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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**

U.S. Patent 7,566,537

**ILLUMINA'S PATENT OWNER RESPONSE**

Columbia Exhibit 2008  
Illumina, Inc. v. The Trustees of Columbia  
University in the City of New York  
IPR2018-00322

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Pursuant to 35 U.S.C. § 316(a)(8) and 37 C.F.R. § 42.120, Illumina Cambridge Ltd. (“Illumina”) responds to the Revised Petition (Paper 7) filed by Intelligent Bio-Systems, Inc. (“IBS”) regarding Claims 1-6 and 8 of U.S. Patent No. 7,566,537 (“the ’537 Patent”).

## **I. Introduction**

Illumina is recognized as the undisputed leader and innovator in the DNA sequencing industry. Illumina has been referred to as the “smartest company in the world” because it “commercializes a truly innovative technology” and causes competitors “to refine or rethink their strategies.” MIT Tech. Review, 117:2 & 27-29 (2014) (Ex. 2004). Illumina’s novel and successful methods of labeling nucleic acid molecules for DNA sequencing use a reversible 3’-O-azidomethyl ether protecting group—a technology subsequently adopted by others, including IBS. *See* Document 71, IBS’s Answer ¶ 24, *Columbia v. Illumina*, No. 12-CV-00376 (D. Del) (admitting that IBS uses Illumina’s azido terminator) (Ex. 2005).

Indeed, where Illumina has succeeded, IBS has failed. For example, prior to adopting Illumina’s technology, IBS’s reversible terminator technology was not commercially viable and could not compete with Illumina’s 3’-O-azidomethyl nucleotides. *See* Interview of Qiagen’s<sup>1</sup> Dietrich Hauffe, Genomeweb, July 17,

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<sup>1</sup> Qiagen acquired IBS in June of 2012.

2013 (admitting that Qiagen “needed to change pretty much everything” that IBS had been working on; “the chemistry has changed, the mechanics have changed, the flow path has changed;” and admitting that “We are not HiSeq<sup>2</sup> competitive, that’s for sure.”) (Ex. 2084). After failing to develop its own technology, IBS now seeks to reap the benefits of Illumina’s labeling methods and reversible azido terminator technology before it is dedicated to the public.

IBS’s petition asserts that DNA sequencing using nucleotides with reversible azido protecting groups is obvious, yet IBS’s own recent arguments to the Patent Office in its pending patent applications assert the nonobviousness of such methods. For example, in response to an Examiner asserting that it would have been obvious to combine Dower, which teaches DNA sequencing methods, with Seo et al. or Zavgorodny et al. (2000), which allegedly teach 3’-OH azido and 3’-OH azidomethyl groups, IBS argued that “there is no basis for the combination” of these references. *See* U.S. Appl. No. 13/305,415, Response to Office Action at 9, filed Nov. 12, 2013 (Ex. 2006). IBS has also repeatedly claimed DNA sequencing methods using azido and azidomethyl protecting groups. *See* U.S. 2012/0052489 at Claims 4-5 (Ex. 2007); U.S. 2011/0124054 at Claims 3-4 & 21-22 (Ex. 2008); U.S.

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<sup>2</sup> The HiSeq instrument is Illumina’s flagship DNA sequencing instrument and, like all of Illumina’s sequencers, uses 3’-O-azidomethyl protected nucleotides.

2010/0159531 at Claims 12-13 (Ex. 2009). The Board should reject IBS's petition inspired arguments that contradict its previous positions before the Patent Office.

IBS fails to carry its burden of proving that Claims 1-6 and 8 of the '537 Patent would have been obvious to a person of ordinary skill in the art at the time of the invention based on the combination of Tsien or Ju with Zavgorodny. 35 U.S.C. § 316(e). IBS's petition merely revisits the same references that the Patent Office fully considered during prosecution of the '537 Patent, and over which the Examiner properly determined that Claims 1-6 and 8 were nonobvious. *See, e.g.*, '537 Patent Prosecution History Excerpts at 5 (Notice of Allowance, mailed April 30, 2009, considering Zavgorodny), 10 (Office Action, mailed May 16, 2008, citing Tsien), and 20 (Office Action, mailed October 30, 2007, citing Ju) (Ex. 2010). The Board should uphold the Examiner's appropriate issuance of these claims over Tsien, Ju and Zavgorodny.

IBS's petition also fails to consider several important teachings in Zavgorodny that render his azidomethyl ether and removal conditions incompatible with Tsien and Ju's express DNA sequencing requirements. In particular, IBS fails to consider that a person of ordinary skill in the art would have thought that:

- Zavgorodny's azidomethyl ether would interfere with DNA polymerases,
- Zavgorodny's removal conditions would not be quantitative or rapid,
- Zavgorodny's removal conditions would denature DNA, and

- Zavgorodny's azidomethyl ether would hinder reinitiation of DNA synthesis.

For any one of the foregoing reasons, a person of ordinary skill in the art would not have been motivated to combine Tsien or Ju with Zavgorodny with a reasonable expectation of success. Furthermore, the patentability of the challenged claims is supported by several secondary considerations of nonobviousness, including long-felt but unsolved need, unexpected results, and copying by others. When one considers all of the relevant evidence, there is only one reasonable conclusion—Claims 1-6 and 8 of the '537 Patent would not have been obvious to a person of ordinary skill in the art in August of 2002.

## **II. Illumina's Claimed Invention Includes a Novel, not Previously Considered 3'-OH Protecting Group for SBS**

In 1991, Tsien identified several criteria for a 3'-protecting group for use in DNA sequencing by synthesis ("SBS"). Tsien at 20:28-21:3, 23:27-24:5 (Ex. 1008). With this information as a guide, researchers immediately set about identifying nucleotide 3'-protecting groups compatible with Tsien's criteria. In 1994, the search for the ideal 3'-protecting group was described as a "formidable obstacle." Metzker et al., Nucl. Acids Res., 22:4259, 4259 (1994) (Ex. 2024, "Metzker"). The same remained true in 1999. Welch et al., Nucl. & Nucl., 18:197,

197-198 (1999) (Ex. 2028, “Welch”). Even as late as 2004, Shendure<sup>3</sup> acknowledged that “developing reversible terminators with the necessary properties has proved to be a difficult problem.” Shendure et al., Nat. Rev. Gen., 5:335, 341 (2004) (Ex. 2029, “Shendure”).

By August of 2002, at least 12 separate research groups had tried to find 3'-protecting groups that were ideal for use in DNA sequencing: (1) Tsien investigated at least 3'-O-allyl and various other groups (Tsien at 21:20-31; Ex. 1008); (2) Canard investigated at least 3'-O-anthranyloyl and 3'-O-anthranyl derivatives (Canard et al., PNAS, 92:10859, 10859; Ex. 2019, “Canard”); (3) Dower contemplated at least “acetyl, tBOC, NBOC, and NVOC” 3'-OH protecting groups (U.S. 5,547,839 at 25:47-51; Ex. 2030); (4) Welch investigated 3'-O-(2-nitrobenzyl) groups with fluorescent labels (Welch at 198; Ex. 2028); (5) Stemple investigated at least 3'-O-(2-nitrobenzyl) groups (WO 00/53805 at 13:18-20; Ex. 2031); (6) Odedra investigated at least acetyl and phenylacetamide groups (WO

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<sup>3</sup> Shendure highlighted Solexa's reversible terminators as “exciting” progress in the field due to “impressive signal-to-noise ratio.” Shendure at 341. Illumina acquired Solexa's reversible terminator patent portfolio in 2006, including the reversible 3'-O-azidomethyl terminators that are the subject of this proceeding. Press Release, Illumina Signs Agreement to Acquire Solexa (Nov. 13, 2006) (Ex. 2078).

01/92284 at Figs. 2 & 11; Ex. 2032); (7) Metzker investigated 3'-O-methyl, 3'-O-acetyl, 3'-O-allyl, 3'-O-tetrahydropyran, 3'-O-4-nitrobenzoyl, 3'-O-2-aminobenzoyl, and 3'-O-2-nitrobenzyl groups (Metzker at 4263; Ex. 2024); (8) Cheeseman investigated at least 3'-O-succinyl and 3'-O-anhydride derivatives (U.S. 5,302,509 at 4:50-63; Ex. 2033); (9) Kwiatkowski investigated at least 3'-O-hydrocarbyldithiomethyl and 3'-O-"functionalized acetal" groups (U.S. 7,279,563 at 2:40-60, Ex. 2034; WO 96/023807 at 15:11-16:24, Ex. 2035); (10) Rosenthal investigated 3'-blocking groups that "generate a 3' hydroxyl group" (WO 93/21340 at 11:3-10; Ex. 2036); (11) Rabani investigated at least a 3'-O-(4-benzoyl)benzoyl group (WO 96/27025 at 2:15-21; Ex. 2037); and (12) Ju investigated at least 3'-O-allyl and 3'-O-methoxymethyl groups (*see, e.g.*, Ju at Fig. 7 (Ex. 1002)). None of these researchers investigated or even suggested a protecting group comprising an azido group that can be modified or removed to expose a 3'-OH group.

These research efforts showed that Tsien's 3'-O-allyl group, as well as a few other groups, were reversible protecting groups that could be effectively incorporated by polymerases. Ex. 1008 at 24:29; Metzker at 4263 (Ex. 2024); Ju at 3:7-22 (Ex. 1002); Manteia I at 9-12 (Ex. 2085); Manteia II at 6, 15 (Ex. 2086). Dr. Ju and Columbia University continued to use Tsien's 3'-O-allyl group through at least 2006. Ju et al., PNAS, 103:19635 (2006) (Ex. 2038, "Ju PNAS").

Despite the aforementioned research efforts, only Illumina identified the azidomethyl group as a protecting group for DNA sequencing. '537 Patent at 19:49-20:4 (Ex. 1001). Illumina found that the azidomethyl protecting group was unexpectedly incorporated by polymerases and was surprisingly cleaved in rapid and quantitative yield under conditions that permit serial polymerase incorporation through numerous rounds of DNA sequencing. These properties were not known at the time the '537 Patent was filed, and no knowledge in the art would have led a person of ordinary skill to expect that an azidomethyl group could be as successful as Illumina later demonstrated. *Eisai Co. v. Dr. Reddy's Labs., Ltd.*, 533 F.3d 1353, 1359 (Fed. Cir. 2008). To date, Illumina's 3'-O-azidomethyl group remains the unsurpassed industry leader even though 12 years have passed since the '537 Patent was filed.

Notably, IBS's expert, Dr. Branchaud, was unaware of anyone other than Solexa suggesting the use of azidomethyl protecting groups until IBS/Ju started using them in 2008. Branchaud '517 Tr. at 162:12-163:22 (Ex. 2039); *see also, infra* Section X.3. But IBS/Ju's first use of azidomethyl groups in SBS came many years after Solexa first published and suggested the use of azidomethyl groups in SBS (*see, e.g.,* Guo et al., PNAS, 105:9145 (2008) (Ex. 2058)), and was not the result of independent invention, as discussed in detail *infra. See, e.g.,* Section X.3; *see also* *Leo Pharm. Prods., Ltd. v. Rea*, 726 F.3d 1346, 1353, 1359 (Fed. Cir.

2013). In view of the above, the nonobviousness of Illumina's azido protecting group in Claims 1-6 and 8 of the '537 Patent should be affirmed.

### **III. The Law of Nonobviousness**

The petitioner bears the burden of proving obviousness. 35 U.S.C. § 316(e).

The analysis under 35 U.S.C. § 103 is objective:

[T]he scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved. Against this background the obviousness or nonobviousness of the subject matter is determined. Such secondary considerations as commercial success, long felt but unsolved needs, failure of others, etc., might be utilized to give light to the circumstances surrounding the origin of the subject matter sought to be patented.

*Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966).

“[A] patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art.” *KSR Int’l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007). Rather, there must be “an apparent reason to combine the known elements in the fashion claimed by the patent at issue.” *Id.* In other words, a patent claim is not obvious unless “a skilled artisan would have been motivated to combine the teachings of the prior art references to achieve the claimed invention, and that the skilled artisan would have had a reasonable expectation of success in doing so.” *ActiveVideo Networks, Inc. v.*



*Verizon Commuc'ns, Inc.*, 694 F.3d 1312, 1327 (Fed. Cir. 2012).

Moreover, the chemical arts are inherently unpredictable and, therefore, a reasonable expectation of success is harder to achieve. *Eisai*, 533 F.3d at 1359 (“To the extent an art is unpredictable, as the chemical arts often are, *KSR*’s focus on these ‘identified predictable solutions’ may present a difficult hurdle because potential solutions are less likely to be genuinely predictable.”).

“A factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon *ex post* reasoning.” *KSR*, 550 U.S. at 421 (citing *Graham*, 383 U.S. at 36). “It is difficult but necessary that the decisionmaker forget what he or she has been taught . . . about the claimed invention and cast the mind back to the time the invention was made (often as here many years), to occupy the mind of one skilled in the art . . . .” *W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1553 (Fed. Cir. 1983).

#### **IV. The Person Of Ordinary Skill in the Art**

Illumina submits that the person of ordinary skill at the time of the invention would have been a member of a team of scientists addressing SBS product development. Such a person 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 includes a person having a doctoral degree in a field related to chemistry, and

at least five years of laboratory experience directed toward the research and development of DNA sequencing technologies.<sup>4</sup> Romesberg Decl. ¶ 26 (Ex. 2011).

## **V. Grounds Of This Proceeding**

The Board instituted this proceeding on the following grounds:

- Claims 1-6 and 8 under 35 U.S.C. § 103(a) over Tsien and Zavgorodny;
- Claims 1-6 and 8 under 35 U.S.C. § 103(a) over Ju and Zavgorodny; and
- Claim 3 under 35 U.S.C. § 103(a) over Tsien, Zavgorodny, and Prober.

Paper 16 at 15. As detailed below, IBS has failed to meet its burden because its petition did not address several issues that would have led a person of ordinary skill in the art to expect that Zavgorodny's removal conditions would be incompatible with the explicit requirements of Tsien and Ju's DNA sequencing methods. Thus, the complete record shows that the labeling methods of Claims 1-6 and 8 of the '537 Patent would not have been obvious to one of ordinary skill in the art in view of Tsien or Ju in combination with Zavgorodny.

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<sup>4</sup> IBS's petition fails to address the level of ordinary skill in the art necessary to support its obviousness arguments. 35 U.S.C. § 103; *Graham*, 383 U.S. at 17. Thus, IBS's assertions regarding the obviousness of Claims 1-6 and 8 of the '537 Patent lack foundation, and IBS fails to carry its burden in this proceeding.

**VI. Tsien's 3'-Azido Group Cannot Be Removed To Expose A 3'-OH Group**

In instituting this proceeding, the Board states that “Tsien discloses using an azido-containing protecting group.” Paper 16 at 11 (*citing* Tsien at 21:12-17). The azido group disclosed in Tsien, however, is not a removable protecting group that will expose a 3'-OH group as required by Claims 1-6 and 8. Instead, the azido group disclosed by Tsien is a 3'-N<sub>3</sub> blocking group that is directly attached to the 3'-carbon of the nucleotide. Romesberg Decl. ¶ 39 (Ex. 2011). Because the Tsien N<sub>3</sub> blocking group is directly attached to the 3'-carbon, its cleavage would not expose a 3'-OH group. *Id.* IBS' expert, Dr. Branchaud, agrees and correctly testified that Tsien's N<sub>3</sub> group is not cleavable to expose a 3'-OH group:

Q. Would a person of ordinary skill in the art have understood what the term “cleavable” meant at the time of the Tsien patent?

A. I think there are a couple of different definitions of “cleavable.” That's why I'm asking the question.

Q. Okay. And what are those potential definitions?

A. First -- okay. One is can you remove the group; the other is can the group be removed to leave a 3' hydroxyl at that position. The second definition is relevant to this topic in general. The first one is more of just a general chemical question.

Q. Yes. Okay. So using your second definition --

A. Yes.

Q. -- which of the blocking groups in Tsien are not cleavable?

A. By that definition, it would be the F, the NH<sub>2</sub>, the N<sub>3</sub>, and the

NHCOCH<sub>3</sub>. The other ones would be cleavable by this second definition to leave a free 3' hydroxyl.

Q. Okay. Now, is the N<sub>3</sub> group cleavable to regenerate a 3'-OH?

A. If, as it's stated here, the N<sub>3</sub> is directly attached to the carbon, which is the way I read it, then that N<sub>3</sub> group could be reacted chemically, but it would not generate a 3' hydroxyl.

Branchaud '128 Tr. at 100:1-101:6 (Ex. 2081) (emphases added). Dr. Romesberg confirms this admission by Dr. Branchaud. Romesberg Decl. ¶ 39 (Ex. 2011). For this reason, Tsien's 3'-N<sub>3</sub> blocking group does not teach an azido protecting group that can be modified or removed to expose a 3'-OH group.

Furthermore, Tsien's disclosure of the non-removable 3'-N<sub>3</sub> blocking group is imbedded in a list of several other potential nucleotide blocking modifications, including other non-removable groups, such as -F, -NH<sub>2</sub>, and -NHCOCH<sub>3</sub>. Tsien at 21:12-17 (Ex. 1008); Romesberg Decl. ¶ 38 (Ex. 2011). Tsien provides no expectation that the 3'-N<sub>3</sub> non-removable blocking group could ever be modified or removed to expose a 3'-OH group as required by Illumina's claims. '537 Patent at 19:57-58 (Ex. 1001). As a result, a person of ordinary skill in the art would not have been motivated to use the non-removable 3'-N<sub>3</sub> group of Tsien to arrive at the instant claims.

## **VII. Tsien's Three 3'-Blocking Group Criteria Are Incompatible With Zavgorodny's Azidomethyl Ether And Removal Conditions**

Tsien is directed to DNA sequencing by synthesis ("SBS"). IBS Revised

Petition at 12 (Paper 7); Branchaud Decl. ¶ 74 (Ex. 1011); Tsien at 6:26-7:3 (Ex. 1008); Romesberg Decl. ¶ 36 (Ex. 2011). Tsien does not teach a protecting group comprising an azido group that can be modified or removed to expose a 3'-OH group. Nor would it have been obvious to the skilled artisan to combine Tsien with Zavgorodny to arrive at the claimed methods. One of ordinary skill in the art would not have been motivated to modify Tsien to incorporate Zavgorodny's azido group because such a modification would not have been expected to work for its intended purpose. *Institut Pasteur v. Focarino*, 738 F.3d 1337, 1346 (Fed. Cir. 2013). Tsien sets forth three specific goals or criteria that must be achieved by a 3'-protecting group for it to work successfully in Tsien's DNA sequencing method:

(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); IBS Revised Petition at 37 (Paper 7); Branchaud Decl. ¶ 76 (Ex. 1011).

Zavgorodny (Ex. 1004) is a general organic chemistry article that was published in 1991. Zavgorodny discloses over 180 compounds that are described as "useful specifically blocked synthons." Zavgorodny at 7595 (Ex. 1004); Romesberg Decl. ¶ 40 (Ex. 2011). Dr. Branchaud testified that Zavgorodny's

compounds have no specified use (Branchaud ‘517 Tr. at 105:23-106:3 (Ex. 2039)), that he does not know the intended purpose of Zavgorodny’s compounds (*id.* at 104:10-14), and that Zavgorodny does not teach DNA sequencing methods. *Id.* at 106:4-6. In contrast, Dr. Romesberg explains why the context of Zavgorodny suggests that it was directed to single-stranded oligonucleotide manufacturing, and would not be understood to be useful in SBS. Romesberg Decl. ¶ 40 (Ex. 2011). As discussed below, Zavgorodny’s azido group does not meet any of Tsien’s stated goals or criteria for a blocking group.

**1. A Person Of Ordinary Skill In The Art Would Not Have Expected Zavgorodny’s Azidomethyl Group To Be Accurately Or Efficiently Incorporated By A Polymerase**

Tsien’s first criteria for a 3’-protecting group is “the ability of a polymerase enzyme to accurately and efficiently incorporate the dNTPs carrying the 3’-blocking groups into the cDNA chain.” Tsien at 20, ll. 28-33 (Ex. 1008). A person of ordinary skill in the art would not have expected Zavgorodny’s 3’-O-azidomethyl ether to be accurately or efficiently incorporated by a polymerase at least because (a) the prior art teaches that the electrophilic characteristics of an azido group would react with strong nucleophiles within the polymerase active site, and (b) similar 3’-O-protecting groups were known to interfere with polymerase incorporation. Romesberg Decl. ¶¶ 31, 58 (Ex. 2011).

**a. The Prior Art Teaches That The Electrophilic Characteristics Of An Azido Group Would React With Strong Nucleophiles In The Polymerase Active Site**

The prior art at the time of the invention taught that the electrophilic characteristics of Zavgorodny's azidomethyl group would likely react with strong nucleophiles within the polymerase active site, thereby preventing polymerase activity. Romesberg Decl. ¶ 31 (Ex. 2011). For example, Ju warns 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 3:15-20 (Ex. 1002); Branchaud '517 Tr. at 73:12-74:23 (Ex. 2039). Ju relies on Canard for this warning. Ju at col. 3, ll. 15-20 (*citing* "Canard et al. 1995"); *see also* Canard at 10863 ("the polymerase-bound 3' end of DNA lies in the vicinity of a powerful nucleophilic group in the active site") (Ex. 2019). A person of ordinary skill would have expected an azido group to have similar electrophilic characteristics to ketone groups. Romesberg Decl. ¶ 31 (Ex. 2011). For example, Canard 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 at 10863 (Ex. 2019). Thus, according to Canard, azido groups contain precisely the type of electrophilic center that Ju teaches is unsuitable for use with polymerases. Ju at 3:16-20 (*citing* "Canard et al. 1995")

(Ex. 1002); Romesberg Decl. ¶ 31 (Ex. 2011). As a result of this teaching, a person of ordinary skill would have expected that the electrophilic characteristics of Zavgorodny's azidomethyl group were incompatible with the polymerase used in Tsien's sequencing method, while still maintaining the ability to be an effective protecting group. Accordingly, a skilled artisan would have been dissuaded from using the electrophilic azidomethyl group of Zavgorodny in Tsien's methods of labeling and sequencing. *DePuy Spine, Inc. v. Medtronic Sofamor Danek, Inc.*, 567 F.3d 1314, 1326 (Fed. Cir. 2009) (explaining that a claim is nonobvious when "the prior art indicated that the invention would not have worked for its intended purpose or otherwise taught away from the invention.") (citing *United States v. Adams*, 383 U.S. 39, 52 (1966)). For at least this reason, a person of skill would not have expected Zavgorodny's azidomethyl group to achieve Tsien's first stated goal of having a polymerase enzyme that accurately and efficiently incorporated dNTPs carrying the 3'-blocking group into a cDNA chain.

**b. Removable 3'-O-Protecting Groups Were Known To Interfere With Accurate And Efficient Polymerase Incorporation**

Without any supporting evidence, IBS and its expert assert that an azidomethyl group would have been expected to "not interfere[] with recognition of the modified nucleotide by polymerase" simply because Zavgorodny's azidomethyl ether can be removed with triphenylphosphine. IBS Revised Petition



at 38-39 (Paper 7); Branchaud Decl. ¶ 81 (Ex. 1011). IBS's unsupported assertion is insufficient to carry its burden in this proceeding. A person of ordinary skill would not have expected polymerase incorporation of nucleotides to correlate to whether a protecting group is removable with triphenylphosphine. Romesberg Decl. ¶ 58 (Ex. 2011). For example, 3'-O-acetyl esters are removable with a triphenylphosphine. Yoshimoto et al., Chem. Lett., 30:934, Table 2 (2001) (Ex. 2025, "Yoshimoto"). Metzker, however, reports that nucleotides bearing removable 3'-O-protecting groups (including a 3'-O-acetyl ester group) would interfere with the recognition of several polymerases. Metzker at 4263 (Ex. 2024). Because it was known that other removable 3'-O-protecting groups would interfere with polymerase incorporation, a person of skill would not have expected a nucleotide incorporating a 3'-O-azidomethyl ether to show accurate and efficient polymerase incorporation, simply because Zavgorodny's 3'-O-azidomethyl ether is removable with triphenylphosphine, as IBS alleges. Romesberg Decl. ¶ 58 (Ex. 2011).

For all of the above reasons, IBS did not establish that modifying Tsien's labeling and sequencing methods to incorporate Zavgorodny's 3'-O-azidomethyl ether would be expected to meet Tsien's criteria for a blocking group that is accurately and efficiently incorporated by a polymerase. Thus, the methods recited in Claims 1-6 and 8 would not have been obvious to one of ordinary skill in the art.

**2. A Person of Ordinary Skill In The Art Would Not Have Expected Zavgorodny's Removal Conditions To Meet Tsien's Requirement for Mild, Rapid, and Quantitative Deblocking**

Tsien's second criteria for a 3'-protecting group is "the availability of mild conditions for rapid and quantitative deblocking." Tsien at 20:28-21:3 (Ex. 1008). A person of ordinary skill in the art would not have expected Zavgorodny's 3'-O-azidomethyl group removal conditions to satisfy this criteria at least because: (a) Zavgorodny's removal conditions are triphenylphosphine in aqueous pyridine, (b) Zavgorodny's removal conditions denature DNA and cannot be "mild" within Tsien's DNA sequencing method, and (c) Zavgorodny's removal conditions would not be quantitative or rapid. Romesberg Decl. ¶¶ 44-56 (Ex. 2011).

**a. Zavgorodny's Removal Conditions Are "Triphenylphosphine In Aqueous Pyridine At 20 °C"**

Zavgorodny states that "[a]zidomethyl is of special interest since it can be removed under very specific and mild conditions, viz. with triphenylphosphine in aqueous pyridine at 20 °C." Zavgorodny at 7595 (emphasis added) (Ex. 1004). A person of ordinary skill in 2002 would have understood that "viz" means "that is to say; namely," and that "viz" is used to explain a previous statement. *See, e.g.*, Merriam-Webster's Collegiate Dictionary, 10th Edition, 1997, p. 1316 ("that is to say; namely") (Ex. 2040); *see also* Ex. 2041; Romesberg Decl. ¶ 41 (Ex. 2011).

During cross examination, IBS's expert, Dr. Branchaud, admitted that he did not know what "viz." means. Instead, his opinions were based on an incorrect

understanding of “viz” meaning “for example.”

Q. Do you know what the term viz, V-I-Z, means?

A. Not specifically. I suspect it means for example, but – well, actually I don’t know. I read it to mean using that as an example.”

Branchaud Tr. ‘517 at 108:16-20 (Ex. 2039). Contrary to Dr. Branchaud’s misunderstanding, a person of ordinary skill in the art would have understood that Zavgorodny’s azidomethyl removal conditions were specifically “triphenylphosphine in aqueous pyridine at 20 °C.” Romesberg Decl. ¶ 41 (Ex. 2011); Zavgorodny at 7595 (Ex. 1004).

**b. Zavgorodny’s Aqueous Pyridine Removal Conditions Would Denature DNA And, Thus, Cannot Be “Mild” Within The Context Of Tsien’s DNA Sequencing Method**

IBS’s petition provides no evidence that Zavgorodny’s “mild” removal conditions would meet Tsien’s requirement for “mild” deprotection. In fact, the evidence shows that (i) Zavgorodny’s removal conditions would have been expected to denature DNA because pyridine is an especially effective DNA denaturing agent, and (ii) Zavgorodny’s general description of its removal conditions as “mild” would not apply to the specific criteria required for Tsien’s DNA sequencing method. Romesberg Decl. ¶¶ 54-56 (Ex. 2011).

**i. Zavgorodny’s Removal Conditions Using Pyridine Would Denature The DNA In Tsien’s Sequencing Method**

Tsien states that its deblocking methods should “not interfere with further

enzyme function or denature the DNA strand.” Tsien at 23:27-24:5 (Ex. 1008); *see also id.* at 24:22-23 (“care must be taken not to denature the DNA”). Indeed, it was well known in the art at the time of invention that 3’-protecting group removal conditions should not denature DNA. *See, e.g.*, Welch at 197-198 (Ex. 2028); Ju at 21:3-16 (Ex. 1002); Romesberg Decl. ¶ 55 (Ex. 2011).

Zavgorodny’s removal conditions use triphenylphosphine in aqueous pyridine. Ex. 1004 at 7595. Pyridine is an especially effective DNA denaturing agent. *See, e.g.*, Kit, *Annu. Rev. Biochem.*, 32:43, 57-58 (1963) (stating that “[a]romatic reagents, such as phenols and pyridine are especially effective” DNA denaturants) (Ex. 2018, “Kit”); Romesberg Decl. ¶ 55 (Ex. 2011). Zavgorodny’s removal conditions require 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); Romesberg Decl. ¶ 54 (Ex. 2011). Moreover, replacing Zavgorodny’s pyridine with a different organic co-solvent would not render Zavgorodny’s removal conditions compatible with Tsien’s sequencing method because most organic co-solvents are also known to denature DNA. *See, e.g.*, Lee et al., *PNAS*, 78:2838, 2840 (1981) (“unwinding of the DNA duplex caused by dehydration is likely to be a common phenomenon in organic solvents”) (Ex. 2021); Romesberg Decl. ¶ 55 (Ex. 2011). Therefore, one of ordinary skill in the art would not have been motivated by Zavgorodny to use an

azidomethyl group as a blocking group in Tsien's sequencing method. Romesberg Decl. ¶ 55 (Ex. 2011).

Dr. Branchaud admits that he did not know that pyridine and other organic solvents denature DNA, and that he did not consider the denaturing effects of pyridine in rendering his opinion in this proceeding. Branchaud '517 Tr. at 113:2-24 (Ex. 2039). A person of ordinary skill in the art would have considered the strong denaturing effects of Zavgorodny's pyridine, and would have expected Zavgorodny's removal conditions to be incompatible with Tsien's deblocking criteria. Romesberg Decl. ¶ 55 (Ex. 2011). IBS did not establish that Zavgorodny's removal conditions were compatible with Tsien's deblocking criteria, and the prior art indicates that combining Zavgorodny's removal conditions with Tsien's DNA sequencing method would produce an inoperable result. *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"). Thus, a person of ordinary skill in the art would not have a reasonable expectation of successfully modifying the sequencing methods in Tsien to use the azidomethyl group of Zavgorodny. Romesberg Decl. ¶ 55 (Ex. 2011).

**ii. Zavgorodny's "Mild" Conditions Would Not Have Been "Mild" In The Context Of Tsien's DNA Sequencing Method**

Zavgorodny's "mild" conditions are triphenylphosphine in aqueous pyridine.

Zavgorodny at 7595 (Ex. 1004); Romesberg Decl. ¶ 56 (Ex. 2011); *supra* Section VII.2.a. IBS asserts, without even a cursory analysis, that Zavgorodny's "mild conditions" would have been mild in Tsien's DNA sequencing methods. IBS Revised Petition at 38-39 (Paper 7). IBS's assertion fails because, as their expert testified, reaction conditions can be mild in one context, but not mild in another. Branchaud '128 Tr. at 85:8-86:1 ("Mild for one thing would not be mild for another.") (Ex. 2081), *id.* at 85:8-86:1 ("mild conditions" is "some generic term, I don't really know what you mean by that except that it's on the mild side of things versus harsh."); *see also* Romesberg Decl. ¶ 56 (Ex. 2011).

IBS's position is undermined by its own example of hydrogenolysis with Pd/C and H<sub>2</sub> as a supposedly "mild reductive condition" for Tsien's sequencing method. IBS Revised Petition at 41 (Paper 7). Hydrogenolysis using Pd/C and H<sub>2</sub> cannot be mild for Tsien's DNA sequencing method because hydrogenolysis chemically degrades pyrimidine bases in DNA. *See, e.g.*, Christensen et al., J. Org. Chem., 37:3398, 3401 (1972) (Ex. 2022); Watkins et al., J. Am. Chem. Soc., 104:5702, 5705 (1982) (Ex. 2023); Branchaud '517 Tr. at 76:4-8 (Ex. 2039); Romesberg Decl. ¶ 56 (Ex. 2011). In fact, various conditions that might be acceptable in some contexts are not acceptable in the DNA sequencing context. Romesberg Decl. ¶ 47 (Ex. 2011).

As previously discussed, Zavgorodny's removal conditions use pyridine,

which cannot be “mild” for Tsien’s DNA sequencing method because it denatures DNA. Kit at 57-58 (Ex. 2018); Branchaud ‘517 Tr. at 76:4-8 (Ex. 2039); Romesberg Decl. ¶ 55 (Ex. 2011). Furthermore, the prior art teaches that it is difficult to cleave ethers under conditions compatible with DNA. *See* Canard et al., *Gene*, 148:1, 4-5 (1994) (“We reasoned that ether bonds would be hard to cleave under mild conditions compatible with DNA chemical stability... .”) (Ex. 2043). Thus, the difficulty in cleaving ether bonds with DNA compatible conditions would have lead a person of ordinary skill in the art to expect that Zavgorodny’s 3’-O-azidomethyl ether would not cleave under conditions compatible with Tsien’s DNA sequencing methods. Romesberg Decl. ¶ 55 (Ex. 2011). For at least the foregoing reasons, the prior art indicates that Zavgorodny’s removal conditions are not mild in the context of DNA sequencing and would be incompatible with Tsien’s requirement for mild deblocking of the 3’-protecting group.

**c. Zavgorodny’s Removal Conditions Would Not Have Been Expected To Be Quantitative Or Rapid**

Tsien’s second criteria for the 3’-protecting group requires “rapid and quantitative deblocking.” Tsien at 20:28-21:3 (Ex. 1008). IBS’s petition fails to provide any empirical data to establish that Zavgorodny’s removal conditions proceed quantitatively or rapidly. The evidence shows that (i) Loubinoux’s azidomethyl ether removal efficiency of 60-80% provides an expectation that

Zavgorodny's azidomethyl ether would not proceed quantitatively, (ii) the prior art repeatedly reports non-quantitative azido conversion using triphenylphosphine in pyridine and water, and (iii) the prior art indicates that azido conversion using triphenylphosphine in pyridine and water would not proceed rapidly.

**i. Loubinoux's Phenolic Azidomethyl Ether Removal Efficiency Of 60-80% Provides An Expectation That Zavgorodny's Aliphatic Azidomethyl Ether Would Not Cleave Quantitatively**

Tsien's second criteria for the 3'-protecting group requires "quantitative deblocking." Tsien at 20:28-21:3 (Ex. 1008). A person of ordinary skill in the art would understand that quantitative means essentially 100% yield. Branchaud '517 Tr. at 98:8-16 (Ex. 2039). Loubinoux reports 60-80% removal efficiency for phenolic azidomethyl groups using triphenylphosphine in tetrahydrofuran and water at 25 °C.<sup>5</sup> Ex. 1006 at 4-5 ("Regardless of the reduction method used, [the products] are obtained as pure products at a yield between 60 and 80%."); Romesberg Decl. ¶ 44 (Ex. 2011).

A person of ordinary skill in the art would have expected Zavgorodny's aliphatic azidomethyl ether to be removed with lower efficiency than the highly variable 60-80% efficiency for Loubinoux's aromatic azidomethyl ethers because

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<sup>5</sup> IBS's petition omits the low cleavage efficiencies reported by Loubinoux using triphenylphosphine. IBS Revised Petition at 29, 42 (Paper 7).



phenolic ethers are generally “more easily cleaved than the analogous ethers of simple alcohols.” Chapter 3 from Greene & Wuts at 248 (Ex. 2026);<sup>6</sup> Romesberg Decl. ¶ 48 (Ex. 2011). Indeed, Greene & Wuts (the leading treatise on protecting groups, *see* Branchaud ‘266 Tr. at 210:5-14 (Ex. 2083)) suggests that azidomethyl protecting groups would not be an acceptable protecting group for aliphatic hydroxyls by excluding the azidomethyl group from the aliphatic chapter (Chapter 2), but including it in the aromatic chapter (Chapter 3). *Compare* Ex. 1005 at 17-23 *with* Ex. 2026 at 246-248; Romesberg Decl. ¶ 49 (Ex. 2011). As Dr. Branchaud explained during cross-examination, phenolic ethers are more easily cleaved than aliphatic ethers because phenols are better leaving groups than aliphatic alcohols. Branchaud ‘517 Tr. at 166:23-168:11, 140:13-24 (Ex. 2039); Romesberg Decl. ¶ 48 (Ex. 2011). Therefore, one of ordinary skill in the art would have expected Zavgorodny’s removal efficiency to be even lower than Loubinoux’s 60-80% removal efficiency. Romesberg Decl. ¶ 48 (Ex. 2011).

In addition, Loubinoux reports highly variable removal efficiencies for phenolic azidomethyl ethers using hydrogenolysis and stannous chloride. Loubinoux at 7, 11 (Ex. 1006). Although also yielding highly variable efficiencies, hydrogenolysis (*id.* at 5) and stannous chloride (*id.* at 11) provide higher removal

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<sup>6</sup> IBS omits page 248 of Greene & Wuts from Exhibit 1005.

efficiencies than triphenylphosphine. Romesberg Decl. ¶ 44 (Ex. 2011). Therefore, Loubinoux's highly variable removal efficiencies for hydrogenolysis and stannous chloride are superior to triphenylphosphine, and a person of ordinary skill in the art would not have expected that Zavgorodny's teachings would lead to cleavage with the quantitative efficiency required for Tsien's DNA sequencing methods. Romesberg Decl. ¶ 44 (Ex. 2011). *Eisai*, 533 F.3d at 1359.

Dr. Branchaud overlooks the 63-67% hydrogenolysis yields reported in Loubinoux and selects the one 95% hydrogenolysis yield to assert that he would expect azidomethyl cleavage efficiency to be high. Branchaud '517 Tr. at 171:10-172:12 (Ex. 2039). Loubinoux reached the opposite conclusion. Loubinoux at 5 (Ex. 1006). Dr. Branchaud's assertion also ignores the more relevant triphenylphosphine yields of 60-80%. *Id.* at 4-5. And as discussed above, removal by hydrogenation is not compatible with Tsien's DNA sequencing method, nor is removal by stannous chloride. Romesberg Decl. ¶¶ 46-47 (Ex. 2011). And cleavage by phosphine would be expected to be less efficient than by hydrogenation or stannous chloride. *Id.* ¶¶ 44, 47.

**ii. The Prior Art Repeatedly Reports Non-Quantitative Azido Conversion Using Triphenylphosphine In Pyridine And Water, And Provides An Expectation That Zavgorodny's Azidomethyl Ether Removal Conditions Would Not Be Quantitative**

IBS's petition fails to establish that Zavgorodny's removal conditions using

triphenylphosphine in aqueous pyridine would meet Tsien's quantitative deblocking criteria. This is not surprising, since the prior art indicates that triphenylphosphine in pyridine with water would not quantitatively convert a nucleoside-related compound bearing an azido group to an amino group, let alone that the much more complicated azidomethyl protecting group removal could be accomplished efficiently to regenerate a 3'-OH. Romesberg Decl. ¶ 50 (Ex. 2011).

In fact, numerous references indicate that the efficiency of azido to amino conversion using triphenylphosphine in pyridine is highly variable and unpredictable, with yields that are significantly less than quantitative (as low as 50%), and that a very long time (*i.e.*, from 3 to 72 hours) is required to realize these non-quantitative yields. Romesberg Decl. ¶ 50 (Ex. 2011); Ranganathan et al., Tet. Lett., 45:4341, 4343 (1978) (Ex. 2013); Mungall et al., J. Org. Chem., 40:1659, 1662 (1975) (Ex. 2014, "Mungall"); U.S. 5,959,089 at 16:1-35, 20:43-21:8 (Ex. 2015); Pilard et al., Tet. Lett., 25:1555-1556 (1984) (Ex. 2016); Tietze et al., Angew. Chem. Int. Ed., 36:1615, 1616 (1997) (Ex. 2017).

The conversion of an azido group to an amino group using triphenylphosphine is similar to the first of two steps required to convert Zavgorodny's azidomethyl ether to an aminomethyl ether. Branchaud '517 Tr. at 110:12-23 (Ex. 2039); Romesberg Decl. ¶ 50 (Ex. 2011). However, deprotection of Zavgorodny's azidomethyl ether requires a second and additional hydrolysis step.

*Id.* Therefore, the low yields reported for triphenylphosphine conversion of nucleoside-related azido compounds would have led a person of ordinary skill in the art to have expected that Zavgorodny's azidomethyl group removal conditions would be even less efficient, and not achieve the quantitative deprotection required by Tsien. Romesberg Decl. ¶¶ 50, 53 (Ex. 2011). In addition, the high variability of azide conversion would have led a person of ordinary skill in the art to conclude that an azidomethyl ether would be removed with unpredictable efficiency. *Id.* ¶ 53; *Eisai*, 533 F.3d at 1359.

Furthermore, Mungall reports lower azide conversion efficiency for nucleotides (78%) than for nucleosides (88-90%). Mungall at 1661-62 (Ex. 2014); Romesberg Decl. ¶ 51 (Ex. 2011). Tsien's DNA sequencing methods use nucleotides. Tsien at 20:28-32 (Ex. 1008). Therefore, the prior art would have led a person of ordinary skill in the art to expect Zavgorodny's nucleoside<sup>7</sup> removal

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<sup>7</sup> IBS and its expert repeatedly mischaracterize Zavgorodny as disclosing "modified nucleotides." IBS Revised Petition at 13, 20, 21, 22, 33, 34, 36, 47 (Paper 7) (emphasis added); *see also* Branchaud Decl. ¶¶ 49, 105 (Ex. 1011). IBS's petition is factually incorrect and misleading, and their expert's testimony should be given little to no weight. Fed. R. Evid. 705; 37 C.F.R. § 42.65; *Rohm & Haas Co. v. Brotech Corp.*, 127 F.3d 1089, 1092 (Fed. Cir. 1997).

conditions to proceed with relatively low yields if combined with Tsien's nucleotide sequencing methods. Romesberg Decl. ¶¶ 52-53 (Ex. 2011); Branchaud '517 Tr. at 67:19-69:8 (Ex. 2039); *In re ICON*, 496 F.3d at 1382. As a result, IBS fails to establish that a person of ordinary skill would expect Zavgorodny's removal conditions to meet Tsien's quantitative deprotection requirement. *Institut Pasteur*, 738 F.3d at 1346.

**iii. The Prior Art Data Indicates That Azido To Amino Conversion Using Triphenylphosphine In Pyridine And Water Would Not Proceed Rapidly**

Tsien's second criteria for the 3'-protecting group also requires "rapid" deblocking. Tsien at 20:28-21:3 (Ex. 1008). The literature, however, indicates that it takes a long time (between 3 to 72 hours) for nucleoside-related compounds bearing an azido group to be converted to an amino group using triphenylphosphine in pyridine and water. *See supra* Section VII.2.c.i; Romesberg Decl. ¶¶ 50, 53 (Ex. 2011); Loubinoux at 7 (7-10 hours) (Ex. 1006). Making matters worse, deprotection of a 3'-azidomethyl group requires additional steps that would have been expected to take even more time. These results would not lead a person of ordinary skill in the art to expect that Zavgorodny's removal conditions would achieve Tsien's rapid deprotection requirement. Romesberg Decl. ¶ 50 (Ex. 2011); *DePuy Spine*, 567 F.3d at 1326. Thus, IBS fails to establish that Zavgorodny's removal conditions would meet Tsien's criteria for at least this

additional reason.

**iv. Zavgorodny's Removal Conditions Do Not Meet Tsien's Requirements For Quantitative And Rapid Cleavage**

During redirect, Dr. Branchaud testified that a person of ordinary skill in the art would discard Zavgorodny's cleavage conditions and conduct new experiments to "find a set of reductive cleavage conditions that would be compatible with all the other parts of the work flow and -- and provide a suitable result." Branchaud '517 Tr. at 180:16-182:1 (Ex. 2039). Dr. Branchaud's analysis discounts the complexity of alternatives facing a person of ordinary skill in the art in 2002, and invokes improper hindsight to retrace Illumina's path to reach an inappropriate obviousness conclusion. *Ortho-McNeil Pharm., Inc. v. Mylan Labs., Inc.*, 520 F.3d 1358, 1364 (Fed. Cir. 2008); *In re Kubin*, 561 F.3d 1351, 1359 (Fed. Cir. 2009).

It was IBS's burden to demonstrate that there was a clear direction for modifying the teachings of Tsien to use the azidomethyl group from Zavgorodny with a reasonable expectation of success. Tsien outlined criteria that required nucleotides to show quantitative and rapid cleavage. IBS, however, offered no evidence that one of ordinary skill in the art would have expected Zavgorodny's azidomethyl group to have quantitative and rapid cleavage when combined with Tsien's methods, and the evidence of record demonstrates that no such expectation existed. Romesberg Decl. ¶¶ 44, 46-48, 50-53 (Ex. 2011).

**3. A Person of Ordinary Skill In The Art Would Not Have Expected Zavgorodny's Azidomethyl Ether To Permit A Polymerase to Reinitiate Synthesis Subsequent To Deblocking**

Tsien's third criteria for the 3'-protecting group is "the ability of a polymerase enzyme to reinitiate the cDNA synthesis subsequent to the deblocking stage." Tsien at 21:1-3 (Ex. 1008). A person of ordinary skill in the art would not have expected Zavgorodny's 3'-O-azidomethyl group to permit a polymerase to reinitiate synthesis after deblocking at least because (i) similar 3'-O-protecting groups reinitiate DNA synthesis *before* (not after) deblocking, and (ii) Zavgorodny's removal conditions use pyridine, which would interfere with reinitiation of DNA synthesis. Romesberg Decl. ¶ 58 (Ex. 2011).

**a. 3'-O-Protecting Groups Can Reinitiate DNA Synthesis Before (Not After) Debblocking**

Without any supporting evidence, IBS and its expert assert that a person of ordinary skill in the art would have expected an azidomethyl group to reinitiate "DNA synthesis following the deblocking step" simply because Zavgorodny states that an azidomethyl ether can be removed with triphenylphosphine. IBS Revised Petition at 38-39 (Paper 7); Branchaud Decl. ¶ 81 (Ex. 1011). This unsupported assertion is insufficient to carry IBS's burden. The evidence shows that the removability of a protecting group provides no indication that a nucleotide will not interfere with reinitiation of DNA synthesis. Romesberg Decl. ¶ 58 (Ex. 2011). In fact, 3'-O-protecting groups can interfere with reinitiation of DNA synthesis. *Id.*

For example, 3'-O-acetyl esters are removable with a triphenylphosphine. Yoshimoto at 934 (Ex. 2025); Romesberg Decl. ¶ 58 (Ex. 2011). It was known that a 3'-O-acetyl ester can cause reinitiation of DNA synthesis *before* (not after) deblocking. Ju at 3:12-15 ("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.") (Ex. 1002); Branchaud '517 Tr. at 72:8-22 (Ex. 2039). Similarly, the electrophilic characteristics of an azide group would be expected by one of ordinary skill in the art to lead to cleavage by nucleophiles in the active site of DNA polymerase before the deblocking step in Tsien's DNA sequencing method. *See supra* at Section VII.1.a; Romesberg Decl. ¶ 31 (Ex. 2011). Thus, the removability of Zavgorodny's 3'-O-protecting group would not have provided an expectation that this group would meet Tsien's requirement for reinitiation of DNA synthesis after deblocking, and IBS fails to establish a reasonable expectation of success for achieving Tsien's protecting group criteria for at least this reason. *Institut Pasteur*, 738 F.3d at 1346.

**b. Zavgorodny's Removal Conditions Use Pyridine, Which Would Interfere With Reinitiation Of DNA Synthesis**

Zavgorodny's deblocking conditions use pyridine, which is an especially effective DNA denaturing agent. Kit at 57-58 (Ex. 2018); Romesberg Decl. ¶ 55 (Ex. 2011). If pyridine were used during Tsien's deblocking step, the template and



complementary DNA strands would denature and separate from one another, thereby preventing the polymerase enzyme from rebinding to the DNA and interfering with reinitiation of DNA synthesis. Romesberg Decl. ¶ 55 (Ex. 2011). Recognizing this issue, Tsien explicitly provides that its deblocking methods should “not interfere with further enzyme function or denature the DNA strand.” Tsien at 23:27-24:5 (Ex. 1008); *see also id.* at 24:22-23. A person of ordinary skill in the art would not expect Zavgorodny’s removal conditions to be compatible with Tsien’s sequencing methods, and therefore, would not be motivated to combine these references with a reasonable expectation of success. Romesberg Decl. ¶ 55 (Ex. 2011); *DePuy Spine*, 567 F.3d at 1326.

#### **VIII. Ju’s Protecting Group Requirements Are Incompatible With Zavgorodny’s Azidomethyl Group And Removal Conditions**

Like Tsien, Ju is directed to DNA sequencing by synthesis (“SBS”). IBS Revised Petition at 12 (Paper 7); Branchaud Decl. ¶ 47 (Ex. 1011); Ju at 4:21-25 (Ex. 1002); *id.* at 21:2-16; Romesberg Decl. ¶ 28 (Ex. 2011). Based on a partial listing of Ju’s protecting group requirements, IBS asserts that it would have been obvious to use Zavgorodny’s teachings to modify nucleotides for Ju’s SBS method. IBS Revised Petition at 23-24 (Paper 7). IBS asserts that Ju’s SBS methods permit the protecting group to be any “small chemical moiety” provided that the group also: “1) is stable during the polymerase reaction, 2) does not

interfere with the recognition of the nucleotide analogue by polymerase as a substrate, and 3) is cleavable.” *Id.* at 24. IBS, however, omits the complete listing of four “fundamental requirements” for Ju’s SBS methods:

(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); Branchaud ‘517 Tr. at 78:17-79:9 (Ex. 2039).<sup>8</sup> IBS’s petition fails to fully address these four fundamental requirements that further explain each of the three properties mentioned in IBS’s petition. One of ordinary skill in the art, reviewing Ju, would have considered the entire reference for guidance on the requirements for successful SBS reactions, and would not have limited their analysis to IBS’s selected portions of this reference. Romesberg Decl.

¶¶ 28-30 (Ex. 2011); *Panduit Corp. v. Dennison Mfg. Co.*, 810 F.2d 1561, 1568,

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<sup>8</sup> Ju’s requirements are highly similar to Tsien’s criteria. *Compare* Ju at 21:3-16 (Ex. 1002) *with* Tsien at 20:24-21: 3 and 23:27-24:5 (Ex. 1008).

1578 (Fed. Cir. 1987) (providing that all parts of a reference must be considered “including portions that would lead away from the invention in suit”).

In addition, IBS fails to address Ju’s explicit exclusion of electrophilic protecting groups because they 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). A person of ordinary skill would have expected Zavgorodny’s azidomethyl group to have the electrophilic characteristics that are expressly prohibited for Ju’s DNA sequencing method. Canard at 10863 (Ex. 2019); Romesberg Decl. ¶ 31 (Ex. 2011). In addition, a person of ordinary skill would not have expected Zavgorodny’s azidomethyl group to satisfy requirements (2)-(4) of Ju’s methods. Romesberg Decl. ¶¶ 32, 44, 50, 55, 58 (Ex. 2011). Thus, there would neither be a motivation to combine Ju and Zavgorodny, nor a reasonable expectation of success. *Institut Pasteur*, 738 F.3d at 1346.

**1. Zavgorodny’s Azidomethyl Group Is Precisely The Type Of Electrophilic Group That Ju Teaches Is Unsuitable For DNA Sequencing**

Ju expressly 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); *see also id.* at 10:5-7 (providing that the protecting group must be “stable during the polymerase reaction”); Branchaud ‘517 Tr. at 73:12-

74:23 (Ex. 2039); IBS Revised Petition at 24 (Paper 7). Ju relies on Canard for this teaching. Ju at 3:16-20 (Ex. 1002) (*citing* “Canard et al. 1995”); *see also* Canard at 10863 (Ex. 2019). As previously discussed, Zavgorodny’s azidomethyl group contains precisely the type of electrophilic center that Ju warns is unsuitable for protecting a 3’-OH group. Canard at 10863 (Ex. 2019); Romesberg Decl. ¶ 31 (Ex. 2011); *see also supra* Section VII.1.a. A skilled artisan would have expected Zavgorodny’s electrophilic azidomethyl group to react with strong nucleophiles within Ju’s polymerases, and would have been dissuaded from combining Zavgorodny and Ju for at least this reason. *DePuy Spine*, 567 F.3d at 1326.

**2. A Person of Ordinary Skill In The Art Would Not Have Expected Zavgorodny’s Azidomethyl Ether To Be Efficiently Incorporated By A Polymerase**

Ju’s second fundamental requirement for DNA sequencing is “the 4 nucleotide analogues (aA, aC, aG, aT) need to be efficiently and faithfully incorporated by DNA polymerase as terminators in the polymerase reaction.” Ju at col. 21:8-11 (Ex. 1002); *see also id.* at col. 10, ll. 5-7 (providing that the protecting group must “not interfere with the recognition of the nucleotide analogue by polymerase as a substrate”); IBS Revised Petition at 24 (Paper 7). Without any support, IBS and its expert assert that a person of ordinary skill in the art would have expected an azidomethyl group to be incorporated by a polymerase simply because an azidomethyl group is small and removable with triphenylphosphine.

IBS Revised Petition at 25-26 (Paper 7); Branchaud Decl. ¶ 57 (Ex. 1011). The removability of a small protecting group with triphenylphosphine, however, provides no indication of efficient or faithful polymerase incorporation. Romesberg Decl. ¶ 58 (Ex. 2011). For example, 3'-O-acetyl esters are small protecting groups that are removable with a triphenylphosphine. Branchaud '266 Tr. at 245:11-22 (Ex. 2083); Yoshimoto at 934 (Ex. 2025). Metzker reports that nucleotides bearing removable 3'-O-protecting groups (including a 3'-O-acetyl ester group) interfere with the recognition of several polymerases. Metzker at 4263 (Ex. 2024). Furthermore, it was known that electrophilic groups (which include esters and azides) were unstable during polymerase reaction due to the presence of nucleophiles in the active site of the polymerase enzyme. Ju at 3:12-20 (Ex. 1002); Canard at 10863 (Ex. 2019); Branchaud '517 Tr. at 72:8-22 (Ex. 2039). Thus, the small size and removability of Zavgorodny's 3'-O-azidomethyl group would not have provided an expectation of efficient and faithful polymerase incorporation because other removable 3'-O-protecting groups were known to interfere with polymerase incorporation. Romesberg Decl. ¶ 58 (Ex. 2011). As a result, a person of ordinary skill would not have a reasonable expectation of success for achieving at least Ju's second fundamental sequencing requirement through the use of Zavgorodny's 3'-O-azidomethyl group. *Institut Pasteur*, 738 F.3d at 1346.

**3. A Person of Ordinary Skill In The Art Would Not Have Expected Zavgorodny's Azidomethyl Group To Be Removed Efficiently Enough To Satisfy Ju's Sequencing Methods**

Ju's third requirement for DNA sequencing 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); *see also id.* at 10:5-7 (providing that the protecting group must be "cleavable"); IBS Revised Petition at 24 (Paper 7). A skilled artisan would not have expected Zavgorodny's 3'-O-azidomethyl group to satisfy this requirement because Zavgorodny's removal conditions would have been expected to proceed too inefficiently for Ju's SBS methods. Romesberg Decl. ¶¶ 32-33, 44, 50, 53 (Ex. 2011).

It is essential to remove the 3'-OH capping group in high yield to achieve Ju's desired read length of more than 30 base pairs, and preferably up to 100 base pairs. Ju at 2:10-25 (disparaging pyrosequencing because it "can only sequence up to 30 base pairs") (Ex. 1002); *see also id.* at 21:63-66 ("Thus, 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"). To achieve Ju's desired read length the 3'-OH capping group must be removed in nearly quantitative yield. Romesberg Decl. ¶¶ 32-33 (Ex. 2011). For example, the removal efficiency must be greater than 98.5% to permit a read length of 100 bases (i.e.,  $0.985^{100} = 22\%$ ). Romesberg Decl. ¶ 33 (Ex.

2011); Branchaud '128 2nd Tr. at 180:24-182:8 (Ex. 2082); Odedra at 2:20-3:5 (requiring greater than 97%) (Ex. 2032); Tsien at 16:21-30 (requiring quantitative cleavage) (Ex. 1008); WO 89/10977 at 6:31-9:21 (Ex. 2087). The removal efficiency needs to be nearly quantitative to account for inefficiencies associated with the other steps in Ju's sequencing cycle (*i.e.*, inefficiencies with tag removal or polymerase incorporation of unnatural nucleotides). Ju at 21:8-13 (Ex. 1002); Romesberg Decl. ¶ 33 (Ex. 2011). Therefore, a skilled artisan would have understood Ju's sequencing method to require nearly quantitative cleavage efficiency for the 3'-O-protecting group. Romesberg Decl. ¶ 33 (Ex. 2011).

As previously discussed, a person of ordinary skill would have expected Zavgorodny's 3'-O-azidomethyl removal conditions to cleave with low yields and high variability. *See supra* at Sections VI.2.c.ii-iii; Romesberg Decl. ¶ (Ex. 2011). Therefore, Zavgorodny's removal conditions would not have been expected to satisfy Ju's fundamental requirement for high cleavage yields. *Eisai*, 533 F.3d at 1359. Therefore, a person of skill in the art would have expected Zavgorodny's azidomethyl group to be incompatible with Ju's sequencing requirements, and would have been dissuaded from combining Zavgorodny with Ju. Romesberg Decl. ¶¶ 32-33, 44, 50, 53 (Ex. 2011).

**4. A Person of Ordinary Skill In The Art Would Have Expected Zavgorodny's Pyridine Solution To Denature DNA, And Therefore Be Incompatible With Ju**

Ju's fourth fundamental DNA sequencing requirement is "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) (emphasis added).

As discussed above, see Section VII.2.b.i, *supra*, it was well known at the time of invention that the removal conditions for the 3'-protecting group cannot denature DNA. *See, e.g.*, Welch at 197-198 (Ex. 2028); Tsien at 24, ll. 1-2 (Ex. 1008).

As previously discussed, the prior art teaches that Zavgorodny's removal conditions use pyridine, which is an especially effective DNA denaturing agent that would cause Ju's DNA template to become unannealed from the growing DNA strand. *See supra* at Section VI.2.b.i; Kit at 57-58 (Ex. 2018); Romesberg Decl. ¶ 55 (Ex. 2011). Thus, a person of skill would have expected that Zavgorodny's 3'-O-azidomethyl removal conditions would be incompatible with Ju's fourth fundamental DNA sequencing requirement, and would not have been motivated to combine Zavgorodny with Ju. *In re ICON*, 496 F.3d at 1382.

**5. IBS's Petition Distorts Dr. Liu's Testimony**

IBS asserts that it would have been obvious to try an azidomethyl group based on Dr. Lui's (one of the inventors of the '537 Patent) previous testimony. IBS Revised Petition at 25 (Paper 7). IBS, however, distorts Dr. Liu's testimony



and omits important parts of his deposition transcript from the record. Dr. Liu testified that the claims are inventive, that he overcame multiple obstacles, and he tried many other protecting groups before he found the azidomethyl group. Dr. Liu Tr. at 39:10-41:15, 45:2-46:1, 79:14-83:1, 276:1-8, 296:17-24 (Ex. 2044). As a result, Dr. Liu's testimony supports the nonobviousness of the claims at issue in this proceeding. *Ortho-McNeil*, 520 F.3d at 1365.

Additionally, IBS's "obvious to try" analysis has been rejected by the Federal Circuit. See *In re Kubin*, 561 F.3d at 1358; *In re O'Farrell*, 853 F.2d 894, 903 (Fed. Cir. 1988). Moreover, as an inventor of the '537 Patent, Dr. Liu's subjective motivation is immaterial to this proceeding. *Life Techs., Inc. v. Clontech Lab., Inc.*, 224 F.3d 1320, 1325 (Fed. Cir. 2000) ("Because patentability is assessed from the perspective of the hypothetical person of ordinary skill in the art, information regarding the subjective motivations of inventors is not material."). The path that led Dr. Liu to arrive at Claims 1-6 and 8 of the '537 Patent is irrelevant to the patentability of these claims. *Id.* Moreover, Dr. Liu testified based on his experience as a person of *extraordinary* skill in the art. *Standard Oil Co. v. Am. Cyanamid Co.*, 774 F.2d 448, 454 (Fed. Cir. 1985) ("Inventors, as a class, according to the concepts underlying the Constitution and the statutes that have created the patent system, possess something – call it what you will – which sets them apart from the workers of *ordinary* skill . . . .") (emphasis in original)).

Additionally, Dr. Liu was testifying based on his collective knowledge as of 2012, not as to what an ordinary person of skill in the art would have known at the time of the invention, back in 2002. For all of the above reasons, IBS's arguments regarding Dr. Liu's prior testimony are both misleading and irrelevant.

**6. Claims 1-6 And 8 Of The '537 Patent Are Nonobvious Over The Combination Of Ju And Zavgorodny**

For any one of the foregoing reasons, a person of ordinary skill in the art would not have expected Zavgorodny's 3'-O-azidomethyl group to meet Ju's fundamental sequencing requirements. A skilled artisan would not have been motivated to combine the SBS teachings of Ju with the azidomethyl group in Zavgorodny to arrive at the claimed methods, because such a combination would not have been expected to work for its intended purpose. *Romesberg Decl.* ¶¶ 31-32, 44, 50, 55, 58 (Ex. 2011). Despite the numerous indications that an azido protecting group would not be compatible with DNA sequencing, Illumina solved a major challenge in the sequencing field by inventing the compounds used in Claims 1-6 and 8 of the '537 Patent. *Institut Pasteur*, 738 F.3d at 1346. IBS did not carry its burden of proving these claims would have been obvious based on the combination of Ju with Zavgorodny. 35 U.S.C. § 316(e). Accordingly, the Board should confirm the nonobviousness of the claims at issue in this proceeding.

**IX. Claim 3 Is Nonobvious Over Tsien, Zavgorodny, and Prober**

Claim 3 depends from independent Claim 1. A dependent claims is

nonobvious if the independent claim from which it depends is nonobvious. *In re Fritch*, 972 F.2d 1260, 1266 (Fed. Cir. 1992). As described above, Claim 1 is nonobvious over Tsien and Zavgorodny. Therefore, for the reasons described above, Claim 3 also is nonobvious over Tsien, Zavgorodny, and Prober. Notably, Prober does not fill in the gaps missing from Tsien and Zavgorodny, nor does it provide the missing motivation to combine those two references.

**X. The Claims are Not Obvious in Light of the Secondary Considerations of Nonobviousness**

Illumina's azido protecting group technology was an important element for advancing SBS from an inefficient process to a breakthrough technology that transformed the genomic industry, and recently led to the announcement of the much sought after \$1,000 genome. MIT Tech. Review at 2 (Ex. 2004). This azido protecting group provided a solution for a significant need in the industry that existed in 2002. *See supra*, Section II.

Objective indicia of nonobviousness "can be the most probative evidence of non-obviousness in the record, and enables the . . . court to avert the trap of hindsight." *Custom Accessories, Inc. v. Jeffery-Allan Indus., Inc.*, 807 F.2d 955, 960 (Fed. Cir. 1986). "Objective indicia of non-obviousness play a critical role in the obviousness analysis." *Leo Pharm. Prods.*, 726 F.3d at 1358. Objective indicia of nonobviousness "may often establish that an invention appearing to have been

obvious in light of the prior art was not.” *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 1538 (Fed. Cir. 1983).” “[E]vidence rising out of the so-called ‘secondary considerations’ must always when present be considered en route to a determination of obviousness.” *Id.* (citations omitted).

Illumina’s claims are not obvious over the applied art because objective evidence demonstrates that (1) Illumina’s claimed method solved a long-felt problem, (2) the claimed method shows unexpectedly superior performance, and (3) others, including IBS, copied Illumina’s claimed method. This objective evidence further confirms the nonobviousness of Claims 1-6 and 8 of the ’537 Patent.

### **1. Illumina’s 3’-O-Azidomethyl Protecting Groups For Labeled Nucleotides Met a Long-Felt, Unmet Need**

Long-felt need serves as evidence of the nonobviousness of a claimed invention. *Procter and Gamble Co. v Teva Pharms. USA*, 566 F.3d 989, 998 (Fed. Cir. 2009); *see also, Leo Pharm. Prods.*, 726 F.3d at 1359. IBS would have the Board believe that combining Tsien and Zavgorodny would have been a trivial matter. That is not the case. The evidence shows that there was a long-felt, unmet need for protecting groups that could be efficiently and rapidly cleaved under conditions compatible with DNA sequencing. Romesberg Decl. at ¶ 60 (Ex. 2011). Although researchers recognized SBS as “highly desirable” (Welch et al., Chem.

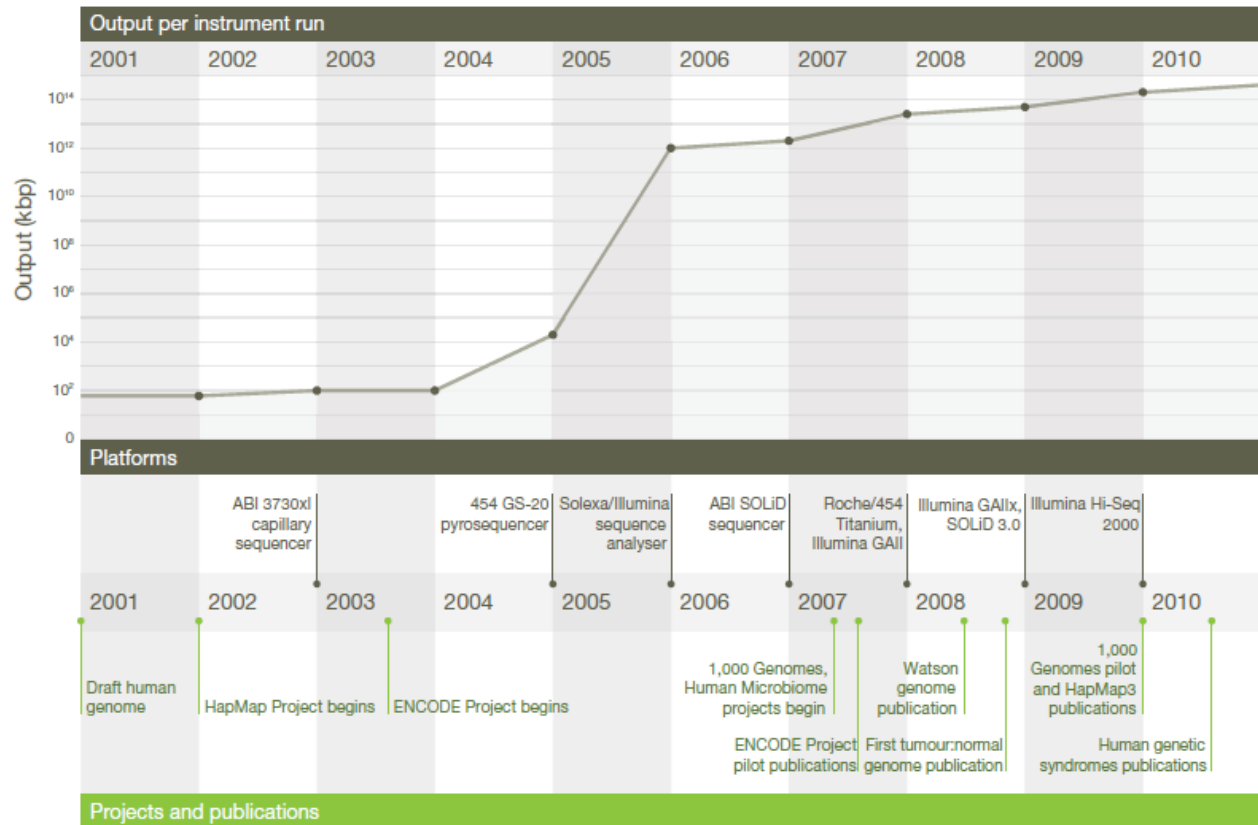
Eur. J., 5:951, 951 (1999), Ex. 2079), this sequencing technology did not achieve widespread application “because of numerous problems, such as inefficient chemistries which prevent determination of any more than a few nucleotides in a complete sequencing operation.” U.S. 6,013,445 at 1:51-61 (Ex. 2045). The search for the ideal 3’-O-protecting group for use in SBS was described as a “formidable obstacle” (Metzker at 4259, Ex. 2024), and a “major challenge” (Welch at 197-198, Ex. 2028), with “many hurdles.” Burgess et al., J. Org. Chem., 62:5165, 5165 (1997) (Ex. 2077).

In spite of these hurdles, researchers had found that Tsien’s 3’-O-allyl group (Ex. 1008 at p. 24, l. 29) was a reversible protecting group that could be effectively incorporated by polymerases. Metzker at 4263 (Ex. 2024); Ju at 3:7-22 (Ex. 1002). Ju merely used Tsien’s 3’-O-allyl group that was already expected to be successful based on the work of Metzker. Ju at 3:23–30 (Ex. 1002); Tsien at 24:29 (Ex. 1008); Metzker at 4263 (Ex. 2024). Notably, Ju (which was filed around the same time as the ’537 Patent) did not even mention azidomethyl as a desirable 3’-O-protecting group. *See* Ex. 1002. In fact, in a 2006 paper touted by Dr. Ju and Columbia, Dr. Ju was still using the 3’-O-allyl group that was first investigated by Tsien. Ju PNAS at 19635 (Ex. 2038); *see also* Burgess Decl., ¶ 36 (Ex. 2089).

Where others failed to find an ideal 3’-OH protecting group, Illumina succeeded in creating fast and efficient SBS methods. Illumina’s reversible azido

group chemistry, as claimed by the '537 Patent, dramatically improved SBS techniques and satisfied a long-felt, unmet need in the field. Bentley et al., *Nature*, 456:53, 53 (2008) (“we used a set of four reversible terminators, 3'-O-azidomethyl 2'-deoxynucleoside triphosphates” to “generate[] several billion bases of accurate nucleotide sequence per experiment at low cost”) (Ex. 2027, “Bentley”); Romesberg Decl. ¶¶ 61-65 (Ex. 2011). Illumina's 3'-O-azidomethyl protected nucleotides demonstrate long read lengths in SBS and can generate superior amounts of sequence information. Mardis, *Nature*, 470:198, 199 (2011) (Ex. 2050, “Mardis”). As shown in Figure 1 below, Illumina's 3'-O-azidomethyl nucleotides were recognized for increasing data output by eight orders of magnitude. The history of SBS development confirms the nonobviousness of Claims 1-6 and 8 of the '537 Patent. It is not surprising that others failed to choose an azidomethyl protecting group for the reasons stated above. *See infra* Sections VII, VIII. Although IBS contends that the claims are obvious, the failure of others to make the claimed invention (before or even around the same time as the inventors of the '537 Patent made their invention) despite a long-felt need in the art for fast and efficient 3'-O-protecting groups for SBS methods indicates otherwise. Romesberg Decl. ¶ 65 (Ex. 2011); *Leo Pharm. Prods.*, 726 F.3d at 1353.

**Figure 1:** Illumina increased sequencing data capacity output by  $10^8$  (kbp).



Mardis at 199 (Ex. 2050).

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

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[REDACTED]

## **2. Illumina’s 3’-O-Azidomethyl Group Performs Unexpectedly Better Than Other Protecting Groups**

The presence of a property not possessed by the prior art is evidence of nonobviousness. *In re Papesch*, 315 F.2d 381 (C.C.P.A. 1963). A showing of “new and unexpected results” must only be “relative to the prior art.” *Iron Grip Barbell Co. v. USA Sports, Inc.*, 392 F.3d 1317, 1322 (Fed. Cir. 2004). There is also no

requirement to test every embodiment that falls within the scope of the claims. *In re Huai-Hung Kao*, 639 F.3d 1057, 1068 (Fed. Cir. 2011); *see also Rambus Inc. v. Rea*, 731 F.3d 1248, 1257 (Fed. Cir. 2013). Evidence of unexpected properties may be in the form of a direct or indirect comparison of the claimed invention with the closest prior art which is commensurate in scope with the claims. *See In re Boesch*, 617 F.2d 272 (C.C.P.A. 1980).

The removable 3'-O-azidomethyl group is an important invention for Illumina's SBS technology. Romesberg Decl. ¶ 65 (Ex. 2011); Bentley at 53 and 58 (Ex. 2027). This protecting group is also a key distinguishing feature of the claims over Ju and Tsien. Romesberg Decl. ¶ 65 (Ex. 2011). In a 2008 Nature article, Illumina published sequencing results using its azido 3'-OH reversible terminators. Bentley (Ex. 2027). 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). Indeed, Nature is 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); Romesberg Decl. ¶ 61 (Ex. 2011). Thus, the Bentley publication evidences the scientific community's recognition of unexpected and surprising results for Illumina's invention. *Id.* Furthermore, Illumina's 3'-O-azidomethyl nucleic acid

molecules demonstrate unexpected and superior results over (a) 3'-O-allyl ethers and (b) the expected combination of Tsien or Ju with Zavgorodny.

**a. Illumina's 3'-O-Azidomethyl Protecting Group Performs Unexpectedly Better Than The 3'-O-Allyl Protecting Group**

SBS methods using nucleotides having a removable azido-containing protecting group at the 3'-OH—as claimed in the '537 Patent—yield dramatically improved results relative to the SBS methods taught in the prior art. As explained below, nucleotides having a 3'-O-azidomethyl group are cleaved with surprisingly greater speed and to a greater extent than those having a 3'-O-allyl group. These properties were not known or suggested by Ju, Tsien or other prior art references, and these properties serve as evidence that the claims are not obvious.

In [REDACTED], scientists for Solexa (later acquired by Illumina) were attempting to optimize conditions for removing 3'-O-allyl groups from nucleosides for use in SBS. Romesberg Decl. ¶ 68 (Ex. 2011). These scientists only obtained [REDACTED] [REDACTED]. *Id.* ¶ 69; SLB-090 at 28 (Ex. 2003). It took [REDACTED] [REDACTED]. Romesberg Decl. ¶ 72 (Ex. 2011); SLB-090 at 36 (Ex. 2003). These cleavage efficiencies for short time frames ([REDACTED]) are insufficient for SBS, and higher efficiency could only be achieved with very long cleavage times. Romesberg Decl. ¶¶ 75-76 (Ex. 2011). For example, the 3'-O-

allyl group data showing [REDACTED]

[REDACTED]. *Id.* ¶ 75.

In [REDACTED], in contrast to the optimized experiments by Solexa for 3'-O-allyl groups, even Solexa scientists' early experiments with 3'-O-azidomethyl groups recorded excellent cleavage data for their new 3'-O-azidomethyl protected nucleosides under SBS compatible conditions. *Id.* ¶ 78-79. Surprisingly, the 3'-O-azidomethyl group cleaved with [REDACTED]. *Id.* ¶ 78; SLB128 at 1 (Ex. 2003). The cleavage efficiency of the 3'-O-azidomethyl group could not have been expected over the teachings of the prior art. Romesberg Decl. ¶ 81 (Ex. 2011); *supra* Section VII.2.b. This cleavage efficiency is also not expected in light of the optimized 3'-O-allyl cleavage data discussed above. Romesberg Decl. ¶ 79 (Ex. 2011).

The cleavage efficiency afforded by the 3'-O-azidomethyl provides a substantial improvement in the context of SBS by allowing a longer read length in a shorter amount of time. *Id.* ¶ 79. For example, using Illumina's methods with a 3'-O-azidomethyl group enables [REDACTED]

[REDACTED]. *Id.* Thus, the use of the 3'-O-

azidomethyl opened the gateway to longer read lengths in SBS that were not achieved with 3'-O-allyl. *Id.* ¶ 80.

These data demonstrate that the cleavage efficiency of the 3'-O-azidomethyl group is substantially superior to the cleavage efficiency of the 3'-O-allyl group under conditions compatible with SBS. *Id.* ¶ 79-80. Accordingly, the unexpected superior cleavage efficiency of 3'-O-azidomethyl from nucleosides supports the nonobviousness of the claims. *Id.*

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

**b. Illumina's 3'-O-Azidomethyl Protecting Group Performs Unexpectedly Better Than The Expected Combination of Tsien or Ju with Zavgorodny**

Illumina's azido protecting group cleavage results are far superior to what one of ordinary skill would have been expected in combining Tsien and Ju with Zavgorodny's teachings. *Id.* ¶ 81. As discussed above, Zavgorodny teaches cleavage of an azidomethyl protecting group using triphenylphosphine in aqueous pyridine. The efficiency of azido to amino conversion using triphenylphosphine in pyridine is highly variable and unpredictable, with yields that are too low for SBS (as low as 50%), and require a long time (3 to 72 hours) to realize these low yields. *Id.* ¶ 50. Thus, one of ordinary skill would have expected an azidomethyl group cleavage to be worse than an allyl group cleavage, both in terms of efficiency and speed. *Id.* ¶ 81. In contrast to this expectation, Illumina found that the 3'-O-azidomethyl group can be cleaved at an efficiency of [REDACTED]. *Id.* ¶ 79; SLB128 at 1 (Ex. 2003). Illumina's results show that the azidomethyl group, which would have been expected to cleave too slowly and too inefficiently to meet Tsien's and Ju's requirements, surprisingly cleaved rapidly and efficiently, opening the gateway to advancements in SBS that were not previously realized. Romesberg Decl. ¶¶ 79-81 (Ex. 2011); Bentley at 53 (Ex. 2027).

### **3. Copying Of Illumina's 3'-O-Azidomethyl Group By IBS And Others Provides Objective Evidence Of Nonobviousness**

The parent application to Illumina's '537 Patent published in 2003 as U.S. 2003/0104437. A few years after this publication, other groups in the sequencing field adopted the 3'-O-azidomethyl group and credited Illumina for this innovation by citing Illumina's patent documents. The adoption of Illumina's technology evidences copying by others and confirms the superiority of Illumina's 3'-O-azidomethyl group over other protecting groups for SBS. *Wyers v. Master Lock Co.*, 616 F.3d 1231, 1246 (Fed. Cir. 2010).

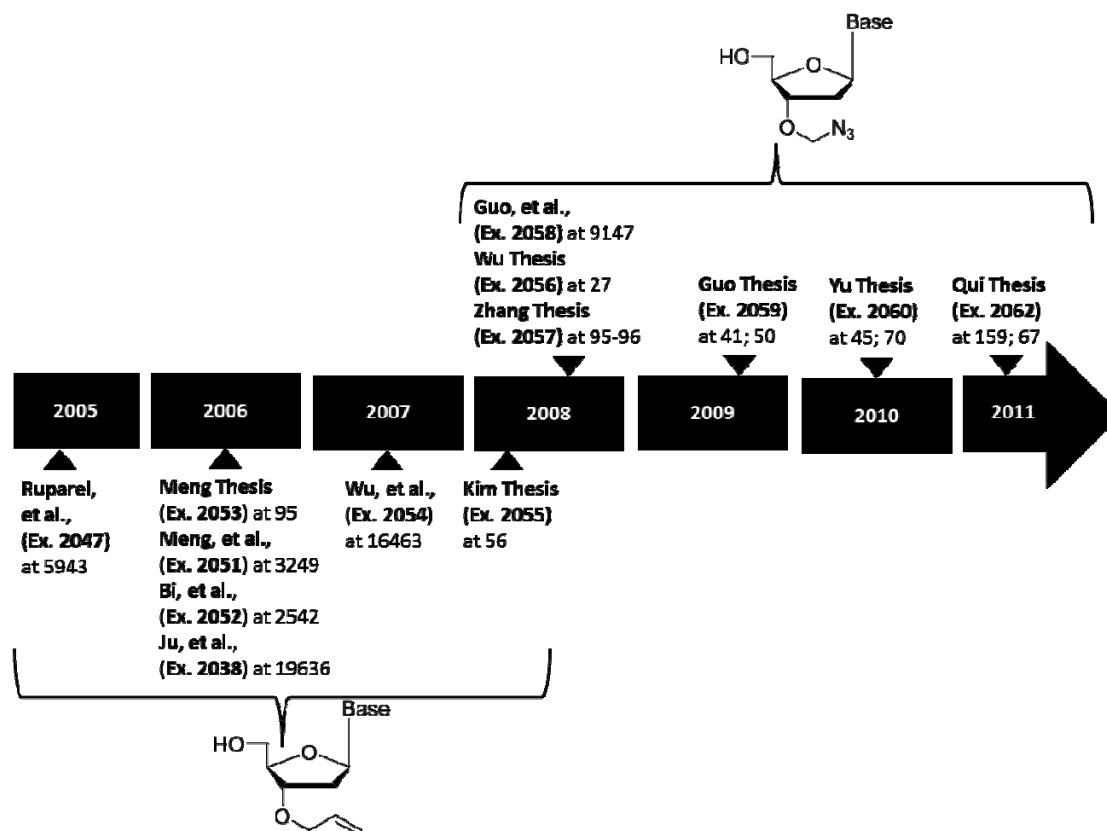
In particular, the evidence shows that Dr. Ju and IBS copied Illumina's 3'-O-azidomethyl compounds and claimed methods. Before copying Illumina's invention, Dr. Ju's lab was investigating SBS methods with Tsien's 3'-O-allyl group. *See, e.g.*, Ruparel et al., PNAS, 102:5932, 5932 (2005) (Ex. 2047, "Ruparel"); Meng et al., J. Org. Chem., 71:3248, 3248 (2006) (Ex. 2051); Bi et al., J. Am. Chem. Soc., 126:2542, 2542 (2006) (Ex. 2052); Meng, Columbia Univ. Thesis at 95 (2006) (Ex. 2053); Ju PNAS at 19636 (Ex. 2038); Kim, Columbia Univ. Thesis at 57 (2008) (Ex. 2055). Dr. Ju spent years trying to optimize the 3'-O-allyl group for SBS but apparently abandoned this group in favor of Illumina's 3'-O-azidomethyl group. *See* Figure 2, below. After adopting Illumina's 3'-O-azidomethyl group, Dr. Ju achieved sequencing results superior to the best results

he realized with the 3'-O-allyl group. *See, e.g.*, Wu, Columbia Univ. Thesis at 27 (2008) (Ex. 2056); Zhang, Columbia Univ. Thesis at 95-96 (2008) (Ex. 2057); Guo at 9146 (Ex. 2058); Guo, Columbia Univ. Thesis at 41, 50 (2009) (Ex. 2059); Yu, Columbia Univ. Thesis at 45, 70 (2010) (Ex. 2060); Qui, Columbia Univ. Thesis at 159 (2010) (Ex. 2062).

Dr. Ju's results did not arise from a simple case of serendipitous development of the same 3'-O-protecting group. The evidence shows that Dr. Ju's laboratory was following Illumina's 3'-O-azidomethyl patent publications as early as 2005. *See* Ruparel at 5937 (*citing* Illumina's WO 2004/018497) (Ex. 2071). In addition, Dr. Ju's earliest publication disclosing a 3'-O-azidomethyl group, and subsequent publications, cite to Illumina's patent documents for the 3'-O-azidomethyl group and its cleavage. *See, e.g.*, Kim Thesis at 99 (*citing* Illumina's WO 2004/018497) (Ex. 2055); Guo at 9145-6 (*citing* Illumina's U.S. 7,057,026 and U.S. 2006/0160081) (Ex. 2058); Zhang Thesis at 101, 111, 114, 174 & 190 (*citing* Illumina's U.S. 7,057,026, U.S. 2006/0160081, and WO 2004/018497) (Ex. 2057); Guo Thesis at 43 & 51 (*citing* Illumina's U.S. 2006/0160081) (Ex. 2059); Yu Thesis at 53, 66, & 95 (*citing* Illumina's U.S. 7,057,026 and U.S. 2006/0160081) (Ex. 2060); and Qui Thesis at 78 (*citing* Illumina's U.S. 2006/0160081) (Ex. 2062). *See also* Illumina's U.S. 2006/0160081 at 17 (Ex. 2072); WO 2004/018497 at 51 (Ex. 2071); WO 2004/018493 at 50 (Ex. 2070).



**Figure 2:** Dr. Ju abandoned 3'-O-allyl for Illumina's 3'-O-azidomethyl.



Moreover Dr. Ju's adoption of Illumina's 3'-O-azidomethyl group permitted Dr. Ju to obtain superior results than he achieved with the 3'-O-allyl group. For example, using Tsien's 3'-O-allyl group, Dr. Ju published data showing sequencing of 13 bases and reported the ability to sequence "≈20 continuous bases." Ju PNAS at 19636, 19638 (Ex. 2038). But, using the 3'-O-azidomethyl group, Dr. Ju reported a "read length of >30 bases." Guo at 9145 & 9149 (Ex. 2058). In addition, Dr. Ju's data showed that the fluorescence intensity for the 3'-O-azidomethyl group was significantly higher than the 3'-O-allyl group. Compare Ju PNAS at Fig. 3 (Ex. 2038) with Guo at Fig. 5 (Ex. 2058). Furthermore, Dr. Ju

reported that the 3'-O-azidomethyl protecting group was "quantitatively cleaved by TCEP" while Dr. Ju did not provide a similar characterization of the 3'-O-allyl group. Guo at 9147 (Ex. 2058). In SBS methods, sequencing >30 bases is a dramatic improvement in kind over sequencing just 20 bases. WO 89/10977 at 6:31-9:21 (Ex. 2087); Romesberg Decl. ¶¶ 79-81 (Ex. 2011). Thus, Dr. Ju's research provides compelling corroboration of Illumina's unexpected results as previously discussed. *Supra* at Section X.2.

The evidence further shows that IBS copied Illumina's claimed methods. In the related district court litigation, IBS admitted that their proposed products utilize "engineered DNA bases that include a removable fluorescent dye and an end cap." IBS Answer at ¶¶ 21-22 (Ex. 2005). The cleavable end cap "includes an azido group." *Id.* at ¶¶ 23-24; *see also id.* at ¶¶ 25, 28-29.

[REDACTED]

[REDACTED]

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[REDACTED]

[REDACTED]

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[REDACTED]

[REDACTED]

[REDACTED]

Accordingly, the objective evidence indicates that Illumina's pioneering technology was copied by others and supports the patentability of the claims.

#### **4. Praise by Others**

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

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## **XI. Conclusion**

In light of the entire record, IBS fails to meet its burden in demonstrating that Claims 1-6 and 8 are obvious over Ju or Tsien in view of Zavgorodny and Prober. Accordingly, the Board should confirm the nonobviousness of Claims 1-6 and 8 of U.S. Patent No. 7,566,537.

Respectfully submitted,

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Dated: May 5, 2014

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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re U.S. Patent of: Balasubramanian et al.  
Title: LABELLED NUCLEOTIDES  
Patent No.: 7,566,537  
Issue Date: July 28, 2009  
IPR Proceeding No.: IPR2013-00517  
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Customer No.: 22922

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**CERTIFICATE OF SERVICE**

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Copies of the following were served electronically this 5th day of May 2014  
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1. Illumina's Patent Owner Response

Dated this 5th day of May 2014.

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