

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

PATENT OWNER'S RESPONSE

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

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I. Introduction

One of the 21st century's most notable scientific achievements was the June 26, 2000 announcement of a sequence draft of the human genome. The announcement came from President Bill Clinton and Prime Minister Tony Blair, underscoring the significance of this watershed achievement. The \$3-billion project paved the way for the emerging field of "molecular medicine," which if realized, promised to revolutionize medicine by allowing the development of individualized therapeutic strategies based on individual genotypes.

Sequencing of the human genome used a form of DNA sequencing called Sanger sequencing. Although powerful, Sanger sequencing had technical limitations that prevented molecular medicine from becoming a practical reality. Researchers understood that advancement required the development of new forms of DNA sequencing. One new form, called Sequencing by Synthesis ("SBS"), began to receive attention during the decade of the human genome project because, in theory, it provided a faster and cheaper method that could take molecular medicine from wish to reality. However, in October 2000, SBS remained only a theoretical possibility.

Dr. Jingyue Ju at Columbia University, a pioneer and internationally acclaimed leader in the development of SBS, designed novel nucleotides with fluorescent labels attached to the base and 3'-OH capping groups that were small

enough to be accommodated within the DNA polymerase active site. While other laboratories tried different strategies and failed, Dr. Ju's approach proved successful, and in October 2000 he filed a patent application describing his novel nucleotides and sequencing methods. The patent-at-issue stems from that application.

Illumina, having competing interests with Patent Owner, asserts in its Petition that Dr. Ju's novel nucleotides would have been obvious to a person of ordinary skill ("POSA"). Illumina bases that on a single assertion—that the prior art would have led a POSA to select the allyl capping group for use in SBS. In considering that assertion, the Board requested the parties to address several questions, including whether the Metzker publication would have discouraged a POSA from selecting the allyl capping group. It would have. Metzker's results showed that the allyl capping group resulted in at best ***inefficient incorporation*** of the capped nucleotide, in direct contrast to the prior art's explicit requirement for ***efficient incorporation***. Dr. Metzker later acknowledged that his 1994 publication showed that the allyl capping group was a "poor substrate[]" with "limited activity" that was "not efficiently incorporated." Ex. 1017 at 6348; Ex. 2025 at 10.

The Board also asked whether the prior art taught that the allyl capping group could be cleaved under SBS-compatible conditions. Illumina's references demonstrate that it could not, because those references teach conditions that do not

satisfy the explicit prior art requirement for ***aqueous, mild*** conditions that result in ***quantitative, rapid*** cleavage of the allyl capping group.

Despite the very references relied upon by Illumina showing that the allyl capping group was not suitable for SBS, Illumina turns a blind eye to the facts. In doing so, Illumina asks the Board to rule in its favor and to preclude Columbia from even offering a defense under an already rejected theory of estoppel. These attempts to circumvent a proper review of the evidence betray the weakness of Illumina's case. As established below, any balanced review of the teachings of the prior art demonstrates that the references would not have provided a POSA any reason to select the allyl capping group. Absent a reason to select the allyl capping group, Illumina's Grounds fail.

II. Level Of Ordinary Skill In The Art

Columbia does not dispute, for purposes of this IPR, Illumina's criteria for defining a POSA.

III. State Of The Art

A. Tsien And Dower Offered Only Speculation

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 Ex. 2116 ¶11.³ 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; Ex. 2116 ¶11.

In 1990, Tsien and Dower prophetically described SBS methods. Ex. 1013; Ex. 1015; Ex. 2116 ¶12. It is undisputed that those hypothetical SBS methods could be conducted only *if* nucleotides that met the stringent requirements set forth in the prior art were discovered. Ex. 2009 at 15, 17 (Illumina stating that Tsien and Dower “constitute[] **speculation** about the existence of” useful nucleotides.);^{4,5} Ex.

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.

³ Columbia submits the Declaration of Steven M. Menchen, Ph.D., a chemist with extensive experience developing DNA sequencing technology, including SBS.

⁴ Ex. 2009 contains submissions by Illumina Inc. to the Patent Office during reexamination of its U.S. Patent No. 5,808,045. See Ex. 2009 at 3 (showing Illumina Inc. as patent assignee); see *also* Ex. 2010 at 1.

⁵ Emphases added throughout unless otherwise indicated.

2116 ¶13. Neither Tsien nor Dower describe any actual experiments or provide any data. Ex. 2116 ¶13. As explained below, as of October 2000 (“Priority Date”), no nucleotides were known to meet these stringent requirements. *Id.*

B. SBS Requires Both Efficient Incorporation And Quantitative, Rapid Cleavage Under Mild, Aqueous Conditions

To perform Tsien’s or Dower’s SBS methods, those references as well as others expressly taught that a number of stringent requirements had to be met. Ex. 2116 ¶14. One requirement was that the nucleotides were *efficiently incorporated* into the growing DNA strand by polymerase:

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.

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.

Ex. 1015 at 26:6-9.

[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; Ex. 2116 ¶14. Thus, the prior art as of the Priority Date clearly taught that efficient incorporation of nucleotides was required to conduct SBS. Ex. 2116 ¶14.

A second stringent requirement for SBS was ***quantitative, rapid cleavage*** of the capping group on the nucleotide under ***mild, aqueous*** conditions. Ex. 2116 ¶15. 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; Ex. 2041 at 197-198; Ex. 2116 ¶15.

A POSA understood those requirements were necessary to perform SBS. Ex. 2116 ¶16; Ex. 1013 at 20 (Tsien noting that the requirements were “criteria for the successful use” of capping groups); IPR2018-00291, Paper 16 at 20 (June 25, 2018) (PTAB recognizing that “[t]hese considerations were . . . known to a person of ordinary skill and would have been considered both individually and as a whole.”); Ex. 2008 at 12-14, 15-33 and Ex. 2026 at 5, 35-56 (Illumina arguing that a POSA ***reading Tsien*** would only be motivated to select a capping group which met the above requirements); Ex. 2027 at 5-6, 29-50 (Illumina arguing that a POSA ***reading Dower*** would only be motivated to select a capping group which

met similar requirements); *see also* Ex. 2035 at 153-56; Ex. 2029 at 6-7; Ex. 2007 ¶¶36-37, 52.⁶

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

Prior to the Priority Date, numerous groups (including Tsien, Dower, Cheeseman, Rabani, Canard, Welch, and Stemple) searched for nucleotides meeting the stringent requirements needed to take SBS from speculation to reality, a task described as a “major challenge” (Ex. 2041 at 197-198) and “formidable obstacle[]” (Ex. 1016 at 4259). Ex. 1013 at 20-25; Ex. 1015 at 25:47-51; Ex. 1020 at 4:50-63; Ex. 2109 at 2:15-21; Ex. 2031 at 1-2; Ex. 1023 at 951-56; Ex. 2013 at 13; Ex. 2116 ¶17. By the Priority Date, despite immense effort, “[t]echnical obstacles includ[ing] a relatively low efficiency of extension and deprotection” continued to prevent the success of SBS as hypothesized by Tsien and Dower. Ex.

⁶ Exs. 2008, 2026, 2027, and 2029 are documents submitted by Illumina Cambridge Ltd. In its Petition, Illumina does not distinguish between Illumina Inc. and Illumina Cambridge Ltd. *See, e.g.*, IPR2018-00291, Petition at 77 (Dec. 8, 2017) (Illumina Inc. representing date on which its preliminary response is due in IPR2017-02172, a proceeding only involving Illumina Cambridge Ltd.); Ex. 2026 at 61 (Illumina Cambridge Ltd. representing that it is the Petitioner in a number of proceedings only involving Illumina Inc.).

2099 at 2:34-36; Ex. 2013 at 3 (noting need for new methods); Ex. 2100 at 1:56-61 (noting problems with SBS); Ex. 2116 ¶17. 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; Ex. 2116 ¶17.

Metzker in 1994 reported success in incorporating an unlabeled 3’-O-(2-nitrobenzyl) nucleotide 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; Ex. 2116 ¶18. While the 3’-O-(2-nitrobenzyl) nucleotide result was promising, further testing revealed that polymerases were incapable of incorporating it after a label was added, as required for SBS. Ex. 1023 at 951-52, 954-56; Ex. 2116 ¶18. This led to the conclusion 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; Ex. 2116 ¶18. As of the Priority Date, the modification of polymerases to better incorporate modified nucleotides was theoretical, and

Illumina has not argued otherwise. Ex. 2116 ¶18; Ex. 2035 at 63-65 (Dr. Romesberg testifying that even in 2002, “people did not believe that you could”); *see also* Ex. 2126 at 252-53.

IV. Claim Construction

Illumina asserts claim term construction is unnecessary. Columbia disagrees. To properly evaluate the differences between the challenged claim⁷ and the cited art, the following claim terms need to be construed.

A. “Small”

The term “small” refers to the ability of the capping group to fit into the active site of the polymerase whose three-dimensional structure is shown in Figure 1 of the patent-at-issue. More specifically, “small” means the group has a diameter less than 3.7Å. This construction is based on the specification of the patent-at-issue. Exs. 1001-1004, each at 2:63-3:54, 5:52-59, Fig. 1, 7:51-8:28. As explained during prosecution of the challenged claim, “[a]s of October 6, 2000, the POSA reading the specification would have understood that ‘small’ referred to the ability to fit into the active site of the polymerase defined by reference to the three-

⁷ Unless otherwise indicated, the “challenged claim” refers to the claim at issue in each of IPR2018-00291, -00318, -00322, and 00385. Similarly, the “patent-at-issue” refers to the patent at issue in those proceedings.

dimensional structure shown in FIG. 1”, and capping group R must have “a diameter less than 3.7Å so that it would fit into the active site of the polymerase.” Exs. 1009, 1062, 1065, each at 16, 17, 27-29; Ex. 1068 at 12, 13, 23-25.⁸ The Examiner adopted this meaning in the Notice of Allowability. Ex. 1009 at 4, 12 (“The declaration of Jingyue Ju . . . is sufficient to explain what is meant by ‘small.’”); Ex. 1062 at 4, 12 (same); Ex. 1065 at 4, 11 (same); Ex. 1068 at 7 (“The declaration of Jingyue Ju . . . explaining what is meant by ‘small’ . . . [is] persuasive.”).

B. “Chemical Linker”

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). Ex. 2116 ¶20. It does not mean merely a covalent bond between the base and the label as disclosed in Dower. *Id.* The specification of the patent-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

⁸ 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.

during prosecution of the challenged claim. Exs. 1009, 1062, 1065, each at 18-19, 30; Ex. 1068 at 14-15, 26; Ex. 2116 ¶20. 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.

During prosecution, Columbia also 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; Ex. 2116 ¶21. The linkers in those references are chemical moieties. See Ex. 1013 at 28:23-29:2; Ex. 2116 ¶21. The Examiner found Columbia’s definition “persuasive.” Ex. 1009 at 4, 12; Ex. 1062 at 4, 12; Ex. 1065 at 4, 11; Ex. 1068 at 7; Ex. 2116 ¶21.

V. Illumina’s Ground 1 Challenge For Obviousness Over Tsien In View Of Prober Fails⁹

Illumina’s Ground 1 challenge 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 challenge fails because:

⁹ In IPR2018-00318 and -00322, Illumina’s Ground 1 challenge is based solely on Tsien.

(A) Illumina's references provided 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 provided 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;

(C) A POSA weighing the allyl capping group's numerous disadvantages 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) Application of the lead compound analysis demonstrates that 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

The Petition argues that a POSA would have selected the allyl capping group from Tsien based on the disclosure of Metzker (Ex. 1016). *E.g.*, IPR2018-00291, Petition at 22-23 (December 8, 2017). In its Institution Decision, the Board encouraged the parties to address whether Metzker would have discouraged a POSA from using the allyl capping group. *E.g.*, IPR2018-00291, Paper 16 at 20 (June 25, 2018). As explained below, Metzker would have done so.

Prior to the Priority Date, Metzker was the only prior art that provided any experimental data using a 3'-O-allyl nucleotide. Ex. 2116 ¶25. A POSA understood from Metzker that the allyl capping group did not meet the stringent requirements set forth in the prior art for performing SBS, one of which was that nucleotides had to be *efficiently incorporated* into the growing DNA strand. See Section III(B); Ex. 2116 ¶25. As explained below, a POSA would have been discouraged from using the allyl capping group because Metzker reported that the 3'-O-allyl nucleotide was at best *inefficiently incorporated*. Ex. 2116 ¶25.

1. Overview Of Metzker

Like others, Metzker was interested in discovering nucleotides that could take SBS from speculation to reality. Ex. 2116 ¶26. In doing so, he first conducted “enzymatic screen[s]” to examine whether any of the commercially

available polymerases tested were capable of efficiently incorporating capped, unlabeled nucleotides. Ex. 1016 at 4259, 4262-63; Ex. 2116 ¶26. Nucleotides that were efficiently incorporated were tested in a second stage for efficient incorporation after a label was added to the nucleotide. See Ex. 1016 at 4267; Ex. 2116 ¶26.

Metzker's results from the first stage are summarized in Table 2:

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

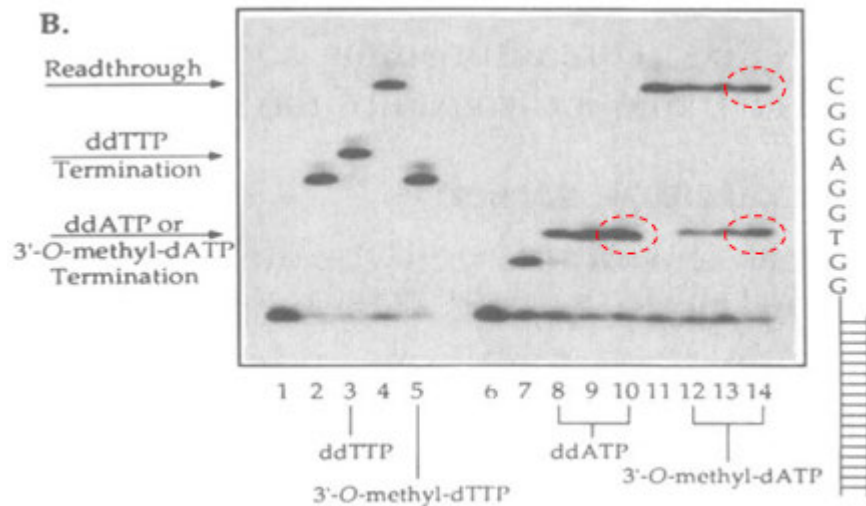
3'-modified-dATP (except compound [8])	AMV-RT	M-MuLV-RT	Klenow fragment	Sequenase®	Bst DNA polymerase	AmpliTag® DNA polymerase	Vent®(exo) DNA polymerase	rTth DNA polymerase
[1] O-methyl	Termination	Termination*	-	-	-	-	Inhibition	Inhibition*
[2] O-acyl	-	-	-	-	-	-	Inhibition	-
[3] O-allyl	-	-	-	-	-	-	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 results using five different terms: – (meaning no activity), Inhibition, Inhibition*, Termination, and Termination*. For purposes of these proceedings, Columbia focuses on the last two terms.

“Termination” indicates efficient incorporation of the 3'-O-capped nucleotide. Ex. 2116 ¶28. As Metzker explains, “Termination” means that “the

termination bands mimic ddNTP termination bands[.]” Ex. 1016 at 4263; Ex. 2116 ¶28. For example, Figure 3(B) of Metzker reports the 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.* (legend of Figure 3(B)); Ex. 2116 ¶28. The termination band in Lane 14 (*i.e.*, across the “T” template base) mimics the termination band in Lane 10 except for the presence of a “readthrough” band in Lane 14.¹⁰ Ex. 2116 ¶28. Metzker reports that the readthrough band was

¹⁰ Readthrough occurs when the solution also contains “trace” amounts of the natural nucleotide, which is preferentially incorporated instead of the 3’-O-capped

the result of “trace” amounts of contaminating dATP in Lane 14. Ex. 1016 at 4263; Ex. 2116 ¶28. 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. Ex. 2116 ¶28. 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; Ex. 2116 ¶28.

Metzker’s assay was designed to permit conclusions regarding a polymerase’s ability to efficiently incorporate the 3’-O-capped nucleotides. Ex. 2116 ¶29. 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; Ex. 2116 ¶29. 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. Ex. 2116 ¶29. The experiment assessed whether the polymerase could incorporate the 3’-O-methyl-dATP whenever it encountered the thymine in the template sequence. *Id.*

nucleotide, resulting in the DNA strand being extended to the end of the template. Ex. 2116 at ¶28.

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). Ex. 2116 ¶30. Lane 14 of Figure 3(B) shows this result. Ex. 1016 at 4264 (see single termination band next to "T"); Ex. 2116 ¶30. Dr. Menchen created an illustration (Figure 1) excising Lanes 10 (*i.e.*, the ddATP control) and 14 (*i.e.*, the 3'-O-methyl-dATP) from Metzker's Figure 3(B), which places them side-by-side to further make evident the single termination band representing efficient incorporation:

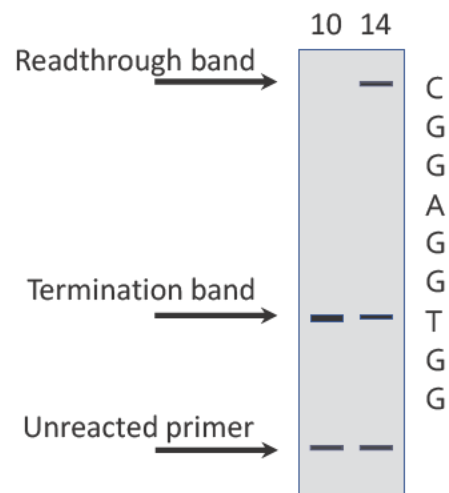


Figure 1

Ex. 2116 ¶30.¹¹ The figure illustrates that the 3'-O-methyl-dATP (lane 14) mimics the termination of the ddATP control (lane 10). *Id.*

If a polymerase *inefficiently* incorporated the 3'-O-capped-dATP, the 3'-O-capped-dATP would only be incorporated into some of the growing DNA strands within the allotted time of the reaction. *Id.* ¶31. Metzker reports this result as “Termination*,” wherein “*” means the activity was “incomplete at a final concentration of 250 μM.” Ex. 1016 at 4263; Ex. 2116 ¶31. Metzker does not show gel images for the polymerase/nucleotide combinations that resulted in “Termination*,” but for explanatory purposes, Dr. Menchen created an illustration (Figure 2) demonstrating the gel image that would exist in the “Termination*” experiments:

¹¹ As Dr. Menchen explains, he purposefully illustrated that the signal intensity of the termination band in lane 14 is less than that of lane 10, which is explained in more detail below. Ex. 2116 at ¶30.

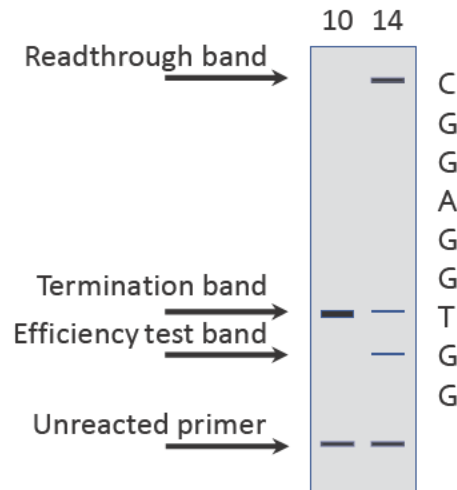


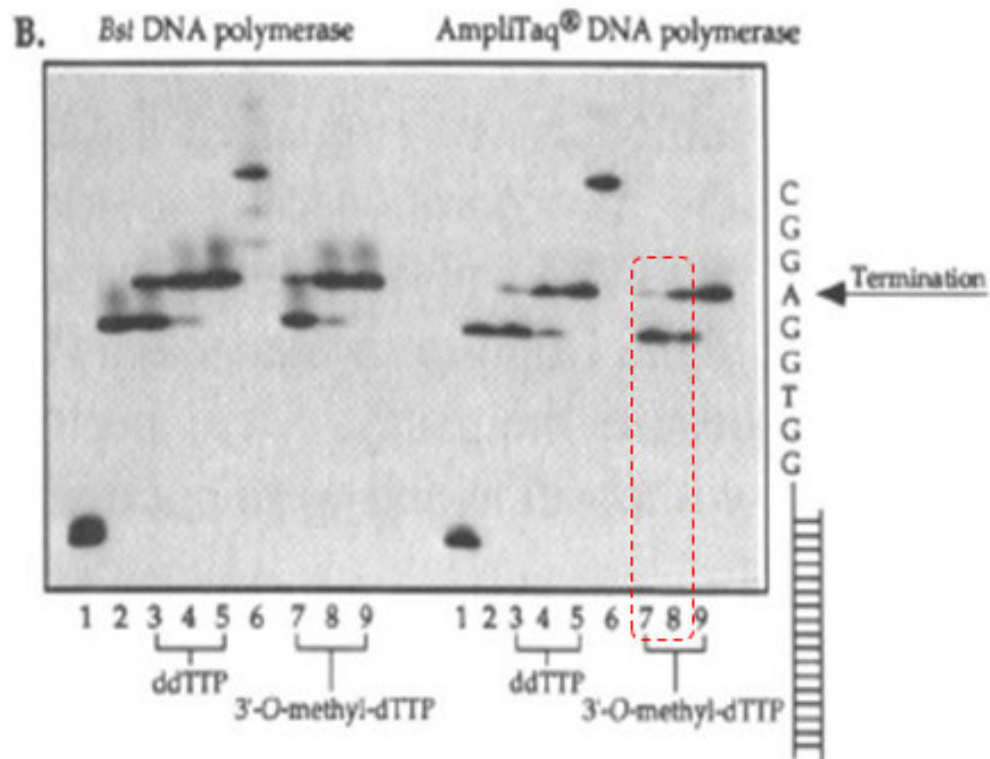
Figure 2

Ex. 2116 ¶31. Figure 2 illustrates that the 3'-O-capped-dATP (lane 14) showed only incomplete termination because a new band appeared, which Dr. Menchen calls the “efficiency test band.”¹² *Id.* ¶31. The termination band at the thymine position consists of DNA strands terminated upon incorporation of the 3'-O-capped-dATP. *Id.* The efficiency test band at the guanine position consists of DNA strands that incorporated natural dCTP (complementary to G in the template) but failed to incorporate the 3'-O-capped-dATP. *Id.* The presence of the efficiency test band indicates the inability of the polymerase to complete

¹² While Metzker does not use the term “efficiency test band,” his experiments show this band, as discussed below, and Dr. Menchen named it solely for explanatory purposes. Ex. 2116 at ¶31.

incorporation of the 3'-O-capped-dATP into the DNA strands during the reaction, *i.e.*, there was inefficient incorporation. *Id.*

The prior art shows examples of the efficiency test band. Ex. 2116 ¶32. 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[.]”); Ex. 2116 ¶32.

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

Metzker tested whether the 3'-O-allyl-dATP could be incorporated by any of eight polymerases. Ex. 1016 at 4263; Ex. 2116 ¶33. For seven polymerases, Metzker reported “no activity[.]” *Id.* It is undisputed that a POSA would not have been motivated to use a 3'-O-allyl nucleotide with these seven polymerases. Ex. 2126 at 115-16; Ex. 2116 ¶33. For the Vent(exo-) polymerase, Metzker reported “Termination*[.]” Ex. 1016 at 4263. Drs. Romesberg and Menchen disagree as to what a POSA understood from this result. Ex. 2116 ¶33. As set forth below, and explained by Dr. Menchen, a POSA understood Termination* to mean that the 3'-O-allyl nucleotide was *inefficiently* incorporated by the Vent(exo-) polymerase and therefore not suitable for SBS. Ex. 2116 ¶33.

First, Dr. Menchen's position is consistent with the Metzker publication and Metzker's own conclusions. Ex. 2116 ¶34. Metzker reports that his use of “*” means the activity was “incomplete at a final concentration of 250 μ M[.]” which was the highest concentration tested. Ex. 1016 at 4263; Ex. 2116 ¶34. As explained in Section V(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. Ex. 2116 ¶34.

Second, Dr. Menchen’s position aligns with Metzker’s own statements regarding his 1994 publication. Ex. 2116 ¶35. 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 Metzker 1994). 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 Metzker 1994). These statements mirror Dr. Menchen’s explanation of “Termination*[.]” Ex. 2116 ¶35.

While these Metzker statements came after the Priority Date, they are nevertheless probative. The Board can rely on non-prior art evidence for its “proper supporting roles, *e.g.*, indicating the level of ordinary skill in the art, what certain terms would mean to one with ordinary skill in the art, and how one with ordinary skill in the art would have understood a prior art disclosure.” *Yeda Research & Dev. Co. v. Mylan Pharms., Inc.*, 2018 U.S. App. LEXIS 28750, *16-18 (Oct. 12, 2018) (internal quotations omitted). Here, Metzker’s 2007 and 2011 statements confirm what Termination* meant and how a POSA would have understood Metzker’s experiments that reported “Termination*” for the allyl capping group.

Third, Dr. Menchen's position comports with the fact Metzker abandoned the allyl capping group after conducting the 1994 experiments. Ex. 2116 ¶36; 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; Ex. 2116 ¶36. He similarly characterized these compounds as "interesting DNA synthesis terminators." Ex. 1016 at 4265; Ex. 2116 ¶36. Not included is the 3'-O-allyl, compound [3]. Ex. 2116 ¶36. A POSA would have understood that the 3'-O-allyl-dATP was excluded from further analysis because it was not suitable for SBS. *E.g.*, Ex. 2126 at 104-105, 130-133; Ex. 2116 ¶36.

Fourth, Dr. Menchen's position aligns with the fact that in the six years between Metzker's publication and the patent-at-issue's Priority Date, there were no reports of any group developing allyl-capped nucleotides for SBS. Ex. 2116 ¶37.

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. This argument fails for numerous reasons. Ex. 2116 ¶38.

First, although Metzker reports that the experiments “showed approximately 1% dATP contamination,” which Metzker characterizes as “trace levels,” the article provides no suggestion that the trace contamination affected his results. Ex. 1016 at 4263; Ex. 2116 ¶39.

Second, Dr. Romesberg’s theory is ***directly at odds*** with Metzker’s 2007 and 2011 statements, reiterating 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; Ex. 2116 ¶40. Dr. Romesberg acknowledged that his theory is 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.

Dr. Romesberg claimed that a POSA would be “confused by” Metzker’s 2007 and 2011 statements (Ex. 2126 at 146), and he presented several far-reaching explanations for them. Ex. 2116 ¶41. 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 my opinion, founded.” Ex. 2126 at 145; Ex. 2116 ¶41. Dr. Romesberg’s explanations are unsupported, and as Dr. Menchen explains, a POSA would not have understood Metzker’s work as a “mistake,” but a rather careful and detailed analysis of the characteristics of 3’-O-

capped nucleotides. Ex. 2116 ¶41. As explained above, there is no evidence that the trace levels of contamination in Metzker's 1994 experiment were a "mistake" that affected his ability to interpret his experiments. Ex. 2116 ¶41. 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 particularly because the statements were made in peer-reviewed journals. Ex. 2116 ¶41.

Another unsupported 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; Ex. 2116 ¶42. 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." *Id.* at 148. Speculative denigration of the authors of peer-reviewed literature is an improper ground for invalidating issued patents.

Third, a POSA would not have expected the trace contamination to affect Metzker's results. Ex. 2116 ¶43. 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; Ex. 2116 ¶43. As explained in Section V(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 efficiency test band and the termination band). Ex. 2116 ¶43.

More specifically, as Dr. Menchen explains, a POSA understood that the nucleotides Metzker reported as Termination* showed polymerase binding but slow incorporation. Ex. 2116 ¶44 (citing Ex. 2059 at 44-45). This meant that in some instances the polymerase was able to bind and incorporate the 3'-O-capped nucleotide (resulting in a termination band), 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). Ex. 2116 ¶44. 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. *Id.* Instead, it affects the **intensity** of those bands and results in a readthrough band. *Id.* In other words, the presence of

trace levels of natural nucleotides would not affect Metzker's ability to accurately report that a nucleotide was efficiently incorporated (*i.e.*, Termination) or inefficiently incorporated (*i.e.*, Termination*). *Id.*

Fourth, the results provided in Metzker are inconsistent with Dr. Romesberg's theory. Ex. 2116 ¶45. As explained in Section V(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; Ex. 2116 ¶45. 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. Ex. 2116 ¶45. This supports the conclusion that when Metzker reports inefficient incorporation, it is not due to dATP contamination. *Id.*

Fifth, Dr. Romesberg's theory that the poor results reported for the 3'-O-allyl nucleotide in Metzker stemmed from contaminating dATP belies 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. Ex. 2116 ¶46.

Sixth, other researchers frequently cited Metzker as evidence that modified nucleotides are ***inefficiently*** incorporated by polymerase. Ex. 2116 ¶47; Ex. 2054

at 1; Ex. 2100 at 1:40-61; Ex. 2099 at 2:21-36. 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. Ex. 2116 ¶47.

Seventh, Dr. Romesberg's position that Metzker taught in 1994 that the 3'-O-allyl-dATP was efficiently incorporated contradicts his own previous statements. Ex. 2116 ¶48. In a grant proposal from 2005, Dr. Romesberg claimed that 3'-O-allyl nucleotides are "**very inefficient substrates** for DNA polymerases[.]" Ex. 2118 at 29. As a result of this claim, Dr. Romesberg obtained an NIH grant to evolve 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; Ex. 2116 ¶48.

Notably, in that same grant proposal, Dr. Romesberg admitted that Dr. Ju "**pioneered the development of novel dNTPs,**" specifically, "the Ju group has developed 3'-O-allyl protecting groups that may be catalytically removed with palladium." Ex. 2118 at 29. Despite making these admissions in 2005, Dr. Romesberg now claims that Dr. Ju's nucleotides would have been obvious to a POSA.¹³

¹³ Illumina, including Dr. Romesberg, had an obligation to disclose any information in its/his possession that is inconsistent with a position advanced in

And eighth, Illumina's reliance on the patent-at-issue's citation to Metzker is misplaced. See IPR2018-00291, Petition at 24 (Dec. 8, 2017). The patent acknowledges that Metzker was the first to evaluate the allyl capping group in a DNA sequencing context, and that the 3'-O-allyl-dATP was "shown to be incorporated" by Vent(exo-) polymerase. Exs. 1001-1004, each at 3:26-30. But the pertinent question is not whether the 3'-O-allyl-dATP was incorporated, it is whether its incorporation was *efficient*, as explicitly required by Tsien and Dower. It was not. Surprisingly, it was not until six years later that Dr. Ju appreciated that the allyl capping group could be used successfully for SBS contrary to Metzker's results, and he was the first to report so in the six years following Metzker's publication.

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

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-)). Ex. 2116 ¶49. A POSA would not have been motivated

this proceeding (37 C.F.R. §42.51(b)(1)(iii)). Dr. Romesberg failed to comply with this duty.

to select the allyl capping group because it did not meet the *efficient incorporation* requirements of Tsien and Dower. See Section III(B); Ex. 2116 ¶49.

A number of other factors about Metzker's experiments would have further confirmed the POSA's lack of motivation. Ex. 2116 ¶50. First, whereas Metzker examined 3'-O-capped nucleotides at 250 μM (Ex. 1016 at 4263), Tsien's methods use concentrations of 1.5-30 μM .¹⁴ Ex. 1013 at 38; Ex. 2116 ¶50. It was known that decreasing the concentration of the 3'-O-capped nucleotides would necessarily decrease their efficiency of incorporation. Ex. 2116 ¶50. Dr. Romesberg agrees. Ex. 2126 at 118:19-24 ("[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 μM), the 3'-O-allyl nucleotide would be incorporated with *even lower efficiency* than reported in Metzker. Ex. 2116 ¶50.

Second, SBS requires modified nucleotides that have both a label and a capping group, whereas the nucleotides tested in Metzker only had a capping group. Ex. 2116 ¶51. Even without a label, the 3'-O-allyl nucleotide in Metzker

¹⁴ 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; Ex. 2116 at ¶50.

was only *inefficiently* incorporated by a single polymerase, Vent(exo-) polymerase. See Section V(A)(2); Ex. 2116 ¶51. A POSA expected that adding a label to the 3'-O-allyl nucleotide would further reduce the polymerase's ability to incorporate it. Ex. 2113 at 102-103; Ex. 2116 ¶51. Indeed, the prior art taught that the Vent(exo-) polymerase inefficiently incorporated labeled nucleotides. 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); Ex. 2116 ¶51.

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

In its Institution Decision, the Board encouraged the parties to address whether the prior art suggested that the allyl capping group could be cleaved under SBS-compatible conditions. *E.g.*, IPR2018-00291, Paper 16 at 14 (June 25, 2018). As explained below, the prior art set forth stringent requirements for performing SBS. One was the *quantitative, rapid* cleavage of the capping group on the nucleotide under *mild, aqueous* conditions. Ex. 2116 ¶52; see Section III(B). 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. Ex. 2116 ¶53.

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. Ex. 2116 ¶53. Instead, Illumina cites references that disclose allyl cleavage reactions that are incompatible with SBS. *Id.* Each of these references is discussed below. It is Illumina's burden to demonstrate that a POSA would have been motivated to select the allyl capping group because it would satisfy the prior art requirement for quantitative, rapid cleavage under mild, aqueous conditions. Illumina has not met its burden. Illumina's failure to identify references teaching SBS-compatible cleavage conditions is especially telling because Illumina previously admitted that *even in 2002* a POSA would not have selected an ether capping group (*e.g.*, the allyl capping group) for use in SBS due to a lack of SBS-compatible cleavage conditions. Ex. 2008 at 23.

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

Tsien states that “[a]llyl ethers are cleaved by treatment with Hg(II) in acetone/water (Gigg and Warren, 1968).” Ex. 1013 at 24.¹⁵ A POSA would have

¹⁵ As explained below in Section V(F), a POSA would not have understood Tsien to disclose the allyl capping group.

understood Tsien's conditions to be incompatible with SBS for several reasons: (i) these conditions are not aqueous; (ii) the use of potassium t-butoxide renders the conditions not mild; and (iii) the use of mercury renders the conditions not mild. Ex. 2116 ¶54. Dr. Romesberg agrees that a POSA would not use Tsien's conditions because there would be no expectation that they could be compatible with SBS. Ex. 2126 at 232.

First, Tsien's conditions are not aqueous. Ex. 2116 ¶55. 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; Ex. 2126 at 230-31 (Dr. Romesberg agreeing); Ex. 2116 ¶55. A POSA understood that potassium t-butoxide in DMSO is incompatible with aqueous conditions and therefore not suitable for SBS. Ex. 2116 ¶55.

Second, potassium t-butoxide is a strong organic base (Ex. 2112 at 914) that would be expected to denature DNA. Ex. 2116 ¶56. These conditions are not mild and therefore are incompatible with SBS. *Id.*

Third, Tsien's conditions are not mild because they use Hg(II), which was a known DNA denaturant. *See* Ex. 2086 at 59; Ex. 2126 at 230 (Dr. Romesberg agreeing that Hg(II) denatures DNA); Ex. 2116 ¶57. 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."); Ex. 2007

¶55 (Dr. Romesberg admitting that Tsien requires cleavage conditions that do not denature DNA); Ex. 2116 ¶57.

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, *e.g.*, Kamal, Boss, and Qian 1998, each of which is discussed below. Ex. 2116 ¶58.

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

Kamal's conditions require acetonitrile, NaI, and chlorotrimethylsilane. Ex. 1037 at 371; Ex. 2116 ¶59. Kamal's conditions are incompatible with SBS because, at least, they (i) are not aqueous, (ii) are not mild, and (iii) do not result in quantitative cleavage of the allyl group. Ex. 2116 ¶59.

a. Kamal's Conditions Are Not Aqueous

Kamal's conditions are incompatible with SBS because they are not aqueous. Ex. 2116 ¶60. Dr. Romesberg agrees. Ex. 2126 at 233-235. More specifically, the non-aqueous conditions in Kamal use acetonitrile (an organic solvent) in the absence of water. Ex. 1037 at 371; Ex. 2116 ¶60. It is undisputed that Kamal's conditions could not be modified to be aqueous, at least because they 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); Ex. 2116 ¶61.

Therefore, regardless of whether Kamal taught quantitative, rapid cleavage of the allyl capping group under mild conditions (which it does not), a POSA understood that Kamal's conditions are incompatible with SBS because they are not aqueous and cannot be conducted in aqueous solution. Ex. 2116 ¶62.

b. Kamal's Conditions Are Not Mild

Kamal's conditions are also incompatible with SBS because they are not mild. Ex. 2116 ¶63. The presence of acetonitrile without water (Ex. 1037 at 371) would denature DNA and, thus, could not provide mild conditions. Ex. 2085 at 171 (Table II); Ex. 2116 ¶63; 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

Kamal's conditions also fail to result in quantitative cleavage. Ex. 2116 ¶64. Kamal reports cleavage yields for nine different compounds, none of which are nucleotides. *Id.* A POSA would have known that compound [8] was the most relevant compound because only it contained a ribose sugar. *Id.* Compound [8] was cleaved with 93% yield. Ex. 1037 at 372; Ex. 2116 ¶64.

There is no dispute that Kamal's 93% cleavage efficiency is incompatible with prior art SBS methods. Dr. Romesberg testified that Tsien's SBS method requires cleavage efficiency greater than 95%. Ex. 2126 at 70; Ex. 2116 ¶65. And Illumina previously admitted Tsien requires greater than 97% cleavage efficiency.

Ex. 2030 at 1, 3; Ex. 2029 at 28; *see also* Ex. 1013 at 16 (Tsien disparaging 98% cleavage efficiency to practice his method). A POSA understood that Kamal's conditions are incompatible with SBS because they do not result in quantitative cleavage. Ex. 2116 ¶65.

Illumina claims that Columbia admitted in the patent-at-issue's specification that Kamal teaches that the allyl capping group "can be cleaved chemically with high yield." *E.g.*, IPR2018-00291, Petition at 23 (Dec. 8, 2017). But there is nothing in the specification suggesting that Kamal's yield is "quantitative" as required by Tsien. Quite the opposite, the specification discloses Kamal's yield as 93%, which as explained above, is not quantitative. Exs. 1001-1004, each at 26:30-33.¹⁶

**d. Illumina's Own Patents Disparage
 Kamal's Conditions**

In the present proceedings, Illumina relies on Kamal to argue that SBS-compatible cleavage conditions for the allyl capping group were known by the Priority Date in **October 2000**, despite having disparaged those same conditions in its own patents with a **2002** priority date:

¹⁶ Even if Kamal's yield could satisfy Tsien's cleavage efficiency requirement, Kamal's conditions are incompatible with SBS because, as explained above, they are not aqueous or mild.

The Kamal methodology is inappropriate to conduct in aqueous media since the TMS chloride will hydrolyse preventing the in situ generation of TMS iodide. Attempts to carry out the Kamal deprotection (in acetonitrile) in sequencing have proven unsuccessful in our hands.

Ex. 2015 at 2:66-3:3, *see also* 2:35-3:3. In fact, in that patent, Illumina claimed that conditions for successful cleavage of the allyl capping group under DNA compatible conditions had not been reported ***before 2002***. Ex. 2015 at 2:60-65. Illumina cannot now credibly take the opposite position in the present proceedings.

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

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; Ex. 2116 ¶66. Boss reports that allyl cleavage yields 95% or greater required reaction times of 6-24 hours. *Id.*; Ex. 2116 ¶66.

Boss's conditions are (i) are not mild and (ii) do not result in rapid cleavage. Ex. 2116 ¶66.

a. Boss's Conditions Are Not Mild

Boss's conditions are incompatible with SBS because they are not mild. Ex. 2116 ¶67. Dr. Romesberg agrees. Ex. 2126 at 200. First, the concentration of

perchloric acid used in Boss will denature DNA. Ex. 2116 ¶67. It was well known that perchloric acid is a powerful DNA denaturing agent. Ex. 2086 at 57; Ex. 2116 ¶67. The pH of Boss's relevant reactions¹⁷ is between 0.4 and 1.1, which would denature DNA because "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; Ex. 2116 ¶67 (calculating pH); Ex. 2126 at 189-90 (Dr. Romesberg agreeing).

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 incompatible with SBS. Ex. 2116 ¶68. The methanol concentration in those reactions is 20.8 M, an amount that would negatively impair the polymerase. Ex. 2126 at 168, 181 (Dr. Romesberg agreeing); Ex. 2116 ¶68 (calculating methanol concentration); Ex. 2007 ¶55. Therefore, Boss's conditions would be incompatible with SBS. Ex. 2116 ¶68.

The methanol concentration would also denature the DNA and therefore would not be compatible with SBS. Ex. 2116 ¶69. 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); Ex. 2116 ¶69. Compared to Levine, the methanol in

¹⁷ Dr. Romesberg only relies on compounds 4-7 (Ex. 2126 at 86-87) because the cleavage efficiencies of compounds 8-10 are too low for SBS. Ex. 2126 at 86-87.

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). Ex. 2116 ¶69. A POSA would therefore expect the methanol in Boss to denature DNA similar to the denaturation reported in Levine. *Id.* 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. 2116 ¶69; Ex. 2126 at 53-54, 170 (Dr. Romesberg agreeing); Ex. 2035 at 177-178.

b. Boss's Cleavage Is Not Rapid

Boss's conditions are also incompatible with SBS because they do not result in rapid cleavage of the allyl capping group. Ex. 2116 ¶70. The reactions relied on by Dr. Romesberg (compounds 4-7 (Ex. 2126 at 86-87)) require between 6 and 24 hours. Ex. 1035 at 559; Ex. 2116 ¶70. Drs. Romesberg and Menchen agree that a POSA understood that cleavage reactions taking two hours or longer are incompatible with SBS. Ex. 2007 ¶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); Ex. 2116 ¶70.

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

Qian 1998 uses PdCl_2 in methanol to cleave the allyl group. Ex. 1036 at 2185; Ex. 2116 ¶71. 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. Ex. 2116 ¶71. 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

The first reason why Qian 1998's conditions are incompatible with SBS is because they are not aqueous. Ex. 2126 at 158 (Dr. Romesberg agreeing that Qian's conditions are not aqueous); Ex. 2116 ¶72. More specifically, the conditions in Qian 1998 involve methanol (an organic solvent) in the absence of water. Ex. 1036 at 2185; Ex. 2116 ¶72.

Dr. Romesberg speculated (at his deposition, not in his Declaration) that a POSA would modify Qian 1998's conditions to include water, with the expectation that the same deprotection reaction and cleavage yield would be obtained. Ex. 2126 at 158, 162-164. This is wrong for several reasons. Ex. 2116 ¶73.

First, Dr. Romesberg has cited no examples in the prior art disclosing the use of PdCl_2 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. Ex. 2116 ¶74.

Second, PdCl_2 is not soluble in water. Ex. 2116 ¶75; *see also* Ex. 2126 at 163 (Dr. Romesberg not disagreeing). Qian's conditions therefore cannot be rendered aqueous by simply adding water. Ex. 2116 ¶75. Dr. Romesberg speculates that a POSA would add a solubilizing agent to make the PdCl_2 soluble in water. Ex. 2126 at 162-163; Ex. 2116 ¶75. A POSA would have had no reason to believe that altering Qian's reaction conditions by adding both water and a solubilizing agent would not affect allyl cleavage efficiency, that is, there would be no expectation that those different conditions would result in quantitative cleavage. Ex. 2116 ¶75. Dr. Romesberg's speculation that the altered conditions would yield quantitative cleavage is contradicted by his agreement that small changes to reaction conditions can affect cleavage efficiency. Ex. 2113 at 72 (“[s]mall differences can have impact”); Ex. 2116 ¶75.

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. Ex. 2116 ¶76. A POSA reading Qian 1998, which used a carbohydrate-allyl-ether and PdCl_2 in **methanol**, **would expect** that deprotection of the allyl protecting group would go according to Bieg (Ex. 2105), *i.e.*, PdCl_2 -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. Ex. 2116 ¶76. However, a POSA **would not expect** to produce prop-1-enyl with PdCl₂ **in an aqueous solution (i.e., in water)**. Instead, a POSA understood that transformation of an olefin using PdCl₂ in water would proceed according to the Wacker process (Ex. 2116 ¶76 (citing Ex. 2115 at 102-104)), resulting in the production of oxidized ether products rather than the isomerized prop-1-enyl product. Ex. 2116 ¶76. 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; Ex. 2116 ¶76. A POSA reading Bieg, Kang and Qian 1998 would expect that Qian's PdCl₂ 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. Ex. 2116 ¶76.

b. Qian 1998's Conditions Are Not Mild

Qian 1998's conditions are also incompatible with SBS because they are not mild. Ex. 2116 ¶77. A POSA would have known that the methanol (without water) used in Qian 1998 would denature DNA. Ex. 2126 at 158 (Dr. Romesberg agreeing that methanol without water is a DNA denaturant); Ex. 2116 ¶77.

c. Qian 1998's Cleavage Is Not Rapid

Qian 1998's conditions are also incompatible with SBS because they do not result in rapid cleavage. Ex. 2116 ¶79. The reaction in Qian 1998 required two hours. Ex. 1036 at 2185. A POSA understood that cleavage reactions taking two hours are incompatible with SBS. Ex. 2007 ¶47; Ex. 2035 at 98 (agreeing that SBS requires cleaving the capping group in no longer than an hour); Ex. 2116 ¶79.

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 PdCl₂ and methanol resulted in 83% cleavage efficiency after four hours. Ex. 2097 at 12065 (reaction (e)); Ex. 2116 ¶80. A POSA reading Qian 1998 and Qian 1999 understood that Qian's allyl deprotection reaction was for two hours (Qian 1998) or four hours (Qian 1999), each of which a POSA understood is too long to be compatible with SBS. Ex. 2116 ¶80.

d. Qian's Cleavage Is Not Reliably Quantitative

Qian's conditions are also incompatible with SBS because they do not reliably result in quantitative cleavage. Ex. 2116 ¶81. As explained above, Qian 1999 reported cleaving the allyl capping group with 83% cleavage efficiency. Ex. 2097 at 12065 (reaction (e)); Ex. 2116 ¶81. 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; Ex. 2030 at 1, 3; Ex. 2029 at 28; Ex. 2116 ¶81.

Therefore, a POSA understood from Qian 1998 and Qian 1999 that Qian's allyl deprotection conditions do not reliably result in quantitative cleavage. Ex. 2116 ¶81.

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

The Board in its Institution Decision 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 because, based on actual data in the prior art, it was understood to be incompatible with SBS. Ex. 2116 ¶82.

As detailed above, a POSA understood that the allyl capping group had disadvantages including that (i) Metzker's actual data showed that the 3'-O-allyl nucleotide was at best *inefficiently incorporated*, and (ii) Illumina's cited references fail to teach conditions for *quantitatively* and *rapidly* cleaving it under *aqueous* and *mild* conditions. Ex. 2116 ¶83. A POSA would have weighed these actual disadvantages against any potential benefits. *Id.* Illumina offers as an alleged benefit of the allyl capping group Tsien's general statement that “deblocking occurs only when the specific deblocking reagent is present and

premature deblocking during incorporation is minimized.” *E.g.*, IPR2018-00291, Petition at 21 (Dec. 8, 2017).

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. Ex. 2116 ¶84. Metzker and the allyl-cleavage references are peer-reviewed papers that provide actual experimental data. *Id.* Tsien’s patent application does not contain any experimental data. *Id.*

Second, a POSA weighing the known disadvantages of the allyl capping group against its one hypothetical benefit would have no motivation to make a 3’-O-allyl nucleotide. Ex. 2116 ¶85. A POSA understood that the disadvantages rendered the allyl capping group incompatible with SBS, making any theoretical benefit moot. *Id.* That is, a POSA would believe that using the allyl capping group for Tsien’s or Dower’s SBS methods would produce an inoperable result. *Id.*; *In re ICON Health & Fitness, Inc.*, 496 F.3d 1374, 1382 (Fed. Cir. 2007) (“[A] reference teaches away from a combination when using it in that combination would produce an inoperative result.”). The single alleged hypothetical benefit (Tsien’s minimization of premature deblocking), if it existed at all for the allyl capping group, would not change this conclusion. Ex. 2116 ¶85. Moreover, Tsien taught that a “wide variety” of capping groups shared the same alleged benefit of minimizing premature deblocking (Ex. 1013 at 24-25), including but not limited to

“[t]etrahydrothiofuranyl ethers” and “2,4-[d]initrobenzenesulphenyl groups[.]” Ex. 1013 at 24-25; Ex. 2116 ¶85. If a POSA were motivated by such a benefit, the POSA would pursue other capping groups disclosed in Tsien that were not understood (based on experimental data) to be incompatible with SBS. Ex. 2116 ¶85.

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

Contrary to Illumina’s assertions (*e.g.*, IPR2018-00291, Petition at 11-12 (Dec. 8, 2017)), a POSA before the Priority Date would not have been motivated to select a capping group that was “small,” Ex. 2116 ¶86, which in the claim means less than 3.7Å in diameter (*see* Section IV(A)).

First, the prior art reported that a nucleotide with a larger, 2-nitrobenzyl capping group was efficiently incorporated by a polymerase. Ex. 1016 at 4263. The 2-nitrobenzyl capping group is 5.0Å in diameter (not small). Exs. 1009, 1062, 1065, each at 23, 29; Ex. 1068 at 18, 27-28. As such, a POSA had no reason to believe that SBS required capping groups smaller than 3.7Å in diameter.

Second, Illumina’s reliance on Pelletier to argue that a POSA would be motivated to use small capping groups is misplaced. Ex. 2116 ¶87. A POSA would not have reviewed Pelletier because it related to HIV drug development, an entirely different field from SBS. *Id.*; Ex. 1021. Additional evidence distinguishing Pelletier from the relevant field is that there is no evidence of any

researchers developing nucleotides for SBS between 1994 and the Priority Date who cited Pelletier. Ex. 2116 ¶87. Illumina’s reliance on Pelletier represents hindsight, driven by Columbia’s citation to that reference in the specification and prosecution history to explain that a POSA ***reading the specification of the patent-at-issue*** would be able to determine whether a capping group was suitably small. Contrary to Illumina’s assertions, Columbia did not concede that a POSA without the benefit of the patent-at-issue’s specification would have consulted Pelletier. Moreover, “[t]he inventor’s own path itself never leads to a conclusion of obviousness; that is hindsight.” *Otsuka Pharm. Co. v. Sandoz, Inc.*, 678 F.3d 1280, 1296 (Fed. Cir. 2012).

Third, Drs. Romesberg and Menchen agree 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 ¶58; Ex. 2126 at 81-84; Ex. 2116 ¶88.

Fourth, contrary to Illumina’s assertions that “Dower disclosed the desirability of nucleotides having ‘small blocking groups’ on the 3’-OH,” *e.g.*, IPR2018-00291, Petition at 11 (Dec. 8, 2017), Dower’s use of the term “small” to describe several capping groups (Ex. 1015 at 25:48-51) does not support a conclusion that Dower teaches that “small” capping groups are “desirable.” Ex. 2116 ¶89. Dower does not state that the four capping groups it characterizes as

small are desirable because of their size. *Id.* Regardless, Dower’s use of “small” when referring to capping groups does not equate to “small” as defined by the patent-at-issue (*i.e.*, smaller than 3.7Å in diameter). For example, the NBOC capping group referred to as small by Dower contains “nitrobenzyl” (“2-**nitrobenzyloxy**carbonyl”; Ex. 1015 at 18:54), which as explained above, is not less than 3.7Å in diameter. Similarly, the tBOC and NVOC are not smaller than 3.7Å in diameter and Metzker showed that the acetyl was not incorporated at all by any polymerase. Ex. 1016 at 4263.

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

Contrary to Illumina’s assertion, a POSA would not have had a reasonable expectation of success with regard to each of the features in the challenged claim, including “each of the recited functional limitations[.]” *E.g.*, IPR2018-00291, Petition at 38 (Dec. 8, 2017). Illumina alleges that a single claimed species is obvious: a nucleotide with the allyl capping group and a base label. As explained below, a POSA would not have had a reasonable expectation that this nucleotide would achieve the challenged claim’s limitations. Ex. 2116 ¶90.

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

In IPR2018-00318, -00322, and -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 reasonably expected that these nucleotides would be “incorporated” by any polymerase. Ex. 2116 ¶94.

The only data that was available to a POSA by the Priority Date for the incorporation of 3'-O-allyl nucleotides is Metzker, and Metzker *only* tested a 3'-O-allyl *adenine* nucleotide. Ex. 1016 at 4263; Ex. 2116 ¶95. Data on incorporation of *adenine* nucleotides would have provided a POSA no expectations regarding *thymine*, *cytosine*, or *guanine* nucleotides. Ex. 2116 ¶95.

For example, using six polymerases, Metzker tested both a 3'-O-methyl *adenine* and a 3'-O-methyl *thymine* nucleotide. Ex. 1016 at 4263; Ex. 2116 ¶96.

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; Ex. 2116 ¶96. As the chart shows, a polymerase’s ability to incorporate an *adenine* nucleotide (3'-O-methyl-dATP) provided no expectation

that the polymerase would also be capable of incorporating a ***thymine*** nucleotide (3'-O-methyl-dTTP) with the same capping group. Ex. 2116 ¶97. In fact, 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; Ex. 2116 ¶97. Based on Metzker’s results, a POSA would have no reason to expect incorporation of a 3'-O-allyl ***thymine, cytosine, or guanine*** nucleotide based on data showing incorporation of the ***adenine*** nucleotide. Ex. 2116 ¶97.

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

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 of the claim. Ex. 2116 ¶98.

Metzker reported that seven polymerases completely failed to incorporate the 3'-O-allyl nucleotide. Ex. 1016 at 4263; Ex. 2116 ¶99. A POSA understood from these results that the allyl capping group interfered with recognition of the 3'-O-allyl nucleotide by these seven polymerases. Ex. 2116 ¶99. With the Vent(exo-) polymerase, the 3'-O-allyl nucleotide’s termination activity was “incomplete at a final concentration of 250 µM[.]” Ex. 1016 at 4263; Ex. 2116 ¶99. 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 V(A)(2). A POSA understood that inefficient incorporation results from the capping group interfering with recognition of the analogue as a substrate by the polymerase. Ex. 2116 ¶99.

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

Illumina’s Ground 1 challenge also fails because neither Tsien nor Prober discloses the allyl capping group, *i.e.*, a capping group with the structure $\text{-CH}_2\text{CH=CH}_2$. The challenged claim includes limitations to “small” capping group that does not contain a ketone and does not form a methoxy or an ester group with the 3'-oxygen of the sugar of the nucleotide. Exs. 1001-1004, each at claim 1. A claim cannot be rendered obvious by a combination of references none of which discloses or suggests a feature of the claim. See *CFMT, Inc. v. Yieldup Int’l Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003) (“[O]bviousness requires a suggestion of all limitations in a claim.”); *Medtronic, Inc., v. Barry*, IPR2014-01210, Paper 10, 21 (Feb. 10, 2015). Prober does not disclose the allyl capping group, and Illumina relies only on Tsien for this element. Ex. 2116 ¶101.

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. There are two reasons why a POSA would not have understood this statement to disclose the allyl capping group. Ex. 2116 ¶101.

A POSA reading Tsien’s sentence that “[a]llyl ethers are cleaved by treatment with Hg(II) in acetone/water” would have found the statement incongruous. Ex. 2116 ¶102. 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. *Id.* 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; Ex. 2116 ¶102. 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. Ex. 2116 ¶102.

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; Ex. 2116 ¶103. Gigg does provide a protocol for removing allyl ether protecting groups from alcohols. Ex. 2116 ¶103. 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; Ex. 2116 ¶103. 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. Ex. 2116 ¶103. 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; Ex. 2116 ¶103. With this information, the POSA would not have been motivated by Tsien to use allyl ethers as protecting groups. Ex. 2116 ¶103.

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. Ex. 2116 ¶104. Allyl ethers describes a large genus of compounds. *Id.* 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; Ex. 2116 ¶104. 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); Ex. 2116 ¶104.

Tsien does not describe the specific allyl capping group unless a POSA would at once envisage each member of the allyl ethers genus. *In re Petering*, 301 F.2d 676, 681 (C.C.P.A. 1962); *Atofina v. Great Lakes Chem. Corp.*, 441 F.3d 991, 999 (Fed. Cir. 2006). A POSA would not at once envisage each member of the unbounded allyl ethers genus. Ex. 2116 ¶105. Any argument by Illumina that it is appropriate to reduce the size of the genus because $\text{-CH}_2\text{CH=CH}_2$ would have “stood out” is misplaced. *Janssen Pharms., Inc. v. Watson Labs., Inc.*, No. 08-5103-SRC, 2012 U.S. Dist. LEXIS 129099, at *18 (D.N.J. Sept. 11, 2012) (“Defendants rely on a key unsupported proposition: if a prior art reference discloses a genus containing a large number of species, a challenger may reduce the size of the genus by asking which species would have ‘stood out’ to the skilled artisan. . . . Defendants’ anticipation case turns entirely on this proposition, and they have shown no legal support for it.”).

Since neither Tsien nor Prober discloses the claimed allyl capping group, neither reference can disclose any motivation for selecting it. Ex. 2116 ¶106. Moreover, 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; Ex. 2116 ¶106.

1. Illumina Previously Argued That Tsien Does Not Disclose The Allyl Capping Group

In the present proceedings, Illumina argues that Tsien discloses the allyl capping group. In a 2007 reexamination proceeding of its own patent, Illumina took the opposite position and argued that Tsien **does not** disclose the allyl capping group. Specifically, Illumina owns U.S. Patent No. 6,232,465 (*see* Ex. 2038 at 1), which claims a nucleotide with the allyl capping group. Ex. 2021 at Reexamination Certificate, claim 1. During a 2007 reexamination of that patent, to overcome **obviousness** and novelty rejections based on Tsien, Illumina cited the same portion of Tsien it relies on in the current proceedings and repeatedly admitted that Tsien **does not** disclose a nucleotide with the allyl capping group. Illumina admitted:

In the . . . section entitled “Deblocking Methods”, *Tsien* references a 1968 publication that allegedly teaches that treatment with Hg(II) in acetone/water will cleave an allyl ether. See, *Tsien*, Page 24, lines 29-30. The mere description of general deblocking methods in this section does not describe a [nucleotide] having an allyl removable blocking moiety, much less one having an allyl removable blocking moiety which is linked specifically at the 3’ carbon[.]

Ex. 2065 at 84-85. Illumina concluded that:

Tsien does not describe using an allyl ether at the 3' position [of a nucleotide].

Id. at 86. And:

Tsien did not envision the compounds which are the subject matter of Claims 1 and 3 as amended [*i.e.*, nucleotides with the allyl capping group].

Id. at 84, 78 (showing amended claims). And:

Tsien does not teach or suggest [a nucleotide] having an allyl removable blocking moiety protecting the 3' position linked to the 3' carbon via an ether linkage.

Id. at 88.¹⁸ These statements contradict Illumina's current position that *Tsien* discloses the allyl capping group.

The Board in IPR2018-00797 invited the parties to address whether judicial estoppel is applicable to IPR proceedings. *E.g.*, IPR2018-00797, Paper 20 at 21 (September 18, 2018). As evidenced by *Data Gen. Corp. v. Johnson*, 78 F.3d 1556, 1565 (Fed. Cir. 1996), judicial estoppel is applicable to administrative

¹⁸ These admissions were made in response to obviousness rejections, or in response to novelty rejections which were then incorporated by reference into obviousness rejection responses. Ex. 2065 at 88.

agency proceedings. Regardless, Illumina's inconsistent statements evidence the lack of credibility of its arguments in this case.

Application of the *New Hampshire* standard shows that Illumina should be judicially estopped here. *New Hampshire v. Maine*, 532 U.S. 742, 750-51 (2001). Illumina's position in this proceeding is clearly inconsistent with its prior positions; Illumina persuaded the Examiner during the '465 reexamination to accept its position that Tsien did not disclose the allyl capping group, Ex. 2065 at 101 (Examiner concluding that "Tsien et al. do not teach explicitly a [nucleotide] with a 3'-allyl protective group"); and allowing Illumina to maintain its new position in this proceeding would impose an unfair detriment on Columbia.

The admissions made in the '465 patent reexamination are attributable to Illumina. The admissions were made by Solexa Inc. (Ex. 2038 at 1-2 (assignment data)) on April 30, 2007 (Ex. 2065 at 91). Illumina and Solexa were operating as a single combined company at the time of the admissions. Specifically, Illumina acquired Solexa on January 26, 2007. Ex. 2024 at 3; Ex. 2055 at 1 (Illumina's CEO stating "We are excited to join the two companies[.]"). In the merged company, Solexa operated "as a business unit of Illumina." Ex. 2119 at 2.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

2. Illumina Previously Argued That Tsien Would Not Motivate A POSA To Select The Allyl Capping Group

In the present proceedings, Illumina argues that Tsien would have motivated a POSA to select the allyl capping group. Illumina previously took the opposite position in the '465 patent reexamination:

[W]hether a person of ordinary skill in the art would or would not have been motivated, on the basis of the teachings in Tsien, to make a compound comprising [a nucleotide] with a 3'-allyl ether group. . . . Basically, the evidence shows that even 6 years after the time the presently claimed invention was made [*i.e.*, 6 years after September 1994, which is ***September 2000***], persons skilled in the art would not have been motivated to prepare the compounds of claims 1, 3 and 7 [nucleotides

with the allyl capping group], or compositions containing them, with a reasonable expectation that they could be used for DNA synthesis.

Ex. 2065 at 90 (in response to obviousness rejections).

All three *New Hampshire* criteria for establishing judicial estoppel are again met. First, Illumina's position in this proceeding—that Tsien would motivate a POSA to select the allyl capping group—contradicts its position in the '465 reexamination that Tsien would not motivate a POSA to do so. Second, Illumina persuaded the Examiner to accept its position in the '465 reexamination. The Examiner withdrew the obviousness rejection based on Illumina's statements and acknowledged that a POSA “would not have expected that a [nucleotide] with a 3'-OH allyl protecting group would have been an acceptable substrate for a DNA polymerase[.]” Ex. 2065 at 127, 124-126. Third, allowing Illumina to maintain its new position in this proceeding would be unjust and impose an unfair detriment on Columbia.

G. The Lead Compound Analysis

Illumina alleges that the Lead Compound Analysis does not apply. *E.g.*, IPR2018-00291, Petition at 38 (Dec. 8, 2017). Regardless of whether it applies or not, Illumina's Petition fails on the merits for the reasons explained in this Response. Therefore, the Board need not address the Lead Compound Analysis to

find that Illumina has failed to meet its burden of demonstrating that the challenged claim is obvious.

Nevertheless, applying the Lead Compound Analysis establishes that 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. Ex. 2116 ¶107; see Sections V(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); Ex. 2116 ¶107.

VI. 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

Illumina's Ground 2 challenge fails for the same reasons as its Ground 1 challenge. Ex. 2116 ¶109. 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 provided 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 V(A));

(B) Illumina's references provided 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 V(B));

(C) A POSA weighing the allyl capping group's numerous disadvantages would have concluded that it was incompatible with SBS (*see* Section V(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 V(D));

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

(F) Application of the lead compound analysis demonstrates that Illumina selected the wrong lead compounds (*see* Section V(G)). Ex. 2116 ¶109.

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

Illumina's Ground 2 challenge also fails because none of its references (Dower, Prober, or Metzker) disclose a "chemically cleavable, chemical linker" as required by the challenged claim. Ex. 2116 ¶110. A claim cannot be rendered obvious by a combination of references none of which discloses or suggests a feature of the claim. *See* Section V(F).

The challenged claim requires 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. Ex. 2116 ¶111. Dower only discloses labels (*e.g.*, FMOC) which are **directly** attached to the base. *E.g.*, Ex. 1015 at Fig. 9; Ex. 2116 ¶111; Ex. 2036 at 14-15 (Board finding that Dower does not disclose a cleavable linker). Prober discloses the attachment of fluorescent labels to the base of ddNTPs by using an acetylenic linker, which is a chemical linker that is **not** cleavable under DNA-compatible conditions. Ex. 1014 at 338; Ex. 2116 ¶111; 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. 2116 ¶111; Ex. 2113 at 125 (Dr. Romesberg agreeing).

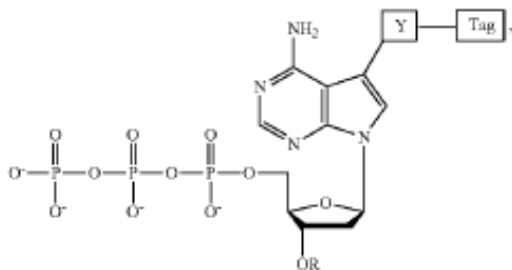
Therefore, the combination of Dower, Prober, and Metzker cannot render the challenged claim obvious because the combination does not disclose or suggest a required claim element, *i.e.*, a “chemically cleavable, chemical linker[.]” Ex. 2116 ¶112.

VII. Neither Patent Owner Estoppel Nor Judicial Estoppel Apply

Illumina asserts Columbia violated the patent owner estoppel provisions of 37 C.F.R. §42.73(d)(3)(i). *E.g.*, IPR2018-00291, Petition at 2 (Dec. 8, 2017). As

the Board recognized, Illumina is wrong. *E.g.*, IPR2018-00291, Paper 16 at 35-36 (June 25, 2018). The challenged claim was specifically drafted to be narrower than the claims previously found unpatentable. Illumina argues that the challenged claim is the same as cancelled claim 16 of the '869 patent (IPR2018-00291, -00385) or cancelled claim 21 of the '869 patent (IPR2018-00318, -00322). But the challenged claim has many features not present in cancelled claims 16 or 21 of the '869 patent. As an example, the following features of the challenged claim in IPR2018-00291 are not present (double underlined below) or are more narrow than (singly underlined below) the features in cancelled claim 16 of the '869 patent:

1. An adenine deoxyribonucleotide analogue having the structure:



wherein R (a) represents a small, chemically cleavable, chemical group capping the oxygen at the 3' position of the deoxyribose of the deoxyribonucleotide analogue, (b) does not interfere with recognition of the analogue as a substrate by a DNA polymerase, (c) is stable during a

DNA polymerase reaction, and (d) does not contain a ketone group;

wherein OR is not a methoxy group or an ester group;

wherein the covalent bond between the 3'-oxygen and R is stable during a DNA polymerase reaction;

wherein tag represents a detectable fluorescent moiety;

wherein Y represents a chemically cleavable, chemical linker which (a) does not interfere with recognition of the analogue as a substrate by a DNA polymerase and (b) is stable during a DNA polymerase reaction;

and wherein the adenine deoxyribonucleotide analogue:

i) is recognized as a substrate by a DNA polymerase,

ii) is incorporated at the end of a growing strand of DNA during a DNA polymerase reaction,

iii) produces a 3'-OH group on the deoxyribose upon cleavage of R,

iv) no longer includes a tag on the base upon cleavage of Y, and

v) is capable of forming hydrogen bonds with thymine or a thymine nucleotide analogue.

The same features of the challenged claim in IPR2018-00318, -00322, and -00385 are missing from cancelled claims 16 and 21 of the '869 patent. *Compare* Ex. 1002-1004, each at claim 1 with Ex. 1010 at claims 16, 21. Patent Owner Estoppel does not apply because it only concerns a party obtaining claims that are “not patentably distinct” from canceled claims. 37 C.F.R. §42.73(d)(3)(i). Here, the challenged claims **are** patentably distinct.

Illumina asserts that the Examiner rejected the challenged claim as not patentably distinct over cancelled claims 17 and 28 of the '869 patent. *E.g.*, IPR2018-00291, Petition at 4-7 (Dec. 8, 2017). This is wrong. The Examiner’s double patenting rejection referenced **17** different claims (Ex. 1009 at 102; Ex. 1062 at 100; Ex. 1065 at 100), 15 of which were not canceled in earlier IPRs. The Examiner’s rejection was not based on **any individual claim**, let alone cancelled claim 17 or 28.

In addition, Columbia’s filing of a terminal disclaimer does not give rise to estoppel under 37 C.F.R. §42.73(d)(3)(i). *See* M.P.E.P. §804.02.

Illumina’s argument also mischaracterizes the prosecution history. Illumina claims that Columbia overcame a lack of written description rejection by asserting the challenged claim “is supported because it covers the **same** subject matter as the previously issued claims” cited in the Examiner’s obviousness-type double patenting rejection. *E.g.*, IPR2018-00291, Petition at 5 (Dec. 8, 2017). Columbia

never said that the challenged claim covers the **same** subject matter as the canceled claims. In fact, Columbia made clear that the challenged claim is narrower than the canceled claims: “The invention was ready for patenting as evidenced by the grant of **broader** claims in related patents[.]” Exs. 1009, 1062, 1065, each at 21; Ex. 1068 at 17.

VIII. Conclusion

For the foregoing reasons, the Board should deny Illumina’s Petitions.

Respectfully submitted,

Dated: October 26, 2018

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CERTIFICATION OF WORD COUNT

Pursuant to 37 C.F.R. § 42.24(d), the undersigned certifies that the foregoing **PATENT OWNER'S RESPONSE**, exclusive of the parts exempted as provided in 37 C.F.R. § 42.24(a), contains 13,618 words and therefore complies with the type-volume limitations of 37 C.F.R. § 42.24(b)(1).

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CERTIFICATE OF SERVICE

I hereby certify that a computer disk containing a true and correct copy of the foregoing **PATENT OWNER'S RESPONSE** and **EXHIBITS 2053-2055, 2057, 2059, 2065, 2066, 2080-2087, 2089-2090, 2094-2126** are being served on October 26, 2018, via Federal Express overnight mail to Counsel for Petitioner at:

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