

Filed on behalf of:

Patent Owner Illumina Cambridge Ltd.

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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

INTELLIGENT BIO-SYSTEMS, INC.

Petitioner,

v.

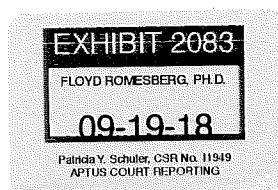
ILLUMINA CAMBRIDGE LTD.

Patent Owner

Case IPR2013-00128 (LMG)

Patent 7,057,026

**SUBSTITUTE DECLARATION OF FLOYD ROMESBERG, PH.D., IN
SUPPORT OF PATENT OWNER'S MOTION TO AMEND**



Columbia Exhibit 2083
Illumina, Inc. v. The Trustees
of Columbia University in the
City of New York
IPR2018-00291, 318, 322, 385 & 797

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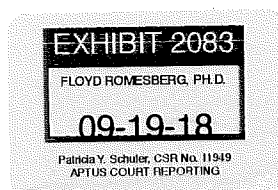
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Illumina Substitute Ex. 2009
IBS v. Illumina
Case IPR2013-00128

1. I, Floyd Romesberg, Ph.D., have been retained by Knobbe, Martens, Olson & Bear, LLP, counsel for Illumina Cambridge Limited (“Illumina”). I understand that the U.S. Patent and Trademark Office Patent Trial and Appeal Board has instituted an inter partes review of the patentability of the claims of U.S. Patent No. 7,056,026 (“the ‘026 patent,” Ex. 1001) based upon a petition filed by Intelligent Bio-Systems, Inc. (“IBS”). The following discussion and analysis provides my opinion as to why the proposed claims submitted in Illumina’s motion to amend are novel and would have been nonobvious.

I. BACKGROUND AND QUALIFICATIONS, PREVIOUS TESTIMONY, AND COMPENSATION

A. Background and Qualifications

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

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

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

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

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

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

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

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

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

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

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

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

14. I have been awarded numerous research grants from the National Institutes of Health, the National Science Foundation, and several other sources, including federal funding for the synthesis and analysis of nucleotide analogues and for the development of DNA polymerases specifically for sequencing by synthesis.

15. I have been the recipient of multiple awards and honors, including the Discover Magazine Technology Innovation Award in 2004, the NSF Career Award in 2004, the Susan B. Komen Breast Cancer Foundation Award in 2003, the Camille Dreyfus Teacher-Scholar Award in 2003, the Baxter Foundation Fellow

Award in 2002, the Mac Nevin Award in 1987, and election to the Defense Science Study Group (DSSG) panel from 2008-2010.

16. I am an expert in the field of nucleotide analogue molecules based on my experience and qualifications in this field. Based on my expertise and qualifications, I am qualified to provide an opinion as to what a person of ordinary skill in the art would have understood, known or concluded as of 2002.

17. Attached as Exhibit 2010 is a copy of my curriculum vitae setting forth my educational experience, employment history, and publications.

B. Previous Testimony

18. I have not previously testified as an expert witness.

C. Compensation

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

II. MATERIALS CONSIDERED

20. Attached as Exhibit 2011 is a listing of the documents that I have considered and reviewed in connection with providing this report.

III. THE PROPOSED CLAIMS

21. Illumina's motion to amend includes two proposed independent claims.

22. Independent proposed claim 9 recites:

9. A nucleotide triphosphate molecule,
having a 7-deazapurine base that is linked to a detectable
label via a cleavable linker,
wherein the cleavable linker is attached to the 7-position
of the 7-deazapurine base and
wherein the cleavable linker contains a disulphide
linkage, and
wherein the nucleotide triphosphate molecule has a
ribose or deoxyribose sugar moiety comprising a
protecting group attached via the 3' oxygen atom,
and
the disulfide linkage of the cleavable linker and the
protecting group are cleavable under identical
conditions.

23. Proposed claim 10 depends from proposed claim 9 and recites:

10. The nucleotide triphosphate molecule of claim 9, wherein
the detectable label is a fluorophore.

24. Independent proposed claim 11 recites:

11. A kit, comprising:
(a) four individual nucleotide triphosphate molecules,
wherein each nucleotide triphosphate molecule has a base that
is linked to a detectable label via a cleavable linker,
wherein the cleavable linker contains a disulphide linkage,
wherein the detectable label linked to each nucleotide
triphosphate molecule can be distinguished upon
detection from the detectable label used for the other
three nucleotide triphosphate molecules,
wherein at least one nucleotide triphosphate molecule has a 7-
deazapurine base that is linked to the detectable label via
the cleavable linker, wherein the cleavable linker is
attached to the 7-position of the 7-deazapurine base, and

each of said individual nucleotide triphosphate molecules has a deoxyribose sugar moiety comprising a protecting group attached via the 3' oxygen atom and the disulfide linkage of the cleavable linker and the protecting group are cleavable under identical conditions; and
(b) packaging materials therefor.

25. Proposed claim 12 depends from proposed claim 11 and recites:

12. The kit of claim 11, further comprising an enzyme and buffers appropriate for the action of the enzyme.

IV. SUPPORT FOR PROPOSED CLAIMS

26. I have read and understand the '026 Patent (Ex. 1001) and U.S. Application No. 10/227,131 ("the '131 Application," Ex. 2008). The '131 Application issued from the United States Patent Office as the '026 Patent. In my opinion, a person of ordinary skill in the art would have understood that the '131 Application as filed conveyed that the inventor possessed the subject matter of proposed claims 9-12 as of at least August 23, 2002.

Support for Proposed Claims 9-10

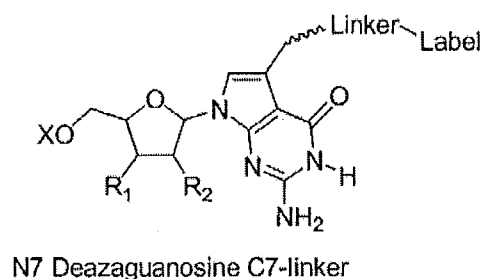
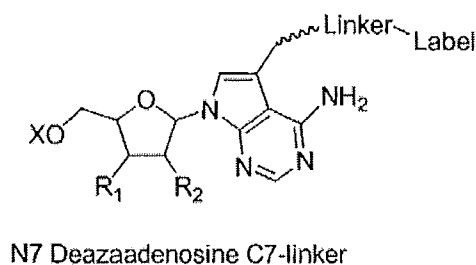
27. The elements that constitute proposed claims 9-10 can be found throughout the '026 Patent (Ex. 1001), as well as the '131 Application (Ex. 2008) as filed with the United States Patent Office on August 23, 2002. Exemplary support for proposed claims 9-10 can be found at the following locations in the '131 Application as filed:

- Figures 1-4 (as detailed below);

- Page 2, l. 27 to Page 3, l. 28 (discussing nucleotides having a deazapurine base, cleavable linkers containing a disulfide linkage, a detectable label attached to the base via a cleavable linker, and a removable protecting group attached to the 3'-OH; and providing that the label can be a fluorophore);
- Page 5, l. 29 to Page 6, l. 15 (explaining Figures 1-4);
- Page 9, l. 18 to Page 10, l. 2 (specifically discussing linker attachment to the 7-position of "the preferred deazapurine analogue," disulfide linkers, nucleotide triphosphates, and 3'-OH protecting groups);
- Page 11, ll. 1-10 (discussing disulfide bond reduction and cleavage in a linker);
- Page 12, ll. 8-13 (discussing protecting the 3'-OH group);
- Page 12, ll. 19-25 (indicating that the "linker may consist of a functionality cleavable under identical conditions" to the 3' protecting group and specifically referencing the functional groups of the linkers of Figure 3);
- Example 1 at page 16, l. 8 to page 20, l. 6 (describing an exemplary synthesis of a dNTP containing a disulfide linker, incorporation of the dNTP into a DNA strand by a polymerase, and subsequent cleavage of the disulfide linker); and

- Original claims 1-8 and 24-25 (claiming nucleotide molecules and kits).

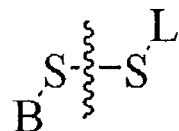
28. Figures 1-4 of the '131 Application (Ex. 2008) are particularly instructive to a skilled artisan with respect to the nucleotide triphosphate molecule described by proposed claims 9-10. For example, Figure 1 as filed illustrates an "N7 Deazaadenosine C7-linker" and an "N7 Deazaguanosine C7-linker," which are reproduced below:



From these structures, as well as the nomenclature used in Figure 1 to describe the structures, a skilled artisan would understand that these molecules include 7-deazapurines. Moreover, the structures and nomenclature indicate that a label is attached to a linker, which in some cases is further attached to the 7-position of the 7-deazapurine base. Furthermore, Figure 1 indicates that the N7 Deazaadenosine C7-linker and the N7 Deazaguanosine C7-linker are either nucleosides (molecules wherein X is hydrogen), nucleotide monophosphates (molecules wherein X is a phosphate), nucleotide diphosphates (molecules wherein X is a diphosphate) or

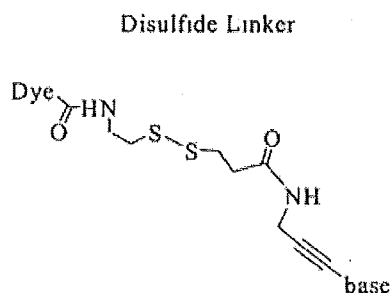
nucleoside triphosphates (molecules wherein X is a triphosphate). Accordingly, Figure 1 of the '131 Application describes a nucleotide triphosphate molecule, having a 7-deazapurine base that is linked to a label via a linker, wherein the linker is attached to the 7-position of the 7-deazapurine base. Figure 1 further indicates that the groups denoted as R_1 and R_2 "are each selected from H, OH, or any group which can be transformed into an OH." A skilled artisan would immediately recognize that if R_1 is OH and R_2 is selected from H or OH, then N7 Deazaadenosine C7-linker and N7 Deazaguanosine C7-linker would have a deoxyribose or ribose sugar moiety, respectively. Moreover, a skilled artisan would understand that R_1 describes a moiety attached to the 3' position of the sugar moiety and R_2 describes a moiety attached to the 2' position of the sugar moiety. A skilled artisan would also understand that if R_1 is a group which can be transformed into an OH, then the ribose or deoxyribose sugar moiety would have a protecting group attached to the 3' oxygen atom. Figure 1 of the '131 Application also directs a skilled artisan's attention to Figure 3 for additional information regarding R_1 and R_2 .

29. Figure 3 of the '131 Application (Ex. 2008) provides additional information regarding cleavable linkers. Figure 3 illustrates that a "Cleavable linker" connects the "Label" to the "Base." Figure 3 further reinforces the description of Figure 1 by indicating that the "Cleavable linker" may include:



From the “S-S” group, a skilled artisan would recognize that the moiety above is a disulfide linkage. A skilled artisan would recognize that the squiggly line indicates where cleavage of the “cleavable linker” is to occur. Moreover, from looking at all the “cleavable linkers” specifically illustrated in Figure 3, it is clear that the squiggly lines are indicative of where cleavage of the “cleavable linker” is to occur. Thus, a skilled artisan would understand from Figure 3 that cleavage of a cleavable linker containing a disulfide linkage is to occur via cleavage of the disulfide bond. Figure 3 also describes several specific protecting groups for R_1 and R_2 that can be transformed into an OH group.

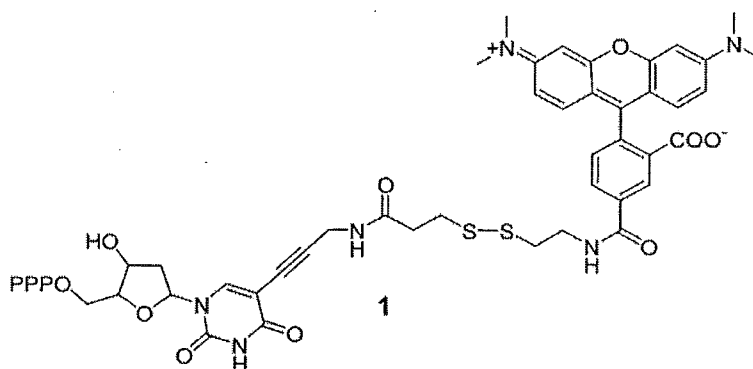
30. Figure 2 of the '131 Application (Ex. 2008) as filed provides an example of a disulfide linker, as reproduced below:



The disulfide moiety, denoted as “S-S”, provides a linkage connecting the “Dye” (Figure 2) or “Label” (Figure 1) to the “base.” Accordingly, a skilled artisan

would understand from Figures 1 and 2 that a linker containing a disulfide linkage is one specific form of a linker described in the '131 Application.

31. Figure 4 of the '131 Application (Ex. 2008) as filed, in conjunction with the description elaborated as Example 1, provides additional exemplary support for a nucleotide triphosphate molecule having a cleavable linker containing a disulfide linkage. Figure 4 illustrates the incorporation of Compound 1 (illustrated below) by a polymerase.



Compound 1, as indicated by both Example 1 and the description in Figure 4, possesses a disulfide cleavable linker connecting a fluorescent label to the base of a 2'-deoxyribonucleoside triphosphate. Figure 4 also indicates that the linker is cleaved with DTT. *See also*, '131 Application at page 20, ll. 1-6¹ (Ex. 2008). DTT

¹ There appears to be a typographical error in the description of Example 1 of the '131 Application at page 20, ll. 2-3, which should refer to "Fig. 4" instead of "Fig. 3." Compare '131 Application at page 20, ll. 2-3 (describing Klenow polymerase

is an abbreviation for dithiothreitol, as is clear to any skilled artisan. Under the conditions described in Figure 4 and Example 1, a skilled artisan would understand that the addition of DTT cleaved the disulfide bond of the disulfide linker in accordance with the cleavage position denoted in Figure 3. In addition, the '131 Application provides that reductive conditions can be used to cleave a disulfide linkage. *Id.* at page 11, ll. 1-4. As discussed in detail below (see ¶¶ 64, 67), reduction of a disulfide bond can be accomplished by, e.g., a phosphine-containing compound. For these reasons, and the reasons discussed above, a person of ordinary skill in the art would have understood that the '131 Application as filed conveyed that the inventor possessed the subject matter of proposed claims 9-10 as of the '131 Application's filing date.

Support for Proposed Claims 11-12

32. The elements that constitute proposed claims 11-12 can be found throughout the '026 Patent (Ex. 1001), as well as the '131 Application (Ex. 2008) as filed with the United States Patent Office on August 23, 2002. The exemplary

incorporation of a nucleotide triphosphate in Example 1) *with* page 6, ll. 6-15 (stating that Fig. 4 “shows a denaturing gel showing the incorporation of the triphosphate of Example 1 using Klenow polymerase”) (Ex. 2008).

evidence cited above regarding proposed claims 9-10 also supports proposed claims 11-12.

33. The '131 Application (Ex. 2008) as filed, particularly at page 5, ll. 14-19, discusses the packaging of nucleotide-containing kits. It is my understanding that proposed claims 11-12 describe a kit that contains each of the four nucleotide triphosphate molecules, at least one of which has a cleavable linker that contains a disulfide linkage. It is also my understanding from the language used in proposed claims 11-12 that one of the nucleotide triphosphate molecules included in the kit is the nucleotide triphosphate molecule of proposed claim 9. For the reasons discussed above with respect to the support of proposed claim 9, and in conjunction with the description of kits in the application as filed and also cited above, a person of ordinary skill in the art would have understood that the '131 Application as filed conveyed that the inventor possessed the subject matter of proposed claims 11-12 as of the '131 Application's filing date. Nucleoside triphosphate molecules possessing the elements recited in proposed claims 11-12 are specifically described in Figures 1-4, and are generally described at page 2, l. 27 to page 3, l. 28; page 5, ll. 14-19; page 5, l. 29 to page 6, l. 15; page 9, l. 18 to page 10, l. 2; page 11, ll. 1-10; page 12, ll. 8-13; page 12, ll. 19-25; and Example 1. As described above with respect to proposed claim 9, a skilled artisan would have understood the '131 Application as filed to describe nucleotide triphosphate

molecules having a cleavable linker that possessed a disulfide linkage, and would have particularly understood this description as applied to “at least one nucleotide triphosphate molecule has a 7-deazapurine base that is linked to the detectable label via the cleavable linker, wherein the cleavable linker is attached to the 7-position of the 7-deazapurine base.” Accordingly, proposed claims 9-12 are supported by the '131 Application.

V. LEGAL STANDARDS

Novelty

34. I understand that 35 U.S.C. § 102 governs the determination of novelty. I understand that under 35 U.S.C. § 102, a prior art reference does not anticipate a claim unless each and every element of a claim is found, either expressly or inherently, within the single prior art reference.

Nonobviousness

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

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

invention pertains. Patentability shall not be negated by the manner in which the invention was made.

36. I understand that hindsight analysis must be avoided to prevent a person from looking back at the path the inventors used to arrive at the claimed invention and inappropriately concluding that the inventor's path would have been obvious. I understand that an analysis under 35 U.S.C. § 103 starts by (1) considering the scope and content of the prior art; (2) determining the differences between the prior art and the claimed invention; and (3) understanding the level of skill that a person of ordinary skill in the art would have had at the time of filing the patent application.

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

Claim Construction

38. I understand that the terms of a claim must be interpreted to determine the scope of the invention and the meaning of the words used in the claim. In interpreting a claim, I understand that one initially applies the plain meaning of the terms as would have been understood by a person of ordinary skill in the art at the time of the invention. Also, I understand that a person of ordinary skill in the art is

deemed to have read the claim terms within the context of the entire patent, including the specification. In addition, I understand that one must review the patent prosecution history to determine if the patentee expressly defined a claim term during prosecution. I understand that one may review other extrinsic evidence if an ambiguity in a claim term exists, or to confirm the accuracy of the interpretation of the claim term. Further, I understand that, in proceedings such as this proceeding, the USPTO applies the broadest reasonable construction of the claims that is consistent with the specification.

VI. A PERSON OF ORDINARY SKILL IN THE ART

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

40. In my opinion, a person of ordinary skill in the art related to the '026 patent would have been a member of a team of scientists addressing sequencing by synthesis product development, such a person of skill in the art would have held a doctoral degree in chemistry, molecular biology, or a closely related discipline, and had at least five years of practical academic or industrial laboratory experience. Thus, a person of ordinary skill in the art includes a person having a doctoral degree in a field related to chemistry, and at least five years of specific laboratory experience directed toward the research and development of DNA sequencing technologies.

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

VII. CONSTRUCTION OF THE TERM “CLEAVABLE UNDER IDENTICAL CONDITIONS”

42. Proposed claim 9 recites a nucleotide triphosphate molecule where “the disulfide linkage of the cleavable linker and the protecting group are cleavable under identical conditions.” Dr. Branchaud and I agree that this recitation limits the types of structures that can be used by the claimed nucleotide molecule. Branchaud Tr. at 170:18-23 (Ex. 2012).

VIII. TECHNICAL BACKGROUND AND STATE OF THE ART

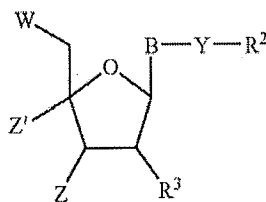
43. In 1977, two methods of DNA sequencing were reported. One of these methods was the chemical sequencing method developed by Maxam and Gilbert. Maxam and Gilbert, Proc. Nat’l. Acad. Sci. USA, 74:560-564, 1977 (Ex. 2026). The second method was the dideoxy method developed by Sanger. Sanger *et al.*, Proc. Nat’l. Acad. Sci. USA, 74:5463-5467, 1977 (Ex. 2019).

44. The concept of DNA sequencing by synthesis (SBS) was well established by 1999. *See e.g.*, Cheeseman 5,302,509 (issued Apr. 12, 1994) (Ex. 2020); Metzker *et al.*, Nucleic Acids Res. 22: 4259-4267, 1994 (Ex. 2021); and Welch *et al.*, Nucleosides and Nucleotides 18:197-201, 1999 (Ex. 2022)).

IX. SCOPE AND CONTENT OF THE PRIOR ART

U.S. 7,078,499 (“Odedra, Ex. 2013) and WO 01/92284 (“Odedra PCT”, Ex. 2014)

45. U.S. 7,078,499 (“Odedra, Ex. 2013) and WO 01/92284 (“Odedra PCT”, Ex. 2014) disclose nucleotide analogues with a reporter moiety and a polymerase enzyme blocking moiety. In particular, Odedra and Odedra PCT disclose compounds of the following general structure:



wherein W is a phosphate group; B is a base; Y is a linker comprising an enzyme-cleavable group; R² is a reporter moiety; R³ is selected from H or OH; Z and Z' are selected from H, OH, or a group X-R¹, wherein X is a linker comprising an enzyme-cleavable group and R¹ is a polymerase enzyme blocking group, provided that at least one of Z and Z' is X-R¹. Odedra at col. 3, ll. 1-23; Odedra PCT at page 4, ll. 1-15.

46. Among several possibilities taught by Odedra for substituent Y, Odedra discloses that Y “may be a chain of up to 30 bond lengths and may include atoms selected from carbon, nitrogen, oxygen and sulphur atoms, the linker group

may be rigid or flexible, unsaturated or saturated as is well known in the field.”
Odedra at col. 3, ll. 32-35; Odedra PCT at page 4, ll. 26-29.

47. I disagree with Dr. Branchaud’s assertion that “a chain of up to 30 bond lengths [that] may include atoms selected from carbon, nitrogen, oxygen and sulphur atoms” (Odedra at col. 3, ll. 32-34; Odedra PCT at page 4, ll. 26-27) would inform a person of ordinary skill in the art “to use a linker comprising a disulfide linkage as a means of attaching the removable detectable label to the base in the modified nucleotide of *Odedra*.” Second Branchaud Declaration² at ¶ 47 (Ex. 2023).

48. Odedra does not provide any exemplary Y linkers that contain a single sulfur atom, much less an example of a linker containing a *di*-sulfide bond. Moreover, the chain of up to 30 bond lengths “may” include carbon, nitrogen, oxygen and sulfur; the linker does not require a single sulfur atom. The number of linkers included within Odedra’s Y linker of from 1 to 30 bond lengths includes a nearly incomprehensible number of compounds (equal to 4^{30} factorial), which exceeds more than a quadrillion compounds. Based on Odedra’s broad linker containing from 1 to 30 bond lengths (which does not even require any sulfur

² The “Second Branchaud Declaration” refers to the Branchaud declaration submitted by IBS in IPR2013-000324, which also involves U.S. 7,057,026.

atoms) one of skill in the art would not understand Odedra to specify a linker containing a disulfide bond. Therefore, the Y linker of Odedra does not teach a person of skill in the art to use a linker containing a disulfide bond. Furthermore, Odedra does not teach a disulfide linker that is cleavable under identical conditions to a 3'-protecting group. Therefore, Illumina's proposed claims are novel over Odedra.

WO 96/27025 ("Rabani, Ex. 2015)

49. WO 96/27025 ("Rabani, Ex. 2015) is directed to a "device, compounds, algorithms, and methods of molecular characterization and manipulation with molecular parallelism." Rabani at Title. In particular, Rabani discloses "[m]ethods and means [that] are provided for the massively parallel characterization of complex molecules and of molecular recognition phenomena with parallelism and redundancy attained through single molecule examination methods." Rabani at Abstract.

50. Rabani states that "[t]he results published [in the prior art] suggests 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." Rabani at page 3, ll. 5-8. Since DNA sequencing by synthesis is an "inherently serial sequencing scheme," this statement in Rabani applies to sequencing by synthesis. A person of ordinary skill

in the art would have been well aware that incorporation of each sequential nucleotide (and any related deprotection of a 3'-protecting group or cleavage of a linker from the nucleotide) in a DNA sequencing by synthesis process must be highly efficient to permit accurate sequencing. For example, if incorporation of each nucleotide (including any 3'-deprotection or linker cleavage) were only 85% efficient, after just 8 iterations there would be $(0.85)^8$, or just 27%, of the sequenced polynucleotide containing the correct sequence of nucleotides. The remaining 73% of the polynucleotides would be one or more bases (or cycles, where one base is read per cycle) "out of phase" because they failed to incorporate a base during a previous iteration. Moreover, analogs that failed to incorporate a nucleotide in one cycle (*i.e.*, due to failed deprotection) can re-enter the process the next cycle, but will then read an incorrect base because it is a round behind the remainder of the nucleotides. With only 27% of the polynucleotides providing the correct signal, and the others providing ambiguous or wrong signals, the noise in the system will overwhelm the actual signal such that detection of the next incorporated nucleotide, or any additional nucleotides in that particular strand would not be possible. After 16 iterations there would be only $(0.85)^{16}$, or just 7%, of the sequenced polynucleotide containing the correct sequence of nucleotides. The remaining 93% of the polynucleotides would be one or more bases "out of phase" because they failed to incorporate a correct base during a previous iteration.

If the efficiency of a DNA sequencing method were limited to just 8 accurate identification iterations, then the method would be insufficient to sequence even a short bacterial genome because 8 bases of information does not provide enough statistical information to uniquely place that particular 8 base sequence within the overall genome. *See, e.g.*, WO 89/10977 (“Southern,” Ex. 2024) at page 6, l. 31 to page 9, l. 21. This problem would be even more pronounced in complex genomes. *Id.* Therefore, Rabani is correct that 3’-OH protecting group cleavage of less than 90% efficiency is unacceptably low for use in iterative DNA sequencing, such as SBS.

51. Rabani discloses several types of linkers, including ester linkages, amide linkages, disulfide linkages, photolabile linkages, and thermolabile linkages. *See, e.g.*, Rabani at page 32, l. 9 to page 33, l. 32. Rabani cites to Ruby³ for an example disulfide linkage that can be cleaved with dithiothreitol. Rabani at page 32, ll. 29-33. As discussed below, Ruby reports that disulfide linkages are cleaved with less than 90% efficiency. *See* Ruby at 117-118.

52. There is no indication in Rabani that a 3’-OH protecting group that is cleavable under identical conditions as a disulfide linkage would be cleaved with any greater efficiency than a disulfide linkage. Since Rabani’s cleavage efficiency

³ Ruby *et al.*, Methods in Enzymology, 181:97-121, 1990 (“Ruby,” Ex. 2016).

is less than 90% (*see* Rabani at page 32, ll. 29-33 (*citing* Ruby); *see also* Ruby at 117-118), Rabani does not provide an expectation that disulfide cleavage conditions would cleave a 3'-OH protecting group with greater than 90% efficiency.

Ruby *et al.*, Methods in Enzymology, 181:97-121, 1990 (“Ruby,” Ex. 2016)

53. Ruby *et al.*, Methods in Enzymology, 181:97-121, 1990 (“Ruby,” Ex. 2016) teaches affinity chromatography with biotinylated RNAs. Ruby at Title. Ruby discloses RNA polynucleotides that contain modified uridine residues. Ruby at 98, l. 16-18; *see also* FIG. 2. The modified uridine residues do not contain a disulfide linker until after the uridine residue has already been incorporated into the RNA polynucleotide. Ruby at FIG. 2. Ruby does not disclose a deoxyribonucleotide triphosphate molecule containing a disulfide linker.

54. Ruby discloses a “biotinylated anchor RNA” containing 32-35 nucleotides, including several modified uridine nucleotides. Ruby at FIG. 2. After the modified uridine residues were incorporated into the anchor RNA, disulfide linkers were installed on the modified uridine residues to connect the uracil bases to biotin in a reversible manner. *Id.* Ruby teaches that a disulfide linker can be cleaved using dithiothreitol (DTT). Ruby at 117-118; *see also* FIG. 4. For example, FIG. 4 of Ruby (which is reproduced below) shows the percent of

biotinylated anchor RNA eluted from a solid support as a function of time after incubation with various concentrations of DTT at two different pH concentrations.

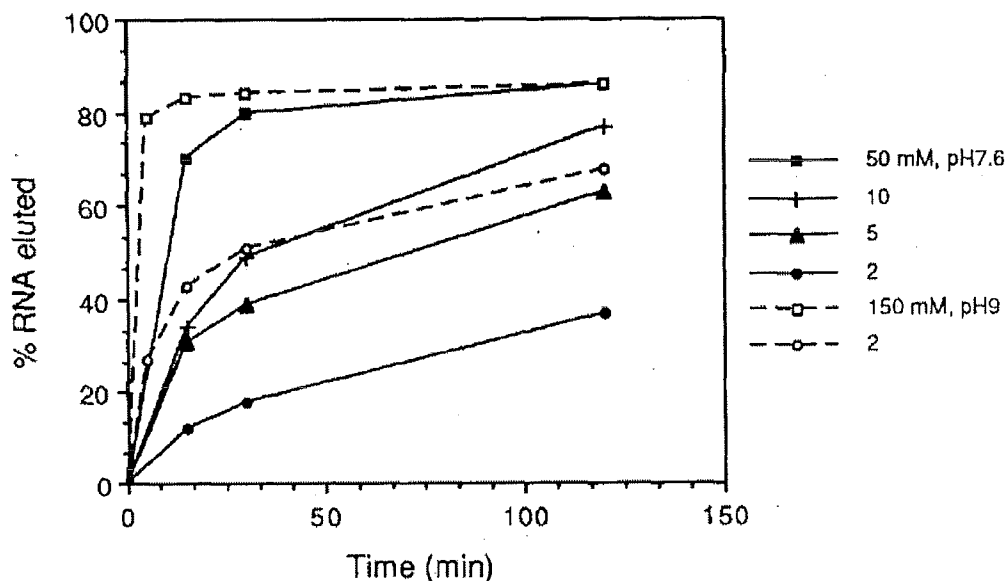


FIG. 4. Eluting biotinylated anchor RNA. Biotinylated anchor RNA 3'-21 was bound to biotin-acryl with succinylavidin as described in Methods. The amounts of RNA eluted with various concentrations of DTT at pH 7.6 (solid lines) or with 2 or 150 mM DTT at pH 9 (dashed lines) were measured as a function of time of incubation in the elution buffers.

The disulfide linker cleavage reported by Ruby provides a maximum recovery of RNA of approximately 86% after more than 100 minutes. Ruby at FIG. 4.

55. As discussed above with Rabani, there is no indication in Ruby that a 3'-OH protecting group that is cleavable under identical conditions as a disulfide linkage would be cleaved with any greater efficiency than the approximately 86% cleavage efficiency reported by Ruby for a disulfide linkage. Since Ruby's cleavage efficiency under disulfide cleavage conditions is less than 90%, Ruby

does not provide an expectation that disulfide cleavage conditions would cleave a 3'-OH protecting group with greater than 90% efficiency.

U.S. 4,772,691 ("Herman," Ex. 2017)

56. U.S. 4,772,691 ("Herman," Ex. 2017) is directed to chemically cleavable nucleotides. Herman at Title. In particular, Herman discloses "biotinylated nucleotides [that] have a chemically cleavable linker between a biotin and an organic basic group." Herman at Abstract.

57. Herman discloses a disulfide linker. *See e.g.*, Herman at cols. 5-6. Herman discloses that the disulfide linker is cleaved by five washes with dithiothreitol to recover a total yield of 87% of the cleavage product. Herman at col. 11, ll. 12-17.

58. As with Ruby and Rabani, there is no indication in Herman that a 3'-OH protecting group that is cleavable under identical conditions as a disulfide linkage would be cleaved with any greater efficiency than the 87% cleavage efficiency reported by Herman for a disulfide linkage. Since Herman's cleavage efficiency under disulfide cleavage conditions is 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.

WO 99/49082 (“Short,” Ex. 2018)

59. WO 99/49082 (“Short,” Ex. 2018) is directed to “modified nucleotides and methods useful for nucleic acid sequencing.” Short at Title. In particular, Short discloses a method for sequencing nucleic acids, including incorporating certain modified nucleotides into nucleic acid strands. Short at Abstract.

60. Short discloses that disulfide-based coupling systems can be utilized as linkers. Short at page 22, 24, and FIG. 2. For example, Short shows a dideoxynucleoside triphosphate that contains a disulfide linker. Short at page 22. Short discloses cleavage of a disulfide bond using “mercaptoethanol dithiothreitol.” Short at page 24. Short does not provide any experimental results reporting the overall yield of a disulfide linker cleavage reaction. Accordingly, Short does not provide a person of skill in the art with an expectation that the disulfide cleavage efficiency would improve upon the results reported by Ruby (~86% yield) or Herman (87% yield).

**X. SECONDARY CONSIDERATIONS SUPPORT THE
NONOBVIOUSNESS OF THE PROPOSED CLAIMS**

61. I understand that Illumina has evaluated the incorporation of nucleotides having disulfide linkers and the cleavage efficiency of such linkers. *See* Vermaas Decl. (Ex. 2028). Illumina has demonstrated that disulfide linkages

can be efficiently cleaved using 2 mM tris(hydroxymethyl)phosphine. *Id.* at ¶¶ 15-19. The Vermaas declaration shows a nucleotide sequencing experiment in which dATP, dTTP, dCTP and dGTP analogs were used, each having a different moiety linked to the nucleobase, as follows:

- linked to the nucleobase of dATP was an azidoalkyl moiety, a disulfide linkage, and a fluorophore (in that order);
- linked to the nucleobase of dTTP was an azidoalkyl moiety and a fluorophore;
- linked to the nucleobase of dCTP was an azidoalkyl moiety and biotin;
- no label was linked to the nucleobase of dGTP.

Id. at ¶ 6. Thus, of the dATP, dTTP, dCTP and dGTP analogs, only dATP and dTTP initially contained fluorophores. Each of these nucleotides also contained an azidomethyl group protecting the 3'-OH.

62. The four nucleotide analogs permitted a single excitation wavelength and a single detection wavelength to be used to determine the identity of the nucleobase that was incorporated into the polynucleotide strand, according to the method described in steps (a)-(e) of paragraph 15 of the Vermaas declaration (Ex. 2028) and shown below:

- a. Adjust temperature to 60° C (15 second duration);

- i. Wash (120 μ L, 3.6 second duration);
 - ii. OCM (60 μ L, 1.8 second duration);
 - iii. Wash (20 μ L, 1 second duration);
 - iv. Dispense contents to waste (6 second duration);
 - v. Wash (250 μ L, 7.5 second duration);
 - vi. Incorporation Mix (OIM) delivery (60 μ L, 1.8 seconds incubation);
 - vii. Wash (20 μ L, 1 second duration);
- b. Adjust temperature to 65° C (25 second duration);
 - i. Wash (5 μ L, 1 second duration);
 - ii. Dispense contents to waste (30 second duration);
- c. Adjust temperature to 22° C (15 second duration);
 - i. Wash (120 μ L, 3.6 second duration);
 - ii. Scan Mix (SRE), scan (60 μ L, 1.8 seconds incubation);
 - iii. Wash (20 μ L, 0.6 seconds duration);
 - iv. Dispense contents to waste (25 second duration);
 - v. Image (60 second duration);
- d. Adjust temperature to 60° C (15 second duration);
 - i. Wash (125 μ L, 3.75 second duration);

- ii. Streptavidin-Binding Mix (SBM) (60 μ L, 1.8 second duration and 5 seconds incubation);
- e. Adjust temperature to 22° C (15 second duration);
 - i. Wash (60 μ L, 1.8 second duration);
 - ii. Scan Mix (SRE), scan at 527 nm (60 μ L, 1.8 seconds incubation);
 - iii. Wash (20 μ L, 0.6 seconds duration);
 - iv. Dispense contents to waste (15 second duration); and
 - v. Image (60 second duration).

63. Specifically, steps (a)(vi)-(b) are the incorporation steps in which DNA polymerase-mediated nucleotide incorporation occurs. Vermaas Decl. at ¶¶ 9 & 15 (Ex. 2028). In step (c), the sample is scanned for incorporation of a fluorophore. Detection of fluorophore incorporation indicates that either an A nucleotide or T nucleotide was incorporated. *Id.* at ¶¶ 11 & 15.

64. In step (d) a solution containing fluorophore-labeled streptavidin and 2 mM of tris(hydroxymethyl)-phosphine is added. *Id.* at ¶¶ 12 & 15. The 2 mM tris(hydroxymethyl)phosphine reacts with and cleaves the disulfide linkage of the A nucleobase, but does not cleave the azidoalkyl moieties linked to the A, T or C nucleobases. Thus, the solution in step (d) selectively cleaves the fluorophore from A nucleobase but does not cleave the fluorophore from the T nucleobase or

the biotin from the **C** nucleobase. The fluorophore-labeled streptavidin in the solution binds the biotin linked to the **C** nucleobase. Thus, after the solution in step **(d)** is added, only CTP and TTP are labeled with a fluorophore.

65. In step **(e)**, the sample is scanned for incorporation of a fluorophore. *Id.* at ¶¶ 11 & 15. Detection of fluorophore incorporation demonstrates that either a **C** nucleotide or **T** nucleotide was incorporated. The azidoalkyl groups of the now-sequenced residue are then removed in step **(a)(ii)** with a buffered solution containing a concentration of tris(hydroxymethyl)phosphine that is greater than 2 mM. *Id.* at ¶¶ 10 & 15.

66. The template is washed, and then primed for the next incorporation steps **(a)(vi)-(b)**. *Id.* at ¶ 15.

67. Both the Cleavage Mix (OCM) and Streptavidin-Binding Mix (SBM) contain tris(hydroxymethyl)phosphine. *Id.* at ¶¶ 10 & 12. The 2 mM concentration of tris(hydroxymethyl)phosphine in the SBM solution is sufficient to cleave the disulfide linkage from the **A** nucleobase in step **(d)**. Generally speaking, if a cleavable moiety is cleaved by exposure to a certain amount of cleavage reagent, a person of ordinary skill in the art would recognize that exposure of a cleavable moiety to additional amounts of the cleavage reagent would also cleave the cleavable moiety. *See* Branchaud Tr. at 183:9-184:20 (Ex. 2012). Because a 2 mM concentration of tris(hydroxymethyl)phosphine cleaved the disulfide linkage

from the **A** nucleobase in the sequencing experiment described by Vermaas, a person of ordinary skill in the art would recognize that the increased concentration of tris(hydroxymethyl)phosphine that cleaved the 3'-OH azidomethyl protecting group (*see* Vermaas Decl. at ¶¶ 10 & 15 (Ex. 2028)) would be a sufficiently high concentration of tris(hydroxymethyl)phosphine to also cleave the disulfide linkage from the **A** nucleobase. Therefore, the increased concentration of tris(hydroxymethyl)phosphine described in the Vermaas declaration provides an exemplary condition for cleaving the disulfide linkage and 3'-OH protecting group under identical conditions.

68. As a result of the scans in steps (c) and (e) and the disulfide cleavage reaction in step (d), the identity of the incorporated base can be determined. *See, e.g.,* Vermaas Decl. at ¶ 19 (Ex. 2028). This determination relies on the ability of the disulfide linkage to be efficiently cleaved. That is, if fluorescence is detected in step (c), the base is an **A** or **T**, and if no fluorescence is detected in step (c), the base is a **C** or **G**. Next, provided that the disulfide cleavage is complete, if fluorescence is detected in step (e), the base is **C** or **T**, and if no fluorescence is detected in step (e), the base is **A** or **G**.

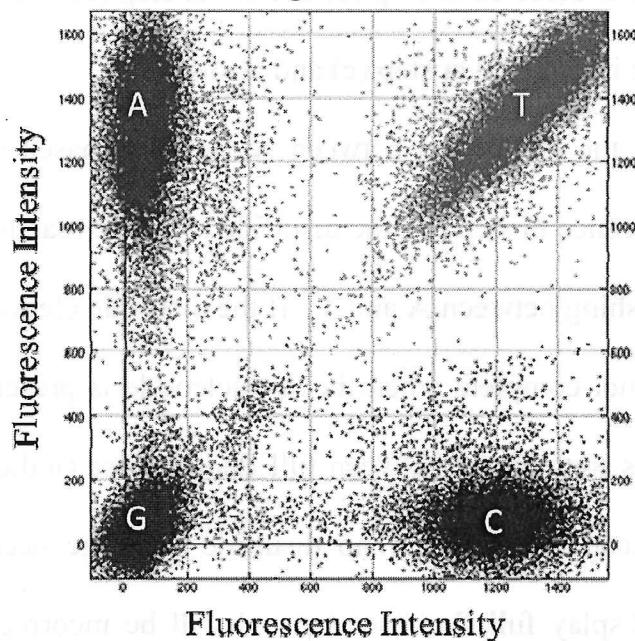
69. Thus, a **G** is present when fluorescence is not detected at step (c) or at step (e). *See, e.g.,* Vermaas Decl. at ¶ 19 (Ex. 2028). A **C** is present when fluorescence is not detected at step (c) but is detected at step (e). An **A** is present

when fluorescence is detected at step (c) but not at step (e). Finally, a T is present when fluorescence is detected at step (c) and at step (e).

70. Since the distinction between A and T is based on the presence or absence of fluorescence in step (e), the disulfide cleavage reaction in step (d) is the basis for distinguishing between A and T. If the disulfide cleavage reaction in step (d) is partial and not complete when the A nucleotide is present, a signal will be measured that falls somewhere between full fluorescence (indicative of T) and no fluorescence (indicative of A). If no disulfide cleavage occurs in step (d), A nucleotides will display full fluorescence and will be incorrectly identified as T nucleotides. If disulfide cleavage in step (d) is complete, A nucleotides will not fluoresce and will be correctly identified.

71. Figure 3 of the Vermaas declaration shows a cloud plot of the fluorescence detection for all sequencing cycles, which is reproduced below:

Figure 3



Id. at ¶ 19. The y-axis is the fluorescence intensity measured at step (c), and the x-axis is the fluorescence intensity measured at step (e). Figure 3 shows a clear separation in the peaks for each of the four nucleotides. As discussed above, if the disulfide cleavage reaction in step (d) is not complete, the measurements associated with A nucleotides will fall somewhere between full fluorescence (indicative of T) and no fluorescence (indicative of A). However, in Figure 3, the A and T peaks are clearly distinct. Importantly, there is no significant number of measurements falling between A and T that is greater than the number of measurements falling between A and G, T and C, or G and C. Further, the clustering of fluorescence intensities that constitutes the A peak is not significantly

different than the clustering of fluorescence intensities that constitute the **C**, **G** and **T** peaks. As such, the cloud plot of Figure 3 indicates that disulfide cleavage occurred efficiently in this sequencing experiment.

72. The Vermaas declaration also reports the percent error rates for basecalls over 150 cycles of steps (a)-(e). *Id.* at ¶ 17, Figure 1. The error rates are generated based on a comparison of the sequence generated using a DNA template with a known sequence. Thus, it was readily possible to assess whether or not each base determination was correct or incorrect. As reported in the Vermaas declaration, the error rate was approximately 0.43% over 150 cycles. *Id.* at ¶ 17. The percent error rate for **A** basecalls was substantially the same as the error rate for **C**, **G**, and **T** basecalls. *Id.* at ¶ 18, Figure 2. The error rates for **C**, **G** and **T** basecalls serve as an indication of the error rate that is independent of the disulfide cleavage reaction in step (d). In addition, the low error rates over 150 cycles also indicates that the azidomethyl 3'-OH protecting group was being cleaved with high efficiency each round.

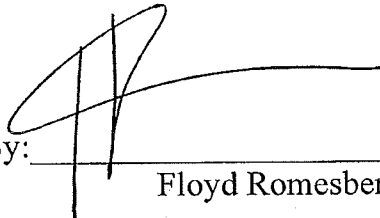
73. As can be seen in Figure 2 of the Vermaas declaration, the error rate for **A** basecalls is nearly identical to, or lower than, the error rate for all **C**, **G** and **T** basecalls. *Id.* at ¶ 18. Thus, no significant amount of error is caused by the disulfide cleavage reaction in step (d). This statistical evidence indicates that the

disulfide cleavage reaction in step (d) was virtually fully (>99%) complete in all instances. In particular, the T->A misreads were less than 0.1%. *Id.*

74. Based on the results presented in the Vermaas declaration, a person of skill in the art would recognize that the yield for cleavage of the disulfide linkage was essentially 100%, because the clustering of fluorescence intensities for the A peak in Figure 3 is substantially the same as the clustering of fluorescence intensities for the C, G and T peaks, and because the error rate for A identification is substantially identical to the error rate for C, G and T identifications. 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.

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

Dated: Jan 9, 2014

By: 
Floyd Romesberg, Ph.D.