

Filed on behalf of:

Patent Owner Illumina Cambridge Ltd.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

INTELLIGENT BIO-SYSTEMS, INC.

Petitioner,

v.

ILLUMINA CAMBRIDGE LTD.

Patent Owner

Case IPR2013-00517

Patent 7,566,537

DECLARATION OF FLOYD ROMESBERG, PH.D.

Columbia Exhibit 2007

Illumina, Inc. v. The Trustees of Columbia
University in the City of New York

IPR2018-00322

1. I, Floyd Romesberg, Ph.D., have been retained by Knobbe, Martens, Olson & Bear, LLP, counsel for Illumina Cambridge Limited (“Illumina”). I understand that the U.S. Patent and Trademark Office Patent Trial and Appeal Board has instituted an inter partes review of the patentability of Claims 1-6 and 8 of U.S. Patent No. 7,566,537 (“the ’537 patent,” Ex. 1001) based upon a petition filed by Intelligent Bio-Systems, Inc. (“IBS”). The following discussion and analysis provides my opinion as to why Claims 1-6 and 8 of the ’537 Patent would have been nonobvious to a person of ordinary skill in the art in August of 2002.

I. BACKGROUND AND QUALIFICATIONS, PREVIOUS TESTIMONY, AND COMPENSATION

A. Background and Qualifications

2. I am currently a professor in the Department of Chemistry at The Scripps Research Institute. I have been a faculty member at The Scripps Research Institute since 1998.

3. I earned a Bachelor of Science in Chemistry from the Ohio State University in 1988.

4. I earned a Master of Science in Chemistry in 1990 and a Doctor of Philosophy in Chemistry in 1994 from Cornell University, where Professor David B. Collum served as my thesis advisor.

5. From 1994 until 1998, I was an NIH postdoctoral research fellow at the University of California, Berkeley, where I studied under Professor Peter G. Schultz.

6. As a principal investigator, I have authored over 100 publications in peer-reviewed journals, including Nature, The Proceedings of the National Academy of Sciences of the United States of America, The Journal of the American Chemical Society, Angewandte Chemie International Edition, Biochemistry, Nucleic Acids Research, and The Journal of Physical Chemistry.

7. I have authored or co-authored at least 18 invited review articles. In addition, I have authored or co-authored at least 17 other publications during my graduate and post-doctoral studies.

8. Over 30 of the publications that I have authored as a principle investigator are related to nucleotide analogues, including nucleotide analogues bearing linkers for attachment of functionalities of interest to polynucleotides.

9. I currently teach several graduate courses in the Department of Chemistry at The Scripps Research Institute, including a Spectroscopy course and a course on Bacteria and Antibiotics.

10. I have mentored numerous graduate students, post-doctoral researchers, interns, and research associates at The Scripps Research Institute.

11. I have been an invited lecturer at multiple universities, symposiums, and conferences throughout the United States and abroad.

12. I have reviewed manuscripts as part of the peer-review process to determine whether they are acceptable for publication for numerous journals, including the Proceedings of the National Academy of Sciences, Science, Nature, the Journal of the American Chemical Society, Angewandte Chemie, Biochemistry, the Journal of Organic Chemistry, Bioorganic and Medicinal Chemistry Letters, Chemistry & Biology, Nucleic Acids Research, and Nucleosides, Nucleotides, and Nucleic Acids.

13. I am a member of the American Chemical Society and the American Society for Microbiology.

14. I am a permanent member of the NIH Synthetic and Biological Chemistry (SBCA) study section that handles a significant percentage of grants dealing with modified nucleotides. Also, I regularly serve on various NSF study sections, and my service on these study sections involves reviewing and determining the merits of numerous grant proposals.

15. I have been awarded numerous research grants from the National Institutes of Health, the National Science Foundation, and several other sources, including federal funding for the synthesis and analysis of nucleotide analogues

and for the development of DNA polymerases specifically for sequencing by synthesis (“SBS”).

16. I have been the recipient of multiple awards and honors, including the Discover Magazine Technology Innovation Award in 2004, the NSF Career Award in 2004, the Susan B. Komen Breast Cancer Foundation Award in 2003, the Camille Dreyfus Teacher-Scholar Award in 2003, the Baxter Foundation Fellow Award in 2002, the Mac Nevin Award in 1987, and election to the Defense Science Study Group (DSSG) panel from 2008-2010.

17. I am qualified to render an opinion in the field of DNA sequencing and methods of labeling nucleic acid molecules based on my experience in this field. Based on my expertise and qualifications, I am qualified to provide an opinion as to what a person of ordinary skill in the art would have understood, known, or concluded as of 2002. I have been doing research in this field since 1999.

18. Attached as Exhibit 2012 is a copy of my curriculum vitae setting forth my educational experience, employment history, and publications.

B. Previous Testimony

19. I previously testified as an expert witness in *Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd.*, IPR2013-00128, regarding U.S. 7,057,026; and in

Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd., IPR2013-00266, regarding U.S. 8,158,346.

C. Compensation

20. I am being compensated for my time at a rate of \$450 per hour or \$3,600 per day, plus actual expenses. My compensation is not dependent in any way upon the outcome of this proceeding.

II. MATERIALS CONSIDERED

21. I have considered and reviewed at least the following in connection with providing this declaration:

- a. U.S. Patent No. 7,566,537 to (“the ’537 patent,” Ex. 1001);
- b. U.S. Patent No. 6,664,079 to Ju et al. (Ex. 1002);
- c. Zavgorodny et al., Tet. Lett., 32:7593 (1991) (Ex. 1004);
- d. Loubinoux et al., Tetrahedron, 44:6055 (1988) (English translation) (Ex. 1006);
- e. PCT Publication WO 91/06678 to Tsien et al. (Ex. 1008);
- f. Declaration of Dr. Bruce Branchaud (Ex. 1011);
- g. Ranganathan et al., Tet. Lett., 45:4341 (1978) (Ex. 2013);
- h. Mungall et al., J. Org. Chem., 40:1659 (1975) (Ex. 2014);
- i. U.S. 5,959,089 to Hannessian et al. (Ex. 2015);
- j. Pilard et al., Tet. Lett., 25:1555 (1984) (Ex. 2016);

- k. Tietze et al., *Angew. Chem. Int. Ed.*, 36:1615 (1997) (Ex. 2017);
- l. Kit, *Annu. Rev. Biochem.*, 32:43 (1963) (Ex. 2018);
- m. Canard et al., *Proc. Natl. Acad. Sci. USA*, 92:10859 (1995) (Ex. 2019);
- n. *The Merck Index*, 13th ed., 2001, p. 9815 (Ex. 2020);
- o. Lee et al., *Proc. Natl. Acad. Sci.*, 78:2838 (1981) (Ex. 2021);
- p. Christensen et al., *J. Org. Chem.*, 37:3398 (1972) (Ex. 2022);
- q. Watkins et al., *J. Am. Chem. Soc.*, 104:5702 (1982) (Ex. 2023);
- r. Metzker, *Nucleic Acids Research*, 22:4259 (1994) (Ex. 2024);
- s. Yoshimoto et al., *Chem. Lett.*, 30:934 (2001) (Ex. 2025);
- t. *Selections from Greene & Wuts*, 1991 (Ex. 1005);
- u. Chapter 3 from *Greene & Wuts*, 1991 (Ex. 2026);
- v. Welch & Burgess, *Nucleosides & Nucleotides*, 18(2) 197-201 (1999) (Ex. 2028);
- w. Bentley et al., *Nature*, 456:53, 53 (2008) (Ex. 2027);
- x. *Nature: The Editorial Process* (2008) (Ex. 2076);
- y. *Nature: The Editorial Process* (2014) (Ex. 2075);
- z. Notebook pages 16, 27-28, 30-34, and 36-40 from SLB-90, and page 1 from SLB-128 (Ex. 2003);
- aa. Kraevski, *Molecular Biology*, 21-25 (1987) (Ex. 2073);

bb.Dantas, Toxicology Letters, 110-129 (1999) (Ex. 2074);

cc. Iyer et al, J. Org. Chem. 60, 5388-5399 (1995) (Ex. 2088);

dd.IBS's Revised Petition (Paper No. 7); and

ee. April 8, 2014, deposition transcript of Dr. Bruce Branchaud (Ex. 2039).

III. LEGAL STANDARD OF NONOBVIOUSNESS

22. I understand that 35 U.S.C. § 103 governs the determination of obviousness. I understand that 35 U.S.C. § 103 states:

A patent for a claimed invention may not be obtained, notwithstanding that the claimed invention is not identically disclosed as set forth in section 102, if the differences between the claimed invention and the prior art are such that the claimed invention as a whole would have been obvious before the effective filing date of the claimed invention to a person having ordinary skill in the art to which the claimed invention pertains. Patentability shall not be negated by the manner in which the invention was made.

23. I understand that hindsight analysis must be avoided to prevent a person from looking back at the path the inventors used to arrive at the claimed invention and inappropriately concluding that the inventor's path would have been obvious. I understand that an analysis under 35 U.S.C. § 103 starts by (1) considering the scope and content of the prior art; (2) determining the differences

between the prior art and the claimed invention; and (3) understanding the level of skill that a person of ordinary skill in the art would have had at the time of filing the patent application.

24. In addition, I understand that objective evidence of nonobviousness must always be considered. I understand that this evidence is referred to as secondary considerations of nonobviousness. I understand that such evidence includes unexpected results.

IV. THE PERSON OF ORDINARY SKILL IN THE ART

25. I understand that obviousness is analyzed from the perspective of a hypothetical person of ordinary skill in the art at the time of the invention.

26. In my opinion, a person of ordinary skill in the art related to the '537 patent would have been a member of a team of scientists working on sequencing by synthesis product development. Such a person of skill in the art would have held a doctoral degree in chemistry, molecular biology, or a closely related discipline, and had at least five years of practical academic or industrial laboratory experience. Thus, a person of ordinary skill in the art includes a person having a doctoral degree in a field related to chemistry, and at least five years of specific laboratory experience directed toward the research and development of DNA sequencing technologies.

27. My opinions concerning the proposed claims, as set forth herein, are from the perspective of a person of ordinary skill in the art, as set forth above.

V. SCOPE AND CONTENT OF THE PRIOR ART

A. U.S. Patent No. 6,664,079 to Ju et al. (Ex. 1002)

28. Ju is directed to DNA sequencing methods. *See, e.g.*, Ju at 4:22-26 (“This invention is directed to a method for sequencing a nucleic acid by detecting the identity of a nucleotide analogue after the nucleotide analogue is incorporated into a growing strand of DNA in a polymerase reaction, which comprises the following steps”) (Ex. 1002); *id.* at 21:1-5 (“Sequencing DNA by synthesis involves the detection of the identity of each nucleotide as it is incorporated into the growing strand of DNA in the polymerase reaction.”). One of ordinary skill in the art would have understood these teachings of Ju as stating that Ju’s invention was nucleotide sequence determination using a DNA polymerase, and in particular, using the method referred to as sequencing by synthesis.

29. Ju’s SBS methods have four “fundamental requirements”:

(1) the availability of 4 nucleotide analogues (aA, aC, aG, aT) each labeled with a unique label and containing a chemical moiety capping the 3'-OH group; (2) the 4 nucleotide analogues (aA, aC, aG, aT) need to be efficiently and faithfully incorporated by DNA polymerase as terminators in the polymerase reaction; (3) the tag and the group capping the 3'-OH need to be removed with high yield to allow the

incorporation and detection of the next nucleotide; and (4) the growing strand of DNA should survive the washing, detection and cleavage processes to remain annealed to the DNA template.

Ju at 21:3-16 (Ex. 1002).

30. Thus, one of ordinary skill in the art would have understood that Ju's sequencing by synthesis methods have the above four fundamental requirements, and if any of these requirements are not satisfied, then Ju's sequencing by synthesis method would not work for its intended purpose.

31. Regarding fundamental requirement (1), Ju teaches that 3'-O-protecting groups "with electrophiles such as ketone groups are not suitable for protecting the 3'-OH of the nucleotide in enzymatic reactions due to the existence of strong nucleophiles in the polymerase." Ju at 3:16-20 (Ex. 1002). Ju cites to "Canard et al. 1995" at col. 3, lines 12-15 of Exhibit 1002. The Canard article further states that "[s]trikingly, the central nitrogen of the 3'-azido group of AZT bears a partial positive charge in a position similar to the carbonyl group of 3'-esterified nucleotides" Canard et al., Proc. Natl. Acad. Sci., 92:10859, 10863 (1995) (Ex. 2019). Thus, a person of skill in the art would have understood Ju's statement regarding the unsuitability of electrophiles to have included azides. These teachings by Ju and Canard would have led one of ordinary skill in the art to expect that a nucleotide containing a 3'-O-azidomethyl group would be reactive

with the strong nucleophiles in the polymerase, even with the inclusion of the O-methyl of the 3'-O-azidomethyl. In particular, if the azido group of the 3'-O-azidomethyl reacted with the strong nucleophiles in the active site of the polymerase, the 3'-OH group could be prematurely deprotected, leading to potential issues with phasing and misidentification of bases. For this reason, Ju teaches that such protecting groups are unsuitable for Ju's SBS methods.

32. Regarding fundamental requirement (3), Ju states that one of the fundamental requirements for DNA sequencing by synthesis is "the tag and the group capping the 3'-OH need to be removed with high yield to allow the incorporation and detection of the next nucleotide." Ju at 21:3-16 (Ex. 1002). In discussing shortcomings of prior art pyrosequencing methods, Ju states that this method "can only sequence up to 30 base pairs (bps) of nucleotide sequences." *Id.* at 2:10-25). Thus, one of ordinary skill would understand Ju as teaching that a method that can only sequence 30 base pairs is undesirable. Regarding Ju's own method, Ju teaches, "in the DNA sequencing system disclosed herein, more than 10,000 bases can be identified after each cycle and after 100 cycles, a million base pairs will be generated from one sequencing chip." *Id.* at 21:63-67. Thus, a person of ordinary skill in the art would understand that the desired read length of Ju's sequencing method is more than 30 base pairs, and preferably up to 100 base pairs.

33. In order to achieve a read length of more than 30 base pairs, one of ordinary skill would have understood that Ju's 3'-OH capping group must be removed in nearly quantitative yield through each cycle of sequencing. For example, the removal efficiency for each cycle must be greater than 95% to permit a read length of 30 bases before phasing errors cause the signal-to-noise ratio to decrease beyond detectable limits (*i.e.*, $0.95^{30} = 21\%$). One of ordinary skill would have known that, in practice, Ju requires a removal efficiency significantly higher than 95% to account for inefficiencies associated with other steps in the sequencing cycle (such as inefficiencies with tag removal and/or polymerase incorporation of unnatural nucleotides). Ju at 21:8-13 (Ex. 1002). Likewise, one of ordinary skill would have known that the removal efficiency must be greater than 98.5% to yield Ju's desired read length of 100 bases (*i.e.*, $0.985^{100} = 22\%$). Therefore, a person of ordinary skill in the art would have understood that Ju's sequencing method requires nearly quantitative cleavage efficiency for the 3'-OH capping group.

34. Ju also states that "The success of this [DNA sequencing by the synthesis] system will allow the development of an ultra high-throughput and high fidelity DNA sequencing system for polymorphism, pharmacogenetics applications and for whole genome sequencing. This fast and accurate DNA resequencing system is needed in such fields as detection of single nucleotide polymorphisms

(SNPS), serial analysis of gene expression (SAGE), identification in forensics, and genetic disease association studies.” Ju. at 4:10-19 (Ex. 1002) (emphases added). Ju’s characterization of its DNA sequencing system as “fast” and “ultra high-throughput” would have led a person of ordinary skill in the art to understand that speed was an important design consideration for Ju’s sequencing system. Thus, one of ordinary skill would have considered that each step of Ju’s sequencing cycle should proceed as quickly as possible to increase the overall speed of the sequencing method. Since removal of the 3’-OH capping group is one step in Ju’s sequencing cycle (Ju at 21:11-13), a person of ordinary skill in the art would understand that rapid removal conditions would be required to develop a “fast” sequencing system capable of the “ultra high-throughput” of Ju’s sequencing method.

35. Regarding potential capping groups, Ju teaches that methoxymethyl (MOM) and allyl ethers can be used to cap the 3’-OH group of a modified nucleotide. Ju at 26:19-30 (Ex. 1002). Ju does not teach or suggest to one of ordinary skill in the art that an azido group could be used to cap the 3’-OH group.

B. PCT Publication WO 91/06678 to Tsien et al. (Ex. 1008)

36. Tsien is directed to DNA sequencing methods. *See, e.g.*, Tsien at title (“DNA Sequencing”) (Ex. 1008); *id.* at Abstract (“The present invention relates to an instrument and a method to determine the nucleotide sequence in a DNA

molecule without the use of a gel electrophoresis step. The method employs an unknown primed single stranded DNA sequence which is immobilized or entrapped within a chamber with a polymerase so that the sequentially formed cDNA can be monitored at each addition of a blocked nucleotide by measurement of the presence of an innocuous marker on specified deoxyribonucleotides.”); *id.* at 6:26-30 (“The present invention provides methods and apparatus for determining the sequence of deoxyribonucleotides in a DNA molecule. ”); *id.* at 6:36 to 7:3 (“This method involves synthesizing, in the presence of a multitude of identical copies of the subject DNA, the DNA molecule which is complementary to it.”). One of ordinary skill in the art would have understood these teachings of Tsien as stating that Tsien’s invention was nucleotide sequence determination using DNA polymerase, and in particular, sequencing by synthesis.

37. According to Tsein “[t]he criteria for the successful use of 3’-blocking groups include: (1) the ability of a polymerase enzyme to accurately and efficiently incorporate the dNTPs carrying the 3’-blocking groups into the cDNA chain, (2) the availability of mild conditions for rapid and quantitative deblocking, and (3) the ability of a polymerase enzyme to reinitiate the cDNA synthesis subsequent to the deblocking stage.” Tsien at 20:28–21:3 (Ex. 1008). Tsien lists a large number of groups that could be used as 3’ protecting groups, some of which were subsequently shown in the art to be incorporated by polymerases. Tsien at 24:5–

25:25 (Ex. 1008); Metzker at Table 2 (Exhibit 2024). Despite providing a long list of potential 3'-OH protecting groups, Tsien does not teach or suggest to one of ordinary skill in the art that an azido group could be used to cap the 3'-OH group and then removed to recover the 3'-OH.

38. Tsien supports the potential polymerase recognition of such removable 3'-blocking groups with well-known examples of polymerase-recognized nucleotides bearing non-removable groups. One of these non-removable groups is an azide. Specifically, Tsien states that “[o]ther blocking modifications to the 3'-OH position of dNTPs include the introduction of groups such as -F, -NH₂, -OCH₃, -N₃, -OPO₃⁻, -NHCOCH₃, 2-nitrobenzene carbonate, 2,4-dinitrobenzene sulfonyl and tetrahydrofuranyl ether.” Tsien at 21:13-17 (Ex. 1008).

39. A person of ordinary skill in the art would have understood that several of the blocking modifications taught in the above quote by Tsien were directly attached to the 3'-carbon of the dNTPs in a non-removable manner. Specifically, one of ordinary skill would have understood that the -F, -NH₂, -N₃, and -NHCOCH₃ blocking groups taught by Tsien were directly attached to the 3'-carbon of the dNTPs. This is further clarified by the next sentence in Tsien, which states, “[i]ncorporation and chain termination have been demonstrated with dNTPs containing many of these blocking groups (Kraevskii et al., 1987).” Tsien at

21:17–19 (Ex. 1008). The full citation for Kraevskii et al., 1987 is on page 4 of Tsien. *Id.* at 4: Kraevskii teaches irreversible terminators as substrates for polymerase enzymes. Kraevskii at p. 27 (Ex. 2073). Kraevskii identifies several irreversible terminators, including dNTPs with -F, -NH₂, -N₃, and -NHCOCH₃ directly attached to the 3'-carbon. *Id.* at Table 1. One of skill in the art reading Tsien's statement of "[i]ncorporation and chain termination have been demonstrated with dNTPs containing many of these blocking groups (Kraevskii et al., 1987)" would have understood this to refer to moieties that cause the nucleotide to act as irreversible inhibitors as these moieties are attached directly to the 3' position, where they replace (not protect) the 3'-OH. Thus, one of ordinary skill would have understood Tsien's teaching of an -N₃ blocking group to be referencing Kraevskii's description of an -N₃ blocking group, where -N₃ is directly attached to the 3'-carbon of the dNTP. One of ordinary skill in the art would have known that an -N₃ blocking group directly attached to the 3'-carbon of the dNTP would not be removable from the dNTP to produce a 3'-OH group, and incorporation of a such a dNTP would irreversibly terminate nucleotide synthesis. Therefore, a person of skill in the art would not have interpreted Tsien as teaching the use of azides as removable blocking groups. Moreover, the function of azides as irreversible terminators directly attached to the 3' carbon as in Kraevskii would not have been expected to be altered by the strong nucleophiles in the polymerase

active site. See ¶ 31, above. An azide directly attached to the 3' carbon of a nucleotide, when reacted with the strong nucleophiles in the polymerase active site would not yield a free 3'-OH, and would continue to function as an irreversible terminator, which is consistent with both Canard's and Kraevskii's teachings. This is in contrast to azidomethyl, as discussed in ¶ 31, above.

C. Zavgorodny et al., Tet. Lett., 32:7593 (1991) (Ex. 1004)

40. Zavgorodny discloses over 180 nucleoside compounds. *See* Zavgorodny at 7594 (showing nucleosides of formula 4-8) (Ex. 1004). Zavgorodny ambiguously states that “[t]he compounds disclosed above are useful specifically blocked synthons” (*id.* at 7595), which does not indicate a specific use for the disclosed compounds. One of ordinary skill in the art would have interpreted the term “synthons” to typically refer to compounds that are used in synthetic organic chemical reactions, such as single-stranded oligonucleotide manufacturing processes (*see, e.g.,* Iyer at 5388-9 (Ex. 2088)), but not in enzymatic reactions. Zavgorodny does not disclose a DNA sequencing method, nor even suggest that its compounds were compatible with the types of enzymes (e.g. polymerases) used in DNA sequencing.

41. Notably, as discussed in more detail in ¶¶ 54-55, the conditions that Zavgorodny discloses for removing protecting groups, including azidomethyl, are not compatible with Tsien's or Ju's DNA sequencing methods. The removal

conditions disclosed by Zavgorodny are triphenylphosphine in aqueous pyridine at 20 °C. Zavgorodny at 7595 (Ex. 1004). Zavgorodny does not teach any other removal conditions. The removal conditions taught by Zavgorodny would not be compatible with DNA sequencing. *See infra* paragraphs ¶¶ 44-53. In contrast, pyridine had been used in single-stranded oligonucleotide manufacturing processes, which is consistent with Zavgorodny's description of these nucleosides as "synthons."

42. In addition, all of Zavgorodny's disclosed compounds are nucleosides. Zavgorodny does not disclose a nucleotide compound. Nucleosides are not substrates of polymerases. Therefore, none of Zavgorodny's 3' substituted groups were intended for use in sequencing by synthesis. Moreover, the differences in reactivity between nucleosides and nucleotides can significantly (and deleteriously) impact the conversion efficiency of an azide to an amine (*see infra* ¶ 51), which is required for cleavage of an azidomethyl ether blocking group.

43. Further, Zavgorodny's use of the term "synthons" would have led one of ordinary skill in the art to view Zavgorodny's teachings in the context of synthetic organic chemical reactions, not in the context of Tsien's or Ju's enzyme-based sequencing by synthesis methods. Thus, Zavgorodny would not have motivated one skilled in the art to use these synthons in Tsien's or Ju's sequencing by synthesis methods.

D. The expected cleavage efficiency for Zavgorodny's removal conditions

44. Zavgorodny does not provide any empirical data for removal of the azidomethyl group. However, the prior art would have led a person of ordinary skill in the art to have expected Zavgorodny's azidomethyl removal conditions (triphenylphosphine and aqueous pyridine) to proceed with efficiency too low for Ju's and Tsien's DNA sequencing methods. In particular, in a systematic study, Loubinoux found no greater than 60-80% removal of the azidomethyl group from phenol derivatives using triphenylphosphine. Loubinoux at 4-5 (Ex. 1006) ("Regardless of the reduction method used, [the products] are obtained as pure products at a yield between 60 and 80%."). Loubinoux also found that triphenylphosphine produced poor yields as compared to other techniques (hydrogenolysis using H₂ with Pd/C or stannous chloride). *Id.* at 5 ("In general, the method that uses hydrogen yields better results than the one that uses triphenylphosphine."); *id.* at 7 & 11 (Tables 2 and 4). In view of the above, one of ordinary skill in the art would have expected that Zavgorodny's azidomethyl removal conditions (triphenylphosphine and aqueous pyridine) would not provide more than 60-80% removal of the azidomethyl group, an efficiency that would not have been expected to meet the requirements of Ju's and Tsien's DNA sequencing methods.

45. I understand that Dr. Branchaud identified one experiment in Table 2 of Loubinoux that showed 95% cleavage when using hydrogenation; however the other seven hydrogenation experiments showed 60%, 63%, 63%, 67%, 73%, 80%, 80% and 88% cleavage, respectively. Focusing on the one experiment that showed 95% cleavage, Dr. Branchaud said that this experiment would cause him to believe that “under the right conditions” azidomethyl could be cleaved at high yields. Branchaud deposition transcript at 171:10-173:10 (Ex. 2039) (commenting on Table 2 of Loubinoux). However, the same reaction conditions for a different compound resulted in only 80% yield. *See* entry “e” of Table 2 of Loubinoux (Ex. 1006). Moreover, a person of ordinary skill in the art would have understood that different substrates can show different cleavage efficiencies and would not have picked just one experiment out of eight and drawn conclusions from just that one experiment while ignoring the other seven experiments. One of ordinary skill would view Table 2 of Loubinoux as teaching that azidomethyl cleavage yields can vary widely, even when the same removal conditions are used. Thus, when the entirety of Table 2 is considered, one skilled in the art would not have expected a yield of 95% or greater.

46. Furthermore, the conditions used by Loubinoux in Table 2 would not be compatible with Tsien’s and Ju’s sequencing by synthesis methods. The experiment in Table 2 that achieved 95% yield used THF as a solvent, which, as

with pyridine, was a known DNA denaturant. See *infra* at ¶ 55; Lee et al., Proc. Natl. Acad. Sci., 78:2838, 2840 (1981) (Ex. 2021). In addition, Loubinoux's reaction proceeded for 7-10 hours (Loubinoux at 7 (Ex. 1006)), which one of ordinary skill in the art would have understood is not sufficiently rapid or fast for Ju's or Tsien's sequencing by synthesis methods. Finally, the experiment referred to by Dr. Branchaud uses hydrogenation to remove the azidomethyl group. *Id.* Hydrogenation would tend to degrade DNA, and would thus be unsuitable for use in the SBS methods of Ju and Tsien. See ¶ 56, *infra*. Therefore, one of ordinary skill would not only have viewed Table 2 of Loubinoux as teaching that yields vary widely, but would also have considered this method of azidomethyl protecting group removal to be incompatible with Tsien's and Ju's requirements for sequencing by synthesis. In addition, Loubinoux states that triphenylphosphine cleavage would be less efficient than hydrogenation-based methods, and thus even less efficient than the variable results reported in Table 2. Loubinoux at 5.

47. In fact, all of the non-triphenylphosphine-based azidomethyl removal conditions tested in Loubinoux would have been expected to be incompatible with Ju's and Tsien's sequencing methods. For example, as Dr. Branchaud recognized at his deposition, lithium aluminum hydride (LiAlH_4 ; Loubinoux at 4) is a very strong reducing agent that would not have been compatible with DNA sequencing by synthesis. Branchaud deposition transcript at 151:15-24 (Ex. 2039).

Loubinoux also used stannous chloride (SnCl_2) for azidomethyl removal. Loubinoux at 10-11 (Ex. 1006). This reaction was performed in methanol (which was known to denature DNA; see *infra* at ¶ 55), required 2 hours, and resulted in only 75%-91% yield. Loubinoux at 9, 11 and 13 (Ex. 1006). Further, stannous chloride (SnCl_2) was known to damage DNA by causing nicks in DNA strands. Dantas, Toxicology Letters 110:129, (1999) (Ex. 2074). Any one of these considerations would have led one of ordinary skill to have expected stannous chloride to be incompatible with Tsien's and Ju's requirements for sequencing by synthesis. In addition, Loubinoux reported variable yields of these compounds (*see, e.g.*, Loubinoux at Table 2), and that triphenylphosphine cleavage provided even lower yields than these methods (*see, e.g.*, Loubinoux at 5).

48. Finally, Loubinoux's azidomethyl groups were attached to hydroxyl groups of phenols, and not to hydroxyl groups of aliphatic hydroxyls such as the 3'-OH of a nucleotide. Loubinoux at Abstract (Ex. 1006). A phenol has an aromatic ring structure which can stabilize the negative charge carried by the oxygen connected to the aromatic ring by sharing that negative charge across the various carbon atoms of the aromatic ring. This sharing of the negative charge makes the deprotonated oxygen of the hydroxyl group (*i.e.* an oxygen without a hydrogen or other protecting group attached) more stable. The result of this stability would have provided an expectation that a protecting group could be

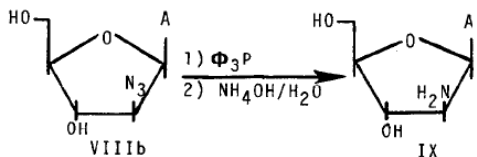
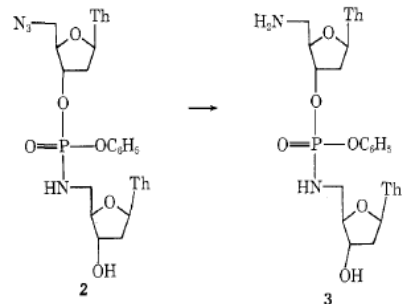
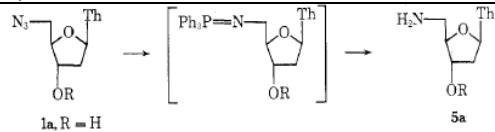
removed more easily from phenol than from an aliphatic alcohol. Chapter 3 from Greene & Wuts at 248 (Ex. 2026) As a result, the cleavage efficiencies described in Loubinoux for phenolic azidomethyl protecting groups would be expected by a person of skill in the art to be higher than the cleavage efficiencies for azidomethyl protecting groups attached to aliphatic alcohols.

49. Supporting my conclusion is the fact that Greene & Wuts, the leading treatise on protecting groups at the time, lists azidomethyl groups as a suitable protecting group for phenols, but does not list it as a suitable protecting group for aliphatic alcohols or polyols. *Compare* Greene & Wuts Chapter 2 table of contents (not listing azidomethyl) (Ex. 1005 at 0026–0031), *with* Chapter 3 table of contents (listing azidomethyl) (Ex. 2026); *see also id.* at 248.

50. The method of removing an azidomethyl group from a -OH is a complex, multi-step reaction that involves more than simply a conversion of an azide to an amine. Thus, a person of skill in the art would view the most relevant evidence for demonstrating the speed and efficiency of azidomethyl group removal, as provided in Loubinoux. However, since the step of converting an azide to an amine has been broadly examined, one may evaluate the efficiency of this step as an upper limit to what could have been expected for the entire azidomethyl group removal reaction. However, other, more general data regarding the conversion of an azide to an amine using triphenylphosphine in pyridine (or other

organic co-solvent) was known at the time and demonstrated that this reaction was highly variable and ranged from 50-90%. *See* Table 1 below. Furthermore, the amount of time required to achieve those yields (3 hours to multiple days) would not have been expected to fulfill the “rapid” deprotection requirement of Tsien’s DNA sequencing method or provide a “fast” sequencing system capable of the “ultra high-throughput” in accordance with Ju’s DNA sequencing method.

Table 1. Conversion of azides to amines on several nucleoside-related analogs using triphenylphosphine in pyridine (or other organic co-solvent) and water.

Reaction	Time	Yield	Reference
 <p>1) PPh₃ in pyridine, 2) concentrated NH₄OH in H₂O</p>	--	50%	Ranganathan et al., Tet. Lett., 45:4341, 4343 (1978) (Ex. 2013).
 <p>1) PPh₃ in pyridine, 2) concentrated NH₄OH</p>	72 h	78%	Mungall et al., J. Org. Chem., 40:1659, 1662 (1975) (Ex. 2014).
 <p>1) PPh₃ in pyridine, 2) 50% saturated methanolic ammonia</p>	3 h	90%	Mungall et al., J. Org. Chem., 40:1659, 1661 (1975) (Ex. 2014).

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51. With respect to nucleotides, Mungall reports the use of triphenylphosphine to convert azides to amines. Mungall at 1659 (Ex. 2014). Mungall provides a yield of 78 percent for the conversion of the azide di-nucleotide derivative labeled **2** to the corresponding amine derivative **3** (*see*, second entry of Table 1 above). The yield of the corresponding tetra-nucleotide derivative (labeled **4** in Mungall at page 1660) is not reported, but a similar yield is likely. Importantly, the conversion of the azide di-nucleotide derivative **2** (78%) was lower than the conversion efficiency of the corresponding nucleosides **1a-c** (88-90%). Mungall at 1661-2 (Ex. 2014). This would have suggested to one skilled in the art that phosphorylation of nucleosides reduces the efficiency of triphenylphosphine conversion of azides to amines.

52. Tsien and Ju's DNA sequencing methods require high and quantitative efficiency using nucleotides. *See, e.g.*, Tsien at 20:28-34 (Ex. 1008) (stating that "[t]he criteria for the successful use of 3'-blocking groups include: (1) the ability of a polymerase enzyme to accurately and efficiently incorporate the dNTPs [*i.e.*, deoxyribonucleotide triphosphates] . . . (2) the availability of mild conditions for rapid and quantitative deblocking"); Ju at 21:5-12 (stating that "[t]he fundamental requirements for [sequencing DNA by synthesis] to work are: (1) the availability of 4 nucleotide analogues . . . (3) the tag and the group capping the 3'-OH need to be removed with high yield" (emphasis added). Because

Tsien and Ju's DNA sequencing methods utilize polynucleotides, Loubinoux's and Mungall's results using triphenylphosphine would have led a person of ordinary skill in the art to expect Zavgorodny's removal conditions would cleave with insufficient efficiency for Tsien and Ju's DNA sequencing methods.

53. In general, the literature would have led a person of ordinary skill in the art to have expected that the yields for azide to amine conversion using triphenylphosphine would have been highly variable, and not acceptable for SBS. Further, these reactions would have required 3 hours to multiple days to achieve those yields, which also would be unacceptable for SBS. In the context of nucleotides bearing azido groups, the analogous nucleoside and nucleotide azide conversion efficiencies reported by Mungall indicate that nucleotide substrates are less efficiently converted than nucleoside substrates. Mungall at 1661-2 (Ex. 2014). Thus, one of ordinary skill would have expected yields at the lower end of the highly variable range of efficiency for Tsien's and Ju's DNA sequencing methods. As a result, a person of ordinary skill in the art would have expected that Zavgorodny's removal conditions would cleave with insufficient efficiency for Tsien's and Ju's DNA sequencing methods.

E. Zavgorodny's Removal Conditions Use Pyridine, Which Was Known To Be An Especially Effective DNA Denaturing Agent

54. Zavgorodny's removal conditions use aqueous pyridine as the solvent for the nucleoside and triphenylphosphine. Zavgorodny at 7959 (Ex. 1004). Zavgorodny's removal conditions would have required significant amounts of pyridine to solubilize triphenylphosphine because triphenylphosphine is practically insoluble in water. *See, e.g.*, The Merck Index, 13th ed., 2001, p. 9815 (Ex. 2020) (providing that triphenylphosphine is practically insoluble in water).

55. Pyridine was known to be an especially effective DNA denaturing agent in aqueous solutions. *See, e.g.*, Kit, *Annu. Rev. Biochem.*, 32:43, 57-58 (1963) (Ex. 2018) (stating that "[a]romatic reagents, such as phenols and pyridine are especially effective" DNA denaturants). Pyridine is not the only organic solvent that was known to be a denaturing agent in aqueous solution. In fact, most organic solvents (including tetrahydrofuran "THF" and methanol) were known to denature DNA. *See, e.g.*, Lee et al., *Proc. Natl. Acad. Sci.*, 78:2838, 2840 (1981) ("such unwinding of the DNA duplex caused by dehydration is likely to be a common phenomenon in organic solvents") (Ex. 2021). As a result, Zavgorodny's use of pyridine to solubilize triphenylphosphine in water would have led a person of ordinary skill in the art to conclude that Zavgorodny's removal conditions would have been incompatible with double stranded DNA. Tsien and Ju state that

their DNA sequencing methods require conditions that do not denature DNA. Tsien at 23:27-24:5 (Ex. 1008) (stating that deblocking methods should “(c) not interfere with further enzyme function or denature the DNA strand”); Ju at 21:3-16 (Ex. 1002) (stating that one of “[t]he fundamental requirements for [sequencing DNA by synthesis] to work” is that “(4) the growing strand of DNA should survive the washing, detection and cleavage processes to remain annealed to the DNA template.”). Accordingly, a person of skill in the art would not have expected use of Zavgorodny’s azidomethyl-containing nucleoside to meet Tsien’s and Ju’s DNA sequencing method requirements because Zavgorodny’s 3’-O-azidomethyl removal conditions required a DNA denaturing agent.

F. Whether Reaction Conditions Would Have Been Considered “Mild” Depended Upon The Context Of The Reaction

56. Zavgorodny states that triphenylphosphine in aqueous pyridine are “mild conditions.” Zavgorodny at 7595 (Ex. 1004). However, whether reaction conditions would have been considered to have been “mild” depends upon the specific context of each chemical reaction. IBS cites hydrogenolysis using Pd/C and H₂ as a “mild” method to remove azidomethyl protecting groups. IBS Revised Petition at 28-29 (*citing* Greene & Wuts at 260 (Ex. 1005)). However, in the context of DNA sequencing, hydrogenolysis using Pd/C and H₂ would not be considered mild, as it was known to reduce pyrimidine bases. *See, e.g.,*

Christensen et al., J. Org. Chem., 37:3398, 3401 (1972) (Ex. 2022) (“Treatment of 2’-O-benzylcytidine (14) under [10% Pd/C and stirred under hydrogen] led to complete debenzylation, but about 10-25% reduction to dihydrocytidine occurred.”); Watkins et al., J. Am. Chem. Soc., 104:5702, 5705 (1982) (Ex. 2023) (“O-benzyl-protected nucleosides 12b-d can be readily deblocked using hydrogen over palladium on carbon or palladium on barium sulfate. However, these conditions also were known to lead to significant reduction of the 5,6 double bond of thymidine. [] Pyrimidine bases are much more easily reduced than purine bases”). Since pyrimidine bases will by definition be present in the DNA template, one of ordinary skill in the art would have expected reduction of pyrimidine bases to interfere with sequencing. Similarly, Zavgorodny’s removal conditions use pyridine, which would not have been considered “mild” in the context of DNA sequencing because pyridine was known to be an especially effective DNA denaturant. *See supra* ¶ 55.

G. Zavgorodny’s azidomethyl ether would not have been expected to have all the desired properties of the Ju and Tsien protecting groups

57. Dr. Branchaud states that Zavgorodny’s azidomethyl protecting group has the properties of being “a small chemical moiety” that “can be removed under very specific and mild conditions.” Branchaud Decl. at ¶ 55-56 (Ex. 1011). Dr. Branchaud then states that “Because Zavgorodny teaches that azidomethyl has the

foregoing chemical and structural properties taught by Ju, one of ordinary skill in the art would also have expected the azidomethyl protecting group to possess the remaining desired functional properties of the Ju protecting groups, such as stability during a polymerase reaction, and not interfering with recognition of the modified nucleotide by a polymerase.” Branchaud Decl. at ¶ 57 (Ex. 1011). Dr. Branchaud makes a similar statement that “Because Zavgorodny teaches that azidomethyl has the foregoing chemical and structural properties taught by Tsien, one of ordinary skill in the art would also have expected the azidomethyl protecting group to possess the remaining desired functional properties of the Tsien protecting groups, such as not interfering with recognition of the modified nucleotide by polymerase, and reinitiating of DNA synthesis following the deblocking step.” Branchaud Decl. at ¶ 81 (Ex. 1011).

58. I disagree with paragraphs 57 and 81 of Dr. Branchaud’s declaration. One skilled in the art would have understood that the physical properties underlying (i) nucleotide stability, (ii) polymerase recognition, and (iii) reinitiation of DNA synthesis following deprotection are not necessarily related to (iv) size and (v) the chemical reversibility. A protecting group can have any subset of the aforementioned properties independent of the other properties. For example, it was known that acetyl esters are small 3’-O-protecting groups that can be cleaved with a derivative of triphenylphosphine. Metzker, *Nucleic Acids Research*, 22:4259,

4260 (1994) (Ex. 2024) (disclosing a nucleotide protected with a 3'-O-acetyl ester); Yoshimoto et al., Chem. Lett., 30:934, Tables 1 & 2 (2001) (Ex. 2025) (using tris(2,4,6-trimethoxy-phenyl)phosphine to cleave a 3'-O-acetyl ester from compound **14**). In fact, acetyl ester is the smallest possible ester. However, Ju taught that esters in general were unstable during polymerase reaction due to the presence of nucleophiles in the active site of the polymerase enzyme. Ju at 3:12-15 (Ex. 1002) ("An ester group was also explored to cap the 3'-OH group of the nucleotide, but it was shown to be cleaved by the nucleophiles in the active site in DNA polymerase."). Furthermore, it was known that a nucleotide having a 3'-O-acetyl ester was not a substrate for polymerases examined by Metzker. Metzker, Nucleic Acids Research, 22:4259, 4263 & 4265 (1994) (Ex. 2024). Thus, 3'-O-acetyl esters were known to interfere with polymerase recognition and incorporation of the modified nucleotide despite the small size and removability of this protecting group. Also, Ju taught that esters in general cannot be used for controlled reinitiation of DNA synthesis due to unintended cleavage in the polymerase active site. Ju at 3:12-15 (Ex. 1002) ("An ester group was also explored to cap the 3'-OH group of the nucleotide, but it was shown to be cleaved by the nucleophiles in the active site in DNA polymerase."). Therefore, it was known that esters can cause reinitiation of DNA synthesis before and after the deblocking step (reinitiation may, for example, cause phasing errors and/or other

misreading errors such as the incorporation of two or more differently labeled bases in a single round). Thus, a person of ordinary skill in the art would not have had an expectation that a small and removable protecting group would also possess all of the listed properties of Ju and Tsien's protecting group.

VI. THE CLAIMED INVENTION WAS A SIGNIFICANT IMPROVEMENT THAT SOLVED A LONG-FELT UNMET NEED IN THE ART

59. I understand that a long-felt need can support the non-obviousness of a claimed invention. I understand that this long-felt need is evaluated as of the filing date of the challenged invention.

60. It is my opinion that there was a need for a fast and efficient SBS method, and this need existed well before Illumina's reversible azido group chemistry was disclosed by the '537 Patent. For example, it was known that "stringent requirements" for nucleotide analogues with removable 3'-OH terminators presented a "formidable obstacle" to developing stable and efficiently deprotected 3'-OH protecting groups for SBS (Metzker at 4259 (Ex. 2024)), and it was a major challenge in the field to develop 3'-OH terminators that were removable under aqueous SBS conditions. *See, e.g.,* Welch at 197-198 (Ex. 2028).

61. In 2008, Bentley et al. published an article in *Nature* entitled, *Accurate whole human genome sequencing using reversible terminator chemistry*. Exhibit 2027. *Nature* is widely recognized as the premiere scientific journal in the

world, and less than 8% of all manuscripts submitted in 2008 made it through Nature's rigorous peer-review process. Nature: The Editorial Process (2014) (Ex. 2075). To meet Nature's strict criteria for publication, the reported results must be "novel, arresting (illuminating, unexpected or surprising)" and have "both immediate and far-reaching implications." Nature: The Editorial Process (2008) (Ex. 2076). The publication of the Bentley article in Nature affirms that Illumina's results were viewed in the scientific community as being unexpected and surprising.

62. In the article, Bentley reported an approach that that generates several billion bases of accurate nucleotide sequence per experiment at low cost. Bentley at 53 (Ex. 2027). In particular, DNA templates were sequenced by repeated cycles of polymerase directed single base extension. *Id.* To ensure base-by-base nucleotide incorporation in a stepwise manner, Bentley used a set of four reversible terminators, 3'-O-azidomethyl 2'-deoxynucleoside triphosphates (A, C, G and T), each labeled with a different removable fluorophore. *Id.* The use of azidomethyl 3'-modified nucleotides allowed their enzymatic incorporation to be driven essentially to completion without risk of over-incorporation. *Id.*

63. In Bentley, the fluorescent dye was removed using tris(2-carboxyethyl)phosphine (TCEP), and this reagent simultaneously removed the 3'-

O-azidomethyl protecting group to regenerate a 3'-hydroxyl group ready for the next cycle of nucleotide addition. *Id.*

64. Using the above-described technique, Bentley characterized four million single-nucleotide polymorphisms and four hundred thousand structural variants, many of which were previously unknown. *Id.* Bentley declared that the approach is effective for accurate, rapid, and economical whole-genome re-sequencing and many other biomedical applications. *Id.*

65. It is my understanding that Illumina's commercial products use modified nucleotides with 3'-O-azidomethyl protecting groups. The removable 3'-O-azidomethyl protecting group distinguishes Illumina's claims and Illumina's commercial products over Ju's and Tsien's teachings. In my opinion, Illumina's methods using novel reversibly terminating 3'-O-azidomethyl protecting group-containing nucleotides were a significant improvement over previous methods and satisfied the long-felt need in the industry for fast and efficient cleavage, which was essential for the development of SBS as a viable technology for sequencing.

VII. UNEXPECTED RESULTS SUPPORT THE NONOBVIOUSNESS OF THE CLAIMS

66. I understand that the presence of a property not possessed by the prior art can be evidence of nonobviousness.

67. I have reviewed several notebook pages provided by Illumina, dated [REDACTED]. The notebook pages are dated and signed by more than one scientist as is common in the field, particularly at companies such as Solexa and Illumina. The name of the organization on the notebooks is Solexa Limited. I have been informed that Solexa Limited was acquired by Illumina, Inc., and Solexa Limited is now called Illumina Cambridge Ltd. After reviewing these pages, I conclude that the times and yields in these experiments demonstrate that the 3'-O-azidomethyl protecting group can be significantly more rapidly and efficiently cleaved from a nucleoside in comparison to a 3'-O-allyl protecting group.

68. Notebook SLB-090 describes several experiments conducted in [REDACTED] where the 3'-O-allyl protecting group was removed from nucleosides under aqueous conditions. In my opinion, these notebook pages show work by Solexa researchers in [REDACTED] to optimize aqueous 3'-O-allyl protecting group cleavage conditions for use in SBS. These cleavage studies provide conditions, product yields, and time points for the removal of the 3'-O-allyl protecting group from a nucleoside. In general, 3'-O-allyl protecting group cleavage conditions described in SLB-090 involved using [REDACTED]. When combined in water with nucleosides having 3'-O-allyl protecting group, the allyl group is cleaved to form a 3'-OH.

69. Pages 27-28 of notebook SLB-090 describe experiment SR 202. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] Time point samples were taken at 5 min, 30 min, 3 hours, (3 hours and thymidine as a reference), and 4.5 hours. The remaining solvent was evaporated from the samples using a freeze drier. The cleavage results for the reaction in water were reported as: [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

70. Pages 30-32 of notebook SLB-090 describe experiment number SR 203. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

71. Pages 33-34 of notebook SLB-090 describe experiment number SR
204. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

72. Pages 36-38 of notebook SLB-090 describe experiment number SR
206. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

73. Page 39 of notebook SLB-090 describes experiment number SR 207.
Four reactions were performed to check reproducibility. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

74. Page 40 of notebook SLB-090 describes experiment number SR 208.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

75. In my opinion, these notebook pages report that even under optimized conditions, long time periods are required to achieve 3'-O-allyl protecting group cleavage efficiencies that are sufficient for SBS. For example, the 3'-O-allyl protecting group was at best only [REDACTED]. This is insufficient for SBS. [REDACTED]

[REDACTED]

[REDACTED]

76. [REDACTED]

[REDACTED]

[REDACTED].

77. I have also reviewed a notebook page from “Notebook SLB-128” dated [REDACTED] which shows investigations into removal of a 3'-O-azidomethyl group from nucleosides. In my opinion, this notebook page shows work by Solexa researchers in [REDACTED] to develop aqueous 3'-O-azidomethyl protecting group cleavage conditions for use in SBS. A review of these results is set forth below. In general, the 3'-O-azidomethyl protecting group cleavage involved using [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

78. Page 1 of Notebook SLB-128 describes [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

79. The cleavage yields and times of the 3'-O-azidomethyl-5'-OH nucleoside are significantly better than those for 3'-O-allyl and represent a substantial improvement over 3'-O-allyl, especially in the context of SBS. Furthermore, in my opinion, this greater cleavage efficiency of the 3'-O-

azidomethyl contributes significantly to advancements in achieving greater read lengths using SBS. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] This data demonstrates that Illumina's 3'-O-azidomethyl protecting group provided a significant advancement in high-throughput SBS relative to 3'-O-allyl cleavage by reducing cleavage time [REDACTED]. Thus, the cleavage efficiency afforded by the 3'-O-azidomethyl protecting group provides a substantial improvement in the context of SBS by allowing a longer read length and in a shorter amount of time relative to the 3'-O-allyl protecting group.

80. Thus, in my opinion, the use of the 3'-O-azidomethyl made possible longer read lengths in shorter time periods, which was unexpectedly superior to that which could be achieved with 3'-O-allyl.

81. In my opinion, the cleavage yield and time achieved with the 3'-O-azidomethyl are also far superior to what one of ordinary skill would have been expected in combining Tsien and Ju with Zavgorodny's teachings. As discussed

above, Zavgorodny disclosed that an azidomethyl protecting group could be cleaved using triphenylphosphine in aqueous pyridine. *Infra* at ¶ 41. The prior art taught that the efficiency of azidomethyl protecting group cleavage using triphenylphosphine ranges from 60-80%. Loubinoux at 5 (Ex. 1006). Thus, one of ordinary skill would have expected an azidomethyl group cleavage to be worse than the efficiency and speed that Solexa characterized for allyl group cleavage.

That the azidomethyl protecting group could be [REDACTED]

[REDACTED] was not expected in view of the prior art.

VIII. CONCLUSION

82. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statement were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statement may jeopardize the validity of the application or any patent issued thereon.

Dated: May 5, 2014

By: 

Floyd Romesberg, Ph.D