

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA, INC.,
Petitioner,

v.

THE TRUSTEES OF COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK
Patent Owner.

IPR2018-00291 (Patent 9,718,852)
IPR2018-00318 (Patent 9,719,139)
IPR2018-00322 (Patent 9,708,358)
IPR2018-00385 (Patent 9,725,480)¹

DECLARATION OF STEVEN M. MENCHEN, PH.D.

Columbia Exhibit 2116
Illumina, Inc. v. The Trustees
of Columbia University in the
City of New York
IPR2018-00291, 318, 322 & 385

¹ An identical Declaration is being entered into each listed proceeding.

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I. Introduction And Qualifications

1. I have been retained on behalf of The Trustees of Columbia University in the City of New York (“Columbia”) in connection with the challenges by Illumina, Inc. (“Illumina”) to the claims of U.S. Patent Nos. 9,718,852 (IPR2018-00291); 9,719,139 (IPR2018-00318); 9,708,358 (IPR2018-00322); and 9,725,480 (IPR2018-00385) (collectively, the “patents-at-issue”). Unless otherwise indicated, my opinions herein apply to each of the patents-at-issue.

2. I am being compensated for my time consulting in this matter at the rate of \$500 per hour. I have no financial interest in the outcome of these proceedings and my compensation is in no way contingent upon my opinions or the outcome of these proceedings.

3. I received a B.S. degree in Chemistry and a M.S. degree in Organic Chemistry from San Jose St. University in 1971 and 1975, respectively. I received my Ph.D. degree in Organic Chemistry from the University of Alberta in 1979 and was a postdoctoral fellow at Princeton University from 1979 through 1981.

4. I have over 30 years of experience developing DNA sequencing technology, including technology for Sequencing by Synthesis (“SBS”). Between 1985 and 2016, I held the positions of Senior Scientist, Staff Scientist, Senior Staff Scientist, Principal Scientist, and Scientific Fellow at Applied Biosystems/Life

Technologies where I developed DNA sequencing technology. In 2016 and 2017, I was the Director of Chemistry at Quantapore, Inc., a company that develops novel, nanopore based sequencing technology, and I am currently a consultant on Next Generation DNA sequencing technology.

5. My *curriculum vitae* lists my publications and patents. Ex. 2066. I am a named inventor on around 100 issued U.S. patents, most related to the development of DNA sequencing technologies using nucleic acid chemistry and organic synthesis.

II. Documents Considered

6. In connection with the preparation of this Declaration, I have considered and reviewed the documents listed in Ex. 2108.

III. The Person Of Ordinary Skill In The Art

7. I have studied the patents-at-issue. Based on my study of the patents, the claims relate to novel nucleotides for use in DNA sequencing, particularly SBS.

8. Based on my experience in, and knowledge of, the field of nucleotides for use in DNA sequencing, the relevant art concerns the design and synthesis of nucleotides that can be incorporated by a polymerase for use in methods for DNA sequencing, such as SBS.

9. I understand that the claims of the patents-at-issue must be assessed from the perspective of a hypothetical person of ordinary skill in the relevant art (“POSA”) as of the date the invention described in the claims was made. For purposes of this Declaration, I have been asked to assume the invention was made by October 6, 2000 (“Priority Date”), when the first application which led to the patents-at-issue was filed. I further understand that a POSA has knowledge of the relevant art, as described in published and issued patents and in scientific publications, and possesses ordinary creativity and a basic skill set.

10. For the purposes of this proceeding, I do not dispute Illumina’s criteria for defining a POSA. Unless indicated otherwise, the opinions I express in this Declaration reflect my understanding of what a POSA would have known and would have thought as of the Priority Date.

IV. State Of The Art

A. Tsien And Dower Offered Only Speculation

11. The claimed invention relates to SBS, a primary purpose of which is to “identify the genes and functional polymorphisms associated with the variability in drug response” to advance therapeutic applications of molecular medicine. Exs.

1001-1004, each at 1:46-55.² As explained below, the invention ended a decade-long search for alternatives to the favored DNA sequencing method in the 1990s, Sanger sequencing, which had limitations that hindered its ability to make sequencing genes and identifying functional polymorphisms practical. Ex. 1016 at 4259; Ex. 1013 at 3; Ex. 2099 at 1:29-45.

12. Tsien and Dower prophetically set forth SBS methods in patent applications in October 1990 and December 1990, respectively, without providing any data or experimentation to guide a POSA as to how to actually achieve SBS. *See* Ex. 1013; Ex. 1015. SBS (sometimes called Base Addition Sequencing Scheme (BASS)) was touted as an alternative to the Sanger scheme for DNA sequencing that could potentially “meet the increasing demands of large scale sequencing of whole genomes[.]” Ex. 1016 at 4259.

13. Importantly, Tsien and Dower describe hypothetical methods for SBS that could theoretically be conducted only *if* nucleotides that met the stringent requirements set forth in the prior art were discovered. Neither Tsien nor Dower

² Citations to Ex. 1001, Ex. 1002, Ex. 1003 and Ex. 1004 are only applicable to IPR2018-00291, IPR2018-00322, IPR2018-00318 and PR2018-00385, respectively.

describe any actual experiments or provide any data. As explained below, as of the Priority Date, no nucleotides were known to meet these stringent requirements.

B. SBS Was Known To Require Both Efficient Incorporation And Quantitative, Rapid Cleavage Under Mild, Aqueous Conditions

14. In order to perform Tsien's or Dower's SBS methods, a number of stringent requirements had to be met. One of the stringent requirements set forth in the prior art was that the nucleotides had to be *efficiently incorporated* into the growing DNA strand by polymerase. Both Tsien and Dower explicitly taught this efficient incorporation requirement. Tsien states:

The criteria for the successful use of 3'-blocking groups include: . . . the ability of a polymerase enzyme to accurately and *efficiently incorporate* the dNTPs carrying the 3'-blocking groups into the cDNA chain[.]

Ex. 1013 at 20.³ Dower states:

The structures of the fluorescently labeled and reversible terminator base analogs are selected to be compatible with *efficient incorporation* into the growing chains by the particular DNA polymerase(s) chosen to catalyze extension.

³ Emphases added throughout unless otherwise indicated.

Ex. 1015 at 26:6-9. Other prior art references similarly taught that SBS requires efficient incorporation:

[SBS] involves repetitive cycles of base incorporation, detection of incorporation, and re-activation of the chain terminator to allow the next cycle of DNA synthesis. . . .

[T]his technique is plagued by any inefficiencies of incorporation and deprotection.

Ex. 2013 at 2-3. Thus, the prior art as of the Priority Date clearly taught that efficient incorporation of nucleotides was required to conduct SBS.

15. A second stringent requirement for SBS set forth in the prior art was the need for *quantitative, rapid* cleavage of the 3'-OH capping group ("capping group") on the nucleotide under *mild, aqueous* conditions. Tsien explicitly taught that SBS requires quantitative, rapid cleavage under mild conditions (Ex. 1013 at 20), and Metzker and Welch each taught that the conditions must be aqueous. Ex. 1016 at 4259 ("The complete scheme demands nucleotide analogs that are tolerated by polymerases, spectroscopically distinct for each base, stable during the polymerization phase, and deprotected efficiently under mild conditions in aqueous solution."); Ex. 2041 at 197-198 ("the 3'-blocking group must be removed in an aqueous medium using reagents/conditions that do not denature or modify double strand DNA").

16. A POSA would have understood that it would not be possible to perform SBS if the above requirements were not met. As the prior art stated, these requirements were “criteria for the successful use” of capping groups. Ex. 1013 at 20.

**C. Identifying Nucleotides Usable For SBS Was
A “Major Challenge” And “Formidable Obstacle”**

17. Prior to the Priority Date in October 2000, numerous groups (including Tsien, Dower, Cheeseman, Rabani, Canard, Welch, and Stemple) set out to discover nucleotides that met the stringent requirements to take SBS from speculation to reality, a task which the prior art described as a “major challenge” (Ex. 2041 at 197-198) and “formidable obstacle[]” (Ex. 1016 at 4259). Ex. 1013 at 20-25 (Tsien); Ex. 1015 at 25:47-51 (Dower); Ex. 1020 at 4:50-63 (Cheeseman); Ex. 2109 at 2:15-21 (Rabani); Ex. 2031 at 1-2 (Canard); Ex. 1023 at 951-56 (Welch); Ex. 2013 at 13 (Stemple). By the Priority Date, despite immense effort, “[t]echnical obstacles includ[ing] a relatively low efficiency of extension and deprotection” continued to prevent the successful application of SBS as hypothesized by Tsien and Dower. Ex. 2099 at 2:34-36; Ex. 2013 at 3 (noting that need for new methods remained); Ex. 2100 at 1:56-61 (noting problems with SBS). As the prior art stated, the “3’-blocking groups [in Tsien (WO 91/06678) and other references] are either not described in any detail, or are not accepted by the

required enzyme, or do not permit desired rapid deblocking of the growing template copy strand after each polymeri[z]ation event.” Ex. 2110 at 3.

18. While in 1994 Metzker reported success in incorporating a 3'-O-(2-nitrobenzyl) nucleotide (without a label) into a DNA strand and then cleaving the 2-nitrobenzyl capping group (which is not a “small” protecting group), he also established that the one protecting group relied upon by Illumina, the allyl capping group, was a poor substrate for the purposes of SBS. See Ex. 1016 at 4259, 4263-67. While the result regarding the 3'-O-(2-nitrobenzyl) nucleotide was promising, further testing revealed that the tested polymerases were incapable of incorporating that nucleotide once a label was added as required for SBS. Ex. 1023 at 951-52, 954-56. This led members of Metzker's lab to conclude in Welch that SBS would not be viable without additional discoveries that would allow modification of polymerases: “[i]t seems clear that *modifications to the polymerase enzyme* as well as to the nucleoside triphosphate *are required* if [SBS] is to be developed into a viable sequencing scheme.” Ex. 1023 at 956, 951 (“We conclude that both the nucleotide triphosphates and the DNA polymerase enzyme must be modified if the proposed DNA sequencing scheme is to be viable”). At the time, the modification of polymerase to better incorporate modified nucleotides was theoretical, and Illumina has not argued otherwise. Ex. 2035 at 63-65 (Dr. Romesberg testifying

that even in 2002, “people did not believe that you could” modify polymerases to improve their ability to incorporate nucleotides); *see also* Ex. 2126 at 252-53 (Dr. Romesberg testifying that a POSA would have viewed modifying polymerases as “very difficult” and “not . . . a likely possibility”). Therefore, the Welch authors were reporting in 1999 that SBS would not be a viable sequencing scheme without additional discoveries that would allow modification of polymerases to incorporate modified nucleotides.

V. Claim Construction

19. I believe that in order to properly evaluate the differences between the claims of the patents-at-issue in IPR2018-00291, IPR2018-00318, IPR2018-00322, and IPR2018-00385 and the cited art, the following claim term needs to be construed for each of the patents-at-issue.

A. “Chemical Linker”

20. For each of the patents-at-issue, in the context of claimed feature Y, “chemical linker” means a chemical moiety attached by covalent bonds at one end to a specified position on the base of a nucleotide and at the other end to a tag (detectable fluorescent moiety). It does not mean merely a covalent bond between the base and the label as disclosed in Dower. The specification of each of the patents-at-issue requires this construction (Exs. 1001-1004, each at 10:64-66, 14:8-10, the structures shown at columns 13-20, and Figs. 7, 8, 10, and 15A), which was

expressly addressed during prosecution of each of the patents-at-issue. Ex. 1009 at 30, 18-19 (“Y is defined as a chemically cleavable, chemical linker and as shown in the structure shown in the pending claim, Y is attached by covalent bonds at one end to the base of a nucleotide analogue at a specific position and at the other end to a detectable fluorescent moiety.”); Ex. 1062 at 30, 18-19 (same); Ex. 1065 at 30, 18-19 (same); Ex. 1068 at 26, 14-15 (same).⁴ Dr. Romesberg agrees that “Y represents a component that is between the fluorophore and the base that is cleavable . . . [the fluorophore is] not directly attached . . . to the base[.]” Ex. 2113 at 117.

21. It is also my understanding that during prosecution of each of the patents-at-issue, Columbia directed the Examiner to Tsien and Stemple for examples of chemical linkers. Exs. 1009, 1062, 1065, each at 19, 30; Ex. 1068 at 14, 26. The linkers in those references are chemical moieties. *See* Ex. 1013 at 28:23-29:2 (noting that “[t]ypical tethers are from about 2 to about 20, and preferably from about 3 to about 10 atoms in length”). The Examiner found

⁴ Citations to Ex. 1009, Ex. 1062, Ex. 1065 and Ex. 1068 are only applicable to IPR2018-00291, IPR2018-00318, IPR2018-00322 and IPR2018-00385, respectively.

Columbia's definitions "persuasive." Ex. 1009 at 4, 12; Ex. 1062 at 4, 12; Ex. 1065 at 4, 11; Ex. 1068 at 7.

[Paragraph Numbers 22-23 Not Used]

VI. Illumina's Ground 1 Challenge For Obviousness Over Tsien In View Of Prober Fails⁵

24. In my opinion, Illumina's Ground 1 challenge in IPR2018-00291, IPR2018-00318, IPR2018-00322, and IPR2018-00385 relies on a single premise—that it would have been obvious to make a base-labeled nucleotide with the 3-carbon, 5-hydrogen allyl capping group ($-\text{CH}_2\text{CH}=\text{CH}_2$) ("the allyl capping group"). Illumina's Ground 1 challenges fail for the following reasons:

(A) Illumina's references provide no motivation to select the allyl capping group because it had been described as being incapable of achieving the efficient incorporation requirement of SBS;

(B) Illumina's references provide no motivation to select the allyl capping group because they do not teach quantitative, rapid cleavage of the allyl capping group under mild, aqueous conditions;

⁵ In IPR2018-00318 and IPR2018-0322, Illumina's Ground 1 challenge is based solely on Tsien.

(C) A POSA weighing the allyl capping group's numerous disadvantages against its one alleged advantage would have concluded that it was incompatible with SBS;

(D) Illumina's references do not teach that capping groups had to be small or that there was any advantage to selecting small capping groups, and a POSA would not have reviewed Pelletier because it related to an entirely different field from SBS;

(E) There was no reasonable expectation of success in achieving the claimed invention;

(F) Tsien does not disclose the allyl capping group, instead it discloses vinyl ethers, and neither does Prober, and Tsien provides no motivation to select the allyl capping group because its cleavage conditions are incompatible with SBS; and

(G) If the lead compound analysis applies, Illumina selected the wrong lead compounds.

A. There Was No Motivation To Select The Allyl Capping Group Because It Was Believed To Be Incompatible With The Efficient Incorporation Requirement Of SBS

25. A POSA would not have been motivated to select the allyl capping group. As of the Priority Date, the only prior art that provided any experimental data using a 3'-O-allyl nucleotide was Metzker (Ex. 1016). A POSA understood

from Metzker that the allyl capping group did not have the properties to meet the stringent requirements set forth in the prior art for performing SBS. *See* Section IV(B). One of those stringent requirements was that the nucleotides had to be *efficiently incorporated* into the growing DNA strand. As explained below, a POSA would have been discouraged from using the allyl capping group because Metzker reported that the 3'-O-allyl nucleotide could not be efficiently incorporated by any of the polymerases tested.

1. Overview Of Metzker

26. Like others throughout the 1990's, Metzker was interested in discovering nucleotides that could take SBS from speculation to reality. He took a two-stage approach to discovering such nucleotides. The first stage consisted of conducting "enzymatic screen[s]" to examine whether any of the commercially-available polymerases tested were capable of efficiently incorporating an unlabeled 3'-O-capped nucleotide. Ex. 1016 at 4259. The second stage consisted of taking the efficiently incorporated 3'-O-capped nucleotides from the first stage, adding a label, and seeing whether those more complex nucleotides could still be efficiently incorporated. *See* Ex. 1016 at 4267 (Metzker concluding his 1994 publication by noting that his lab was planning to take the most promising nucleotide, the 3'-O-(2-nitrobenzyl) nucleotide, add a label to it, and test the capped, labeled nucleotide for enzymatic activity).

27. In the first stage, Metzker tested whether any of eight polymerases were capable of incorporating any of eight different unlabeled 3'-O-capped nucleotides. Ex. 1016 at 4259, 4262-63. The results from Metzker's first stage were summarized in Table 2 of his 1994 publication:

Table 2. Activity matrix of RP-HPLC purified 3'-protecting dNTPs challenged against commercially available polymerases

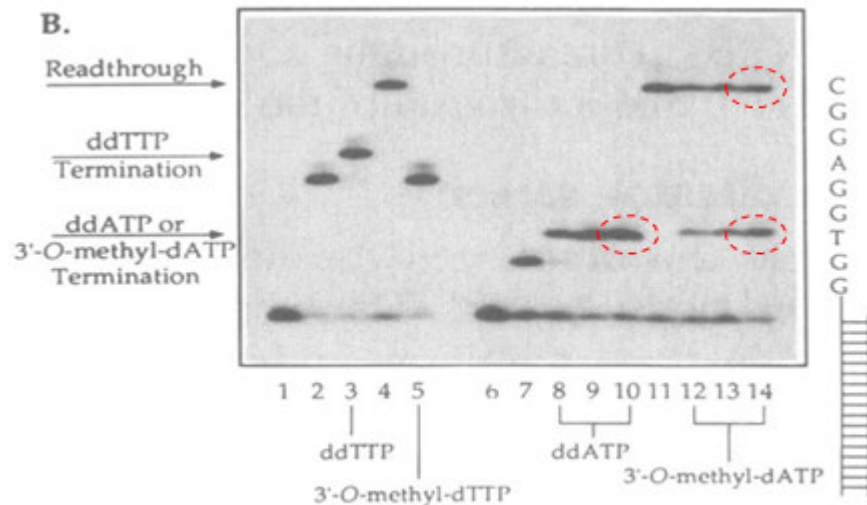
3'-modified-dATP (except compound [8])	AMV-RT	M-MuLV-RT	Klenow fragment	Sequenase®	Bst DNA polymerase	AmpliTaQ® DNA polymerase	Vent®(exo-)® DNA polymerase	rTth DNA polymerase
[1] O-methyl	Termination	Termination*	-	-	-	-	Inhibition	Inhibition*
[2] O-acyl	-	-	-	-	-	-	Inhibition	-
[3] O-allyl	-	-	-	-	-	-	Termination*	-
[4] O-tetrahydropyran	-	-	-	-	-	-	-	-
[5] O-(4-nitrobenzoyl)	-	-	-	-	-	-	-	-
[6] O-(2-aminobenzoyl)	-	-	-	-	-	-	-	-
[7] O-(2-nitrobenzyl)	-	-	-	Inhibition	Termination	Termination*	Termination*	-
[8] 3'-O-methyl-dTTP	-	Inhibition	-	Inhibition	Termination	Termination	Termination	Termination

All compounds were assayed at a final concentration of 250 μ M according to the conditions specified in Table 1. '-' means no activity was detected, 'Termination' means that the termination bands mimic ddNTP termination bands, and 'Inhibition' means the rate of DNA synthesis is reduced in a nonspecific manner. '*' means the activity was incomplete at a final concentration of 250 μ M.

Ex. 1016 at 4263. Table 2 reports the results using five different terms: – (meaning no activity), Inhibition, Inhibition*, Termination, and Termination*. For purposes of these proceedings, I focus on the last two terms.

28. “Termination” indicates efficient incorporation of the 3'-O-capped nucleotide. As Metzker explains, “Termination” means that “the termination bands mimic ddNTP termination bands[.]” Ex. 1016 at 4263. For example, Figure

3(B) of Metzker reports the Oligo-assay results for 3'-O-methyl-dATP with AMV-RT:



Metzker's Figure 3(B) (excerpt)

Ex. 1016 at 4264 (circles added for emphasis). Lane 14 contains 250 μ M of 3'-O-methyl-dATP and Lane 10 contains 2.5 μ M of ddATP. *Id.* The termination band in Lane 14 (across the "T" template base) mimics the termination band in Lane 10 except for the presence of a "readthrough" band in Lane 14.⁶ Metzker reports that the readthrough band was the result of "trace" amounts of contaminating dATP in

⁶ Readthrough occurs when the solution also contains "trace" amounts of the natural nucleotide, which is preferentially incorporated instead of the 3'-O-capped nucleotide, resulting in the DNA strand being extended to the end of the template.

Lane 14. Ex. 1016 at 4263. The presence of the readthrough band does not affect the assessment of whether the termination band observed in Lane 14 mimics that of Lane 10. For example, in Table 2, Metzker reported “Termination” for the 3’-O-methyl-dATP/AMV-RT combination, indicating that he concluded that Lane 14 mimics Lane 10. Ex. 1016 at 4263.

29. Metzker’s assay was designed to permit conclusions regarding a polymerase’s ability to efficiently incorporate the 3’-O-capped nucleotides. For example, in Figure 3(B), the template DNA had the sequence GGTGGAGGC and Lane 14 contained dCTP, dTTP, ddGTP, and the 3’-O-methyl-dATP. Ex. 1016 at 4263. Based on this design, the critical base in the template sequence was the thymine (*i.e.*, “T”), because the solution did not contain natural dATP (apart from trace amounts of contamination) to serve as the complementary base to the thymine. The experiment assessed whether the polymerase could incorporate the 3’-O-methyl-dATP whenever it encountered the thymine in the template sequence.

30. Because the polymerase efficiently incorporated 3’-O-methyl-dATP, it was able to incorporate the 3’-O-methyl-dATP into the many growing DNA strands in the solution, resulting in a single termination band (apart from the readthrough band). Lane 14 of Figure 3(B) shows this result. Ex. 1016 at 4264 (see single termination band next to “T”). I have created an illustration (Figure 1,

below) excising Lanes 10 (*i.e.*, the ddATP control) and 14 (*i.e.*, the 3'-O-methyl-dATP) from Metzker's Figure 3(B) and placing them side-by-side to further make evident the single termination band representing efficient incorporation:

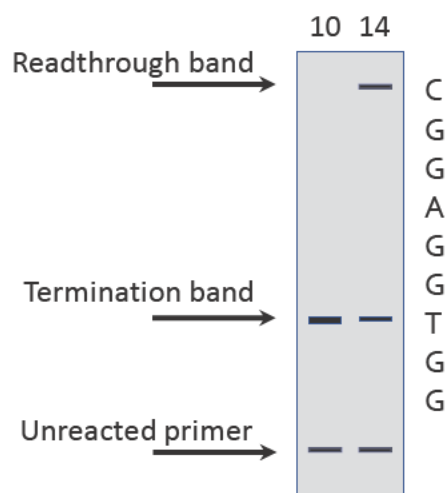


Figure 1

Figure 1 illustrates that the 3'-O-methyl-dATP (lane 14) mimics the termination of the ddATP control (lane 10).⁷

31. If a polymerase *inefficiently* incorporated a 3'-O-capped-dATP, it would only be incorporated into some of the growing DNA strands within the allotted time of the reaction. Metzker reports this result as "Termination*," wherein "*" means the activity was "incomplete at a final concentration of 250

⁷ I have purposefully illustrated that the signal intensity of the termination band in lane 14 is less than that of lane 10, which I explain in more detail below.

μM.” Ex. 1016 at 4263. Metzker does not show gel images for the polymerase/nucleotide combinations that resulted in “Termination*,” but for explanatory purposes, I have created an illustration (Figure 2, below) demonstrating the gel image that would exist in the “Termination*” experiments:

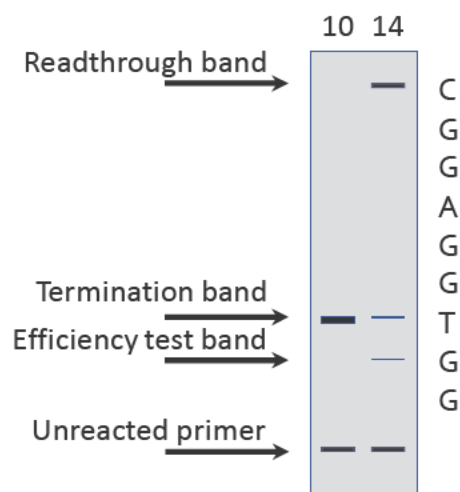


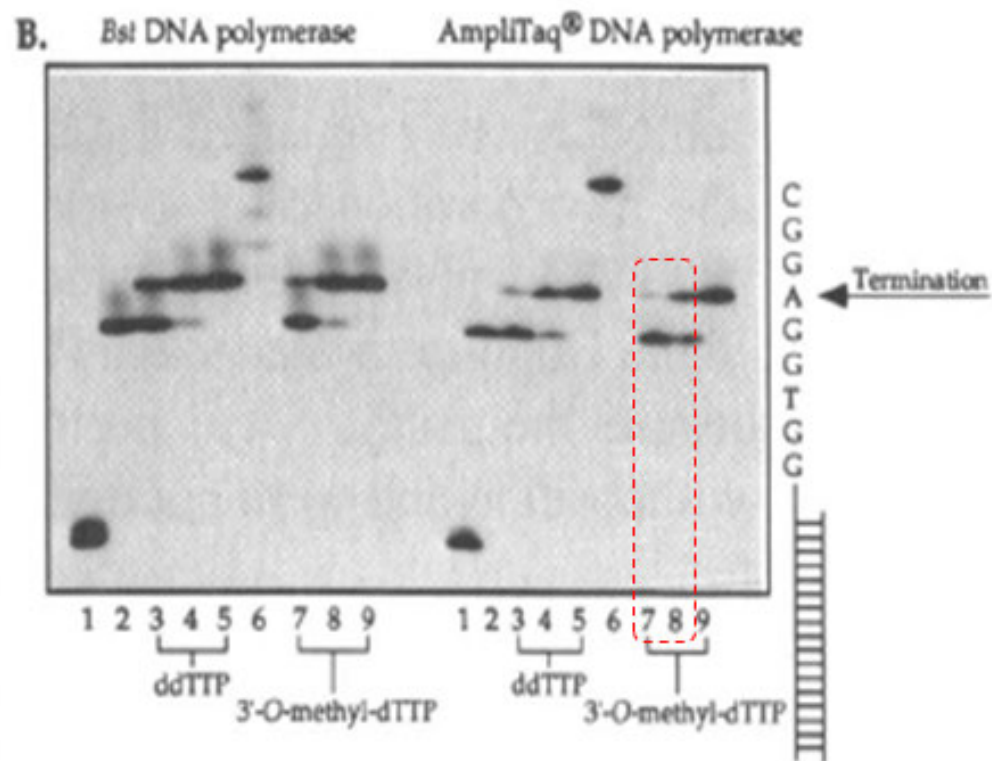
Figure 2

Figure 2 illustrates that the 3'-O-capped-dATP (lane 14) showed only incomplete termination because a new band appeared, which I refer to here as the “efficiency test band.”⁸ The termination band at the thymine position consists of DNA strands terminated upon incorporation of the 3'-O-capped-dATP. The efficiency test band at the guanine position consists of DNA strands that incorporated natural dCTP

⁸ While Metzker does not use the term “efficiency test band,” I have provided it a name solely for explanatory purposes.

(complementary to G in the template) but failed to incorporate the 3'-O-capped-dATP. The presence of the efficiency test band indicates the inability of the polymerase to complete incorporation of the 3'-O-capped-dATP into the DNA strands during the reaction, *i.e.*, there was inefficient incorporation.

32. The prior art shows examples of the efficiency test band. In Figure 4B of Metzker, lanes 7 and 8 show an efficiency test band at the guanine position, indicating that the 3'-O-methyl-dTTP nucleotide incorporated inefficiently at the concentrations tested in lanes 7 and 8:



Metzker's Figure 4(B) (excerpt)

Ex. 1016 at 4265 (dotted shape added for emphasis). *See also* Ex. 2111 at 3015 (Lane 17 of Figure 1 showing termination band and efficiency test band, leading authors to conclude that the tested nucleotide/polymerase combination showed “only partial termination[.]”).

2. Metzker Taught That The 3'-O-Allyl Nucleotide Was Inefficiently Incorporated

33. Metzker tested whether the 3'-O-allyl-dATP could be incorporated by any of eight polymerases. Ex. 1016 at 4263. For seven polymerases, Metzker reported “no activity[.]” *Id.* It is my understanding that Dr. Romesberg does not dispute that a POSA would not have been motivated to use a 3'-O-allyl nucleotide with these seven polymerases. Ex. 2126 at 115-16. For the Vent(exo-) DNA polymerase, Metzker reports that there was “Termination*[.]” Ex. 1016 at 4263. Dr. Romesberg and I disagree on what this result would have taught a POSA. For the reasons set forth below, a POSA would have understood this result to mean that the 3'-O-allyl nucleotide was *inefficiently* incorporated by the Vent(exo-) DNA polymerase and therefore not suitable for SBS.

34. First, my position is consistent with the Metzker publication and Metzker's own conclusions. Metzker reports that his use of “*” means that the activity was “incomplete at a final concentration of 250 μ M[.]” which was the highest concentration tested. Ex. 1016 at 4263. As explained in Section VI(A)(1),

the inability of the polymerase to complete incorporation of the 3'-O-allyl nucleotide into the DNA strands (reported as Termination*) indicates inefficient incorporation.

35. Second, my position aligns with Metzker's own statements regarding his 1994 publication. In 2007, Metzker stated that his 1994 publication taught that the 3'-O-allyl-dATP was a "*poor substrate*[]," and that "screening 3'-O-allyl-dATP with eight different DNA polymerases revealed *limited activity* at high micromolar concentrations with only Vent(exo-) DNA polymerase." Ex. 1017 at 6348 (citing the Metzker 1994 publication). In 2011, Metzker further stated that his 1994 publication taught that the "3'-O-allyl-dATP is *not efficiently incorporated* by Vent(exo-) polymerase[.]" Ex. 2025 at 10 (citing the Metzker 1994 publication).

36. Third, my position comports with the undisputed fact that Metzker abandoned the allyl capping group after conducting his 1994 experiments. *See* Ex. 2126 at 130-133. Metzker concluded that "[c]ompounds [1], [8], and [7] showed specific termination and were further evaluated with respect to their concentration dependent effects." Ex. 1016 at 4263. He similarly characterized these three compounds as "interesting DNA synthesis terminators." Ex. 1016 at 4265. Not included in this group of compounds was the 3'-O-allyl-dATP, compound [3]. A

POSA would have understood that the 3'-O-allyl-dATP was excluded from further analysis because it was not suitable for the SBS method. *E.g.*, Ex. 2126 at 104-105, 130-133.

37. Fourth, my position aligns with the fact that in the six years between publication of Metzker and the patents-at-issue's Priority Date of October 2000, there were no reports of any group attempting to develop nucleotides with the allyl capping group for SBS.

38. At his deposition in this trial, in an attempt to explain away the poor results reported in Metzker for the 3'-O-allyl nucleotide (*i.e.*, "Termination*"), Dr. Romesberg alleged that flaws in Metzker's experiments caused those results, shifting blame away from the 3'-O-allyl nucleotide and to the presence of contaminating dATP in the experiments. Ex. 2126 at 120-121, 129-130, 136-149, 265. I disagree.

39. First, while it is true that Metzker reports that the experiments "showed approximately 1% dATP contamination," which Metzker characterizes as "trace levels," the article provides no suggestion that the contamination affected the results reported in Table 2. Ex. 1016 at 4263.

40. Second, Dr. Romesberg's theory is *directly at odds* with Metzker's 2007 and 2011 statements, in which Metzker reiterated that his 1994 publication

showed that the 3'-O-allyl nucleotide was a “*poor substrate*[.]” with “*limited activity*” which was “*not efficiently incorporated.*” Ex. 1017 at 6348; Ex. 2025 at 10. Dr. Romesberg acknowledged that his theory is directly at odds with Metzker’s statements. When asked “So you disagree with Metzker’s interpretation of his own data,” Dr. Romesberg responded, “I am.” Ex. 2126 at 138.

41. Dr. Romesberg claimed that a POSA would be “confused by” Metzker’s 2007 and 2011 statements (Ex. 2126 at 146), and he presented several explanations for Metzker’s statements. One was that Metzker in 2007 and 2011 understood that the contaminating dATP in his 1994 experiments was a “mistake” that he “may not have been proud of” and may have “wanted to forget[,]” resulting in Metzker “drawing conclusions that are really not, in [Dr. Romesberg’s] opinion, founded.” Ex. 2126 at 145. I disagree with Dr. Romesberg’s position. I also submit that a POSA would not have understood the work of Metzker as a “mistake,” but a rather careful and detailed analysis of the characteristics of 3'-O-capped nucleotides. As explained above, there is no evidence that the trace levels of contamination in Metzker’s 1994 experiments were a “mistake” that affected his ability to interpret his experiments. Further, Dr. Romesberg’s position implies that each of Metzker’s multiple co-authors in 2007 and 2011 were willing participants in perpetuating the “mistake,” a position that is not credible.

42. Another explanation provided by Dr. Romesberg is that Metzker had ulterior motives for denigrating the 3'-O-allyl nucleotide in 2007 and 2011. Ex. 2126 at 137, 140-41, 148. Dr. Romesberg testified that Metzker was motivated by his desire to justify his decision to pursue different nucleotides. *Id.* According to Dr. Romesberg, Metzker in 2007 and 2011 “decided that he’s pursuing a different analog [using a 2-nitrobenzyl], and he is then saying that . . . all the old ones [*i.e.*, the 3'-O-allyl nucleotide] were not incorporated efficiently” in order to “justify” his pursuit of different nucleotides. Ex. 2126 at 137. Dr. Romesberg apparently believes that “[u]nfortunately, everyone in science has to do this. We have to justify what we are doing relative to other things.” Ex. 2126 at 148.

43. Third, a POSA would not have expected the trace contamination to affect the results reported in Table 2. The only substantive statement in Metzker regarding the contaminating dATP is that “some readthrough was . . . observed due to the presence of contaminating dATP.” Ex. 1016 at 4263. As explained in Section VI(A)(1), contaminating dATP results in a readthrough band, which does not impair the assessment of whether a 3'-O-capped nucleotide is efficiently incorporated (indicated by the presence of a single termination band) or inefficiently incorporated (indicated by the presence of the efficiency test band and the termination band).

44. More specifically, incorporation of nucleotides by polymerases comprises multiple steps, the first being binding of the nucleotide substrate to the polymerase-DNA complex, followed by steps leading to the incorporation of the bound substrate. Ex. 2059 at 44-45. As explained in Johnson (Ex. 2059), the binding of the polymerase to the nucleotide results in a rate-limiting conformational change of the polymerase which ultimately leads to nucleotide incorporation. *Id.* A POSA would have understood that the substrates in Metzker reported as Termination* showed polymerase binding but slow nucleotide incorporation. This meant that in some instances the polymerase was able to bind and incorporate the 3'-O-capped nucleotide (resulting in a termination band at the T position of the template in Figure 2, above), and in other instances the polymerase was able to bind the 3'-O-capped nucleotide but failed to incorporate it (resulting in the efficiency test band at the G position in Figure 2, above). The presence of contaminating natural nucleotide does not affect the rate at which the 3'-O-capped nucleotide is incorporated, and therefore does not affect the presence or absence of the termination band and efficiency test band. Instead, it only affects the *intensity* of the termination band and/or efficiency test band, as well as produces a readthrough band. In other words, the presence of trace levels of natural nucleotides would not affect Metzker's ability to accurately report that a

substrate was inefficiently incorporated (*i.e.*, Termination*) or efficiently incorporated (*i.e.*, Termination).

45. Fourth, the results provided in Metzker are inconsistent with Dr. Romesberg's theory. As explained in Section VI(A)(1), Metzker shows that the 3'-O-methyl-dATP was *efficiently incorporated* (*i.e.*, "Termination") by AMV-RT *despite the presence of contaminating dATP*. Ex. 1016 at 4263-64. As explained in that Section, the dATP contamination did not lead Metzker to report that the 3'-O-methyl-dATP was inefficiently incorporated (*i.e.*, "Termination*"), as would be expected under Dr. Romesberg's theory. This supports the conclusion that when Metzker reports inefficient incorporation, it is not due to dATP contamination.

46. Fifth, Dr. Romesberg's theory that the poor results reported for the 3'-O-allyl nucleotide in Metzker are due to the presence of contaminating dATP is inconsistent with the fact that instead of removing the contamination and retesting the allyl capping group, Metzker abandoned the allyl capping group and there are no reports of any laboratory developing 3'-O-allyl nucleotides for SBS prior to the Priority Date in October 2000.

47. Sixth, Metzker was frequently cited by other researchers as evidence that modified nucleotides are *inefficiently* incorporated by polymerase. In 1998, Ronaghi concluded from Metzker that "[t]he level of incorporation of . . .

[f]luorescent nucleotides is low,” and “therefore, [SBS] protocols only permit detection of a few bases.” Ex. 2054 at 1. In 1999, Jones concluded based on Metzker and other publications that “[t]echnical obstacles include a relatively low efficiency of extension and deprotection[.]” Ex. 2099 at 2:21-36. And in 2000, Albrecht concluded based on Metzker and other publications that SBS methods suffered from “numerous problems, such as inefficient chemistries which prevent determination of any more than a few nucleotides in a complete sequencing operation.” Ex. 2100 at 1:40-61. Contrary to Dr. Romesberg’s position, none of these authors dismissed Metzker’s results as being flawed due to the “trace” levels of dATP contamination.

48. Seventh, Dr. Romesberg’s position that Metzker taught that the 3’-O-allyl-dATP was efficiently incorporated contradicts his successful pursuit of an NIH grant in 2006 to “[e]volve polymerases” to “efficiently incorporate reversibly fluorophore tagged dNTPs with 3’-O-allyl protecting groups and then continue synthesis after double deprotection.” Ex. 2113 at 98-108.

**3. A POSA Would Have Understood That
The Allyl Capping Group Was Incompatible
With The Efficient Incorporation Requirement Of SBS**

49. As explained above, the 3’-O-allyl nucleotide examined in Metzker was not incorporated by seven polymerases and inefficiently incorporated by one polymerase (Vent(exo-) DNA polymerase). A POSA would not have been

motivated to select the allyl capping group because it was not believed to be compatible with the prior art SBS methods, which require *efficient* incorporation. See Section IV(B).

50. A number of other factors about Metzker's experiments would have further confirmed the POSA's lack of motivation. First, whereas Metzker examined 3'-O-capped nucleotides at 250 μ M (Ex. 1016 at 4263), Tsien's methods use concentrations of 1.5-30 μ M.⁹ Ex. 1013 at 38. It was known that decreasing the concentration of the 3'-O-capped nucleotides would necessarily decrease their efficiency of incorporation. Dr. Romesberg agrees. Ex. 2126 at 118 ("[t]hat is a physical law"). As a result, a POSA understood that at the nucleotide concentration used in Tsien's method (1.5-30 μ M), the 3'-O-allyl nucleotide would be incorporated with even lower efficiency than reported in Metzker.

51. Second, SBS methods require nucleotides that have both a label and a capping group, whereas the nucleotides tested in Metzker only had a capping

⁹ It was known that "high dNTP concentrations decrease polymerase fidelity by decreasing the amount of error discrimination at the extension step, while low dNTP concentrations increase fidelity by reducing the rate of mispair extension." Ex. 2125 at 21.

group. Even without such a label, the 3'-O-allyl nucleotide in Metzker was only capable of being *inefficiently* incorporated by a single polymerase, Vent(exo-) DNA polymerase. See Section VI(A)(2). A POSA would have expected that adding a label to the 3'-O-allyl nucleotide would further reduce the polymerase's ability to incorporate the nucleotide. The prior art taught that the Vent(exo-) DNA polymerase inefficiently incorporated labeled nucleotides, *e.g.*:

Can the CircumVent Kit be used for sequencing templates to be used on automated fluorescent sequencers?

Yes, given the following: In any automated sequencing system which uses end-labeled primers, the CircumVent Kit gives excellent results. However, when fluorescent tagged dideoxy dNTPs are used in the sequencing method, the results can be mixed. ***Inefficient incorporation*** leads to different intensities in peak heights on the resultant fluorograms; thus, the software has more difficulty calling correct bases.

Ex. 2084 at 13; Ex. 2126 at 121-129 (Dr. Romesberg agreeing that Ex. 2084 teaches that “there can be some fluorophore link or modifications that reduce efficiency” with Vent(exo-) DNA polymerase).

**B. There Was No Motivation To Select The
Allyl Capping Group Because Illumina's References
Do Not Satisfy The Cleavage Requirements Of SBS**

52. As explained in Section IV(B), the prior art set forth stringent requirements for performing SBS, one of which was the *quantitative, rapid* cleavage of the capping group on the nucleotide under *mild, aqueous* conditions.

53. A POSA would not have been motivated to select the allyl capping group based on Illumina's cited references (*e.g.*, Tsien (Ex. 1013), Kamal (Ex. 1037), Boss (Ex. 1035), Qian 1998 (Ex. 1036)), none of which other than Tsien are part of Illumina's Grounds, because they do not teach conditions for quantitative, rapid cleavage under mild, aqueous conditions. Illumina has not provided any empirical data that would have supported an expectation that the allyl capping group could be cleaved quantitatively and rapidly under mild, aqueous conditions. Instead, Illumina cites references that disclose allyl cleavage reactions that are incompatible with SBS. I address each of these references below.

**1. Tsien Did Not Teach Quantitative,
Rapid Cleavage Under Mild, Aqueous Conditions**

54. Tsien states that "Allyl ethers are cleaved by treatment with Hg(II) in acetone/water (Gigg and Warren, 1968)." Ex. 1013 at 24.¹⁰ A POSA would have

¹⁰ As explained in Section VI(F), Tsien does not disclose the allyl capping group.

understood that Tsien's conditions were incompatible with SBS for several reasons: (1) these conditions are not aqueous; (2) the use of potassium t-butoxide renders the conditions not mild; and (3) the use of mercury renders the conditions not mild. Dr. Romesberg agrees that a POSA would not use Tsien's conditions because there would be no expectation that they could be used in a manner that is compatible with SBS. Ex. 2126 at 232.

55. First, Tsien's conditions are not aqueous. Tsien cites Gigg for the cleavage reactions, and Gigg's mechanism to cleave allyl ethers requires first converting the allyl ether to vinyl ether using potassium t-butoxide in DMSO. Ex. 1046 at 1907. Dr. Romesberg agrees. Ex. 2126 at 230-231. A POSA understood that potassium t-butoxide in DMSO is incompatible with aqueous conditions and therefore not suitable for SBS.

56. Second, potassium t-butoxide is a strong organic base (Ex. 2112 at 914) that would be expected to denature DNA and therefore is not mild and is incompatible with SBS.

57. Third, Tsien's conditions are not mild because they require the use of Hg(II), which was a known DNA denaturant. *See* Ex. 2086 at 59 ("Additional evidence that partial and reversible denaturation of DNA molecules may occur has been derived from experiments on the reaction of HgCl₂ with DNA."); Ex. 2126 at

230 (Dr. Romesberg agreeing that Hg(II) denatures DNA). A POSA understood that conditions that denature DNA are not mild. Ex. 1013 at 23-24 (Tsien teaching that “[t]he deblocking method should . . . not . . . denature the DNA strand.”).

58. Because Tsien does not disclose that the allyl capping group is cleaved quantitatively and rapidly under mild, aqueous conditions, Illumina resorts to other non-primary references for those conditions, *e.g.*, Kamal, Boss, Qian 1998, each of which is discussed below.

2. Kamal Did Not Teach Quantitative, Rapid Cleavage Under Mild, Aqueous Conditions

59. Kamal’s conditions require acetonitrile, NaI, and chlorotrimethylsilane. Ex. 1037 at 371. Kamal’s conditions are incompatible with SBS for at least three reasons: the conditions (i) are not aqueous, (ii) are not mild, and (iii) do not result in quantitative cleavage.

a. Kamal’s Conditions Are Not Aqueous

60. The first reason why Kamal’s conditions are not compatible with SBS is because they are not aqueous. I note that Dr. Romesberg agrees. Ex. 2126 at 233-235. More specifically, the conditions in Kamal use acetonitrile, which is an organic solvent, in the absence of water. Ex. 1037 at 371.

61. It is not the case that Kamal’s conditions could be modified in a manner that rendered them aqueous. This is at least because Kamal’s conditions

use chlorotrimethylsilane (Ex. 1037 at 371), which is incompatible with water. Ex. 2112 at 1136-37 (noting that chlorotrimethylsilane “reacts violently with water”); Ex. 2126 at 235 (Dr. Romesberg agreeing that “chlorotrimethylsilane is not compatible with water[.]”).

62. Therefore, regardless of whether Kamal taught quantitative, rapid cleavage of the allyl capping group under mild conditions (which it does not), a POSA would have understood that Kamal’s conditions are incompatible with SBS because they are not aqueous and cannot be conducted in aqueous solution.

b. Kamal’s Conditions Are Not Mild

63. The second independent reason why Kamal’s conditions are not compatible with SBS is because they are not mild. The presence of acetonitrile in Kamal’s conditions (Ex. 1037 at 371) would denature DNA and are therefore not mild. Ex. 2085 at 171 (Table II); Ex. 2126 at 234-235 (Dr. Romesberg agreeing that acetonitrile in the absence of water would denature DNA).

c. Kamal’s Cleavage Is Not Quantitative

64. The third independent reason why Kamal’s conditions are not compatible with SBS is because they do not result in quantitative cleavage. Kamal reports cleavage yields for nine different compounds, none of which are nucleotides. A POSA would have known that compound [8] was the most relevant

of the nine compounds because it was the only compound that contained a ribose sugar. Compound [8] was cleaved with 93% yield. Ex. 1037 at 372.

65. It is my understanding that there is no dispute that Kamal's 93% cleavage efficiency is incompatible with prior art SBS methods. Dr. Romesberg and I agree that Tsien's SBS method requires cleavage efficiency greater than 95%. Ex. 2126 at 70; Ex. 1013 at 16 (Tsien disparaging 98% cleavage efficiency to practice his method). A POSA understood that Kamal's conditions are incompatible with prior art SBS methods because they do not result in quantitative cleavage.

**3. Boss Did Not Teach Quantitative,
Rapid Cleavage Under Mild, Aqueous Conditions**

66. Boss's conditions require 60°C to 80°C in a mixed solvent (including methanol) for a few hours in the presence of perchloric acid and palladium on activated charcoal (Pd/C). Ex. 1035. Boss reports that allyl deprotection yields 95% or greater required reaction times of 6-24 hours. *Id.*

Boss's conditions are incompatible with SBS for at least two reasons: the conditions (i) are not mild and (ii) do not result in rapid cleavage.

a. Boss's Conditions Are Not Mild

67. The first reason why Boss's conditions are not compatible with SBS is because they are not mild. Dr. Romesberg agrees that Boss's conditions are not

mild. Ex. 2126 at 200. First, the concentration of perchloric acid used in Boss's allyl cleavage reactions will denature DNA. A POSA would have known that "DNA is stable over the approximate pH range of 2.7 to 12, but denatures at higher or lower pH's" (Ex. 2086 at 56) and would have understood that Boss's conditions result in a pH outside of this range. Boss uses "perchloric acid in concentrations of 1 to 5 mmol/ether" in each of the relevant reactions, Ex. 1035 at 559,¹¹ meaning that the total amount of perchloric acid ranges from 83 mM to 417 mM.¹² pH is defined as $\text{pH} = -\log[\text{H}^+]$. When $[\text{H}^+] = 0.083\text{M}$ (*i.e.*, 83 mM), the pH will be $\text{pH} = -\log[0.083]$, or about 1.1. When $[\text{H}^+] = 0.417\text{M}$ (*i.e.*, 417 mM), the pH will be $\text{pH} = -\log[0.417]$, or about 0.4. A pH of 0.4 to 1.1 as taught by Boss would denature DNA because it is outside the range in which DNA is stable at room temperature. Ex. 2086 at 56. Dr. Romesberg agrees. Ex. 2126 at 189-90. That

¹¹ Dr. Romesberg testified that his reliance on Boss only relates to compounds 4-7 (Ex. 2126 at 86-87), each of which uses perchloric acid. Dr. Romesberg did not rely on compounds 8-10 because the cleavage efficiencies of those reactions are too low to be compatible with SBS. Ex. 2126 at 86-87.

¹² $1 \text{ mmol perchloric acid} / 12 \text{ ml} = 83 \text{ mM}$; $5 \text{ mmol perchloric acid} / 12 \text{ ml} = 417 \text{ mM}$.

Boss's conditions denature DNA and are incompatible with SBS is not surprising, given that it was well known that perchloric acid is a powerful DNA denaturing agent. Ex. 2086 at 57.

68. In addition, Boss's conditions are not mild because the amount of methanol used in three of the four reactions relied on by Dr. Romesberg (compounds 4, 5 and 7) is not compatible with SBS. The concentration of methanol can be calculated from Boss's experimental section where he states that Reaction 7 uses 10 mL of methanol and 2 mL of water, and the molecular weight of methanol (32 g/mol) and its density (0.79 g/mL). 10 mL of methanol weighs 7.9 grams ($0.79 \text{ g/mL} \times 10 \text{ mL}$) and equals 0.25 mol ($7.9 \text{ g} / 32 \text{ g/mol}$). The molarity of methanol in a mixture of 10 mL methanol and 2 mL water is 20.8 M ($0.25/0.012 = 20.8 \text{ mol/L}$, or 20.8M). This amount of methanol would negatively impair the polymerase, as Dr. Romesberg agrees. Ex. 2126 at 168, 181. Therefore, Boss's conditions would be incompatible with SBS.

69. The methanol concentration would also denature the DNA. Levine taught that 3.5 M methanol caused 50% denaturation of bacteriophage DNA in 30 minutes at 73°C. Ex. 2085 at 171 (Table II). Compared to Levine, the methanol in Boss is six-fold higher (20.8 M vs. 3.5 M), the reaction time is much longer (6-24 hours vs. 30 minutes), and the temperature is similar (64.7°C (reflux) vs. 73°C). A

POSA would therefore expect the methanol in Boss to denature DNA similar to the denaturation reported in Levine. A POSA also understood that the DNA templates used in SBS are even more susceptible to denaturation than bacteriophage DNA (as used in Levine) because they are shorter fragments. Ex. 2126 at 53-54, 170 (Dr. Romesberg agreeing).

b. Boss's Cleavage Is Not Rapid

70. The second independent reason why Boss's conditions are not compatible with SBS is because they do not result in the rapid cleavage of the allyl capping group. The four reactions that Dr. Romesberg relies on in Boss (compounds 4-7 (Ex. 2126 at 86-87)) each require between 6 and 24 hours. Ex. 1035 at 559. A POSA would have understood that cleavage reactions taking 2 hours or longer are incompatible with SBS. Ex. 2007 at ¶47; *see also* Ex. 2126 at 58, 202-203 (agreeing that Boss's reactions are incompatible with Tsien's methods); Ex. 2035 at 98 (agreeing that SBS requires cleavage of the capping group in no longer than an hour).

4. Qian 1998 Did Not Teach Quantitative, Rapid Cleavage Under Mild, Aqueous Conditions

71. Qian 1998 uses palladium chloride (PdCl₂) in methanol to cleave the allyl group. Ex. 1036 at 2185. Qian 1998's conditions are incompatible with SBS for at least four reasons: the conditions (i) are not aqueous, (ii) are not mild, (iii) do

not result in rapid cleavage, and (iv) do not reliably result in quantitative cleavage. Dr. Romesberg agrees that Qian 1998's conditions are incompatible with SBS. Ex. 2126 at 158, 162-164.

a. Qian 1998's Conditions Are Not Aqueous

72. The first reason why Qian 1998's conditions are not compatible with SBS is because they are not aqueous. Ex. 2126 at 158 (Dr. Romesberg agreeing that Qian's conditions are not aqueous). More specifically, the conditions in Qian 1998 involve methanol (an organic solvent) in the absence of water. Ex. 1036 at 2185.

73. It appears to be Dr. Romesberg's speculation (raised at his deposition but not in his Declaration) that a POSA would have understood that Qian 1998's conditions could be modified to include water, with the expectation that the same deprotection reaction and cleavage yield would be obtained. Ex. 2126 at 158, 162-164. I disagree with Dr. Romesberg's speculation for several reasons.

74. First, Dr. Romesberg has not cited any examples in the prior art disclosing the use of palladium chloride in water to cleave an allyl capping group, let alone an example that results in quantitative, rapid cleavage of the allyl capping group under mild, aqueous conditions, and I am not aware of any.

75. Second, palladium chloride is not soluble in water. Dr. Romesberg does not disagree. Ex. 2126 at 163. Qian's conditions therefore cannot be

rendered aqueous by simply adding water. Dr. Romesberg speculates that a POSA could add a solubilizing agent to make the palladium chloride soluble in water. Ex. 2126 at 162-163. A POSA would have had no reason to believe that altering Qian's reaction conditions by adding both water and a palladium solubilizing agent would not affect allyl cleavage efficiency, that is, there would be no expectation that those different conditions would result in the allyl capping group being cleaved quantitatively. Dr. Romesberg and I agree that small changes to a reaction's conditions can affect its cleavage efficiency. Ex. 2113 at 72 (“[s]mall differences can have impact”). Dr. Romesberg offers only speculation that the cleavage efficiency of such an altered reaction would be quantitative.

76. Third, the prior art suggested that carrying out Qian 1998's reaction in an aqueous environment would yield different products compared to carrying out the reaction in methanol. A POSA reading Qian 1998, which used a carbohydrate-allyl-ether and PdCl₂ in *methanol*, would expect that deprotection of the allyl protecting group would go according to Bieg (Ex. 2105), *i.e.*, PdCl₂-catalyzed isomerization of the allyl group to the prop-1-enyl group, followed by *in situ* loss of that group in the methanol solvent to yield the desired carbohydrate alcohol. However, a POSA would not expect to produce prop-1-enyl with PdCl₂ in *water*. Instead, a POSA understood that transformation of an olefin using PdCl₂ in water

would proceed according to the Wacker process (Ex. 2115 at 102-104), resulting in the production of oxidized ether products rather than the isomerized prop-1-enyl product. The oxidized ether products obtained from the Wacker process, which did not yield any prop-1-enyl ether products, are disclosed in Kang. Ex. 2098 at 4678 n.6. A POSA reading Bieg, Kang and Qian 1998 would expect that Qian's PdCl_2 deprotection of an allyl capping group in water would not result in production of a deprotected alcohol (*i.e.*, 3'-OH), but would instead result in the production of oxidized ether products that are incompatible with SBS.

b. Qian 1998's Conditions Are Not Mild

77. The second independent reason why Qian 1998's conditions are not compatible with SBS is because they are not mild.

78. A POSA would have known that the methanol used in Qian 1998 would denature DNA. Ex. 2126 at 158 (Dr. Romesberg agreeing that methanol without water (as used in Qian 1998) is a DNA denaturant).

c. Qian 1998's Cleavage Is Not Rapid

79. The third independent reason why Qian 1998's conditions are not compatible with SBS is because they do not result in rapid cleavage of the allyl capping group. The reaction in Qian 1998 required 2 hours. Ex. 1036 at 2185. A POSA understood that cleavage reactions taking 2 hours are incompatible with

SBS. Ex. 2007 at ¶47; Ex. 2035 at 98 (agreeing that SBS requires cleaving of the capping group in no longer than an hour).

80. Further, a second publication by Qian in 1999 reports that cleaving the allyl capping group from a saccharide substrate similar to that in Qian 1998 with PdCl_2 and methanol resulted in 83% cleavage efficiency after 4 hours. Ex. 2097 at 12065 (reaction (e)). A POSA reading Qian 1998 and Qian 1999 would have understood that Qian's allyl deprotection reaction was for two hours (Qian 1998) or four hours (Qian 1999), each of which a POSA would have understood is too long to be compatible with SBS.

d. Qian's Cleavage Is Not Reliably Quantitative

81. Qian 1998 reports "quantitative" cleavage of the allyl capping group. Ex. 1036 at 2185. However, Qian 1999 reports that cleavage of the allyl capping group with the same conditions as Qian 1998 (PdCl_2 and methanol) resulted in 83% cleavage efficiency. Ex. 2097 at 12065 (reaction (e)). A POSA understood that this was lower than the efficiency necessary for prior art SBS methods (*i.e.*, at least 95%). Ex. 2126 at 87; Ex. 1013 at 16. Therefore, a POSA would have understood from Qian 1998 and Qian 1999 that Qian's allyl deprotection conditions do not reliably result in quantitative cleavage.

C. A POSA Weighing The Allyl Capping Group's Evidence-Based Disadvantages Against Its Alleged Advantage Would Conclude That It Was Incompatible With SBS

82. I understand that the Board in its Institution Decisions for IPR2018-00291, IPR2018-00318, IPR2018-00322, and IPR2018-00385 stated that “[w]e are mindful that this is a situation in which a person of ordinary skill would have been weighing the disadvantages of a compound against its benefits.” *E.g.*, IPR2018-00291, Paper 16 at 20 (June 25, 2018). After conducting such an assessment, a POSA would not be motivated to select the allyl capping group on a nucleotide for SBS because, based on actual data in the prior art, it was understood to be incompatible with SBS.

83. As detailed above, a POSA understood that the allyl capping group had disadvantages due to (i) Metzker’s teaching that the 3’-O-allyl nucleotide was at best *inefficiently incorporated*, and (ii) Illumina’s cited references’ failure to teach conditions for quantitatively and rapidly cleaving it under aqueous and mild conditions. A POSA would have weighed these disadvantages against any potential benefits of the allyl capping group. It is my understanding that Dr. Romesberg offers as an alleged benefit of the allyl capping group Tsien’s statement that “deblocking occurs only when the specific deblocking reagent is present and premature deblocking during incorporation is minimized.” *E.g.*, IPR2018-00291, Romesberg Declaration (Exhibit 1012) at ¶64.

84. First, a POSA would have given much more weight to Metzker and the references relied on by Illumina for cleavage of the allyl capping group than the Tsien patent application. Metzker and the allyl-cleavage references are peer-reviewed papers that actually provide actual experimental data. Tsien's patent application does not contain any experimental data.

85. Second, a POSA weighing the many experimental data-driven disadvantages of the allyl capping group against its one hypothetical potential benefit would have had no motivation to make a 3'-O-allyl nucleotide. A POSA would have understood that the disadvantages rendered the allyl capping group incompatible with SBS, making any theoretical benefit moot. That is, a POSA understood that using the allyl capping group for Tsien's or Dower's SBS methods would produce an inoperable result. The single alleged benefit (Tsien's minimization of premature deblocking), if it existed at all for the allyl capping group, would not change this conclusion. Tsien taught that a "wide variety" of capping groups shared the alleged benefit of minimizing premature deblocking (Ex. 1013 at 24-25), including but not limited to "[t]etrahydrothiofuranyl ethers" and "2,4-[d]initrobenzenesulfonyl groups[.]" Ex. 1013 at 24-25. If a POSA were motivated by such a benefit, the POSA would pursue other capping groups disclosed in Tsien that were not understood to be incompatible with SBS.

**D. A POSA Would Not Have Been Motivated
To Select Small Capping Groups**

86. Contrary to Illumina's assertions (*e.g.*, IPR2018-00291, Petition at 11-12, 22 (Dec. 8, 2017)), a POSA before the Priority Date would not have been motivated to select a capping group that was small.

87. First, Illumina's reliance on Pelletier to argue that a POSA would be motivated to use small capping groups is misplaced. I do not believe that a POSA would have reviewed Pelletier. Pelletier was published in 1994 and related to HIV drug development, an entirely different field from SBS. *See* Ex. 1021. Additional evidence that Pelletier was not in the field is that there is no evidence of any researchers developing nucleotides for SBS between 1994 and the Priority Date in October 2000 who cited Pelletier.

88. Second, Dr. Romesberg testified that a POSA would not have expected a capping group to possess the characteristics necessary for SBS (*e.g.*, efficient incorporation of the capped nucleotide) simply because it was small. Ex. 2007 at ¶58; Ex. 2126 at 81-84.

89. Third, contrary to Illumina's assertions, Dower's use of the term "small" to describe several capping groups (*see* Ex. 1015 at 25:48-51) does not support a conclusion that Dower teaches that "small" capping groups are

“desirable.” Dower does not state that the four capping groups it characterizes as small are desirable because of their size.

**E. A POSA Would Not Have Had A Reasonable
 Expectation Of Success In Achieving The Claimed Invention**

90. I understand that Illumina and Dr. Romesberg allege that a single claimed species of each of the patents-at-issue is obvious: in IPR2018-00291, an *adenine* nucleotide with the allyl capping group and a base label; in IPR2018-00318, a *thymine* nucleotide with the allyl capping group and a base label; in IPR2018-00322, a *cytosine* nucleotide with the allyl capping group and a base label; and in IPR2018-00385, a *guanine* nucleotide with the allyl capping group and a base label. As explained below, I do not believe that a POSA would have had a reasonable expectation that these nucleotides would achieve the claim limitations of the patents-at-issue.

[Paragraph Numbers 91-93 Not Used]

**1. There Was No Reasonable Expectation
 That A 3'-O-Allyl Thymine, Cytosine, Or Guanine
 Nucleotide Would Be Incorporated**

94. In IPR2018-00318, IPR2018-00322, and IPR2018-00385, the challenged claim requires that the claimed *thymine* nucleotide, *cytosine* nucleotide, or *guanine* nucleotide, respectively, “is incorporated at the end of a growing strand of DNA during a DNA polymerase reaction.” Exs. 1002-1004, each at claim 1. A

POSA would not have had a reasonable expectation that these nucleotides would be “incorporated” by any polymerase.

95. The only data that was available to a POSA prior to the Priority Date in October 2000 for the incorporation of 3'-O-allyl nucleotides is Metzker, and Metzker *only* tested a 3'-O-allyl *adenine*. Ex. 1016 at 4263. As explained below, data on incorporation of *adenine* nucleotides would have provided a POSA no expectations regarding *thymine*, *cytosine*, or *guanine* nucleotides.

96. In six polymerases, Metzker tested both a 3'-O-methyl *adenine* nucleotide and a 3'-O-methyl *thymine* nucleotide. Ex. 1016 at 4263. The results are summarized below (wherein ✓ indicates incorporation and X indicates no incorporation):

	AMV-RT	M-MuLV-RT	<i>Bst</i>	AmpliTaq	Vent(exo-)	<i>rTth</i>
3'-O-methyl-dATP	✓	✓	X	X	X	X
3'-O-methyl-dTTP	X	X	✓	✓	✓	✓

Ex. 1016 at 4263.

97. As the chart shows, a polymerase's ability to incorporate an *adenine* nucleotide provided no expectation that the polymerase would also be capable of incorporating a *thymine* nucleotide with the same capping group. Metzker

concluded that: “[w]e have not identified a polymerase that will incorporate both 3’-O-methyl-dATP and 3’-O-methyl-dTTP.” Ex. 1016 at 4266. Based on Metzker’s results, a POSA would have no reason to expect that a 3’-O-allyl *thymine*, *cytosine*, or *guanine* nucleotide would be “incorporated at the end of a growing strand of DNA during a DNA polymerase reaction,” as required by the challenged claim in IPR2018-00318, IPR2018-00322, and IPR2018-00385.

**2. There Was No Reasonable Expectation That
The Allyl Capping Group Would Not
Interfere With Recognition Of The Nucleotide**

98. In IPR2018-00291, IPR2018-00318, IPR2018-00322, and IPR2018-00385, the challenged claim requires that the capping group of the nucleotide “does not interfere with recognition of the analogue as a substrate by a DNA polymerase[.]” Exs. 1001-1004, each at claim 1. A POSA would not have had an expectation that the allyl capping group would satisfy this limitation.

99. Metzker reported that seven polymerases completely failed to incorporate the 3’-O-allyl nucleotide. Ex. 1016 at 4263. A POSA understood from these results that the allyl capping group interfered with recognition of the 3’-O-allyl nucleotide by these seven polymerases. With the Vent(exo-) polymerase, the 3’-O-allyl nucleotide’s termination activity was “incomplete at a final concentration of 250 μ M[.]” Ex. 1016 at 4263. As explained above, this meant that the 3’-O-allyl nucleotide was “not efficiently incorporated” by the polymerase,

i.e., it was a “poor substrate” with “limited activity.” *See* Section VI(A). A POSA understood that inefficient incorporation results from the capping group interfering with recognition of the analogue as a substrate by the polymerase.

**F. Neither Tsien Nor Prober Would Have
Motivated A POSA To Select The Allyl Capping Group**

100. It is my understanding that a claim cannot be rendered obvious by a combination of references none of which discloses or suggests a feature of the claim.

101. Prober does not disclose the allyl capping group, and Illumina relies only on Tsien for this element. As support for its position, Illumina cites a single sentence in Tsien: “Allyl ethers are cleaved by treatment with Hg(II) in acetone/water (Gigg and Warren, 1968).” Ex. 1013 at 24:29-30. As explained below, there are two reasons why a POSA would not have understood this statement to disclose the allyl capping group, *i.e.*, a capping group with the structure $\text{-CH}_2\text{CH=CH}_2$.

102. A POSA reading Tsien’s sentence that “Allyl ethers are cleaved by treatment with Hg(II) in acetone/water” would have found the statement surprising and out of place. The POSA would have known that Hg(II) in acetone/water would not cleave allyl ethers, and thus would not have been certain what Tsien was intending to convey. A POSA reviewing Gigg for clarity would confirm that the

reaction conditions using Hg(II) in acetone/water in fact were not being applied to cleave allyl ethers, but rather were being applied to cleave vinyl ethers. *E.g.*, Ex. 1046 at 1907. The POSA would thus contemplate that Tsien may have intended to refer to vinyl ethers as suitable capping groups, rather than allyl ethers as written.

103. The allyl/vinyl mix-up interpretation would have been reinforced by the first sentence of the Tsien paragraph in which “allyl ethers” appears, which states: “A wide variety of hydroxyl blocking groups are cleaved selectively using chemical procedures other than base hydrolysis.” Ex. 1013 at 24. Gigg does provide a protocol for removing allyl ether protecting groups from alcohols. But the first step of this protocol involves treating allyl ethers with the exceedingly strong base, potassium *t*-butoxide, to generate the vinyl ethers that are cleaved using the Hg(II) conditions. *E.g.*, Ex. 1046 at 1907. Based on this, the POSA would conclude that Tsien did not intend to refer to allyl ethers as a non-base cleavable capping group class in this paragraph, and rather that vinyl ethers were intended. For the same reason, the sentence at the end of the paragraph—“These protecting groups . . . have some advantages over groups cleavable by base hydrolysis . . .”—would reinforce this interpretation. Ex. 1013 at 24. With this information, the POSA would not have been motivated by Tsien to use allyl ethers as protecting groups.

104. Second, even if a POSA entertained that Tsien could have intended to reference allyl ether capping groups despite the mixup regarding chemical reactivity noted above, the POSA understood that the “[a]llyl ethers” in Tsien is not $\text{-CH}_2\text{CH=CH}_2$ itself. Allyl ethers describes a large genus of compounds. Although Dr. Romesberg testified that the use of “[a]llyl ethers” in Tsien does not refer to a genus of compounds (Ex. 2126 at 208-209), he agreed it is “common” for scientists to use the term “allyl ethers” to refer to a genus of compounds, and that the structure $\text{-CH}_2\text{CH=CH}_2$ is not disclosed in Tsien. Ex. 2113 at 127, 130, 134. For example, in each of Tsuji, Ochiai, and Katritzky, “allyl ethers” refer to groups of compounds that vary in size from 3-12 carbon atoms and 5-15 hydrogen atoms. Ex. 2101 at 2680-81; Ex. 2106 at 7719; Ex. 2107 at 1319; Ex. 2113 at 126-130 (Dr. Romesberg agreeing).

105. A POSA would have understood that Tsien’s “[a]llyl ethers” refer to a large, unbounded genus and not the specific $\text{-CH}_2\text{CH=CH}_2$ structure. Nor would a POSA reading Tsien’s disclosure of “[a]llyl ethers” at once envisage each member of the genus.

106. Because neither Tsien nor Prober discloses the allyl capping group, *i.e.*, a capping group with the structure $\text{-CH}_2\text{CH=CH}_2$, neither reference can disclose any motivation for selecting that capping group. Even if Tsien disclosed

the allyl capping group, a POSA would not have been motivated to select it because Gigg teaches that cleavage of allyl ethers requires strong base and hydrolysis (Ex. 1046 at 1907), contrary to Tsien's explicit teaching that cleavage conditions "other than base hydrolysis" are advantageous. Ex. 1013 at 24.

G. The Lead Compound Analysis

107. A 3'-O-allyl nucleotide would not have been selected as a lead compound because the prior art demonstrated that it was incompatible with SBS. *See* Sections VI(A)-(C). The 3'-O-acetyl nucleotide would also not have been a lead compound because the prior art demonstrated that it was incompatible with SBS, at least because it was not incorporated by any polymerase in Metzker's experiments. Ex. 1016 at 4263; Ex. 2126 at 96 (Dr. Romesberg agreeing).

[Paragraph Number 108 Not Used]

VII. Illumina's Ground 2 Challenge For Obviousness Over Dower In View Of Prober And Metzker Fails

A. Illumina's Ground 2 Challenge Fails For The Same Reasons As Its Ground 1 Challenge

109. Illumina's Ground 2 challenge in IPR2018-00291, IPR2018-00318, IPR2018-00322, and IPR2018-00385 fails for the same reasons as its Ground 1 challenge in those proceedings. Regardless of whether Illumina's primary references consist of Tsien and Prober (Ground 1) or Dower, Prober, and Metzker (Ground 2), the prior art as a whole taught that:

(A) Illumina's references provide no motivation to select the allyl capping group because it had been described as being incapable of achieving the efficient incorporation requirement of SBS (*see* Section VI(A));

(B) Illumina's references provide no motivation to select the allyl capping group because they do not teach quantitative, rapid cleavage of the allyl capping group under mild, aqueous conditions (*see* Section VI(B));

(C) A POSA weighing the allyl capping group's numerous disadvantages against its one alleged advantage would have concluded that it was incompatible with SBS (*see* Section VI(C));

(D) Illumina's references do not teach that capping groups had to be small or that there was any advantage to selecting small capping groups, and a POSA would not have reviewed Pelletier because it related to an entirely different field from SBS (*see* Section VI(D));

(E) There was no reasonable expectation of success in achieving the claimed invention (*see* Section VI(E));

(F) If the lead compound analysis applies, Illumina selected the wrong lead compounds (*see* Section VI(G)).

**B. Illumina’s Ground 2 Challenge Fails Because
None Of Its References Disclose The Claimed
“Chemically Cleavable, Chemical Linker”**

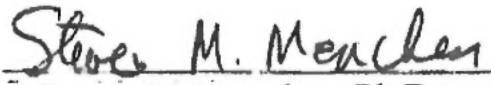
110. Illumina’s Ground 2 challenge in IPR2018-00291, IPR2018-00318, IPR2018-00322, and IPR2018-00385 also fails because none of its references (Dower, Prober, or Metzker) disclose a “chemically cleavable, chemical linker” as required by the challenged claim of the patents-at-issue. It is my understanding that a claim cannot be rendered obvious by a combination of references none of which discloses or suggests a feature of the claim.

111. The challenged claim of the patents-at-issue require the presence of “Y,” a “chemically cleavable, chemical linker” between the label and the base. Exs. 1001-1004, each at claim 1. Dower does not disclose any chemical linker attaching a label to the base, let alone a chemically cleavable, chemical linker. Dower only discloses labels (*e.g.*, Fmoc) which are ***directly*** attached to the base. *E.g.*, Ex. 1015 at Fig. 9. Prober discloses the attachment of labels to the base of ddNTPs by using an acetylenic linker, which is a chemical linker that is ***not*** cleavable under conditions compatible with DNA. Ex. 1014 at 338; Ex. 2113 at 124 (Dr. Romesberg agreeing). Metzker does not disclose any nucleotide with a label attached to the base, let alone a label attached by a chemically cleavable, chemical linker. *See generally* Ex. 1016; Ex. 2113 at 125 (Dr. Romesberg agreeing).

112. Therefore, the combination of Dower, Prober, and Metzker cannot render the challenged claim in IPR2018-00291, IPR2018-00318, IPR2018-00322, and IPR2018-00385 obvious because the combination does not disclose a required claim element, *i.e.*, a “chemically cleavable, chemical linker[.]”

I hereby declare under penalty of perjury under the laws of the United States of America that the foregoing is true and correct, and that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true. I understand that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. §1001.)

Executed on October 25, 2018


Steven M. Menchen, Ph.D.