Although Quake does not provide experimental results from performing PCR amplification in its droplets, Quake expressly suggests doing so and conceptually reduces this to practice by expressly describing PCR and by incorporating Kopp. This suggestion by Dr. Quake would have been highly motivating to a POSA. In contrast to the '193 specification and its Provisional Applications (*see* paragraph 43 above), Quake not only squarely describes PCR but also provides detailed information to one of ordinary skill on how PCR is performed, including by citing a PCR reference (EX1011) and incorporating it in its entirety. *Id.*, 1:10-12, 2:25-3:6, 23:26-30, 89:32. As explained in Section VII

above, benefits of miniaturizing PCR were well known and included conserving reagents, decreasing the reaction time, and the ability to successfully perform DNA PCR with a reduced copy number of beginning DNA template (even one template molecular per reaction).

164. A POSA following would have had good reason to look to Holliger for further information on successful PCR amplification in microscopic (*e.g.*, sub-microliter) w/o droplets formed in standard hydrocarbons such as decane and mineral oil. Indeed, Holliger teaches successful PCR amplification of DNA in microscopic w/o droplets formed in mineral oil, just as suggested by Quake. EX1005, Abstract, 2:4-11, 3:14-20, 8:21-9:6, 9:12, 18:3-7, 19:3, 19:22-20:12, 21:8-9, 21:23-22, 27:19-2049:20-27, 52:25-53:2, 59:4-23, 63:9-13 & Figs. 3-4, and 7. A POSA thus had good reasons to combine the teachings of Quake and Holliger to perform PCR in water-in-oil (w/o) droplets in a microfluidic device because both references create microscopic w/o droplets for performing enzymatic reactions and teach they were useful for performing PCR analyses.

165. The Quake and Holliger references expressly contemplate performing PCR in the w/o droplets in a microfluidic device in a manner that satisfies each element of the challenged claims. As discussed in paragraphs 161-164 above, Quake discloses the claimed microfluidic device and using it to make the microfluidic plugs at the junction by continuously flowing the aqueous solution

into the continuously flowing oil. Quake suggests the enzymatic reaction in its droplets should be PCR by citing and incorporating Kopp, with its description of performing PCR using thermocycling. EX1005, 23:26-27, 89:31; EX1011, 36-37. Holliger included the PCR reagents and DNA substrate in the aqueous fluid *before* droplet formation. *Id.*, 8:5-13 & Fig. 1B, 50:21-22, 59:10-16, 49:20-27. Holliger also confirmed Quake's oils and surfactants were suitable for PCR amplification. EX1006, Abstract, 2:4-11, 3:14-20, 6:11-15, 7:16-20, 8:21-9:6, 9:12, 18:3-7, 19:3, 19:22-20:12, 21:8-9, 21:23-22, 27:19-20, 49:20-27, 52:25-53:2, 52:7-15, 59:4-23, 63:9-13 & Figs. 3-4, and 7.

166. The combined teachings of Quake and Holliger also illustrate that combining the PCR reaction mixture in the aqueous fluid before droplet formation was a reasonable and convenient approach for performing PCR in the droplets. As was usual in the literature, Holliger included the PCR reagents and DNA substrate in the aqueous fluid *before* droplet formation and initiated PCR amplification by thermocycling as *Taq* polymerase is inactive at low temperatures prior to thermocycling. *Id.*, 8:5-13 50:21-22, 59:10-16, 49:20-27, 18:3-7, 19:3-4, 21:23-22:23; 59:4-8, 75:20-26. Any POSA would have known that PCR, unlike many other enzymatic reactions, does not spontaneously proceed upon the combination of PCR reagents with DNA template but instead requires thermocycling repeatedly through the three distinct temperature stages to

induce amplification. *See, e.g.*, EX1011, 1046. Because PCR amplification does not occur spontaneously upon combination of enzyme with substrate and other reagents, a POSA would have recognized easily that it was simple and straightforward to combine the DNA template and PCR reagents before droplet formation. Quake's practice of merging substrate droplets with reactant droplets would have had good application for reactions different than PCR, but a POSA would have recognized this practice was not necessary for PCR amplification.

167. Holliger evidences this understanding by disclosing the successful performance of PCR amplification in sub-microliter aqueous droplets formed in mineral oil using an aqueous fluid already containing both the DNA substrate and the other PCR reagents at droplet formation. Based on the disclosures of Quake and Holliger, a POSA thus would have had good reason to form Quake's PCR droplets at the junction of Quake's microchannels by continuously flowing an aqueous solution that already contained at least one DNA substrate and PCR reagents through an inlet or injection channel into the oil continuously flowing in the main channel and to provide conditions suitable for the autocatalytic reaction PCR in at least one droplet such that the at least one substrate molecule is amplified, including by thermocycling the droplet after droplet formation, as disclosed in Holliger. A POSA would have had motivation to combine the PCR reagents before droplet formation, as in Holliger, when forming PCR droplets at

the channel junction, as in Quake, to provide a simple, direct, and efficient means for PCR droplet formation in which droplets PCR subsequently could be performed.

168. A POSA reading Holliger would have recognized Quake's droplet-formation microfluidic device was quite attractive for high-throughput formation of PCR droplets because of Quake's ability to form generally monodisperse (*i.e.*, uniformly-sized) droplets. EX1005, 27:27-32 & Figs. 17A, C and 19J, L.

Forming many uniformly-sized droplets would mean that roughly the same amount of template and reactants would be found in each droplet. A POSA would have recognized that this uniformity even at high throughput, as reported by Quake, was beneficial for w/o droplet PCR. Holliger recognized the potential for w/o droplets created on a massive scale for digital and multiplex PCR.

EX1006, 12:21-26. Quake's ability to create these droplets uniformly and with high throughput thus would have provided good reason to combine the references as discussed herein.

169. Holliger also discloses, and confirms that Quake discloses, conditions suitable for PCR amplification in the droplet. For example, Holliger discloses that PCR amplification was successful in sub-microliter aqueous droplets formed in mineral oil in the presence of non-ionic surfactants (*e.g.*, Span/Tween), the same droplet materials disclosed in Quake. Like the PCR reference Quake

incorporated in its entirety (EX1011), Holliger teaches how to perform PCR amplification using DNA *Taq* polymerase by thermocycling repeatedly through the three temperature stages to denature the DNA, anneal the primers to the DNA, and extend the DNA. *Id.*, 5:7-10, 5:19-23, 6:20-21, 10:20-25, 21:23-22:20, 25:24-26:22. Holliger thus discloses providing conditions suitable for PCR amplification of DNA in sub-microliter w/o droplets and also confirms that this information was already available to a POSA from Quake's teachings. Holliger was well aware of the importance of preserving polymerase functionality in the droplets and taught that doing so in microscopic droplets formed in traditional hydrocarbons was well-within the skill of the art. For example, Holliger observes that the composition of the compartments (the aqueous droplets in the emulsion) must preserve the function of the enzymes in amplifying the DNA in the compartment and discloses that this function was preserved in Holliger's droplets. *Id.*, 44:19-21. 47:7-48:5, 48:22-26. Holliger specifically teaches that mineral oil was a suitable oil. *Id.*, 47:16-48:5. Holliger discloses that the droplets are produced by adding the aqueous phase to the oil phase in the presence of a surfactant comprising Span 80 and Tween 80, both of which are disclosed in Quake. *Id.*, 6:11-15, 7:16-20, 52:7-15.

170. Holliger discloses that the w/o droplets "are generally stable with little if any exchange of polypeptides or nucleic acids between compartments." *Id.*

48:22-26, 53:1-6. Holliger teaches its use of emulsion PCR (ePCR), with picoliter-scale or smaller ( $> 10^9$  microcompartments/ml) droplets, “offers the possibility of parallel PCR multiplexing on a[n] unprecedented scale” and “may also have applications for large-scale diagnostic PCR applications like “Digital PCR.” *Id.*, 12:21-26; *see also Id.*, 13:30-14:8, 43:11-44:18, 53:1-6 & Figs. 2-3. Holliger thus confirms a POSA would have had a reasonable expectation of success in combining the substrate and PCR reagents in the aqueous solution before droplet formation in Quake’s microfluidic device and also in providing conditions suitable for successful PCR amplification in Quake’s microfluidic w/o droplets.

171. Although Holliger’s sub-microliter-sized w/o PCR droplets were not formed in a channel, a POSA would have recognized that such droplets also could be formed beneficially and with high-throughput using Quake’s microfluidic device. In each case, mechanical force is used to generate the droplets (stirring in Holliger and shear force in Quake). Quake and Holliger each teach ways to optimize the droplet formation, including varying concentrations of water, oil, and surfactant and the mechanical force imposed on the emulsion (e.g., varying relative fluid pressures in Quake). Moreover, Litborn (EX1007) and Schneegaß (EX1009) confirm that flowing the microscopic w/o droplets through a microfluidic channel presented no obstacle to successful PCR amplification.



See Section VIII.C, E above. In other words, moving Holliger's droplets into a microfluidic device did not present a difficult obstacle of a technical nature for a POSA.

172. As discussed above in Section VII, a POSA would have known that microfluidic PCR could be performed in a stationary PCR thermocycling chamber or in a flow-through thermocycling channel. A POSA would have recognized that both options remained available for thermocycling Quake's PCR droplets. For a stationary reaction chamber, a POSA would have recognized that droplet formation could be paused during thermocycling or that the droplets could be directed into one of several parallel reaction chambers at a time. When a given reaction chamber was full, additional droplets could be directed to a different reaction chamber to proceed in parallel with the earlier chamber(s). A POSA would have had a reasonable expectation of successfully performing PCR amplification in Quake's w/o droplets in a microfluidic reaction chamber just as thermocycling in microfluidic reaction chambers on chips had been performed previously. See Section VII (discussing, *e.g.*, EX1012).

173. A POSA also would have had a reasonable expectation of successfully performing PCR amplification in Quake's w/o droplets using flow-through thermocycling. As discussed above in Section VII, practitioners had successfully

done so already in standard hydrocarbons such as octane and mineral oil. *See, e.g.,* EX1009, 109; EX1007, 451-453.

174. The speed of the flow-through thermocycling would not present a difficult obstacle to PCR amplification. Multiple options were well-known to practitioners, as evidenced by the success of the Schneegaß reference (EX1009) in doing this specifically on a microfluidic chip. Moreover, as discussed above, droplets of the desired size could be obtained at slower flow speeds by altering the surfactant amounts, droplets could be formed at high speed with thermocycling performed at slower speeds simply by widening the channel. Moreover, the prior art even taught available reaction time on-chip could be increased using stacked chips and other methods. EX1007, 451. Quake and Holliger's teachings that PCR can, should, and could be performed in microfluidic w/o droplets formed in standard hydrocarbons such as mineral oil and decane are thus confirmed by the knowledge of the POSA at the time, as evidenced by other references available to the POSA.

175. As I discuss in detail below, the combination of teachings from Quake and Holliger as discussed above renders obvious each of claims 1-9 and 11. Below, I analyze each claim over these disclosures of Quake in view of Holliger and find that each and every element of each of claims 1-9 and 11 is disclosed in these references and that these claims are each obvious as a whole. I have

included charts below to show exemplary text from the prior art references showing the disclosure of the claim elements below.

*1. Claim 1*

- a) 1.[preamble] A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:*

176. Quake in view of Holliger discloses a method for conducting an autocatalytic reaction in plugs in a microfluidic system. Quake discloses “[a] microfluidic device for analyzing and/or sorting biological materials,” including specifically polynucleotides and viruses. EX1005, Abstract; *see also id.*, 1:15 (“This invention relates to microfluidic devices and methods”), 26:30-27:32 (channels and droplets of micron dimensions), 29:7-32 (discussing pressure-driven and electro-osmotic flow in channels of microfluidic device). Quake’s microfluidic droplets preferably have a diameter smaller than the diameter of the microchannel and a volume of 0.1-100 picoliters. *Id.*, 1:15-28, 27:27-30.

177. As discussed above, Quake suggests performing the autocatalytic reaction PCR in the droplets in a microfluidic system by teaching that its microfluidic devices may be used to conduct on-chip PCR and by incorporating a PCR reaction reference in its entirety. EX1005, 1:29-2:9. 2:29-32, 23:26-30 (citing EX1011). Quake teaches performing enzyme-catalyzed bioreactions within microfluidic aqueous droplets formed in an immiscible fluid. *Id.*,

Abstract, 5:1-12, 5:29-32, 6:10-16, 7:6-15, 8:14-15, 35:9-14, 36:9-28, 85:9-86:6 & Fig. 22. Quake specifically teaches these reactions include reactions with biological materials such as DNA polynucleotides. *Id.*, 8:19-23, 13:16-26, 15:18-21.

178. Also as discussed above, Holliger discloses successfully performing PCR amplification in microscopic water-in-oil droplets comparable to Quake's droplets. EX1006, 8:21-9:6, 18:3-7, 49:20-27, 52:25-53:2, 59:4-23, 63:9-13 & Figs. 3-4, and 7. Holliger discloses compartmentalizing the nucleic acids encoding polymerase enzymes together with the enzyme in water-in-oil (w/o) droplets, allowing the processing (*e.g.*, PCR amplification) to occur in the droplets, and detecting the processing (*e.g.*, PCR amplification) of the nucleic acid member by the enzyme. *Id.*, Abstract, 2:4-11, 3:14-20, 19:3, 19:22-20:12, 21:8-9, 21:23-22, 27:19-20). Holliger teaches its use of emulsion PCR (ePCR), with picoliter-scale or smaller ( $> 10^9$  microcompartments/ml) droplets, "offers the possibility of parallel PCR multiplexing on a[n] unprecedented scale" and "may also have applications for large-scale diagnostic PCR applications like "Digital PCR." *Id.*, 12:21-26; *see also id.*, 13:30-14:8, 43:11-44:18, 53:1-6 & Figs. 2-3.

179. In view of Holliger's disclosure that PCR amplification could be performed successfully in sub-microliter w/o droplets such as those formed in

Quake, a person of ordinary skill in the art would have had good reason and a reasonable expectation of success for conducting an autocatalytic reaction in plugs in a microfluidic system. Quake and Holliger thus disclose a method for conducting an autocatalytic reaction in plugs in a microfluidic system, as recited in claim element 1.[preamble].

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND HOLLIGER
<p>1[preamble] A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:</p>	<p><b><u>Quake discloses w/o droplets for conducting enzymatic reactions in a microfluidic system:</u></b></p> <p>“<b>A microfluidic device</b> for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides ...) ... The <b>microfluidic device</b> comprises a main channel and an inlet region in communication with the main channel at a droplet extrusion region. <b>Droplets of solution containing the biological material are deposited into the main channel through the droplet extrusion region.</b>” (EX1005, [Abstract]; <i>see</i> EX1030, Abstract.)</p> <p>“<b>The sample fluid</b> is, specifically, a fluid which is incompatible with the extrusion fluid and <b>contains the biological material or sample. Thus, droplets of the sample fluid containing the biological material for analysis, reaction</b> or sorting are sheared at the droplet extrusion region into the flow of the extrusion fluid in the main channel.” (EX1005, 8:1-5; <i>see</i> EX1030, 8:16-20.)</p> <p>“For example, the first biological material may be an <b>enzyme</b> and the second biological material may be a <b>substrate for the enzyme</b>. The interaction of</p>

	<p>the first and second biological materials may produce a signal that can be detected, <i>e.g.</i>, as the droplet passes through a detection region associated with the device.” (EX1005, 7:17-21; <i>see</i> EX1030, 8:1-5).</p> <p>“[T]he amount of the signal can be measured and can correspond to the degree to which a characteristic is present. For example, the strength of the signal may indicate...<b>a chemical reaction of a substrate catalyzed by an enzyme.</b>” (EX1005, 22:30-23:3; <i>see</i> EX1030, 23:15-20.)</p> <p><b><u>Quake teaches the enzymatic reaction may be PCR, which is an autocatalytic reaction:</u></b></p> <p><b>“Microfabrication permits other technologies to be integrated</b> or combined with flow cytometry on a single chip, <b>such as PCR</b> ([M.U. Kopp et al., <i>Science</i>, 280: 1046 (1998)]”) (EX1005, 23:26-27, 89:31; <i>see</i> EX1030, 24:12-13, 90:13.)</p> <p>“Molecular techniques such as DNA probes or the <b>polymerase chain reaction (PCR)</b> are used for <b>the detection of Viruses</b> where cell culture or Serological methods are difficult, expensive or unavailable. <b>PCR is also generally the method of choice to detect viral DNA or RNA</b> directly in clinical specimens.” (EX1005, 2:29-3:6; <i>see</i> EX1030, 3:1-14.)</p> <p><b><u>Holliger discloses a method for conducting the autocatalytic PCR amplification reaction in microfluidic w/o droplets:</u></b></p> <p>“Figure 3A is a diagram showing [dNTP] crossover between <b>emulsion compartments</b>. Two <b>standard PCR reactions</b>, differing in template size...and presence of Taq...are amplified individually or combined. When combined in solution, both</p>
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	<p>templates are amplified. When <b>emulsified</b> separately, prior to mixing, only <b>PCR1 is amplified.</b>" (EX1006, 8:21-9:6.)</p> <p>"PCR thermocycling then leads to the <b>amplification of the polymerase genes</b> by the polypeptides they encode....Furthermore, the copy number of a polymerase gene X after <b>self-amplification</b> will be directly proportional to the catalytic activity of the polymerase X it encodes. (see Figures 1A and 1B)." (EX1006, 18:3-7.)</p> <p>"The methods of our invention involve the <b>templated amplification of desired nucleic acids.</b> 'Amplification' refers to the increase in the number of copies of a particular nucleic acid fragment (or a portion of this) resulting either from an <b>enzymatic chain reaction</b> (such as a <b>polymerase chain reaction</b>....Preferably, the amplification according to our invention is an exponential amplification, as exhibited by for example the polymerase chain reaction. (EX1006, 25:24-26:22.)</p> <p>"The water-in-oil emulsion according to the invention has advantageous properties of increased thermal stability....In addition <b>polymerase chain reaction proceeded efficiently within the compartments of this water-in-oil composition</b>, to approach the rates observed in solution PCR. Average aqueous compartment dimensions in the water-in-oil emulsion according to our invention are on average <b>15µm [&lt;2 picoliters]</b> in size." (EX1006, 52:25-53:2.)</p> <p>"Emulsified mixtures are then transferred...and PCR carried out....<b>Amplified product</b> is visualized on by gel electrophoresis on agarose gels using standard methods[.]" EX1006, 59:16-21</p>
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	<p><b>“Example 9. Standard PCR in Aqueous Compartments Within an Emulsion</b></p> <p>To establish whether conditions in the aqueous compartment present in an emulsion are permissive for catalysis, <b>a standard reaction mix is emulsified and PCR carried out.</b> This leads to <b>amplification</b> of the correct sized <i>Taq</i> polymerase gene present in the plasmid template, with yields sufficient yields to allow visualization using standard agarose gel electrophoresis.”</p> <p>(EX1006, 63:9-13.)</p> <p><b>“With &gt; 10<sup>9</sup> microcompartments/ml, emulsion PCR (ePCR</b> offers the possibility of parallel PCR multiplexing on a[n] unprecedented scale.... It may also have applications for <b>large-scale diagnostic PCR applications like ‘Digital PCR.’”</b> EX1006, 12:21-26.</p>
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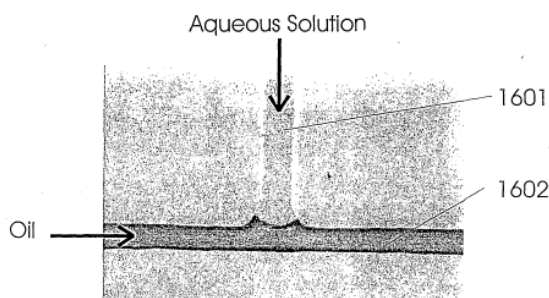
*1.[a] providing the microfluidic system comprising at least two channels having at least one junction;*

180. Quake discloses providing the microfluidic system comprising at least two channels having at least one junction. For example, Quake discloses forming droplets at the junction of two microfluidic channels (a main channel and at least one sample inlet channel). EX1005, 1:18-24, 6:4-8, 7:22-26, 11:17-21, 22:6-12& Figs. 16A-B, 17C. Quake discloses the junction is preferably a T-junction. *Id.*, 6:23-27. Quake thus discloses providing the microfluidic system comprising at least two channels having at least one junction, as required by claim element 1.[a].



CLAIM ELEMENT	OBVIOUS OVER QUAKE AND HOLLIGER
<p>[1.a] Providing the microfluidic system comprising at least two channels having at least one junction;</p>	<p style="text-align: center;"><b><u>Quake teaches forming the droplets by providing a microfluidic system comprising at least two channels having at least one junction:</u></b></p> <p>“A <b>microfluidic device</b> provided by the invention comprises a <b>main channel</b> and at least one <b>inlet region</b> which is in communication with the main channel at a <b>droplet extrusion region.</b>” (EX1005, 6:4-6; <i>see</i> EX1030, 6:17-19.)</p> <p>“The invention also <b>provides a device</b> for sorting biological material comprising: a microfabricated substrate; a detection region; and a flow control region. In more detail, <b>the micro fabricated substrate has at least one main channel, an inlet which meets the main channel at a droplet extrusion region,</b> and at least two branch channels meeting at a junction downstream from the droplet extrusion region.” EX1005, 7:22-26; <i>see</i> EX1030, 8:6-10.)</p> <p>“In preferred embodiments, the droplet extrusion region comprises a <b>T-shaped junction</b> between the inlet region and the main channel....” (EX1005, 6:23-25; <i>see</i> EX1030, 7:6-10.)</p> <p>“The devices and methods of the invention comprise a <b>main channel</b>, through which a pressurized stream of oil is passed, and at least <b>one sample inlet channel</b>, through which a pressurized stream of aqueous solution is passed. <b>A junction or ‘droplet extrusion region’ joins the sample inlet channel to the main channel</b> 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.” (EX1005, 1:18-24; <i>see</i> EX1030, 1:14-19.)</p>

“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  $\mu\text{m}$ ) **intersects the main channel 1602** (inner diameter 30  $\mu\text{m}$ ) 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. The channel architecture for the second exemplary extrusion region is shown in FIG. 16B. In this device, both the inlet channel 1603 and the outlet channel 1604 have inner diameters of 60  $\mu\text{m}$ . However, the channels 1605 and 1606 taper to inner diameters of 30  $\mu\text{m}$  at the droplet extrusion region.” (EX1005, 79:23-31]; *see* EX1030, 83:8-16.)



**FIG. 16A**

(EX1005, Fig. 16A; *see* EX1030, Fig. 16A.)

*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;*

181. Quake in view of Holliger discloses continuously 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. For

example, Quake discloses continuously flowing an aqueous fluid containing a substrate or reagents for conducting an enzymatic reaction through a first channel of the at least two channels. EX1005, 1:15-28, 5:21-23, 6:4-8, 7:30-8:5, 11:20-12:12, 22:6-16. Quake discloses that the aqueous fluid may comprise a biological sample such as DNA, a substrate, an enzyme, buffer such as phosphate buffer saline, and reaction reagents. EX1005, 6:10-16, 8:14-15, 8:19-23, 13:16-26, 15:18-21, 35:9-14. Quake teaches using its droplets for enzyme-catalyzed bioreactions reactions. EX1005, 7:17-21, 8:1-5, 22:30-23:3.

182. Holliger discloses forming microscopic PCR w/o droplets from an aqueous fluid containing a substrate and reagents for conducting PCR, an autocatalytic reaction. EX1006, 8:5-13 & Fig. 1B, 50:21-22, 59:10-16, 49:20-27 (combining PCR reagents before droplet formation). Holliger teaches that *Taq* polymerase is inactive at low temperature and that PCR thermocycling activates polymerase-catalyzed amplification. EX1006, 18:3-7, 19:3-4, 21:23-22:23; 59:4-8, 75:20-26. Holliger thus confirms the knowledge of a POSA that a POSA may combine the DNA substrate and PCR reagents for convenient droplet formation because PCR will not initiate upon this combination but instead requires thermocycling.

183. The convenience of forming ready-made droplets at the junction with all required ingredients for PCR amplification provided a POSA with good

reason to combine the substrate and PCR reagents in Quake's aqueous fluid before forming the w/o droplets by continuously flowing the aqueous fluid through the first channel into the oil continuously flowing in the second channel. As discussed above, Holliger, Litborn and Schneegaß all support a reasonable expectation of being able to do this successfully. For the reasons discussed above, Quake in view of Holliger thus teach continuously flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel, as required by claim element 1.[b].

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND HOLLIGER
<p>[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;</p>	<p style="text-align: center;"><b><u>Quake discloses forming the droplets by continuously flowing an aqueous fluid through a first channel of the at least two channels</u></b></p> <p>“The devices and methods of the invention comprise a main channel, through which a pressurized stream of oil is passed, and at least one <b>sample inlet channel, through which a pressurized stream of aqueous solution is passed.</b>” (EX1005, 1:18-24; <i>see</i> EX1030, 1:14-17.)</p> <p>“<b>Fluid flows continuously through the system</b> and there is no need for charged droplets, so that many difficult technical issues associated with traditional, e. g., FACS devices are avoided.” (EX1005, 5:21-23; <i>see</i> EX1030, 6:3-5.)</p> <p>“[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 <b>a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid,</b></p>

**passes or flows through the inlet region. The sample fluid** is, specifically, a fluid which is incompatible with the extrusion fluid and **contains the biological material** or sample.” (EX1005, 8:1-5 ; *see* EX1030, 8:14-18.)

**Quake suggests performing PCR in the droplets:**

“**Microfabrication permits other technologies to be integrated** or combined with flow cytometry on a single chip, **such as PCR** ([EX1011])” (EX1005, 23:26-27, 89:31; *see* EX1030, 24:12-13, 90:13.

“Molecular techniques such as DNA probes or the **polymerase chain reaction (PCR)** are used for the **detection of Viruses** where cell culture or Serological methods are difficult, expensive or unavailable. **PCR is also generally the method of choice to detect viral DNA or RNA** directly in clinical specimens. (EX1005, 2:29-3:6; *see* EX1030, 3:1-14.)

“**The sample fluid** is, specifically, a fluid which is incompatible with the extrusion fluid and **contains the biological material or sample. 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.” (EX1005, 8:1-5; *see* EX1030, 8:16-20.)

“For example, the first biological material may be an **enzyme** and the second biological material may be a **substrate for the enzyme**. The interaction of the first and second biological materials may produce a signal that can be detected, *e.g.*, as the droplet passes through a detection region associated with the device.” (EX1005, 7:17-21; *see* EX1030, 8:1-5).

“[T]he amount of the signal can be measured and can correspond to the degree to which a characteristic is present. For example, the strength of the signal may indicate...**a chemical reaction of a substrate catalyzed by an enzyme.**”  
(EX1005, 22:30-23:3; *see* EX1030, 23:15-20)

**Holliger teaches the aqueous PCR reaction mixture (*i.e.*, DNA substrate and PCR reagents in water) is combined in the aqueous fluid before forming the droplet in the oil:**

“200 µl of **PCR reaction mix** (*Taq* expression plasmid (200ng), primers 3 and 4 (1µM each), dNTPs (0.25mM), *Taq* polymerase (10 units)) is **added dropwise (12 drops/min) to the oil phase (mineral oil (Sigma))** in the presence of 4.5% (v/v) Span 80 (Fluka), 0.4% (v/v) Tween 80 (Sigma) and 0.05% (v/v) Triton X100 (Sigma) under constant stirring (1000rpm) in 2ml round bottom biofreeze vials (Costar, Cambridge MA)...Emulsified mixtures are then transferred to 0.5 ml thin-walled PCR tubes (100µl/tube) and PCR carried out[.]”  
(EX1006, 59:10-16; *see also id.*, 49:20-27 (polymerase gene, *Taq* polymerase, and PCR reagents colocalized in a single emulsion compartment)).

“Bacterial cells containing the **polymerase and encoding gene** are suspended in **reaction buffer containing flanking primers and nucleotide triphosphates (dNTPS)** and segregated into aqueous compartments.” (EX1006, 8:5-13 & Fig. 1B.)

*1.[c] flowing an oil through the second channel of the at least two channels;*

184. Quake discloses continuously flowing an oil through the second channel of the at least two channels. For example, Quake discloses forming microfluidic w/o droplets at the junction by continuously flowing an aqueous fluid through a first channel into an oil flowing in a second channel. EX1005, 1:15-28, 5:21-23, 6:4-8, 7:30-8:5, 11:20-12:12, 22:6-16. Quake thus discloses flowing an oil through the second channel of the at least two channels, as required by claim element 1.[c].

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND HOLLIGER
[1.c] Flowing an oil through the second channel of the at least two channels;	<p><b><u>Quake discloses forming the droplets by continuously flowing an oil through the second channel of the at least two channels</u></b></p> <p>“<b>Fluid flows continuously through the system</b> and there is no need for charged droplets, so that many difficult technical issues associated with traditional, e. g., FACS devices are avoided.” (EX1005, 5:21-23; <i>see</i> EX1030, 6:3-5.)</p> <p>“[T]he first phase or fluid which <b>flows through the main channel</b> can be a non-polar solvent, such as decane (e.g., tetradecane or hexadecane) or another <b>oil</b> (for example, mineral oil).” (EX1005, 6:8-10; <i>see also id.</i>, 8:12-13; EX1030, 6:21-23.)</p> <p>“[A] <b>first fluid, which may be referred to as an ‘extrusion’ or ‘barrier’ fluid, passes (i.e., flows) through the main channel of the device</b> and a second fluid, referred to as a ‘sample’ or ‘droplet’ fluid, passes</p>

or flows through the inlet region.” (EX1005, 7:30-8:1; *see* EX1030, 8:14-16.)

“More specifically, droplets of a solution (preferably an aqueous solution or buffer), containing a sample of molecules, cells or virions, are introduced through a droplet extrusion region **into a stream of fluid (preferably a non-polar fluid such as decane or other oil) in the main channel.**” (EX1005, 34:17-21; *see* EX1030, 34:26-29.)

**Holliger discloses conducting PCR in microfluidic w/o droplets formed in oil:**

“200 µl of PCR reaction mix (*Taq* expression plasmid (200ng), primers 3 and 4 (1µM each), dNTPs (0.25mM), *Taq* polymerase (10 units)) is added dropwise (12 drops/min) to the **oil phase (mineral oil (Sigma))** in the presence of 4.5% (v/v) Span 80 (Fluka), 0.4% (v/v) Tween 80 (Sigma) and 0.05% (v/v) Triton X100 (Sigma) under constant stirring (1000rpm) in 2ml round bottom biofreeze vials (Costar, Cambridge MA)...Emulsified mixtures are then transferred to 0.5 ml thin-walled PCR tubes (100µl/tube) and PCR carried out using 25 cycles of the profile 94 °C (1 min), 60 °C (1 min), 72 °C (3min) after an initial 5 minute incubation at 94 °C. (EX1006, 59:10-16.)

“Preferably the emulsion of the present invention has water (containing the biochemical components) as the phase present in the form of finely divided droplets (the disperse, internal or discontinuous phase) and a **hydrophobic, immiscible liquid (an ‘oil’) as the matrix in which these droplets are suspended** (the nondisperse, continuous or external phase). Such emulsions are terms ‘water-in-oil’ (W/O). This has the advantage that the entire aqueous phase containing the biochemical components is compartmentalized in



	<p>discrete droplets (the internal phase). The external phase, being a <b>hydrophobic oil</b>, generally contains none of the biochemical components and hence is inert.” (EX1006, 47:16-24.)</p> <p>“Suitable oils include <b>light white mineral oil....</b>” (EX1006, 47:25-48:5.)</p>
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*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,*

185. Quake in view of Holliger discloses 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 continuously flowing oil at the junction of the at least two channels. Quake discloses forming at least one plug of aqueous fluid for performing an enzymatic reaction on a substrate molecule by partitioning the aqueous fluid with the continuously flowing oil at the junction of the at least two channels.

186. For example, Quake discloses forming microfluidic w/o droplets at the junction by continuously flowing an aqueous fluid through a first channel into an oil flowing in a second channel. EX1005, 1:15-28, 5:21-23, 6:4-8, 7:30-8:5, 11:20-12:12, 22:6-16. Quake discloses adjusting the pressures of the oil and aqueous solutions so that the stream of aqueous solution is sheared off at a regular frequently as it enters the continuously flowing oil stream to form the w/o

droplets. *Id.*, 1:15-28, 19:26-32, 78:17-79:31, 81:31-82:8, Figs. 16-22 & accompanying text.

187. Quake also discloses that the aqueous fluid may contain reagents for an enzymatic reaction and teaches using the droplets formed from the aqueous fluid for conducting enzyme-catalyzed bioreactions. EX1005, 6:10-16, 8:14-15, 8:19-23, 13:16-26, 15:18-21, 35:9-14 (aqueous fluid); *Id.*, 7:17-21, 8:1-5, 22:30-23:3 (enzyme-catalyzed bioreaction). Quake thus teaches forming at least one plug of the aqueous fluid containing reagents for an enzymatic reaction by partitioning the aqueous fluid with the continuously flowing oil at the junction of the at least two channels. As discussed above for element 1.[b], Quake suggests the enzymatic reaction may be PCR.

188. As discussed above for element 1.[b], Holliger discloses forming microscopic w/o droplets from an aqueous fluid already containing a substrate and reagents for conducting autocatalytic PCR and subsequently initiating PCR via thermocycling. EX1006, 8:5-13 & Fig. 1B, 50:21-22, 59:10-16, 49:20-27 (PCR mixture before droplet formation); *id.*, 18:3-7, 19:3-4, 21:23-22:23; 59:4-8, 75:20-26 (thermocycling initiates PCR).

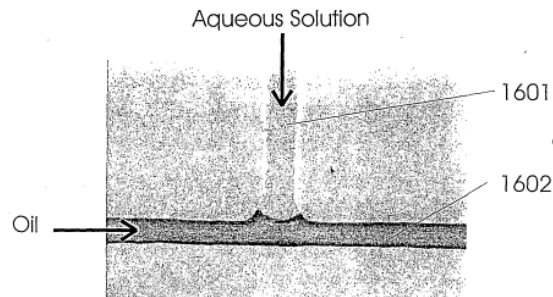
189. As I explained for element 1.[b], Holliger confirms a POSA had good reasons (e.g., simplicity and convenience for reactions that do not spontaneously proceed absent thermocycling) and a reasonable expectation of success for

combining the substrate and PCR reagents in Quake’s aqueous fluid before forming the w/o droplets by continuously flowing the aqueous fluid through a first channel into the oil continuously flowing in the second channel. Quake in view of Holliger thus teach 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 continuously flowing oil at the junction of the at least two channels, as required by claim element 1.[d].

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND HOLLIGER
<p>[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,</p>	<p><b><u>Quake discloses forming the droplets by partitioning the aqueous fluid with the continuously flowing oil at the junction of the at least two channels:</u></b></p> <p>“A microfluidic device for analyzing and/or sorting biological materials (e.g., molecules such as polynucleotides and polypeptides ...) ... The microfluidic device comprises a main channel and an inlet region in communication with the main channel at a droplet extrusion region. <b>Droplets of solution containing the biological material are deposited into the main channel through the droplet extrusion region.</b>” (EX1005, Abstract; <i>see</i> EX1030, Abstract.)</p> <p>“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 <b>such that the stream of aqueous solution is sheared off at a</b></p>

	<p><b>regular frequency as it enters the oil stream, thereby forming droplets.”</b> (EX1005, 1:21-27; <i>see</i> EX1030, 1:17-2:2.)</p> <p>“In a preferred embodiment, <b>water droplets are extruded into a flow of oil</b>, but any fluid phase may be used as a droplet phase and any other incompatible or immiscible fluid or phase may be used as a barrier phase.” (EX1005, 5:32-6:3; <i>see</i> EX1030, 6:14-16.)</p> <p>“Thus, for example, in preferred embodiments wherein <b>droplets of aqueous solution</b> are extruded into a pressurized stream of oil, the pressures of the oil and/or fluid lines are adjusted so that the pressure difference of the oil and water channels at the droplet extrusion region is zero, and the oil and water are in a state of equilibrium. This can be visually observed. <b>Droplet extrusion can then be initiated by slightly adjusting the pressure difference between the different fluids (i.e., at the different inlet lines) so that the droplet fluid (e.g., water) enters the main channel and is sheared off at a fixed frequency.</b> A preferred frequency is 1 Hz because the frequency with which droplets are sheared off into the main channel depends on the pressure difference between the different fluids, the frequency can be readily adjusted by simply adjusting the pressures of the individual fluid lines.” (EX1005, 79:7-17; <i>see</i> EX1030, 82:23-83:4.)</p> <p>“The devices of this invention are useful for <b>partitioning a first fluid into droplets within a second, incompatible fluid.</b>” (EX1005, 78:18-19; <i>see</i> EX1030, 82:2-3.)</p> <p>“In preferred embodiments, <b>the droplet extrusion region comprises a T-shaped junction between the inlet region and the main channel</b>, so that the second</p>
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fluid enters the main channel at an angle perpendicular to the flow of the first fluid, and is **sheared off** into the flow of the first fluid in the main channel.” (EX1005, 6:23-27; *see* EX1030, 7:7-10.)



**FIG. 16A**

EX1005, Fig. 16A; *see* EX1030, Fig. 16A.)

**Holliger discloses forming microscopic w/o droplets from an aqueous fluid containing the at least one substrate molecule and reagents for conducting PCR:**

“200  $\mu$ l of **PCR reaction mix** (*Taq* expression plasmid (200ng), primers 3 and 4 (1 $\mu$ M each), dNTPs (0.25mM), *Taq* polymerase (10 units)) is **added dropwise (12 drops/min) to the oil phase (mineral oil (Sigma))** in the presence of 4.5% (v/v) Span 80 (Fluka), 0.4% (v/v) Tween 80 (Sigma) and 0.05% (v/v) Triton X100 (Sigma) under constant stirring (1000rpm) in 2ml round bottom biofreeze vials (Costar, Cambridge MA)...Emulsified mixtures are then transferred to 0.5 ml thin-walled PCR tubes (100 $\mu$ l/tube) and PCR carried out using 25 cycles of the profile 94 °C (1 min), 60 °C (1 min), 72 °C (3min) after an initial 5 minute incubation at 94 °C. (EX1006, 59:10-16; *see also id.*, 49:20-27 (polymerase gene, *Taq* polymerase, and PCR reagents colocalized in a single emulsion compartment).

“Bacterial cells containing the **polymerase** and *encoding gene* are suspended in **reaction buffer containing flanking primers and nucleotide triphosphates (dNTPS)** and segregated into aqueous compartments.” (EX1006, 8:5-13 & Fig. 1B.)

“Non-membranous compartmentalization systems based on phase partitioning of an aqueous environment in a colloidal system, such as an emulsion, may also be used. Preferably, the compartments of the present invention are formed from emulsions; heterogeneous systems of two immiscible liquid phases with one of the phases dispersed in the other as **droplets of microscopic or colloidal size**[.]” (EX1006, 47:7-13.)

“Preferably the emulsion of the present invention has **water (containing the biochemical components)** as the phase present in the form of finely divided droplets (the disperse, internal or discontinuous phase) and a **hydrophobic, immiscible liquid (an ‘oil’)** as the matrix in which **these droplets are suspended** (the nondisperse, continuous or external phase). Such emulsions are terms ‘water-in-oil’ (W/O). This has the advantage that **the entire aqueous phase containing the biochemical components is compartmentalized in discrete droplets** (the internal phase). The external phase, being a hydrophobic oil, generally contains none of the biochemical components and hence is inert.” (EX1006, 47:16-24.)

*1.[d.1] the plug being substantially surrounded by an oil flowing through the channel,*

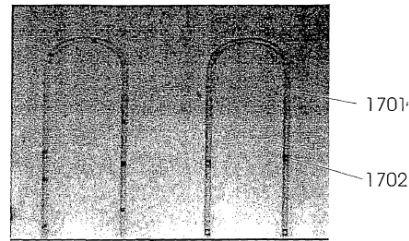
190. Quake discloses the plug being substantially surrounded by an oil continuously flowing through the channel. For example, Quake teaches forming microfluidic aqueous droplets in an immiscible fluid such that biological materials (such as DNA) within the droplets do not uncontrollably mix. *Id.*, Abstract, 5:1-12; *Id.*, 5:29-32, 7:6-15, 36:9-28, 85:9-86:6 & Fig. 22. The aqueous droplets are substantially surrounded by oil, and are thus separated from each other and from the channel wall by oil. *Id.*, 29:33-30:2, Figs. 17A, 17B, 17C, 19J, 19L. Quake thus discloses claim element 1.[d.1].

191. Holliger's microscopic aqueous PCR droplets were substantially surrounded by oil. EX1006, 8:14-9:6, 47:7-13, 47:16-24 & Fig. 2-4. Holliger thereby confirms a POSA would have had a reasonable expectation of success.

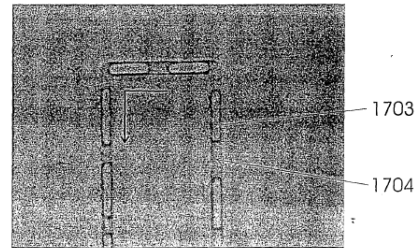
CLAIM ELEMENT	OBVIOUS OVER QUAKE AND HOLLIGER
[1.d.1] the plug being substantially surrounded by an oil flowing through the channel,	<p><b><u>Quake discloses the plug being substantially surrounded by the oil continuously flowing through the channel</u></b></p> <p>“In embodiments of the invention where an aqueous phase is combined with an oil phase, <b>aqueous droplets are encapsulated or separated from each other by oil.</b>” (EX1005, 29:32-30:2; <i>see</i> EX1030, 30:2-3.)</p> <p>“In one particularly preferred embodiment, the extrusion fluid is an oil... that contains a surfactant....</p>

In such an embodiment, a user preferably causes the extrusion fluid to flow through channels of the microfluidic device so that the surfactant in the **extrusion fluid coats the channel walls.**” (EX1005, 28:24-29; *see* EX1030, 28:21-26.)

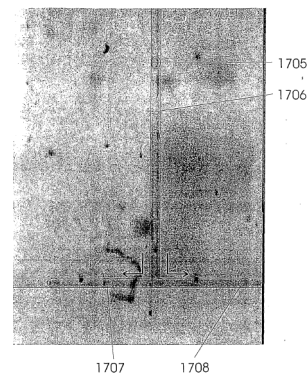
*See also* EX1005, Figs. 17A, 17B, 17C, 19J, 19L



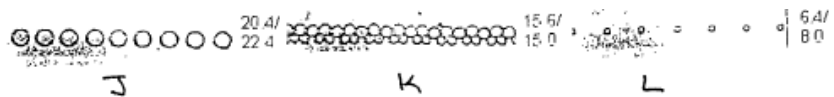
**FIG. 17A**



**FIG. 17B**



**FIG. 17C**



**FIG. 19**

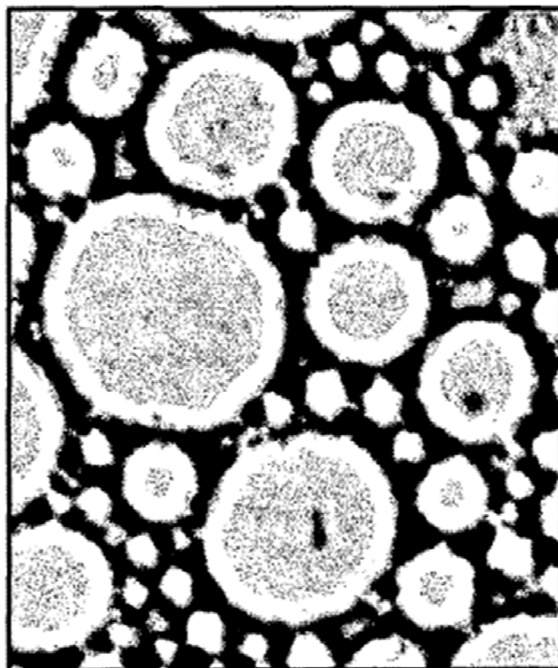


**Holliger discloses the oil encapsulates the droplets, preventing macromolecule migration between droplets:**

“Figure 3A is a diagram showing [dNTP] crossover between **emulsion compartments**. Two standard PCR reactions, differing in template size...and presence of Taq...are amplified individually or combined. When combined in solution, both templates are amplified. When **emulsified** separately, prior to mixing, only **PCR1 is amplified.**” (EX1006, 8:21-9:6.)

“Preferably the emulsion of the present invention has water (containing the biochemical components) as the phase present in the form of **finely divided droplets** (the disperse, internal or discontinuous phase) and a **hydrophobic, immiscible liquid (an ‘oil’)** as the matrix in which these droplets are **suspended** (the nondisperse, continuous or external phase). Such emulsions are termed ‘water-in-oil’ (W/O). This has the advantage that **the entire aqueous phase containing the biochemical components is compartmentalized in discrete droplets** (the internal phase). The external phase, being a hydrophobic oil, generally contains none of the biochemical components and hence is inert.” (EX1006, 47:16-24.)

B



15.7 $\mu$ m

FIG. 2

EX1006, Fig. 2.

*1.[d.2] 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*

192. As I explained above for element 1.[b] and 1.[d], Quake in view of Holliger discloses the plug fluid comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule. Once the plug fluid is sheared off into individual plugs, as discussed above for element 1.[d], the extruded plug comprises the substrate molecule and

reagents for conducting the autocatalytic reaction that were already present in the plug fluid. Indeed, Holliger teaches performing PCR amplification in the microscopic w/o droplets, which would not proceed if the droplets did not comprise substrate molecule and PCR reagents after droplet formation.

Encapsulating the substrate molecule together with the PCR reactions in the droplet was exactly the purpose of including them in the same aqueous fluid used to make the droplets, as discussed above for element 1.[b] and 1.[d].

193. Moreover, a POSA could reasonably expect success in encapsulating DNA template (substrate molecule) and PCR reagents together in a single microfluidic-sized droplets, as evidenced by Holliger, Litborn, and Schneegaß, and in a microfluidic-sized volume, as evidenced by, for example, Lagally (EX1012) and He (EX1013), discussed in Section VII above.

194. For the reasons discussed above, Quake in view of Holliger discloses 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 required by claim element 1.[d.2].

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND HOLLIGER
[1.d.2] wherein the at least one plug comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction	<p style="text-align: center;"><b><u>See discussion of element 1.[b] and 1.[d] above.</u></b></p>

with the at least one substrate molecule; and	
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*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.*

195. Quake in view of Holliger discloses providing conditions suitable for the autocatalytic reaction PCR in the at least plug such that the at least one substrate molecule is amplified.

196. As discussed in detail below, the prior art details conditions suitable for PCR in the plugs such that the DNA molecule is amplified, which conditions were well-known in the art. The prior art thus confirms that a POSA would have had a reasonable expectation of success for performing PCR in the droplets under the same conditions suggested by Quake. In contrast, the '193 patent specification contains considerably less detail than the prior art concerning specific conditions that would be necessary or suitable for performance of PCR and autocatalytic reactions generally, and none that was not already disclosed in the prior art.

197. For example, Holliger discloses providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified. Holliger discloses successfully performing PCR amplification in sub-microliter scale water-in-oil droplets just like Quake's.

EX1006, 8:21-9:6, 9:12, 18:3-7, 49:20-27, 52:25-53:2, 59:4-23, 63:9-13 & Figs. 3-4, and 7. Holliger specifically discloses combining the nucleic acid substrate, the polymerase, and other PCR reagents in the aqueous solution **before** forming the droplets in oil. *Id.*, 8:5-13 & Fig. 1B, 50:21-22, 59:10-16, 49:20-27.

198. Holliger discloses compartmentalizing the nucleic acids encoding polymerase enzymes together with the enzyme in water-in-oil (w/o) droplets, allowing the processing (*e.g.*, PCR amplification) to occur in the droplets, and detecting the processing (*e.g.*, PCR amplification) of the nucleic acid member by the enzyme. *Id.*, Abstract, 2:4-11, 3:14-20, 19:3, 19:22-20:12, 21:8-9, 21:23-22, 27:19-20). Holliger teaches how to perform PCR amplification in sub-microliter w/o droplets, including PCR thermocycling to repetitively denature and rehybridize the DNA, and also explains how PCR works using DNA *Taq* polymerase. *Id.*, 5:7-10, 5:19-23, 6:20-21, 10:20-25, 21:23-22:20, 25:24-26:22. Holliger discloses that the composition of the compartments must not abolish the function of the enzymes in amplifying the DNA in the compartment and discloses that the w/o emulsion may be used for this purpose. *Id.*, 44:19-21. 47:7-48:5, 48:22-26. Holliger teaches that mineral oil was a suitable oil. *Id.*, 47:16-48:5. Holliger thus teaches claim element 1.[e] and confirms that thermocycling Quakes droplets comprising DNA template and PCR reagents provided conditions suitable for PCR amplification

199. Even without Holliger, Quake provided POSAs with all the information they needed to perform PCR amplification in Quake's droplets. For example, Quake specifically teaches its microfluidic devices may be used to conduct on-chip PCR (an autocatalytic reaction). EX1005, 23:26-30. Quake cites and incorporate Kopp (EX1011) in its entirety for its teachings about PCR reactions. EX1005, 1:10-12, 23:26-30, 89:31.

200. Kopp discloses that PCR amplification may be accomplished in microfluidic systems in the same manner as it is performed outside microfluidic systems. For example, Kopp discloses that the aqueous fluid must contain at least one substrate molecule and PCR reagents, such as polymerase, primers, and dNTPs in aqueous buffer, to perform PCR. EX1011, 1046-47. Kopp also discloses that PCR is performed by thermocycling the aqueous PCR mixture for repeated cycles of unzipping the DNA, annealing the primers, and extending the primers. *Id.* Kopp thus teaches conditions that are, in fact, suitable for droplet PCR amplification. These conditions were well-known and widely-practiced by persons of ordinary skill in the art at the relevant times. By incorporating Kopp's disclosure of PCR reagents and thermocycling, Quake disclosed the conditions that would have allowed PCR to proceed in Quake's droplets. Quake thus discloses element 1.[e].

201. For the reasons discussed above, Quake in view of Holliger discloses each and every element of claim 1 and renders claim 1 obvious as a whole.

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND HOLLIGER
<p>[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.</p>	<p><b><u>Quake suggests amplifying DNA substrate in the w/o droplets by PCR thermocycling :</u></b></p> <p><b>“Microfabrication permits other technologies to be integrated</b> or combined with flow cytometry on a single chip, <b>such as PCR</b> [citing EX1011].” (EX1005, 23:26-27, 89:31; <i>see</i> EX1030, 24:12-13, 90:13.)</p> <p>“PCR...doubles the number of specific DNA molecules during each cycle of melting of the double-stranded DNA (dsDNA), binding of the specific primers to their target sites (annealing), and extending the primers with the thermostable DNA polymerase (such as Taq). The individual steps are <i>simply performed by heating or cooling the sample to the characteristic temperatures:</i> 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.” (EX1011, 1046.)</p> <p>Molecular techniques such as DNA probes or the <b>polymerase chain reaction (PCR)</b> are used for <b>the detection of Viruses</b> where cell culture or Serological methods are difficult, expensive or unavailable. <b>PCR is also generally the method of choice to detect viral DNA or RNA</b> 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. However, this Sensitivity of detection can also cause significant problems in routine Viral</p>

diagnostics. The Significant risk of cross-contamination from Sample to Sample can outweigh the benefits of detecting Small quantities of a target viral nucleic acid. Cross-contamination can also result in false positives, making interpretation of epidemiological data impossible. (EX1005, 2:29-3:6; *see* EX1030, 3:1-14.)

**Holliger discloses providing conditions suitable for PCR in the at least one w/o droplet such that the at least one substrate molecule is amplified:**

“Gene amplification techniques **requiring thermal cycling** such as PCR and LCR may also be used....” (EX1006, 50:21-22.)

“200 µl of PCR reaction mix (*Taq* expression plasmid (200ng), primers 3 and 4 (1µM each), dNTPs (0.25mM), *Taq* polymerase (10 units)) is added dropwise (12 drops/min) to the oil phase (mineral oil (Sigma)) in the presence of 4.5% (v/v) Span 80 (Fluka), 0.4% (v/v) Tween 80 (Sigma) and 0.05% (v/v) Triton X100 (Sigma) under constant stirring (1000rpm) in 2ml round bottom biofreeze vials (Costar, Cambridge MA)...Emulsified mixtures are then transferred to 0.5 ml thin-walled PCR tubes (100µl/tube) and **PCR carried out using 25 cycles of the profile 94 °C (1 min), 60 °C (1 min), 72 °C (3min)** after an initial 5 minute incubation at 94 °C. (EX1006, 59:10-16.

**Holliger discloses w/o droplets formed in traditional hydrocarbons (e.g., mineral oil) were suitable for a successful PCR amplification reaction:**

“Figure 3A is a diagram showing [dNTP] crossover between **emulsion compartments**. Two standard



	<p>PCR reactions, differing in template size...and presence of Taq...are amplified individually or combined. When combined in solution, both templates are amplified. When <b>emulsified</b> separately, prior to mixing, only <b>PCR1 is amplified.</b>" (EX1006, 8:21-9:6.)</p> <p>"[T]he formation and the composition of the compartments <b>must not abolish the function of the machinery for the expression of the nucleic acids</b> and the activity of the polypeptides." (EX1006, 44:19-21.)</p> <p>"Suitable oils include <b>light white mineral oil</b> and <b>non-ionic surfactants</b>...such as sorbitan monooleate (Span<sup>TM</sup>80, ICI) and polyoxyethylenesorbitan monooleate (Tween<sup>TM</sup> 80; ICI)...." (EX1006, 47:25-48:5.)</p> <p>"The water-in-oil emulsion according to the invention has advantageous properties of increased thermal stability....In addition <b>polymerase chain reaction proceeded efficiently within the compartments of this water-in-oil composition</b>, to approach the rates observed in solution PCR. Average aqueous compartment dimensions in the water-in-oil emulsion according to our invention are on average 15µm [&lt;2 picoliters] in size." (EX1006, 52:25-53:2.)</p> <p>"Emulsified mixtures are then transferred...and PCR carried out....<b>Amplified product</b> is visualized on by gel electrophoresis on agarose gels using standard methods[.]" EX1006, 59:16-21</p> <p><b>"Example 9. Standard PCR in Aqueous Compartments Within an Emulsion</b> To establish whether conditions in the aqueous compartment present in an emulsion are permissive for catalysis, <b>a standard reaction mix is emulsified</b></p>
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**and PCR carried out. This leads to amplification** of the correct sized *Taq* polymerase gene present in the plasmid template, with yields sufficient yields to allow visualization using standard agarose gel electrophoresis.”  
(EX1006, 63:9-13.)

“With > 10<sup>9</sup> microcompartments/ml, emulsion **PCR (ePCR** offers the possibility of parallel PCR multiplexing on a[n] unprecedented scale.... It may also have applications for **large-scale diagnostic PCR applications like ‘Digital PCR.’**” EX1006, 12:21-26.

**Holliger discloses PCR is an autocatalytic amplification reaction:**

“PCR thermocycling then leads to the **amplification of the polymerase genes** by the polypeptides they encode....Furthermore, the copy number of a polymerase gene X after **self-amplification** will be directly proportional to the catalytic activity of the polymerase X it encodes. (see Figures 1A and 1B).”  
(EX1006, 18:3-7.)

“The **amplification reaction** is preferably selected from...**polymerase chain reaction (PCR)**....”  
(EX1006, 5:19-20.)

“An example of a replicase is DNA polymerase. DNA polymerase enzymes...are used by a cell to **replicate a nucleic acid strand using a template molecule to manufacture a complementary nucleic acid strand.** Enzymes having **DNA polymerase activity catalyze** the formation of a bond between the 3’ hydroxyl group at the growing end of a nucleic acid primer and the 5’ phosphate group of a nucleotide triphosphate.... The *in-vitro* use of enzymes having DNA polymerase activity has in recent years become more common in a

	<p>variety of biochemical applications...<b>amplification of nucleic acid</b> by methods such as the <b>polymerase chain reaction</b> (PCR) (Mullis et al., U.S. Pat. Nos. 4,683,195, 4,683,202, and 4,800,159, hereby incorporated by reference herein)....” (EX1006, 21:23-22:20.)</p> <p>“The methods of our invention involve the <b>templated amplification of desired nucleic acids</b>. ‘Amplification’ refers to the increase in the number of copies of a particular nucleic acid fragment (or a portion of this) resulting either from an <b>enzymatic chain reaction</b> (such as a <b>polymerase chain reaction</b>....Preferably, the amplification according to our invention is <b>an exponential amplification</b>, as exhibited by for example the polymerase chain reaction.” (EX1006, 25:24-26:22.)</p>
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202. For the reasons discussed above, Quake in view of Holliger discloses each element of claim 1 in the claimed configuration and renders claim 1 obvious.

## 2. Claim 2

*2. The method of claim 1, wherein the at least one substrate molecule is a single biological molecule.*

203. As I discussed above in Section IX.1, claim 1 is obvious over Quake and Holliger. Quake and Holliger also each disclose the substrate molecule is a single biological molecule. For example, Quake discloses that each droplet preferably encapsulates only a single biological particle. EX1005, 5:7-8, 6:16-21, 8:5-8, 9:5-9, 36:29-37:28. Holliger teaches its use of emulsion PCR (ePCR), with

picoliter-scale or smaller ( $> 10^9$  microcompartments/ml) droplets, may have application for large-scale “Digital PCR” and that each water-in-oil compartment preferably contains substantially one nucleic acid member. EX1006, 12:21-26, 13:30-14:1. Accordingly, Quake in view of Holliger thus renders obvious the method of claim 1 wherein the at least one substrate molecule is a single biological molecule, as recited by claim 2. The claim chart below reproduces text from exemplary portions of the prior art that demonstrate the obviousness of the claim.

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND HOLLIGER
2. The method of claim 1, wherein the at least one substrate molecule is a single biological molecule.	<p><i>See discussion of claim 1 above.</i></p> <p>“[T]he sample concentration should be dilute enough that most of the droplets <b>contain no more than a single molecule</b>... with only a small statistical chance that a droplet will contain two or more molecules.... This is to ensure that for the large majority of measurements, the level of reporter measured in each droplet as it passes through the detection region corresponds to a single molecule... and not to two or more molecules[.]” (EX1005, 36:29-37:4; <i>see</i> EX1030, 37:12-19.)</p> <p>“In preferred embodiments the second fluid includes a <b>biological sample</b> that comprises one or more molecules, cells, virions or particles. In exemplary embodiments for detecting and sorting droplet contents, the droplets of the second fluid each contains, on average, <b>no more than one particle</b>. For example, in preferred embodiments where the biological material comprises viral particles, each droplet contains, on</p>

	<p>average, no more than one viral particle.” EX1005, 6:16-20; <i>see</i> EX1030, 6:29-7:5;</p> <p>“With &gt; 10<sup>9</sup> microcompartments/ml, emulsion PCR (ePCR)...may also have applications for large-scale diagnostic PCR applications like ‘<b>Digital PCR</b>[.]’” EX1006, 12:21-26.</p> <p>“In preferred embodiments, each compartment contains <b>substantially one nucleic acid member....</b>” EX1006, 13:30-14:1.</p>
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### 3. Claim 3

*3. The method of claim 2, wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.*

204. As I discussed in Section IX.2 above, claim 2 is obvious over Quake and Holliger. Quake and Holliger also each disclose the substrate molecule is DNA and the autocatalytic reaction is PCR. *See, e.g.*, EX1005, 2:29-3:6, 23:26-27, 89:31; EX1006, 5:19-20, 8:5-13, 8:21-9:6, 12:21-26, 18:3-7, 21:23-22:20, 25:24-26:22, 50:21-22, 52:25-53:2, 59:10-16, 59:16-21, 63:9-13. Accordingly, Quake in view of Holliger thus renders obvious the method of claim 2 wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase chain reaction, as recited by claim 3. The claim chart below reproduces text from exemplary portions of the prior art that demonstrate the obviousness of the claim.

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND HOLLIGER
<p>3. The method of claim 2, wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.</p>	<p><i>See</i> discussion of claim 2 above.</p> <p><i>See</i> discussion of claim element 1.[e] above.</p> <p>“<b>PCR</b> is also generally the method of choice to detect viral <b>DNA</b> or RNA directly in clinical specimens.” (EX1005, 2:31-32; <i>see</i> EX1030, 3:1-14.)</p> <p>“<b>Microfabrication permits other technologies to be integrated</b> or combined with flow cytometry on a single chip, <b>such as PCR</b>” (EX1005, 23:26-27, 89:31 (citing and incorporating EX1011); <i>see</i> EX1030, 24:12-13, 90:13; EX1011, 1046-47.)</p> <p>“Figure 3A is a diagram showing [dNTP] crossover between emulsion compartments. Two standard PCR reactions, differing in template size... <b>PCR1 is amplified.</b>” (EX1006, 8:21-9:6.)</p> <p>“<b>PCR</b> thermocycling then leads to the <b>amplification of the polymerase genes</b> by the polypeptides they encode....” (EX1006, 18:3-7.)</p> <p>“The <i>in-vitro</i> use of enzymes having <b>DNA polymerase activity</b> has in recent years become more common in a variety of biochemical applications...<b>amplification of nucleic acid</b> by methods such as the <b>polymerase chain reaction</b> (PCR) (Mullis et al., U.S. Pat. Nos. 4,683,195, 4,683,202, and 4,800,159, hereby incorporated by reference herein)....” (EX1006, 21:23-22:20.)</p> <p>“200 µl of PCR reaction mix (<i>Taq</i> expression <b>plasmid</b> (200ng)...and <b>PCR carried out</b>.... (EX1006, 59:10-16.)</p>

	<p><b>“[A] standard reaction mix is emulsified and PCR carried out. This leads to amplification of the correct sized <i>Taq</i> polymerase gene present in the plasmid template, with yields sufficient yields to allow visualization using standard agarose gel electrophoresis.”</b>  <b>(EX1006, 63:9-13.)</b></p>
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#### 4. Claim 4

##### 4. The method of claim 1, wherein the providing step includes heating.

205. As I discussed in Section IX.1 above, claim 1 is obvious over Quake and Holliger. Quake and Holliger also each disclose the providing step includes heating. Heating was well-known to POSAs before the '193 patent for performing the PCR reaction. For example, Quake cites and incorporates by reference Kopp for its discussion of microfluidic PCR. EX1005, 23:26-27, 89:31 (citing EX1011). Kopp discloses that PCR is “simply performed by heating and cooling” and that the optimal temperatures are 95°C for melting the DNA, 50° to 65°C for primer annealing, and 72° to 77°C for primer extension. EX1011, 1046.

206. Holliger also discloses that PCR requires thermal cycling and used temperatures within the ranges provided in Kopp to successfully perform PCR amplification of DNA in microfluidic w/o droplets. EX1006, 50:21-22, 59:10-16. Accordingly, Quake in view of Holliger thus renders obvious the method of claim 1 wherein the providing step includes heating, as recited by claim 4. The

claim chart below reproduces text from exemplary portions of the prior art that demonstrate the obviousness of the claim.

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND HOLLIGER
<p>4. The method of claim 1, wherein the providing step includes heating.</p>	<p><i>See discussion of claim 1 above.</i></p> <p><i>See discussion of claim element 1.[e] above.</i></p> <p><b>“Microfabrication permits other technologies to be integrated</b> or combined with flow cytometry on a single chip, <b>such as PCR</b>” (EX1005, 23:26-27, 89:31 (citing and incorporating EX1011); <i>see</i> EX1030, 24:12-13, 90:13.)</p> <p>“The individual steps are simply performed by heating or cooling the sample to characteristic temperatures <b>95°C</b> for dsDNA melting, <b>50° to 65°C</b> for primer annealing, and <b>72° to 77°C</b> for primer extension at the optimum enzyme temperature. EX1011, 1046.</p> <p>“Gene amplification techniques <b>requiring thermal cycling</b> such as PCR and LCR may also be used....” (EX1006, 50:20-23.)</p> <p>“PCR carried out using 25 cycles of the profile <b>94 °C (1 min), 60 °C (1 min), 72 °C (3min)</b> after an initial 5 minute incubation at 94 °C. (EX1006, 59:10-16.)</p>



5. *Claim 5*

*5. The method of claim 1, further comprising the step of providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.*

207. As I discussed in Section IX.1 above, claim 1 is obvious over Quake and Holliger. Quake discloses providing a detector to detect, analyze, characterize and monitor one or more properties of the reaction during and after it has occurred. For example, Quake discloses the microfluidic device comprises a detector, preferably an optical detector downstream of the junction. EX1005, 6:28-31.

208. Quake also discloses that an enzyme and a biological material substrate of the enzyme may react with one another within a droplet and the reaction may produce a signal that is detected as the droplet passes through the detection region. *Id.*, 7:15-21, 22:27-23:3 (discussing detection region and detection of, for example, “a chemical reaction of a substrate catalyzed by an enzyme”), 32:1-34:11 (discussing detection, including using fluorescent reporters), 17:3-25 (discussing fluorescent labels and other reporters), 51:13-21 (same), 52:6-21 (same); *Id.*, 15:2-6 (defining enzyme as a catalyst and substrate as any substance on which an enzyme acts), 49:13-25 (discussing using reporters to quantitate nucleic acid content for cellular DNA/RNA analysis), 50:7-23

(discussing fluorescent reporters activated by interaction of biological material with one or more reagents added to the fluid medium—any reporter or combination of substrate, enzyme and product can be used for detection). Quake discloses that the biological materials may thus be “analyzed and/or identified” but also may be sorted. *Id.*, 9:10-13.

209. Quake in view of Holliger thus renders obvious the method of claim 1 further comprising the step of providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred, as recited by claim 5. The claim chart below reproduces text from exemplary portions of the prior art that demonstrate the obviousness of the claim.

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND HOLLIGER
5. The method of claim 1, further comprising the step of providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.	<p><i>See</i> discussion of claim 1 above.</p> <p>“The 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 <b>device may also have a detector</b>, preferably an optical detector such as a microscope, associated with the detection region.” (EX1005, 6:28-31, 7:19-21; <i>see</i> EX1030, 7:11-14, 8:3-5.)</p> <p>“[T]he first biological material may be an enzyme and the second biological material may be a substrate for the enzyme. The interaction of the first and second biological materials may produce <b>a signal that can be detected, e.g., as the droplet passes through a</b></p>

	<p><b>detection region</b> associated with the device.” (EX1005, 7:17-21; <i>see</i> EX1030, 8:1-5.)</p> <p>“As each droplet passes into the detection region, it is examined for a predetermined characteristic (<i>i.e.</i>, using the detector) and a corresponding signal is produced, for example indicating that “yes” the characteristic is present, or “no” it is not. The signal may correspond to a characteristic qualitatively or quantitatively. That is, the amount of the signal can be measured and can correspond to the degree to which a characteristic is present. For example, the <b>strength of the signal may indicate...a chemical reaction of a substrate catalyzed by an enzyme.</b>” (EX1005, 22:27-23:3; <i>see</i> EX1030, 23:12-20.)</p>
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6. *Claim 7*

*7. The method of claim 1, wherein the carrier fluid further comprises a surfactant.*

210. As I discussed above in Section IX.1, claim 1 is obvious over Quake and Holliger. Quake teaches the oil carrier (extrusion) fluid comprises a surfactant. EX1005, 8:15-18, 9:4-5; 28:24-29, 35:18-24. Quake teaches including surfactant in the carrier fluid prevents adherence of particles to the sides of the channel and also promotes droplet formation. For example, Quake teaches putting a surfactant in the oil to help form the w/o droplets. EX1005, 35:18-24, 35:27-29). Quake also discloses the surfactants may be used in the carrier oil to coat the channel walls as the oil flows through the channels. EX1005, 28:6-29, 35:18-24, 35:27-29.

211. Quake discloses that the oil carrier fluid can be a non-polar solvent, and gives decane, tetradecane, hexadecane, and mineral oil as examples. EX1005, 6:8-10, 8:12-13, 9:2-4, 21:26-22:5, 28:23-29, 35:14-17, 82:1-2. Quake discloses exemplary surfactants, including Tween, Span, and fluorinated oils. *Id.*, 28:7-29, 35:18-24, 35:27-29, 82:2-3. Holliger discloses successful PCR amplification in w/o droplets formed using these same surfactants. EX1006, 47:25-48:5, 52:7-15. Quake in view of Holliger thus renders obvious the method of claim 1, wherein the carrier fluid further comprises a surfactant, as required by claim 7. The claim chart below reproduces text from exemplary portions of the prior art that demonstrate the obviousness of the claim.

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND HOLLIGER
7. The method of claim 1, wherein the carrier fluid further comprises a surfactant.	<p><i>See discussion of claim 1 above.</i></p> <p>“In one particularly preferred embodiment, the extrusion fluid is an <b>oil (e.g., decane, tetradecane or hexadecane) that contains a surfactant</b> (e.g., a non-ionic surfactant such as a Span surfactant) as an additive (preferably between about 0.2 and 5% by volume, more preferably about 2%). In such an embodiment, a user preferably causes the extrusion fluid to flow through channels of the microfluidic device so that <b>the surfactant in the extrusion fluid</b> coats the channel walls.” (EX1005, 28:24-29; <i>see</i> EX1030, 28:21-26.)</p> <p>“The fluids used in the invention may contain additives, such as agents which reduce surface</p>

	<p>tensions (surfactants). <b>Exemplary surfactants include Tween, Span, fluorinated oils</b>, 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. This may <b>affect droplet volume and periodicity, or the rate or frequency</b> at which droplets break off into an intersecting channel.” (EX1005, 35:18-24, 35:27-29; <i>see also id.</i>, 8:15-18, 9:2-5, 28:7-29; <i>see</i> EX1030, 28:21-23, 35:28-36:5, 36:8-10)</p> <p>“[T]he channels may be coated with a <b>surfactant</b>.” (EX1005, 28:1-6; <i>see</i> EX1030, 28:21-26.)</p> <p>“The emulsion may be stabilized by addition of one or more <b>surface-active agents (surfactants)</b>. These surfactants are termed emulsifying agents and act at the water/oil interface to prevent (or at least delay separation of the phases. Many oils and many emulsifiers can be used for the generation of water-in-oil emulsions....Suitable oils include light white mineral oil and non-ionic surfactants...such as sorbitan monooleate (<b>Span<sup>TM</sup>80, ICI</b>) and polyoxyethylenesorbitan monooleate (<b>Tween<sup>TM</sup> 80; ICI</b>)....” (EX1006, 47:25-48:5.)</p> <p>“In a highly preferred embodiment of the present invention, the emulsion is a water-in-oil emulsion. The water-in-oil emulsion is made by adding an aqueous phase dropwise to an <b>oil phase in the presence of a surfactant</b>....” (EX1006, 52:7-10.)</p> <p>“200 µl of PCR reaction mix (<i>Taq</i> expression plasmid (200ng), primers 3 and 4 (1µM each), dNTPs (0.25mM), <i>Taq</i> polymerase (10 units)) is added dropwise (12 drops/min) to the <b>oil phase (mineral oil (Sigma)) in the presence of 4.5% (v/v) Span 80 (Fluka), 0.4% (v/v) Tween 80</b></p>
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	<b>(Sigma) and 0.05% (v/v) Triton X100...PCR carried out[.]” (EX1006, 59:10-16.)</b>
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7. *Claims 6 and 8*

6. *The method of claim 1, wherein the oil is fluorinated oil.*

8. *The method of claim 7, wherein the surfactant is fluorinated surfactant.*

212. As I discussed above in Sections IX.1 and IX.6, claims 1 and 7 are each obvious over Quake and Holliger. As discussed above for claim 7, Quake discloses the surfactant may be fluorinated oil and that the surfactants may be used in the carrier oil to coat the channel walls and to form droplets. EX1005, 28:6-29, 35:18-24, 35:27-29. To a POSA, Quake’s disclosure is directed to using a fluorinated oil that is a surfactant, not a non-surfactant fluorinated oil.

Generally, such a surfactant would have a hydrophilic head and a oleophilic or fluorophilic tail. A POSA would understand Quake’s disclosure to refer fluorinated oil surfactants with a hydrophilic head and a oleophilic or fluorophilic tail, as was customary. This meaning of fluorinated surfactant was well known in the prior art. *See, e.g.*, EX1017, 209, 212-14; EX1018, Summary. POSAs also knew full well that these fluorinated surfactants prevent protein adherence to the interface. *Id.* As understood by a POSA, Quake thus discloses the method of claim 7 wherein the surfactant is fluorinated surfactant, as required by claim 8.

213. By disclosing using a fluorinated oil surfactant that is soluble in the oil, Quake suggests using carrier fluid oils that would be compatible with fluorinated oil surfactants, and most especially using fluorinated oil as the carrier fluid. Even an undergraduate student of chemistry should know that using a fluorinated oil surfactant provides good reason to use a fluorinated oil as the carrier fluid. A POSA also certainly would have been aware of this.

214. It was well known that fluorinated oils are not only immiscible with water, but also have miscibility difficulties with non-fluorinated oils. Indeed, perfluorinated oils were known to be essentially immiscible with non-fluorinated oils and were even referred to in the art as oleophobic. *See*, for example, my discussion of Exhibits 1017 and 1018, in Section VII above. On the other hand, fluorinated oils were well known as being compatible with other fluorinated compounds. *Id.* For example, it was well-known at the time that perfluorinated oils and fluorinated surfactants, including fluorinated surfactants with glycol (e.g., PEG or OEG) headgroups that could interact with the aqueous side of an emulsion, were useful for emulsions to contain aqueous solutions of biological materials both because of the high degree of biocompatibility of fluorinated oils and because they formed very strong emulsions. *Id.*

215. The combination of fluorinated oils with fluorinated surfactants when making an emulsion, such as is described in Quake, was so natural at the time

that a POSA would have understood Quake's disclosure about using fluorinated oil surfactants as entailing the use of fluorinated oil as the oil phase of the emulsion. By disclosing the use of a fluorinated oil surfactant, Quake thus provided a POSA with very good reason to use a fluorinated oil as the carrier fluid because fluorinated oils are chemically compatible with fluorinated oil surfactants and because fluorinated oil emulsions were well known to be biologically compatible. Accordingly, Quake in view of Holliger renders obvious the method of claim 1 wherein the oil is fluorinated oil, as recited by claim 6.

216. For the reasons discussed above, Quake and Holliger render each of claims 6 and 8 obvious. The claim chart below reproduces text from exemplary portions of the prior art that demonstrate the obviousness of the claims.

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND HOLLIGER
<p>Claim 6. The method of claim 1, wherein the oil is fluorinated oil.</p> <p>Claim 8. The method of claim 7, wherein the surfactant is fluorinated surfactant.</p>	<p><i>See</i> discussion of claims 1 and 7 above.</p> <p>“Exemplary <b>surfactants</b> include ... <b>fluorinated oils</b>, and other agents that are soluble in oil relative to water.” (EX1005, 35:18-24, 35:27-29; <i>see also Id.</i>, 8:15-18, 9:2-5, 28:7-29; <i>see</i> EX1030, 28:21-23, 35:28-36:5, 36:8-10.)</p>



8. *Claim 9*

9. *The method of claim 1, wherein the at least one plug is a merged plug.*

217. As I discussed in Section IX.1 above, claim 1 is obvious over Quake in view of Holliger. Quake further discloses forming a merged plug. For example, Quake discloses combining w/o droplets with other droplets to facilitate a reaction or analysis in the merged plug. EX1005, 5:11-12, 7:15-17, 15:22-25, 36:9-28, 85:9-86:6. Even where a plug already contained DNA template and PCR reagents at droplet formation at the junction, a POSA would have recognized based on Quake's disclosure that it would be advantageous for certain applications for the plug to be a merged plug. As just one example, a POSA would have recognized that Quake's disclosure of merging two droplets could be useful to increase the concentration of PCR reagents in the plug or add additional reagents, as needed. Accordingly, Quake in view of Holliger renders obvious the method of claim 1, wherein the at least one plug is a merged plug, as recited in claim 9. The claim chart below reproduces text from exemplary portions of the prior art that demonstrate the obviousness of the claim.

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND HOLLIGER
9. The method of claim 1, wherein the at least one plug is a merged plug.	See discussion of claim 1 above.

	<p>“These droplets can be analyzed, combined with other droplets (e.g. to react droplet contents) and/or sorted, as desired.” (EX1005, 5:11-13; <i>see</i> EX1030, 5:21-22,16.)</p> <p>“[T]hese droplets are then sorted, combined and/or analyzed in a microfabricated device.” (EX1005, 15:22-25; <i>see</i> EX1030, 16:4-7.) <i>See also</i> EX1005, 7:15-17, 36:21-28, 85:9-86:6; EX1030, 7:22-8:1, 37:3-5, 84:28-85:10.)</p>
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9. *Claim 11*

*11. The method of claim 1, wherein the at least one plug is substantially spherical in shape.*

218. As I discussed above in Section IX.1, claim 1 is obvious over Quake in view of Holliger. Quake also discloses the at least one plug is substantially spherical in shape. For example, Quake discloses using rounded channels and plugs that substantially conform to the channel shape as substantially spherical droplets. EX1005, 26:30-27:3, 27:9-32.

219. Quake depicts substantially spherical droplets (e.g., 1702, 1705) in Figures 17 and 19:

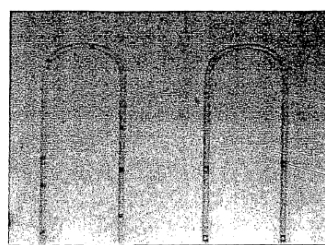


FIG. 17A

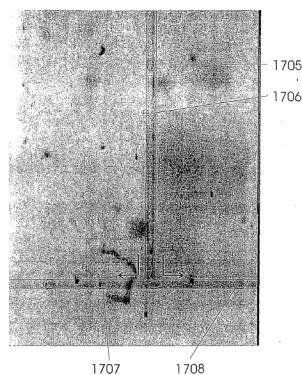


FIG. 17C

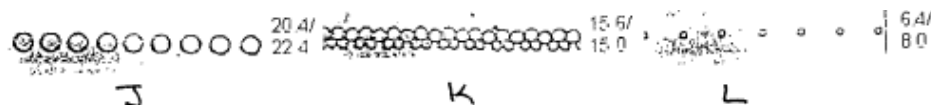
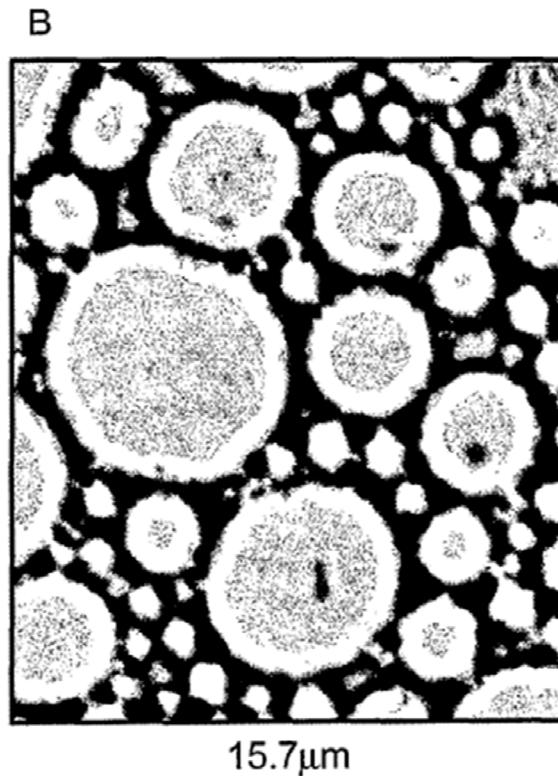


FIG. 19

EX1005, 80:8-14, 81:6-11, 82:19-30 & Figs. 17A, 17C, 19J, 19L.

220. Quake discloses that making the round, monodisperse droplets in Figs. 19J and 19L are preferred, especially in high throughput devices. *Id.*, 27:27-32. Quake discloses that these “round” droplets preferably have a diameter that is smaller than the diameter of the microchannel. *Id.*

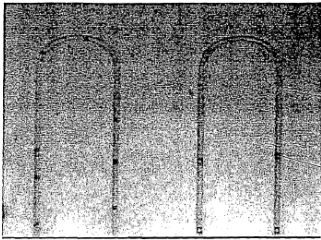
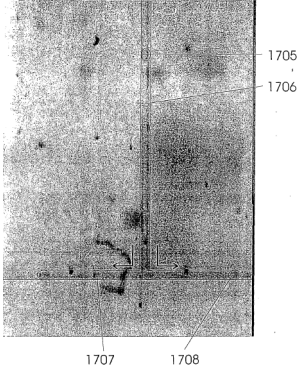
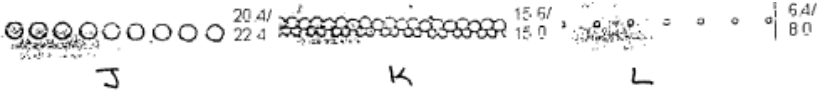
221. Holliger confirmed that PCR amplification in substantially spherical w/o droplets formed in standard hydrocarbons was eminently achievable. Holliger Figure 2, for example, confirms that Holliger’s PCR droplets were substantially spherical:



**FIG. 2**

EX1006, Fig. 2.

222. For the reasons I have just discussed, Quake in view of Holliger renders obvious the method of claim 1 wherein the at least one plug is substantially spherical in shape, and recited by claim 11. The claim chart below reproduces text from exemplary portions of the prior art that demonstrate the obviousness of the claim.

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND HOLLIGER
<p>11. The method of claim 1, wherein the at least one plug is substantially spherical in shape.</p>	<p>See discussion of claim 1 above.</p> <p>“[D]roplets at these dimensions tend to conform to the size and shape of the channels, while maintaining their respective volumes.... ¶More preferably, however, the microfabricated devices of this invention generate round, monodisperse droplets (such as those illustrated in Frames J and L of FIG. 19).” (EX1005, 27:9-32, Fig. 19 (Panels J &amp; L); see EX1030, 28:19-20, Fig. 17A.)</p> <p>“For particles...or molecules that are in droplets...within the flow of the main channel, the channels of the device are preferably round....This geometry facilitates an orderly flow of droplet sin the channels.” See e.g. FIG. 16B.” (EX1005, 26:30-27:3; see EX1030, 27:22-27.)</p> <p>See also EX1005, Figs. 17A, 17C, 19J, 19L &amp; accompanying text.</p> <div data-bbox="638 1283 956 1520"></div> <p data-bbox="651 1530 781 1562"><b>FIG. 17A</b></p> <div data-bbox="1049 1163 1344 1528"></div> <p data-bbox="1053 1547 1146 1575"><b>FIG. 17C</b></p> <div data-bbox="610 1623 1422 1713"></div> <p data-bbox="971 1778 1084 1810"><b>FIG. 19</b></p>

223. For the reasons discussed above, Quake in view of Holliger renders obvious each of claims 1-9 and 11.

**X. GROUND 2: CLAIMS 1-9 AND 11 ARE OBVIOUS OVER QUAKE AND HOLLIGER IN FURTHER VIEW OF SHAW STEWART.**

224. As discussed above in Sections IX, claims 1-9 and 11 are obvious over Quake and Holliger. My discussion from Ground 1 above applies equally here. Shaw Stewart provides additional teachings that render these claims obvious.

225. As discussed above in Section VIII.D, Shaw Stewart discloses a microfluidic system designed to reduce or eliminate contamination by performing chemical reactions, including biological assays, in nanoliter-sized droplets. EX1008, Abstract, 2 (objects 1 and 5), 8. Similar to Quake, Shaw Stewart discloses substantially spherical droplets, stating that the conduit (channel) size is “of the same order of magnitude as the cross-section of the droplets.” *Id.*, 4. Shaw Stewart discloses creating the droplets by injecting a small volume or droplet of reactant through a constriction and into a conduit at a right angle. *Id.*, Abstract, 3. The discrete reactant droplets are thus separated from the parent volume by passing carrier phase along the conduit such that the droplets are separated from each other by an inert, immiscible liquid. *Id.*, Abstract, 3.

226. Shaw Stewart discloses the invention can be used to perform biological or chemical assays. *Id.*, 8. Shaw Stewart discloses mineral oil/spirit or

a fluorinated hydrocarbon may be used as the carrier. *Id.*, Abstract, 4, 8. For example, Shaw Stewart states:

This reactant liquid, hereafter referred to as a reactant, will be supported, defined and moved by another, immiscible liquid, referred to hereafter as the carrier phase. ***Suitable carrier phases include mineral oils***, light silicon oils, water, and ***fluorinated hydrocarbons***.

EX1008, 4:3-7 (emphasis added).

227. Shaw Stewart thus discloses that fluorinated carrier oil was a readily accessible and desirable alternative to the traditional hydrocarbons (*e.g.*, decane and mineral oil) Quake identifies as carrier fluids, especially where Quake identifies fluorinated oil surfactant for use in forming its droplets. In view of Shaw Stewart's disclosure that fluorinated oils were a known alternative to using mineral oil as the immiscible carrier fluid for forming microfluidic droplets for biological reactions, a POSA would have had good reason to use a fluorinated oil as the carrier fluid for forming aqueous droplets containing biological materials, such as in Quake, to provide a chemically compatible carrier oil for use in combination with Quake's fluorinated surfactant.

228. A POSA would have had a reasonable expectation of success for using a fluorinated oil as the carrier fluid because of the known properties of fluorinated oils. For example, fluorinated oils were known to be compatible with biological materials, to be sufficiently hydrophobic to encapsulate aqueous

droplets, and to be desirable for forming w/o emulsions for containing biological materials. EX1017, 209, 212-213; EX1018, Summary. As evidenced by Shaw Stewart, fluorinated oils were also known to be capable of forming microfluidic aqueous droplets when the aqueous solution is flowed into the flowing fluorinated oil. Accordingly, a POSA would have had good reason and reasonable expectation of success in view of Quake, Holliger, and Shaw Stewart to perform PCR amplification in Quake's droplets using fluorinated oil as the carrier fluid and using a fluorinated surfactant.

229. Shaw Stewart is, of course, very relevant to claims 6 and 8, but it is also relevant to the obviousness of the remaining claims discussed herein, as all of these claims require providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified. A POSA would recognize a fluorinated oil carrier fluid would provide a suitable carrier fluid for forming PCR in droplets as described in Quake because of the biocompatibility and miscibility characteristics discussed above. Shaw Stewart's disclosure of fluorinated oil as a suitable carrier fluid for microfluidic biological droplet reactors thus provides additional evidence for the obviousness of each of claims 1-9 and 11 over Quake and Holliger.



**XI. GROUND 3: CLAIMS 1-9 AND 11 ARE OBVIOUS OVER QUAKE IN VIEW OF LITBORN.**

230. It is my opinion that each of claims 1-9 and 11 would have been obvious before May 9, 2002, based on the disclosures of Quake and Litborn. It is my opinion that Quake and Litborn together teach each element of each of these claims and render each claim obvious as a whole. It also is my opinion that a POSA would have had strong motivation to apply the below-cited teachings of Litborn in microfluidic droplets such as those described by Quake and would have, in the process, obtained exactly what is claimed in claims 1-9 and 11. It is my opinion that a POSA not only would have had motivation to do so, but also would have had a reasonable expectation of success in obtaining, exactly what is claimed.

231. A POSA would have looked to combine the teachings of Quake and Litborn to perform PCR in water-in-oil (w/o) droplets in a microfluidic device. Indeed, both references create microscopic w/o droplets for performing enzymatic reactions and teach they were useful for performing PCR analyses. Quake teaches a microfluidic device for making microfluidic water-in-oil (w/o) droplets, including picoliter-sized w/o droplets, for analysis, reaction or sorting. Quake's aqueous droplets are made in standard hydrocarbons such as decane or mineral oil at the junction of two microfluidic channels by continuously flowing the aqueous solution from the injection channel into the oil continuously flowing

in the main flow channel. EX1005, 1:18-24, 5:6-7, 5:21-23, 6:4-8, 6:23-27, 7:30-8:5, 11:20-12:12, 22:6-16, 30:1-2.

232. Quake teaches its microfluidic w/o droplets are useful for microanalysis and as enzyme bioreactors EX1005, 6:10-16, 8:14-15, 8:19-21, 35:9-14 (aqueous fluid is phosphate buffered saline and may contain biological sample, substrate, and/or enzyme); *id.*, 7:15-21, 15:2-6, 22:27-23:3 (discussing enzymatic reaction of biological substrate within the droplets). Quake specifically teaches these droplet bioreactions include reactions using DNA polynucleotides as the biological material. *Id.*, 8:19-23, 13:16-26, 15:18-21. Quake also specifically teaches its microfluidic devices should be used to conduct on-chip PCR, which is an autocatalytic reaction, such as for identification of viral DNA. *Id.* 2:25-3:6, 23:26-30.

233. Although Quake does not provide experimental results from performing PCR amplification in its droplets, Quake expressly suggests doing so and conceptually reduces this to practice by expressly describing PCR and by incorporating Kopp. This suggestion by Dr. Quake would have been highly motivating to a POSA. In contrast to the '193 patent specification and its Provisional Applications (*see* paragraph 43 above), Quake not only squarely describes PCR but also provides detailed information to one of ordinary skill on how PCR is performed, including by citing a PCR reference (EX1011) and

incorporating it in its entirety. *Id.*, 1:10-12, 2:25-3:6, 23:26-30, 89:32. As explained in Section VII above, benefits of miniaturizing PCR were well known and included conserving reagents, decreasing the reaction time, and the ability to successfully perform DNA PCR with a reduced copy number of beginning DNA template (even one template molecular per reaction).

234. A POSA would have had good reason to look to Litborn for further information on successful PCR amplification in microfluidic w/o droplets formed in standard hydrocarbons such as decane and mineral oil. Indeed, Litborn teaches successful nanoscale PCR amplification reactions in aqueous droplets in standard hydrocarbons (*e.g.*, octane) flowing through a microfluidic channel. EX1007, 447, 451-53; *see also* EX1009, 109, 120. Litborn teaches that performing PCR in w/o droplets helps to address cross-contamination between samples and also provides “effective mixing” within each sample. *Id.*, 451-53. A POSA thus had good reasons to combine the teachings of Quake and Litborn to perform PCR in water-in-oil (w/o) droplets in a microfluidic device because both references create microscopic w/o droplets for performing enzymatic reactions and teach they were useful for performing PCR analyses.

235. The Quake and Litborn references expressly contemplate performing PCR in the w/o droplets in a microfluidic device in a manner that satisfies each element of the challenged claims. As discussed in paragraphs 231-34 above,

Quake discloses the claimed microfluidic device and using it to make the microfluidic plugs at the junction by continuously flowing the aqueous solution into the continuously flowing oil. Quake suggests the enzymatic reaction in its droplets should be PCR by citing and incorporating Kopp, with its description of performing PCR using thermocycling. EX1005, 23:26-27, 89:31; EX1011, 36-37. Litborn also discloses the PCR reaction mixture is preferably included in the aqueous fluid before droplet formation, after which Litborn suggests to a POSA that the PCR amplification reaction is induced by thermocycling. EX1007, 451 (“simplest version of a fluidic reactor is the transport of a pre-mixed sample through a channel, which has the desired reaction conditions, *e.g.*, an elevated temperature zone”). Litborn also confirmed aqueous droplets formed in standard hydrocarbon were suitable for PCR amplification by reporting successful PCR amplification. EX1007, 451-53.

236. The combined teachings of Quake and Litborn also illustrate that combining the PCR reaction mixture in the aqueous fluid before droplet formation was a reasonable and convenient approach for performing PCR in the droplets. As was usual in the literature, Litborn taught using a “pre-mixed” reaction mixture and suggested to a POSA initiating PCR amplification by thermocycling. *Id.*, 451. Any POSA would have known that PCR, unlike many other enzymatic reactions, does not spontaneously proceed upon the combination

of PCR reagents with DNA template but instead requires thermocycling repeatedly through the three distinct temperature stages to induce amplification. *See, e.g.,* EX1011, 1046.

237. In view of Litborn's teachings, a POSA would have had good reasons to perform PCR in Quake's droplets formed at the junction as claimed and to provide conditions suitable for PCR amplification in the droplet. Because PCR amplification does not occur spontaneously upon combination of enzyme with substrate and other reagents, a POSA would have recognized easily that it was simple to combine the DNA template and PCR reagents before droplet formation. Quake's practice of merging substrate droplets with reactant droplets would have had good application for reactions different than PCR, but a POSA would have recognized this practice was not strictly necessary for PCR amplification. A POSA would have had motivation to combine the PCR reagents before droplet formation, as in Litborn, when forming PCR droplets at the channel junction, as in Quake, to provide a simple, direct, and efficient means for PCR droplet formation in which droplets PCR subsequently could be performed.

238. Litborn evidences this understanding by disclosing the successful performance of PCR amplification in sub-microliter aqueous droplets formed in standard hydrocarbon oil and by stating that pre-mixed aqueous fluid already containing both the DNA substrate and the other PCR reagents was "the simplest

version of a fluidic reactor.” EX1007, 451. Based on the disclosures of Quake and Litborn, a POSA thus would have had good reason to form Quake’s PCR droplets at the junction of Quake’s microchannels by continuously flowing an aqueous solution that already contained at least one DNA substrate and PCR reagents through an inlet or injection channel into the oil flowing continuously in the main channel and to provide conditions suitable for the autocatalytic reaction PCR in at least one droplet such that the at least one substrate molecule is amplified, including by thermocycling the droplet after droplet formation, as disclosed in Litborn.

239. A POSA reading Litborn would have recognized Quake’s droplet-formation microfluidic device was quite attractive for high-throughput formation of PCR droplets because of Quake’s ability to form generally monodisperse (*i.e.*, uniformly-sized) droplets. EX1005, 27:27-32 & Figs. 17A, C and 19J, L. Forming many uniformly-sized droplets would mean that roughly the same amount of template and reactants would be found in each droplet. A POSA would have recognized that this uniformity even at high throughput, as reported by Quake, was beneficial for w/o droplet PCR. Litborn recognized the potential for w/o droplets created on a massive scale for PCR reactions. EX1007, 453. Holliger did as well. EX1006, 12:21-26. Quake’s ability to create these droplets

uniformly and with high throughput thus would have provided additional reason to combine the references as discussed herein.

240. Litborn also confirms that Quake discloses conditions suitable for PCR amplification in the droplet. The PCR reference Quake incorporated in its entirety (EX1011 at 1046-47) teaches how to perform PCR amplification using DNA *Taq* polymerase by thermocycling repeatedly through the three temperature stages to denature the DNA, anneal the primers to the DNA, and extend the DNA. Litborn discloses that PCR amplification was successful in sub-microliter aqueous droplets formed in standard hydrocarbon oil, the same kind of droplet materials disclosed in Quake. Litborn thus confirms a POSA would have had a reasonable expectation of success in successful PCR amplification in microfluidic aqueous droplets formed in standard hydrocarbons such as disclosed in Quake. As discussed above in Section VII, other references also confirmed reasonable expectation of success for PCR amplification in microfluidic aqueous droplets formed in standard hydrocarbons. *See, e.g.*, EX1006, 8:21-9:6, 52:25-53:2, 59:16-21, 63:9-13; EX1009, 109.

241. As discussed above in Section VII, a POSA would have known that microfluidic PCR could be performed in a stationary PCR thermocycling chamber or in a flow-through thermocycling channel. A POSA would have recognized that both options remained available for thermocycling Quake's PCR

droplets. For a stationary reaction chamber, a POSA would have recognized that droplet formation could be paused during thermocycling or that the droplets could be directed into one of several parallel reaction chambers at a time. When a given reaction chamber was full, additional droplets could be directed to a different reaction chamber to proceed in parallel with the earlier chamber(s). A POSA would have had a reasonable expectation of successfully performing PCR amplification in Quake's w/o droplets in a microfluidic reaction chamber just as thermocycling in microfluidic reaction chambers on chips had been performed previously. *See* Section VII (discussing, *e.g.*, EX1012); *see also* EX1006, 8:21-9:6, 52:25-53:2, 59:16-21, 63:9-13.

242. A POSA also would have had a reasonable expectation of successfully performing PCR amplification in Quake's w/o droplets using flow-through thermocycling. As discussed above in Section VII, practitioners had successfully done so already in standard hydrocarbons such as octane and mineral oil. *See, e.g.*, EX1009, 109; EX1007, 451-453.

243. The speed of the flow-through thermocycling would not present a difficult obstacle to PCR amplification. Multiple options were well-known to practitioners, as evidenced by the success of the Schneegaß reference (EX1009) in doing this specifically on a microfluidic chip. Moreover, as discussed above, droplets of the desired size could be obtained at slower flow speeds by altering



the surfactant amounts, droplets could be formed at high speed with thermocycling performed at slower speeds simply by widening the channel. Moreover, the Litborn reference even taught available reaction time on-chip could be increased using stacked chips and other methods. EX1007, 451. Quake's and Litborn's teachings that PCR can, should, and could be performed in microfluidic w/o droplets formed in standard hydrocarbons are thus confirmed by the knowledge of the POSA at the time, as evidenced by other references available to the POSA.

244. As I discuss in detail below, the combination of teachings from Quake and Litborn as discussed above renders obvious each of claims 1-9 and 11. Below, I analyze each claim over these disclosures of Quake in view of Litborn and find that each and every element of each of claims 1-9 and 11 is disclosed in these references and that these claims are each obvious as a whole. I have included charts below to show exemplary text from the prior art references showing the disclosure of the claim elements below.

## **1. Claim 1**

*1.[preamble] A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:*

245. Quake in view of Litborn discloses a method for conducting an autocatalytic reaction in plugs in a microfluidic system. Quake discloses “[a]

microfluidic device for analyzing and/or sorting biological materials,” including specifically polynucleotides and viruses. EX1005, Abstract; *see also id.*, 1:15 (“This invention relates to microfluidic devices and methods”), 26:30-27:32 (channels and droplets of micron dimensions), 29:7-32 (discussing pressure-driven and electro-osmotic flow in channels of microfluidic device). Quake’s microfluidic droplets preferably have a diameter smaller than the diameter of the microchannel and a volume of 0.1-100 picoliters. *Id.*, 1:15-28, 27:27-30.

246. As discussed above, Quake suggests performing the autocatalytic reaction PCR, in the droplets in a microfluidic system by teaching that its microfluidic devices may be used to conduct on-chip PCR and by incorporating a PCR reaction reference in its entirety. EX1005, 1:29-2:9. 2:29-32, 23:26-30 (citing EX1011). Quake teaches performing enzyme-catalyzed bioreactions within microfluidic aqueous droplets formed in an immiscible fluid. *Id.*, Abstract, 5:1-12, 5:29-32, 6:10-16, 7:6-15, 8:14-21 35:9-14, 36:9-28, 85:9-86:6 & Fig. 22. Quake specifically teaches these reactions include reactions with biological materials such as DNA polynucleotides. *Id.*, 8:19-23, 13:16-26, 15:18-21.

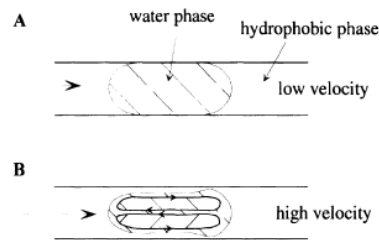
247. Litborn discloses successfully performing PCR amplification in sub-microliter scale water-in-oil droplets like Quake’s. EX1007, 447, 451-53 & Fig. 7; *see also* EX1009, 109, 120. Regarding PCR amplification in these w/o

droplets, Litborn reports that “[a]mplification was successful, and the results were fully comparable with the results of the commercial standard procedure.” EX1007, 453.

248. In view of Litborn’s disclosure that PCR amplification could be performed successfully in sub-microliter w/o droplets such as those formed in Quake, a person of ordinary skill in the art would have had good reason and a reasonable expectation of success for conducting an autocatalytic reaction in plugs in a microfluidic system. Quake and Litborn thus disclose a method for conducting an autocatalytic reaction in plugs in a microfluidic system, as recited in claim element 1.[preamble].

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND LITBORN
<p>1.[preamble] A method for conducting an autocatalytic reaction in plugs in a microfluidic system, comprising the steps of:</p>	<p><i>See discussion of Quake’s disclosures for claim element 1.[preamble] in Ground 1 above.</i></p> <p><b><u>Litborn discloses a method for conducting an autocatalytic reaction (PCR) in w/o droplets a microfluidic system:</u></b></p> <p>“A concept which should have one of the best potentials for high throughput sequential flow reactions is the use of systems with two immiscible fluids. A <b>droplet of water in a continuous flow of a hydrocarbon</b> in a hydrophobized channel will keep well separated from <b>an adjacent water droplet</b>, even at high velocities. Instead of disintegration into smaller droplets, the water droplet detaches from the surface while the</p>

hydrocarbon creates a layer between the channel surface and the droplet (Figure 7).



**Figure 7:** A: Transport of a water droplet in a hydrophobic solvent at low velocity. B: Same as in A, but at a high velocity.

...We have carried out preliminary experiments with a **flow based PCR reaction, where the reaction mixture is interspaced with a hydrocarbon, as described above. Amplification was successful**, and the results were fully comparable with the results of the commercial standard procedure. It seems that this mode of operation could hold the potential to carry out a massive number of **PCR reactions in nanoliter or even sub-nanoliter plugs of solution.** This would have fundamental impacts on throughput bottlenecks in molecular biology.” EX1007, 452-53 & Fig. 7.

*1.[a] providing the microfluidic system comprising at least two channels having at least one junction;*

249. Quake discloses providing the microfluidic system comprising at least two channels having at least one junction. For example, Quake discloses forming droplets at the junction of two microfluidic channels (a main channel and at least one sample inlet channel). EX1005, 1:18-24, 6:4-8, 7:22-26, 11:17-21, 22:6-12 & Figs. 16A-B, 17C. Quake discloses the junction is preferably a T-junction. *Id.*, 6:23-27. Quake thus discloses providing the microfluidic system comprising at

least two channels having at least one junction, as required by claim element

1.[a].

*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;*

250. Quake in view of Litborn discloses continuously 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. For example, Quake discloses continuously flowing an aqueous fluid containing a substrate or reagents for conducting an enzymatic reaction through a first channel of the at least two channels. EX1005, 1:15-28, 5:21-23, 6:4-8, 7:30-8:5, 11:20-12:12, 22:6-16. Quake discloses that the aqueous fluid may comprise a biological sample such as DNA, a substrate, an enzyme, buffer such as phosphate buffer saline, and reaction reagents. EX1005, 6:10-16, 8:14-15, 8:19-23, 13:16-26, 15:18-21, 35:9-14. Quake teaches using its droplets for enzyme-catalyzed bioreactions reactions. EX1005, 7:17-21, 8:1-5, 22:30-23:3.

251. Litborn discloses forming microfluidic droplets from an aqueous fluid containing a substrate and reagents for conducting an autocatalytic reaction (*e.g.*, PCR). For example, Litborn describes formation of “a ***droplet of water*** in a continuous flow of a hydrocarbon in a hydrophobized channel...at high velocities.” *Id.*, 452 (emphasis added) & Fig. 7. Litborn discloses the PCR

reaction mixture is preferably included in the aqueous fluid *before* droplet formation, after which the PCR amplification reaction is induced by thermocycling. EX1007, 451 (“simplest version of a fluidic reactor is the transport of a pre-mixed sample through a channel, which has the desired reaction conditions, *e.g.*, an elevated temperature zone....”). Litborn thus teaches that the substrate and PCR reagents conveniently may be combined *before* w/o droplet formation because the autocatalytic PCR reaction requires thermocycling. A POSA thus would have had good reason to combine the substrate and PCR reagents in Quake’s aqueous fluid before forming the w/o droplets.

252. The convenience of forming ready-made droplets at the junction with all required ingredients for PCR amplification provided a POSA with good reason to combine the substrate and PCR reagents in Quake’s aqueous fluid before forming the w/o droplets by continuously flowing the aqueous fluid through the first channel into the oil flowing continuously in the second channel. As discussed above, Litborn, Holliger, and Schneegaß all support a reasonable expectation of being able to do this successfully. For the reasons discussed above, Quake in view of Litborn thus teach continuously flowing an aqueous fluid containing at least one substrate molecule and reagents for conducting an autocatalytic reaction through a first channel, as required by claim element 1.[b].

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND LITBORN
<p>[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;</p>	<p>See discussion of Quake’s disclosures for claim element 1.[b] in Ground 1 above.</p> <p>“4. Flow-based reactors Microfluidic systems also offer exciting possibilities for nanoscale reactions. The simplest version of a fluidic reactor is the transport of a <b>pre-mixed sample through a channel</b>, which has the desired reaction conditions, e.g., an elevated temperature zone....” EX1007, 451.</p> <p>“A <b>droplet of water in a continuous flow of a hydrocarbon</b> in a hydrophobized channel will keep well separated from <b>an adjacent water droplet</b>, even at high velocities.</p> <p>Figure 7 consists of two diagrams, A and B, illustrating the transport of a water droplet in a hydrophobic solvent. Diagram A shows a water droplet (labeled 'water phase') moving through a hydrophobic phase (labeled 'hydrophobic phase') at 'low velocity'. The droplet is elongated and has a tail. Diagram B shows the same droplet at 'high velocity', where it is more elongated and has a more pronounced tail. Arrows indicate the direction of flow from left to right.</p> <p>Figure 7: A: Transport of a water droplet in a hydrophobic solvent at low velocity. B: Same as in A, but at high velocity.</p> <p>We have carried out preliminary experiments with a flow based PCR reaction, where <b>the reaction mixture</b> is interspaced with a hydrocarbon, as described above.” EX1007, 453 &amp; Fig. 7.</p>

*1.[c] flowing an oil through the second channel of the at least two channels;*

253. Quake discloses continuously flowing an oil through the second channel of the at least two channels. For example, Quake discloses forming

microfluidic w/o droplets at the junction by continuously flowing an aqueous fluid through a first channel into an oil flowing continuously in a second channel. EX1005, 1:15-28, 5:21-23, 6:4-8, 7:30-8:5, 11:20-12:12, 22:6-16. Quake thus discloses flowing an oil through the second channel of the at least two channels, as required by claim element 1.[c].

*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,*

254. Quake in view of Litborn discloses 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. Quake discloses forming at least one plug of aqueous fluid for performing an enzymatic reaction on a substrate molecule by partitioning the aqueous fluid with the flowing oil at the junction of the at least two channels.

255. For example, Quake discloses forming microfluidic w/o droplets at the junction by continuously flowing an aqueous fluid through a first channel into an oil continuously flowing in a second channel. EX1005, 1:15-28, 5:21-23, 6:4-8, 7:30-8:5, 11:20-12:12, 22:6-16. Quake discloses adjusting the pressures of the oil and aqueous solutions so that the stream of aqueous solution is sheared off at a regular frequently as it enters the oil stream to form the w/o droplets. *Id.*, 1:15-28, 19:26-32, 78:17-79:31, 81:31-82:8, Figs. 16-22 & accompanying text.



256. Quake also discloses that the aqueous fluid may contain reagents for an enzymatic reaction and teaches using the droplets formed from the aqueous fluid for conducting enzyme-catalyzed bioreactions. EX1005, 6:10-16, 8:14-15, 8:19-23, 13:16-26, 15:18-21, 35:9-14 (aqueous fluid); *Id.*, 7:17-21, 8:1-5, 22:30-23:3 (enzyme-catalyzed bioreaction). Quake thus teaches forming at least one plug of the aqueous fluid containing reagents for an enzymatic reaction by partitioning the aqueous fluid with the continuously flowing oil at the junction of the at least two channels. As discussed above for element 1.[b], Quake suggests the enzymatic reaction may be PCR.

257. As discussed above for element 1.[b] and included here, Litborn discloses forming microfluidic droplets from an aqueous fluid already containing a substrate and reagents for conducting autocatalytic PCR and initiating PCR via thermocycling. *See* discussion of Litborn's disclosures for forming w/o droplets containing at least one substrate molecule and reagents for claim element 1.[b] above.

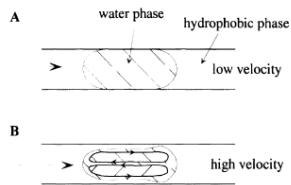
258. As I explained for element 1.[b], Litborn confirms a POSA had good reasons (e.g., simplicity and convenience for reactions that do not spontaneously proceed absent thermocycling) and a reasonable expectation of success for combining the substrate and PCR reagents in Quake's aqueous fluid before forming the w/o droplets by continuously flowing the aqueous fluid through a

first channel into the oil continuously flowing in the second channel. Quake in view of Litborn thus teach 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, as required by claim element 1.[d].

*1.[d.1] the plug being substantially surrounded by an oil flowing through the channel,*

259. Quake discloses the plug being substantially surrounded by an oil flowing through the channel. For example, Quake teaches forming microfluidic aqueous droplets in a continuously flowing immiscible fluid such that biological materials (such as DNA) within the droplets do not uncontrollably mix. *Id.*, Abstract, 5:1-12, 5:29-32, 7:6-15, 36:9-28, 85:9-86:6 & Fig. 22. The aqueous droplets are substantially surrounded by oil, and are thus separated from each other and from the channel wall by oil. *Id.*, 29:33-30:2, Figs. 17A, 17B, 17C, 19J, 19L. Quake thus discloses claim element 1.[d.1].

260. Litborn's discloses successful PCR amplification in microfluidic aqueous droplets that are substantially surrounded by an oil flowing through the microchannel. EX1007, 451-53 & Fig. 7. Litborn thereby confirms a POSA would have had a reasonable expectation of success.

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND LITBORN
<p>[1.d.1] the plug being substantially surrounded by an oil flowing through the channel,</p>	<p>See discussion of Quake’s disclosures for claim element 1.[d.1] in Ground 1 above.</p> <p><b><u>Litborn discloses the w/o droplet being substantially surrounded by an oil flowing through the channel:</u></b></p> <p>“A concept which should have one of the best potentials for high throughput sequential flow reactions is the use of systems with two immiscible fluids. A <b>droplet of water in a continuous flow of a hydrocarbon</b> in a hydrophobized channel <b>will keep well separated from an adjacent water droplet</b>, even at high velocities. Instead of disintegration into smaller droplets, <b>the water droplet detaches from the surface while the hydrocarbon creates a layer between the channel surface and the droplet</b> (Figure 7).</p>  <p>Figure 7 consists of two diagrams, A and B, illustrating the transport of a water droplet in a hydrophobic solvent. Diagram A shows a water droplet (labeled 'water phase') in a hydrophobic phase (labeled 'hydrophobic phase') at low velocity. The droplet is in contact with the channel surface. Diagram B shows the same system at high velocity. The droplet is elongated and has a layer of hydrocarbon between it and the channel surface, preventing contact. Arrows indicate the direction of flow in both diagrams.</p> <p><b>Thus, there is no more surface contact of the water (and no more risk for surface interactions).”</b> EX1007, 452-53 &amp; Fig. 7.</p>

*1.[d.2] 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*

261. As I explained above for element 1.[b] and 1.[d], Quake in view of Litborn discloses the plug fluid comprises at least one substrate molecule and reagents for conducting an autocatalytic reaction with the at least one substrate molecule. Once the plug fluid is sheared off into individual plugs, as discussed above for element 1.[d], the extruded plug comprises the substrate molecule and reagents for conducting the autocatalytic reaction that were already present in the plug fluid. Indeed, Litborn teaches performing PCR amplification in the microfluidic w/o droplets, which would not proceed if the droplets did not comprise substrate molecule and PCR reagents after droplet formation. Encapsulating the substrate molecule together with the PCR reactions in the droplet was exactly the purpose of including them in the same aqueous fluid used to make the droplets, as discussed above for element 1.[b] and 1.[d].

262. Moreover, a POSA could reasonably expect success in encapsulating DNA template (substrate molecule) and PCR reagents together in a single microfluidic-sized droplets, as evidenced by Litborn, Holliger, and Schneegaß, and in a microfluidic-sized volume, as evidenced by, for example, Lagally (EX1012) and He (EX1013), discussed in Section VII above.

263. For the reasons discussed above, Quake in view of Litborn discloses 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 required by claim element 1.[d.2].

*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.*

264. Quake in view of Litborn discloses providing conditions suitable for the autocatalytic reaction PCR in the at least plug such that the at least one substrate molecule is amplified. Even without Litborn, Quake provided POSAs with all the information they needed to perform PCR amplification in Quake's droplets. For example, Quake specifically teaches its microfluidic devices may be used to conduct on-chip PCR (an autocatalytic reaction). EX1005, 23:26-30. Quake cites and incorporates Kopp (EX1011) in its entirety for its teachings about PCR reactions. EX1005, 1:10-12, 23:26-30, 89:31.

265. Kopp discloses that PCR amplification may be accomplished in microfluidic systems in the same manner as it is performed outside microfluidic systems. For example, Kopp discloses that the aqueous fluid must contain at least one substrate molecule and PCR reagents, such as polymerase, primers, and dNTPs in aqueous buffer, to perform PCR. EX1011, 1046-47. Kopp also discloses that PCR is performed by thermocycling the aqueous PCR mixture for

repeated cycles of unzipping the DNA, annealing the primers, and extending the primers. *Id.* Kopp thus teaches conditions that are, in fact, suitable for droplet PCR amplification. These conditions were well-known and widely-practiced by persons of ordinary skill in the art at the relevant times. By incorporating Kopp's disclosure of PCR reagents and thermocycling, Quake disclosed the conditions that would have allowed PCR to proceed in Quake's droplets. Quake thus discloses element 1.[e].

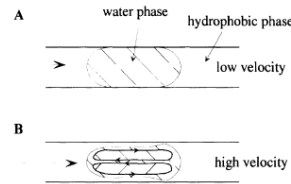
266. Litborn also discloses providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified. For example, Litborn teaches successful nanoscale PCR amplification reactions in microliter scale aqueous droplets in standard hydrocarbons (*e.g.*, octane) flowing through a microfluidic channel. EX1007, 447, 451-53 & Fig. 7; *see also* EX1009, 109, 120. Regarding PCR amplification in these w/o droplets, Litborn reports that "[a]mplification was successful, and the results were fully comparable with the results of the commercial standard procedure." EX1007, 453. Litborn states that "this mode of operation could hold the potential to carry out a massive number of PCR reactions in nanoliter or even sub-nanoliter plugs of solution." *Id.*, 453. Litborn teaches the reaction proceeds simply by transporting the pre-mixed sample through a channel having the desired conditions, "*e.g.*, an elevated temperature zone." EX1007, 451. Litborn

thus also teaches claim element 1.[e] and confirms that thermocycling Quake’s droplets comprising DNA template and PCR reagents provided conditions suitable for PCR amplification. Litborn, like Holliger discussed above in Ground 1, provides more evidence about conditions suitable for PCR in plugs than the ’193 patent itself.

267. For the reasons discussed above, Quake in view of Litborn discloses each and every element of claim 1 and renders claim 1 obvious as a whole.

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND LITBORN
<p>[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.</p>	<p><i>See</i> discussion of Quake’s disclosures for claim element 1.[e] in Ground 1 above.</p> <p><b><u>Litborn discloses providing conditions suitable for the PCR autocatalytic reaction in the w/o droplet such that the substrate molecule is amplified:</u></b></p> <p>“4. Flow-based reactors Microfluidic systems also offer exciting possibilities for <b>nanoscale reactions</b>. The simplest version of a fluidic reactor is the transport of a pre-mixed sample through a channel, which has <b>the desired reaction conditions, e.g., an elevated temperature zone....</b>” EX1007, 451.</p> <p>“A <b>droplet of water in a continuous flow of a hydrocarbon</b> in a hydrophobized channel will keep well separated from <b>an adjacent water droplet</b>, even at high velocities. Instead of</p>

disintegration into smaller droplets, the **water droplet detaches from the surface** while the hydrocarbon creates a layer between the channel surface and the droplet (Figure 7).



**Figure 7:** A: Transport of a water droplet in a hydrophobic solvent at low velocity. B: Same as in A, but at high velocity.

Thus, there is no more surface contact of the water (and no more risk for surface interactions). An additional benefit of this situation is that the friction between the hydrocarbon and the water leads to an intrasegmental movement within the droplet, which means **that an effective mixing action is obtained**. The principles of this phenomenon have been described in detail.

**We have carried out preliminary experiments with a flow based PCR reaction, where the reaction mixture is interspaced with a hydrocarbon, as described above. Amplification was successful**, and the results were fully comparable with the results of the commercial standard procedure. It seems that this mode of operation could hold the potential to carry out a massive number of **PCR reactions in nanoliter or even sub-nanoliter plugs of solution**. This would have fundamental impacts on throughput bottlenecks in molecular biology.” EX1007, 452-53 & Fig. 7.

268. For the reasons discussed above, Quake in view of Litborn discloses each and every element of claim 1 and renders claim 1 obvious as a whole.



**i. Claim 2**

*2. The method of claim 1, wherein the at least one substrate molecule is a single biological molecule.*

269. As I discussed above in Section XI.1, claim 1 is obvious over Quake and Litborn. Quake also discloses the substrate molecule is a single biological molecule. For example, Quake discloses that each droplet preferably encapsulates only a single biological particle. EX1005, 5:7-8, 6:16-21, 8:5-8, 9:5-9, 36:29-37:28. Accordingly, Quake in view of Litborn renders obvious the method of claim 1 wherein the at least one substrate molecule is a single biological molecule, as recited by claim 2.

**ii. Claim 3**

*3. The method of claim 2, wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.*

270. As I discussed in Section XI.2 above, claim 2 is obvious over Quake and Litborn. Quake and Litborn also each disclose the substrate molecule is DNA and the autocatalytic reaction is PCR. *See, e.g.*, EX1005, 2:29-3:6, 23:26-27, 89:31; EX1007, 447, 451-53; *see also* EX1009, 109, 120 (“Litborn and co-workers recently reported a successful DNA amplification”). Accordingly, Quake in view of Litborn thus renders obvious the method of claim 2 wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase chain reaction, as recited by claim 3. The claim chart below

reproduces text from exemplary portions of the prior art that demonstrate the obviousness of the claim.

CLAIM ELEMENT	OBVIOUS OVER QUAKE AND LITBORN
3. The method of claim 2, wherein the at least one substrate molecule is DNA and the autocatalytic reaction is a polymerase-chain reaction.	<p><i>See</i> discussion of claim 1 above.</p> <p><i>See</i> discussion of Quake’s disclosures for claim 3 in Ground 1 above.</p> <p>“We have carried out preliminary experiments with a flow based <b>PCR reaction</b> where the reaction mixture is interspaced with a hydrocarbon, as described above. <b>Amplification</b> was successful, and the results were fully comparable with the results of the commercial standard procedure.” EX1007, 453.</p>

**iii. Claim 4**

*4. The method of claim 1, wherein the providing step includes heating.*

271. As I discussed in Section XI.1 above, claim 1 is obvious over Quake and Litborn. Quake and Litborn also each disclose the providing step includes heating. Heating was well-known to POSAs before the ’193 patent for performing the PCR reaction. For example, Quake cites and incorporates by reference Kopp for its discussion of microfluidic PCR. EX1005, 23:26-27, 89:31 (citing EX1011). Kopp discloses that PCR is “simply performed by heating and cooling” and that the optimal temperatures are 95°C for melting the DNA, 50° to 65°C for primer annealing, and 72° to 77°C for primer extension. EX1011, 1046.

272. Litborn also discloses that PCR employs thermocycling. EX1007, 451 (“elevated temperature zone”). Accordingly, Quake in view of Litborn thus renders obvious the method of claim 1 wherein the providing step includes heating, as recited by claim 4.

**iv. Claim 5**

*5. The method of claim 1, further comprising the step of providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred.*

273. As I discussed in Section XI.1 above, claim 1 is obvious over Quake and Litborn. Quake discloses providing a detector to detect, analyze, characterize and monitor one or more properties of the reaction during and after it has occurred. For example, Quake discloses the microfluidic device comprises a detector, preferably an optical detector downstream of the junction. EX1005, 6:28-31.

274. Quake also discloses that an enzyme and a biological material substrate of the enzyme may react with one another within a droplet and the reaction may produce a signal that is detected as the droplet passes through the detection region. *Id.*, 7:15-21, 22:27-23:3 (discussing detection region and detection of, for example, “a chemical reaction of a substrate catalyzed by an enzyme”), 32:1-34:11 (discussing detection, including using fluorescent

reporters), 17:3-25 (discussing fluorescent labels and other reporters), 51:13-21 (same), 52:6-21 (same); *Id.*, 15:2-6 (defining enzyme as a catalyst and substrate as any substance on which an enzyme acts), 49:13-25 (discussing using reporters to quantitate nucleic acid content for cellular DNA/RNA analysis), 50:7-23 (discussing fluorescent reporters activated by interaction of biological material with one or more reagents added to the fluid medium—any reporter or combination of substrate, enzyme and product can be used for detection). Quake discloses that the biological materials may thus be “analyzed and/or identified” but also may be sorted. *Id.*, 9:10-13.

275. Quake in view of Litborn thus renders obvious the method of claim 1 further comprising the step of providing a detector to detect, analyze, characterize, or monitor one or more properties of the autocatalytic reaction during and/or after it has occurred, as recited by claim 5.

**v. Claim 7**

*7. The method of claim 1, wherein the carrier fluid further comprises a surfactant.*

276. As I discussed above in Section XI.1, claim 1 is obvious over Quake and Litborn. Quake teaches the oil carrier (extrusion) fluid comprises a surfactant. EX1005, 8:15-18, 9:4-5; 28:24-29, 35:18-24. Quake teaches including surfactant in the carrier fluid prevents adherence of particles to the sides of the

channel and also promotes droplet formation. For example, Quake teaches putting a surfactant in the oil to help form the w/o droplets. EX1005, 35:18-24, 35:27-29. Quake also discloses the surfactants may be used in the carrier oil to coat the channel walls as the oil flows through the channels. EX1005, 28:6-29, 35:18-24, 35:27-29.

277. Quake discloses that the oil carrier fluid can be a non-polar solvent, and gives decane, tetradecane, hexadecane, and mineral oil as examples. EX1005, 6:8-10, 8:12-13, 9:2-4, 21:26-22:5, 28:23-29, 35:14-17, 82:1-2. Quake discloses exemplary surfactants, including Tween, Span, and fluorinated oils. *Id.*, 28:7-29, 35:18-24, 35:27-29, 82:2-3; *see also* EX1009, 110 (using Tween surfactant for flow-through droplet PCR to stabilize the sample droplets in the oil flow and to simultaneously stabilize the polymerase during PCR). Quake in view of Litborn thus renders obvious the method of claim 1, wherein the carrier fluid further comprises a surfactant, as required by claim 7.

**vi. Claims 6 and 8**

*10. Claims 6 and 8*

*6. The method of claim 1, wherein the oil is fluorinated oil.*

*8. The method of claim 7, wherein the surfactant is fluorinated surfactant.*

278. As I discussed above in Sections XI.1 and XI.6, claims 1 and 7 are each obvious over Quake and Litborn. As discussed above for claim 7, Quake

discloses the surfactant may be fluorinated oil and that the surfactants may be used in the carrier oil to coat the channel walls and to form droplets. EX1005, 28:6-29, 35:18-24, 35:27-29. Quake thus discloses the method of claim 7 wherein the surfactant is fluorinated surfactant, as required by claim 8.

279. By disclosing using a fluorinated oil surfactant that is soluble in the oil, Quake suggests using carrier fluid oils that would be compatible with fluorinated oil surfactants, and most especially using fluorinated oil as the carrier fluid. Even an undergraduate student of chemistry should know that using a fluorinated oil surfactant provides good reason to use a fluorinated oil as the carrier fluid. A POSA also certainly would have been aware of this.

280. It was well known that fluorinated oils are not only immiscible with water, but also have miscibility difficulties with non-fluorinated oils. Indeed, perfluorinated oils were known to be essentially immiscible with non-fluorinated oils and were even referred to in the art as oleophobic. *See*, for example, my discussion of Exhibits 1017 and 1018, in Section VII above. On the other hand, fluorinated oils were well known as being compatible with other fluorinated compounds. *Id.* For example, it was well-known at the time that perfluorinated oils and fluorinated surfactants, including fluorinated surfactants with glycol (e.g., PEG or OEG) headgroups that could interact with the aqueous side of an emulsion, were useful for emulsions to contain aqueous solutions of biological

materials both because of the high degree of biocompatibility of fluorinated oils and because they formed very strong emulsions. *Id.*

281. The combination of fluorinated oils with fluorinated surfactants when making an emulsion, such as is described in Quake, was so natural at the time that a POSA would have understood Quake's disclosure about using fluorinated oil surfactants as entailing the use of fluorinated oil as the oil phase of the emulsion. By disclosing the use of a fluorinated oil surfactant, Quake thus provided a POSA with very good reason to use a fluorinated oil as the carrier fluid because fluorinated oils are chemically compatible with fluorinated oil surfactants and because fluorinated oil emulsions were well known to be biologically compatible. Accordingly, Quake in view of Litborn renders obvious the method of claim 1 wherein the oil is fluorinated oil, as recited by claim 6.

282. For the reasons discussed above, Quake and Litborn render each of claims 6 and 8 obvious.

**vii. Claim 9**

*9. The method of claim 1, wherein the at least one plug is a merged plug.*

283. As I discussed in Section XI.1 above, claim 1 is obvious over Quake in view of Litborn. Quake further discloses forming a merged plug. For example, Quake discloses combining w/o droplets with other droplets to facilitate a

reaction or analysis in the merged plug. EX1005, 5:11-12, 7:15-17, 15:22-25, 36:9-28, 85:9-86:6. Even where a plug already contained DNA template and PCR reagents at droplet formation at the junction, a POSA would have recognized based on Quake's disclosure that it would be advantageous for certain applications for the plug to be a merged plug. As just one example, a POSA would have recognized that Quake's disclosure of merging two droplets could be useful to increase the concentration of PCR reagents in the plug or add additional reagents, as needed. Accordingly, Quake in view of Litborn renders obvious the method of claim 1, wherein the at least one plug is a merged plug, as recited in claim 9.

**viii. Claim 11**

*11. The method of claim 1, wherein the at least one plug is substantially spherical in shape.*

284. As I discussed above in Section XI.1, claim 1 is obvious over Quake in view of Litborn. Quake also discloses the at least one plug is substantially spherical in shape. For example, Quake discloses using rounded channels and plugs that substantially conform to the channel shape as substantially spherical droplets. EX1005, 26:30-27:3, 27:9-32.

285. Quake depicts substantially spherical droplets (e.g., 1702, 1705) in Figures 17 and 19:



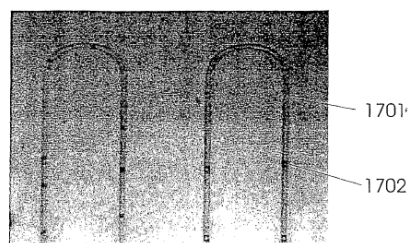


FIG. 17A

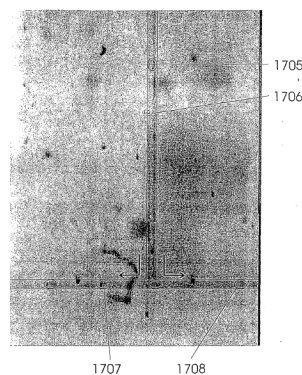


FIG. 17C

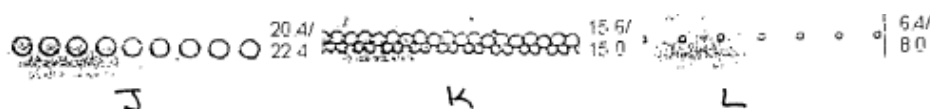


FIG. 19

EX1005, 80:8-14, 81:6-11, 82:19-30 & Figs. 17A, 17C, 19J, 19L.

286. Quake discloses that making the round, monodisperse droplets in Figs. 19J and 19L are preferred, especially in high throughput devices. *Id.*, 27:27-32. Quake discloses that these “round” droplets preferably have a diameter that is smaller than the diameter of the microchannel. *Id.*

287. For the reasons I have just discussed, Quake in view of Litborn renders obvious the method of claim 1 wherein the at least one plug is substantially spherical in shape, and recited by claim 11.

**XII. GROUND 4: CLAIMS 1-9 AND 11 ARE OBVIOUS OVER QUAKE AND LITBORN IN FURTHER VIEW OF SHAW STEWART.**

288. As discussed above in Sections XI, claims 1-9 and 11 are obvious over Quake and Litborn. My discussion from Ground 3 above applies equally here. Shaw Stewart provides additional teachings that render these claims obvious.

289. As discussed above in Section VIII.D, Shaw Stewart discloses a microfluidic system designed to reduce or eliminate contamination by performing chemical reactions, including biological assays, in nanoliter-sized droplets. EX1009, Abstract, 2 (objects 1 and 5), 8. Similar to Quake, Shaw Stewart discloses substantially spherical droplets, stating that the conduit (channel) size is “of the same order of magnitude as the cross-section of the droplets.” *Id.*, 4. Shaw Stewart discloses creating the droplets by injecting a small volume or droplet of reactant through a constriction and into a conduit at a right angle. *Id.*, Abstract, 3. The discrete reactant droplets are thus separated from the parent volume by passing carrier phase along the conduit such that the droplets are separated from each other by an inert, immiscible liquid. *Id.*, Abstract, 3.

290. Shaw Stewart discloses the invention can be used to perform biological or chemical assays. *Id.*, 8. Shaw Stewart discloses mineral oil or a fluorinated hydrocarbon may be used as the carrier. *Id.*, Abstract, 4. For example, Shaw Stewart states:

This reactant liquid, hereafter referred to as a reactant, will be supported, defined and moved by another, immiscible liquid, referred to hereafter as the carrier phase. *Suitable carrier phases include mineral oils*, light silicon oils, water, and *fluorinated hydrocarbons*.

EX1008, 4:3-7 (emphasis added).

291. Shaw Stewart thus discloses that fluorinated carrier oil was a readily accessible and desirable alternative to the traditional hydrocarbons (*e.g.*, decane and mineral oil) Quake identifies as carrier fluids, especially where Quake identifies fluorinated oil surfactant for use in forming its droplets. In view of Shaw Stewart's disclosure that fluorinated oils were a known alternative to using mineral oil as the immiscible carrier fluid for forming microfluidic droplets for biological reactions, a POSA would have had good reason to use a fluorinated oil as the carrier fluid for forming aqueous droplets containing biological materials, such as in Quake, to provide a chemically compatible carrier oil for use in combination with Quake's fluorinated surfactant.

292. A POSA would have had a reasonable expectation of success for using a fluorinated oil as the carrier fluid because of the known properties of fluorinated oils. For example, fluorinated oils were known to be compatible with biological materials, to be sufficiently hydrophobic to encapsulate aqueous droplets, and to be desirable for forming w/o emulsions for containing biological materials. EX1017, 209, 212-213; EX1018, Summary. As evidenced by Shaw

Stewart, fluorinated oils were also known to be capable of forming microfluidic aqueous droplets when the aqueous solution is flowed into the flowing fluorinated oil. Accordingly, a POSA would have had good reason and reasonable expectation of success in view of Quake, Litborn, and Shaw Stewart to perform PCR amplification in Quake's droplets using fluorinated oil as the carrier fluid and using a fluorinated surfactant.

293. Shaw Stewart is, of course, very relevant to claims 6 and 8, but it is also relevant to the obviousness of the remaining claims discussed herein, as all of these claims require providing conditions suitable for the autocatalytic reaction in the at least one plug such that the at least one substrate molecule is amplified. A POSA would recognize a fluorinated oil carrier fluid would provide a suitable carrier fluid for forming PCR in droplets as described in Quake because of the biocompatibility and miscibility characteristics discussed above. Shaw Stewart's disclosure of fluorinated oil as a suitable carrier fluid for microfluidic biological droplet reactors thus provides additional evidence for the obviousness of each of claims 1-9 and 11 over Quake and Litborn.

### **XIII. SECONDARY CONSIDERATIONS**

294. I understand that Patent Owner argued during 10X's IPR and reexamination that there was a "long felt need for methods of conducting biological reactions in [microfluidic] droplets" that was solved by "Ismagilov and

co-workers.” EX1026, 48-49. I understand that Patent Owner argued that Ismagilov invented specific fluorinated surfactants including a hydrophilic OEG head group and that these specific surfactants were “crucial” to preventing harmful protein-surface interactions and “unlocked microfluidic droplets as systems for studying autocatalytic biological reactions.” EX1026, 44, 55-58; EX1028, 254-260. I understand that Patent Owner also argued that scientists praised the inventors for using these surfactants in combination with perfluoramines. EX1028, 277.

295. These argument appear to be premised on Patent Owner’s argument that autocatalytic biological reactions were not and could not be successfully performed in microfluidic droplets before the ’193 patent and that the inventors of the ’193 patent were the first to do so. As discussed above, these premises are not correct. As I have explained above, at least Holliger, Litborn, and Schneegaß demonstrate successful PCR amplification in microfluidic droplets before the ’193 patent. Similarly, the properties of fluorinated oils and surfactants and their utility for emulsions for biological materials were well-known, as I explained in Section VII when discussing EX1017 and EX1018. Moreover, Shaw Stewart had already disclosed the use of fluorinated oils as carrier fluids for microfluidic droplet reactors for biological reactions. Thus, the need Patent Owner identifies did not exist at the time of the ’193 patent, was certainly not unmet or long-felt,

and was not solved by the claims of the '193 patent. As demonstrated by at least Holliger, Litborn and Schneegaß, it also is not correct to say that fluorinated oils, fluorinated surfactants, or perfluoroamines were crucial to performing autocatalytic biological reactions in microfluidic droplets or “unlocked microfluidic droplets as systems for studying autocatalytic biological reactions.”

296. Patent Owner had hypothesized in its proceedings against 10X that the “tiny size” of “microfluidic droplets” ranging from femtoliter to nanoliter range presented “prevented researchers from using microfluidic droplets as chemical reactors.” EX1026, 7. Among other things, Patent Owner argued that difficulties with mixing reagents, adherence of droplet material to channel walls and the droplet/carrier-fluid interface, and “environments incompatible with biological molecules” prevented researchers from using microfluidic droplets as chemical reactors. *Id.*, 7-8. As discussed above, however, Litborn discloses that the w/o droplet itself assists with mixing the reagents. EX1007, 453 & Fig. 7B. Quake discloses coating the channel walls and using the carrier oil containing a surfactant to coat the channel walls and prevent adherence of materials in the aqueous droplets from the channel walls. EX1005, 28:1-29. Schneegaß similarly discloses coating the channel walls to prevent adherence of materials in the aqueous droplets from the channel walls. EX1009, 110 (describing channel silanization, use of oil and surfactants, and addition of PEG for preventing

protein adsorption and stabilizing the polymerase in the droplet). As discussed above, each of Holliger, Litborn, and Schneegaß confirm that adherence of droplet material to channel walls and the water/oil interface of microfluidic aqueous droplets formed in standard hydrocarbon oils did not preclude successful PCR amplification. They similarly demonstrate that w/o droplets formed in traditional hydrocarbon oils did not create “environments incompatible with biological molecules” and were instead suitable for conducting an autocatalytic reaction such that the DNA substrate molecule is amplified.

297. In addition to these prior art references that affirmatively disprove Patent Owner’s hypothetical concerns, it is my opinion that the questions Patent Owner raises would have motivated a POSA to optimize droplet parameters using known methods. A POSA would not have been dissuaded by such questions because varying oil type, surfactant type, and the relative concentration of water, oil, and surfactant to optimize microfluidic droplets was well-within the ability of a POSA before the ’193 patent. The prior art references discussed above reflect a high level of skill in the art and confirm that POSA were well equipped to address, and already had in fact, succeeded in addressing, the academic questions raised by the Patent Owner.

298. Even if Patent Owner’s specific fluorinated surfactants or fluorinated amines were praised by others, there appears to be no relationship between those

specific compounds and the purported invention of the '193 patent. Indeed, I have reviewed the '193 patent, including its claims. Although the '193 patent generically claims the method of claim 1 wherein the surfactant is fluorinated surfactant, it does not claim the smaller genus of OEG-containing fluorinated surfactants or the specific OEG-containing fluorinated surfactants that Patent Owner mistakenly believes was required for performing autocatalytic biological reactions in microfluidic droplets.

299. I also have reviewed the Provisional applications for the '193 patent, as discussed above in paragraph 43. These Provisional applications do not even disclose OEG-containing fluorinated surfactants. EX1021; EX1022. Moreover, neither the claims nor the specification of the '193 patent discuss perfluoramines. To whatever extent some in the field may have praised Patent Owner's specific surfactants and their use in conjunction with perfluoroamines, these statements would appear to have no connection to the claims of the '193 patent.

300. Moreover, Patent Owner was not the first to use PEG and OEG compounds to reduce non-specific adsorption of proteins on microfluidic surfaces. The '193 patent itself admits that "[p]olyethylene glycols (PEG) and oligoethylene glycols (OEG) are known to reduce non-specific adsorption of proteins on surfaces" and were useful in the claimed invention for the same purpose. EX1001, 35:63-65. Schneegaß specifically discloses that such



compounds were useful in microfluidic w/o droplet PCR. EX1009, 109. What Patent Owner argues were its advancements over the prior art were, without exception, merely using known things for their known uses to achieve their expected results. In my opinion, doing so would have been obvious to a POSA before the '193 patent.

301. I understand that Patent Owner argued that Thorsen was unsuccessful in his 2003 Thesis attempts to conduct model enzymatic assay in droplets because he did not account for protein adhesion on the droplet surface. EX1026, 58-60 (citing EX1036, 94-108). I have reviewed this Thesis, which did not report any failed attempt at performing PCR in the droplets. To the contrary, the Thorsen Thesis did not attempt PCR in droplets. Instead, Thorsen performed pNB esterase enzyme experiments wherein entire cells expressing the pNB esterase enzyme were encapsulated in a droplet to hydrolyze fluorescein. Thorsen reported that inclusion of the cell in the droplet was “not entirely successful” because there were problems with “substrate autohydrolysis and inefficient diffusion of the substrate across the cell membrane.” EX1036, 95, 100, 107. In other words, the substrate was hydrolyzed by materials in the intact cell and the substrate had difficulty crossing the membrane of the cell. Neither problem is implicated in the proposed combination of Quake with Holliger or Litborn

discussed above. Indeed, the claims do not require injecting entire cells into the droplets or using pNB esterase as the enzyme.

302. Moreover, it would not be accurate to characterize Thorsen's experiments as a failure. Thorsen disclosed that "good examples of enzymatic catalysis inside the formed droplets were still acquired" despite the difficulties associated with using the cell as the delivery vehicle to the droplet for the enzyme EX1036, 100, 104. Thorsen thus indicates that even that there was a reasonable expectation of successful enzymatic reactions in droplets using Thorsen's cell-based delivery of enzymes into the droplets. To the extent Thorsen is considered in evaluating the claims of the '193 patent, it shows that enzymatic activity could proceed in the droplets despite the additional obstacles posed by cell encapsulation in the droplets. When the cellular barrier is not present, as proposed in the Grounds and as demonstrated in Litborn and Schneegaß and certain embodiments of Holliger, PCR amplification of DNA already was successfully performed in microfluidic w/o droplets before the '193 patent. Thorsen's Thesis certainly does not undermine the reasonable expectation of success for practicing what is claimed.

#### **XIV. CONCLUDING STATEMENTS**

303. In signing this declaration, I understand that the declaration will be filed as evidence in a contested case before the Patent Trial and Appeal Board of

the United States Patent and Trademark Office. I acknowledge that I may be subject to cross-examination in this case and that cross-examination will take place within the United States. If cross-examination is required of me, I will appear for cross-examination within the United States during the time allotted for cross-examination.

304. I declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: October 21, 2020

By: /Carl D. Meinhart/  
Carl D. Meinhart, Ph.D.

**XV. APPENDIX – LIST OF EXHIBITS**

<b>Exhibit No</b>	<b>Description</b>
1001	U.S. Patent No. 8,304,193 to Ismagilov, <i>et al.</i>
1003	<i>Curriculum Vitae</i> of Carl D. Meinhart, Ph.D.
1004	Prosecution History of U.S. Patent No. 8,304,193 to Ismagilov, <i>et al.</i>
1005	International Publication No. WO 02/23163 to Quake and Thorsen.
1006	International Publication No. WO 02/022869 to Holliger and Gahdessy.
1007	Litborn <i>et al.</i> , <i>Synthesis and Analysis of Chemical Components in Nanoscale</i> , MICRO TOTAL ANALYSIS SYSTEMS 2000, Enschede, The Netherland, 447-454 (2000).
1008	International Publication No. WO 84/02000 to Shaw Stewart
1009	Ivonne Schneegaß & Johann Michael Köhler, <i>Flow-through polymerase chain reactions in chip thermocyclers</i> , 82 REV. MOL. BIOTECH. 101-121 (2001)
1010	U.S. Patent No. 6,033,880 to Haff <i>et al.</i>
1011	Kopp <i>et al.</i> , <i>Chemical Amplification: Continuous-Flow PCR on a Chip</i> , 280 SCIENCE 1046-1048 (1998)
1012	Lagally <i>et al.</i> , <i>Monolithic integrated microfluidic DNA amplification and capillary electrophoresis analysis system</i> , 63 SENSORS AND ACTUATORS B: CHEMICAL 136-146 (2000).

Exhibit No	Description
1013	He <i>et al.</i> , <i>Capillary-based fully integrated and automated system for nanoliter polymerase chain reaction analysis directly from cheek cells</i> , 924 J. CHROMATOGR. A 271-284 (2001).
1014	U.S. Patent No. 6,171,850 to Nagle <i>et al.</i>
1015	U.S. Patent Appl. Pub. No. 2001/0041357 to Fouillet <i>et al.</i>
1016	Burns <i>et al.</i> , <i>An Integrated Nanoliter DNA Analysis Device</i> , 282 Science 484-487 (1998).
1017	Rudiger <i>et al.</i> , <i>Perfluorocarbons—Useful Tools for Medicine</i> , 5 EUR. J. MED. RES. 209-216 (2000).
1018	Krafft <i>et al.</i> , <i>Highly fluorinated amphiphiles and colloidal systems, and their applications in the biomedical field</i> , 80 BIOCHIMIE 489-514 (1998).
1019	<i>Reserved</i>
1020	UK Patent Application GB 2,097,692
1021	U.S. Provisional Application No. 60/379,927
1022	U.S. Provisional Application No. 60/394,544
1023	Shaw Stewart, P., <i>The Development and Applications of a New Liquid Handling Device</i> , Univ. of London Master's Thesis (1988).
1024	<i>Bio-Rad Labs., Inc. and The University of Chicago v. Dropworks, Inc.</i> , Case No. 20-cv-506-UNA, District of Delaware, Complaint, D.I. 1 (April 14, 2020).

Exhibit No	Description
1025	<i>10X Genomics, Inc. v. The University of Chicago</i> , IPR2015-01163, Paper 2.
1026	<i>10X Genomics, Inc. v. The University of Chicago</i> , IPR2015-01163, Paper 9.
1027	<i>10X Genomics, Inc. v. The University of Chicago</i> , IPR2015-01163, Paper 14.
1028	Reexamination File for Control No. 90/014,043
1029	U.S. Patent Application 2002/0058332 to Quake and Thorsen
1030	U.S. Provisional Application 60/233,037
1031	<i>Bio-Rad Labs., Inc. and The University of Chicago v. 10X Genomics, Inc.</i> , Case No. 15-cv-00152-RGA, District of Delaware, Memorandum Order re Claim Construction, D.I. 116 (Jan. 26, 2017).
1032	<i>Bio-Rad Labs., Inc. and The University of Chicago v. 10X Genomics, Inc.</i> , Case No. 15-cv-00152-RGA, District of Delaware, Memorandum Order re Summary Judgment, D.I. 351 (June 26, 2018).
1033	<i>Bio-Rad Labs., Inc. and The University of Chicago v. 10X Genomics, Inc.</i> , Case No. 15-cv-00152-RGA, District of Delaware, Claim Construction Order, D.I. 179 (May 30, 2017).
1034	<i>Bio-Rad Labs., Inc. and The University of Chicago v. 10X Genomics, Inc.</i> , Case No. 15-cv-00152-RGA, District of Delaware, Claim Construction Order, D.I. 469 (Nov. 13, 2018).

Exhibit No	Description
1035	<i>Bio-Rad Labs., Inc. and The University of Chicago v. 10X Genomics, Inc.</i> , Appeal Nos. 2019-2255, 2019-2285, Slip Op., (Fed. Cir. Aug. 3, 2020).
1036	Thorsen, T., <i>Microfluidic Technologies for High-Throughput Screening Applications</i> , Caltech Ph.D Thesis (2003).
1037	U.S. Patent No. 6,524,456 to Ramsey <i>et al.</i>