

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

INTELLIGENT BIO-SYSTEMS, INC.,
Petitioner,

v.

ILLUMINA CAMBRIDGE LIMITED,
Patent Owner.

Case IPR2013-00266
Patent 8,158,346 B2

Before LORA M. GREEN, SCOTT E. KAMHOLZ, and
CHRISTOPHER L. CRUMBLEY, *Administrative Patent Judges*.

CRUMBLEY, *Administrative Patent Judge*.

FINAL WRITTEN DECISION
35 U.S.C. § 318(a) and 37 C.F.R. § 42.73

I. BACKGROUND

A. Introduction

Petitioner, Intelligent Bio-Systems, Inc. (“IBS”), filed a Petition (Paper 1, “Pet.”) for *inter partes* review of claims 1, 2, 4, 11, 12, 17, 18, and 19 of U.S. Patent No. 8,158,346 B2 (Ex. 1001, “the ’346 patent”) pursuant to 35 U.S.C. §§ 311–319 and 37 C.F.R. §§ 42.1–42.123.

On October 28, 2013, the Board instituted *inter partes* review of claims 1, 2, 4, 11, 12, 17, 18, and 19 of the ’346 patent on the following three grounds of unpatentability:

1. Whether claims 1, 2, 4, 11, 12, 17, 18, and 19 are unpatentable under 35 U.S.C. § 102(a) or (e) as anticipated by Ju;¹
 2. Whether claims 1, 2, 4, 11, 12, 17, 18, and 19 are unpatentable under 35 U.S.C. § 102(b) as anticipated by Tsien;² and
 3. Whether claims 1, 2, 4, 11, and 12 are unpatentable under 35 U.S.C. § 102(b) as anticipated by Stemple.³
- Paper 20 (“Dec.”), 13.

Following institution of *inter partes* review, Patent Owner, Illumina Cambridge Limited (“Illumina”), filed a Motion to Amend Claims (Paper 31, “Mot.”), but did not file a response under 37 C.F.R. § 42.120 to the Decision instituting *inter partes* review. IBS filed an opposition to Illumina’s Motion to Amend (Paper 37), and both parties filed Motions to Exclude Evidence (Papers 46, 49).

¹ As used in our Decision to Institute, “Ju” collectively referred to both Ju, U.S. 6,664,079 B2 (Dec. 16, 2003) (Ex. 1002) and Ju, WO 02/29003 A2 (Apr. 11, 2002) (Ex. 1003).

² Tsien, WO 91/06678 A1 (May 16, 1991) (Ex. 1006).

³ Stemple, WO 00/53805 A1 (Sept. 14, 2000) (Ex. 1007).

Pursuant to requests by both parties, an oral hearing was held on May 28, 2014, and the transcript of the hearing was entered into the record. Paper 69, “Tr.”

The Board has jurisdiction under 35 U.S.C. § 6(c). This final written decision is issued pursuant to 35 U.S.C. § 318(a) and 37 C.F.R. § 42.73. For the reasons that follow, Illumina’s Motion to Amend is *granted* to the extent it requests to cancel claims 1, 2, 4, 11, 12, 17, 18, and 19; Illumina’s Motion to Amend is *denied* to the extent that it requests entry of substitute claims 20–26.

B. The ’346 Patent

The ’346 patent relates to DNA sequencing using nucleotides that are labeled and blocked. Ex. 1001, 2:18–22. A detectable label is attached to the base of a nucleotide by a cleavable linker, and a polymerase-blocking group is removably attached at the 3’ (or 2’) position of the sugar moiety of the nucleotide. *Id.* at 2:38–44. A target DNA is sequenced by synthesizing its complement polynucleotide using the labeled and blocked nucleotides. *Id.* at 9:3–7. The blocking group prevents the polymerase from adding more than one nucleotide at a time. *Id.* at 8:13–20. The label then is detected, thereby identifying the newly-added nucleotide. *Id.* at 3:17–19. The label and the blocking group then are removed from the added base under identical conditions. *Id.* at 8:27–28. The process repeats with the next base. *Id.* at 3:20–22. The sequence of the target DNA then may be determined from the complementary sequence. *Id.* at 3:21–22.

C. Related Proceedings

The '346 patent is asserted in the following copending district court case: *Trustees of Columbia University in the City of New York v. Illumina, Inc.*, 1:12-cv-00376-GMS (D. Del.). Pet. 5.

II. ORIGINAL CLAIMS

As noted above, Illumina did not file a Response following our Decision instituting *inter partes* review of claims 1, 2, 4, 11, 12, 17, 18, and 19. Instead, Illumina filed a Motion to Amend pursuant to 35 U.S.C. § 316(d)(1) (“During an inter partes review . . . , the patent owner may file 1 motion to amend the patent in 1 or more of the following ways: (A) Cancel any challenged patent claim. (B) For each challenged claim, propose a reasonable number of substitute claims.”). In its Motion, Illumina requested cancellation of claims 1, 2, 4, 11, 12, 17, 18, and 19 and proposed substitute claims 20–26 to replace the cancelled claims, and asserted that each of the grounds upon which the *inter partes* review was instituted “is rendered moot in light of Illumina’s proposed substitute claims.” Mot. 1. We shall *grant* Illumina’s Motion to Amend to the extent it requests to cancel claims 1, 2, 4, 11, 12, 17, 18, and 19.

III. PROPOSED SUBSTITUTE CLAIMS

In the Motion to Amend, Illumina proposed substitute claim 20 to replace claim 2. The claim, as annotated by Illumina to show the differences between original claim 2 and proposed substitute claim 20, is reproduced below:

20. A method ~~according to claim 1~~ for determining the sequence of a target single-stranded polynucleotide, comprising

monitoring the sequential incorporation of complementary nucleotides, the method further comprising the steps of

(a) providing said nucleotides, wherein the nucleotides each have a base that is linked to a detectable label via a cleavable linker, wherein the cleavable linker contains a disulfide linkage, wherein each of the nucleotides has a ribose or deoxyribose sugar moiety and the ribose or deoxyribose sugar moiety comprises a protecting group attached via the 3' oxygen atom; and wherein said monitoring comprises

(b) incorporating a nucleotide of (a) into the complement of the target single stranded polynucleotide;

(c) detecting the label linked to the base of the nucleotide of (b), thereby determining the identity type of the nucleotide incorporated;

(d) subsequently removing the label and the protecting group of the nucleotide of (b) under a single set of chemical cleavage conditions, wherein the chemical cleavage conditions cleave the disulfide linkage and permit further nucleotide incorporation into the complement of the target single stranded polynucleotide to occur; and

(e) optionally repeating steps (b)-(d) one or more times; thereby determining the sequence of a target single-stranded polynucleotide.

Mot. 2.

Proposed substitute claim 20 combines the limitations found in original claims 1 and 2, and also recites a newly added limitation that the cleavable linker “contains a disulfide linkage,” which was not present in the original claims.

For illustrative purposes, an annotated generic nucleotide from Figure 1B of Stemple is reproduced below to show the main parts of a nucleotide used in sequencing-by-synthesis (“SBS”) processes such as the one of proposed claim 20:

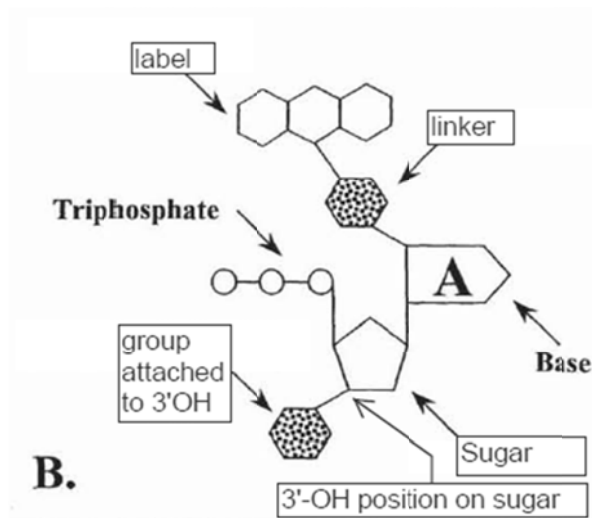


Figure 1B of Stemple shows a generic nucleotide's main parts, including a detectable label ("label"), a linker, and a chemical group attached to the 3'-OH position.

Original claim 1 was drawn to an SBS process using a nucleotide with, *inter alia*, the following features:

1. "a base that is linked to a detectable label via a cleavable linker" (Ex. 1001, 2:18–20) (illustrated as "label," "linker," and "base" in Figure 1B of Stemple reproduced above);

2. "a ribose or deoxyribose sugar moiety . . . compris[ing] a protecting group attached via the 2' or 3' oxygen atom" (*Id.* at 2:43–45) (illustrated in the figure above as a protecting group attached to the oxygen of the 3'-OH group); and

3. "removal of the label and the protecting group under a single set of conditions."

Proposed substitute claim 20 contains these features, but further recites that the linker contains a disulfide linkage. The obviousness of using a disulfide linkage is the main issue to be decided in whether to grant the Motion to Amend.

Proposed substitute dependent claims 21–26 replace claims 4, 11, 12, and 17–19, respectively. The sole changes to these claims are to change their dependency from claim 1 or 17 to claims 20 and 24. Mot. 3.

As movant, Illumina bears the burden of proof to establish that it is entitled to the relief requested in the Motion to Amend. 37 C.F.R. § 42.20(c). In other words, Illumina bears the burden of showing the patentability of the amended claims. Illumina must, therefore, show that the conditions for novelty and non-obviousness are met for the prior art available to one of ordinary skill in the art at the time the invention was filed, not just for the prior art cited in the Petition or the grounds upon which trial was instituted. *See Idle Free Sys., Inc. v. Bergstrom, Inc.*, Case IPR 2012-00027, slip op. at 7 (PTAB June 11, 2013) (Paper 26).

IV. PATENTABILITY OF CLAIMS 20–26

A. Claim Interpretation

In an *inter partes* review, “[a] claim in an unexpired patent shall be given its broadest reasonable construction in light of the specification of the patent in which it appears.” 37 C.F.R. § 42.100(b). Under this standard, we construe claim terms using “the broadest reasonable meaning of the words in their ordinary usage as they would be understood by one of ordinary skill in the art, taking into account whatever enlightenment by way of definitions or otherwise that may be afforded by the written description contained in the applicant’s specification.” *In re Morris*, 127 F.3d 1048, 1054 (Fed. Cir. 1997). We presume that claim terms have their ordinary and customary meaning. *See In re Translogic Tech., Inc.*, 504 F.3d 1249, 1257 (Fed. Cir. 2007) (“The ordinary and customary meaning is the meaning that the term

would have to a person of ordinary skill in the art in question.”) (internal quotation marks and citations omitted). A patentee may rebut this presumption, however, by acting as his own lexicographer, providing a definition of the term in the specification with “reasonable clarity, deliberateness, and precision.” *In re Paulsen*, 30 F.3d 1475, 1480 (Fed. Cir. 1994).

1. the cleavable linker and the protecting group are cleavable under identical conditions

Proposed substitute claim 20 recites, *inter alia*, “a base that is linked to a detectable label via a cleavable linker,” a “protecting group attached via the 3' oxygen atom” of the nucleotide, and a step of “removing the label and the protecting group of the nucleotide . . . *under a single set of chemical cleavage conditions*.” Mot. 2 (emphasis added). These limitations are similar to those present in original claim 1, which we construed in our Decision on Institution. Specifically, we construed *removal of the label and the protecting group under a single set of conditions* “to mean what its plain language indicates: removal of the label and the protecting group under a single set of conditions.” Dec. 6. Neither of the parties contested this construction subsequent to institution, and we discern no reason to modify our initial construction for the purposes of this decision.

Proposed claim 20 also requires that the cleavable linker comprise a disulfide linkage, but does not specify the protecting group. Thus, “a single set of chemical cleavage conditions,” under the broadest reasonable interpretation standard, limits the structure of the protecting group to one which can be cleaved under the same conditions as a disulfide linkage, but does not require a specific structure, such as a disulfide linkage.

2. cleavable linker contains a disulfide linkage

All proposed substitute claims contain a new limitation, not present in the original claims, that the cleavable linker contains a disulfide linkage. Neither party proposes a specific construction for this new claim element. We note that a disulfide linkage contains a bond between two sulfur atoms. Ex. 1001, Fig. 2.

B. Illumina's Burden to Show Nonobviousness

As noted above, the primary remaining issue in this *inter partes* review is the obviousness of using a cleavable disulfide linker to attach a label to the nucleotide base, where the linker and protecting group of the nucleotide are cleaved under identical conditions.

Because Illumina bears the burden of showing that it is entitled to entry of its proposed substitute claims, it must show that one of ordinary skill in the art would not have considered the proposed substitute claims obvious in view of the prior art available before the filing date of the claimed invention. More specifically, the issue is whether it would have been nonobvious at the time of the invention to have attached a detectable label to a base using a disulfide linkage, where the base is present in a nucleotide having "ribose or deoxyribose sugar moiety compris[ing] a protecting group attached via the 3' oxygen atom" and where the disulfide linkage and the protecting group are removed "under a single set of chemical cleavage conditions."

A patent claim is invalid for obviousness "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.” 35 U.S.C. § 103.

The underlying factual considerations in an obviousness analysis include the scope and content of the prior art, the differences between the prior art and the claimed invention, the level of ordinary skill in the art, and any relevant secondary considerations. Relevant secondary considerations include commercial success, long-felt but unsolved needs, failure of others, and unexpected results.

Allergan, Inc. v. Sandoz Inc., 726 F.3d 1286, 1291 (Fed. Cir. 2013) (internal citations omitted).

An important consideration is “whether a person of ordinary skill in the art would, at the relevant time, have had a ‘reasonable expectation of success’ in pursuing the possibility that turns out to succeed and is claimed.” *Institute Pasteur & Universite Pierre et Marie Curie v. Focarino*, 738 F.3d 1337, 1344 (Fed. Cir. 2013) (citations omitted).

C. Prior Art

Before turning to the specific arguments presented by both parties, we summarize some of the prior art of record that was known at the time of the invention claimed in the ’346 patent. This discussion is not meant to be exhaustive, but rather to provide a brief description of what was known about modified nucleotides prior to the priority date of the ’346 patent.

The claimed method is nucleic acid sequencing-by-synthesis (“SBS”), a process in which 3’-OH protected and detectably labeled nucleotides are added stepwise to a nucleic acid primer during sequencing. In that process, it was known to use a nucleotide labeled at its base with a detectable label in order to identify when the nucleotide is incorporated into the newly

synthesized strand. Ex. 1006, 27:33–28:2; Ex. 1010,⁴ 18:64–19:2. It also was known to attach a protecting group to the 3'-OH of the nucleotide. Ex. 1006, 9:32–10:3. During DNA synthesis, nucleotides are added sequentially to the 3'-OH group of the nucleotide sugar. The 3'-OH group contains a removable protecting group so the labeled nucleotides can be added one at a time. After each addition, the label is detected and the 3'-OH group is deblocked and new nucleotide (with its own 3'-OH protecting group) is added. *Id.* at 13:14–35. In sum, it was not new to employ a nucleotide in sequencing which comprised a detectable label on the nucleotide base and a 3'-OH protecting group.

The prior art also described attaching a label to the nucleotide base using a cleavable linker as recited in the proposed claims. *Id.* at 28:20–23; Ex. 1002, Abstract, 2:50–53. Furthermore, as we discussed in the Decision on Institution, Tsien described removing the detectable label and the protecting group simultaneously. Ex. 1006, 28:5–8; Dec. 9.

The newly added limitation that the cleavable linker is a disulfide bond also is described in the prior art. As discussed in more detail below, Rabani⁵ and Church⁶ both describe attaching a detectable label to a nucleotide base via a disulfide linkage, where the nucleotide is used in nucleic acid sequencing. An example is shown in Figure 5 of Church, reproduced below, which we have annotated to identify specific structures.

⁴ Dower, W.J. & Fodor, S.P.A. U.S. 5,547,839 (Aug. 20, 1996).

⁵ Rabani, E. WO 96/27025 A1 (Sept. 6, 1996) (Ex. 2017).

⁶ Church, G.M. WO 00/53812 A2 (Sept. 14, 2000) (Ex. 1019).

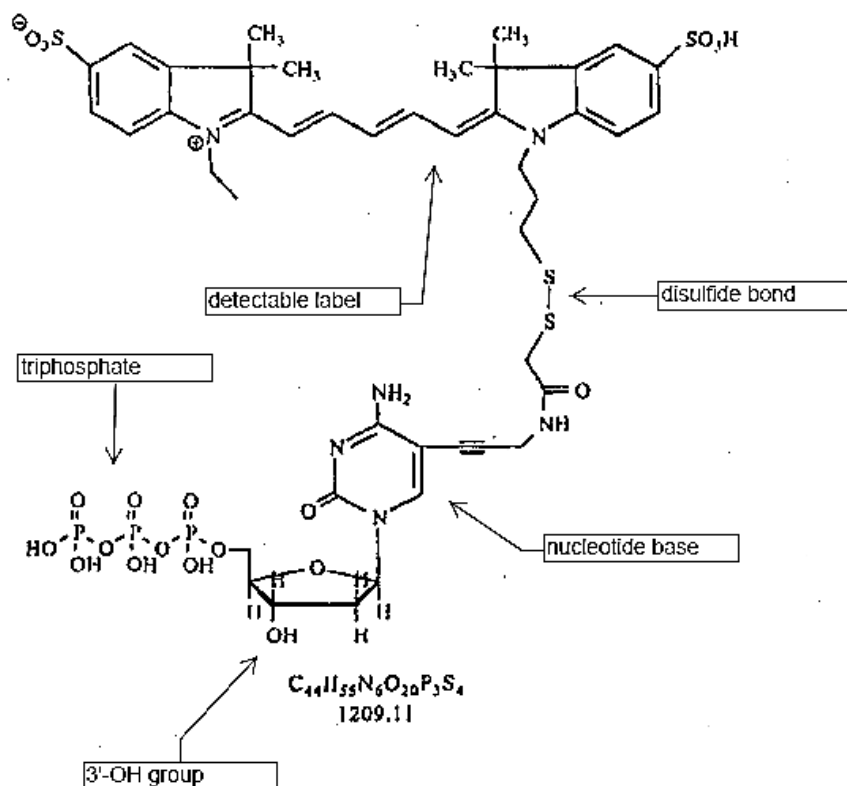
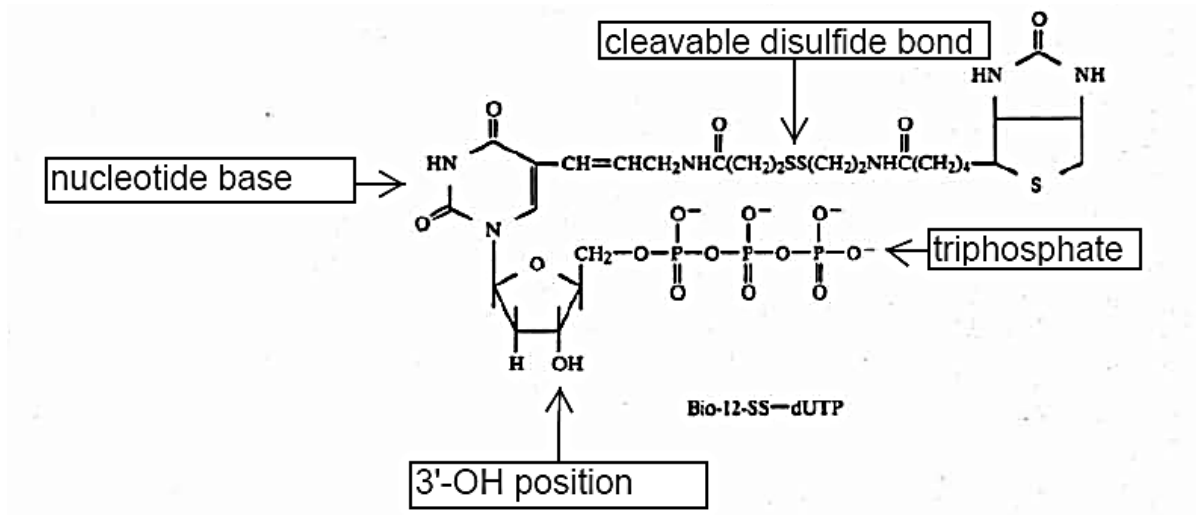


Figure 5 of Church is annotated to identify the structures of the nucleotide, including a detectable label, a triphosphate, and a disulfide bond. Ex. 1019, 17:10–11 (“Figure 5 is a schematic drawing of a disulfide-bonded cleavable nucleotide fluorophore complex.”). The nucleotide, however, lacks the claimed protecting group on the 3'-OH.

In addition to Church, six additional publications⁷ are cited in this *inter partes* review for their description of nucleotides comprising cleavable

⁷ (1) Herman, U.S. Patent No. 4,772,691 (Sept. 20, 1988) (Ex. 2019).
 (2) S.W. Ruby, et al., *Affinity Chromatography with Biotinylated RNAs*, METHODS IN ENZYMOLOGY, vol. 181, 97–121 (1990) (Ex. 2016).
 (3) Short, WO 99/49082 A2 (Sept. 30, 1999) (Ex. 2020).
 (4) Barbara A. Dawson, et al., *Affinity Isolation of Transcriptionally Active Murine Erythroleukemia Cell DNA Using a Cleavable Biotinylated*

linkers with disulfide bonds. One of these, Herman, shows a nucleotide with disulfide bond attaching a biotin to a nucleotide base. Ex. 2019. In the figure from Herman, reproduced below, orientation of the nucleotide is flipped 180 degrees from Church's nucleotide, reproduced above.



Herman's Figure (col. 5) shows a nucleotide with a cleavable linker comprising a disulfide bond joining a biotin ("detectable label") to a nucleotide base. Ex. 2019, 7:24–27. The nucleotide lacks the protecting group on the 3'-OH as required in proposed claim 20.

Nucleotide Analog, THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 264, no. 22, 12830–37 (1989) (Ex. 1027).

(5) Barbara A. Dawson, et al., *Affinity Isolation of Active Murine Erythroleukemia Cell Chromatin: Uniform Distribution of Ubiquitinated Histone H2A Between Active and Inactive Fractions*, JOURNAL OF CELLULAR BIOCHEMISTRY, vol. 46, 166–173 (1991) (Ex. 2038).

(6) Basil Rigas, et al., *Rapid plasmid library screening using RecA-coated biotinylated probes*, PROC. NATL. ACAD. SCI. USA, vol. 83, 9591–9595 (1986) (Ex. 2039).

D. Reason to Use a Disulfide Bond on Nucleotides

In the Decision on Institution, we instituted *inter partes* review on three grounds based on Ju, Tsien, and Stemple. Those publications, however, do not describe using a cleavable disulfide linker for attaching the detectable label to a base. A disulfide bond as a linker is described in the prior art (*see* discussion, *supra*), however, along with a reason to have used one.

1. *Rabani*

Rabani, in the section titled “Cleavable linkers,” teaches that “[l]abeling moieties are favorably in communication with or coupled to nucleotides via a linker of sufficient length to ensure that the presence of said labeling moieties on said nucleotides will not interfere with the action of a polymerase enzyme on said nucleotides.” Ex. 2017, 32:10–13. Rabani specifically mentions disulfide linkages as useful when a cleavable linker is desired:

Linkages comprising disulfide bonds within their length have been developed to provide for cleavability²⁴; reagents comprising such linkages are commercially available²⁵ and have been used to modify nucleotides²⁶ in a manner which may be conveniently reversed by treatment with mild reducing agents such as dithiothreitol.

Id. at 32:29–33. Footnotes 24 and 26 of the above quoted passage cite to Ruby.⁸ *Id.* at 49. Footnote 25 references “for example, from Pierce Chemical Co., [] of Rockford, IL., U.S.A.” *Id.*

⁸ Ruby, S.W. et al., *Affinity Chromatography with Biotinylated RNAs*, METHODS IN ENZYMOLOGY, 181:97 (1990) (Ex. 2016).

Given these disclosures, we find that Rabani would have given a skilled worker reason to have used a cleavable linker with a disulfide bond to ensure that the labeling moieties on the nucleotides will not interfere with the action of a polymerase enzyme during the synthesis reaction.

2. *Church*

IBS cites Church as evidence of the obviousness of using a disulfide linker in a sequencing reaction. Paper 55, 2. Church provides another example of the use of a disulfide linker to attach a label to base of a nucleotide, further establishing its conventionality at the time of the invention. Ex. 1019, 17:10–18, 68:12–21. Church describes a working example in which a label was attached to a nucleotide base using a disulfide linker and then cleaving it off with DTT. *Id.* at 86:6–30.

Dr. Bruce P. Branchaud,⁹ a declarant for IBS, testified:

Church teaches a SBS [sequencing by synthesis] method termed fluorescent *in situ* sequencing extension quantification (FISSEQ). In one embodiment, *Church* teaches the sequential addition of fluorescently labeled nucleotides in which the label is attached to the base via a “cleavable linkage.”

Ex. 1021 ¶ 12 (citing Ex. 1019 at 67:30–68:11).

⁹ To support its obviousness challenge, IBS provided two Declarations by Bruce P. Branchaud, Ph.D. Exs. 1011 & 1021. Dr. Branchaud is Professor Emeritus in the Department of Chemistry at the University of Oregon. Ex. 1011 ¶ 5. He has a Ph.D. in Organic Chemistry from Harvard University, and has held positions in industry, including as an internal consultant and advisor for DNA sequencing projects. Ex. 1011 ¶¶ 5, 7, 12–15. Dr. Branchaud has the requisite familiarity with DNA sequencing to qualify as one of ordinary skill in the art at the time of the invention. Consequently, we conclude that Dr. Branchaud is qualified to testify on the matters addressed in his Declarations.

E. 90% Cleavage Efficiency of the Detectable Label Is Not Required

As discussed in the preceding sections, it was known as of the filing date of the '346 patent to use disulfide linkers, and there was a reason to use such linkers in SBS reactions. Illumina argues, however, that a person of ordinary skill in the art would not have used a disulfide bond. At oral hearing, counsel for Illumina conceded that the claimed method does not recite a particular cleavage efficiency. Tr. 20. Citing Rabani, however, Illumina contends sequencing by synthesis processes require that the disulfide linker joined to the detectable label be cleaved with 90% efficiency, because of the iterative nature of the process. Mot. 10–12. According to Illumina, greater than 90% efficiency in the cleavage reaction could not be achieved at the time of the invention if a disulfide linkage were used. *Id.*

Rabani teaches published results “suggest[ing] that the rate of chemical removal of *3'-hydroxy protecting groups* (less than 90% removal after 10 minutes of treatment with 0.1M NaOH) will be unacceptably low for such an inherently serial sequencing scheme.” Ex. 2017, 3:5–8 (emphasis added). Illumina reasons that since Rabani teaches that less than 90% removal of the *protecting group from the 3'-OH* “will be unacceptably low for . . . [a] serial sequencing scheme,” (Ex. 2017, 3:5–8) and since the claims require that the disulfide linkage of the cleavable linker and the protecting group are cleavable under a single set of chemical conditions, *the disulfide linker joining the detectable label to the nucleotide base* must also achieve 90% or more cleavage. Mot. 10–12. Illumina provides evidence that the prior art teaches less than 90% efficiency in cleaving the 3'-OH protecting group, leading Illumina to reason “the expectation of

unacceptably low 3'-OH protecting group cleavage efficiency when using 'a single set of chemical conditions' would not lead a skilled artisan to believe that one could efficiently cleave 3'-OH protecting groups under the same single set of chemical conditions with a disulfide linkage during SBS." Mot. 12.

In other words, Illumina argues that because 90% efficiency in cleaving the 3'-OH group could not be achieved, there would not have been a reason to use a disulfide linkage to attach the detectable label to the base, because the detectable label must be cleaved under identical conditions to the 3'-OH protecting group. Cleavage of the 3'-OH group requires 90% efficiency, Illumina argues, thus 90% cleavage efficiency must be achieved at the disulfide bond of the detectable label, as well.

Illumina's argument is flawed. Rabani's disclosure is directed to cleavage of the *protecting groups*, not the claimed detectable label. Illumina's arguments are based on the logic that if no better than 90% cleavage of the disulfide bond on the protecting group can be achieved, the skilled worker would not have used it as a cleavable linker for attaching a detectable label to a nucleotide in DNA sequencing, because the proposed substitute claims require it be cleaved under identical conditions to the 3'-OH group, which requires 90% efficiency.

The proposed substitute claims do not require the linkage between the 3'-OH and protecting group to comprise a disulfide bond, nor does Illumina argue that the "single set of chemical cleavage conditions" requires that the protecting group and detectable label be attached to the nucleotide using the same linker. We, therefore, discern no reason to apply Rabani's protecting

group cleavage efficiency requirement to the disulfide linkage of the proposed substitute claims.

Significantly, Dr. Floyd Romesberg,¹⁰ Illumina's declarant, conceded that he chose a 90% efficiency requirement not because of Rabani, but rather because "it was a round number slightly above the values reported by Ruby and Herman." Ex. 1022, 198:16–18. Furthermore, we note that Dr. Romesberg's rationale that high cleavage efficiency is necessary in SBS processes uses examples involving 8 or 16 iterations of the process. Ex. 2004 ¶ 63. Yet, as counsel for Illumina conceded at oral hearing, the claimed process requires far fewer iterations: at most, two and a half cycles. Tr. 7.

Illumina has not persuaded us that cleavage of the disulfide bond that attaches the detectable label to the base with less than 90% efficiency would be unacceptable for SBS processes, such that a person of ordinary skill in the art would not investigate linkers with less efficient cleavage.

F. Cleavage Efficiency of the Disulfide Bond

Even were we persuaded by Illumina's argument that 90% cleavage of the 3'-OH protecting group is necessary for sequencing, Illumina did not provide adequate evidence that the skilled worker would have been unable to

¹⁰ Declarations by Floyd Romesberg, Ph.D. were submitted by Illumina in support of its Motion to Amend. Exs. 2004 & 2037. Dr. Romesberg is a professor in the Department of Chemistry at The Scripps Research Institute, where he has been a faculty member since 1998. Ex. 2004 ¶ 2. Dr. Romesberg testified that he is "qualified to render an opinion in the field of nucleotide analogue molecules based on [his] experience in this field." *Id.* ¶ 17. Dr. Romesberg was deposed twice; the transcripts of his depositions are Exhibits 1022 & 1042.

choose conditions and linkages that would achieve 90% cleavage of the 3'-OH group under the same conditions required for cleavage of the label, e.g., using a reducing agent (Ex. 1001, 6:32–43).

The '346 patent suggests that choosing cleavage conditions for the 3'-OH group was conventional to one of ordinary skill in the art:

Suitable protecting groups will be apparent to the skilled person, and can be formed from any suitable protecting group disclosed in Green and Wuts, *supra*. [Some examples of such protecting groups are shown in FIG. 3.] The protecting group should be removable (or modifiable) to produce a 3' OH group. The process used to obtain the 3' OH group can be any suitable chemical or enzymic reaction.

Id. at 8:21–26.

Dr. Branchaud testified that:

even if one of ordinary skill in the art considered the elution percentages of *Ruby* . . . in deciding whether to use such a linker for SBS, one of ordinary skill in the art would know that such elution percentages could be improved by routine experimentation to improve the cleavage efficiency of the disulfide linkers and thus would not be dissuaded from using such a linker.

Ex. 1021 ¶ 40. As discussed below, this conclusion is supported by the prior art of record.

1. Ruby

Ruby is cited expressly by Rabani for its teaching of a disulfide bond that is cleavable with a reducing agent, such as dithiothreitol (“DTT”), and describes attaching a biotin molecule attached to a nucleotide base of a RNA “via a linker containing a disulfide bond.” Ex. 2016, 98. The RNA is bound to a column containing avidin, based on the affinity of the biotin for the

avidin. *Id.* at 98–99 (Fig. 1). The RNA is “eluted [from the column] by adding dithiothreitol (DTT) to reduce the disulfide bonds linking biotin to the anchor RNA.” *Id.* at 98; Ex. 1021 ¶ 31. Relying on testimony by Dr. Romesberg, Illumina states that “Ruby reports that disulfide linkages are cleaved with only ~86% efficiency after more than 100 minutes, which is significantly less than 90% efficient.” Mot. 11 (citing Ex. 2016, 117–18; Ex. 2004 ¶ 60). The “~86% efficiency” comes from Figure 4 of Ruby, a graph of % RNA eluted using DTT under different conditions. Ex. 2016, 117. Dr. Branchaud did not dispute that Ruby recovered “approximately 86% of the RNA.” Ex. 1021 ¶ 31.

Dr. Romesberg testified that, because “[t]here is no indication in Ruby that a 3'-OH protecting group that is cleavable under a single set of conditions with a disulfide linkage would be cleaved with any greater efficiency than the approximately 86% cleavage efficiency reported by Ruby for a disulfide linkage,” a person of ordinary skill “would have had no reason to expect cleavage efficiency to be greater than 86%.” Ex. 2004 ¶ 61.

In response, Dr. Branchaud testified that:

even if one of ordinary skill in the art considered the elution percentages of *Ruby* . . . in deciding whether to use such a linker for SBS, one of ordinary skill in the art would know that such elution percentages could be improved by routine experimentation to improve the cleavage efficiency of the disulfide linkers and thus, would not be dissuaded from using such a linker.

Ex. 1021 ¶ 40.

Dr. Branchaud’s testimony is supported factually. Ruby teaches “[e]lution by reduction of the disulfide bonds on the biotinylated anchor RNA depends on the pH of the buffer, the DTT concentration, and the time

of incubation in DTT (Fig. 4) in addition to the type of avidin binding.”
Ex. 2016, 117–118. Ruby describes manipulating the conditions to alter the elution profile: “By increasing the pH and DTT concentration of the elution buffer slightly, one can effectively elute the RNA during longer incubation times.” *Id.* at 118. Thus, Ruby expressly teaches conditions that modify cleavage of the disulfide bond, and that cleavage of the bond can be manipulated by adjusting these conditions. In view of this teaching, one of ordinary skill in the art reasonably would have believed that disulfide bond cleavage could be modified to achieve the desired amount of cleavage.

Dr. Romesberg testified that the 86% value of Ruby could not be exceeded, but did not provide sufficient factual evidence to support this testimony. It is true that Ruby describes an experiment in which, apparently, a maximum of 86% cleavage was obtained, but Ruby did not characterize it as a limit. As Dr. Branchaud testified, it was not critical for Ruby to achieve higher efficiency, so it was not evident why Ruby would have done experimentation to achieve even higher cleavage of the 86% value shown in Figure 4. Ex. 1021 ¶¶ 39–40.

2. *Herman*

Illumina also cites Herman as evidence that 90% cleavage could not be achieved with a disulfide linkage attaching a detectable label to the base of a nucleotide. Mot. 11–12. Herman describes a similar system to Ruby, where RNA is immobilized to a column using a biotin-avidin interaction. Ex. 2019, Abstract, 3:20–25. Herman describes using a biotin attached to a nucleotide base through linker comprising a disulfide bond, as required by the proposed substitute claims. *Id.* at 7:24–34. The disulfide S-S bond is

cleaved with a reducing agent, such as DTT or 2-mercaptoethanol. *Id.* at 7:47–48, 10:3–19. Herman describes the results of one experiment:

Bio-SS-DNA with buffer containing 50 mM dithiothreitol resulted in the recovery of a total of 87% of the DNA from the affinity column. Only 7.3% of the ³²P-labeled Bio-SS-DNA remained bound to the resin.

Id. at 11:14–17.

Dr. Romesberg makes the same conclusions for Herman that he did for Ruby. That is, since Herman’s cleavage was less than 90%, “Herman does not provide an expectation that disulfide cleavage conditions would cleave a 3’-OH protecting group with greater than 90% efficiency.” Ex. 2004 ¶ 67. IBS challenged the conclusion that one of ordinary skill in the art reading Herman would have determined the cleavage efficiency to be 87%, providing testimony by Dr. Branchaud that the real efficiency value was above 90% when calculated properly. Ex. 1021 ¶ 38. Dr. Romesberg acknowledged that Herman’s values did not “add up,” but he testified that there were several possible explanations, of which Dr. Branchaud’s was only one. Ex. 1022 (Romesberg Tr.), 193:6–194:13. We are not persuaded, therefore, that one of ordinary skill in the art would have understood Herman to describe disulfide bond cleavage efficiency above 90%.

The parties cited two additional publications, both co-authored with the same Timothy M. Herman who is listed as inventor of Herman, U.S. Patent No. 4,772,691: Dawson (1989) (Ex. 1027) (*see supra* n.7), cited by IBS, and Dawson (1991) (Ex. 2038) (*see supra* n.7), cited by Illumina. IBS cites Dawson (1989) for its statement in the abstract that “[c]leavage of the disulfide bond in the linker arm of the biotinylated nucleotide resulted in elution of virtually all of the affinity isolated sequences.” Ex. 1021 ¶ 40.

Illumina identifies Dawson (1991) for its disclosure that “[r]eduction of the disulfide bond in the biotinylated nucleotide eluted approximately one-half of the affinity isolated chromatin.” Ex. 2038, 166.

In other words, the “Herman” publications report varying degrees of cleavage: from “virtually all” in Dawson (1989) (Ex. 1027), to 87% in Herman (Ex. 2019), to approximately 50% in Dawson (1991) (Ex. 2038). It is evident, consistent with Ruby, that the conditions can be routinely varied to achieve a desired level of disulfide bond cleavage.

Moreover, as stated by Dr. Branchaud, in the experiments described in Ruby and Herman:

[I]t is not crucial that such elution percentage be greater than 90%. One of ordinary skill in the art would be aware that *Ruby* and *Herman* were not necessarily motivated to achieve a high elution percentage and thus a higher cleavage efficiency. Thus, one of ordinary skill in the art would not be dissuaded from using a disulfide linkage in a modified nucleotide by the elution percentages shown in *Ruby* and *Herman*.

Ex. 1021 ¶ 39.

In an attempt to rebut this testimony, Illumina cites a 1986 publication by a different group which stated: “Release of the nick-translated probe-plasmid complex from avidin by reduction of the disulfide bond of Bio-19-SS-dUTP gave variable results and was not pursued rigorously.” Ex. 2039, 9594. In the ensuing years, however, the Herman group (Exs. 1027, 2019, 2038) did pursue disulfide linkers and showed that higher cleavage rates could be achieved. Exs. 1027, 2019.

3. *Church*

Church does not numerically disclose cleavage efficiency, but shows the results of an experiment in Figure 6. Illumina and IBS dispute the

degree of cleavage shown in Figure 6. Because the quality of Figure 6 is so poor, however, the extent of cleavage cannot be determined reliably. We, therefore, give originally filed Figure 6 no weight.

A later-filed version of Figure 6 was provided by Illumina to support their claim that cleavage was incomplete, but this Figure was not available until after the August 23, 2002 filing date of the '346 patent. Ex. 2033 (showing that the Figure was not available until October 31, 2002). The later-filed Figure, therefore, does not establish how one of ordinary skill in the art would have interpreted the results of Church at the time the '346 patent was filed. Nonetheless, Church suggested disulfide linkers in a sequencing reaction, and carried out an example to show their utility (Ex. 1019, 85–87), providing a reason to have used them in sequencing and a reasonable expectation of success.

4. Summary

The record contains numerous publications that utilize a disulfide bond linker to join a label to a nucleotide base. Rabani and Church used the linker in the context of DNA sequencing, the primary use for the claimed nucleotides described by Illumina. Ruby, Herman, Dawson (1989), Dawson (1991), Short (referenced in footnote 7), and Rigas, each used disulfide linkers to attach a label to a base, but not for sequencing purposes. While the prior art reported variability in the disulfide cleavage rates, Illumina has not established by a preponderance of the evidence that efficiency yields above 90% could not be achieved, even if there were a 90% efficiency threshold required for SBS.

In particular, Ruby and other publications had no reason to go above whatever cleavage rate was achieved, because it was not critical to their

experiments. Herman, in at least one case, described “virtually all” the bond was cleaved. Ex. 1027. Thus, even if 90% efficiency were required for a reasonable expectation of success of using the claimed compounds in an SBS reaction, the ordinary artisan reasonably would have expected that such cleavage efficiency could be achieved.

In sum, the art (*e.g.*, Ju and Tsien) teaches SBS processes using a 3'-OH protecting group and a single set of chemical cleavage conditions; the proposed substitute claim substitutes a known cleavable linker for the linkers used in the prior processes, for the art-recognized purpose of linking the detectable label to the nucleotide (*e.g.*, Rabani and Church). In other words, the improvement claimed is no more than “the predictable use of prior art elements according to their established functions.” *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 417 (2007).

G. Objective Evidence of Nonobviousness

Factual considerations that underlie the obviousness inquiry include the scope and content of the prior art, the differences between the prior art and the claimed invention, the level of ordinary skill in the art, and any relevant secondary considerations. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966). Relevant secondary considerations include commercial success, long-felt but unsolved needs, failure of others, and unexpected results. *KSR*, 550 U.S. at 406; *In re Soni*, 54 F.3d 746 (Fed. Cir. 1995). Secondary considerations are “not just a cumulative or confirmatory part of the obviousness calculus but constitute independent evidence of nonobviousness . . . [and] enable[] the court to avert the trap of hindsight.” *Leo Pharm. Prods., Ltd. v. Rea*, 726 F.3d 1346, 1358 (Fed. Cir. 2013) (internal quotation marks and citations omitted). “This objective evidence

must be ‘considered as part of all the evidence, not just when the decisionmaker remains in doubt after reviewing the art.’” *Transocean Offshore Deepwater Drilling, Inc. v. Maersk Drilling USA, Inc.*, 699 F.3d 1340, 1349 (Fed. Cir. 2012) (internal citations omitted).

In the Motion to Amend, as objective evidence of nonobviousness, Illumina provided a Declaration by Mr. Eric Vermaas, Illumina’s Director of Consumables Product Development, describing sequencing experiments performed under Mr. Vermaas’s supervision while employed by Illumina. Ex. 2023 ¶¶ 4–5. The sequencing experiments used the four nucleotides dATP, dTTP, dGTP, and dCTP, only one of which—dATP—contained a disulfide linker attaching a fluorophore to the nucleotide base. *Id.* ¶ 6; Ex. 2004 ¶ 71. Each of these nucleotides also contained an azidomethyl group protecting the 3’-OH. Ex. 2004 ¶ 71.

Mr. Vermaas describes sequential sequencing reactions on PhiX Control DNA for over 150 cycles in which nucleotides were added one at a time. Ex. 2023 ¶¶ 13–17; Ex. 2004 ¶ 73. After each scan for the fluorophore incorporated, a solution comprising 2 mM tris(hydroxymethyl)-phosphine was added. Ex. 2004 ¶ 74. “The 2 mM tris(hydroxymethyl)-phosphine . . . reacts with and cleaves the disulfide linkage of the A [adenine of the dATP] nucleobase,” but does not cleave the detectable groups on the other nucleotides. *Id.* According to Dr. Romesberg, “[b]ased on the results presented in the Vermaas declaration, a person of ordinary skill in the art would recognize that the yield for cleavage of the disulfide linkage was essentially 100%.” *Id.* ¶ 84. Dr. Romesberg concluded:

Therefore, the disulfide cleavage yield achieved by Illumina was essentially 100%. This is a significant and unexpected improvement over the disulfide cleavages of Ruby (~86%) and

Herman (87%). Accordingly, Illumina's proposed claims are nonobvious over the prior art for at least this reason.
Id.

To establish unexpected results, the claimed subject matter must be compared with the closest prior art. *In re Baxter Travenol Labs.*, 952 F.2d 388, 392 (Fed. Cir. 1991). Illumina does not state what references are the closest prior art to the claims. Dr. Romesberg, however, in his declaration, compared the cleavage efficiency reported by Mr. Vermaas with Ruby and Herman. Ex. 2004 ¶ 84.

There are at least two differences between the experiments described in Ruby and Herman, and the experiment described by Mr. Vermaas. First, while Ruby, Herman, and Vermaas each used a nucleotide with a cleavable disulfide bond, the Ruby and Herman references do not use the nucleotide in a sequencing reaction as it has been used in the experiment described by Mr. Vermaas. Ruby involved RNA binding to a column and using the disulfide linkage to release the bound RNA. Ex. 2016, 98–99. Herman used a system similar to Ruby. Ex. 2019, Abstract, col. 3.

Secondly, the cleavage agents are different. The cleavage agent used in Ruby is DTT (Ex. 2016, 103, “Elution buffer” A and B) and in Herman, the cleavage agent is described as “a reducing agent such as DTT” (Ex. 2019, 7:47–48) with DTT being used in its example (*id.* at 10:47–54, 11:11–22; 12:3–8). Mr. Vermaas describes an experiment that utilized another cleavage agent, 2 mM tris(hydroxymethyl)phosphine. Ex. 2023 ¶ 12. Illumina did not offer an explanation as to why the phosphine compound was used instead of DTT as used by both Ruby and Herman. Indeed, Church used DTT in a DNA sequencing reaction, similar to the sequencing carried out in the Vermaas experiments, providing an additional reason to

have used DTT in Dr. Vermaas's comparison to the prior art. Ex. 1019, Fig. 5, 68:12–13, 86:20–23.

Dr. Romesberg testifies that “the disulfide cleavage yield achieved by Illumina was essentially 100%. This is a significant and unexpected improvement over the disulfide cleavages of Ruby (~86%) and Herman (87%).” Ex. 2004 ¶ 84. There is no testimony, however, that the stated results were due to the claimed nucleotide rather than the reducing agent. While Dr. Romesberg refers to the “disulfide cleavage yield” as being unexpected, he does not testify that this yield was attributable to the claimed nucleotide configuration, rather than the reducing agent which performs the disulfide bond cleavage.

Illumina has not distinguished the Vermaas results from the prior art by showing that the nucleotide is responsible for the disulfide yield, rather than it being a property of the disulfide bond and the yield being “the mere recognition” of the bond’s “latent properties,” which “does not render nonobvious an otherwise known invention.” *Baxter*, 952 F.2d at 392; *In re Geisler*, 116 F.3d 1465, 1468 (Fed. Cir. 1997). Absent such evidence, Illumina has not shown that the claimed subject matter possesses “unexpected results relative to the prior art.” *Galderma Labs., LP v. Tolmar, Inc.*, 737 F.3d 731, 738 (Fed. Cir. 2013). “The combination of familiar elements according to known methods is likely to be obvious when it does no more than yield predictable results.” *KSR*, 550 U.S. at 416.

H. Summary

After considering all the evidence as a whole, we conclude that Illumina has not met its burden in showing that the proposed substitute claims are patentable over the prior art considered in this Decision. The

Motion to Amend is DENIED to the extent that it requests entry of substitute claims 20–26.

V. MOTIONS TO EXCLUDE EVIDENCE

Both IBS (Paper 46) and Illumina (Paper 49) filed Motions to Exclude Evidence. Both motions are *dismissed* as moot.

A. Illumina's Motion

Illumina requests that Figure 6 from Ex. 1019 (Church), all characterizations of Figure 6 from Ex. 1019, and all arguments based on it be excluded as evidence from this *inter partes* review. Paper 49, 1. Illumina argues that the Figure should be excluded because the Exhibit “is not the best evidence of the data contained in Fig. 6. It is a poor quality copy that has drawn objections from IBS’ own counsel, and it cannot be used to draw conclusions regarding cleavage efficiency.” *Id.* at 1. As discussed above, we agree with Illumina that the figure of Church is of poor quality and gave it no weight. We, therefore, did not rely on Figure 6 of Church in reaching our decision, and for this reason Illumina’s motion is moot.

B. IBS's Motion

IBS requests that Illumina’s Exhibits 2033, 2034, 2035, 2041, and 2042, as well as a portion of IBS’s Exhibits 1030 and 1031 relied upon by Illumina, be excluded from evidence. Paper 46, 1. As we did not rely on Exhibits 2034, 2035, 2041, 2042, or 1031, we dismiss this part of the motion as moot.

Exhibits 2033 and 1030 relate to Figure 6 of Church (Ex. 1019). We did not rely on Figure 6 of Church because it is of such poor quality that the amount disulfide cleavage cannot be determined reliably. To remedy this

deficiency, Illumina sought to introduce a substitute Figure 6 from another patent publication by Church. Exs. 2033, 1030. Illumina did not establish that this substitute figure was available prior to the filing date of the '346 patent. We, therefore, did not consider it. Consequently, we dismiss this part of the motion as moot.

VI. ORDER

In consideration of the foregoing, it is

ORDERED that Illumina's Motion to Amend is *denied in part*, to the extent it seeks to add substitute claims 20–26, and *granted in part*, to the extent it seeks to cancel claims 1, 2, 4, 11, 12, 17, 18, and 19;

FURTHER ORDERED that Illumina's motion to exclude evidence is *dismissed* as moot; and

FURTHER ORDERED that IBS's motion to exclude evidence is *dismissed* as moot.

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