

Paper No. _____
Filed: April 13, 2023

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

PETITIONER'S REPLY
U.S. PATENT NO. 10,760,127

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I. INTRODUCTION

The petition established that the consensus sequencing method developed by Pacific Biosciences and described in the Travers Publications is plainly encompassed by the challenged claims, as expressly indicated by both the claims and specification of the '127 patent itself. *See, e.g.*, DI, 8, 15-19. The petition further established that Patent Owner ("PO") acknowledged—within the '127 patent and before the Examiner—that the Travers Publications teach amplification of templates prior to sequencing as required by the claims. PO again admits as much here, repeatedly acknowledging Travers disclosure of amplification prior to sequencing—which PO refers to as "Travers-'075's rolling circle replication embodiment."

PO never grapples with its admissions regarding the scope and content of the Travers Publications, or that the '127 patent critically relies on Travers to claim the very subject matter PO now frivolously argues is not enabled. PO even acknowledges that its claims encompass rolling circle replication of templates prior to sequencing as Travers describes. PO also does not dispute the claims encompass Travers' adapter-DNA template molecules, acknowledges Travers' adapters comprise barcodes, and does not dispute Travers' barcodes are "selected from a plurality of distinct barcode sequences." That is, PO either acknowledges or does not dispute issues sufficient to find claims 1-30 unpatentable.

PO's admissions and concessions are fatal to the claims of the '127 patent and cannot be overcome by PO confining its discussion to non-limiting embodiments (Y-shaped adapters, PCR amplification, Illumina sequencing) that even PO's expert concedes do not limit the '127 patent claims. Nor can patentability be shown with PO's frivolous allegations that Travers rolling circle replication embodiment is inoperable. Finally, PO's evidence of secondary considerations lack nexus at least because Duplex Consensus Sequencing (DCS) is not required by the '127 patent claims.

II. THE TRAVERS PUBLICATIONS DISCLOSE EACH AND EVERY ELEMENT OF CLAIMS 1-30

PO does not dispute that its claims encompass Travers' U-shaped linkers, rolling circle amplification of templates, and broadly include sequencing using the Pacific Biosciences sequencing platform. Nor does PO dispute the Travers Publications discuss the same sequencing methodology and expressly disclose template amplification prior to sequencing, which PO (18) refers to as "Travers-'075's rolling circle replication embodiment." Nor could PO dispute any of this in view of the '127 patent claims and specification, which plainly confirm as much and even critically rely on Travers for descriptive support. *See, e.g.*, EX1001, claims 10, 13, 10:61-11:18; *see also* EX1039, ¶¶1-5; DI, 8.

PO, however, inconsistently argues the Travers Publications fail to disclose “first and second strand sequence reads,” “grouping,” and distinguishing strands based on adapter-DNA asymmetry. But none of its arguments are commensurate with the scope of the claims, the specification, or the scope and content of the prior art. PO’s arguments lack merit—particularly so in view of its contrary admissions and concessions. EX1039, ¶¶6-24.

A. Claims 1-30: amplification followed by sequencing

PO argues (5-8) that the ’127 patent claims recite an amplification step that is followed by sequencing. Such an interpretation of claims 1-30 fails to distinguish the Travers Publications which expressly disclose an embodiment where adapters are attached to a template molecule, followed by amplification then sequencing. *E.g.*, EX1018, ¶122; *see also* DI, 17-18. PO has repeatedly admitted this prior art disclosure in the Travers Publications. *E.g.*, EX1001, 10:63-11:18; EX1024, 550-551; *see also* DI, 8; EX1039, ¶2.

PO falsely suggests (29-31) that Travers’ rolling circle embodiment is inoperable. But PO acknowledges the Travers Publications disclose such an embodiment and that same embodiment is encompassed by the ’127 patent claims. EX2014, ¶214; EX1037, 94:24-95:15 (confirming claims encompass but are not limited to Travers’ amplification embodiment); *see also* EX1039, ¶2. That is, PO’s claims do not “distinguish from the alleged inoperative subject matter in

[Travers].” *Ex Parte Coyle*, No. 2020-004797, 2021 Pat. App. LEXIS 2416, at *12 (P.T.A.B. April 29, 2021) (citing *In re Crosby*, 157 F.2d 198 (C.C.P.A. 1946)).

Nor could PO dispute Travers’ disclosure—the ’127 patent acknowledges it and even critically relies on Travers to support claims 1-30. The ’127 patent specifically acknowledges that Travers 2010 discloses amplification followed by sequencing, and that such amplification was a recognized step in the Pacific Biosciences workflow.

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. ...

Additionally, closed circles may be pre-amplified using rolling circle amplification or serve as the substrate for continuous loop sequencing [49].

EX1001, 10:63-11:18; *see also* EX1024, 550-551; EX1037, 109:19-110:23 (confirming disclosure in ’127 patent), 153:21-154:8 (describing the ’127 patent inventors as credible scientists and POSAs); DI, 8; EX1039, ¶3. *Compare with* EX1018, ¶¶80, 122.

PO's "admissions about the scope and content of [Travers] provide a factual foundation as to what a skilled artisan would have known at the time of invention." *Qualcomm Inc. v. Apple Inc.*, 24 F.4th 1367, 1376 (Fed. Cir. 2022)). Furthermore, PO's admissions are confirmed by the Travers Publications which disclose amplifying adapter-DNA molecules as part of sequencing. Travers 2010 describes amplifying DNA molecules having ligated U-shaped linkers. For instance, Figure 3 shows products (i.e., concatemers) resulting from rolling circle replication of a circular SMRTbell templates. EX1021, Figure 3; *see also id.*, 4. Travers '075 expressly discusses amplification prior to sequencing, which the Board has already agreed and PO repeatedly acknowledges. EX1018, ¶122; *see also* EX1020, 24:36-44 (discussing sequence redundancy in the context of concatemer molecules); DI, 13-14 (quoting EX1018, ¶122); EX1039, ¶4.

Despite PO's claim construction, there does not appear to be any dispute that the Travers Publications describe amplifying adapter-DNA molecules prior to sequencing. Nor does PO dispute that the '127 patent claims encompass Travers' U-shaped adapters, amplifying adapter-DNA molecules (e.g., rolling circle amplification), and sequencing. EX1039, ¶5.

PO identifies no disclosure in the '127 patent regarding rolling circle replication and sequencing that is not also found in Travers. *Compare* EX2014, ¶¶214-215; EX1037, 95:10-100:8 *with* EX1018, ¶¶80, 122. Accordingly, PO's

inoperability arguments should not be entertained. *See In re Paulsen*, 30 F.3d 1475, 1481 n.9 (Fed. Cir. 1994) (noting the specification would not be enabling if held to the same standard urged for the reference); *SRI Int’l, Inc. v. Internet Sec. Sys.*, 511 F.3d 1186, 1192-94 (Fed. Cir. 2008) (same).

B. Claims 1-21: “first and second strand sequence reads”

PO argues (11-13) the Travers Publications do not teach first strand sequence reads and second strand sequence reads, as recited in claims 1-21.¹ PO’s argument fails for at least the reasons discussed below. EX1039, ¶6.

PO’s argument regarding “first and second strand sequence reads” is not commensurate with the language of the claims or guidance in the ’127 patent specification. As confirmed by Dr. Quackenbush, claim 1 plainly encompasses use of U-shaped adapters, rolling circle amplification, and broadly encompasses sequencing on the Pacific Biosciences sequencing platform. *See* EX1001, claims 10, 13. The specification specifically instructs use of Travers’ adapters, amplification, and continuous loop sequencing as suitable for use with the disclosed methods. EX1001, 10:63-11:15, 15:29-39; *see also* EX2014, ¶214; EX1037, 94:24-95:15; EX1039, ¶7.

¹ PO does not dispute that “strand sequence reads,” as required by claims 22-30 is described by the Travers Publications.

PO argues (8-9) that the language of claim 1 “indicates”—but does not require—“multiple, distinct sequencing reactions, each of which results in a sequence read.” However, PO does not, and cannot, identify language in claim 1 reciting multiple, distinct sequencing reactions or any definition in the specification expressly limiting a “sequence read” in such a manner because there is no such disclosure. *See* EX1039, ¶8; *cf.* EX1001, 10:63-11:15, 15:33-39 (“Pacific Biosciences sequencing platform”).

Like much of the POR, PO’s analysis (8-9) is limited to discussion of non-limiting embodiments utilizing Y-shaped adapters, PCR amplification, and sequencing on the Illumina sequencing platform. But Dr. Satija conceded the claims are not limited to those embodiments. EX1037, 26:9-17, 76:10-14, 77:14-17; *see also* EX1039, ¶9.

PO’s remaining argument (9-10) amounts to an observation of no moment—Travers discusses distinguishing between a longer raw sequence read of the concatemer and discrete sequence reads corresponding to the sense and antisense strand. This argument fails for at least two reasons. First, as explained by Dr. Quackenbush, the “subreads” corresponding to the sense and antisense strand described by Travers 2010 meet PO’s own interpretation of a sequence read. POR, 9 (citing EX2014, ¶60). Dr. Satija states “[a] POSA reading [claim 1] would understand that a ‘sequence read’ refers to a contiguous set of DNA nucleotides

that correspond to the output of a DNA sequencing reaction...” EX2014, ¶60. The so-called “subreads” of Travers 2010 are a contiguous set of DNA nucleotides” and clearly “correspond” to output of the sequencing process performed on the Pacific Biosciences sequencing platform. For example, Figure 4 of Travers 2010 (EX1021) depicts an example of sequence data generated for a template molecule. Read data is demarcated and colored to identify each respective contiguous set of DNA nucleotides corresponding to the sense and antisense strands, and such data corresponds to the output of a DNA sequencing reaction. EX1039, ¶¶10, 11. PO does not argue otherwise.

Second, PO’s limited linguistic argument focuses only on Travers 2010’s use of the term “subread” but ignores that Travers ’075 refers to the sense and antisense reads as “sequence reads.” EX1018, ¶61 (“[A] consensus is first established for one sequence read, e.g., a sense strand, and another consensus sequence is established for another read, e.g., the an[ti]sense strand.”); *see also id.*, ¶¶55, 62. This further illustrates that a POSA would recognize the sense and antisense reads discussed in the Travers Publications are sequence reads as ordinarily understood by those in the art. EX1039, ¶12.

In summary, PO’s argument regarding “first and second strand sequence reads” is inconsistent with the language of the claims and guidance in the specification. The Travers Publications demonstrate disclosure of sequence reads

corresponding to the sense and antisense strands that meets the requirements of claims 1-21. EX1002, ¶¶80-82; EX1039, ¶13.

C. Claims 22-30: “grouping the strand sequence reads”

PO falsely argues (13-15) the Travers Publications do not describe “grouping the strand sequence reads into families based on i) the barcode sequence, and ii) DNA fragment-specific information” as required by claims 22-30. EX1001, claim 22. Again, PO does not dispute the ’127 patent claims encompass Travers U-shaped adapters or that those adapters contain barcodes. Nor does PO dispute that Travers’ barcodes are “selected from a plurality of distinct barcode sequences” as recited in either claims 1 or 22. DI, 9. PO argues the Travers Publications, while disclosing barcoded adapters, do not specifically utilize the barcodes for grouping as recited in claim 22.² This argument is not commensurate with the language of the ’127 patent claims or the guidance in the specification. EX1039, ¶14.

Claims 22-30 of the ’127 patent generically recite “barcode.” But PO’s argument ignores the broad language of the claims and draws distinction between sample barcodes (also known as index or multiplex barcodes) and molecular or

² A tacit admission that claim 1 does not require grouping reads into families for determining a consensus sequence. *See also* EX1037, 80:7-10.

fragment-specific barcodes. EX1037, 92:23-93:13 (acknowledging the '127 patent discloses multiple different types of barcode). Claims 22-30 do not recite “molecular” or “fragment-specific” barcodes. The claims simply recite “barcode.” In fact, the only usage of the term “barcode” in the specification of the '127 patent is in connection with an index barcode. EX1001, 20:34-39 (“where XXXXXX indicates the position of a fixed multiplexing barcode”); *see also* EX1037, 59:8-16 (arguing a nucleotide sequence can be considered a barcode even when not used for identification purposes), 90:25-91:13 (confirming the '127 patent only mentions “barcode” once); EX1039, ¶¶15, 16.

The '127 patent does not use the term “molecular barcode” and neither do the claims. Claim 22 generically recites “barcodes” and requires grouping based on “the barcode sequence.” EX1037, 86:15-87:10 (confirming “barcode” broadly includes both molecular barcodes and index barcodes). Travers discloses such a grouping. For example, Travers describes including barcodes in template molecules such that sequence data may be “pooled for analysis.” EX1018, ¶100; *see also* EX1037, 86:4-10 (“The important thing is that those indexing barcodes enable material from different sources to be pooled together and then effectively indexed after the sequencing data is collected so that each molecule can be returned back or grouped together with molecules from the same original source”); EX1002, ¶¶99-104; EX1039, ¶17.

Travers discloses redundantly sequencing both strands of DNA molecules. EX1018, Abstract, ¶58, Figure 3B; EX1021, Abstract, Figure 1. Travers further describes “assembling the sequence data from multiple reads of a given sequence ... to [] provide an overall consensus sequence.” EX1018, ¶58. Where multiplexing is employed, distinguishing sequence reads based on sample origin (index or multiplex barcodes) is a bioinformatics component to Travers’ process of assembling the sequence data from multiple reads to determine consensus sequence. EX1002, ¶¶111-115; EX1039, ¶18.

Accordingly, Travers expressly describes determining consensus sequences based on assembled (e.g., grouped) redundant sequence reads as required by claims 22-30. Certainly, when multiplexing, sequence reads are assembled or grouped based both on barcode sequence information and DNA fragment-specific information in the manner recited in claims 22-30. EX1039, ¶19.

D. Claim 19: “asymmetrical double-stranded adapter-DNA molecules”

PO argues (15-18) that the Travers Publications do not teach distinguishing first and second strand sequence reads as recited in claim 19 “using the asymmetric nature of the adapters.” This argument fails at least because it is not commensurate with the language of claim 19. EX1039, ¶20.

PO's misplaced argument (15-18) is directed only to the adapter. *E.g.*, EX2014, ¶¶89, 93-94 (“using the asymmetric nature of the adapters”). But the plain language of claim 19 recites distinguishing first and second strand sequence reads based on the “asymmetry of the asymmetrical double-stranded adapter-DNA molecules.” Even the portion of the specification PO identifies as supporting its arguments expressly states that it is the “asymmetric nature of adapted fragments” that allows the strands to be distinguished. POR, 16 (quoting EX1001, 3:64-4:3); *see also* EX1039, ¶21.

As was explained by Dr. Quackenbush in his first declaration, the U-shaped adapters of Travers are asymmetric, are encompassed by the '127 patent claims, and generate asymmetric double-stranded adapter-DNA molecules. *E.g.*, EX1002, ¶¶49-52, 69. Independent claim 1 requires attaching adapters to the DNA molecules to make “partially-complementary, asymmetrical double-stranded adapter-DNA molecules.” Accordingly, the claims would be reasonably understood to encompass adapter-DNA molecules generated by attachment of Travers' U-shaped adapters comprising barcodes to each end of double-stranded DNA molecules. PO does not dispute any of this, nor could it. EX1001, 10:63-11:2; *see also* DI, 8; EX1039, ¶22.

Indeed, PO affirmatively argues that Travers 2010 describes asymmetry associated with its adapter-DNA molecules. Dr. Satija points to disclosure in

Travers 2010 of using two different hairpin adapters such that the “circular DNA template [has] one and only one sequencing primer binding site.” EX2014, ¶129; *see also* EX1021, 2; EX1018, ¶92 (“asymmetric linking oligonucleotides”); DI, 12-13 (quoting EX1018, ¶¶50, 55); EX1039, ¶23. That is, Dr. Satija argues, rather than disputes, that Travers discloses adapter-DNA molecules that are asymmetric.

Nor does PO dispute Travers’ use of the U-shaped linkers and corresponding asymmetric molecules in the sequencing process to distinguish sequence reads corresponding to the sense and antisense strands. PO argues Travers describes using adapter sequences as landmarks or “‘registration sequences’ that mark the portion of the sequence read where one strand ends and the next strand begins.” POR, 17 (quoting EX1018, ¶54). This argument offers no distinction from what is claimed. That is, PO acknowledges Travers describes asymmetric adapter-DNA molecules and that Travers uses adapter structure (e.g., position and sequence of the two adapters) to distinguish sequence reads for each of the two strands of an original DNA molecule. EX1037, 114:1-10 (confirming registration sequences in ligated adapters distinguish sense and antisense sequence reads). The disclosure of the Travers Publications meets the requirements of claim 19. PO fails to demonstrate otherwise. EX1039, ¶24.

E. Claims 13, 14, 29, and 30: “degenerate” barcodes and barcode length

PO argues (65-67) that a POSA would not have used McCloskey’s barcodes in in Travers’s adapters because McCloskey’s barcodes are “designed and used exclusively for amplicon products that are separate molecules (e.g., PCR products).” PO relies solely on Dr. Satija for this incredible assertion who, in turn, identifies no corroborating evidence. *See* EX2014, ¶221; *cf.* 37 C.F.R. §42.65(a). In any event, Dr. Satija is wrong as established by express disclosure in McCloskey. *E.g.*, EX1023, Title (“Molecular Encoding of Nucleic Acid Templates For PCR and Other Forms of Sequence Analysis”), Abstract, ¶¶2, 7.

As the explained in the petition (64), a POSA would have been motivated with an expectation of success to use McCloskey’s barcodes in Travers’ adapters to facilitate identification of unique double-stranded molecules. EX1018, ¶100; EX1023, ¶21; EX1002, ¶¶195-97. PO does not credibly argue otherwise.

III. PATENT OWNER’S FRIVOLOUS INOPERABILITY ARGUMENTS SHOULD BE REJECTED

Relying on Dr. Satija’s testimony, PO concocts an inoperability narrative regarding Travers’ rolling circle replication embodiment that ignores the ’127 patent disclosure and is wholly without merit. This narrative should be rejected as based on testimony that is not only uncorroborated but is in direct conflict with express disclosure in both the ’127 patent and the prior art. *In re Am. Acad. of Sci.*

Tech Ctr., 367 F.3d 1359, 1368 (Fed. Cir. 2004) (“[T]he Board is entitled to weigh the declarations and conclude that the lack of factual corroboration warrants discounting the opinions expressed in the declarations.”). As Dr. Quackenbush and Dr. Larrea explain, a POSA would not consider any of alleged Problems 1-4 to preclude performance of a method within the scope of the claims. EX1033, ¶¶8-34; EX1039, ¶¶25-31.

A. Alleged Problem 1

PO argues (31-35) that rolling circle replication would result in multiple primer/polymerase binding sites, which would allegedly prevent sequencing on the Pacific Biosciences sequencing platform. But PO’s alleged “Problem 1” is directly contradicted by the express content of the prior art, including the Travers Publications and the manufacturer’s sequence guide (“Template Preparation Guide,” EX1035),³ and rests on a fundamental misapprehension of how SMRT sequencing operates. EX1033, ¶¶8, 9; EX1039, ¶28.

PO’s own evidence confirms that the presence of multiple primer/polymerase binding sites presented no bar to sequencing but is actually

³ Exhibit 1035 bears PO’s Bates numbering from the district court proceeding where it represented that the document was publicly available at least as of 2010.

preferred for SMRT sequencing. The Pacific Biosciences Template Preparation Guide, for example, states the following:

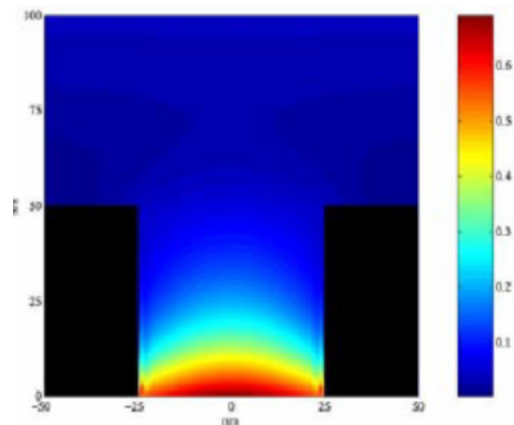
The stoichiometric optimum for the polymerase:template ratio is 2 polymerases bound to each template molecule (one to each hairpin adapter).

EX1035, 34. As Dr. Larrea explains, this recommendation reflects a gain in efficiency realized from minimizing the number of templates that lack a bound polymerase. EX1033, ¶10.

The Travers Publications expressly describe templates with more than one primer/polymerase binding sites. For example, Travers '075 expressly provides for templates which include one or more primer/polymerase binding site. EX1018, ¶¶17 (“...one or more of a primer sequence capable of hybridizing to at least a portion of the template nucleic acid and initiating polymerase mediated nucleic acid synthesis...”), 14, 67. The Travers Publications further disclose ligating identical hairpin linkers—each of which has a primer/polymerase binding site—to both ends of double-stranded nucleic acid fragments. EX1018, ¶¶103, 105, 144, Figure 7; EX1021, 3; *see also* EX1036, 12-13; EX1037, 117:19-24 (stating Travers discloses adapter complex having two different adapters, or two identical adapters and, therefore, multiple polymerase binding sites). Discussion in Travers of providing “a” site or “the” site on the template for primer binding does not imply

that the template provides only one site, despite PO's erroneous suggestion to the contrary. Accordingly, the Travers Publications confirm that template molecules for sequencing routinely had multiple primer/polymerase binding sites. EX1033, ¶¶11-15; EX1039, ¶28.

Templates with multiple polymerases routinely generate useable reads. This is because typically only one polymerase becomes bound to the bottom of the ZMW. EX2021, 1, 4; *see also id.*, Figure 6. It is the polymerase bound to the bottom of the ZMW whose activity is detected to provide sequencing data. To the extent additional polymerases are complexed with the template, these generally do not contribute signal because the excitation beam used for visualizing nucleotide incorporation penetrates only a short distance into the ZMW. EX2021, 4, Figure 4 (reproduced herein). Any polymerases outside the range of the excitation beam would not contribute signal. EX1033, ¶¶16-18.



In contrast to the binding of multiple polymerases on a single template, which was not a problem, it was beneficial to avoid loading multiple templates in a single ZMW. For example, the Template Preparation Guide cautions against loading “multiple polymerase-template complexes in the same ZMW.” EX1035,

39. As explained by Dr. Larrea, loading of multiple templates is easily mitigated. *Id.*; EX1033, ¶19.

In sum, PO's alleged Problem 1 was no problem at all. Pacific Biosciences sequencing platforms would routinely generate useable sequence when multiple polymerases were bound to a template. EX1033, ¶20.

B. Alleged Problems 2 and 3

PO's alleged Problems 2 and 3 (35-45) each relate to the presence of different sized molecules and diffusion bias favoring smaller template molecules for faster loading into ZMW. But despite copious discussion of how diffusion and its implications were known in the art, EX2014, ¶¶139-163, 165-169, Dr. Satija unreasonably assumes that POSAs would ignore known and routine methods to address it. EX1037, 40:2-5 ("Q. In 2012 were any DNA fragment size selection methods known in the art? A. Yes, there were size selection methods that were known in the art."). As Dr. Larrea explains, this assumption is nonsensical and wrong. EX1033, ¶21.

The Template Preparation Guide describes multiple ways in which slower diffusion of large molecules was routinely addressed. For example, it was routine to adjust the diffusion time based on template size. *E.g.*, EX1035, 38. It was also routine to perform size selection such that sequencing is directed to templates of the desired size. *E.g.*, EX1035, 27, 35 ("It is highly recommended to select

amplicons of the same size (+/- 10%) to minimize loading bias.”), (“highly recommended to perform a size selection step”), 48 (“We strongly encourage performing at least a double-AMPure PB bead purification step....”). As taught in the Travers Publications, a POSA would have known that a variety of effective size selection means were readily—and even commercially—available. *E.g.*, EX1018, ¶¶145, 150 (“ChromaSpin 1000”); EX1021, Figure 3 (gel electrophoresis of amplified concatemers); *see also* EX1035, 3 (“magnetic bead purification step with size-selective conditions”), 24 (“AMPure PB Size-Selection”); EX1036, 12 (“AMPure XP™”); EX1037, 43:16-45:3 (confirming AMPure beads were a commercially available size selection product); EX1033, ¶¶22, 23; EX1039, ¶29. Simply following such known protocols (e.g., the manufacturer’s protocol) would eliminate the alleged “Problems 2 and 3” that PO imagines. EX1037, 50:1-51:5 (arguing that a POSA would not ignore the manufacturer’s published protocol).

PO’s arguments, however, ignore the level of skill in the art and unreasonably assume that a POSA would forgo routine processing steps. EX1037, 37:13-23 (arguing it would be improper to ignore the perspective of a POSA when reading a document); EX2014, ¶29 (arguing a POSA would have had knowledge of methods for “DNA sample preparation for sequencing”). As Dr. Larrea explains, a POSA would have regarded size selection of template molecules to be a routine aspect of sequencing on the Pacific Biosciences platform described in the

manufacturer's own guidance and publications. *E.g.*, EX1018, ¶¶145, 150; EX1035, 27, 35, 48; EX1036, 12; EX1033, ¶24.

Even if one accepts PO's unreasonable assumption that a POSA would forgo routine size selection processes or selecting an appropriate loading/diffusion time, PO's argument still fails to establish any diffusion bias that would be so problematic so as to preclude collection of sequencing data. Loading bias is only a bias, it does not mean that larger templates are not loaded into any ZMW and sequenced. *E.g.*, EX1035, 35 (showing loading and sequencing of templates ranging from 160 bp to 4,251 bp). The existence of bias certainly does not mean that only small, uninformative templates are exclusively loaded. EX1033, ¶25. PO does not demonstrate otherwise.

PO's arguments (43-44) relating to insufficient copies of the target sequence does not dispute that informative templates would nonetheless still be loaded and sequenced despite loading bias. For example, PO invokes "shorter, truncated products" lacking a complete copy of each strand and template molecules that "would not provide a sufficient number of target DNA copies to be able to re-sequence the target DNA sufficiently." *Id.* But PO does not address that valuable sequencing data would still be obtained for informative templates. EX1033, ¶26.

Furthermore, to the extent redundancy is required by the claims, Travers '075 describes a method for circularizing linear concatemer templates to increase

redundancy. EX1018, ¶122 (incorporating U.S. Pat No. 7,476,503 [EX1020] by reference); EX1020, 24:36-44 (“[R]edundancy is additionally accomplished by circularizing this linear, multi-copy template and iteratively sequencing multiple copies, multiple times.”). PO’s hypothetical concerns (43) about shorter templates that would not provide “sufficient number of target DNA copies” has no practical bearing on the operability of the Pacific Biosciences sequencing platform described in the Travers Publications. EX1033, ¶27.

POSAs do not unreasonably ignore or forgo routine and commercially-available size selection protocols, particularly when specifically recommended in the manufacturer’s protocol. Even if one is to unreasonably underestimate the intelligence and capabilities of a POSA, as PO does, Pacific Biosciences sequencing platforms would still generate useable sequence even if rolling circle amplification produced a range of concatemer sizes. In sum, PO’s alleged Problems 2 and 3 are not problems at all. EX1033, ¶28.

C. Alleged Problem 4

PO argues (47) that “a POSA would have expected the narrow ZMWs to present physical size constraints on the larger concatemer products.” Specifically, PO argues (50-51) that hypothetical concatemer products of 1,008, 1,680, and 3,360 nucleotides are too large to fit in ZMW. *See* EX1037, 124:6-14, 125:21-126:3, 126:24-127:1 (confirming size of concatemers). This argument is directly

contradicted by the content of the prior art, including the Travers Publications, which expressly describe sequencing far larger templates and “no evidence of an intrinsic limit in the size of template.” EX1021, 1; *see also* DI, 20; EX1033, ¶29.

The Travers Publications disclose that much larger templates would fit into a ZMW and even provide examples of sequencing much larger templates. For example, Travers '075 explains “templates that are from about 200 to about 50,000 bases in length may be used.” EX1018, ¶83; *see also id.*, ¶85. Travers '075 further provides working examples in which templates containing inserts in the range of 500-2,560 bp were sequenced. EX1018, ¶¶144, 150. Converting the templates of the examples into nucleotide length (i.e., # base pairs * 2 nucleotides/base pair = # nucleotides) yields templates in the range of 1,000-5,120 nucleotides. Travers '075 thus directly contradicts PO's argument. EX1033, ¶¶30-31; EX1039, ¶30.

Travers 2010 also contradicts PO's argument. Travers 2010 discloses that templates with “inserts as short as 40 bp, and as large as 25 000 bp [have been created], and we currently see no evidence of an intrinsic limit in the size of template.” EX1021, 1. Converting Travers templates expressed in bp into nucleotide length yields templates between 80 and 50,000 nucleotides. Travers 2010 further describes sequencing a 1,000 bp/2,000 nucleotide template. EX1021, 5, Figure 4B. Travers 2010 is thus consistent with Travers '075 in contradicting PO's argument. EX1033, ¶32; EX1039, ¶30.

The Template Preparation Guide similarly undermines Dr. Satija’s argument. It describes “sequencing large insert libraries (e.g. 20 kb library) for generating long read lengths.” EX1035, 35; *see also id.*, 6. Converting the exemplary large insert library to nucleotides yields templates of 40,000 nucleotides. The Template Preparation Guide is thus consistent with the Travers Publications in contradicting PO’s argument. EX1033, ¶33.

PO’s argument appears to be based on a false premise that templates assume rigid conformations that prevent them from entering ZMW. But even PO’s expert agrees that the template conformation would “fluctuate in solution.” EX2014, ¶188; EX1037, 120:15-18 (“Q. But DNA in solution doesn’t assume a rigid conformation like a piece of lumber; correct? A. That’s correct.”). PO’s hypothetical conformations present no bar to SMRT sequencing in practice and fail to account for the reality of how these molecules fluctuate in solution. In sum, PO’s alleged Problem 4 is no problem at all. EX1033, ¶34; EX1039, ¶30.

IV. NO NEXUS

PO argues for nexus to evidence discussing DCS but fails to demonstrate (because it cannot) a “legally and factually sufficient connection” to the claims. *Fox Factory, Inc. v. SRAM, LLC*, 944 F.3d 1366, 1373 (Fed. Cir. 2019). PO’s claims are not directed to DCS of the Schmitt reference—as discussed in the Kirsch reference—and, as such, its evidence uniformly relates to unclaimed

features. *Ormco Corp. v. Align Tech., Inc.*, 463 F.3d 1299, 1311-12 (Fed. Cir. 2006) ([I]f the commercial success is due to an unclaimed feature of the device, the commercial success is irrelevant.”). Moreover, the broad claims of ’127 patent (admittedly) encompass the Travers Publications and, as established by PO’s own evidence, additional prior art approaches. *Id.* (“[I]f the feature that creates the commercial success was known in the prior art, the success is not pertinent.”). Accordingly, there is no nexus and PO’s secondary considerations are entitled no weight.

PO points to the Kirsch reference and argues (68-74) that praise, long-felt need, and unexpected results associated with “duplex sequencing” provides objective evidence of nonobviousness of claims 2-9, 16, 18, 19. For example, PO (69) relies on Kirsch (EX2007) to establish praise of “the duplex sequencing approach.” However, Kirsch is all about duplex consensus sequencing (“DCS”). EX1037, 127:15-129:18. Even PO characterizes the secondary considerations as pertaining to DCS. *E.g.*, EX2014, ¶229 (“Dr. Kirsch praises Patent Owner’s duplex consensus sequencing”). As, Dr. Quackenbush explains, none of claims 2-9, 16, 18, 19 require performance of DCS. Indeed, PO’s evidence relates exclusively to unclaimed features and to features found in the prior art. Accordingly, there is no nexus between the claims and the alleged secondary considerations. EX1039, ¶32.

The '127 patent describes DCS as a two-stage process, where the second stage (termed “DCS”) includes matching (by SMI) previously constructed single-strand consensus sequences (SSCSs). *E.g.*, EX1001, 16:19-28. Kirsch similarly describes the DCS process described in the Schmitt reference. EX2007, 1 (“[Schmitt] also identifies the complementary DNA strand by searching for the complementary tag sequences among the SSCS reads [duplex consensus sequences (DCS)].”); *see also* EX1037, 145:17-146:8 (confirming Kirsch describes DCS of Schmitt as comparing SSCSs); EX1039, ¶33.

None of the claims of the '127 patent requires performing DCS as that method is described either in the specification or by Kirsch. Claims 2, 3, 9, 16, 18, and 19, which depend from claim 1, do not require generating a consensus sequence much less a duplex consensus sequence or DCS. Claims 4-8 recite “consensus sequence” but require neither a single-strand consensus sequence, matching SSCSs, nor a duplex consensus sequence. EX1039, ¶34; *see also* EX1037, 80:7-83:17 (acknowledging claim 1 does not recite consensus sequence and claim 22 recites neither single-strand consensus sequence nor comparing SSCSs).

Furthermore, the claims of the '127 patent are so broad as to encompass the prior art, including prior art methods described by the Travers Publications. Furthermore, the Board has previously found that Kinde (EX1006) in view of

Craig (EX1038) teach each limitation of claim 1 of the '127 patent. IPR2022-00816, DI, 13. That the claims recite only features found in the prior art is further confirmed by Kirsch. Kirsch explains Kinde's Safe-SeqS, like DCS, uses tags (i.e., UIDs) to group original strands into families and generate a single-strand consensus sequence (SSCS). EX2007, 1. Indeed, Kinde instructs "High-quality reads were grouped into UID families on the basis of their endogenous or exogenous UIDs." EX1006, 9535; *see also id.*, 9531; EX1037, 137:1-6. Kinde further describes calling mutations when 95% of reads within a family have the same mutation. EX1006, 9530, 9531 (Figure 1); *see also* EX1039, ¶¶35, 36.

Kirsch ascribes no novelty over Kinde with respect to tagging sample DNA fragments with adapters comprising barcodes, and indeed there is none. There can be no dispute that barcoded adapters were known in the art and were even commercially available prior to the '127 patent. *E.g.*, EX1012, Figure 4; EX1037, 30:3-33:24 (acknowledging use of commercially available Illumina adapters). Indeed, Kinde identifies prior art barcoded Y-shaped adapters (e.g., Craig, EX1038) in its discussion of options for assigning UIDs to molecules. EX1006, 9531 ("UIDs, sometimes called barcodes or indexes, can be assigned to nucleic acid fragments using a variety of methods. These methods include the introduction of exogenous sequences through ...ligation (42, 43).") (Reference 42 is Craig); *see also* EX1022, ¶22; EX1039, ¶¶37, 38.

Kirsch identifies the difference between DCS and one embodiment of Kinde as “searching for the complementary tag sequences among the SSCS reads [duplex consensus sequences (DCS)]. A sequence base at a given position is then kept only if the read data from each of the two strands match perfectly....” EX2007, 1. However, the claims of the ’127 patent do not require DCS. Furthermore, searching for and comparing consensus sequences for both strands of an original DNA molecule was known in the art. EX1006, SI3 (“[T]he specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation....”); *see also* EX1022, ¶¶42, 62; EX1039, ¶39.

V. CONCLUSION

For the reasons set forth above, claims 1-30 of the ’127 patent are unpatentable and should be canceled.

Respectfully submitted,

Dated: April 13, 2023

/ Michael T. Rosato /
Michael T. Rosato, Lead Counsel
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VI. CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. §42.24(d), the undersigned certifies that this Reply complies with the type-volume limitation of 37 C.F.R. §42.24(c). The word count application of the word processing program used to prepare this Petition indicates that the Reply contains 5,584 words, excluding the parts of the brief exempted by 37 C.F.R. §42.24(c).

Respectfully submitted,

Dated: April 13, 2023

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VII. APPENDIX – PETITIONER’S UPDATED EXHIBIT LIST

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)

1032	Second Affidavit of Nathaniel E. Frank-White (served but not filed)
1033	Declaration of Andres Larrea
1034	<i>Curriculum Vitae</i> of Andres Larrea, Ph.D.
1035	Pacific Biosciences Template Preparation and Sequencing Guide
1036	Chin et al., “The Origin of the Haitian Cholera Outbreak Strain,” <i>New England Journal of Medicine</i> 364(1) (2011)
1037	Deposition Transcript of Rahul Satija, D. Phil (March 21, 2023)
1038	Craig et al., “Identification of Genetic Variants Using Bar-Coded Multiplexed Sequencing,” <i>Nature Methods</i> 5(10) (2008)
1039	Rebuttal Declaration of Dr. John Quackenbush

CERTIFICATE OF SERVICE

The undersigned certifies that the foregoing Petitioner's Reply to Patent Owner's Response and Exhibits 1033 – 1039 were served on April 13, 2023, at the following electronic service addresses:

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