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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

**PETITION FOR INTER PARTES REVIEW OF
U.S. PATENT NO. 10,760,127**

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LISTING OF CHALLENGED CLAIMS

1. [**Preamble**] A method of sequencing DNA comprising:

[**Element 1.1**] a) attaching adapters to double-stranded DNA fragments to generate a plurality of partially-complementary, asymmetrical double-stranded adapter-DNA molecules,

[**Element 1.2**] wherein the adapters comprise barcodes selected from a plurality of distinct barcode sequences;

[**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;

[**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

[**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;

[**Element 1.6**] comparing at least one of the confirmed first and second strand sequence reads to a reference sequence; and

[**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.

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, and wherein, for at least some of the adapter-DNA molecules, 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

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.

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.
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.
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 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.
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.
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.

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.

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.

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.

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.

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.

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.

17. The method of claim 1, wherein the double-stranded DNA fragments were at least partially generated by nuclease cleavage.

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.

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.

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.

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.

22. [**Preamble**] A method of sequencing DNA comprising:

[**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;

[**Element 22.2**] b) amplifying strands from a plurality of the double-stranded adapter-DNA molecules in the library to produce strand copies;

[**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

[**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;

[**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;

[**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.

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 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.

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.

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.

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.

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.

Guardant Health, Inc., (“Petitioner”) hereby requests review of United States Patent No. 10,689,127 to Jesse Salk et al. (hereinafter “the ’127 patent,” EX1001), which is currently assigned to The University of Washington Through Its Center for Commercialization (“Patent Owner”). This petition demonstrates by a preponderance of the evidence, that claims 1-30 of the ’127 patent are unpatentable for failing to distinguish over prior art.

I. INTRODUCTION

The ’127 patent specification purports to have invented duplex consensus sequencing (“DCS”). But many claims do not even require consensus sequencing, much less duplex consensus sequencing, the purported point of novelty in the specification. The ’127 patent instead claims a far simpler concept—attaching partially-single-stranded adapters comprising barcodes to DNA fragments to detect sequence variants in the DNA fragments as compared to a reference sequence. The ’127 patent describes use of commercially available Y- or U-shaped adapters (e.g., Illumina, Pacific Biosciences), and claims the use of Y- or U-shaped adapters with barcode sequences for otherwise standard sequencing processes. EX1001, 3:52-4:11, 6:4-41, 10:63-11:2, 18:49-57, 25:64-26:1.

As established in this petition, the Travers Publications describe a consensus sequencing method developed by Pacific Biosciences which is indistinguishable from the claimed method. Travers discloses U-shaped hairpin adapters which

generate partially single-stranded and partially-complementary, asymmetrical double-stranded adapter DNA molecules. Travers discloses that these adapters include a barcode so that template molecules may be traced back to their origin. Travers further discloses redundantly sequencing sense and antisense strands of original molecules. From these redundant reads, Travers describes generating a consensus sequence that represents the “true” sequence of the molecule. The Travers Publications teach each and every limitation of claims 1-10, 12, and 15-28. Claims 11, 13, 14, 29, and 30 are obvious in further view of NEB Expression or McCloskey.

Accordingly, Petitioner respectfully request institution as to all challenged claims and cancellation of the claims as unpatentable.

A. Background in the Art

This petition is supported by the Declaration of Dr. John Quackenbush who is the Henry Pickering Walcott Professor of Computational Biology and Bioinformatics and Chair of the Department of Biostatistics at Harvard University. EX1002; EX1003 (Dr. Quackenbush’s *curriculum vitae*, providing a summary of his education, training, and experience). As Dr. Quackenbush explains and as addressed in further detail below, the involved claims would not have been considered new or non-obvious to a person of ordinary skill in the art (“POSA”) at the relevant time.

i. Consensus sequencing and “asymmetric” adapters

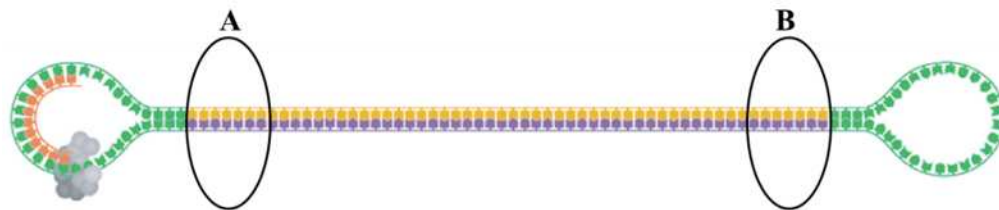
Before March 20, 2012, POSAs were aware that sequencing read errors may arise as artifacts of next-generation sequencing (“NGS”), and that such errors were mitigated by redundantly sequencing multiple copies of a molecule and evaluating resulting sequence data for consensus. EX1031, Abstract; EX1021, Abstract; EX1006, Abstract. The prior art consensus sequencing methods conventionally used barcodes to uniquely “label” original DNA molecules. A consensus sequence generated using multiple reads of a target molecule 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 is nothing new regarding NGS, adapters comprising barcodes, or consensus sequencing in the ’127 patent. EX1002, ¶¶30-31.

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. Partially single-stranded, partially-

complementary sequencing adapters that rendered adapter-DNA molecules asymmetric were not only commercially available, but were conventionally used to convert sample DNA into sequence-ready DNA molecules. EX1012, Figure 4; EX1013, Figure 1; EX1018, Figure 7; EX1021, Figure 1. As explained 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. Moreover, 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.

PacBio’s hairpin adapters are partially single-stranded, partially-complementary adapter. These adapters include sequences in the non-complementary portion that are necessary for sequencing double-stranded DNA molecules. 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.

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).¹



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; *see also* EX1002, ¶¶32-37.

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

¹ 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).

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.

As explained 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. EX1002, ¶¶38-39.

ii. The Travers Publications

This petition relies on a patent publication and a paper describing sample preparation and sequencing methods developed at PacBio. These publications are collectively referred to as “The Travers Publications” and are described below.

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. Travers ’075 was published on December 3, 2009 and qualifies as prior art under 35 U.S.C. §102(a)(1) and pre-AIA 35 U.S.C. §102(b). EX1002, ¶¶40-41.

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, 7 (“All the authors are employees of Pacific Biosciences.”), Figure 1. The Travers Paper was published online on June 22, 2010 and qualifies as a prior art printed publication under 35 U.S.C. §102(a)(1) and pre-AIA 35 U.S.C. §102(b).

As discussed herein, Travers provides disclosure relevant to all aspects of the challenged claims. Travers discloses “asymmetric” U-shaped or hairpin adapters used 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; EX1024, 550-551; Section VI. 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...”); EX1002, ¶¶42-43.

iii. McCloskey

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. McCloskey was published on January 25, 2007 and qualifies as a prior art under 35 U.S.C. §102(a)(1) and pre-AIA 35 U.S.C. §102(b). EX1002, ¶44.

iv. NEB Expressions

NEB Expressions was published by New England Biolabs in Spring 2007 and also distributed publicly through the U.S. Postal Service. EX1014, 1, 8. Its public availability is confirmed by an Internet Archive WayBack Machine capture of the publication in its entirety on the NEB website at least as early as November 19, 2008 and of the index page for the publication at least as early as March 21, 2008. EX1015; EX1016. Its prior art status also is confirmed by a citation of the publication in a review published in September 2011. EX1017, 28, 31 (reference

20). NEB Expressions qualifies as a prior art under 35 U.S.C. §102(a)(1) and pre-AIA 35 U.S.C. §102(b). EX1002, ¶45.

B. The '127 Patent

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, 3:17-4:3.

As with prior art consensus sequencing methods, DCS generally includes tagging polynucleotides with identifier sequences (“SMIs”), amplifying the SMI-tagged molecules using PCR, and sequencing the amplified products. EX1001, 2:15-30. The tagged amplified molecules are sequenced and then the data is grouped into families based on common identifier sequences. *Id.*, 3:17-39, FIG.1, 3:52-4:3, 19:20-25. A “consensus sequence” is determined from the collection of redundant sequence reads which allows true mutations to be distinguished from sequencing errors. *Id.*, 3:33-39, FIG.3, 4:12-37; *see also* Section I.A.

The '127 patent specification cites to commercially available Y- and U-shaped adapters as within the scope of the invention. EX1001, 10:63-11:2, 25:64-67; EX1002, ¶¶21-29.

C. Prosecution History

The '127 patent was filed as U.S. Application No. 16/503,382 (“the '382 application”) on July 3, 2019, claiming priority, via U.S. Application Nos. 16/120,072, 15/660,785, 14/386,800 and PCT/US2013/032665, to U.S. Provisional Patent Application Nos. 61/613,413, 61/625,623, and 61/625,319 filed Mar. 20, 2012; Apr. 17, 2012; and Apr. 17, 2012, respectively.

The claims of the '382 application were rejected as anticipated by Otwinowski (WO2013/181170) which Patent Owner overcame by arguing that the reference is not prior art. EX1004, 360-369, 407. In a subsequent telephonic interview, Examiner and Patent Owner discussed “how the barcoded adapters label individual strands.” *Id.*, 422, 435. No further Office actions were issued.

The Examiner explained in the reasons for allowance that she did not understand the art as describing adapters that enable strand-specific labeling.

Further, as established in the interview, the method of the claims, as amended, provides the ability to label each strand of a target with asymmetric adaptors that enable strand specific labeling. With these adaptors, as depicted in Figure 1 and 3, the adaptors allow for specific detection and identification of genuine mutations as opposed to sequence variations that are artifacts or side-effects of the sequencing process.

Further, a careful search of the prior art does not find any references which teach adapters of the format as claimed by Applicant.

EX1004, 432-433. As explained above, adapters comprising barcodes — such as depicted in Figures 1 and 3 of the '127 patent — were known and well-documented in the prior art. Accordingly, the Examiner's failure to identify such art and apply it to the claims represents material error.

D. Level of Skill in the Art

As Dr. Quackenbush explains, a person of ordinary skill in the art by 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. *See also* EX1002, ¶¶46-47.

II. GROUNDS FOR STANDING

Petitioner certifies that, under 37 C.F.R. §42.104(a), the '127 patent is available for *inter partes* review, and Petitioner is not barred or estopped from requesting *inter partes* review of the '127 patent on the grounds identified.

III. MANDATORY NOTICES UNDER 37 C.F.R. §42.8

Real Party-in-Interest (37 C.F.R. §42.8(b)(1)): Guardant Health, Inc. is the real party-in-interest.

Related Matters (37 C.F.R. §42.8(b)(2)): Patent Owner has asserted the '127 patent against Petitioner in the United States District Court in Delaware (1:21-cv-

01126-LPS). Concurrently filed with this petition is another petition also requesting *inter partes* review of the '127 patent in IPR2022-00816.

Lead and Back-Up Counsel (37 C.F.R. §42.8(b)(3))

Lead Counsel: Michael T. Rosato (Reg. No. 52,182)

Back-Up Counsel: Jad A Mills (Reg. No. 63,344); Sonja R. Gerrard (Reg. No. 72,802)

Service Information – 37 C.F.R. §42.8(b)(4). Petitioner hereby consents to electronic service.

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IV. STATEMENT OF THE PRECISE RELIEF REQUESTED FOR EACH CLAIM CHALLENGED

Petitioners request review of claims 1-30 of the '127 patent under 35 U.S.C. §311 and AIA §6. The grounds for relief are as follows:

Ground	Claims	Description
1	1-10, 12, 15-28	Unpatentable under 35 U.S.C. §103 as obvious over the Travers Publications
2	11	Unpatentable under 35 U.S.C. §103 as obvious

		over the Travers Publications in view of NEB Expressions
3	13, 14, 29, and 30	Unpatentable under 35 U.S.C. §103 as obvious over the Travers Publications in view of McCloskey

V. CLAIM CONSTRUCTION

No terms need be construed to find the claims unpatentable in view of the prior art. *Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co.*, 868 F.3d 1013, 1017 (Fed. Cir. 2017). In mapping the prior art to the claims, Petitioner has applied the “the meaning that [a] term would have to a person of ordinary skill in the art in question at the time of the invention.” *Phillips v. AWH Corp.*, 415 F.3d 1303, 1313 (Fed. Cir. 2005) (en banc). While no construction is necessary to find the claims unpatentable, below provides context for understanding the scope of the term “partially-complementary, asymmetrical double-stranded adapter-DNA molecules” which limits claims 1-21.

Independent claim 22 recites attaching “partially single-stranded adapters” to double-stranded DNA fragments to generate a library of tagged double-stranded adapter-DNA molecules. Independent claim 1 recites that 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 (e.g., Illumina) adapters (when those adapters comprise barcodes selected from a plurality of distinct barcode sequences). *E.g.*, EX1012, Figure 4.

As Dr. Quackenbush explains, and as explained above in Section I.A., 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, ¶¶14-16, 55. The same is true when using hairpin adapters, which also are partially single-stranded and partially-complementary adapters. EX1002, ¶¶48-50.

This 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).

Accordingly, the plain meaning of these claims 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. EX1002, ¶¶51-52.

VI. DETAILED EXPLANATION FOR GROUNDS OF UNPATENTABILITY

As discussed in detail below, the Travers Publications render claims 1-10, 12, and 15-28 obvious. 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.

There can be no novelty of the claimed invention residing in the requirement for partially-complementary, asymmetric adapter-DNA molecules (claim 1) or the requirement for determining a consensus sequence (claim 22). Section I.A. Each and every feature of claims 1-10, 12, and 15-28—including any alleged points of novelty—is taught or suggested in the prior art, as illustrated by the Travers Publications. EX1002, ¶¶53-55.

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 I.A.



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

The SMRTbell templates are amplified by primer extension with a strand displacing polymerase to produce a concatemer comprising repeating copies of the sense and antisense strands separated by a copy of the adapter. Use of the

SMRTbell 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* EX1021, 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; EX1002, ¶¶56-57.

Patent Owner acknowledged that virtually every aspect of the claimed subject matter is disclosed by Travers. In responding to an anticipation rejection in a related case, Patent Owner characterized Travers ’075 as generating a “consensus sequence of the double stranded molecule by redundant sequencing of the entire single template.” EX1024, 551; *see also* EX1001, 33:22-25 (citing the Travers Paper “A flexible and efficient template format for circular consensus sequencing and SNP detection”). Patent Owner further acknowledged that Travers ’075 discloses attaching adapters, 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.”

EX1024, 551 (original emphasis); *see also* EX1001, 10:63-11:18, 15:29-39.

Patent Owner was able to overcome Travers '075 by arguing 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” as recited in the claims under examination in that case. EX1024, 581-582; *see also id.*, 556. The claims of the '127 patent have no such limitation. *See also* EX1002, ¶¶58-60.

That the current claims of the '127 patent read on 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 amplification and sequencing templates as “adequate for use in the assay.” *Id.*, 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.” *Id.*, 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 I.A.

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. EX1002, ¶¶61-62.

A. [Ground 1] Claims 1-10, 12, 15-28 are unpatentable as obvious under 35 U.S.C. §103 over the Travers Publications

As explained herein, 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). As explained by Dr. Quackenbush, a POSA would

have understood the Travers Paper to disclose applications of the PacBio workflow described in Travers '075. A POSA 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 POSA would have been motivated and expected success in combining the teachings of the Travers Publications because each describe aspects of the PacBio workflow. *See also* EX1026 (explaining that the methodologies described in the Travers Publications became commercially available in 2010); EX1002, ¶¶51-63.; Section I.A.

i. Claim 1

Preamble

“A method of sequencing DNA comprising:”

The Travers Publications disclose a method for sequencing double-stranded nucleic acids, including DNA. As Dr. Quackenbush explains, the Travers Publications disclose the “PacBio” 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, 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, 10:63-11:18, 15:29-39; EX1024, 551; EX1002, ¶¶64-66.

Element 1.1

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

The Travers Publications disclose Element 1.1. Patent Owner acknowledged that Travers describes attaching adapters to double stranded polynucleotide molecules. EX1024, 551 (citing EX1018, ¶51). Indeed, Travers discloses 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....”).

Travers further 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 POSA would have understood that this structure (*i.e.*, the SMRTbell template) is within the scope of “partially-complementary, asymmetrical double-stranded adapter-DNA molecules.” *See* Section V. 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); EX1002, ¶¶67-70.

Element 1.2

“wherein the adapters comprise barcodes selected from a plurality of distinct barcode sequences;”

As an initial matter, 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. Accordingly, Patent Owner cannot distinguish over the prior art based on the utility of the barcode. *WesternGeco LLC v. ION Geophysical Corp.*, 889 F.3d 1308, 1323-24 (Fed. Cir. 2018) (citing *Comaper Corp. v. Antec, Inc.*, 596 F.3d 1343, 1348 (Fed. Cir. 2010)) (“It is well established that claims are not limited to preferred embodiments, unless the specification clearly indicates otherwise.”).

Travers teaches Element 1.2. As Travers explains, “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”).

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 POSA would have understood that multiple different barcodes (*i.e.*, a plurality) would sensibly be used to uniquely identify each sample. *E.g.*, *Id.*, ¶101 (describing the use of barcodes to identify “different starting materials” and “different portions of the same original material”).

Moreover, 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. As Dr. Quackenbush explains, a POSA would have understood 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 POSA

would understand that such a sample of sheared *E. coli* genomic DNA comprise a plurality of DNA molecules. EX1002, ¶¶71-75.

Accordingly, a POSA would have understood the Travers Publications to teach selecting barcodes from a plurality of distinct barcode sequences. *See also* EX1002, ¶¶71-76.

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;”

The Travers Publications disclose Element 1.3. Patent Owner acknowledged that Travers describes “amplification of these linked original strands ‘to ensure complete, and preferably redundant sequencing of the entire template.’” EX1024, 551 (citing EX1018, ¶83²); *see also* EX1001, 10:63-11:18.

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

² Patent Owner cites to paragraph 81. EX1024, 551. This appears to be a clerical error as the quoted language appears at paragraph 83.

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. Accordingly, 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. *See also* EX1002, ¶¶77-79.

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”

The Travers Publications disclose Element 1.4. Patent Owner acknowledged that Travers describes “redundant sequencing” and “a single template molecule ... [that] can be sequenced in one integrated process.” EX1024, 551 (quoting EX1018, ¶¶54, 83); *see also* EX1001, 10:63-11:18, 15:29-39. Travers discloses that either SMRTbell templates or replication products therefrom (*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, 102, 122, 141-143, 145-147, 151, claim 13; EX1021, 4-5 (“Generation of a long SMRTbell™ template for sequencing”); EX1002, ¶¶80-82.

Element 1.5

“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;

As explained above with respect to Elements 1.2-1.4, the Travers Publications discloses amplification and sequencing of adapter-DNA molecules comprising barcodes. Patent Owner has also admitted that Travers discloses linking the sense and antisense strands together such that they may be “sequenced in one integrated process.” EX1024, 551. Patent Owner has further characterized Travers method as providing a “consensus sequence of the double stranded molecule by redundant sequencing of the entire single template.” *Id.*; *see also* EX1018, ¶54 (“...providing sense and antisense sequence reads...”), ¶55 (“...replicate sequence read data...”), ¶57 (“...iterative sequence reads...”), ¶62 (“...repeated sequence reads...”).

The Travers Publications disclose Element 1.5. 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). Moreover, Travers expressly discloses confirming that reads derived from the first and second strands are present.

EX1021, Figure 4 (“...an alternating pattern of sense strand, first hairpin, antisense strand and second hairpin alignments,...”), Figure 4; *see also* EX1002, ¶¶83-86.

Element 1.6

- ii) comparing at least one of the confirmed first and second strand sequence reads to a reference sequence; and

Patent Owner has conflated comparing reads to a reference sequence as stated in Element 1.6 with comparing consensus sequence reads to a reference. The was directly addressed by the Examiner in a related continuation application. Specifically, the Examiner found that the specification (which is shared with the ’127 patent) only discusses the alignment of the consensus sequence. EX1025, 138.

Nevertheless, the Travers Publications disclose Element 1.6. For example, 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. 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 ...”). A POSA would have understood that “subreads” refers to the redundant reads derived from the sense and antisense strands. EX1021, Figure 4 (“...there are two subreads that correspond to the sense strand and one to the antisense strand...”). Accordingly, Travers discloses aligning and comparing reads from the sense and antisense strands to the reference sequence. *See also* EX1002, ¶¶87-90.

Element 1.7

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.”

Travers discloses Element 1.7. For example, 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).”). 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.

In summary, the Travers Publications render obvious claim 1. EX1002, ¶¶91-95.

ii. Claim 22

Preamble

“A method of sequencing DNA comprising:”

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; EX1002, ¶¶96-98.; Claim 1 Preamble above.

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;

The Travers Publications disclose Element 22.1. Patent Owner has admitted that Travers describes attaching adapters to double stranded polynucleotide molecules. EX1024, 551 (citing EX1018, ¶51); *see also* EX1001, 10:63-11:18. 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 is 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.

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. As Dr. Quackenbush explains, a POSA would have understood that double-stranded DNA could be obtained from the same sources that Travers identifies as sources for mRNA. Furthermore, as Dr. Quackenbush explains, 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 application of the consensus sequencing method of Travers to genomic DNA 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); EX1002, ¶¶99-102_.

As described above 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. As Dr. Quackenbush explains, a POSA 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; *see also* EX1002, ¶¶99-104.

Element 22.2

b) amplifying strands from a plurality of the double-stranded adapter-DNA molecules in the library to produce strand copies;

As disclosed above with respect to Element 1.3, Patent Owner acknowledged that Travers describes “amplification of [] linked original strands ‘to

ensure complete, and preferably redundant sequencing of the entire template.”

EX1024, 551 (citing EX1018, ¶83); *see also* EX1001, 10:63-11:18. Travers further 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; EX1002, ¶¶105-107.

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

The Travers Publications disclose Element 22.3. As explained above with respect to Element 1.4, Patent Owner acknowledged that Travers describes “redundant sequencing” and “a single template molecule ... [that] can be sequenced in one integrated process.” EX1024, 551 (quoting EX1018, ¶¶54, 83). 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”); EX1002, ¶¶108-110.

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;

The Travers Publications teach Element 22.4. 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 I.A.ii.

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”). Further, 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.

Furthermore, Travers 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 [the disclosed] template dependent sequencing process.” EX1018, ¶122; *see* Element 1.3 above. A POSA 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; EX1002, ¶¶111-15.

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;

The Travers Publications teach Element 22.5. Based on the '127 patent, a POSA would have understood “collapsing” to be the process by which consensus sequence information is drawn from redundant sequence reads derived from the

same molecule. *E.g.*, EX1001, 21:35-38 (“Reads having common (i.e., identical) SMI sequences were grouped together, and were collapsed to generate a consensus read.”). The Travers Publications disclose such a process.

[T]he 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.”); EX1002, ¶¶116-19.

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.

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.

The Travers Publications also teach Element 22.6. For example, 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.

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”). Thus, a skilled artisan would understand that Travers discloses comparing the consensus sequence to a reference sequence. POSA would also have 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...”).

In summary, the Travers Publications render obvious claim 22. EX1002, ¶¶120-25.

iii. Claim 2

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

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.

As explained above, Patent Owner has characterized the Travers method as providing a “consensus sequence of the double stranded molecule by redundant sequencing of the entire single template.” EX1024, 551. 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.

As Dr. Quackenbush explains, a POSA would have understood “consensus” to refer to sequence information that is supported by most of the redundant reads. A POSA would further have understood 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 POSA would have understood that processing errors and true variants are identified during the comparison process used to generate a consensus sequence, as is discussed by Travers. EX1002, ¶¶126-28.

A POSA 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. Moreover, EX1020 (incorporated by reference into Travers '075) directly acknowledges that the Travers' consensus sequencing identifies and removes sporadic errors derived from the sequencing process (*i.e.*, processing errors). *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[en]t 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...”); EX1002, ¶¶129-30.

Accordingly, a POSA would find claim 2 obvious in view of the Travers Publications. See also EX1002, ¶¶126-31.

iv. Claim 3 and 16

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 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.

Regardless of the lack of support in the '127 patent, 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. Patent Owner acknowledged that the sense and antisense strands of the original template molecule are linked together and sequenced in one integrated process. EX1024, 551; EX1001, 10:63-11:18. 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.

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.

Accordingly, a POSA would understand Travers to disclose associating the strands of an original molecule using an adapter sequence or original strand information as required by claims 3 and 16. *See also* EX1002, ¶¶132-36.

v. Claims 4 and 8

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.

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.

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. As Dr. Quackenbush explains, a POSA 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 (“...a sequencing read produces multiple observations of each base, and these multiple observations can then be used to generate high-accuracy consensus sequence”), 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).

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, ...”); EX1021, 6-7 (describing identification of A and T alleles at a particular position).

Accordingly, the Travers Publications teaches the limitations of claims 4 and 8. *See also* EX1002, ¶¶137-41.

vi. Claims 5-7

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 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.

The Travers Publications disclose or teach the additional subject matter recited in claims 5-7. For example, 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, ...”).

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 POSA would have understood that “variants” in consensus sequences are determined relative to a reference sequence.

With respect to claim 7, the ’127 patent does not describe identifying a structural rearrangement or an insertion using DCS. To the extent Patent Owner argues that these were art known types of variation or mutation that a POSA would have been motivated to detect using DCS — that is, it would have been obvious to apply DCS to detect such mutations — this fails to establish written description support. *Research Corp. Techs. v. Microsoft Corp.*, 627 F.3d 859, 870 (Fed. Cir.

2010) (“Entitlement to a filing date extends only to subject matter that is disclosed; not to that which is obvious.”). Based on this same logic, a POSA would have been motivated to apply the method described by Travers to detect such mutations.

EX1002, ¶¶142-44.

Travers expressly discloses that single nucleotide variants may be identified with its methods. EX1018, ¶143. Moreover, a POSA would have understood the terms “transition” and “transversion” as simply types of single nucleotide variants. Similarly, a POSA would have understood 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 POSA 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); EX1002, ¶¶145-47.

Accordingly, a POSA would understand the Travers Publications disclose or teach the additional limitations recited in claims 5-7. EX1002, ¶¶142-47.

vii. Claim 9

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.

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. As Dr. Quackenbush explains, a POSA would have understood 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.”). EX1002, ¶149.

Accordingly, Travers teaches the limitations of claim 9. *See also* EX1002, ¶¶148-50.

viii. Claim 10

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.

Patent Owner acknowledged that Travers describes “amplification of these linked original strands ‘to ensure complete, and preferably redundant sequencing of the entire template.’” EX1024, 551 (citing EX1018, ¶83). The ’127 further patent acknowledges that Travers discloses “rolling circle amplification.” EX1001, 11:3-17. Moreover, 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; *see also* EX1002, ¶¶151-54.

ix. Claim 12 and 21

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 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.

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.

With respect to claim 21, Travers discloses that sample DNA may be divided into different portions, such as “different size-selected portions,” and that 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.

Accordingly, claims 12 and 21 are disclosed or taught by the Travers Publications. EX1002, ¶¶155-59.

x. Claim 15

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.

Claim 15 specifies that the biological source or bodily sample of claims 1 and 22, respectively, comprise tissue. This is a feature of the source of the DNA sample used in the method, not the method itself. Such sources were well known in the art and are disclosed by the Travers Publications. For example, 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. As Dr. Quackenbush explains, a POSA would have understood 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. *See also* EX1026, 2 (describing the applicability of the Travers method to detection of “structural rearrangements” in the genome of tumor cells). EX1002, ¶¶161-62.

Accordingly, claim 15 is obvious. EX1002, ¶¶160-63.

xi. Claim 17

17. The method of claim 1, wherein the double-stranded DNA fragments were at least partially generated by nuclease cleavage.

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); *see also* EX1002, ¶¶164-66.

xii. Claim 18

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.

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.

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.

Accordingly, claim 18 is obvious in view of the Travers Publications. *See also* EX1002, ¶¶167-70.

xiii. Claim 19

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.

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; *see also* EX1002, ¶¶171-73.

xiv. Claims 20, 27, and 28

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.

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.

Patent Owner has conflated comparing reads to a reference sequence as recited in Element 1.6 with comparing consensus sequence reads to a reference. The was directly addressed by the Examiner in a related continuation application. Specifically, the Examiner found that the specification (which is shared with the '127 patent) only discusses the alignment of the consensus sequence. EX1025, 138.

Nevertheless, 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 POSA would have understood 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. EX1002, ¶¶174-76.

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. As Dr. Quackenbush explains, a POSA would have been motivated to enrich single-stranded concatemer molecules for sequences of interest prior to sequencing. *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 POSA 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. EX1018, ¶124; EX1002, ¶¶176-77.

Accordingly, claim 20, 27, and 28 are obvious in view of the Travers Publications. *See also* EX1002, ¶¶174-78.

xv. Claims 23-25

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 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.

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. Accordingly, Patent Owner cannot distinguish the Travers Publications based on the source of the double-stranded DNA fragments.

Travers discloses that the double-stranded DNA may be obtained from a bodily sample. EX1018, ¶101 (“...different samples (cells, cell cultures, patients, etc.) ...”); *see also id.*, ¶¶5, 102. Travers further discloses that the bodily sample may comprise tissue as required by claim 23. For example, Travers 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. As Dr. Quackenbush explains, a POSA would have understood that double-stranded DNA could be obtained from the same sources that Travers identifies as sources for mRNA. EX1002, ¶¶180-81. 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.

With respect to claim 24 and 25, a POSA 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).

Accordingly, the Travers Publications renders each of claims 23-25 obvious. EX1002, ¶¶179-182.

xvi. Claim 26

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.

Patent Owner cannot distinguish the prior art from the claims based on the recited “non-unique tagging” scheme. 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, 19:20-25 (“the use of SMI tags...allows every molecule to be *uniquely* labeled”); *see also id.*, 6:67-7:4, 16:11-15, 22:44-46, 23:2-3, 23:8-10, 28:51-55, 27:45-47; EX1002, ¶183.

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. As Dr. Quackenbush explains, a POSA would have understood 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 POSA would have understood that the molecules are non-uniquely tagged within the meaning of claim 26. EX1002, ¶184.

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.

Accordingly, claim 26 is obvious in view of the Travers Publications. *See also* EX1002, ¶¶183-86.

B. [Ground 2] Claim 11 is unpatentable as obvious under 35 U.S.C. §103 over 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.

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.

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).

A POSA 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 POSA would have been motivated to repair these damaged bases using enzymes such as with the PreCR™ Repair Mix described in NEB Expressions. A POSA 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.

Accordingly, the Travers Publications in further view of NEB Expressions renders obvious claim 11. EX1002, ¶¶187-90.

C. [Ground 3] Claims 13, 14, 29, and 30 are unpatentable as obvious under 35 U.S.C. §103 over the Travers Publications in view of McCloskey

i. 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.

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.

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. As Dr. Quackenbush explains, a POSA would have understood Travers to teach using barcodes to distinguish molecules of the sample mixture. A POSA 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. 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. EX1002, ¶¶191-93, 198-200.

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.

McCloskey discloses “the present invention permits identification of valid sequences, and distinguishes the valid sequences from contaminants and redundant sequences.” *Id.* 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. *Id.* (“... ‘random barcode’ refers to an arbitrary sequence that can uniquely identify a target nucleic acid ...”). 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.*; EX1002, ¶¶194-95.

McCloskey discloses that its random barcodes may be included in hairpin adapters. EX1023, Figure 3, ¶15. McCloskey further discloses linkers that are ligated to genomic DNA fragments prior to amplification. EX1023, ¶5; *see also id.*, ¶¶10, 15. McCloskey discloses that the barcodes are in the single-stranded portion of the adapter. EX1023, Figure 3.

A POSA 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. A POSA would have expected success in using the random barcodes described by McCloskey because methods for making such oligonucleotides were well-known in the art. EX1021, ¶21; *see also* EX1002, ¶¶195-97.

Accordingly, the Travers Publications in further view of McCloskey renders obvious claims 13 and 14. EX1002, ¶¶191-200.

ii. 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.

Patent Owner cannot distinguish over the Travers Publications based on any established criticality of the barcode lengths recited in claims 29 and 30. The specification of the '127 patent does not exemplify, or otherwise indicate, that the recited lengths of barcodes are preferable relative to the other disclosed lengths of barcodes. *E.g.*, EX1001, 7:4-10 (“...may be between approximately 3 to 20

nucleotides in length. ...may be approximately 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 nucleotides in length. ...is 12 nucleotides in length.”).

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.* As Dr. Quackenbush explains, a POSA would have understood based on McCloskey that the length of the barcode is chosen such that there are sufficient barcodes to uniquely identify each target DNA molecule. EX1002, ¶¶201-202.

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 POSA would have been motivated to follow the guidance in McCloskey and would have chosen a 6-nucleotide barcode, as required by claim 29, 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. EX1002, ¶203.

With respect to claim 30, McCloskey discloses specific species within the claimed range and ranges that overlap with the claimed range. *In re Peterson*, 315 F.3d 1325, 1329 (Fed. Cir. 2003) (“A *prima facie* case of obviousness typically exists when the ranges of a claimed composition overlap the ranges disclosed in the prior art.”); *E.I. du Pont de Nemours & Co. v. Synvina C.V.*, 904 F.3d 996, 1006 (Fed. Cir. 2018).

Accordingly, the Travers Publications in view of McCloskey renders obvious each of claims 29 and 30. EX1002, ¶¶201-204.

VII. THE SAME OR SUBSTANTIALLY THE SAME ARGUMENTS HAVE NOT BEEN PREVIOUSLY PRESENTED

Discretionary denial of institution under 35 U.S.C. §325(d) is not appropriate here. McCloskey and NEB Expressions were not previously presented to the Office. Travers '075 and the Travers Paper were included in an IDS together with over a hundred references, but no apparent substantive prior art analysis was applied by the Examiner. *See, e.g., Vudu, Inc. v. IndeaHub, Inc.*, IPR2020-01689, Paper 16, 18-20 (§325(d) not applied where asserted references were cited repeatedly in the body of the patent and considered in IDS but never applied in a rejection); *Dish Network L.L.C., v. Sound View Innovations, LLC*, IPR2020-01041, Paper 13, 16-22 (similar); *Cellco P'ship D/B/A Verizon Wireless v. Huawei Device Co., Ltd.*, IPR2020-01117, Paper 10, 12-15; *Draftkings Inc. v. Interactive Games*

LLC, IPR2020-01107, Paper 10, 15-16 (examiner erred by overlooking relevant teachings in reference listed on IDS).

Moreover, the Examiner here did not have the benefit of Dr. Quackenbush's testimony and there was no apparent consideration of Patent Owner's admissions regarding Travers '075 (discussed above) in a related case (Application No. 16/118,306) before a different Examiner. *Mylan Pharmaceuticals Inc. v. Sanofi-Aventis Deutschland GMBH*, IPR2017-01528, Paper 12, 6-9 (declining to exercise discretionary denial in view of inconsistent action before a different Examiner during prosecution of a related application). The prosecution history of the 16/118,306 case was not cited by the Examiner in this case or submitted in an IDS. Furthermore, the materials presented here demonstrate material error in that the '127 patent should never have issued.

Limited activity during *ex parte* prosecution indicates the prior examination was cursory at best. See Section I.C. The Examiner never made comment, let alone any rejection, based on the Travers publications or a comparable consensus sequencing reference. *Advanced Bionics, LLC v. MED-EL Elektromedizinische Geräte GmbH*, IPR2019-01469, Paper 6, 10 (explaining "if the record of the Office's previous consideration of the art is not well developed or silent, then a petitioner may show the Office erred"). Furthermore, the Examiner erred when she stated that "the ability to label each strand of a target with asymmetric adaptors

that enable strand specific labeling” was not found in the art. EX1004, 432-433. As explained above, adapters that enable strand specific labeling were known in the art, widely used, and even commercially available as of the earliest claimed filing date of the ’127 patent—this is acknowledged in the ’127 patent. *See* Section I.B. EX1001, 10:63-11:2 (Travers’ hairpin adapters “adequate for use in the assay.”).

Accordingly, the issuance of the ’127 patent was substantive error.

VIII. THERE ARE NO *FINTIV* ISSUES

The parties appear to be in agreement that the present petition does not implicate the Board’s discretion according to *Apple Inc. v. Fintiv, Inc.*, IPR2020-00019, Paper 11 (PTAB Mar. 20, 2020 (precedential)). Patent Owner recently filed two petitions (IPR2022-00746, -00747) challenging patents at issue in the same district court proceeding in which it argued that there are no *Fintiv* issues. The corresponding district court case is at a very early stage and is not currently assigned to a judge.

Indeed, the “Fintiv factors” favor institution here. The potential of a stay exists in the district court case if this proceeding is instituted, as Petitioner intends to request a stay if the IPR is instituted. The co-pending district court litigation is in its early stages. The trial date is uncertain, and the proximity to the projected statutory deadline for a final written decision is highly speculative. The parties filed infringement contentions one week ago. The parties have not exchanged

invalidity contentions, have not briefed claim construction, have not filed dispositive motions, and have not begun expert discovery. Expert reports and dispositive motions will not have come due by the time an institution decision has been rendered. Furthermore, Petitioner has diligently prepared the IPR petitions well in advance of the 12-month statutory deadline. Thus, there will be no significant investment of resources wasted upon the Board’s institution. Also, despite Petitioner being the defendant in the litigation—something out of Petitioner’s control—other circumstances weigh against discretionary denial. The present challenges are strong on the merits and there is a significant public interest against “leaving bad patents enforceable.” *Thryv, Inc v. Click-To-Call Techs., LP*, 140 S. Ct. 1367, 1374 (2020). Moreover, Petitioner is the sole party to challenge this patent before the Board.

IX. CONCLUSION

For the reasons set forth above, claims 1-30 of the ’127 patent are unpatentable. Petitioners therefore request that a *inter partes* review of these claims be instituted.

Respectfully submitted,

Dated: April 8, 2022

/ Michael T. Rosato /
Michael T. Rosato, Lead Counsel
Reg. No. 52,182

X. CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. §42.24(d), the undersigned certifies that this Petition complies with the type-volume limitation of 37 C.F.R. §42.24(a). The word count application of the word processing program used to prepare this Petition indicates that the Petition contains 13,927 words, excluding the parts of the brief exempted by 37 C.F.R. §42.24(a).

Respectfully submitted,

Dated: April 8, 2022

/ Michael T. Rosato /
Michael T. Rosato, Lead Counsel
Reg. No. 52,182

XI. PAYMENT OF FEES UNDER 37 C.F.R. §§42.15(A) AND 42.103

The required fees are submitted herewith. If any additional fees are due at any time during this proceeding, the Office is authorized to charge such fees to Deposit Account No. 23-2415.

XII. APPENDIX – LIST OF EXHIBITS

Exhibit No.	Description
1001	U.S. Patent No. 10,760,127 to Salk et al.
1002	Declaration of Dr. John Quackenbush
1003	<i>Curriculum Vitae</i> of John Quackenbush, Ph.D.
1004	File History for Application No. 16/503,382
1005	RESERVED
1006	Kinde et al., “Detection and quantification of rare mutations with massively parallel sequencing,” <i>PNAS</i> 108(23) (2011)
1007	RESERVED
1008	RESERVED
1009	U.S. Patent Publication No. 2012/0071331 to Casbon et al.
1010	Shiroguchi et al., “Digital RNA sequencing minimizes sequence-dependent bias and amplification noise with optimized single-molecule barcodes,” <i>PNAS</i> 109(4) (2012)
1011	RESERVED
1012	Illumina, TruSeq RNA and DNA Sample Preparation Kits, Data Sheet: Illumina Sequencing (2010)
1013	Bentley et al., “Accurate whole human genome sequencing using reversible terminator chemistry,” <i>Nature</i> 456 (2008)
1014	Evans et al., “NEB Expressions” New England BioLabs (2007)
1015	NEB Expressions, New England BioLabs, https://web.archive.org/web/20080321144426/http://www.neb.com/nebecomm/tech_reference/neb_transcripts.asp (Feb. 23, 2022)

1016	Affidavit of Nathaniel E. Frank-White
1017	Nisha et al., “Antioxidants and Their Protective Action Against DNA Damage,” <i>International Journal of Pharmacology and Pharmaceutical Sciences</i> 3 (2011)
1018	U.S. Patent Publication No. 2009/0298075 to Travers et al.
1019	RESERVED
1020	U.S. Patent No. 7,476,503 to Turner et al.
1021	Travers et al., “A flexible and efficient template format for circular consensus sequencing and SNP detection,” <i>Nucleic Acids Research</i> 38(15) (2010)
1022	International Publication No. WO 2012/142213 to Vogelstein et al.
1023	U.S. Patent Publication No. 2007/0020640 to McCloskey et al.
1024	File History for Application No. 16/118,306
1025	File History for Application No. 17/392,185
1026	McCarthy, “Third Generation DNA Sequencing: Pacific Biosciences’ Single Molecule Real Time Technology,” <i>Chemistry & Biology</i> 17 (2010)
1027	Song et al., “Sensitive and specific single-molecule sequencing of 5-hydroxymethylcytosine,” <i>Nature Methods</i> 9(1) (2012)
1028	Hodges et al., “Genome-wide <i>in situ</i> exon capture for selective resequencing,” <i>Nature Genetics</i> 39(12) (2007)
1029	RESERVED
1030	Clark et al., “Direct Detection and Sequencing of Damaged DNA Bases,” <i>Genome Integrity</i> 2 (2011)
1031	Casbon et al., “A method for counting PCR template molecules with application to next-generation sequencing,” <i>Nucleic Acids Research</i> 39(12)(2011)

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §§42.6(e) and 42.105(a), this is to certify that I caused to be served a true and correct copy of the foregoing Petition for Inter Partes Review of U.S. Patent No. 10,760,127 (and accompanying Exhibits 1001-1031) by overnight courier (Federal Express or UPS), on this 8th day of April, 2022, on the Patent Owner at the correspondence address of the Patent Owner as follows:

Perkins Coie LLP – University of Washington
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Seattle WA 98111-1247

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4311 11th Ave NE
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Respectfully submitted,

Dated: April 8, 2022

/ Michael T. Rosato /

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