

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

GUARDANT HEALTH, INC.,
Petitioner,

v.

UNIVERSITY OF WASHINGTON THROUGH ITS CENTER FOR
COMMERCIALIZATION,
Patent Owner.

Case No. IPR2022-00817
Patent No. 10,760,127

DECLARATION OF DR. JOHN QUACKENBUSH

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I, John Quackenbush, declare as follows:

I. QUALIFICATIONS

1. I have been retained by Counsel on behalf of Guardant Health, Inc. to provide opinions in this matter related to my experience and expertise. My opinions are based on my skills, knowledge, training, education, and experience in matters of this nature, and my examination of the materials utilized in preparing this report.

2. I am a Henry Pickering Walcott Professor of Computational Biology and Bioinformatics and Chair of the Department of Biostatistics at the Harvard T.H. Chan School of Public Health. I am Director of the MS Program in Computational Biology and Quantitative Genetics, Director of the Big Data to Knowledge Training Program, and co-Director of the Cancer Genomics Training Program. I hold appointments as Professor of Biostatistics and Computational Biology at the Dana-Farber Cancer Institute and at the Channing Division of Network Medicine of Brigham and Women's Hospital.

3. In addition to my academic appointments, I co-founded Genospace LLC. Genospace produces software solutions to operationalize precision medicine through the integration of clinical and genomic data and its presentation to clinical and research users in an intuitive format. Genospace was purchased by Hospital Corporation of America in January of 2017.

4. In addition to my academic and research responsibilities, over the years I have taken significant regional and national research leadership responsibilities. I have served on the Scientific Advisory Board of the St. Jude's Children's Research Hospital and the Board of Scientific Counselors of the Environmental Protection Agency. I have also served on numerous scientific review panels for the National Institutes of Health, the National Science Foundation, the Department of Energy, the National Aeronautics and Space Administration, the Wellcome Trust, Genome British Columbia, Genome Canada, the National Sciences and Engineering Council of Canada, and the European Union. And I have served on numerous study panels for the National Research Council, the National Academy of Sciences, and the Institute of Medicine.

5. I have presented more than 300 talks, courses, and workshops since joining the Harvard School of Public Health and Dana-Farber in 2005, including the Master's Core Course, Biostatistics 280, Genomics and Genetics for Health Research. I have also taught numerous courses and workshops on the analysis of genomic data and served as an organizer of the EMBO course on DNA microarray analysis for more than five years.

6. Prior to joining the faculty at Dana-Farber and Harvard, I was a Professor of Chemical Engineering at the University of Maryland from 2003-2005; an Investigator with The Institute for Genomic Research from 2002-2006; a

Professor in the Department of Biochemistry at The George Washington University from 2000 – 2005; a Lecturer in The Department of Biostatistics at The Johns Hopkins University from 1998 – 2005; an Associate Investigator at The Institute for Genomic Research from 1997 – 2001; and an Assistant Investigator at The Institute for Genomic Research in 1997.

7. I received my Ph.D. in Theoretical Particle Physics at the University of California, Los Angeles in 1990. I also hold a Master of Science in Physics from University of California, Los Angeles and a Bachelor of Science in Physics from the California Institute of Technology.

8. I have studied, taught, practiced, and conducted research involving the use of genomic technologies, including DNA sequencing and DNA microarrays, and involving computational analysis of data related to genomic technologies. I have expertise in genomic technologies, computational analysis, and modeling of a plant, animal, and microbial systems with an emphasis on the study of human diseases, such as cancer.

9. I have received numerous awards and honors including being recognized as a White House Open Science Champion of Change, an Australia Fellow, and a Distinguished International Advisor for the International Conference of BioInformatics and BioEngineering sponsored by the Institute of Electrical and Electronics Engineers.

10. I have published more than 320 peer reviewed scientific papers that have been cited more than 84,000 times. I have had continuous grant support for my research from the National Institutes of Health and other organizations for nearly twenty-eight years. In 2018, I received an NCI Outstanding Investigator Career Award, a seven-year research grant recognizing a lifetime record of substantial contributions to the field.

11. A copy of my *Curriculum Vitae*, attached as EX1003, contains further details on my education, experience, publications, and other qualifications to render an expert opinion in this matter.

II. SCOPE OF WORK

12. I understand that petitions are being filed with the United States Patent and Trademark Office for Inter Partes Review of U.S. Patent No. 10,760,127 to Salk, et al. (“the ’127 patent,” attached as EX1001), entitled “Methods Of Lowering The Error Rate Of Massively Parallel DNA Sequencing Using Duplex Consensus Sequencing.”

13. I have been retained by Guardant Health, Inc. (“Guardant”) to offer an expert opinion on the validity of claims 1-30 of the ’127 patent assigned to University of Washington Through Its Center For Commercialization (“UW”). I receive \$750 per hour for my services. No part of my compensation is dependent on my opinions or on the outcome of this proceeding.

14. I have been specifically asked to provide my opinion on the unpatentability, of claims 1-30 of the '127 patent. In connection with this analysis, I have reviewed the '127 patent. I have also reviewed and considered various other documents in arriving at my opinions and may cite to them in this declaration.

III. LEGAL STANDARDS

15. I understand that hindsight must not be used when opining on the patentability of claims of a patent. Therefore, my opinion will reflect the understanding of a person of skill in the art as of the relevant patent application filing dates, which I have been instructed is March 20, 2012 for this matter, the earliest date to which applicants have claimed priority for the claims of the '127 patent.

16. I have been advised that a claimed invention is not patentable under an anticipation theory (35 U.S.C. § 102) if all claim elements are found in a single prior art reference. I further understand that anticipation is about prior invention and therefore the single prior art reference must be found to disclose all elements of the claimed invention arranged as in the claim. I also understand that picking, choosing, and combining various embodiments disclosed within a single reference is not proper under an anticipation theory.

17. I understand that differences between the prior art reference and a claimed invention, however slight, invoke the question of obviousness, not anticipation.

18. I understand that for a claimed invention to be considered obvious, there must be some rational underpinning for combining cited references as proposed.

19. I understand that obviousness may also be shown by demonstrating that it would have been obvious to modify what is taught in a single piece of prior art in view of what is general knowledge in the art of a skilled artisan at the time to create the patented invention. Obviousness may be shown by demonstrating that it would have been obvious to combine the teachings of more than one item of prior art. In determining whether a piece of prior art could have been combined with other pieces of prior art or with other information within the knowledge of one of ordinary skill in the art, the following are examples of approaches and rationales that may be considered:

- Combining prior art elements according to known methods to yield predictable results;
- Simple substitution of one known element for another to obtain predictable results;

- Use of a known technique to improve similar devices (methods, or products) in the same way;
- Applying a known technique to a known device (method, or product) ready for improvement to yield predictable results;
- Applying a technique or approach that would have been “obvious to try” (choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success);
- Known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations would have been predictable to one of ordinary skill in the art; or
- Some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior art reference or to combine prior art reference teachings to arrive at the claimed invention.

20. Finally, I have been advised that any obviousness rationale for modifying or combining prior art must include a showing that a person of ordinary skill would have had a reasonable expectation of success.

IV. THE '127 PATENT

21. The '127 patent is entitled “Methods of lowering the error rate of massively parallel DNA sequencing using duplex consensus sequencing.” EX1001, cover (54). The '127 patent specification proposes correcting sequencing errors with a tag-based method referred to as “Duplex Consensus Sequencing” or “DCS.” *E.g.*, EX1001, Abstract, 3:52-4:37.

22. The '127 patent's DCS method makes use of “Single Molecule Identifiers” or “SMIs” which are used to uniquely identify original sample molecules. The '127 explains that SMIs may comprise exogenous and/or endogenous sequences. *E.g.*, EX1001, 6:67-7:2 (“The first and/or second degenerate or semi-degenerate nucleotide n-mer sequences may be any suitable length to produce a sufficiently large number of unique tags....”), 9:38-42 (“A hybrid method using a combination of sheared ends and a shorter n-mer tag ... in the adaptor may also serve as unique molecular identifiers.”), 26:38-41 (“Example 3: Demonstration of Error-Correction by DCS Using Randomly Sheared DNA Ends as Single Molecule Identifiers”).

23. Similar to other consensus sequencing methods of the relevant time, DCS generally includes tagging polynucleotides with SMI sequences, amplifying the SMI-tagged molecules using PCR, and sequencing the amplified products. EX1001, 2:15-30. The patent discloses that the prior art taught, for example,

“techniques whereby DNA fragments to be sequenced are each uniquely tagged [34, 35] prior to amplification [36-41].” *Id.*, 2:16-19. Skilled artisans used SMI sequences so that “all amplicons derived from a particular starting molecule will bear its specific tag” and “any variation in the sequence or copy number of identically tagged sequencing reads can be discounted as technical error.” *Id.*, 2:19-23. The sequenced tagged molecules are then grouped into families based on a common SMI sequence. EX1001, 3:17-39, FIG.1, 3:52-4:3, 19:20-25. A “consensus sequence” is determined from the grouped sequence reads which allows true mutations to be distinguished from sequencing errors. EX1001, 3:33-39, FIG.3, 4:12-37; *see also* Section V.

24. The '127 patent recognizes that tag-based methods for generating error corrected consensus sequences were known in the art at the time.

For example techniques whereby DNA fragments to be sequenced are each uniquely tagged [34, 35] prior to amplification [36-41] have been reported. Because all amplicons derived from a particular starting molecule will bear its specific tag, any variation in the sequence or copy number of identically tagged sequencing reads can be discounted as technical error. This approach has been used to improve counting accuracy of DNA [38, 39, 41] and RNA templates [37, 38, 40] and to correct base errors arising during PCR or sequencing [36, 37, 39].

EX1001, 2:17-26; *see also id.*, 2:26-30 (“Kinde et al. reported a reduction in error frequency...based on labeling single-stranded DNA fragments”), 26:46-48 (to compare to Kinde, “reads were grouped into families by SMI tag”).

25. References 36 and 39 that the ’127 patent identifies as disclosing methods for correcting base errors arising during PCR or sequencing are the Kinde (EX1006) and Casbon (EX1031) articles that I will discuss in further detail below.

26. According to the ’127 patent the purported novelty of DCS is individual tagging and analysis of both strands of a double-stranded DNA.

Limitations related to sequencing single-stranded DNA (e.g., sequencing errors) may therefore be overcome...by individually tagging and sequencing each of the two strands of a double-stranded (or duplex) target nucleic acid molecule and comparing the individual tagged amplicons derived from one half of a double-stranded complex with those of the other half of the same molecule.

EX1001, 17:1-8; *see also id.*, Abstract, 17:13-26; Section V.

27. The ’127 patent, however, also acknowledges that labeling both strands of double-stranded DNA was known in the art and was provided by commercially available sequencing adapters. For example, the ’127 patent describes performing DCS on sheared DNA ligated to Illumina TruSeq adapters. EX1001, 25:53-55, 25:64-26:1 (“The DNA was randomly sheared ..., followed by end-repair, A-tailing, and ligation of Illumina TruSeq DNA sequencing adapters,

....”). The ’127 patent further describes TruSeq adapters as providing individual labelling of the DNA duplex strands.

The first 10 nucleotides of each sequencing read pair, corresponding to the randomly sheared DNA ends, were combined, such that the first 10 nucleotides of read 1, referred to as A, was combined with the first 10 nucleotides of read 2, referred to as B, to yield an SMI tag of form AB. Reads were grouped according to SMI sequence, and nucleotide reads were considered only if they agreed among at least 90% of family members sharing a given tag sequence. For DCS analysis, a tag of form AB1 is partnered with the corresponding tag of form BA2, and nucleotide positions are considered only when the sequence is in agreement among read pairs with matching tags AB1 and BA2.

EX1001, 26:5-17.

28. The ’127 patent describes SMI adapters as Illumina adapters which are modified to include an SMI sequence in the double-stranded portion. EX1001, 22:19-24 (describing SMI adapters as “contain[ing] the standard sequences required for the Illumina HiSeq system ... with [the] addition of a double-stranded, complementary SMI sequence (or “tag”) of 12 random nucleotides ...”); *see also id.*, 3:52-4:11, 6:4-41, 10:63-11:2, 18:49-57, 25:64-26:1; EX1005, ¶5, 6 (showing that SMI adapters are Illumina TruSeq adapters modified to include an SMI sequence in the double-stranded portion).

29. As I explain in detail below, analysis of both strands of a double-stranded DNA was known in the art and well-documented in the literature.

V. BACKGROUND IN THE ART

A. Consensus sequencing and “asymmetric” adapters

30. Before March 20, 2012, skilled artisans were aware that sequencing read errors may arise as artifacts of next-generation sequencing (“NGS”), and that such errors were mitigated by redundantly sequencing multiple copies of a molecule and evaluating resulting sequence data for consensus. EX1031, Abstract; EX1021, Abstract; EX1006, Abstract.

31. The prior art consensus sequencing methods conventionally used barcodes to uniquely “label” original DNA molecules. With such labeling, methods could identify the amplification progeny (*i.e.*, copies) of original DNA molecules by virtue of inclusion of the same barcode also being copied during amplification. *E.g.*, EX1006, 9530 (describing copies as “UID” families); EX1031, 4 (“Identification of PCR duplicates using the DBR”); EX1010, 1348, Figure 1. Prior art methods used the barcodes to group corresponding sequence reads into a family corresponding to a particular DNA molecule. *Id.* A consensus sequence could then be generated for each family that better represented the “true” sequence of the original molecule because the sporadic errors found in individual reads would not find consensus compared reads from other copies of the same molecule.

EX1006, 9530 (“...at least 95% of family members ...”); EX1031, 4 (“...we derived a consensus sequence...”); EX1018, ¶15; EX1020, 24:9-20, 33:24-37; EX1021, Abstract, 3 (“The ability to read both strands on a single DNA molecule therefore enables correction for sequence context-dependent variation.”). There was nothing new about NGS, adapters comprising barcodes, or consensus sequencing at the relevant time. The ’127 patent specification is consistent with this perspective.

32. The purported novelty of the claimed sequencing methods relates to the use of partially-complementary adapters that, when ligated to double-stranded DNA, enables reads associated with the strands (*i.e.*, top and bottom) of double-stranded DNA molecules to be distinguished.

33. As I explain below, partially single-stranded sequencing adapters that rendered adapter-DNA molecules asymmetric were not only commercially available from companies like Illumina and Pacific Biosciences, but were routinely used to convert sample DNA into sequence-ready DNA molecules. EX1012, Figure 4; EX1013, Figure 1; EX1018, Figure 7; EX1021, Figure 1. Numerous examples of such adapters are found in the literature at the time.

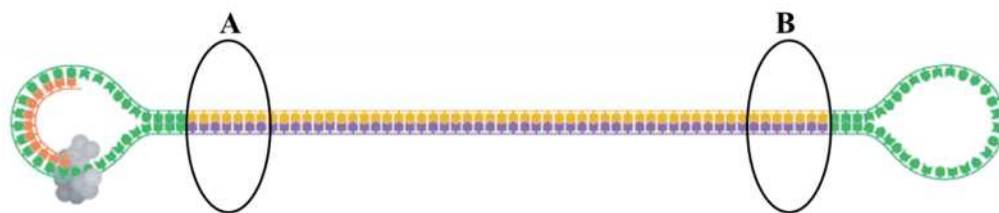
34. As I explain in detail below, Pacific Biosciences (“PacBio”) U-shaped hairpin adapters render adapter-DNA molecules asymmetric and thus make each strand of double-stranded DNA identifiable. Prior art methods for reducing

sequencing errors and amplification bias took advantage of this property of hairpin-shaped adapters to confirm the presence of reads associated with both strands of double-stranded DNA molecules.

35. PacBio adapters are partially single-stranded and include a binding site for a sequencing primer and may also include a barcode so that “each template sequence [may be] traced back to its origin. EX1018, ¶102; *see also id.*, ¶¶100, 101; EX1021, Abstract (explaining that the primer binding site is in a “single-stranded hairpin loop on either end”), 2 (“Prior to ligation, the hairpins were annealed in stem–loop structures ...”), Figure 1.

36. Typical PacBio sequencing workflows include ligation of hairpin adapters to sample DNA prior to amplification. As shown below, by virtue of the asymmetric nature of the adapted fragments, the molecule will be replicated and sequenced in the following order A (antisense), B (antisense), adapter, B (sense), A (sense). Note that, while not shown, had the sequencing primer bound to the adapter on the right, the molecule will be replicated and sequenced in the following order B (sense), A (sense), adapter, A (antisense), B (antisense).

37.



EX1021, Figure 1 (reproduced above, modified to include annotations); *see also* Figure 4, (showing “an alternating pattern of sense strand, first hairpin, antisense strand and second hairpin alignments,”). This asymmetry is similarly described in the ’127 patent. EX1001, 3:64-4:3.

38. Skilled artisans used the asymmetry of PacBio adapter-DNA molecules to confirm the presence of reads associated with each strand of double-stranded DNA molecules. Specifically, the strand of the reference sequence that the read corresponds to is determined as part of the standard PacBio read analysis workflow. EX1021, 2 (“This is done by *aligning a raw read sequence* to the known adapter sequence and a putative *reference sequence* of the insert regions.”), 6, Figure 4 (“there are two sub-reads that correspond to the sense strand and one to the antisense strand”); *see also* EX1018, ¶¶15, 146, 147, 150, Figure 16, claim 14.

39. As I explain in detail below, hairpin (*i.e.*, U-shape) adapters render adapter-DNA molecules asymmetric and thus make each strand of double-stranded DNA identifiable. Moreover, prior art methods (*e.g.*, PacBio) for reducing sequencing errors took advantage of this property of hairpin adapters to confirm the presence of reads associated with both strands of double-stranded DNA molecules.

B. Travers Publications

40. I rely on a patent publication and a paper describing sample preparation and sequencing methods developed at PacBio. I refer to these publications collectively as “the Travers Publications.”

41. **Travers ’075 (EX1018):** U.S. Patent Publication No. 2009/0298075 to Travers, et al. is entitled “Compositions and Methods for Nucleic Acid Sequencing” and is assigned on its face to Pacific Biosciences. I understand that Travers ’075 is prior art.

42. **The Travers Paper (EX1021):** “A flexible and efficient template format for circular consensus sequencing and SNP detection,” by Travers, et al. was published in *Nucleic Acids Research*. The Travers Paper is authored by scientists employed by PacBio (including inventors of Travers ’075) and describes applications of the sequencing method described in Travers ’075. *E.g.*, EX1021, Abstract, Figure 1, 7 (“All the authors are employees of Pacific Biosciences.”). I understand that the Travers Paper is prior art.

43. As discussed in greater detail herein, Travers provides disclosure relevant to all aspects of the challenged claims. Travers discloses use of “asymmetric” U-shaped or hairpin adapters for redundant sequencing of a target molecule. EX1018, Abstract (discussing “redundant sequence determination” and “iteratively sequencing the entire construct multiple times”), ¶57, Figure 3B;

EX1021, Abstract (determining “consensus sequence” from “...multiple passes on a single molecule...”), Figure 1. Travers’ method includes collectively analyzing (grouping) multiple reads of the target molecule and determining consensus sequence of the target molecule based on the multiple reads. EX1018, ¶58 (“...assembling the sequence data from multiple reads of a given sequence ... to provide an overall consensus sequence for that segment ...”). This has all been admitted by Patent Owner. EX1001, 10:63-11:18. Moreover, Travers discloses use of barcodes together with its U-shape or hairpin adapters in determining a consensus sequence. EX1018, ¶100 (“...template constructs that include sequence markers like a barcode...”). There’s no meaningful distinction between the ’127 patent claims and Travers. *See also* EX1018, ¶¶15, 54, 101, 102, 122; EX1021, 3 (“...ability to read both strands...”), Figure 4 (“...generating 4-fold coverage...”).

C. McCloskey

44. U.S. Patent Publication 2007/0020640 to McCloskey et al. (“McCloskey,” EX1023) is entitled “Molecular encoding of nucleic acid templates for PCR and other forms of sequence analysis.” McCloskey describes hairpin adapters comprising barcodes for molecular identification of sample molecules. EX1023, ¶5. I understand that McCloskey is prior art.

D. NEB Expressions

45. Volume 2.1 of NEB Expressions was published by New England Biolabs in Spring 2007. EX1014, 1, 8. I understand that NEB Expressions is prior art. NEB Expressions discloses that damage commonly found in double-stranded DNA fragments includes deaminated nucleic acid bases and nucleic acid bases having oxidative damage. EX1014, 1-2 & Table 1. NEB Expressions discloses the commercial product, PreCR™ Repair Mix. EX1014, 1. PreCR™ Repair Mix is marketed as providing enzymes that “repair a broad range of DNA damage” that “increase[s] your chances for successful PCR amplification.” *Id.* NEB Expressions discloses that the commercially available PreCR™ Repair Mix uses enzymes specifically to repair oxidized guanine, and deaminated cytosine, among other damage. EX1014, 3 & Table 2.

VI. BRIEF OVERVIEW OF THE LEVEL OF SKILL

46. I have been advised that “a person of ordinary skill in the relevant field” is a hypothetical person to whom one could assign a routine task with reasonable confidence that the task would be successfully carried out. I have been advised further that, in determining the level of ordinary skill in the art that someone would have had at the time the claimed invention was made, I should consider: (1) the levels of education and experience of persons working in the

field; (2) the types of problems encountered in the field; and (3) the sophistication of the technology.

47. In my opinion, a person of ordinary skill in the relevant field as of March 20, 2012, would typically have an advanced degree, such as a Ph.D., with research experience in genomics, molecular biology, bioinformatics, or a related field, or could have less education but significant professional experience in one or more of these fields.

VII. CLAIM CONSTRUCTION

48. I have been advised that the claims terms of the '127 patent claim are to be given the plain and ordinary meaning that the terms would have to a person of ordinary skill in the art in question at the relevant priority date. I understand that the analysis focuses on how the patentee used the claim term in the claims, specification, and prosecution history. I also understand that dictionaries or other extrinsic sources may assist in determining the plain and ordinary meaning but cannot override a meaning that is unambiguous from the intrinsic evidence. I have followed these principles in my analysis throughout this declaration. While it appears that no terms need to be expressly construed for the claims of the '127 patent to be found unpatentable, I provide additional discussion of some claim terms below.

49. Independent claim 22 requires attaching “partially single-stranded adapters” to double-stranded DNA fragments to generate a library of tagged double-stranded adapter-DNA molecules. Independent claim 1 requires attaching adapters to the DNA fragments makes them “partially-complementary, asymmetrical double-stranded adapter-DNA molecules.” The claims also require that the adapters comprise barcodes selected from a plurality of distinct barcode sequences. Claim 13 depends from claim 1 and recites that the adapters comprise a Y-shape or a U-shape. In each case, the claims encompass the use of standard Y-shaped Illumina adapters (when those adapters include barcodes selected from a plurality of distinct barcode sequences). *E.g.*, EX1012, Figure 4.

50. As I explained above, standard Illumina Y-shaped adapters are partially single-stranded (the arms of the Y) and also are partially-complementary (the trunk of the Y). When the adapters are ligated to a double-stranded DNA molecule, the resulting adapter-DNA molecule is partially-complementary and asymmetric. EX1010, Figure S5 (“We are able to distinguish the different strands of an original cDNA molecule because of the design of the paired-end sequencing adapters (Fig. 1B).”); EX1009, ¶55. The same is true when using U-shape hairpin adapters, which also are partially single-stranded and partially-complementary adapters.

51. My understanding of the claims is consistent with the specification of the '127 patent which characterizes the hairpin adapters of the Travers Paper (Reference 49) as compatible with DCS.

Amplification conditions that are compatible with amplification of hairpin DNA are thus already established. Moreover, ligation and amplification with circularizing “linkers” (i.e. hairpin linkers affixed to both ends of a fragment) has been demonstrated as a step in the Pacific Biosciences sample preparation workflow [49]. As the sequence of the linker itself does not matter in the workflow, the published linker sequences from either of these references would be adequate for use in the assay.

EX1001, 10:63-11:2; *see also id.*, 15:29-39 (describing “non-PCR” amplification methods and “Pacific Biosciences sequencing platform” as within the scope of the invention).

52. Accordingly, the plain meaning of “partially single-stranded adapters” and “partially-complementary, asymmetrical double-stranded adapter-DNA molecules” is reasonably understood to encompass adapter-DNA molecules generated by attachment of Y-shaped or U-shaped adapters comprising barcodes to each end of double-stranded DNA molecules.

VIII. GROUND 1 - CLAIMS 1-10, 12, 15-28 ARE OBVIOUS AS ILLUSTRATED BY THE TRAVERS PUBLICATIONS

53. As I explain in detail below, it is my opinion that each of claims 1-10, 12, and 15-28 of the '127 patent are obvious in view of the prior art at the time, as illustrated by the Travers Publications. Travers exemplifies consensus sequencing based on redundant sequencing of the sense and antisense strands of an original DNA molecule. Travers describes the generation of circular template molecules by attaching hairpin adapters to double-stranded DNA molecules — thus generating partially single-stranded and partially-complementary, asymmetrical double-stranded adapter DNA molecules. Travers further discloses that the hairpin adapters may include barcodes so that each template sequence may be traced back to its origin.

54. The '127 patent specification is directed to Duplex Consensus Sequencing (“DCS”), which purports to “reduce[] errors by independently tagging and sequencing each of the two strands of a DNA duplex.” EX1001, Abstract. According to the '127 patent, “true mutations are found at the same position in both strands.” *Id.* The method of claim 1 requires:

- Attaching adapters comprising barcodes to double-stranded DNA to produce partially-complementary, asymmetric adapter-DNA molecules;
- Amplifying original strands to produce first and second strand copies;

- Sequencing at least some amplified first and second strand progeny; and
- For at least some of the sequences, confirming the presence of at least one sequence read derived from each of the original first and second strands, comparing at least one of the confirmed first and second strand sequences reads to a reference sequence, and analyzing one or more correspondences between at least one of the confirmed first and second strand sequence reads and the reference sequence to identify a sequence variation.

55. Each of these aspects of claims 1-10, 12, and 15-28 is taught or suggested in the Travers Publications.

56. The Travers Publications disclose a method for generating consensus sequences from both strands of an original DNA duplex molecule. According to Travers, adapters (green) are attached to double-stranded DNA to produce partially-complementary, asymmetric adapter-DNA molecules. *See* Section V.



EX1021, Figure 1; *see also id.* (“Production of SMRTbell™ templates”); EX1018, Figure 2, 3, 7, ¶¶51-55, ¶¶16, 100-102 (describing adapters comprising barcodes).

Travers refers to these circular templates as SMRTbell™ templates. EX1021, Figure 1.

57. The SMRTbell™ templates are amplified by primer extension using a strand displacing polymerase to produce concatemers comprising repeating copies of the sense and antisense strands separated by a copy of the adapter. EX1021, Figure 4; EX1018, ¶122. Travers explains that the concatemer copies generated from SMRTbell™ templates may be directly sequenced. EX1018, ¶122. Use of SMRTbell™ templates allows for generation of a consensus sequence based on redundant reads of the sense and antisense strands of the original molecule.

EX1021, Abstract; *see also id.*, 1 (explaining that the method “produces multiple observations of each base, and these multiple observations can then be used to generate high-accuracy consensus sequence from single molecules.”), Figure 4; EX1018, ¶¶53, 54, 122.

58. I understand that Patent Owner has characterized Travers '075 as generating a “consensus sequence of the double stranded molecule by redundant sequencing of the entire single template.” As indicated below, Patent Owner also acknowledged that Travers '075 discloses attaching adapters to double-stranded DNA, amplifying, and sequencing both strands of an original double-stranded nucleic acid molecule.

Travers describes method steps for use in single molecule sequencing reactions that includes: linking sense and antisense strands of a

double-stranded nucleic acid molecule together with a “linking segment” (e.g., using exogenous hairpin adapters (see, e.g., Travers, ¶[0051])) and amplification of these linked original strands “to ensure complete, and preferably redundant sequencing of the entire template” (¶[0081]). By linking the sense and antisense strands together, Travers ensures that only a single set of amplified products are generated from any one particular double-stranded molecule, with every member of the set being an identical sequence. As stated in cited paragraph [0054], Travers discloses that each template molecule includes sequence segments corresponding to (1) the sense strand, (2) the antisense strand and (3) the linking segment(s) of the hairpin adapter(s), such that “a single template molecule ... can be sequenced in one integrated process.”

59. I have reviewed Travers '075, including the paragraphs cited by Patent Owner, and agree with this assessment of the reference.

60. I understand that Patent Owner was able to overcome Travers '075 by asserting that the reference does not disclose “amplifying each original strand of the double-stranded target nucleic acid molecule resulting in each original strand generating a distinct yet related set of amplified target nucleic acid products” which was a requirement of the claims under examination in that case. The claims of the '127 patent, however, do not have that requirement.

61. That the claims of the '127 patent would cover the workflow described by the Travers Publications is supported by the specification. The '127 patent cites the Travers Paper (Reference 49) and describes it as disclosing the “Pacific Biosciences sample preparation workflow.” EX1001, 10:63-11:2; *see also id.*, 11:3-17 (citing Travers for disclosure of “rolling circle amplification” and

“continuous loop sequencing”); 19:3-5 (incorporating all cited disclosures by reference); 33:22-25. The ’127 patent describes the hairpin linkers used in the PacBio workflow for generating amplification and sequencing templates as “adequate for use in the assay.” EX1001, 10:63-11:2; *see also id.* (explaining that “the sequence of the linker itself does not matter in the workflow”), 6:31-32, 8:44-53, 10:63-11:2, 11:3-17, 11:3-9, 14:4-8 (using the terms “hairpin” and “U-shaped” interchangeably to describe adapters having a double-stranded portion and a single-stranded loop). The ’127 patent also discloses that the sequencing step of DCS may be accomplished “using any suitable method known in the art” including the “Pacific Biosciences sequencing platform.” EX1001, 15:22-39. Moreover, dependent claims 10 and 13 establish that rolling circle amplification and U-shaped adapters — such as disclosed by Travers—are within the scope of claims 1-21. *See* Section V.

62. An element-by-element discussion of the claims, together with discussion illustrating exemplary prior art disclosure and how each and every aspect of the challenged claims is found in the prior art, is provided below.

A. Claim 1

63. In my opinion, the combination of Travers ’075 and the Travers Paper disclose or teach every element of claims 1-10, 12, 15-28. Travers ’075 describes the PacBio workflow (*e.g.*, sample preparation, sequencing, and bioinformatic

analysis of sequence reads). A person of ordinary skill in the art would understand the Travers Paper to disclose applications of the same PacBio workflow described in Travers '075. In my opinion a person of ordinary skill in the art would have been motivated to look to both Travers '075 and the Travers Paper because together the references provide descriptions of the same PacBio methodology. Moreover, a person of ordinary skill in the art would have been motivated and expected success in combining the teachings of the Travers Publications because each are describing aspects of the PacBio workflow. *See also* EX1026 (explaining that the methodologies described in the Travers Publications became commercially available in 2010); Section V.

i. Preamble – “A method of sequencing DNA comprising:”

64. The Travers Publications disclose the preamble of claim 1.

'127 Patent	Travers
1. A method of sequencing DNA comprising:	“Compositions and methods for nucleic acid sequencing include template constructs that comprise double stranded portions in a partially or completely contiguous constructs, to provide for redundant sequence determination through one or both of sequencing sense and antisense strands, and iteratively sequencing the entire construct multiple times.” EX1018, Abstract. “[F]or diagnostic sequencing applications, it may be necessary only

	to provide sequence data for a small fragment of DNA, but do so in an extremely accurate sequencing process.” EX1018, ¶89 “In a typical sequencing process, a double stranded target nucleic acid... is converted to a template configuration of the invention. Typically, this involves fragmentation of a population of a larger double stranded nucleic acid... into pools of smaller, overlapping double stranded target nucleic acids.” EX1018, ¶51. “A novel template design for single-molecule sequencing is introduced, a structure we refer to as a SMRTbell™ template. ...When placed into a single-molecule sequencing reaction, the SMRTbell template format enables a consensus sequence to be obtained from multiple passes on a single molecule. Furthermore, this consensus sequence is obtained from both the sense and antisense strands of the insert region.” EX1021, Abstract, Figure 1, Figure 4. <i>See also</i> EX1018, ¶¶9, 51, 89, 105, 112, 124, claim 1; EX1021, Abstract, 1.
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65. The Travers Publications discuss methods for sequencing double-stranded nucleic acids, including DNA. Travers discloses the “Pacific Biosciences” sequencing workflow which generates sequence reads for “a sense and an antisense strand of the template nucleic acid segment [from] a contiguous nucleic acid

molecule.” EX1018, ¶9; *see also id.*, Abstract, ¶¶15, 51, 89, 105, 112, 124, claim 1; EX1021, Title (“A flexible and efficient template format for circular consensus sequencing and SNP detection”), Abstract, 1 (“In this application, a sequencing read produces multiple observations of each base, and these multiple observations can then be used to generate high-accuracy consensus sequence from single molecules.”), Figure 1, Figure 4; *see also* EX1001, 15:22-39.

66. Accordingly, it is my opinion that the Travers Publications disclose the Preamble.

ii. Element 1.1 – “a) attaching adapters to double-stranded DNA fragments to generate a plurality of partially-complementary, asymmetrical double-stranded adapter-DNA molecules,”

67. The Travers Publications disclose Element 1.1.

'127 Patent	Travers
a) attaching adapters to double-stranded DNA fragments to generate a plurality of partially-complementary, asymmetrical double-stranded adapter-DNA molecules,	“In one simple example, exogenous hairpin adapter sequences are used and simply ligated to the end(s) of the double stranded fragments to form such linking oligonucleotides. Such exogenous hairpin adapters will typically include two complementary nucleic acid sequence segments at opposing ends of a longer sequence, where they are separated by another stretch of non-complementary nucleotides. The result is a structure that includes a double stranded stem structure coupled to the single stranded loop.” EX1018, ¶51.

	“As alluded to above, however, the connecting or linking oligonucleotide may be selected or generated to include sequence with identifiable sequence characteristics to facilitate its identification, both with respect to the contiguous template sequence with which it is associated, and also with respect to other template sequences that may exist in the same sample mixture.” EX1018, ¶100 “This structure consists of a double-stranded portion, containing the insert of interest, and a single-stranded hairpin loop on either end, which provides a site for primer binding. Structurally, this format resembles a linear double-stranded molecule, and yet it is topologically circular.” EX1021, Abstract <i>See also</i> EX1018, Figure 2, 7, ¶¶22, 27, 50, 51, 92, 105, claim 35; EX1021, 2-4 (“Production of SMRTbell™ templates”), Figures 1 and 2.
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68. Travers describes attaching hairpin adapters to double-stranded DNA fragments using ligation. EX1018, ¶51 (“In one simple example, exogenous hairpin adapter sequences are used and simply ligated to the end(s) of the double stranded fragments ...”), ¶105; EX1021, 1 (“Due to the nature of the ligation reactions used to generate these templates....”).

69. Travers discloses that ligation of the hairpin adapters “result[s] [in] a structure that includes a double stranded stem structure coupled to the single

stranded loop.” EX1018, ¶51; *see also* EX1021, Figure 1. As explained above, a person of ordinary skill in the art would understand that this structure (*i.e.*, the SMRTbell template) is within the scope of “partially-complementary, asymmetrical double-stranded adapter-DNA molecules.” *See* Section VII. Moreover, Travers describes adapters that are both functionally and physically asymmetric. EX1018, ¶92 (“...asymmetric linking oligonucleotides, e.g., having different numbers of nucleotides joining the sense and antisense strands, may be used within a single template construct.”); *see also* EX1030, Supplementary File 1 (exemplifying use of physically asymmetric adapters).

70. Accordingly, it is my opinion that the Travers Publications disclose Element 1.1.

iii. Element 1.2 – “wherein the adapters comprise barcodes selected from a plurality of distinct barcode sequences;”

71. The Travers Publications teach Element 1.2.

'127 Patent	Travers
wherein the adapters comprise barcodes selected from a plurality of distinct barcode sequences;	“In particular, one can utilize connecting oligonucleotides in the template constructs that include sequence markers like a barcode, to indicate the origin of a given template sample. Different template samples including distinguishable connecting oligonucleotide tags could then be pooled for analysis in a single sequencing process. Sequence data

	derived from individual templates is then attributable to the originating sample preparation process through the identification of the connecting oligonucleotide tag.” EX1018, ¶100 “In particular, a number of discrete sample preparation processes are performed for different nucleic acid containing samples. ...The template deriving from each discrete process is bar-coded through the use of a unique, identifiable and discrete linking nucleotide sequence in the template construct.” EX1018, ¶101. <i>See also</i> EX1018, ¶¶16, 100-102, 144, claim 34.
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72. While claims 1-21 require attachment of adapters comprising barcodes to double-stranded DNA, none of these claims require that the barcode be used for any purpose. Thus, in my opinion independent claim 1 cannot distinguish over the prior art based on any utility of the barcode.

73. Travers discloses “because each sequence read is derived from a single molecule, the sequence of the insert portion of the template can be unambiguously linked to the sequence of its attached adapter sequence that includes the bar code sequence within that adapter sequence.” EX1018, ¶102; *see also id.*, ¶¶16, 100, claim 34 (describing linkers with “identifiable sequence characteristics”).

74. Travers also discloses that templates derived from multiple preparation processes or sources may be pooled for sequencing and that “each template sequence [may be] traced back to its origin” using the barcode. EX1018, ¶102. Because Travers explicitly describes the pooling of different samples, a person of ordinary skill in the art would have understood that multiple different barcodes (*i.e.*, a plurality) would be necessary to uniquely identify each sample. *E.g.*, EX1018, ¶101 (describing the use of barcodes to identify “different starting materials” and “different portions of the same original material”).

75. Travers describes the use of barcodes to facilitate “identification, both with respect to the contiguous template sequence with which it is associated, and also with respect to other template sequences that may exist in the same sample mixture.” EX1018, ¶100. In my opinion, a person of ordinary skill in the art would understand that distinguishing template sequences with respect to others that may exist in the sample mixture would require a plurality of barcodes because the samples described by Travers comprise a plurality of template sequences. Specifically, Travers discloses shearing genomic DNA derived from a culture of *E. coli* into 500 to 1500 bp fragments. *E.g.*, EX1018, ¶144. A person of ordinary skill in the art would understand that such a sample of sheared *E. coli* genomic DNA comprises a plurality of DNA molecules.

76. Accordingly, a person of ordinary skill in the art would understand the Travers Publications to teach selecting barcodes from a plurality of distinct barcode sequences as required by Element 1.2.

iv. Element 1.3 – “b) amplifying original strands of at least a portion of the double-stranded adapter-DNA molecules to produce first and second strand copies;

77. The Travers Publications disclose Element 1.3.

'127 Patent	Travers
b) amplifying original strands of at least a portion of the double-stranded adapter-DNA molecules to produce first and second strand copies;	“[T]he structurally circular nucleic acid segments described herein, may be used as templates in a rolling circle replication process to produce concatamer molecules that include repeating copies of both the sense and antisense strands of the originating double stranded segment included within the circular nucleic acid. These replicated products may then be employed directly as template molecules in a template dependent sequencing process, ... Likewise, duplication processes may be employed to produce multiple copies of a prepared circular construct using methods previously described” EX1018, ¶122. <i>See also</i> EX1018, ¶83; EX1021, Figure 1, Figure 4.

78. Travers explains that template molecules are “subjected to a polymerase mediated sequencing process.” EX1018, ¶83. Travers further describes

amplification of template molecules prior to redundant sequencing. For example, Travers explains that “rolling circle replication” of SMRTbell templates produces concatemer molecules comprising “repeating copies of both the sense and antisense strands of the originating double stranded segment.” EX1018, ¶122; *see also* EX1021, Figure 1, Figure 4. Travers further explains that these “replicated products may then be employed directly as template molecules in a template dependent sequencing process.” EX1018, ¶122. Thus, the Travers Publications disclose an amplification method in which copies of the original strands (*i.e.*, the sense and antisense strands) are produced for at least a portion of the adapter-DNA molecules.

79. Accordingly, it is my opinion that the Travers Publications disclose Element 1.3.

v. Element 1.4 – “c) sequencing a plurality of first and second strand copies to obtain first and second strand sequence reads for at least a portion of the adapter-DNA molecules; and”

80. The Travers Publications disclose Element 1.4.

'127 Patent	Travers
c) sequencing a plurality of first and second strand copies to obtain first and second strand sequence reads for at least a portion of the adapter-DNA molecules; and	13. The method of claim 12, wherein the sequencing process sequences both the first and second complementary nucleic acid strands of the template nucleic acid segment.

	“[T]he resulting sequence is derived from both sense and anti-sense strands.” EX1021, Figure 1. “In addition to providing sense and antisense sequence reads from a single template molecule that can be sequenced in one integrated process,...” EX1018, ¶54. “By way of example, where a given template is being subjected to a polymerase mediated sequencing process, limitations on the read length for the particular system may be factored into the selection of the overall template size, e.g., to ensure complete, and preferably redundant sequencing of the entire template.” EX1018, ¶83. “These replicated products may then be employed directly as template molecules in a template dependent sequencing process, as described elsewhere herein...” EX1018, ¶122. <i>See also</i> EX1018, ¶13, 102, 141-143, 145-147, 151; EX1021, 4-5 (“Generation of a long SMRTbell™ template for sequencing”).
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81. Travers discloses that either SMRTbell templates, or their replication products (*i.e.*, concatemers), may be sequenced using the disclosed template-dependent sequencing process. EX1018, ¶122 (“...structurally circular nucleic acid segments described herein, may be used as templates in a rolling circle replication process to produce concatamer molecules that include repeating copies of both the

sense and antisense strands...”); *see also* EX1018, ¶13, 54, 83, 102, 122, 141-143, 145-147, 151, claim 13; EX1021, 4-5 (“Generation of a long SMRTbell™ template for sequencing”).

82. Accordingly, it is my opinion that the Travers Publications disclose Element 1.4.

- vi. **Element 1.5 – “d) for at least some of the adapter-DNA molecules comprising barcodes – confirming the presence of at least one sequence read derived from each of the original first and second strands of the adapter-DNA molecules;”**

83. The Travers Publications disclose Element 1.5.

'127 Patent	Travers
d) for at least some of the adapter-DNA molecules comprising barcodes— i) confirming the presence of at least one sequence read derived from each of the original first and second strands of the adapter-DNA molecules;	“In addition to providing sense and antisense sequence reads from a single template molecule that can be sequenced in one integrated process, the presence of linking segment 206 also provides an opportunity to provide a registration sequence that permits the identification of when one segment, e.g., 202, is completed and the other begins, e.g., 204. Such registration sequences provide a basis for alignment sequence data from multiple sequence reads from the same template sequences, e.g., the same molecule, or identical molecules in a template population.” EX1018, ¶54.

84. As I explain above with respect to Elements 1.2-1.4, the Travers Publications disclose amplification and sequencing of adapter-DNA molecules.

E.g., EX1018, ¶54 (“...providing sense and antisense sequence reads...”), ¶55 (“...replicate sequence read data...”), ¶57 (“...iterative sequence reads...”), ¶62 (“...repeated sequence reads...”).

85. Travers explains that the sequence of the hairpin adapter may be used as a “registration sequence” to define when the sequence for one strand ends and the next one begins. EX1018, ¶54, Figure 2, claim 1 (claiming a method for determining a consensus sequence from the sense and antisense strand sequences). Travers also reports confirming that reads derived from the first and second strands are present. EX1021, 5 (describing Figure 4 as showing “an alternating pattern of sense strand, first hairpin, antisense strand and second hairpin alignments,”), Figure 4.

86. Accordingly, it is my opinion that the Travers Publications disclose Element 1.5.

vii. Element 1.6 – “comparing at least one of the confirmed first and second strand sequence reads to a reference sequence; and”

87. The Travers Publications disclose Element 1.6.

'127 Patent	Travers
ii) comparing at least one of the confirmed first and second strand sequence reads to a reference sequence; and	“This is done by <i>aligning a raw read sequence</i> to the known adapter sequence and a putative <i>reference sequence</i> of the insert regions.” EX1021, 2.

	“The sequenced fragments were aligned to the K12 MG1665 reference sequence.” EX1018, ¶146.
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88. I understand that a Patent Office Examiner in examining a related patent application determined that the specification does not describe comparing reads to a reference sequence. I have reviewed the '127 patent specification and agree that it does not describe comparing reads to a reference sequence as required by Element 1.6.

89. Travers discloses aligning reads to a reference sequence. EX1021, 2 (“This is done by *aligning a raw read sequence* to the known adapter sequence and a putative *reference sequence* of the insert regions.”), 6; *see also* EX1018, ¶¶15, 146, 147, 150, Figure 16, claim 14. Travers further discloses comparing first and second strand reads to a reference sequence. *Id.* (“Once all subreads are identified, we test at each base position whether the subreads are generated by the putative reference sequence of the template or some variation of that sequence). A person of ordinary skill in the art would have understood that Travers to refer to the redundant reads derived from the sense and antisense strands as “subreads.” EX1021, Figure 4 (“there are two sub-reads that correspond to the sense strand and one to the antisense strand”). Thus, Travers discloses aligning and comparing reads from the sense and antisense strands to the reference sequence.

90. Accordingly, it is my opinion that the Travers Publications disclose Element 1.6.

viii. Element 1.7 – “analyzing one or more correspondences between at least one of the confirmed first and second strand sequence reads and the reference sequence to identify a sequence variation”

91. The Travers Publications disclose Element 1.7.

'127 Patent	Travers
iii) analyzing one or more correspondences between at least one of the confirmed first and second strand sequence reads and the reference sequence to identify a sequence variation.	“Once all subreads are identified, we test at each base position whether the subreads are generated by the putative reference sequence of the template or some variation of that sequence (e.g. a single-nucleotide polymorphism or an indel).” EX1021, 2.

92. Travers discloses comparing first and second strand reads to a reference sequence to identify sequence variation. EX1021, 2 (“Once all subreads are identified, we test at each base position whether the subreads are generated by the putative reference sequence of the template or some variation of that sequence (e.g. a single-nucleotide polymorphism or an indel).”). *See* Element 1.6 above.

93. Travers further describes identifying sequence variation. EX1018, ¶143 (“This molecular redundant sequencing was applied in the identification of a single nucleotide variation, e.g., a SNP.”); EX1021, 5 (“To test the sensitivity of SNP detection ...”); *see also* Elements 1.5 and 1.6 above.

94. A person of ordinary skill in the art would have understood that SNPs and indels are identified relative to the corresponding positions on a reference sequence. Accordingly, by disclosing the identification of SNPs and indels, Travers discloses analyzing correspondences between the sense and antisense reads to the reference sequence.

95. Accordingly, it is my opinion that the Travers Publications teach or suggests each of the limitations of independent claim 1, such that this claim would have been obvious to a person of ordinary skill in the art.

B. Claim 22

i. Preamble – “A method of sequencing DNA comprising:”

96. The Travers Publications disclose the Preamble.

'127 Patent	Travers
22. A method of sequencing DNA comprising:	“Compositions and methods for nucleic acid sequencing include template constructs that comprise double stranded portions in a partially or completely contiguous constructs, to provide for redundant sequence determination through one or both of sequencing sense and antisense Strands, and iteratively sequencing the entire construct multiple times.” EX1018, Abstract. “A novel template design for single-molecule sequencing is introduced, a structure we refer to as a SMRTbell™ template.” EX1021, Abstract.

	<i>See also</i> EX1018, Abstract, ¶¶9, 51, 89, 105, 112, 124, claim 1; EX1021, Abstract, 1, Figure 1, Figure 4; EX1001, 15:22-39; EX1024, 551.
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97. As described above with respect to the Preamble of claim 1, the Travers Publications disclose a method for sequencing double-stranded nucleic acids, including DNA. EX1018, Abstract, ¶¶9, 51, 89, 105, 112, 124, claim 1; EX1021, Abstract, 1, Figure 1, Figure 4; *see also* EX1001, 15:22-39; EX1024, 551.

98. Accordingly, it is my opinion that the Travers Publications disclose the Preamble.

- ii. **Element 22.1 – “a) attaching partially single-stranded adapters comprising barcodes selected from a plurality of distinct barcode sequences to double-stranded DNA fragments obtained from a bodily sample, wherein attachment of the adapters to double-stranded DNA fragments generates a library of tagged double-stranded adapter-DNA molecules”**

99. The Travers Publications disclose Element 22.1.

'127 Patent	Travers
a) attaching partially single-stranded adapters comprising barcodes selected from a plurality of distinct barcode sequences to double-stranded DNA fragments obtained from a bodily sample, wherein attachment of the adapters to double-stranded DNA fragments generates a library of tagged	“The hairpin loops present a single-stranded region to which a sequencing primer can bind (orange).” EX1021, Figure 1. “As shown, the attachment of the hairpin adapters 710 relies upon the presence of unique overhang sequences 720 at the 3' end of each strand of the

double-stranded adapter-DNA molecules;	double stranded fragment 700.” EX1018, ¶105. “Prior to ligation, the hairpins were annealed in stem–loop structures ...” EX1021, 2. “These discrete sample preparation processes may be performed on different starting materials, e.g., different samples (cells, cell cultures, patients, etc.), different portions of the same original material, i.e., different size-selected portions or the like, or different portions of the same population, cell culture, etc.” EX1018, ¶101. “In particular, because each sequence read is derived from a single molecule, the sequence of the insert portion of the template can be unambiguously linked to the sequence of its attached adapter sequence that includes the bar code sequence within that adapter sequence. Accordingly, each template sequence is then traced back to its origin, e.g., a particular sample, patient, etc.” EX1018, ¶102. <i>See also</i> EX1018, ¶¶5, 51, 100-105, 126, Figure 7; EX1021, 2, Figure 1.
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100. Travers discloses “attaching partially single-stranded adapters ... to double-stranded DNA fragments obtained from a bodily sample” as required by Element 22.1. Travers explains that “Complementary overhang sequences [] are provided on the hairpin adapters, to provide for specific annealing and ligation of

the hairpin adapters [] to the double stranded fragments []." EX1018, ¶105, Figure 7; *see also* EX1021, 3 ("Production of SMRTbell™ templates"). Travers further discloses partially single-stranded adapters. For example, Travers discloses that ligation of the hairpin adapters "result [in] a structure that includes a double stranded stem structure coupled to the single stranded loop." EX1018, ¶51, Figure 7; *see also* EX1021, 2, Figure 1.

101. Travers discloses that the double-stranded DNA fragments may be obtained from a bodily sample. EX1018, ¶101 ("sample preparation processes may be performed on different starting materials, e.g., different samples (cells, cell cultures, patients, etc.), different portions of the same original material, i.e., different size-selected portions or the like, or different portions of the same population, cell culture, etc."), ¶102; *see also id.*, ¶5 (explaining that sequencing has been applied to the "entire human genome"). Travers also discloses that mRNA may be obtained from "various sources, e.g., bovine, mouse, and human, and tissues e.g. brain, blood, and heart." EX1018, ¶126.

102. A person of ordinary skill in the art would have understood that double-stranded DNA could be obtained from the same sources that Travers identifies as sources for mRNA. Furthermore, it would have been obvious to apply the consensus sequencing method of Travers to double-stranded DNA obtained from a bodily sample. *See also* EX1027, 1, 10 (describing genomic DNA samples

isolated from brain tissue and embryonic stem cells of mice); EX1026, 2 (explaining that the consensus sequencing method of Travers is particularly advantages for analyzing “structural rearrangements” in the genome of tumor cells).

103. As I explained with respect to Element 1.2, Travers discloses adapters comprising “barcodes selected from a plurality of distinct barcode sequences.” For example, Travers discloses that templates derived from multiple preparation processes or sources may be pooled for sequencing and that “each template sequence [may be] traced back to its origin” using the barcode. EX1018, ¶102. Travers also describes the use of barcodes to facilitate “identification, both with respect to the contiguous template sequence with which it is associated, and also with respect to other template sequences that may exist in the same sample mixture.” EX1018, ¶100. A person of ordinary skill in the art would understand that either of the applications of barcodes described by Travers would require selection of a barcode from a plurality of barcodes. *See* Element 1.2 above.

104. Accordingly, it is my opinion that the Travers Publications disclose Element 22.1.

iii. Element 22.2 – “b) amplifying strands from a plurality of the double-stranded adapter-DNA molecules in the library to produce strand copies;”

105. The Travers Publications disclose Element 22.2.

'127 Patent	Travers
b) amplifying strands from a plurality of the double-stranded adapter-DNA molecules in the library to produce strand copies;	EX1018, ¶122; <i>see also</i> EX1021, Figure 1, Figure 4.

106. Travers describes an amplification method (*i.e.*, rolling circle) that produces concatemer molecules comprising “repeating copies of both the sense and antisense strands of the originating double stranded segment.” EX1018, ¶122; *see also id.*, ¶83; EX1021, Figure 1, Figure 4; Element 1.3 above.

107. Accordingly, it is my opinion that the Travers Publications disclose Element 22.2.

iv. Element 22.3 – “c) sequencing a plurality of the strand copies to obtain strand sequence reads comprising one or more barcode sequences and DNA fragment-specific information; and”

108. The Travers Publications disclose Element 22.3.

'127 Patent	Travers
c) sequencing a plurality of the strand copies to obtain strand sequence reads comprising one or more barcode sequences and DNA fragment-specific information; and	EX1018, ¶122; <i>see also</i> EX1018, ¶13, 54, 102, 122, 141-143, 145-147, 151, claim 13; EX1021, 4-5 (“Generation of a long SMRTbell™ template for sequencing”).

109. Travers further discloses that either concatemers produced from an intermediate amplification step or SMRTbell templates may be sequenced using the disclosed template-dependent sequencing process. EX1018, ¶122; *see also*

EX1018, ¶13, 54, 102, 122, 141-143, 145-147, 151, claim 13; EX1021, 4-5

(“Generation of a long SMRTbell™ template for sequencing”); Element 1.4 above.

110. Accordingly, it is my opinion that the Travers Publications disclose Element 22.3.

- v. **Element 22.4 – “d) for at least some of the double-stranded adapter-DNA molecules in the library— grouping the strand sequence reads into families based on i) the barcode sequence, and ii) DNA fragment-specific information”**

111. The Travers Publications teach Element 22.4.

'127 Patent	Travers
d) for at least some of the double-stranded adapter-DNA molecules in the library— grouping the strand sequence reads into families based on i) the barcode sequence, and ii) DNA fragment-specific information;	As alluded to above, however, the connecting or linking oligonucleotide may be selected or generated to include sequence with identifiable sequence characteristics to facilitate its identification, both with respect to the contiguous template sequence with which it is associated, and also with respect to other template sequences that may exist in the same sample mixture. EX1018, ¶100. FIG. 11 shows a sequencing trace showing 700 bases of sequence data around the template construct providing 154 bases of consensus information. EX1018, ¶141.

112. As discussed above, Travers discloses redundant sequencing of target DNA molecules. *E.g.*, EX1018, Abstract (discussing “redundant sequence determination” and “iteratively sequencing the entire construct multiple times”),

¶57, Figure 3B; EX1021, Abstract (determining “consensus sequence” from “...multiple passes on a single molecule...”), Figure 1; Section V.

113. Travers further discloses the grouping of redundant sequence reads. Specifically, Travers describes collectively analyzing multiple reads of the target molecule. EX1018, ¶55 (describing assembly of “replicate sequence read data” for determining consensus), ¶58 (“...assembling the sequence data from multiple reads of a given sequence ... to provide an overall consensus sequence for that segment ...”); ¶100 (describing the use of “oligonucleotide tags” or barcodes to “pool[data] for analysis”). Travers further discloses that the assembled reads are used to determine a consensus sequence. EX1018, ¶57 (“...a consensus base call for that particular position based upon multiple reads at that position ... These multiple reads are then assembled or compared to provide a consensus ...”). That is, Travers expressly describes determining consensus sequences based on assembled (*i.e.*, grouped) redundant sequence reads.

114. Travers describes amplification of template molecules prior to redundant sequencing. Travers explains that “rolling circle replication” of SMRTbell templates produces concatemer molecules comprising “repeating copies of both the sense and antisense strands of the originating double stranded segment.” EX1018, ¶122; *see also* EX1021, Figure 1, Figure 4. Travers further explains that these “replicated products may then be employed directly as template

molecules in [the disclosed] template dependent sequencing process.” EX1018, ¶122; *see* Element 1.3 above. A person of ordinary skill in the art would have understood that the concatemer molecules would have the same sequence (e.g., barcode and target molecule) and thus could be assembled and used to determine a consensus sequence in the manner described above. EX1018, ¶¶55, 57, 58, 100.

115. Accordingly, it is my opinion that the Travers Publications teach Element 22.4.

vi. Element 22.5 – “collapsing a plurality of strand sequence reads within the families to provide a consensus sequence for each of at least some of the double-stranded DNA molecules in the library”

116. The Travers Publications disclose Element 22.5.

'127 Patent	Travers
collapsing a plurality of strand sequence reads within the families to provide a consensus sequence for each of at least some of the double-stranded DNA molecules in the library;	“In particular, as with circular templates, the template configurations of the invention allow for single molecular consensus sequence determination, where sequencing a given template provides duplicative or replicate data of the sequence information obtained, and thereby improves accuracy over linear templates by providing multiple reads for a given template sequence or sequence portion, that can be used to derive consensus sequence data from a given template sequence and/or for specific base locations within such sequence.” EX1018, ¶53.

117. Based on the '127 patent, a person of ordinary skill in the art would understand “collapsing” to be the process by which consensus sequence information is drawn from redundant sequence reads derived from the same molecule. *E.g.*, EX1001, 20:53-55 (“Reads having common (i.e., identical) SMI sequences were grouped together, and were collapsed to generate a consensus read.”).

118. The Travers Publications disclose such a collapsing process.

In particular, as with circular templates, the template configurations of the invention allow for single molecular consensus sequence determination, where sequencing a given template provides duplicative or replicate data of the sequence information obtained, and thereby improves accuracy over linear templates by providing multiple reads for a given template sequence or sequence portion, that can be used to derive consensus sequence data from a given template sequence and/or for specific base locations within such sequence.

EX1018, ¶53; *see also* EX1021, 6 (“We have illustrated the application of this DNA format by sequencing a polymorphic region of the MRSA genome and in doing so demonstrated reads as high as QV40 at the single-molecule level, by generating a consensus sequence from multiple reads of the same molecule.”).

119. Accordingly, it is my opinion that the Travers Publications disclose Element 22.5.

- vii. **Element 22.6 – “comparing the consensus sequence to a reference sequence; and analyzing one or more correspondences between the consensus sequence and the reference sequence to identify a sequence variation”**

120. The Travers Publications teach Element 22.6.

'127 Patent	Travers
comparing the consensus sequence to a reference sequence; and analyzing one or more correspondences between the consensus sequence and the reference sequence to identify a sequence variation.	“Once all subreads are identified, we test at each base position whether the subreads are generated by the putative reference sequence of the template or some variation of that sequence (e.g. a single-nucleotide polymorphism or an indel).” EX1021, 2. “In other words, the identification of a true variant requires that the observed variant could not occur by chance. The circular consensus sequencing approach described here allows one to tune the quality values of consensus base calls to the level demanded by the expected rate of variation.” EX1021, 6-7.

121. As explained above with respect to Element 1.6, Travers discloses aligning and comparing reads from the sense and antisense strands to the reference sequence.

122. Travers “demonstrate[s] the generation of high-quality consensus accuracy from single molecules, as well as the use of SMRTbell templates in the identification of rare sequence variants.” EX1021, Abstract; *see also id.*, 1 (“In this work, we demonstrate the utility of this template format in a SMRT sequencing

system by sequencing variants of a targeted region of the *Staphylococcus aureus* genome.”), 2 (“These markers were selected as a source of genetic diversity to test the ability of SMRTbell™ templates to identify variants when applied in a circular consensus sequencing mode.”). Travers also explains that “identification of a true variant requires that the quality values of the base calls are high enough that the observed variant could not occur by chance. The circular consensus sequencing approach described here allows one to tune the quality values of consensus base calls to the level demanded by the expected rate of variation.” EX1021, 6-7.

123. Travers explains that generation of consensus sequences begins with aligning reads for the sense and antisense strands to the reference sequence, then “each base position” is tested for exhibiting variation (e.g., “single-nucleotide polymorphism or an indel”). EX1021, 2. Travers explains that a consensus is generated for potential variant positions relative to the reference sequence by determining if the variant represents the most likely sequence of the original molecule. EX1021, 2-3 (describing methodology for “detect[ing] the potential single-nucleotide polymorphism at a given location of the template”). A skilled artisan would have understood that Travers’ analytic process allows for identification of sequence variation, supported by a consensus, in the sample molecule at positions corresponding to the reference sequence. Thus, a skilled artisan would understand that Travers discloses comparing the consensus sequence

to a reference sequence. Such a skilled artisan would have also understood that Travers’ analytic process allows for identification of sequence variation in the sample molecule at positions corresponding to the reference sequence. EX1018, ¶58 (“...assigning a consensus determination to a particular base call from multiple reads...”).

124. It is my opinion that the Travers Publications teach the Element 22.6.

125. Accordingly, it is my opinion that the Travers Publications teach or suggest each of the limitations of independent claim 22, such that this claim would have been obvious to a person of ordinary skill in the art.

C. Claim 2

126. The Travers Publications disclose or teach the limitations of claim 2.

'127 Patent	Travers
2. The method of claim 1, wherein the sequence variation is either (i) a processing error or a site of DNA damage, or (ii) a true variant, the method further comprises: comparing the confirmed first strand sequence read and confirmed second strand sequence read; identifying nucleotide bases that are not consistent between the confirmed first and second strand sequence reads; and	“Although referred to herein as comparing or assembling the sequence data from multiple reads of a given sequence, and/or from the sense and antisense strands of the sequence, it will be appreciated that any method of assigning a consensus determination to a particular base call from multiple reads of that position of sequence, and/or to a provide an overall consensus sequence for that segment, will be envisioned and encompassed by the term ‘compare.’” EX1018, ¶58. As previously described (18), the identification of sequence

identifying nucleotide bases that are consistent between the confirmed first and second strand sequence reads, wherein— if the sequence variation is not a consistent nucleotide base, then categorizing the sequence variation as (i) a processing error or a site of DNA damage, and if the sequence variation is a consistent nucleotide base, then categorizing the sequence variant as (ii) a true variant.	polymorphisms requires that allelic variation can be separated from sequencing error. In other words, the identification of a true variant requires that the quality values of the base calls are high enough that the observed variant could not occur by chance. The circular consensus sequencing approach described here allows one to tune the quality values of consensus base calls to the level demanded by the expected rate of variation.” EX1021, 6-7.
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127. Travers describes the process of generating a consensus sequence as including a comparison of multiple reads derived from the sense and antisense strands.

Although referred to herein as comparing or assembling the sequence data from multiple reads of a given sequence, and/or from the sense and antisense strands of the sequence, it will be appreciated that any method of assigning a consensus determination to a particular base call from multiple reads of that position of sequence, and/or to a provide an overall consensus sequence for that segment, will be envisioned and encompassed by the term “compare.”

EX1018, ¶58.

128. A person of ordinary skill in the art would understand “consensus” to refer to sequence information that is supported by most of the redundant reads. A person of ordinary skill in the art would further understand that sporadic errors

(*i.e.*, processing errors) will not be part of the consensus sequence because they will not be supported by most of the redundant reads. EX1021, 3 (“The ability to read both strands on a single DNA molecule therefore enables correction for sequence context-dependent variation.”). On the other hand, if the molecule has a nucleotide different than the reference sequence (*i.e.*, it has a variant base), this will be represented in the consensus sequence because the variant will have support from the sense and antisense strands. A person of ordinary skill in the art would understand that processing errors and true variants are identified during the comparison process used to generate a consensus sequence, as is discussed by Travers.

129. A person of ordinary skill in the art would further understand that the accuracy achieved by consensus sequencing according to the Travers Publications reflects the identification of (1) sporadic errors derived from the sequencing process (*i.e.*, processing errors) and (2) true variants. For example, Travers discloses that the quality of the consensus sequence increases with the number of redundant reads of the molecule. EX1021, 6-7 (“...the choice of template size determines the number of reads that can be obtained from that template and therefore the final empirical [quality] of the consensus data...”). Travers explains that the quality of the consensus sequence can be “tuned” such that the consensus sequence allows for true variants to be distinguished from sequencing errors.

As previously described (18), the identification of sequence polymorphisms requires that allelic variation can be separated from sequencing error. In other words, the identification of a true variant requires that the quality values of the base calls are high enough that the observed variant could not occur by chance. The circular consensus sequencing approach described here allows one to tune the quality values of consensus base calls to the level demanded by the expected rate of variation.

Id.; see also EX1018, ¶¶7, 53, 89, 142.

130. That the consensus sequencing described by Travers, identifies and removes sporadic errors derived from the sequencing process (*i.e.*, processing errors) is directly acknowledged in EX1020 which was incorporated by reference into Travers '075. *E.g.*, EX1020, 24:14-17 (“In particular, the systems of the invention typically provide for redundancy in numerous ways, to correct for any errors that may arise in template depend[e]nt synthesis by the polymerase enzyme.”), 33:32-37 (“Resequencing the same DNA template greatly enhances the chances to detect any errors made by the polymerase, because the same errors unlikely would be repeated by the polymerase...”).

131. Accordingly, it is my opinion that the Travers Publications teach or suggest each and every element of claim 2 such that the claim as a whole would have been obvious.

D. Claims 3 and 16

132. The Travers Publications disclose the limitations of claims 3 and 16.

'127 Patent	Travers
3. The method of claim 2, wherein prior to comparing the confirmed first strand sequence read and confirmed second strand sequence read, the method comprises associating the confirmed first strand sequence read with the confirmed second strand sequence read using one or more of an adapter sequence, a sequence length, sequence alignment information and original strand information. ----- 16. The method of claim 1, wherein prior to the comparing step, the method comprises associating the confirmed first strand sequence read with the confirmed second strand sequence read using one or more of an adapter sequence and original strand information.	As noted, because the [linker] sequence may be exogenously provided, and thus known, it may also provide a registration sequence indicating a point in the sequence determination at which the sequencing reaction, and thus, the sequence data being obtained from the overall template construct, transitions from the sense to the antisense strands.” EX1018, ¶54. “[T]he target sequence portion of the template, as a double stranded segment, will have, within the same sequence data, an internal complement, i.e., will have both a sense and an antisense strand.” EX1018, ¶98.

133. As an initial matter, the specification of the '127 patent does not describe using “sequence alignment” to “associate” first and second strand reads. *See* Element 1.6 above.

134. The Travers Publications disclose “associating the confirmed first strand sequence read with the confirmed second strand sequence read using one or more of an adapter sequence” as required by claims 3 and 16. Travers further

discloses using the sequence of the hairpin adapter linking the sense and antisense strands as a “registration sequence” to define when the sequence for one strand ends and the next one begins.

As noted, because the [linker] sequence may be exogenously provided, and thus known, it may also provide a registration sequence indicating a point in the sequence determination at which the sequencing reaction, and thus, the sequence data being obtained from the overall template construct, transitions from the sense to the antisense strands.

EX1018, ¶54.

135. Travers discloses associating first and sequence strand reads using the “original strand information” as required by claims 3 and 16. For example, Travers discloses that the sequence of the sense and antisense strands is distinguishable from the adapter by virtue of their complementarity. *E.g.*, EX1018, Figure 11, ¶¶31, 98 (“[T]he target sequence portion of the template, as a double stranded segment, will have, within the same sequence data, an internal complement, i.e., will have both a sense and an antisense strand.”), ¶141. Travers also discloses using alignment to distinguish “signals originating from a base and signals originating from other reads at that position or from its complement.” EX1018, ¶60.

136. Accordingly, it is my opinion that the Travers Publications teach or suggest each and every element of claims 3 and 16 such that the claim as a whole would have been obvious.

E. Claims 4 and 8

137. The Travers Publications disclose or teach the limitations of claims 4 and 8.

'127 Patent	Travers
4. The method of claim 1, further comprising generating a consensus sequence for a plurality of adapter-DNA molecules, wherein each consensus sequence comprises nucleotide bases at which both the confirmed first strand sequence read and the confirmed second strand sequence read are in agreement. 8. The method of claim 4, further comprising identifying a nucleotide sequence occurring at a particular position or region in the consensus sequence as a true nucleotide sequence.	“This molecular redundant sequencing was applied in the identification of a single nucleotide variation, e.g., a SNP. Two SMRTbell™ templates were generated that differ at only a single nucleotide (indicated as the T allele and the A allele. The two templates are illustrated in FIG. 12, with the T allele shown in Panel A and marked at the variant base with the arrow, and the A allele shown in panel B. The two templates were mixed together at known ratios, ranging from 0% ‘A’:100% ‘T’ to 100% ‘A’:0% ‘T’.” EX1018, ¶143. “Resequencing the same DNA template greatly enhances the chances to detect any errors made by the polymerase, because the same errors unlikely would be repeated by the polymerase and the same error certainly would not be exponentially amplified as in a polymerase chain reaction.” EX1020, 33:32-37.

138. As explained above, with respect to claim 2, the Travers Publications disclose a consensus sequence method that distinguishes processing errors from true mutations. EX1024, 551; EX1018, ¶58; EX1020, 24:14-17, 33:32-37.

139. Travers discloses forming a consensus sequence wherein “both the confirmed first strand sequence read and the confirmed second strand sequence read are in agreement,” as required by claim 4. As explained above with respect to claim 2, Travers discloses generating consensus sequences derived from the sense and antisense strands of an original adapter-DNA molecule. A person of ordinary skill in the art would have understood that the consensus sequence reflects nucleotide bases that are consistent across the redundant sequences of the sense and antisense strands. *E.g.*, EX1018, Figure 12; ¶¶32, 143. The consensus sequence of Travers represents those nucleotide bases which are consistent across the redundant sequence reads for the sense and antisense strands. *E.g.*, EX1018, ¶142; *see also* EX1020, 24:14-17, 33:32-37; EX1021, 1 (“In this application, a sequencing read produces multiple observations of each base, and these multiple observations can then be used to generate high-accuracy consensus sequence from single molecules.”), 3 (“The ability to read both strands on a single DNA molecule therefore enables correction for sequence context-dependent variation.”), 6 (describing relationship between the number of times the molecule is read and the quality of the consensus sequence).

140. Travers further discloses identifying nucleotide sequence occurring at a particular position or region in the consensus sequence as a true nucleotide sequence. Using this method, Travers identified a true single nucleotide variant. EX1018, ¶143 (“This molecular redundant sequencing was applied in the identification of a single nucleotide variation, e.g., a SNP.”); EX1021, 6-7 (describing identification of A and T alleles at a particular position).

141. Accordingly, it is my opinion that the Travers Publications teach or suggest each and every element of claims 4 and 8 such that the claim as a whole would have been obvious.

F. Claims 5-7

142. The Travers Publications disclose or teach the additional subject matter recited in claims 5-7.

'127 Patent	Travers
5. The method of claim 4, further comprising aligning the consensus sequence to the reference sequence and identifying a variant or a mutation occurring at a particular position or region in the consensus sequence as a true variant or mutation. ----- 6. The method of claim 5, further comprising comparing the consensus sequence to the reference sequence and	“This molecular redundant sequencing was applied in the identification of a single nucleotide variation, e.g., a SNP.” EX1018, ¶143. “In this manner, the choice of template size determines the number of reads that can be obtained from that template and therefore the final empirical QV (EQV) of the consensus data. This trade-off is illustrated in Figure 7.” EX1021, 6.

identifying a mutation type for each of the true mutations. ----- 7. The method of claim 6, wherein the mutation type is selected from one or more of a transition, a transversion, a point mutation, a structural rearrangement, a single-nucleotide substitution, a single-nucleotide variation, and an insertion.	In other words, the identification of a true variant requires that the quality values of the base calls are high enough that the observed variant could not occur by chance. The circular consensus sequencing approach described here allows one to tune the quality values of consensus base calls to the level demanded by the expected rate of variation.” EX1021, 6-7.
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143. Travers also discloses identifying a variant in the consensus sequence as a true variant as required by claim 5. Travers explains that the quality of the consensus sequence can be “tuned” such that the consensus sequence allows for true variants to be distinguished from sequencing errors. EX1021, 6-7 (“...by generating a consensus sequence from multiple reads of the same molecule...”). Using this method, Travers identified true single nucleotide variants. EX1018, ¶143 (“This molecular redundant sequencing was applied in the identification of a single nucleotide variation, e.g., a SNP.”).

144. Travers teaches that the consensus sequence may be compared to a reference sequence to identify a mutation type for true mutations as required by claim 6. As explained above, Travers also discloses identifying true single nucleotide variants using consensus sequencing. EX1018, ¶143; EX1021, 6-7 (“...we were able to accurately call the frequency of the polymorphism...”). A

person of ordinary skill in the art would have understood that “variants” in consensus sequences are determined relative to a reference sequence.

145. With respect to claim 7, the ’127 patent does not describe identifying a structural rearrangement or an insertion using DCS.

146. Travers expressly discloses that single nucleotide variants may be identified with its methods. EX1018, ¶143. Moreover, a person of ordinary skill in the art would understand the terms “transition” and “transversion” as simply types of single nucleotide variants. Similarly, a person of ordinary skill in the art would understand transitions and transversions to be types of “point mutation” or “single-nucleotide substitutions.” That Travers discloses identifying single-nucleotide variants, at the very least, renders obvious using its consensus sequencing method to identify transitions, transversions, point mutations and single-nucleotide substitutions. Travers further discloses that its consensus sequencing method may be used to identify indels, EX1021, 2, which a person of ordinary skill in the art would understand to include insertions. *See also* EX1026, 2 (describing the applicability of the Travers method to detection of “structural rearrangements” in the genome of tumor cells).

147. Accordingly, it is my opinion that the Travers Publications teach or suggest each and every element of claims 5-7 such that the claim as a whole would have been obvious.

G. Claim 9

148. The Travers Publications disclose or teach the additional subject matter recited in claim 9.

'127 Patent	Travers
9. The method of claim 1, further comprising determining nucleotide bases at which the confirmed first strand sequence read and the confirmed second strand sequence read are not complementary, and identifying non-complementary bases as either an experimental error or a site of DNA damage.	“Resequencing the same DNA template greatly enhances the chances to detect any errors made by the polymerase, because the same errors unlikely would be repeated by the polymerase and the same error certainly would not be exponentially amplified as in a polymerase chain reaction.” EX1020, 33:32-37. “The ability to read both strands on a single DNA molecule therefore enables correction for sequence context-dependent variation.” EX1021, 3.

149. As described above with respect to claim 2, Travers teaches identifying sporadic errors (*i.e.*, processing or experimental errors) through comparison of confirmed first and second reads. Travers further teaches “identifying non-complementary bases as either an experimental error or a site of DNA damage,” as required by claim 9. A person of ordinary skill in the art would understand that sporadic errors are inconsistent between reads from the first and second strand. *E.g.*, EX1020, 33:32-37. Nucleotide bases that are not consistent (*i.e.*, complementary) between the confirmed first and second strand sequence reads are not incorporated into the consensus sequence for the DNA molecule. As

Travers explains, such inconsistent bases are presumed to be the result of experimental error. *E.g.*, EX1021, 3 (“The ability to read both strands on a single DNA molecule therefore enables correction for sequence context-dependent variation.”).

150. Accordingly, it is my opinion that the Travers Publications teach or suggest each and every element of claim 9 such that the claim as a whole would have been obvious.

H. Claim 10

151. The Travers Publications disclose or teach the limitations of claim 10.

'127 Patent	Travers
10. The method of claim 1, wherein amplifying original strands comprises amplifying original strands via PCR amplification, cluster amplification, rolling circle amplification or a combination thereof.	“[T]he structurally circular nucleic acid segments described herein, may be used as templates in a rolling circle replication process to produce concatamer molecules that include repeating copies of both the sense and antisense strands of the originating double stranded segment included within the circular nucleic acid.” EX1018, ¶122.

152. The '127 patent acknowledges that Travers discloses “rolling circle amplification.” EX1001, 11:3-17.

153. The Travers Publications expressly disclose amplifying template molecules using rolling circle replication. EX1018, ¶122 (“[T]he structurally circular nucleic acid segments described herein, may be used as templates in a

rolling circle replication process to produce concatamer molecules that include repeating copies of both the sense and antisense strands of the originating double stranded segment included within the circular nucleic acid.”); EX1021, 4.

154. Accordingly, it is my opinion that the Travers Publications teach or suggest each and every element of claim 10 such that the claim as a whole would have been obvious.

I. Claims 12 and 21

155. The Travers Publications disclose or teach the limitations of claims 12 and 21.

'127 Patent	Travers
12. The method of claim 1, wherein following step (a), the method further comprises selectively enriching the adapter-DNA molecules based on size to provide enriched adapter-DNA molecules. 21. The method of claim 1, wherein prior to attaching adapters to double-stranded DNA fragments, the method further comprises selectively enriching double-stranded DNA fragments by size selection.	“The size of the target portion may also be varied depending upon the application in which the template is being used. For example, in genomic sequencing applications, e.g., de novo or resequencing processes, longer target segment will be desired in order to reduce the level of duplicate coverage that is required among different fragments.” EX1018, ¶86. “The ligation products were concentrated by ethanol precipitation and then applied to a ChromaSpin 1000 column to remove any templates that contained no insert or <i>short inserts</i>.” EX1018, ¶145. “The final template construct included a 244 nucleotide duplex segment and

	an overall length of 546 nucleotides (including linking/hairpin segments).” EX1018, ¶139. “The genomic DNA was sheared using a nebulizer to generate fragments ranging from 500 - 1500 bp in size.” EX1018, ¶144.
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156. The Travers Publications disclose the limitations of claims 12 and 21. For example, Travers discloses that the size of the template molecules may varied depending on the application.

The size of the target portion may also be varied depending upon the application in which the template is being used. For example, in genomic sequencing applications, e.g., de novo or resequencing processes, longer target segment will be desired in order to reduce the level of duplicate coverage that is required among different fragments.

EX1018, ¶86.

157. Travers discloses selectively enriching the adapter-DNA molecules based on size to provide enriched adapter-DNA molecules as required by claim 12. For example, Travers discloses that adapter-DNA molecules may be “concentrated by ethanol precipitation and then applied to a ChromaSpin 1000 column to remove any templates that contained no insert or *short inserts*.” EX1018, ¶145.

158. With respect to claim 21, Travers discloses that sample DNA may be divided into different portions, such as “different size-selected portions,” and these portions may be barcoded to facilitate “linking nucleotide sequence in the template

construct” with the different portions of the sample. EX1018, ¶¶101, 102. Travers also discloses that double-stranded DNA fragments may be generated by PCR to produce fragments of a particular size. EX1018, ¶139. Travers further discloses that genomic DNA may be sheared “to generate fragments ranging from 500-1500 bp in size.” EX1018, ¶144.

159. Accordingly, it is my opinion that the Travers Publications teach or suggest each and every element of claims 12 and 21 such that the claim as a whole would have been obvious.

J. Claim 15

160. The Travers Publications disclose or teach the limitations of claim 15.

'127 Patent	Travers
15. The method of claim 1, further comprising providing a sample comprising the double-stranded DNA fragments from a biological source, wherein the biological source comprises tissue.	The nucleic acids that can be prepared by the methods described herein, e.g., for use with high-throughput sequencing systems can also be derived from a cDNA, e.g. cDNAs prepared from mRNA obtained from, e.g., a eukaryotic subject or a specific tissue derived from a eukaryotic subject. EX1018, ¶125.

161. Travers discloses that “[g]enomic DNA can be prepared from any source” and describes use of DNEasy™ Tissue Kit for isolation of such DNA.” EX1018, ¶124. In the context of describing embodiments directed to mRNA sequencing, Travers discloses that such nucleic acids can be prepared from a

variety of sources including “a eukaryotic subject or a specific tissue derived from a eukaryotic subject.” EX1018, ¶125.

162. A person of ordinary skill in the art would understand tissue derived from a eukaryotic subject would be a source of genomic DNA and it would have been obvious to apply the consensus sequencing method of Travers to genomic DNA derived from tissue from a eukaryotic subject.

163. Accordingly, it is my opinion that the Travers Publications teach or suggest each and every element of claim 15 such that the claim as a whole would have been obvious.

K. Claim 17

164. The Travers Publications disclose or teach the limitations of claim 17.

'127 Patent	Travers
17. The method of claim 1, wherein the double-stranded DNA fragments were at least partially generated by nuclease cleavage.	There exist a plethora of ways of generating nucleic acid fragments from a genomic DNA, a cDNA, or a DNA concatamer. These include, but are not limited to, mechanical methods, such as Sonication, mechanical shearing, nebulization, hydroshearing, and the like; chemical methods, such as treatment with hydroxyl radicals, Cu(II): thiol combinations, diazonium salts, and the like; enzymatic methods, such as exonuclease digestion, restriction endonuclease digestion, and the like; and electrochemical cleavage. These methods are further explicated in

	Sambrook and Ausubel.” EX1018, ¶128
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165. The Travers Publications disclose that double-stranded DNA fragments may be generated using exonucleases and restriction endonucleases. EX1018, ¶92 (describing use of restriction endonucleases), ¶128 (describing use of exonuclease digestion and restriction endonuclease digestion).

166. Accordingly, it is my opinion that the Travers Publications teach or suggest each and every element of claim 17 such that the claim as a whole would have been obvious.

L. Claim 18

167. The Travers Publications disclose or teach the limitations of claim 18.

'127 Patent	Travers
18. The method of claim 1, wherein the first strand sequence reads derived from a particular original adapter-DNA molecule and the second strand sequence reads derived from the same particular original adapter-DNA molecule can be related based on a barcode sequence, a fragment-specific feature, or a combination thereof.	“[T]he target sequence portion of the template, as a double stranded segment, will have, within the same sequence data, an internal complement, i.e., will have both a sense and an antisense strand.” EX1018, ¶98. “As alluded to above, however, the connecting or linking oligonucleotide may be selected or generated to include sequence with identifiable sequence characteristics to facilitate its identification, both with respect to the contiguous template sequence with which it is associated, and also with respect to other template sequences that may exist in the same sample mixture.

	In particular, one can utilize connecting oligonucleotides in the template constructs that include sequence markers like a barcode, to indicate the origin of a given template sample. Different template samples including distinguishable connecting oligonucleotide tags could then be pooled for analysis in a single sequencing process. Sequence data derived from individual templates is then attributable to the originating sample preparation process through the identification of the connecting oligonucleotide tag.” EX1018, ¶100.
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168. The Travers Publications disclose relating strands of an original DNA molecule for the purpose of forming a consensus sequence as required by claim 18. For example, Travers discloses that the adapter may include a barcode (or “identifiable sequence”) to “facilitate” identification of the read “both with respect to the contiguous template sequence with which it is associated, and also *with respect to other template sequences that may exist in the same sample mixture.*” EX1018, ¶100.

169. Travers further discloses relating strands of an original DNA molecule based on a fragment-specific feature. For example, Travers discloses that the *sequence* of the sense and antisense strands is distinguishable from the adapter by virtue of their complementarity. *E.g.*, EX1018, Figure 11, ¶¶31, 98 (“[T]he target sequence portion of the template, as a double stranded segment, will have, within

the same sequence data, an internal complement, i.e., will have both a sense and an antisense strand.”), ¶141.

170. Accordingly, it is my opinion that the Travers Publications teach or suggest each and every element of claim 18 such that the claim as a whole would have been obvious.

M.Claim 19

171. The Travers Publications disclose or teach the limitations of claim 19.

'127 Patent	Travers
19. The method of claim 1, wherein the sequence reads derived from a particular original adapter-DNA molecule can be differentiated from the second strand sequence reads derived from the same particular original adapter-DNA molecule at least in part by an asymmetry of the asymmetrical double-stranded adapter-DNA molecules.	“The process then continues along the antisense strand to the A sequence, and provides the nucleotide sequence (A'), which sequence can be used to derive or determine a consensus sequence for the sense strand, as its antisense counterpart.” EX1018, ¶54. “Demonstration of single-molecule traces that include both sense and antisense strands of a single molecule. The pulses within the sequencing traces are colored according to location within the template, with blue corresponding to the sense strand, orange corresponding to the antisense strand, and light blue and green corresponding to the hairpin adapters.” EX1021, Figure 4.

172. As discussed above, Travers discloses adapter-DNA molecules within the scope of “partially-complementary, asymmetrical double-stranded adapter-

DNA molecules.” *See* Section V. Travers further discloses that the asymmetry of adapter-DNA molecules imbued by the hairpin linker allows the first and second strands of an original adapter-DNA molecules to be distinguished. EX1018, ¶54; EX1021, Figure 1, Figure 4.

173. Accordingly, it is my opinion that the Travers Publications teach or suggest each and every element of claim 19 such that the claim as a whole would have been obvious.

N. Claims 20, 27, and 28

174. The Travers Publications disclose or teach the limitations of claims 20, 27, and 28.

'127 Patent	Travers
20. The method of claim 1, wherein prior to sequencing, the method further comprises selectively enriching adapter-DNA molecules or copies thereof for a subset of said adapter-DNA molecules that map to one or more genetic loci in a reference genome. ----- 27. The method of claim 22, further comprising selectively enriching double-stranded adapter-DNA molecules or copies thereof to enrich for a subset of DNA molecules that map to one or more genetic loci in the reference sequence.	“Alternatively, or additionally, target nucleic acid segments may be obtained through targeted capture protocols where target nucleic acids are obtained initially as single stranded segments on microarrays or other capture techniques, followed by amplification of the captured material to generate double stranded sample materials.” EX1018, ¶124.

----- 28. The method of claim 22, wherein, prior to sequencing, double-stranded adapter-DNA molecules or copies thereof are selectively enriched using a hybridization capture method to provide target DNA molecules that map to one or more genetic loci in the reference sequence.	
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175. As an initial matter, the specification of the '127 patent does not describe “adapter-DNA molecules that map to one or more genetic loci in a reference genome” at least because it does not disclose mapping molecules to a reference genome.

176. The Travers Publications teach the selective enrichment of single-stranded molecules using a hybridization capture method. For example, Travers discloses that “microarrays or other capture techniques” may be used to “target nucleic acid segments.” EX1018, ¶124. Travers further discloses that these methods are applied to “single stranded segments.” *Id.* A person of ordinary skill in the art would understand microarrays to be a type of hybridization capture method because it allows for sequence-specific enrichment of single-stranded molecules based on hybridization to immobilized oligonucleotides.

177. As explained above with respect to Element 1.3, Travers discloses that SMRTbell templates may be amplified prior to sequencing to produce single-

stranded concatemer molecules comprising “repeating copies of both the sense and antisense strands of the originating double stranded segment.” EX1018, ¶122. A person of ordinary skill in the art would have been motivated to enrich single-stranded concatemer molecules prior to sequencing in order to enrich for sequences of interest. *E.g.*, EX1028, abstract (“Roughly 98% of the human genome is composed of repeats and intergenic or non–protein coding sequences. Thus, it is crucial to focus resequencing on high-value genomic regions.”). A person of ordinary skill in the art would have had a reasonable expectation of success in enriching concatemer molecules because, as Travers explains, these were well-established methods commonly used in conjunction with high-throughput sequencing.

178. Accordingly, it is my opinion that the Travers Publications teach or suggest each and every element of claims 20, 27, and 28 such that the claim as a whole would have been obvious.

O. Claims 23-25

'127 Patent	Travers
23. The method of claim 22, wherein the bodily sample comprises tissue obtained from a subject. ----- 24. The method of claim 22, wherein at least some of the double-stranded DNA	“mRNA can typically be isolated from almost any source using protocols and methods described in, e.g., Sambrook and Ausubel. The yield and quality of the isolated mRNA can depend on, e.g., how a tissue is stored prior to RNA extraction, the means by which the

fragments are derived from a tumor or circulating neoplastic cells. ----- 25. The method of claim 22, wherein the bodily sample is derived from a human subject having a tumor cell population, and wherein following step (d), the method further comprises identifying a genetic mutation conferring drug resistance present in one or more of the consensus sequences derived from the double-stranded DNA fragments obtained from the tumor cell population present in the bodily sample.	tissue is disrupted during RNA extraction, or on the type of tissue from which the RNA is extracted. RNA isolation protocols can be optimized accordingly. Many mRNA isolation kits are commercially available, e.g., the mRNA-ONLY™ Prokaryotic mRNA. Isolation Kit and the mRNA-ONLY™ Eukaryotic mRNA. Isolation Kit (Epicentre Biotechnologies), the Fast Track 2.0 mRNA. Isolation Kit (Invitrogen), and the Easy mRNA Kit (BioChain). In addition, mRNA from various Sources, e.g., bovine, mouse, and human, and tissues, e.g. brain, blood, and heart, is commercially available from, e.g., BioChain (Hayward, Calif.), Ambion (Austin, Tex.), and Clontech (Mountainview, Calif.).” EX1018, ¶126.
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179. Claims 23-25 depend from claim 22 and further require the “double-stranded DNA fragments” recited in Element 22.1 be derived from “tissue obtained from a subject,” “tumor or circulating neoplastic cells” or “a tumor cell population,” respectively. Claims 23 and 24 are directed only to features of the bodily sample being used in the method, not the method itself.

180. As explained above with respect to claim 22, Travers discloses that the double-stranded DNA fragments may be obtained from a bodily sample. EX1018, ¶101, ¶102; *see also id.*, ¶5. Travers also discloses that mRNA may be obtained from “various sources, e.g., bovine, mouse, and human, and *tissues* e.g. brain, blood, and heart.” EX1018, ¶126. A person of ordinary skill in the art would

have understood that double-stranded DNA could be obtained from the same sources that Travers identifies as sources for mRNA. Accordingly, it would have been obvious to apply the consensus sequencing method of Travers to double-stranded DNA obtained from a tissue sample as required by claim 23. *See also* EX1027, 1, 10 (describing application of the Travers method to genomic DNA samples isolated from brain tissue and embryonic stem cells of mice); EX1026, 2.

181. With respect to claim 24 and 25, a person of ordinary skill in the art would have considered application of Travers to sequence analysis of tumors, including drug resistance therein, as within the scope of the method or, at minimum, application of Travers to detect such mutations would have been obvious. The '127 patent admits that genetic analysis of tumors generally, and drug resistance in particular, was well-documented in the art. EX1001, 1:43-54. In fact, this prior art is the only discussion of cancer and drug resistance in the treatment of cancer in the '127 patent. Travers expressly discusses use of the method to detect variation and mutation and does not limit its disclosure to any particular type of mutation. EX1021, 2 (describing genotyping "to identify variants"). McCarthy confirms that Travers is not narrowly limited to the analysis of any particular mutation type and describes the applicability of the Travers method to detection of "structural rearrangements" in the genome of tumor cells. EX1026, 2 (pointing to the particular advantages of PacBio's "long read" format).

182. Accordingly, it is my opinion that the Travers Publications teach or suggest each and every element of claims 23-25 such that the claim as a whole would have been obvious.

P. Claim 26

'127 Patent	Travers
26. The method of claim 22, wherein the library comprises at least a subset of non-uniquely tagged double-stranded adapter-DNA molecules, and wherein non-uniquely tagged double-stranded adapter-DNA molecules are substantially identifiable with respect to other non-uniquely tagged double-stranded adapter-DNA molecules in the bodily sample using the one or more barcode sequences and DNA fragment-specific information.	“As the sequencing output from each run is based upon individual molecules, the sequencing data is then parsed out according to its origin by virtue of the barcode sequence that is integrated into the template molecules. In particular, because each sequence read is derived from a single molecule, the sequence of the insert portion of the template can be unambiguously linked to the sequence of its attached adapter sequence that includes the bar code sequence within that adapter sequence. Accordingly, each template sequence is then traced back to its origin, e.g., a particular sample, patient, etc.” EX1018, ¶102.

183. The '127 patent never mentions “non-unique tagging.” The '127 specification describes using a sufficient diversity of “SMI tags” to promote labeling different DNA fragments in the sample with a different SMI tag—an aspect of the DCS method that the '127 patent repeatedly describes as “unique” tagging or labeling. EX1001, 18:37-42 (“the use of SMI tags...allows every

molecule to be *uniquely* labeled”); *see also id.*, 6:67-7:4, 15:29-33, 21:61-63, 22:19-20, 22:25-27, 27:63-67, 26:59-61.

184. The Travers Publications describe using barcodes to identify sample molecules. For example, Travers discloses that templates derived from multiple preparation processes or sources may be pooled for sequencing and that “each template sequence [may be] traced back to its origin” using the barcode. EX1018, ¶102. A person of ordinary skill in the art would understand that Travers to be describing sample barcoding wherein each molecule in the sample receives the same barcode. Because all molecules in a sample receive the same barcode, A person of ordinary skill in the art would understand that the molecules are non-uniquely tagged within the meaning of claim 26.

185. Travers discloses that the combination of the barcode and the insert sequence renders the identity of the molecule “unambiguous.”

In particular, because each sequence read is derived from a single molecule, the sequence of the insert portion of the template can be unambiguously linked to the sequence of its attached adapter sequence that includes the bar code sequence within that adapter sequence.

EX1018, ¶102.

186. Accordingly, claim 26 is obvious in view of the Travers Publications.

IX. GROUND 2 - CLAIM 11 IS OBVIOUS AS ILLUSTRATED BY THE TRAVERS PUBLICATIONS IN VIEW OF NEB EXPRESSIONS.

11. The method of claim 1, wherein the double-stranded DNA fragments comprise at least one of a deaminated nucleic acid base and a nucleic acid base having oxidative damage, and wherein prior to or following step (a) the method further comprises chemically or enzymatically treating the double-stranded DNA fragments to remove or repair one or more damaged bases.

187. As I explain above, the Travers Publications render claim 1 obvious.

Claim 11 depends from claim 1 and further requires that the DNA sample comprise fragments with “a deaminated nucleic acid base” or “a nucleic acid base having oxidative damage,” and chemically or enzymatically treating the DNA to remove or repair one or more damaged base. The additional subject matter of claim 11 is disclosed by NEB Expressions.

188. NEB Expressions discloses that deaminated cytosine and oxidized bases (e.g., 8-oxo-guanine) are two common types of DNA damage that may be present in typical DNA samples. *E.g.*, EX1014, 1-2 & Table 1 (listing deaminated cytosine and oxidized bases as types of damage typically found in DNA samples). NEB Expressions explains that the commercial product “PreCR™ Repair Mix” is “a cocktail of enzymes formulated to repair damaged DNA *in vitro* prior to PCR,” including specifically deaminated cytosine and oxidized guanine. EX1014, 1, 3 & Table 2 (listing deaminated cytosine and oxidized bases as types of damage enzymatically repaired by PreCR™ Repair Mix).

189. In my opinion, a person of ordinary skill in the art would have been motivated to use PreCR™ Repair Mix as described in NEB Expressions on the samples of the Travers Publications because it allows the “Unlock[ing of] genetic information that was previously inaccessible due to damaged DNA template.” EX1014, 1. As NEB Expressions explains, deaminated cytosine residues “may result in inhibition of PCR or [production of] mutagenic DNA products.” EX1014, 2. NEB Expressions explains that “most DNA samples are susceptible to oxidation” and that oxidation of guanine to 8-oxo-guanine may occur *in vitro* (e.g., during the DNA extraction process) or *in vivo* (e.g., oxidation of mitochondrial DNA) and produces “mutagenic” DNA products. EX1014, 2. Because deaminated and oxidized residues are mutagenic (e.g., cause base mispairing during replication), a person ordinary skill in the art would have been motivated to repair these damaged bases using enzymes such as with the PreCR™ Repair Mix described in NEB Expressions. Further, a person ordinary skill in the art would have had a reasonable expectation of success because, as NEB Expressions explains, PCR™ Repair Mix is effective in repairing deaminated cytosines and oxidized guanine. EX1014, 3.

190. Accordingly, it is my opinion that the Travers Publications in further view of NEB Expressions render obvious claim 11 as a whole.

X. GROUND 3 - CLAIMS 13, 14, 29, AND 30 ARE OBVIOUS AS ILLUSTRATED BY THE TRAVERS PUBLICATIONS IN VIEW OF MCCLOSKEY

A. Claims 13 and 14

13. The method of claim 1, wherein the adapters comprise a Y-shape or a U-shape, and wherein the barcodes comprise a degenerate or semi-degenerate sequence containing between 3 and 20 nucleotide bases.

14. The method of claim 13, wherein the adapters comprise the barcodes in at least one of a double-stranded portion or a single-stranded portion of the adapter.

191. The Travers Publications in view of McCloskey teach U-shape adapters comprising “a degenerate or semi-degenerate sequence containing between 3 and 20 nucleotide bases” as required by claim 13. Claim 14 depends from claim 13 and requires the barcode to be located in either the single- or double-stranded portion of the adapter.

192. Travers also discloses “the connecting or linking oligonucleotide may be selected or generated to include sequence with identifiable sequence characteristics to facilitate its identification, both with respect to the contiguous template sequence with which it is associated, and also with respect to other template sequences that may exist in the same sample mixture.” EX1018, ¶100.

193. A person of ordinary skill in the art would understand Travers to teach using barcodes to distinguish molecules of the sample mixture. A person of ordinary skill in the art would have been motivated to include an identifying

sequence (i.e., barcode) in the adapter, as taught by Travers, when making multiple copies of original circular templates. EX1018, ¶122 (“Although the constructs of the invention are described primarily, and preferably, for use directly as templates for, e.g., sequencing applications, it will be appreciated that these structures may also serve as intermediate structures in the preparation of templates that provide for sequence redundancy in line with that provided by such constructs.”).

Incorporation of a barcode into the circular template facilitates identification of the template with respect to other template sequences that may exist in the same sample mixture.

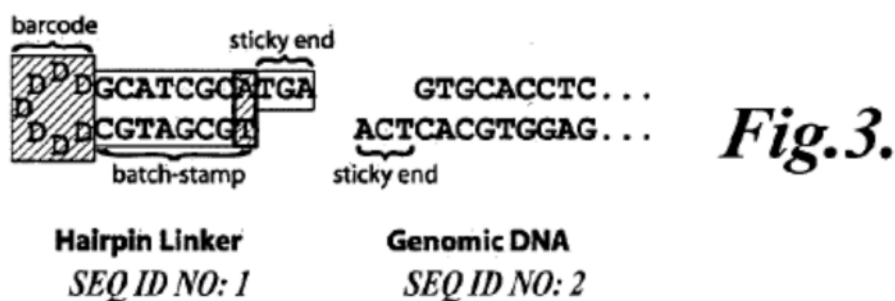
194. The ’127 patent uses the term degenerate barcode to refer to barcodes comprising random sequences. *E.g.*, EX1001, 7:14-16 (“the SMI tag nucleotide sequence may be completely **random** and degenerate, wherein each sequence position may be any nucleotide”). Travers does not place any restrictions on the types of barcodes that may be included in its hairpin adapters and used for identification of template molecules. Moreover, the use of degenerate barcodes in the context of hairpin adapters for distinguishing sample molecules was well documented in the prior art and described by McCloskey among other references.

195. McCloskey discloses “the present invention permits identification of valid sequences, and distinguishes the valid sequences from contaminants and redundant sequences.” EX1023, ¶5 (“Thus, the present invention permits

identification of valid sequences, and distinguishes the valid sequences from contaminants and redundant sequences arising from template re-cloning.”).

196. McCloskey further explains that the barcodes used for this purpose may be “random.” EX1023, ¶21. McCloskey describes its barcodes as comprising random sequences that are unknown in advance that allow for assigning a unique identity to the molecule it is ligated to. “The term “random barcode” refers to an arbitrary sequence that can uniquely identify a target nucleic acid in an experiment, and whose sequence is unknown at the start of the experiment. Methods of synthesizing oligonucleotides containing random sequences are well known in the art. Typically, oligonucleotides containing random sequences are synthesized by randomly incorporating a nucleotide at each of the positions of the second sequence. ... The second sequence is located 5’ to the first sequence in the bar-coded oligonucleotide. The length of the second sequence is sufficient to provide, with high probability, a unique identity to each target nucleic acid molecule in the sample prior to amplification.” EX1023, ¶21. McCloskey also explains that the length of the random barcodes may be “between 3 and 30 nucleotides, such as between 5 and 25 nucleotides or between 7 and 13 nucleotides.” *Id.*

197. McCloskey discloses that its random barcodes may be included in hairpin adapters. EX1023, Figure 3 (reproduced below), ¶15 (“FIG. 3 shows a schematic representation of a bar-coded and batch-stamped hairpin linker (SEQ ID NO:1), designed for ligation to DraIII-cut genomic DNA of FMR1 (SEQ ID NO:2), as described in EXAMPLE 6.”). According to McCloskey, the disclosed



linkers are ligated to genomic DNA fragments prior to amplification. EX1023, ¶5; *see also id.*, ¶¶10, 15. “In the practice of the present invention nucleic acid molecules (e.g., genomic DNA fragments) are labeled with distinct sequence tags prior to PCR amplification.

198. A person of ordinary skill in the art would have been motivated to use the barcodes of McCloskey with the hairpin adapters of the Travers Publications to facilitate identification of unique double-stranded molecules. Travers ’075 suggests that barcodes may be used to facilitate identification of sample sequences relative to other sequences in the mixture and McCloskey indicates that random barcodes may be used for this purpose. EX1018, ¶100; EX1023, ¶21. “As alluded to above, however, the connecting or linking oligonucleotide may be selected or generated to

include sequence with identifiable sequence characteristics to facilitate its identification, both with respect to the contiguous template sequence with which it is associated, and also with respect to other template sequences that may exist in the same sample mixture. In particular, one can utilize connecting oligonucleotides in the template constructs that include sequence markers like a barcode, to indicate the origin of a given template sample.” EX1018, ¶100.

199. A person of ordinary skill in the art would have expected success in using the random barcodes described by McCloskey because methods for making such oligonucleotides were well-known in the art. EX1023, ¶21. “Methods of synthesizing oligonucleotides containing random sequences are well known in the art. Typically, oligonucleotides containing random sequences are synthesized by randomly incorporating a nucleotide at each of the positions of the second sequence.” EX1023, ¶21.

200. Accordingly, it is my opinion that the Travers Publications in further view of McCloskey render obvious claims 13 and 14 as a whole.

B. Claims 29 and 30

29. The method of claim 22, wherein the barcode sequences are 6 nucleotides in length.

30. The method of claim 22, wherein the barcode sequences are 3, 4, 5, 6, 7 or 8 nucleotides in length.

201. The specification of the '127 patent does not indicate that the claimed lengths of barcodes are preferable to the other lengths of barcodes. *E.g.*, EX1001, 7:4-10 (“Each n-mer sequence may be between approximately 3 to 20 nucleotides in length. Therefore, each n-mer sequence may be approximately 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 nucleotides in length. In one embodiment, the SMI sequence is a random degenerate nucleotide n-mer sequence which is 12 nucleotides in length.”).

202. As discussed above with respect to claims 13 and 14, McCloskey discloses that the length of the random barcodes may be “between 3 and 30 nucleotides, such as between 5 and 25 nucleotides or between 7 and 13 nucleotides.” EX1022, ¶21. McCloskey also discloses a random barcode comprising 7 nucleotides. *Id.* McCloskey explains that “The length of the [barcode] is sufficient to provide, with high probability, a unique identity to each target nucleic acid molecule in the sample prior to amplification.” *Id.* A person of ordinary skill in the art would understand based on McCloskey that the length of the barcode is chosen such that there are sufficient barcodes to uniquely identify each target DNA molecule.

203. Much like McCloskey, the '127 patent explains that the barcode “may be any suitable length to produce a sufficiently large number of unique tags to label a set of sheared DNA fragments.” EX1001, 6:67-7:4. Accordingly, a person

of ordinary skill in the art would have been motivated to follow the guidance in McCloskey and would have chosen a 6-nucleotide barcode from the ranges described by McCloskey, if that length of barcode was sufficient to assign a unique identity to each target nucleic acid molecule in the sample.

204. Accordingly, it is my opinion that the Travers Publications in further view of McCloskey render obvious claims 29 and 30 as a whole.

XI. CONCLUDING STATEMENTS

205. In signing this declaration, I understand that the declaration will be filed as evidence in a contested case before the Patent Trial and Appeal Board of the United States Patent and Trademark Office. I acknowledge that I may be subject to cross-examination in this case and that cross-examination will take place within the United States. If cross-examination is required of me, I will appear for cross-examination within the United States during the time allotted for cross-examination.

206. I declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements 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.

Dated: April 8, 2022

By: / John Quackenbush /
John Quackenbush