

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

GUARDANT HEALTH, INC.
Petitioner,

v.

UNIVERSITY OF WASHINGTON,
Patent Owner.

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

**PATENT OWNER RESPONSE
UNDER 37 C.F.R. § 42.120**

Mail Stop “PATENT BOARD”
Patent Trial and Appeal Board
U.S. Patent & Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

TABLE OF CONTENTS

I.	INTRODUCTION	1
II.	LEVEL OF SKILL IN THE ART	5
III.	CLAIM CONSTRUCTION	5
A.	“Amplifying” adapter-DNA molecules to produce “strand copies.”	5
B.	“First and second strand sequence reads.”	8
C.	District court litigation	10
IV.	THE PETITION FAILS TO ADDRESS ALL THE ELEMENTS OF THE CHALLENGED CLAIMS	11
A.	The Petition fails to demonstrate that the asserted references disclose first strand sequence reads and second strand sequence reads, as recited in claims 1-21.	11
B.	The Petition fails to demonstrate that the asserted references disclose grouping the strand sequence reads into families (claims 22-30).....	13
C.	The Petition fails to demonstrate that the asserted references disclose additional elements of claim 19.	15
V.	A POSA WOULD NOT HAVE COMBINED TRAVERS-075’S ROLLING CIRCLE REPLICATION EMBODIMENT WITH TRAVERS-2010’S SMRT SEQUENCING APPLICATIONS.....	18
A.	Petitioner fails to explain <i>why</i> a POSA would have combined Travers-’075’s unnecessary and problematic RCR embodiment with the SMRT sequencing applications of Travers-2010.....	19
B.	A POSA would not have had any reason to modify the PacBio SMRT sequencing workflow—which does not use a template amplification step—with Travers-’075’s RCR step.....	24

C.	A POSA would have known that Petitioner’s rolling circle replication embodiment is incompatible with SMRT sequencing on zero mode waveguides (ZMWs).	29
1.	Problem 1: The linear concatemer template in Petitioner’s RCR embodiment would have multiple, repeating copies of the primer/polymerase binding site, which would result in unreadable sequence data.	31
2.	Problems 2 and 3: A POSA would have expected Petitioner’s RCR embodiment to produce a vast range of concatemer sizes, resulting in unwanted diffusion bias towards small templates into the ZMWs.	35
a.	Problem 2: SMRT sequencing’s DNA diffusion bias for small templates would result in unreliable data.	40
b.	Problem 3: Large concatemer products would lose the diffusion race for ZMW occupancy.	44
3.	Problem 4: The geometry of SMRT sequencing’s ZMWs posed physical constraints on Petitioner’s RCR embodiment.	45
D.	Petitioner provides no explanation of <i>how</i> a POSA would have combined its rolling circle replication embodiment with the SMRT sequencing applications of Travers-2010.	55
1.	Petitioner’s conclusory, unsupported arguments cannot meet its burden of persuasion.	56
2.	Travers-’075 does not enable Petitioner’s RCR embodiment.	59
VI.	EVEN IF A POSA WERE TO COMBINE PETITIONER’S ROLLING CIRCLE REPLICATION EMBODIMENT WITH THE SMRT SEQUENCING APPLICATIONS OF TRAVERS-2010, THE POSA WOULD NOT HAVE HAD A REASONABLE EXPECTATION OF SUCCESS.	61
A.	A POSA would have known that Petitioner’s rolling circle replication embodiment was fraught with technical obstacles.	62

B.	The '127 patent's claimed rolling circle replication embodiment does not support Petitioner's embodiment.....	63
VII.	A POSA would not have had any reason to combine McCloskey with Travers-'075 and Travers 2010 (claims 13-14, 29-30).	65
VIII.	OBJECTIVE INDICIA OF NONOBVIOUSNESS FURTHER SUPPORTS A CONCLUSION OF PATENTABILITY	68
A.	Industry praise	69
B.	Long-felt need	72
C.	Unexpectedly superior results	73
IX.	CONCLUSION.....	74

PATENT OWNER'S UPDATED EXHIBIT LIST

Exhibit No.	Description
2001	Eid, J., et al., "Real-Time DNA Sequencing from Single Polymerase Molecules," <i>Science</i> 323:133-138 (2009)
2002	Niedringhaus, T.P., et al., "Landscape of Next-Generation Sequencing Technologies," <i>Anal. Chem.</i> 83:4327-4341 (2011)
2003	Thompson, J.F. and Milos, P.M., "The Properties and Applications of Single-Molecule DNA Sequencing," <i>Genome Biology</i> 12:217 (2011)
2004	Stranneheim, H. and Lundeberg, J., "Stepping Stones In DNA Sequencing," <i>Biotechnol</i> 7:106-1073 (2012)
2005	Quail, M.A., et al., "A Tale of Three Next Generation Sequencing Platforms: Comparison of Ion Torrent, Pacific Biosciences and Illumina Miseq Sequencers," <i>BMC Genomics</i> 13:341 (2012)
2006	Egan, A.N., et al., "Applications of Next-Generation Sequencing In Plant Biology," <i>American Journal of Botany</i> 99(2):175-185 (2012)
2007	Kirsch, S. and Klein, C.A., "Sequence Error Storms and the Landscape of Mutations in Cancer," <i>Proc. Natl. Acad. Sci.</i> 109(36):14289-14290 (2012)
2008	Turner, S., et al., "Apparatus and Method for Performing Nucleic Acid Analysis," U.S. Patent No. 7,479,503 B2 (filed Sept. 16, 2005; issued Jan 13, 2009)
2009	Miner, B.E., et al., "Molecular barcodes detected redundancy and contamination in hairpin-bisulfite PCR," <i>Nucleic Acids Research</i> 32(17): s135 (2004)
2010	Lindahl, T. and Wood, R.D., "Quality Control by DNA Repair," <i>Science</i> , 286:1897-1905 (1999)

Exhibit No.	Description
2011	Shibutani, S., et al., “Insertion of Specific Bases During DNA Synthesis Past the Oxidation-Damaged Base 8-oxodG,” <i>Nature</i> , 349:431-434 (1991)
2012	Stiller, M., et al., “Patters of Nucleotide Misincorporations During Enzymatic Amplification and Direct Large-Scale Sequencing of Ancient DNA,” <i>Proc. Natl. Acad. Sci.</i> , 103(37):13578-13584 (2006)
2013	McCloskey, M.L., et al., “Encoding PCR Products with Batch-stamps and Barcodes,” <i>Biochem Genet</i> 45:761-767 (2007)
2014	Declaration of Rahul Satija, D.Phil. in Support of Patent Owner Response
2015	Complaint, <i>Twinstrand Biosciences, Inc. v. Guardant Health, Inc.</i> , Case No. 1:21-cv-01126-UNA (D. Del.), filed Aug. 3, 2021
2016	Decision Denying Institution, <i>Guardant Health, Inc. v. University of Washington</i> , Case No. IPR2022-00816 (P.T.A.B.), Paper No. 16, filed Oct. 13, 2022
2017	Johne, R., et al., “Rolling-Circle Amplification Of Viral DNA Genomes Using Phi29 Polymerase,” <i>Trends in Microbiology</i> , 17(5):205-11 (2009)
2018	Report and Recommendation, <i>Twinstrand Biosciences, Inc. v. Guardant Health, Inc.</i> , Case No. 1:21-cv-01126-UNA (D. Del.), filed Dec. 29, 2022
2019	Guardant Health, Inc. Response to Notice of Opposition, European Patent No. 2,893,040, filed May 29, 2020
2020	Prosecution History of U.S. Patent No. 10,760, 127 (“127 Prosecution History”)

Exhibit No.	Description
2021	“Single Molecule Real Time (SMRT™) DNA Sequencing,” Technology Backgrounder, <i>Pacific Biosciences</i> (2009)
2022	Fichot, E. B. and Norman, R. S, “Microbial Phylogenetic Profiling With The Pacific Biosciences Sequencing Platform,” <i>Microbiome</i> 1(10):1-5 (2013)
2023	Shadt, E. E., et al., “A Window Into Third-Generation Sequencing,” <i>Pacific Human Molecular Genetics</i> , 19(2):R277-R240 (2010)
2024	Liu, L., et al., “A Brief Utilization Report On The Illumina Hiseq 2000 Sequencer,” <i>Mycology</i> , 2(3):169-91 (2011)
2025	Koren, S., et al., “Hybrid Error Correction And <i>De Novo</i> Assembly Of Single-Molecule Sequencing Reads,” <i>Nature Biotechnology</i> (2012)
2026	Eid, J, et al., “Supporting Online Material for: Real-Time DNA Sequencing from Single Polymerase Molecules,” <i>Science</i> (2008)
2027	Gupta, P. K., “Single-Molecule DNA Sequencing Technologies For Future Genomics Research,” <i>Trends in Biotechnology</i> , 26(11):602-11 (2008)
2028	Zhang, X., et al., “Improving Genome Assemblies By Sequencing PCR Products With Pacbio,” <i>BioTechniques</i> , 53(1):61-2 (2012)
2029	Levene, M. J., et al., “Zero-Mode Waveguides For Single-Molecule Analysis At High Concentrations,” <i>Science</i> , 299:682-86 (2003)
2030	Korlach, J., et al., “Selective Aluminum Passivation For Targeted Immobilization Of Single DNA Polymerase Molecules In Zero-Mode Waveguide Nanostructures,” <i>PNAS</i> , 105(4):1176-81 (2008)

Exhibit No.	Description
2031	McCarthy, A., “Third Generation DNA Sequencing: Pacific Biosciences’ Single Molecule Real Time Technology,” <i>Chemistry & Biology</i> , 17:675-76 (2010)
2032	Shendure, J. A., et al., “Overview of DNA Sequencing Strategies,” <i>Current Protocols in Molecular Biology</i> , Supp. 96:7.1.1-7.1.23 (2011)
2033	Mardis, E. R., “Next-Generation Sequencing Platforms,” <i>Annual Review of Analytical Chemistry</i> , 6:287-303 (2013)
2034	Glenn, T. C., “Field Guide To Next-Generation DNA Sequencers,” <i>Molecular Ecology Resources</i> , 11:759-69 (2011)
2035	Ozsolak, F., “Third Generation Sequencing Techniques and Applications to Drug Discovery,” <i>Expert Opinion on Drug Discovery</i> , 7(3):231-43 (2012)
2036	Demidov, V. V., “Rolling-Circle Amplification In DNA Diagnostics: The Power Of Simplicity,” <i>Expert Review of Molecular Diagnostics</i> , 2(6):542-48 (2002)
2037	Liu, D., et al., “Rolling Circle DNA Synthesis: Small Circular Oligonucleotides as Efficient Templates for DNA Polymerases,” <i>Journal of American Chemical Society</i> , 118:1587-94 (1996)
2038	Lizardi, P. M., et al., “Mutation Detection And Single-Molecule Counting Using Isothermal Rolling-Circle Amplification,” <i>Nature Genetics</i> , 19:225-32
2039	Smith, D. E., et al., “Dynamical Scaling of DNA Diffusion Coefficients,” <i>Macromolecules</i> , 29:1372-73 (1996)
2040	Robertson, R. M., “Diffusion Of Isolated DNA Molecules: Dependence On Length And Topology,” <i>Proceedings of the National Academy of Sciences</i> , 103(19):7310-14 (2006)

Exhibit No.	Description
2041	Grosberg, A. Y. and Rabin, Y., “DNA Capture Into A Nanopore: Interplay Of Diffusion And Electrohydrodynamics,” <i>The Journal of Chemical Physics</i> , 133: 165102-1-15 (2010)
2042	Coupland, P., et al., “Direct Sequencing Of Small Genomes On The Pacific Biosciences RS Without Library Preparation,” <i>BioTechniques</i> , 53(6): 365-72 (2012)
2043	Patent Owner’s Pre-Institution Sur-Reply, <i>TwinStrand Biosciences, Inc. v. Guardant Health, Inc</i> , Case No. IPR2022-00746 (P.T.A.B.), Paper No. 13, filed Aug. 15, 2022
2044	Patent Owner’s Pre-Institution Sur-Reply, <i>TwinStrand Biosciences, Inc. v. Guardant Health, Inc</i> , Case No. IPR2022-00747 (P.T.A.B.), Paper No. 13, filed Aug. 15, 2022
2045	Deposition Transcript of John Quackenbush, Ph.D., <i>Guardant Health, Inc. v. University of Washington</i> , Case No. IPR2022-00817 (P.T.A.B), Dec. 19, 2022
2046	Loomis, E. W., et al., “Sequencing The Unsequenceable: Expanded CGG-Repeat Alleles Of The Fragile X Gene,” <i>Genome Research</i> , 23:121-28 (2014)
2047	Drmanac, R., et al., “Human Genome Sequencing Using Unchained Base Reads on Self-Assembling DNA Nanoarrays,” <i>Science</i> , 327: 78-81 (2010)
2048	Hui, P., “Next Generation Sequencing: Chemistry, Technology and Applications,” <i>Topics in Current Chemistry</i> (2012)
2049	U.S. Provisional Application No. 61/072,160 to Patel et al., filed Mar. 28, 2008

Exhibit No.	Description
2050	Schmitt, M. W., et al., “Detection Of Ultra-Rare Mutations By Next-Generation Sequencing,” <i>Proceedings of The National Academy of Sciences</i> , 109(36):14508-13 (2012)
2051	Kelvey, J., “Liquid Biopsies Generate Ample Insights from Sparse Clues,” Genetic Engineering & Biotechnology News (2020)
2052	LeMieux, J., “Sequencing’s Stars Urge Exploration of New Dimensions” Genetic Engineering & Biotechnology News, 40(4) (2020)
2053	“TwinStrand Biosciences to Showcase Recent Applications of its Duplex Sequencing Technology at the 2022 AGBT Annual Meeting,” Businesswire (2022)
2054	Ribeiro, F. J., et al., “Finished Bacterial Genomes From Shotgun Sequence Data,” <i>Genome Research</i> , 22:2270-77 (2012)
2055	“Quality Scores for Next-Generation Sequencing,” Illumina (2011)
2056	“12A: Nomenclature,” Encyclopedia of Separation Science, Academic Press, 4712-53 (2000) (excerpt)
2057	Larkin, J., et al., “Length-Independent DNA Packing into Nanopore Zero-Mode Waveguides for Low-Input DNA Sequencing,” <i>Nature Nanotechnology</i> , 12(12) 1169-75 (2017)
2058	Kalendar, R., et al., “Java Web Tools For PCR, In Silico PCR, And Oligonucleotide Assembly And Analysis,” <i>Genomics</i> , 98:137-44 (2011)
2059	Kalendar, R., et al., “Java Web Tools For PCR, In Silico PCR, And Oligonucleotide Assembly And Analysis,” <i>Genes, Genomes and Genomics</i> , 3(1):1-14 (2009)

Exhibit No.	Description
2060	Vincze, T., et al., “NEBcutter: A Program To Cleave DNA With Restriction Enzymes,” <i>Nucleic Acids Research</i> , 31(13):3688-91 (2003)
2061	Reuter, J. S., “RNAstructure: Software For RNA Secondary Structure Prediction And Analysis,” <i>BMC BioInformatics</i> , 11(129):1-9 (2010)
2062	Kennedy, S. R., et al., “Detecting ultralow-frequency Mutations by Duplex Sequencing,” <i>Nature Protocols</i> , 9(11):2586-2606 (2014)
2063	Ng, D. W. and Wong, C. K. B, “Self-Recognition Of DNA From Life Processes To DNA Computation,” <i>Biophysical Reviews and Letters</i> , 2(2):123-37 (2007)
2064	<i>Curriculum Vitae</i> of Rahul Satija, D.Phil
2065	“TwinStrand’s Duplex Sequencing Goes Deep,” GenEdge, Genetic Engineering & Biotechnology News (2020)
2066	Semsarian, C., et al., “Molecular Medicine in the 21 st Century,” <i>Internal Medicine Journal</i> , 31:53-59 (2001)
2067	Forthofer, R. N., et al., “Biostatistics: A Guide to Design, Analysis, and Discovery,” Chapter 5: Probability Distributions, <i>Elsevier Academic Press</i> , 103-133 (2007) (excerpt)
2068	Esteban, J. A., et al., “Fidelity of Φ 29 DNA Polymerase,” <i>The Journal of Biological Chemistry</i> , 268(4):2719-26 (1993)
2069	DNA Secondary Structures for 6x Product

Exhibit No.	Description
2070	DNA Secondary Structures for 10x Product
2071	DNA Secondary Structures for 20x Product

I. INTRODUCTION

The '127 patent covers groundbreaking duplex sequencing methods developed at the University of Washington. Before the invention of the '127 patent, next generation sequencing was plagued with sequencing errors. EX1001, 1:60-2:10. The inventors of the '127 patent discovered a method of overcoming these errors, providing the ability achieve unprecedented sequence accuracy and to detect mutations in DNA target molecules that are present in extremely low abundance. EX1001, 1:60-2:62. Peers in the field hailed this inventive breakthrough, proclaiming that “the Gordian knot in sequencing accuracy has apparently been cut by the duplex sequencing approach” and that duplex sequencing “may open doors to clinical applications in which diagnostic accuracy is the sine qua non for ethical treatment decisions.” EX2007, 1, 2. Indeed, duplex sequencing can “detect a single mutation among $>10^7$ sequenced bases.” EX2062, 1.

In response to an infringement suit, Petitioner Guardant Health filed two IPR petitions against the '127 patent. EX2015. The Board denied institution of Petitioner's first ranked petition (EX2016, 17) but instituted the present IPR on Petitioner's second-ranked petition. However, as demonstrated herein, Petitioner's second-ranked petition is fraught with fundamental legal and technical flaws, which are fatal to its case.

All three of Petitioner's grounds rely upon the combination of two references, Travers-'075 and Travers-2010. Petitioner alleges that "*the combination* of Travers '075 and [Travers-2010] disclose or teach every element of the claims." Pet., 19.

But Petitioner's combinations fail to disclose several claim elements:

- The combination's sequencing methods produce one read, not separate reads derived from the first strand and second strand, as claimed. (Claims 1-21).
- The combination does not disclose grouping the sequence reads into families based on a barcode sequence and DNA fragment-specific information.
(Claims 22-30)
- The combination does not disclose using any asymmetric feature of an adapter DNA molecule to differentiate the strands. (Claim 19).

Moreover, although Petitioner unequivocally relies on a combination of Travers-'075 and Travers-2010, nowhere does Petitioner articulate why or how a POSA would have combined these references in the way imagined by Petitioner. The Petition is simply *devoid* of any rationale for doing so.

The SMRT sequencing workflow disclosed in Travers-'075 and Travers-2010 is based on a real-time, sequencing by synthesis technique that does not utilize a DNA template amplification step. Paper 10, 11-15, 20-23; Paper 14, 10, 16. The claimed methods of the '127 patent, however, expressly require a separate

template amplification step *after* adapters are attached to the target DNA fragments and before sequencing. *Id.* Petitioner approaches this claim element by pure hindsight arguing that a POSA would have first used a hypothetical rolling circle replication (RCR) technique mentioned in Travers-'075 to produce linear concatemer templates, and then sequenced the linear concatemers using the SMRT sequencing applications of Travers-2010. Pet., 24-25, 31-32; Paper 14, 19. But the evidence Patent Owner provides here shows multiple, independent reasons why a POSA never would have sought out Petitioner's proposed embodiment in the first place, much less implemented it in Travers 2010's workflow with a reasonable expectation of success.

First, Petitioner's RCR generated concatemer templates would contain multiple primer/polymerase binding sites, leading to *multiple polymerases binding on different sites within a single concatemer template*. But when multiple polymerases carry out sequencing by synthesis reactions in a single SMRT sequencing nanowell, no useful sequencing data can be obtained because multiple polymerases each would produce signals from the same nanowell at the same time, thereby interfering with each other's signals. Sequencing simply would not work.

Second, RCR generates a wide assortment of product sizes, ranging from small fragments only a few hundred nucleotides long to large concatemer molecules several thousand nucleotides long. This broad range of product sizes

would lead to several significant technical problems because smaller template molecules diffuse more efficiently and would outcompete larger template molecules for occupancy and sequencing in the nanowells. Yet, these smaller templates would lack sufficient concatemer copies for *sequencing redundancy* in SMRT sequencing's error correction. On the flip side, the larger concatemer molecules, which might possess more copies of the target DNA for the required sequencing redundancy, would have lost the diffusion race for nanowell occupancy to the smaller templates and not even be sequenced.

Third, even if the large concatemer molecules: 1) were somehow only bound to a single polymerase; and 2) could somehow overcome the DNA diffusion bias issue, the narrow opening of the nanowell would have *physically precluded* them from fitting into the wells. The concatemer molecules would form large and complex secondary structures that are much too bulky to facilitate entry into the nanowells. And of course, without entry into the nanowells, these concatemers could not have been sequenced at all. Nowhere do Petitioner or its expert recognize or deal with any of these technical issues.

At bottom, the Patent Owner's evidence shows that a POSA would not have had any reason to combine Petitioner's RCR embodiment with the SMRT sequencing applications of Travers-2010. *To do so only would have made a worse sequencing method*, if it was even workable at all. That Petitioner fails to even

attempt to articulate an actual reason to combine the references only underscores its hindsight-based approach to its challenge and shows it cannot meet its burden.

II. LEVEL OF SKILL IN THE ART

Solely for the purposes of this proceeding, Patent Owner does not dispute Petitioner's articulation of the level of skill in the art. EX2014, ¶¶28-30.

III. CLAIM CONSTRUCTION

Claim terms must be given their “ordinary and customary meaning as understood by one of ordinary skill in the art” in view of the specification and prosecution history. 37 C.F.R. § 42.100(b); *Phillips v. AWH Corp.*, 415 F.3d 1303, 1313 (Fed. Cir. 2005) (en banc).

A. “Amplifying” adapter-DNA molecules to produce “strand copies.”

Independent claims 1 and 22 each requires separate steps of (a) attaching adapters to double-stranded DNA fragments; *then* (b) amplifying the adapter-DNA fragments to produce “strand copies”; *then* (c) sequencing the “strand copies” (i.e., sequencing the amplified adapter-DNA fragments); and *then* assessing sequence information via different steps. EX1001, 37:31-52, 39:56-40:25.

Claim 1 recites the following steps (a), (b) and (c):

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

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

b) *amplifying* original strands of at least a portion of the double-stranded *adapter-DNA molecules* to produce *first and second strand copies*;

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;

EX1001, 37:31-52 (emphasis added)¹. Claim 1 recites that the adapter attachment step (a) generates “adapter-DNA molecules,” the amplification step (b) amplifies the “adapter-DNA molecules” from step (a) to produce “first and second strand copies,” and the sequencing step (c) sequences the “first and second strand copies” that were produced in the amplification step (b). *Id.*; EX2014, ¶¶48-59.

The method of claim 22 uses similar language. Claim 22 recites the following steps (a), (b), and (c):

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

¹ Emphasis added throughout unless noted.

to double-stranded DNA fragments generates a library of tagged double-stranded *adapter-DNA molecules*;

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

c) *sequencing* a plurality of *the strand copies* to obtain strand sequence reads comprising one or more barcode sequences and DNA fragment-specific information;

EX1001, 39:56-40:25.

Like claim 1, claim 22 recites that the adapter attachment step (a) generates “adapter-DNA molecules,” the amplification step (b) amplifies the “adapter-DNA molecules” from step (a) to produce “strand copies,” and the sequencing step (c) sequences the “strand copies” that were produced in the amplification step (b). *Id.*; EX2014, ¶52.

The plain language of the claims is consistent with the disclosures in the specification. *Phillips*, 415 F.3d at 1313. For example, the specification discloses:

[E]ach end of the double-stranded target nucleic acid molecule is *ligated* to an SMI *adaptor molecule*. The double-stranded target nucleic acid complex *is then amplified* by a method known in the art (e.g., a PCR or non-PCR method known in the art), resulting in a set of uniquely labeled, amplified SMI-target nucleic acid products. These *products are then sequenced....*

EX1001, 15:27-39; *see also, id.*, Fig. 1 (depicting PCR amplification step after adapter ligation step but prior to “massively parallel sequencing”), 3:21-27 (disclosing steps of “ligating” target DNA molecules to adapters, “amplifying” the adapter-DNA molecules, and then “sequencing the amplified ... products.”); EX2014, ¶¶54-56.

Thus, based on the plain language of the claims, which is confirmed by the disclosures in the specification, a POSA would understand that (i) the claimed methods require that the amplification step occurs after adapter attachment; but before the sequencing step; and (ii) the sequencing step requires sequencing the amplified adapter-DNA products from the amplification step. *Id.*

In its Decision on Institution, the Board agreed with this construction, stating that “Patent Owner’s argument concerning the sequential order of the steps is *supported by the claim language and the disclosures in the specification.*” Paper 14, 10; *see also, id.*, 16.

B. “First and second strand sequence reads.”

Claim 1 recites, “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.” EX1001, 37:39-41. Claims 2, 3, 4, 16, 18, and 19 also recite this limitation. *Id.*, 37:53-38:45, 39:27-31, 39:35-46.

A POSA reading the plain language of the claims in view of the

specification and file history would have understood that the term “sequence reads” (plural), indicates multiple and distinct sequencing reactions, each of which results in a sequence read, rather than a single sequencing reaction that produces a single sequence read containing multiple subparts (which a POSA would know are *subreads*, not sequence reads). EX2014, ¶¶60-65. For example, the specification discloses, “Figure 3 ... shows *sequence reads* (brown) sharing a unique set of SMI tags are grouped into paired families....” EX1001, 4:12-18. The specification also discloses that, “[w]hen these molecules are amplified with PCR and sequenced, they will yield the following *sequence reads*” *Id.*, 4:45-49; *see also, id.*, 9:5-9 (depicting two distinct sequence reads, “*Read 1*” and “*Read 2*”), 9:11-12 (“[t]he sequences of *the two duplex strands* seen in *the two sequence reads* may then be compared....”); EX2014, ¶62.

Extrinsic evidence further supports this construction. Travers-2010 (EX1021) refers to the sense and antisense target DNA sequences as *subreads*, which are distinct from the “raw read sequence” or “raw read” that comprises all of the subreads physically linked together. EX1021, 2; EX2014, ¶¶63-64. As Travers-2010 explains:

The first step is to classify the different regions in the raw circular molecule reads into *adapter and insert subreads*. This is done by *aligning a raw read sequence* to the known adapter sequence and a putative reference sequence of the

insert regions. *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[.]

EX1021, 2; *see also, id.* (“we align all the insert subreads identified from a single-molecule *raw read*”); *id.*, 5 (“[w]e call the set of called bases corresponding to the insert region a *subread*.”); EX2014, ¶¶63-64; EX2001, 4.

C. District court litigation

In concurrent litigation, Petitioner argued unsuccessfully that the term “DNA fragment-specific information” recited in claim 22 is indefinite. *See e.g.*, EX2018, 23-25. The court rejected Petitioner’s argument, concluding that the term is not indefinite and recommended adopting Patent Owner’s plain and ordinary meaning construction. *Id.*

The court also recommended adopting Patent Owner’s construction of the terms “degenerate” and “semi-degenerate” sequences, recited in claim 13. *Id.*, 3, 15-20. The court concluded that a “degenerate sequence” of claim 13 should be construed as “[a] nucleotide sequence that is known or unknown in which every nucleotide position is unrestricted in its nucleotide variability,” and a “semi-degenerate sequence” of claim 13 should be construed as “[a] nucleotide sequence that is known or unknown in which at least one, but not all, nucleotide positions are fixed or restricted in their nucleotide variability.” *Id.*, 3, 15-20, 32.

The court also recommended applying the plain and ordinary meaning to '127 patent terms “partially complementary, asymmetrical double-stranded adapter-DNA molecules” (claim 1) and “partially single-stranded adapters” (claim 22). *Id.*, 2-3.

IV. THE PETITION FAILS TO ADDRESS ALL THE ELEMENTS OF THE CHALLENGED CLAIMS

Petitioner's obviousness theory fails on numerous technical and legal levels, which are fatal to the entire Petition, across all challenged claims.

A. The Petition fails to demonstrate that the asserted references disclose first strand sequence reads and second strand sequence reads, as recited in claims 1-21.

The Petition fails to demonstrate that either of Travers-'075 or Travers-2010 discloses obtaining “first and second strand sequence reads,” as recited in the method of claim 1, step (c), and required for claims 1-21. Discussed above in Section III, a POSA would understand that first strand sequence reads and second strand sequence reads recited in claims 1-21 result from *separate, distinct* sequencing reactions. EX2014, ¶¶75-80; *supra* Section III. Neither Travers-2010 nor Travers-'075 discloses this limitation, and the Petition fails to show otherwise.

Travers-2010 discloses obtaining “sequence reads,” but in Travers-2010, the sequence reads comprise subreads *physically linked together in a single read produced from a single sequencing reaction*, not as separate first and second strand sequence reads as claimed. EX2014, ¶76; EX1021, 2, 5. Specifically, Travers-2010

refers to the sense and antisense target DNA sequences as *subreads*, which are distinct from the “raw read sequence” or “raw read” that comprises all of the subreads from sequences physically linked together and sequenced in the same sequencing reaction. EX1021, 2; *see also, id.*, 5; EX2014, ¶76. This teaching in Travers-2010 is further confirmed in Eid, PacBio’s seminal SMRT sequencing paper. EX2001, 4 (referring to a sequence “read” as comprising repeating copies of the target sequences, rather than separate and distinct reads from different sequencing reactions); EX2014, ¶78.

Claim 1 and its dependent claims, however, require obtaining *separate* sequence reads for the first strand sequence read and the second strand sequence read, and “confirming the presence of *at least one sequence read* derived from *each* of the original first and second strands” respectively. This is different from the single sequence read comprising subreads linked together as disclosed in Travers-’075 and Travers-2010. EX2014, ¶79; *supra* Section III.

The Petition ignores this claim limitation, instead only providing an unsupported, conclusory allegation that “Travers discloses that either SMRTbell templates or replication products therefrom (i.e., concatemers) may be sequenced using the disclosed template-dependent sequencing process.” Pet., 25. Dr. Quackenbush merely parrots the Petition, without offering anything more than the Petition’s conclusory statement above. EX1002, ¶81. But neither of these

embodiments shows separate sequence reads derived *from each* of the original first and second strands, as claimed.

These deficiencies in the Petition are fatal, and infect Petitioner's challenges to at least claims 1-21 which each recite the claim elements "first strand sequence read" and "second strand sequence read." Pet., 25-26, 38-44, 47, 51-53.

B. The Petition fails to demonstrate that the asserted references disclose grouping the strand sequence reads into families (claims 22-30).

The Petition also fails to establish that either of the Travers publications discloses step (d) of the method of claim 22, which recites "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...*" EX1001, 40:12-16; EX2014, ¶¶81-87.

When addressing this element of claim 22, Dr. Quackenbush argues that ¶[0100] in Travers-'075 discloses "the use of 'oligonucleotide tags' or barcodes to 'pool[data] for analysis.'" See EX1002, ¶113 (citing EX1018, ¶[0100]). However, Dr. Quackenbush's overuse of excerpted quotes, brackets, and ellipses when quoting Travers-'075 places the disclosures entirely out of context. EX2014, ¶¶82-83. In the proper context, a POSA would have known that Travers-'075 discloses adding *index barcodes* to track sample origins, *not* grouping the strands into

families based on barcode sequence *and* DNA fragment-specific information. *Id.*.

Travers-'075 discloses:

In particular, one can utilize connecting oligonucleotides in the template constructs that include sequence markers like a *barcode*, to indicate the origin of a given template sample. Different template samples including distinguishable connecting oligonucleotide tags *could then be pooled* for analysis in a single sequencing process. Sequence data derived from individual templates is then attributable to the originating *sample* preparation process through the identification of the connecting oligonucleotide tag.

EX1018, ¶[0100]; EX2014, ¶83.

As Dr. Satija explains, Travers-'075's "pooling" of indexed barcoded *samples* to track a *sample's* source is far different from the claimed step of grouping the strands of sequence reads into families based on barcode and DNA fragment-specific information. EX2014, ¶84.

Petitioner argued as much in a European Opposition proceeding involving one of Petitioner's European patents. In that proceeding, Petitioner argued that that index sequences (sample barcodes) are "used to permit multiplexing of several different samples in a single sequencing experiment," and that a POSA would *not* "consider using the index sequence for collapsing sequences into sets [] or 'UID

families’....” EX2019, 18; EX2014, ¶85. Thus, Travers-’075’s disclosure of pooling samples using index barcodes fails to meet claim 22’s requirement of grouping the strand sequence reads into families based on barcode sequence and DNA fragment-specific information. EX2014, ¶¶84-85.

Petitioner also ignores claim 22’s requirement to use both the barcode sequence *and* “DNA fragment-specific information” for grouping the sequence reads into families. Dr. Quackenbush argues that “Travers expressly describes determining consensus sequences *based on assembled (i.e., grouped)* redundant sequence reads,” but conveniently glosses over *how* Travers-’075 and Travers-2010 “group” subreads. EX1002, ¶113. As Dr. Satija explains, the subreads in Travers-’075 and Travers-2010 are only “grouped” because they are physically connected to one another, and are *not* grouped *based on DNA fragment-specific information* (or barcode sequence), as required in claim 22. EX2014, ¶86.

This deficiency is fatal to the Petition’s challenge to claim 22 and dependent claims 23-30 which depend from claim 22 and thus require the same grouping step of claim 22. *In re Magnum Oil Tools Int’l, Ltd.*, 829 F.3d 1364, 1380 (Fed. Cir. 2016).

C. The Petition fails to demonstrate that the asserted references disclose additional elements of claim 19.

Claim 19 recites that “the [*first strand*] *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.*²” EX1001, 39:4-41; EX2020, 482.

As Dr. Satija explains, POSA would have understood that the method of claim 19 leverages the asymmetric nature of the adapters to differentiate the first strand sequence read and second strand sequence read. EX2014, ¶¶88-95. This is consistent with the disclosures in the specification. *See e.g.*, EX1001, 3:64-4:3 (distinguishing “two types of PCR products” – “those derived from one strand” and “products originating from the complementary strand” – “[b]y virtue of *the asymmetric nature of adapted fragments*”).

Neither Travers-2010 nor Travers-’075 discloses or suggests using any aspect of adapter asymmetry to distinguish the first and second strands, as claimed in claim 19. EX2014, ¶¶90-91. When discussing claim 19, Dr. Quackenbush relies upon ¶[0054] in Travers-’075. *See* EX1002, ¶¶171-172. Paragraph [0054] in Travers-’075 discloses nothing about using the *asymmetric nature* of the adapter-DNA fragments to distinguish first and second strands. At most, Travers-’075 discloses identifying the *mere presence* of *any* adapter sequences as landmarks or

² Guardant’s petition failed to accurately recite the claims of the ’127 patent which were corrected by a Certificate of Correction on March 9, 2021.

“registration sequences” that mark the portion of the sequence read where one strand ends and the next strand begins. EX1018, ¶[0054]; EX2014, ¶¶91-92. In other words, Travers-’075 relies upon *the order of sequence segments* (e.g., target-adapter-target-adapter-target-adapter-etc.), rather than any asymmetrical adapter-DNA configuration. *Id.* In Travers-’075 and Travers-2010, the adapter sequence need not provide any asymmetry to the adapter-DNA molecule.

For example, Travers-’075 refers to the adapter segments as “*a registration sequence that permits the identification of when one segment ... is completed and the other begins ...*” EX1018, ¶[0054]. Travers-’075 further explains that the “registration sequence indicat[es] a point in the sequence ... [that] transitions from the sense to the antisense strands.” *Id.* According to Travers-’075, “because the connecting oligonucleotide is known or knowable, one can readily exploit this pre-known sequence in aligning long strings of sequence data from such template or templates, e.g., *as a landmark or registration sequence....*” *Id.*, ¶[0098]. These disclosures have nothing to do with adapter asymmetry, and everything to do with the *order of sequence segments* in the sequence read. EX2014, ¶92. Petitioner only glosses over this critical deficiency in the art, proffering a paltry two sentences for its entire discussion of claim 19. Pet., 52-53; EX1002, ¶¶172-173.

Dr. Quackenbush also relies upon Figures 1 and 4 in Travers-2010. *See* EX1002, ¶¶172-173 (citing EX1021, Fig. 1, 4). As with Travers-’075, Figure 1 in

Travers-2010 discloses nothing about using adapter asymmetry to distinguish first strand and second strand sequence reads. EX2014, ¶93; EX1021, Fig. 1. And Figure 4 in Travers-2010 merely exemplifies using the adapter segments as *landmarks* to identify when one strand ends and the next strand begins. EX1021, Fig. 4; EX2014, ¶93. Thus, nothing in Travers-'075 or Travers-2010 discloses or even suggests using any adapter-DNA asymmetry in a method to distinguish first and second strand sequence reads, as claimed.

Further, neither the Petition nor Dr. Quackenbush provides *any reason* a POSA attempting Petitioner's RCR embodiment would have supposedly *used* an asymmetric adapter-DNA configuration to differentiate the first strand sequence reads and second strand sequence reads, as claimed. As the Federal Circuit made clear in *Personal Web*, merely arguing that the art "allows" a POSA to practice the claimed invention is not enough to show obviousness—there must be a motivation to do it. *Personal Web Techs., LLC v. Apple, Inc.*, 848 F.3d 987, 993 (Fed. Cir. 2017). This alone is sufficient to reject Petitioner's challenge to claim 19.

V. A POSA WOULD NOT HAVE COMBINED TRAVERS-075'S ROLLING CIRCLE REPLICATION EMBODIMENT WITH TRAVERS-2010'S SMRT SEQUENCING APPLICATIONS.

The Petition relies on the following combinations of references:

- Ground 1: Travers-'075 (EX1018) combined with Travers-2010 (EX1021). *See* Pet., 19 (alleging "as explained herein, *the*

combination of Travers '075 and the Travers Paper disclose or teach every element of claims 1-10, 12, 15-28.”).

- Ground 2: Travers-'075 combined with Travers-2010 and NEB Expressions (EX1014). *See* Pet., 60 (alleging “the *Travers Publications in further view of NEB expressions* renders obvious claim 11”).
- Ground 3: Travers-'075 combined with Travers-2010 and McCloskey. *See* Pet., 63 (alleging “the *Travers Publications in further view of McCloskey* renders obvious claims 13 and 14; *id.*, 65 (claims 29-30)).

All three grounds fail to establish that any of the challenged claims are unpatentable.

A. Petitioner fails to explain *why* a POSA would have combined Travers-'075's unnecessary and problematic RCR embodiment with the SMRT sequencing applications of Travers-2010.

The Petition relies entirely on disclosures of sample preparation and sequencing methods developed by Pacific Biosciences of California, Inc.

(“PacBio”): “This petition relies on a patent publication and a paper describing sample preparation and sequencing methods developed at PacBio.” Pet., 6.

Petitioner further admits that “[Travers-2010] is authored by scientists employed by PacBio (including inventors of Travers '075) and describes applications of the sequencing method described in Travers '075.” *Id.*, 7.

Both Travers-2010 and Travers-'075 disclose PacBio's DNA sequencing template design called "SMRTbell" for use in PacBio's "single-molecule real-time (SMRT™)" sequencing methods. EX1021, 1; EX1018, ¶[0141]. Travers-2010 and Travers-'075 disclose ligating hairpin adapters to each end of a template DNA molecule to generate a circular SMRTbell template, which is then ready for single-molecule sequencing in a nanowell called a zero mode waveguide (ZMW). EX1021, 4, Fig. 2; EX1018, ¶¶[0105], [0141]-[0148], Fig. 7; EX2014, ¶¶31-40.

The premise behind SMRT sequencing is monitoring and recording in real-time the incorporation of fluorescently labeled nucleotides by a single DNA polymerase as it moves along the template molecule, with each nucleotide incorporation event emitting a different color fluorophore that is captured by a camera. EX2001, 2-3; EX1021, 4; EX1018, ¶¶[0042]-[0043], Fig. 1; EX2014, ¶¶36-40. Thus, in SMRT sequencing, the strand extension of the SMRTbell template molecule (performed by a DNA polymerase enzyme that incorporates fluorescently labeled nucleotides) *is* the sequencing reaction (fluorescent flashes captured by a camera), with no prior template amplification step. *Id.* The art referred to this process as "sequencing-by-synthesis." EX2003, 5; EX2004, 8; EX2021, 1, 6; EX2022, 1; EX1018, ¶[0083]; EX2014, ¶¶41-47. A POSA would have known that SMRT sequencing's real-time sequencing by synthesis is not a template amplification step. *Id.* Travers-2010 expressly discloses that "this

protocol for template preparation requires a minimal number of steps and *does not depend on amplification*.” EX1021, 5. Travers-’075 also confirms that the SMRT sequencing methods do not amplify the SMRTbell templates before sequencing. For example, none of the working examples or claims in Travers-’075 discloses amplifying SMRTbell template molecules before SMRT sequencing. *See* EX1018, ¶¶[0139]-[0140], [0142], [0145], [0151], claim 12; EX2014, ¶¶45-46.

According to Petitioner, “Travers ’075 describes the PacBio workflow (e.g., sample preparation, sequencing, and bioinformatics analysis of sequence reads)” and Travers-2010 “disclose[s] applications of the PacBio workflow described in Travers ’075.” Pet., 19-20. The PacBio SMRT sequencing workflow, however, is different from the claimed methods, which require a separate amplification step prior to the sequencing step. *See supra* Section III; EX2014, ¶¶41-47. In an attempt to address this amplification requirement in the claimed methods, Petitioner erroneously relies on disclosures in Travers-’075 paragraph [0083] to argue that the SMRTbell template molecules are “subjected to a polymerase mediated sequencing process.” Pet., 24 (quoting EX1018, ¶[0083]; EX1002, ¶78 (same). As Dr. Satija explains, Travers-’075’s reference to a “polymerase mediated sequencing process” is an unequivocal reference to the “sequencing by synthesis” process of SMRT sequencing—*not* a template amplification step. EX2014, ¶96; EX2003, 5; EX2004, 8; EX2021, 1, 6; EX2022, 1. The Petition fails to appreciate

this basic concept of SMRT sequencing, which would have been well known to a POSA. *Id.*

Petitioner then proposes combining a RCR step purportedly disclosed in Travers-'075 with the SMRT sequencing applications of Travers-2010. *See e.g.*, Pet., 24-25 (“[Travers-'075] explains that ‘rolling circle replication’ of SMRTbell templates produces concatemer molecules ... these ‘replicated products may then be employed directly as template molecules in a template dependent sequencing process.’” (citing EX1018, ¶[0122])); *see also, id.*, 32 (“concatemers produced from an intermediate amplification step ... may be sequenced using the disclosed template-dependent sequencing process.” (citing EX1018, ¶[0122])). In its Decision on Institution, the Board confirmed that Petitioner’s obviousness theory depends on this RCR embodiment:

As we understand it, these molecules may be subject to SMRT sequencing directly, or they *may be amplified to produce concatemer molecules*, which are then subject to SMRT sequencing. *Petitioner relies on the latter in this case*, and we find that showing sufficient for institution purpose.

Paper 14, 19 (internal citation omitted).

Discussed below, neither Petitioner nor Dr. Quackenbush provides any explanation of *why* a POSA would have been motivated to combine the PacBio

SMRT sequencing applications of Travers-2010 with the unnecessary and problematic RCR embodiment mentioned in Travers-'075. Indeed, Petitioner *does not even allege any benefit* in implementing the mentioned RCR embodiment from Travers-'075 with the teaching of Travers-2010. *Winner Int'l. Royalty Corp. v. Wang*, 202 F.3d 1340, 1349 n8 (Fed. Cir. 2000) (for obviousness, “the benefits [of a proposed art combination], both lost and gained, should be weighed against one another.”) Here, there are no benefits to Petitioner’s proposed RCR embodiment because it destroys the ability to even use SMRT sequencing.

Patent Owner’s expert Dr. Satija identifies at least four critical technical problems with Petitioner’s RCR embodiment that would render it incompatible with the SMRT sequencing applications of Travers-2010 and would require undue experimentation to achieve an embodiment within the scope of any challenged claim—critical issues that Petitioner and Dr. Quackenbush completely ignored. These issues render Petitioner’s proposed embodiment unworkable and would have dissuaded a skilled artisan from combining the RCR embodiment mentioned in Travers-'075 with Travers-2010, in the first place. EX2014, ¶¶96-100. At bottom, all three of Petitioner’s grounds rely explicitly on the *combination* of an RCR embodiment mentioned in Travers-'075 together with Travers-2010 (Pet., 19, 60, 63, 65)—a combination that a POSA would have rejected in view of a multitude of problems. And without any reason to combine these references, Petitioner’s entire

Petition should likewise be rejected.

B. A POSA would not have had any reason to modify the PacBio SMRT sequencing workflow—which does not use a template amplification step—with Travers-'075's RCR step.

Before 2012, artisans understood that a major *advantage* of the PacBio SMRT sequencing workflow was that it did not use a template amplification step. EX2014, ¶¶101-112. Travers-2010 itself discloses that “this protocol for template preparation requires a minimal number of steps and *does not depend on amplification.*” EX1021, 5. Travers-'075 further discloses that “the constructs of the invention are described primarily, and preferably, for *use directly as templates* for, e.g., sequencing applications....” EX1018, ¶[0122].

This advantage of PacBio's SMRT sequencing workflow was well known in the art. For example, Niedringhaus disclosed that an “advantage” of SMRT sequencing is that there is “*no amplification* of sequencing fragments. EX2002, 4330, Table 2; EX2014, ¶102. Schadt similarly disclosed that “*SMRT sequencing does not require routine PCR amplification* needed by most SGS systems, thereby *avoiding systematic amplification bias.*” EX2023, 7. As Liu explained in 2011: “[t]he key difference between NGS and single-molecular sequencing technology [SMRT sequencing] is that, in NGS, DNA molecules have to be amplified before sequencing, while *the latter* enables us to sequence a single DNA molecule; thus, *avoid the time, error and cost associated with sample preparation and*

amplification.” EX2024, 4. Liu also explained that by eliminating a template amplification step, SMRT sequencing “*lowers the risk of having systematic amplification bias.*” *Id.*, 171; *see also*, EX2025, 1; EX2054, 1; EX2022, 5; EX2006, Table 1; EX2004, 1065, Table 1; EX2005, 2, 9; EX2014, ¶¶101-112.

In its Decision on Institution, the Board agreed with this indisputable fact, stating “we agree with Patent Owner that the Travers references describe *SMRT sequencing, which does not require amplification before sequencing*, as the primary and preferred embodiment.” Paper 14, 18.

Nowhere in the Petition or Quackenbush declaration does Petitioner even attempt to explain *why* a POSA would have allegedly tossed aside the established and advantageous SMRT sequencing workflow—which does not use a template amplification step—in favor of modifying it with Travers-’075’s RCR step. For obviousness, it is not enough for Petitioner to argue that a POSA *could* incorporate RCR into the PacBio SMRT sequencing workflow; Petitioner must show that a POSA *would* have done so. *Personal Web*, 848 F.3d at 994; *In re Gordon*, 733 F.2d 900, 902 (Fed. Cir. 1984).

Petitioner may argue that its RCR embodiment need not be “combined” with PacBio’s SMRT sequencing workflow because Travers-’075 discloses a hypothetical RCR embodiment. This argument fails both scientifically and legally. First, Petitioner unequivocally relies on “the *combination* of Travers ’075 and the

Travers Paper,” arguing that “Travers ’075 describes the PacBio workflow” while Travers-2010 discloses “applications of the PacBio workflow described in Travers ’075,” and that a POSA would have thus “*combined* the teachings of the Travers Publications.” Pet., 19-20. Accordingly, Petitioner’s grounds all indisputably rely on a *combination* of Travers ’075 and Travers-2010; a combination Petitioner completely fails to support.

It is indisputable that the PacBio SMRT sequencing workflow does *not* use a template amplification step—not using an amplification step was a feature that the prior art consistently hailed as an advantage, including both Travers-’075 and Travers-2010. EX2014, ¶¶101-112; EX1018, ¶[0122]; EX1021, 5. “[A reference’s] statements regarding *preferences are relevant* to a finding regarding *whether a skilled artisan would be motivated* to combine that reference with another reference.” *Polaris Indus. v. Arctic Cat, Inc.*, 882 F.3d 1056, 1069 (Fed. Cir. 2018).

Moreover, a POSA would have known that introducing an unnecessary amplification step into the amplification-free SMRT sequencing workflow as Petitioner proposes would only introduce *more errors* into the system, making SMRT sequencing *worse*. EX2014, ¶¶110-111. For example, the polymerases used in rolling circle replication reactions would certainly introduce errors in the amplification process. EX2017, 3; EX2068, 6; EX2014, ¶111. A POSA would

have had no reason to introduce this additional likelihood of generating sequence errors into the SMRT sequencing workflow. This is especially so when considering that SMRT sequencing was already known in the art for having the “highest raw error rates of NGS methods.” EX2006, 5; EX2014, ¶¶113-116.

Thus, the art’s explicit preference for omitting template amplification is directly relevant to a POSA’s *lack of a reason* to combine Petitioner’s RCR embodiment with the SMRT sequencing workflow of Travers-’075 and Travers-2010. *Polaris*, 882 F.3d at 1069. Whether “combining disclosures from multiple references, combining multiple embodiments from a single reference, or selecting from large lists of elements in a single reference, there *must be a motivation to make the combination and a reasonable expectation that such a combination would be successful*, otherwise a skilled artisan would not arrive at the claimed combination.” *In re Stepan Co.*, 868 F.3d 1342, 1346 n.1 (Fed. Cir. 2017).

In its Decision on Institution, the Board relied on *Merck & Co., Inc. v. Biocraft Labs., Inc.*, 874 F.2d 804 (Fed. Cir. 1989), when noting that “unpreferred embodiments[] must be considered” for obviousness. Paper 14, 18. The facts in *Merck* are distinguishable on multiple levels. First, in *Merck*, the issue on appeal was a *single reference* obviousness challenge in which Biocraft argued that the disclosures in a single reference (the ’813 patent) rendered Merck’s patent claims obvious. *Merck*, 874 F.3d at 806. Here, however, Petitioner expressly relies on the

combination of Travers-'075 and Travers-2010 for all of its grounds. Pet., 6, 19.

Thus, while a motivation to combine may not have been an issue for the single reference '813 patent in *Merck*, such is not the case here. Petitioner was required to articulate which specific disclosures in Travers-'075 a POSA would have modified or combined with which specific disclosures in Travers-2010 (or vice versa), and why the POSA would have done so. *Personal Web*, 848 F.3d at 994; *Metalcraft of Mayville, Inc. v. The Toro Co.*, 848 F.3d 1358, 1367 (Fed. Cir. 2017). The Petition makes no such showing.

Second, in *Merck*, the single reference '813 patent showed that *all* of the disclosed embodiments—even the so-called “unpreferred embodiments”—would successfully produce the claimed diuretic formulation, without requiring “random variation of numerous parameters.” *Merck*, 874 F.2d at 807. That is, the prior art showed not only that the unpreferred embodiments *could* be tried, but that they *would* work. *Id.* That is not the case here, because as detailed below, the combination of Travers-'075 and Travers-2010 fails to provide any teaching of how to successfully incorporate an RCR step into the SMRT sequencing workflow. *Id.*; EX2014, ¶¶122-127. Indeed, Travers-'075 would not have enabled a POSA to practice Petitioner's rolling circle replication embodiment without undue experimentation. *Raytheon Tech. v. General Electric Co.*, 993 F.3d 1374, 1381 (Fed. Cir. 2021); *see infra*, Section V(D)(2); EX2014, ¶¶206-210.

Even if applying *Merck*, the rule is that unpreferred embodiments must be *considered*, not that they must be *accepted*. As discussed in detail below, a POSA “considering” Petitioner’s RCR embodiment would have rejected it because it would “destroy the basic objective of the [invention].” *Trivascular Inc. v. Samuels*, 812 F.3d 1056, 1068 (Fed. Cir. 2016).

A POSA would have expected Petitioner’s concatemer embodiment to have numerous problems associated with multiple polymerase binding sites, complex concatemer structures, DNA diffusion principles, and geometrical constraints of SMRT sequencing’s zero mode waveguides. EX2014, ¶¶122-127. Petitioner fails to deal with these critical issues or even attempt to explain how a POSA relying on the asserted references would have allegedly practiced the proposed combination embodiment.

C. A POSA would have known that Petitioner’s rolling circle replication embodiment is incompatible with SMRT sequencing on zero mode waveguides (ZMWs).

Before 2012, SMRT sequencing was known for having the highest error rate of all NGS platforms, which was only overcome by re-sequencing the same template multiple times using the circle sequencing of Travers-2010 and Travers-’075. *See e.g.*, EX2003, 5 (SMRT sequencing’s “high raw error rate can be overcome by ligating a hairpin oligonucleotide to each end of the DNA, creating a circular template (called SMRTbell for single molecule real time), and then

repeatedly sequencing the same molecule.”); EX2006, 5; *see also* EX2032, 12; EX2033, 14; EX2034, Table 3; EX2004, 9; EX2002, 5; EX2035, 6; EX2014, ¶¶113-116.

Petitioner’s hindsight-based RCR embodiment attempts to accomplish this resequencing by generating linear concatemer templates containing numerous copies of the target DNA in order to effectively apply it in the SMRT sequencing applications of Travers-2010. However, Petitioner’s RCR embodiment would have created critical problems when used in SMRT sequencing. EX2014, ¶¶122-127.

- *Problem 1:* the RCR linear concatemers would have contained repeating copies of the sequencing primer/polymerase binding site and would bind multiple polymerases per concatemer molecule. Multiple polymerases catalyzing sequencing by synthesis reactions in the same ZWM renders the sequencing data completely unreadable.
- *Problem 2:* the smaller-sized RCR replication products would not possess sufficient copies of the target DNA required for SMRT sequencing, rendering them unsuitable for Travers-2010’s SMRT sequencing applications.
- *Problem 3:* due to their slower diffusion, the large linear concatemers would not efficiently compete for occupancy in the ZMW wells for

SMRT sequencing; smaller unusable templates would swamp out the larger molecules for ZMW occupancy.

- *Problem 4*: the large linear concatemers would form complex secondary structures, posing additional physical constraints on their ability to even fit into the narrow ZMW wells for SMRT sequencing.

See EX2014, ¶¶14-27, 122-127.

Clearly, Petitioner's linear concatemer approach introduces several new problems—with absolutely no upside—compared to the circular template approach. *Id.* As a result, a POSA would not had any reason to jettison the straightforward resequencing available on circular templates to implement a sequencing method using concatemers where such resequencing would be rarely if ever be achieved. EX2014, ¶127.

1. Problem 1: The linear concatemer template in Petitioner's RCR embodiment would have multiple, repeating copies of the primer/polymerase binding site, which would result in unreadable sequence data.

Petitioner's linear concatemer products of its RCR embodiment would have had multiple primer/polymerase binding sites throughout the concatemer—at least one site for each copy of the SMRTbell template in the concatemer. EX2014, ¶¶128-137; EX1021, Abstract; EX1002, ¶35. This would have created a critical problem with SMRT sequencing's single-molecule design. EX2014, ¶¶128-137.

The SMRT sequencing workflow requires first pre-incubating the SMRTbell

DNA template with the sequencing primer and polymerase enzyme, allowing the molecules to form a 1:1:1 primer/template/polymerase complex, and then distributing the complexes onto a ZMW array for sequencing. EX2014, ¶129.

Travers-2010 discloses incubating biotinylated DNA polymerases with primer and template to form a “*ternary* complex” which is then immobilized onto the ZMW arrays. EX1021, 2; EX1018, ¶[0043]; EX2026, 2; EX2014, ¶129.

Of paramount importance, the SMRT sequencing workflow requires distributing the primer/template/polymerase ternary complex at one complex per ZMW well. EX2001, 3 (“A *single molecule of DNA template-bound Φ29 DNA polymerase* is immobilized at the bottom of a ZMW.”); EX2002, 4 (“immobilizing a *single functioning DNA polymerase* at the bottom of *each ZMW*.”); EX2021, 4; EX2014, ¶130. If two or more polymerases generate synthesis products in a single ZMW, the multiple florescent pulses from the different polymerases would be indistinguishable and produce unusable data. EX2001, 4; EX2014, ¶133. After all, “SMRT sequencing” stands for “*single-molecule* real-time [] sequencing.” EX1021, 1; EX1018, ¶[0043].

A POSA would have known that the SMRTbell circular template of Travers-’075 and Travers-2010 contains a *single* primer/polymerase binding site, and thus can form the desired 1:1:1 ternary complex to enable *single molecule* sequencing. EX1021, Abstract (SMRT bell template “provides a *site* for primer binding.”), Fig.

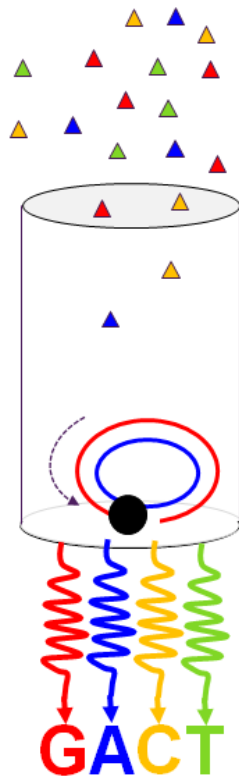
1 (SMRTbell template includes “a single-stranded *region* to which a *sequencing primer* can bind.”); EX1018, ¶[0067] (SMRTbell templates “provide *the primer* and polymerase *binding site*.”) EX2014, ¶131.

However, in Petitioner’s RCR embodiment, the linear concatemer template would contain multiple, repeating copies of the primer/polymerase binding site. EX2014, ¶132. A POSA reading Travers-2010 would have expected even the *smallest* RCR concatemer to have at least two primer/polymerase binding sites. *Id.*, ¶133. For example, Travers-2010 discloses a range of RCR product sizes, the smallest of which is about 500 nucleotides long. EX1021, Fig. 3. Given the SMRTbell circle template length of 336 nucleotides, this 500 nucleotide product represents about 1.5 times around the circle (about 3 target copies), and thus would include at least two copies of the primer/polymerase binding site. *Id.*; EX1021, 4. In Petitioner’s RCR embodiment, the primer and polymerase molecules would bind the linear concatemer template at *multiple sites* along the concatemer. *Id.* This would result in a multi-complex of DNA template bound by multiple primer and polymerase molecules. *Id.*

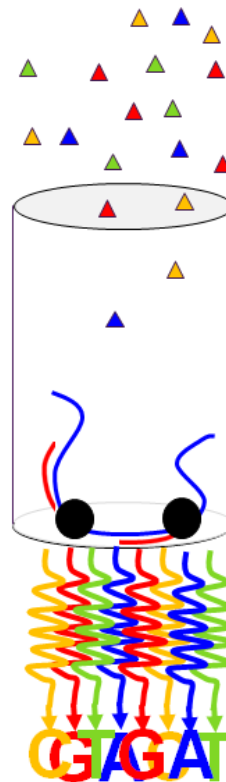
A POSA would have immediately recognized the futility of attempting to sequence this multi-primed, multi-polymerase concatemer multi-complex in the SMRT sequencing ZMW array. EX2014, ¶¶134-136. The SMRT sequencing photodetector would be presented with numerous, concurrent nucleotide flashes

occurring from the multiple, simultaneous polymerase extension reactions taking place in each ZMW. *Id.*; EX1021, 4-5 (“The fluorescence signal for *each* of the analogs is observed by a CCD camera.”); EX1018, ¶¶[0045], [0141]. These concurrent nucleotide flashes would be detected *not* as amplification of the signal, but rather as readings of different nucleotides all at once and therefore the order of the nucleotides (i.e., the *actual sequence*) would be *impossible* to determine. EX2014, ¶¶134-137. This is shown in Dr. Satija’s figure, copied below:

SMRTbell circle sequencing
(no template amplification)



Petitioner's RCR embodiment



EX2014, ¶135.

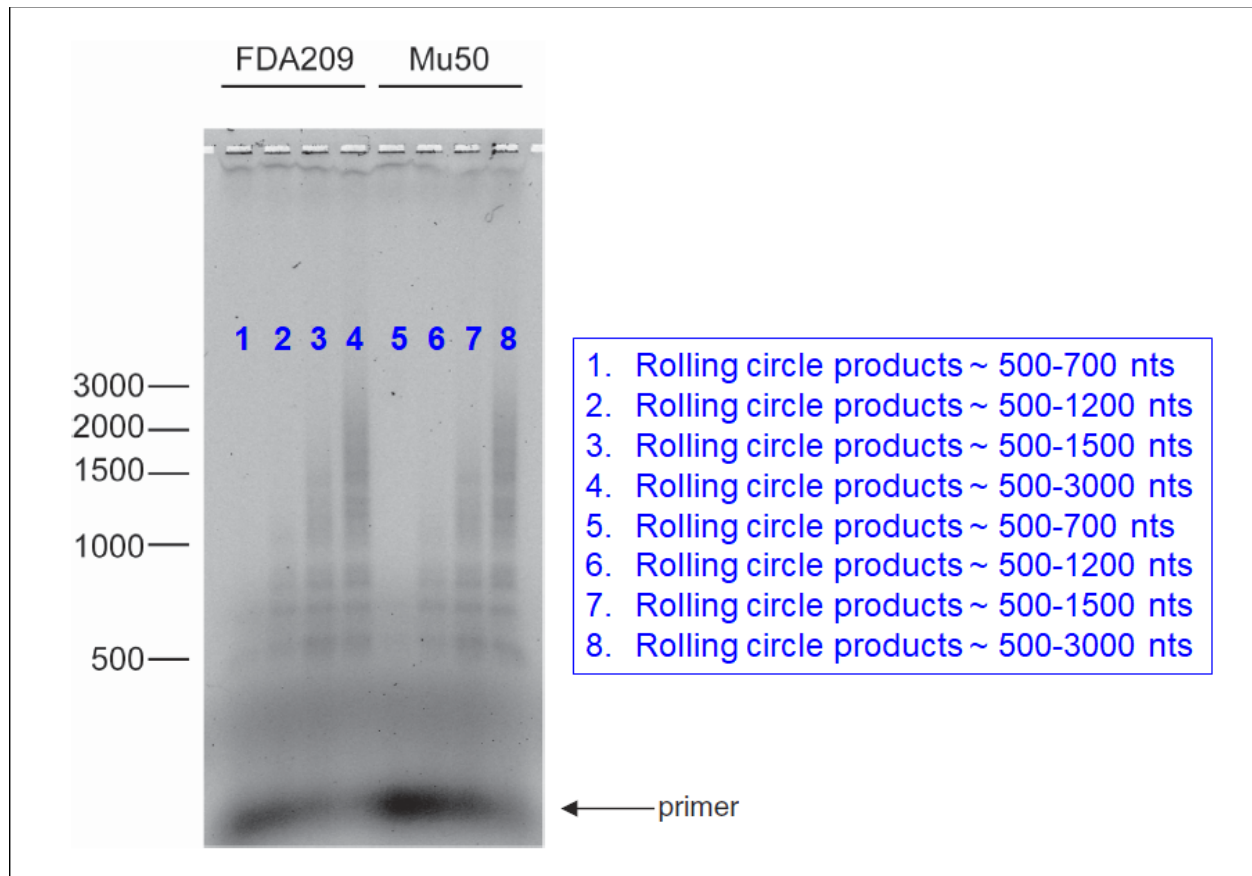
Indeed, the SMRT sequencing system would register *every* incorporation

event detected within an individual ZMW as part of the same linear sequence.

EX2014, ¶136. Obviously, if those signals were coming from different polymerases, the signals would create a jumbled mess. *Id.* And this problem only gets worse for larger concatemers with more polymerase binding sites. *Id.* This alone would have been reason enough for a POSA to *not* incorporate Petitioner's RCR embodiment into SMRT sequencing. *Id.*, ¶137. No POSA would be motivated to modify a sequencing workflow to *make it unusable. Id.*

2. Problems 2 and 3: A POSA would have expected Petitioner's RCR embodiment to produce a vast range of concatemer sizes, resulting in unwanted diffusion bias towards small templates into the ZMWs.

A POSA considering Petitioner's RCR embodiment would have immediately recognized problems with DNA diffusion and SMRT sequencing's ZMW arrays. EX2014, ¶¶138-169. Petitioner and Dr. Quackenbush fail to account for the fact that a RCR reaction produces a vast mixture of product sizes. *Id.*, ¶¶139-144. This is evident in Petitioner's own references. For example, Dr. Satija annotated Figure 3 from Travers-2010, which depicts a range of RCR product sizes from as small as 500 nucleotides to 3,000 nucleotides long:

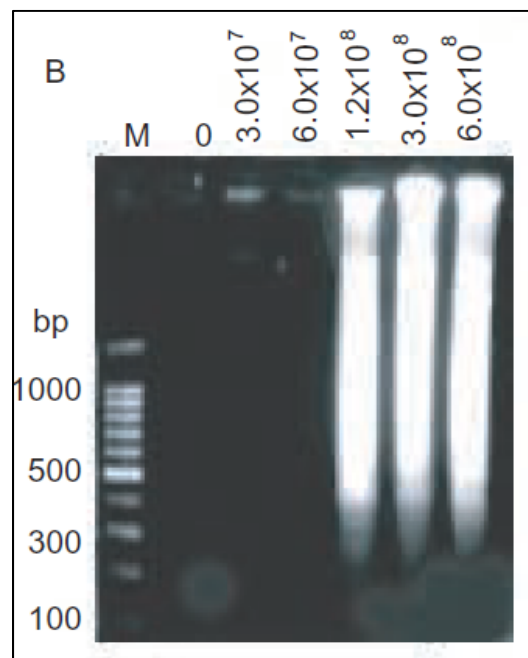


EX1021, Fig. 3 (annotated); EX2014, ¶141.

As Dr. Satija explains, a POSA would have known that RCR initially produces smaller products (e.g., 500 nucleotides long in Travers-2010's Figure 3), with gradual accumulation in product sizes over time (e.g., up to 700, 1,200, 1,500, and 3,000 nucleotides in Travers-2010's Figure 3). EX1021, Fig. 3 (annotated); EX2014, ¶141. As Travers-2010 shows, the smaller species are present at every time point, even as longer products form and accumulate at later time points. EX1021, Fig. 3 (annotated); EX2014, ¶141. Ultimately, this results in a smear of product sizes representing a vast size difference in potential templates for a

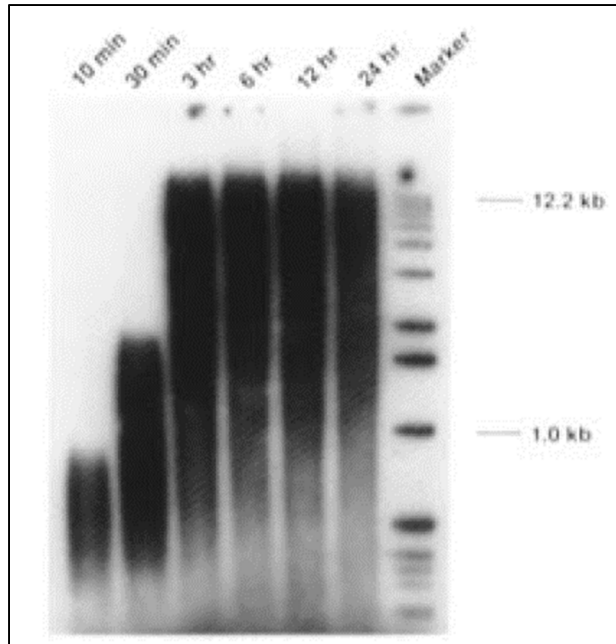
sequencing reaction. EX1021 Fig. 3, lanes 4 and 8; EX2014, ¶¶141-142.

Travers-2010's RCR disclosure is consistent with the prior art. For example, Demidov explained in 2002 that "these [rolling circle replication] products generally exhibit a *wide essentially continuous distribution over length* and therefore cannot be resolved by gel electrophoresis *yielding a broad smear* of the high molecular weight DNAs (Figure 3B)." EX2036, 2. Demidov's Figure 3 looks very similar to Travers-2010's Figure 3, depicting a smear of product sizes accumulating from rolling circle replication:



EX2036, Fig. 3 (excerpted); EX2014, ¶142.

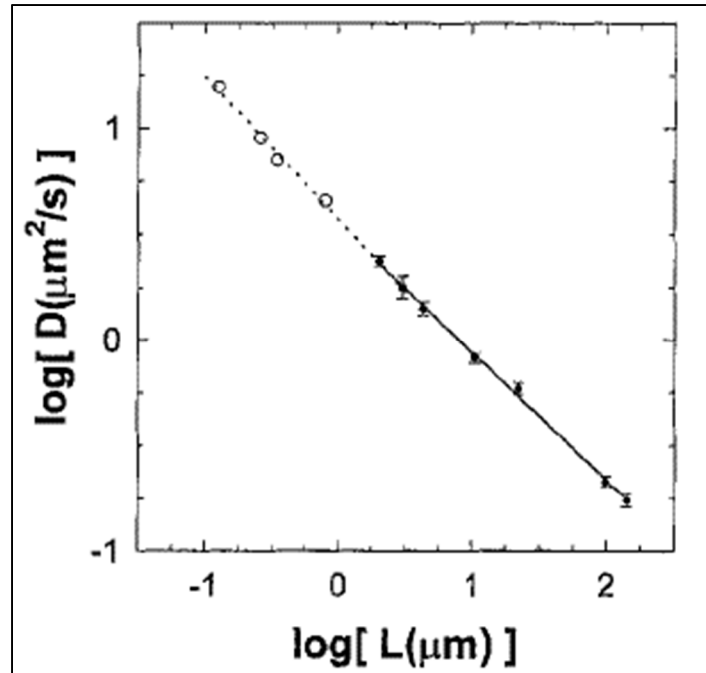
Liu disclosed a similar smear of RCR products that accumulates over time, with product sizes ranging from hundreds of nucleotides to over 12,000 nucleotides long:



EX2037, Fig. 6; *see also*, EX2038, Fig. 3; EX2014, ¶143.

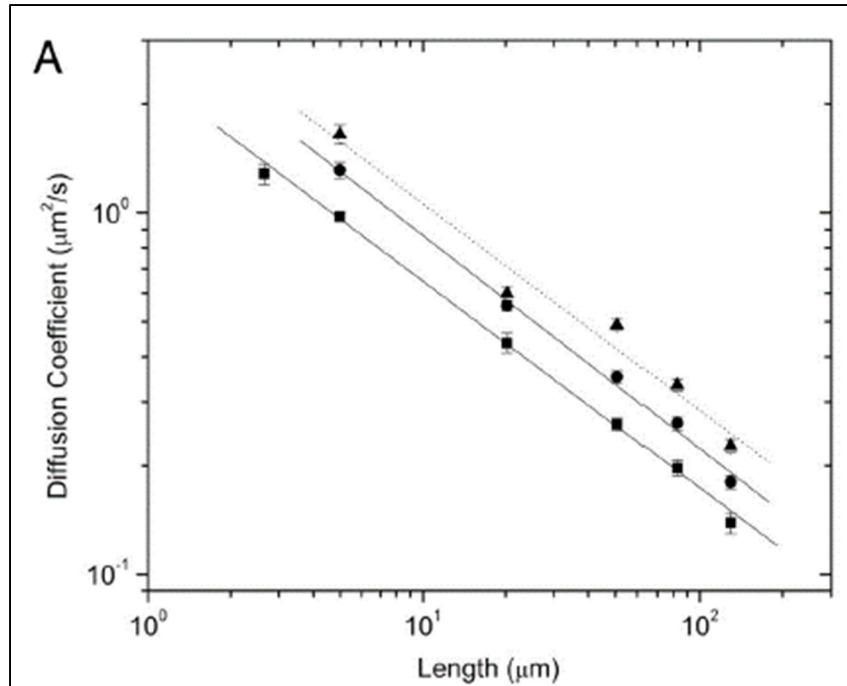
A POSA would have known that this smear of reaction products from RCR would create significant problems if combined with the SMRT sequencing applications of Travers-2010, due (at least in part) to basic principles of DNA diffusion. EX2039, Table 1, Fig. 1; EX2040, 4, Fig. 1; EX2041, 3; EX2014, ¶145.

Before 2012, a POSA would have known that longer DNA molecules have lower diffusion coefficients and diffuse less efficiently than smaller DNA molecules. EX2014, ¶145; EX2056, 20. As Grosberg disclosed in 2010, DNA's “*diffusion coefficient, which we denote as D, decreases with DNA length N.*” EX2041, 3. Smith 1996 disclosed this relationship graphically, demonstrating that a 10-fold increase in DNA molecule length “L” (1 log on x-axis) results in approximately a 5-fold decrease in diffusion coefficient “D” (0.5 log on y-axis):



EX2039, Fig. 1; *see also, id.*, Table 1; EX2014, ¶¶145-149.

Robertson 2006 further demonstrated that the relationship between diffusion coefficient and DNA molecule length remained consistent in linear, relaxed circular, or supercoiled structures. EX2040, 4. Robertson also presented this relationship graphically, again showing that an increase in DNA molecule length (x-axis) directly corresponds with a decrease in diffusion coefficient (y-axis):



EX2039, Fig. 1 (depicting linear DNA (squares), relaxed circular DNA (circles), and supercoiled DNA (triangles)); EX2014, ¶149.

a. Problem 2: SMRT sequencing’s DNA diffusion bias for small templates would result in unreliable data.

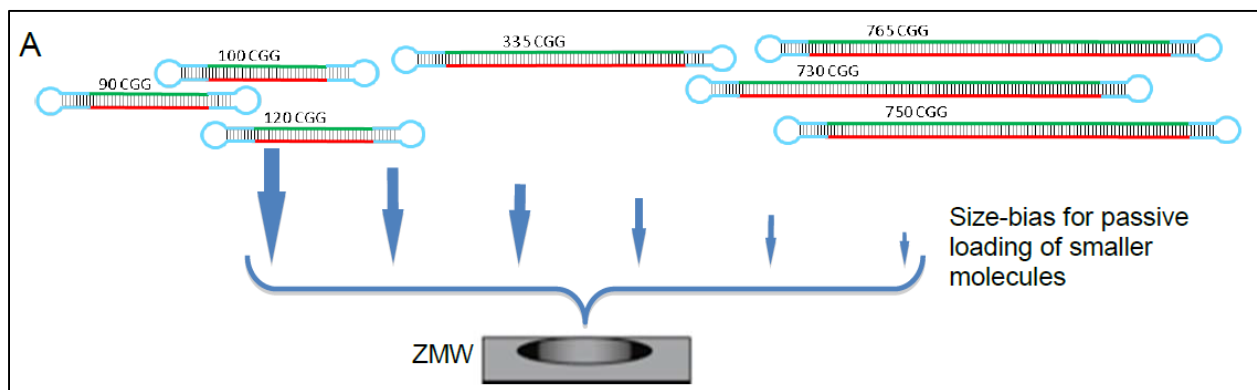
Given this understanding in the art, and in addition to the multi-polymerase issue of Problem 1, *supra*, a POSA would have expected Petitioner’s RCR embodiment to be incompatible with SMRT sequencing. Because of the diffusion characteristics of shorter DNA templates, the majority of potentially useable ZMW would be occupied by smaller DNA templates, without sufficient redundancy for resequencing. EX2014, ¶¶150-164; EX2067, 11-12. The art taught that in SMRT sequencing, “the DNA polymerase and DNA template to be sequenced are delivered to ZMWs via a *random diffusion process*.” EX2023, 5; EX2014, ¶158. In

view of the known relationship between a DNA molecule's size and its diffusion rate, a POSA would have expected that the diffusion process for loading ZMWs is a race for ZMW occupancy between the vast range of different-sized RCR products, with the much smaller products generally winning the diffusion race. EX2023, 5; EX2014, ¶¶158-163. Yet, these small templates would lack sufficient concatemer copies to conduct redundant sequencing required to overcome the very high error rate of the PacBio system, rendering the sequencing data they produce unreliable. EX2014, ¶163.

Indeed, contemporaneous art³ reflected these very concerns. For example, Zhang described “*a loading bias against large DNA fragments in the PacBio*

³ While some of this evidence is dated after March 20, 2012, Petitioner has repeatedly argued in other IPRs that post-filing evidence is relevant for “assessing obviousness, including ... factors such as *enablement*, the *state of the art* in general, and a *POSA's understanding of the prior art*.” EX2043, 3 (citing *Teva Pharms. USA, Inc. v. Sandoz Inc.*, 906 F.3d 1013, 1025 (Fed. Cir. 2018); *Bristol-Myers Squibb Co. v. Ben Venue Labs., Inc.*, 246 F.3d 1368, 1379 (Fed. Cir. 2001); *Gould v. Quigg*, 822 F.2d 1074, 1078 (Fed. Cir. 1987)); EX2044, 3. In any event, the relationship between diffusion coefficient and DNA length was indisputably known before the critical date.

sequencing process.” EX2028, 1. As Zhang explained, “[s]maller PCR products will load into the PacBio wells with a greater efficiency than larger PCR products” and that this ultimately “result[s] in *poor quality or incomplete sequences for the larger PCR products.*” *Id.*; EX2014, ¶¶159-160. Coupland similarly taught that “[t]here is a *loading inefficiency of larger molecules because of their lower diffusion coefficient*, as well as the disparity between the molecule’s *hydrodynamic radius and the very small zero-mode waveguide (ZMW).*” EX2042, 4. Loomis provided a schematic depicting this known diffusion issue:



EX2046, Fig. S4 (excerpted); *see also* EX2005, 7; EX2014, ¶¶161-162.

SMRT sequencing’s DNA diffusion bias issues persisted well after 2012. Fichot disclosed that “one *known bias of the PacBio platform is the preferential loading of shorter sequences into zero-mode waveguides ... thus biasing the resulting community toward members having shorter sequences...*” EX2022, 4. Even as late as 2017, Larkin explained, “[a] critical *limiting step of SMRT sequencing is the loading of long DNA templates into ZMW confinements.*”

EX2057, 2. Larkin further disclosed that “[m]ismatch between the equilibrium hydrodynamic diameter of long DNAs ... and the ZMW diameter ... creates an *entropic barrier to molecular entry under diffusive conditions*. Under diffusive conditions this barrier *biases entry of short DNA templates over long ones*,” *Id.*; EX2014, ¶163.

Thus, a POSA would have known that in the milieu of Petitioner’s RCR products, basic DNA diffusion principles would create a bias for the smaller DNA products to diffuse into Travers’ ZMW wells over the larger products. *Id.* A POSA would have known that the smaller DNA products, however, would not provide a sufficient number of target DNA copies to be able to re-sequence the target DNA sufficiently and achieve a higher EQV, as taught in Travers-2010. EX1021, Fig. 7; EX2014, ¶¶117-121, 163; EX2055, 1. This further undermines any reason to use an RCR embodiment because these problems simply are not present when using similarly sized circular templates for sequencing as disclosed in Travers-2010. EX2014, ¶167.

Moreover, sequencing shorter, truncated RCR products would not even meet the elements of claim 1-21, which require “*confirming the presence* of at least one sequence read derived from *each* of the original first *and* second strands of the adapter-DNA molecules.” EX1001, 37:44-46; EX2014, ¶164. The shorter, truncated products would not offer the ability to confirm the presence of a

sequence read from *each* strand, as claimed, because they would not contain a complete copy of each strand. *Id.*

b. Problem 3: Large concatemer products would lose the diffusion race for ZMW occupancy.

A POSA would have known that the smaller, truncated products from RCR would essentially be useless in the SMRT sequencing applications of Travers-2010. *See supra*, “Problem 2.” The other end of the RCR product spectrum is no better. While the larger RCR concatemers *might* contain multiple copies of the target DNA, they would be outcompeted for ZMW occupancy by the smaller products due to SMRT sequencing’s known DNA diffusion bias. EX2041, 3; EX2039, Fig. 1; EX2040, Fig. 1; EX2014, ¶¶165-169. A POSA would have viewed combining Petitioner’s RCR embodiment with the SMRT sequencing applications of Travers-2010 as futile, and simply would not have had any reason to combine the embodiments. *Gordon*, 733 F.2d at 902; *Trivascular*, 812 F.3d at 1068.

On the other hand, if the POSA were to follow the normal SMRT sequencing workflow of Travers-2010 and Travers-’075 that uses single molecule circle sequencing *without an amplification* step, the issues of multi-complexes, RCR product sizes, diffusion bias, and target DNA copy number could be avoided. EX2014, ¶167.

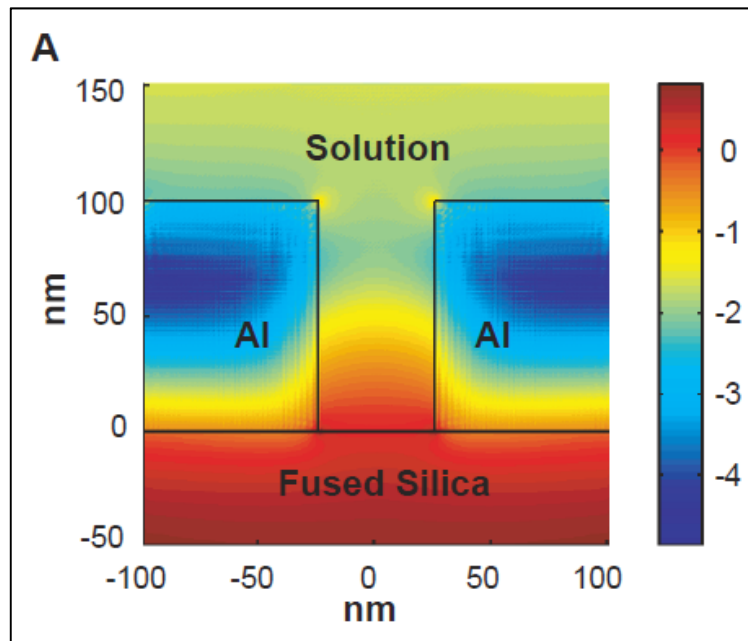
Neither Petitioner nor Dr. Quackenbush address these issues. *Raytheon*, 93

F.3d at 1381. In fact, on cross-examination, Dr. Quackenbush adamantly and repeatedly testified that he did *not* address basic principles of DNA diffusion in his declaration, and he certainly did not address SMRT sequencing's known DNA diffusion bias issues. *See e.g.*, EX2045, 15:24-25 ("I don't believe I talk about diffusion coefficients in my declaration...."); 18:6-7 ("Again, I don't believe I discussed DNA diffusion coefficients in the context of my report...."); *See also id.*, 18:16-17; 23:8-9; 24:1-2; 30:12-13; 31:12-18; EX2014, ¶168. These unaddressed issues alone are sufficient for the Board to conclude that Petitioner has failed to meet its burden.

3. Problem 4: The geometry of SMRT sequencing's ZMWs posed physical constraints on Petitioner's RCR embodiment.

Before March 20, 2012, artisans were aware that SMRT sequencing's ZMWs have a very narrow diameter, mere tens of nanometers across. *See e.g.*, EX2029, Fig. 2 ("a [ZMW] 50 nm in diameter"); EX2027, 5 ([ZMWs] ... are cavities of 10–50 nm in diameter"); *see also* EX2030, Abstract; EX2001, 134; EX2021, 1; EX2023, 5; EX2031, 1; EX2014, ¶¶170-197. The narrow diameter of ZMWs was by design, as their narrow geometry prevented the excitation laser light from passing up through the entire ZMW well and exciting any unincorporated fluorescently labeled nucleotides (which thus reduced background fluorescence). EX2021, 3; EX2023, 5; EX2014, ¶¶170-176. As Eid explained in 2009, the ZMW

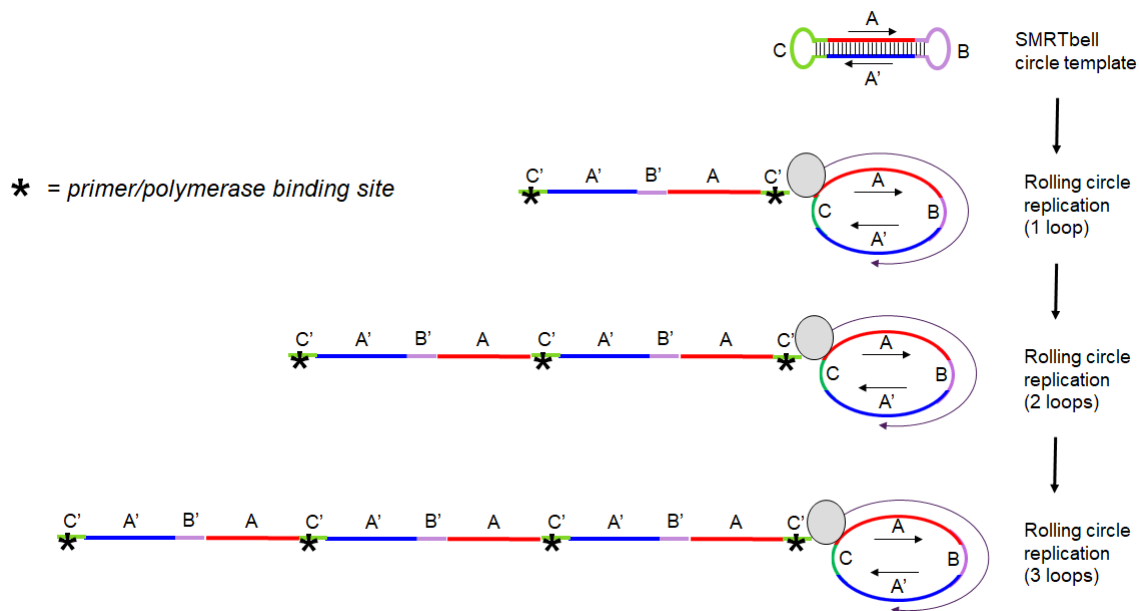
geometry “provides excitation confinement in the zeptoliter (10^{-21} liter) regime, enabling detection of individual phospholinked nucleotide substrates against the bulk solution background as they are incorporated into the DNA strand by the polymerase.” EX2001, 3. Levene provided a figure depicting laser light excitation in a 50 nm ZMW:



EX2029, Fig. 2 (excerpt); EX2014, ¶174; *see also* EX2001, Fig. 1; EX2023, Fig. 2.

In the SMRT sequencing workflow, single molecule circular templates (SMRTbell templates) are combined with a single polymerase enzyme are then added to the ZMW array, after which the complex diffuses through the aqueous medium into the ZMW well, where it is immobilized on the bottom of the well for sequencing (one complex per ZMW well). EX1021, 2; EX2001, 3; EX1018, ¶¶[0140]-[0149]; EX2014, ¶175.

In addition to critical Problems 1-3 above, a POSA would have expected the narrow ZMWs to present physical size constraints on the larger concatemer products of Petitioner's RCR embodiment. EX2014, ¶¶177-178. As Dr. Satija explains, a POSA would have known that linear concatemers produced from Petitioner's RCR embodiment would contain many repeating stretches of complementary DNA sequences. EX2014, ¶178. Petitioner also admits that "the concatemer molecules would have the same sequence (e.g., barcode and target molecule)...." Pet., 34. Dr. Satija's figure below depicts these repeating complementary stretches as A and A' coming off a rolling circle in linear fashion:



EX2014, ¶178.

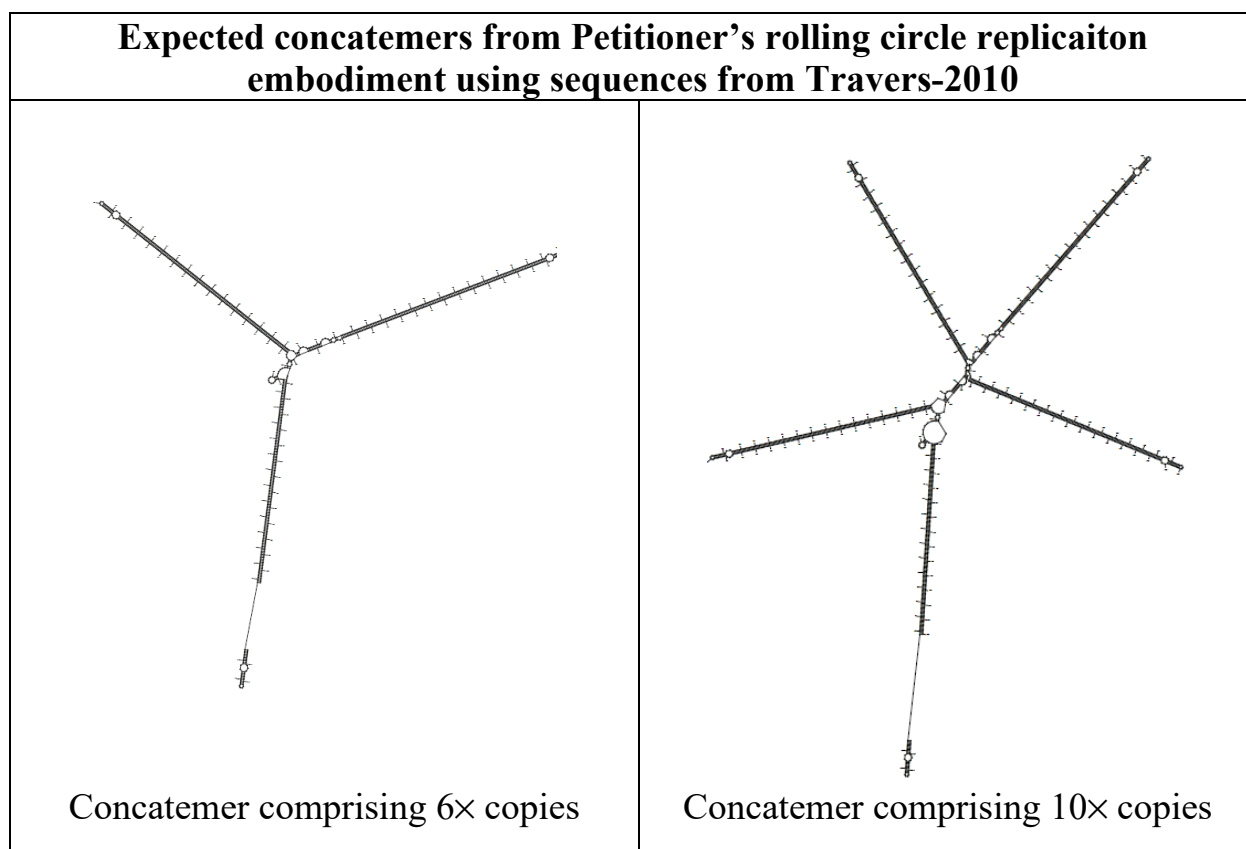
As discussed above, because of SMRT sequencing's known 10-20% error rate, a POSA using the PacBio approach would have understood the need to

sequence many copies of the template DNA to achieve sufficiently high sequencing redundancy and thus a higher EQV. EX2014, ¶179; EX1021, 6; EX2006, 5. In SMRT sequencing with the circular templates of Travers-'075 and Travers-2010, this is achieved by re-sequencing *the same SMRTbell circular template* multiple times. *Id.* To achieve this in Petitioner's RCR embodiment, however, would have required sequencing a long, linear concatemer molecule comprising many repeating sequences of complementary DNA. *Id.* As Dr. Satija explains, a POSA would have known that these repeating complementary stretches of DNA would hybridize to form complex secondary structures that would have significant difficulties even fitting into the narrow ZMW wells for SMRT sequencing. EX2014, ¶179.

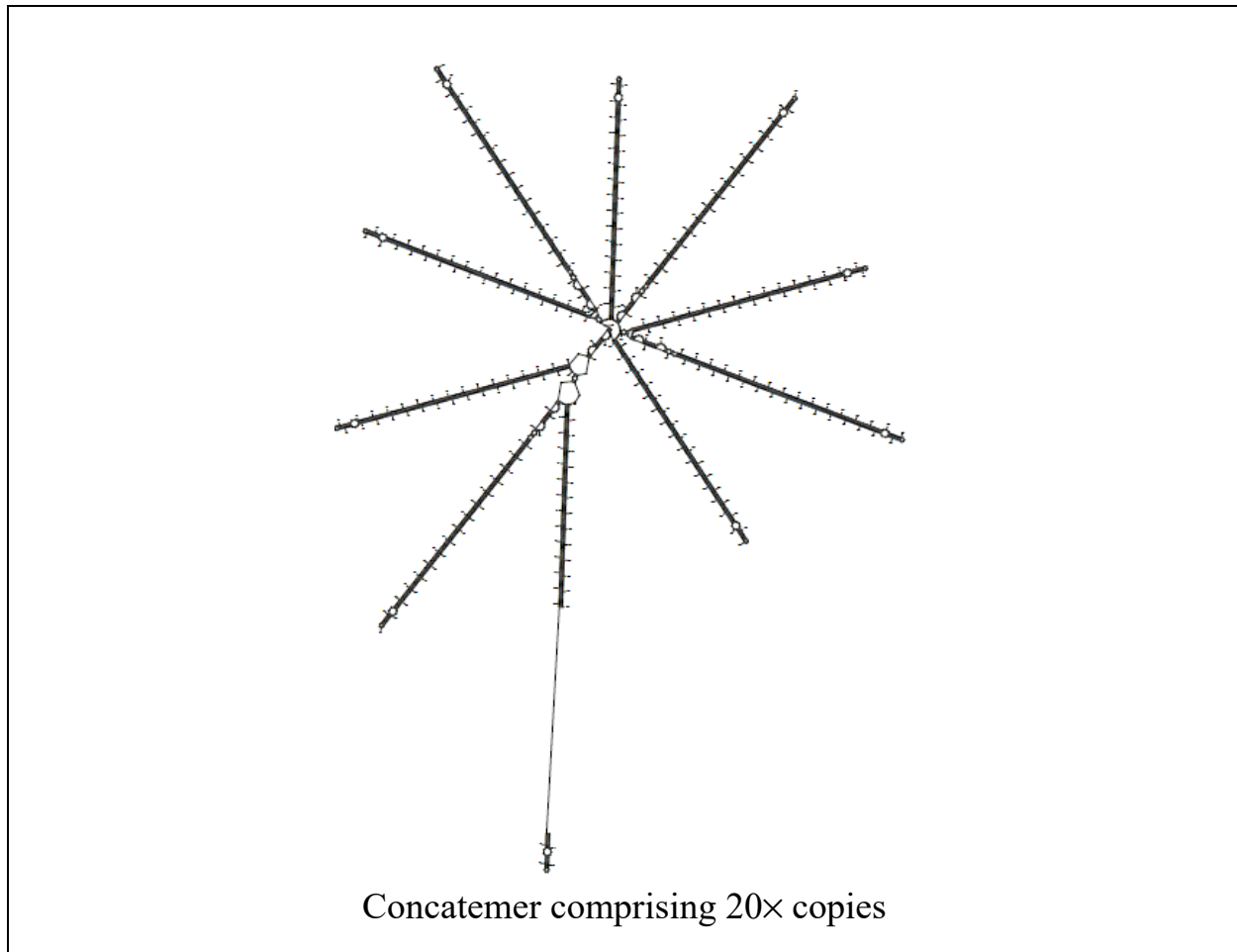
Using in silico DNA structure modeling with the same template sequences disclosed in Travers-2010, Dr. Satija confirms this problem, which would have been readily appreciated by a POSA, but was ignored by Petitioner. EX2014, ¶¶180-189; EX1021, 2. Using Travers-2010's "short" (*S. aureus* fragment) template, Dr. Satija generated⁴ the concatemer sequences a POSA would have

⁴ Pursuant to 37 C.F.R. § 42.65, Dr. Satija explains in detail the underlying facts, data, and methodology he used, all of which would have been available to a POSA before March 20, 2012. EX2014, ¶¶180-184; EX2058-2061.

expected from RCR of varying lengths, from three full loops (6× copies) to ten full loops (20× copies) around Travers-2010's SMRTbell templates. EX2014, ¶¶183-185; EX1021, 4-5, Fig. 3. Dr. Satija then used in silico DNA modeling software⁵ to predict the secondary structures of the RCR concatemers. *Id.* As Dr. Satija's data show, the numerous repeating stretches of complementary DNA would have been expected to hybridize with each other and form a variety of complex, star-shaped structures. *Id.*; EX2069; EX2070; EX2071. The predicted structures of the 6×, 10×, and 20× concatemers are shown below:



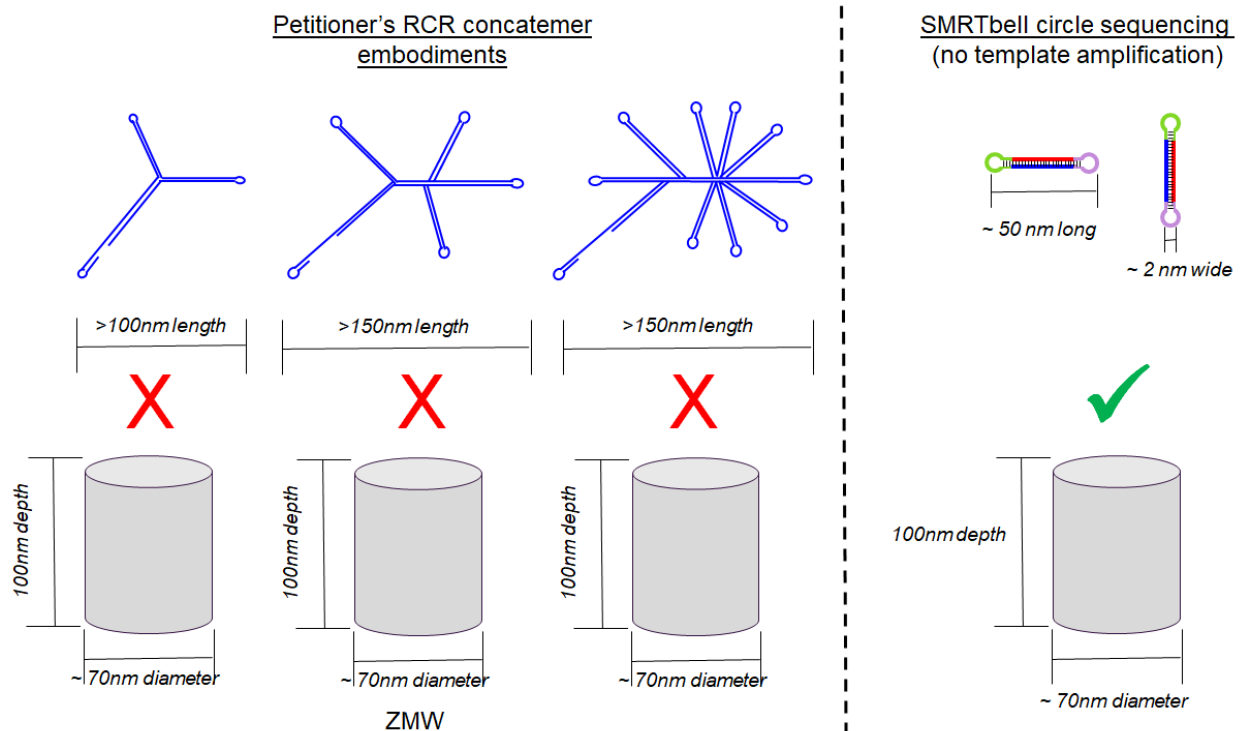
⁵ See *supra* footnote 2.



See EX2014, ¶185; EX2069; EX2070; EX2071; EX1021, 2.

A POSA would have known that these large concatemer molecules would be well over 100nm in size and would have significant difficulties physically fitting into the narrow ZMWs of Travers-'075 and Travers-2010, which are ~50-70nm in diameter. EX2014, ¶¶186-188; EX2029, Fig. 2; EX2030, Abstract; EX2027, 5; EX2001, 134; EX2021, 1; EX2023, 5; EX2031, 1 ; EX2063, 2. While the depicted structures are only two-dimensional secondary structures, Dr. Satija explains that, even when considering DNA tertiary structures in solution, a POSA still would not have expected the large size of Petitioner's concatemer molecules to facilitate

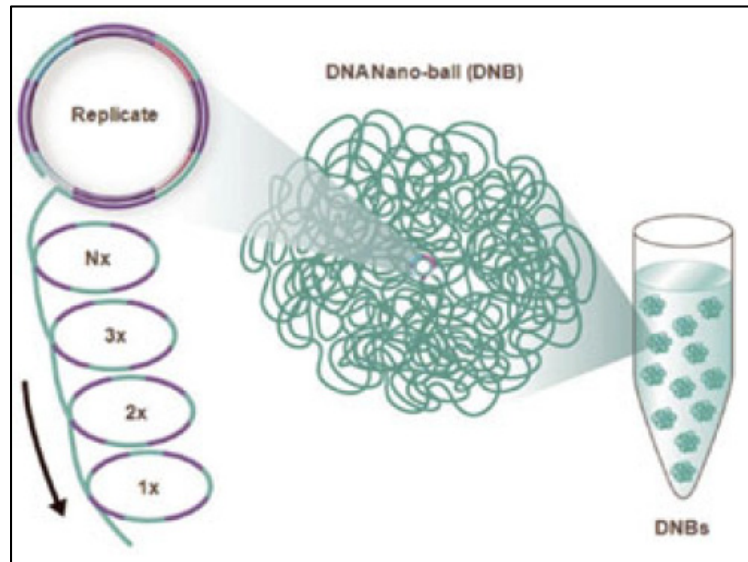
diffusion into ZMW wells. EX2014, ¶188. Dr. Satija's schematic below depicts large concatemers shown to scale with ZMWs:



EX2014, ¶¶188-189. As show in the figure above (left panel), the narrow ZMW diameter would physically limit the ability the large RCR concatemers to fit into the wells, while Travers-2010's single molecule SMRTbell templates (right panel), which are *not* amplified prior to sequencing, were designed to easily fit into the ZMWs. *Id.*; EX1021, 3 (SMRTbell template designed for "compatibility with ZMW geometry.').

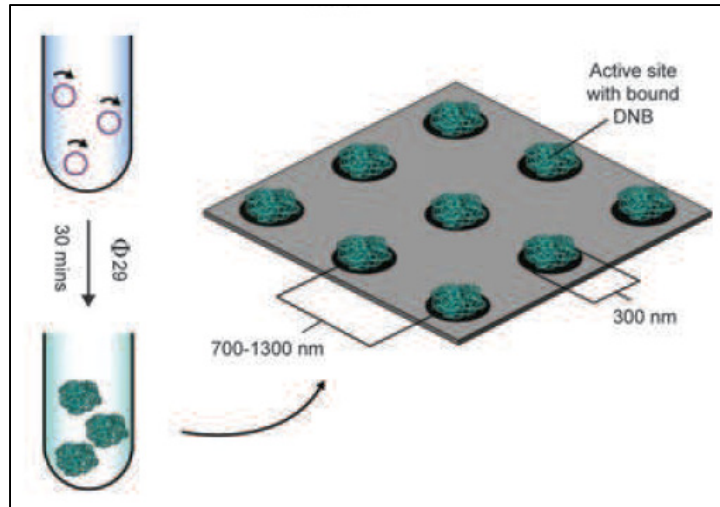
Petitioner's geometry problem is further confirmed by the prior art workflow of Complete Genomics. EX2047 1-2; EX2004, 7; EX2002, 5, 7; EX2014, ¶¶190-192. Complete Genomics' system relied upon a RCR step similar to what

Petitioner proposes here, in that circular template molecules were copied using RCR to produce long concatemers of repeating copies of template DNA, known as “DNA nanoballs.” Hui depicted these DNA nanoballs as extremely large, complex structures:



EX2048, Fig. 3 (excerpted); EX2014, ¶190.

Drmanac showed that these DNA nanoballs were indeed massive, and thus were only sequenced in Complete Genomics’ fabricated arrays of wells at least 300nm in diameter — an opening over 18 times larger than the tiny openings of PacBio’s ZMWs:



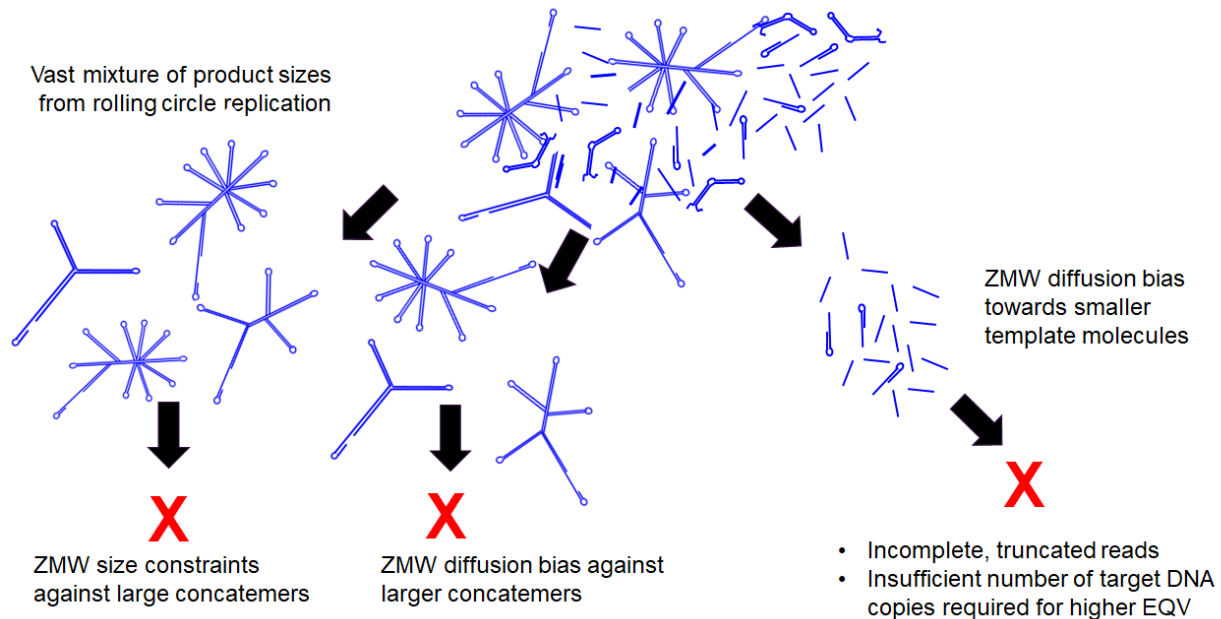
EX2047, Fig. 1 (exerpted); EX2002, Fig. 3; EX2014, ¶191.

Additionally, sequencing large nanoballs in Complete Genomics' system required many additional and different steps from the SMRT sequencing workflow. EX2014, ¶192. For example, sequencing Complete Genomics' nanoballs required a special form of sequencing by ligation called "combinatorial probe-anchor ligation (cPAL) technology." EX2004, 7; EX2002, 6; EX2014, ¶192. As Drmanac explained, "cPAL is based on unchained hybridization and ligation technology." EX2047, 1. Petitioner's proposes no such additional steps or technology here, relying solely on PacBio's SMRT sequencing in tiny ZMWs. Pet., 6.

In sum, the POSA would have faced a predicament. The larger concatemer molecules in Petitioner's RCR embodiment would not readily diffuse into or fit into the SMRT sequencing ZMW wells, meanwhile smaller template molecules lacking the requisite template redundancy for SMRT sequencing would

dramatically out compete larger molecules for ZMW occupancy. EX2014, ¶¶193-

195. This predicament is depicted in the figure below:



EX2014, ¶195.

Petitioner and Dr. Quackenbush fail to address any of the foregoing problems, nor do they account for how a POSA would have allegedly resolved them. These unexplained and irreconcilable deficiencies in Petitioner's proposed combination of embodiments only further demonstrate that Petitioner has not met its burden. *Oticon Med. AB v. Cochlear Ltd.*, IPR2019-00975, Paper 52, 26-27, 50 (P.T.A.B., Oct. 14, 2020).

* * *

In sum, the POSA would have expected Petitioner's RCR embodiment to be unsequenceable in SMRT sequencing ZMWs due to each template concurrently

binding multiple primers and polymerase molecules, all but eliminating the prospect of a single polymerase per ZMW. Moreover, the artisan would have expected the RCR step to produce a slurry of reaction product sizes that, even if they could somehow be sequenced in the PacBio ZMWs, would produce a vast mixture of truncated, incomplete templates—templates that are too short to contain enough copies of the target DNA for SMRT sequencing’s required sequencing depth. EX2014, ¶¶150-164. The POSA would have known that longer concatemer templates might possess more target DNA copies, but the longer concatemers (i) would not readily diffuse into the ZMWs, and (ii) would not easily fit into the ZMWs even if diffusion were not an issue. EX2014, ¶¶165-197. Again, nowhere in the Petition or in Dr. Quackenbush’s declaration are these issues addressed.

And moreover, a POSA would have appreciated that all of these problems could be completely avoided by using the circular SMRT sequencing applications of Travers-2010 and not the RCR embodiment proposed by Petitioner. This is further evidence of Petitioner’s ill-conceived and hindsight-based approach to obviousness here.

D. Petitioner provides no explanation of *how* a POSA would have combined its rolling circle replication embodiment with the SMRT sequencing applications of Travers-2010.

It is telling that Travers-2010 (published a year after Travers-’075, and actually putting Travers-’075’s method into practice) makes no mention of

applying SMRT sequencing to a RCR concatemer. This is further evidence that, in practice, no POSA would be motivated to use Petitioner's proposed RCR step before sequencing. Shown below, Petitioner provides no explanation as to *how* a POSA would have allegedly practiced this purported embodiment. EX2014, ¶¶198-205; *Raytheon*, 93 F.3d at 1381.

1. Petitioner's conclusory, unsupported arguments cannot meet its burden of persuasion.

After arguing that the asserted art purportedly discloses all the elements of the challenged claims, Petitioner jumps to the conclusion, "[i]n summary, the Travers Publications render obvious claim 1," with no further explanation. Pet., 28-29. Petitioner uses the same short shrift, conclusory approach in its challenge to independent claim 22, Pet., 37, as well as all of its challenges to the dependent claims, *see e.g.*, Pet., 40, 42, 44, 46-47, 50-52, 54, 57-58, 60, 65. A petitioner, however, must articulate "*how* specific references could be combined" and "*how* any specific combination would operate or read on the asserted claims." *Qiagen N. Amer. Holdings, Inc. v. Handylab, Inc.*, IPR2019-00490, Paper 51, 36 (P.T.A.B., July 14, 2020); *Stepan*, 868 F.3d at 1346, n1. Nowhere does Petitioner or Dr. Quackenbush articulate *how* a POSA would have purportedly practiced Petitioner's RCR embodiment, or *why* a POSA would have supposedly had a reasonable expectation of success. Petitioner does not mention, much less explain how to overcome Problems 1-4 discussed above. EX2014, ¶¶198-205. Instead, Petitioner

seems to be oblivious to these fundamental issues with its proposed approach.

These deficiencies are fatal to the entire Petition, because the Petitioner must explain “*how* the combination ... was supposed to work.” *Personal Web*, 848 F.3d at 994. In *Personal Web*, as is the case here, “a clear, evidence-supported *account of the contemplated workings of the combination* is a prerequisite to adequately explaining and supporting a conclusion that a relevant skilled artisan would have been motivated to make the combination and reasonably expect success in doing so.” *Id.* Neither Petitioner nor Dr. Quackenbush provides any account of the workings in the proposed combination or offers any reason to use a RCR-based embodiment. And neither Petitioner nor Dr. Quackenbush devote any analysis to why a POSA would have had a reasonable expectation of success.

References cited in ¶[0122] of Travers-’075 do not cure this deficiency. Travers-’075 cites U.S. Pat. No. 7,476,503 (“the ’503 patent”) when disclosing, “[t]hese replicated products may then be employed as template molecules in a template dependent sequencing process....” EX1018, ¶[0122] (citing EX1020). Like Travers-2010 and Travers-’075, the ’503 patent does not disclose *how* to combine Petitioner’s RCR embodiment with SMRT sequencing, and only reinforces the fact that SMRT sequencing does *not* utilize a template amplification step. EX2014, ¶204. The ’503 patent discloses:

This method also allows the use of target nucleic acid molecules taken *directly* from a biological sample, *minimizing the need for* cloning, subcloning, or *amplification* of the target nucleic acids *before sequencing* can take place.

EX2008, 23:51-54; *see also, id.*, 33:24-27 (disclosing that *sequencing*—not template amplification—is performed with RCR.); EX2014, ¶204.

Likewise, Travers-'075 cites U.S. Provisional Application No. 61/072,160 (“the ’160 application”) when disclosing, “duplication processes may be employed to produce multiple copies of a prepared circular construct...” EX1018, ¶[0122] (citing EX2049). The ’160 application—which Petitioner did not include as an exhibit nor even mention in its Petition or expert declaration—merely discloses methods of producing various circular template formats for sequencing, most of which involve a complicated processes of ligating exonuclease-sensitive and exonuclease-resistant tags to the different strands of DNA, followed by exonuclease digestion to produce templates for sequencing. EX2049, ¶[0005], claims 1, 3, 8; EX2014, ¶205. Indeed, the ’160 application discloses a method of generating circular DNA templates by ligating hairpin adapters to each end of a target DNA fragment, similar to the SMRTbell templates of Travers-'075 and Travers-2010. EX2049, ¶¶[0071]-[0073], Fig. 6; EX2014, ¶205. But nowhere in the ’160 application are any details on how to practice Petitioner’s RCR

embodiment in the SMRT sequencing applications of Travers-2010 and Petitioner certainly provided none. EX2014, ¶205.

2. Travers-'075 does not enable Petitioner's RCR embodiment.

In view of the litany of problems with Petitioner's RCR embodiment, and the absence of any details in Travers-'075, Travers-2010, or the general knowledge in the art on how to actually practice it—especially in view of the multi-primed, multi-polymerase concatemer multi-complex issue (Problem 1)—Travers-'075 is not enabling. EX2014, ¶¶206-210. “To render a claim obvious, the prior art, taken as a whole, *must enable* a skilled artisan to make and use the claimed invention.” *Raytheon*, 993 F.3d at 1380. As *Raytheon* explained, when there is no supporting evidence to establish enablement in the primary reference or general knowledge in the art—such as the case here—“the reference must necessarily enable the relied-upon portion of its own disclosure.” *Id.*, 1381. Indeed, this is the “same standard applied to anticipatory references.” *Id.* (citing *In re Antor Media Corp.*, 689 F.3d 1282, 1290 (Fed. Cir. 2012)).

Weighing the *Wands* factors confirms Travers-'075 does not enable Petitioner's embodiment. *In re Wands*, 858 F.2d 731 (Fed. Cir. 1988). Petitioner's RCR embodiment is fraught with *uncertainties and unpredictability*. EX2014. ¶¶207-208. Neither Travers-'075 nor Travers-2010 (nor any other art cited by Petitioner) provides any *direction or guidance* on how to practice Petitioner's RCR

embodiment or resolve any of Problems 1-4 discussed above. *Id.* Nor do any of the references provide any *working examples* of the RCR embodiment. *Id.*, ¶209. And, as Dr. Satija's in silico modeling experiments further demonstrate, applying Petitioner's RCR embodiment in the SMRT sequencing applications of Travers-2010 would have required excessive and *undue quantities of experimentation*, if it could even be practiced at all. *Id.* Thus, Travers-'075 does not enable Petitioner's embodiment, and therefore cannot render obvious any of the challenged claims, whether considered alone or combined with Travers-2010.

But even if Travers-'075's RCR embodiment were enabled, a POSA still would have found *no reason to combine* this RCR embodiment with the workflow Travers-2010. Petitioner unquestionably relies on the combination of Travers-'075 and Travers-2010. Petitioner offers no reason at all to make the proposed combination to implement an RCR embodiment into Travers-2010 except for the mere fact that it is mentioned in Travers-'075. In order to swap an RCR embodiment into SMRT sequencing, a POSA would have to discard the many advantages of the SMRTbell workflow and embark on a trial and error enterprise to attempt to needlessly get an RCR embodiment to work – all to perform (at best) not as well. Petitioner does not even allege a single advantage to implementing an RCR approach here compared to the tried and true SMRTbell workflow using circular templates and no amplification step. *Winner*, 202 F.3d at 1349 n8. As

such, Petitioner's challenge here fails.

VI. EVEN IF A POSA WERE TO COMBINE PETITIONER'S ROLLING CIRCLE REPLICATION EMBODIMENT WITH THE SMRT SEQUENCING APPLICATIONS OF TRAVERS-2010, THE POSA WOULD NOT HAVE HAD A REASONABLE EXPECTATION OF SUCCESS.

The Petition is further defective in that it fails to demonstrate by a preponderance of the evidence that a POSA would have had a reasonable expectation of success. For example, *the entire extent* of Petitioner's discussion of reasonable expectation of success for independent claim 1 amounts to a single sentence: "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." Pet., 20-29. Petitioner's argument for independent claim 22 contains *no discussion at all* of a POSA's reasonable expectation of success. *Id.*, 29-37. Likewise, the Petition's challenges to the dependent claims in Ground 1 (claims 2-10, 12, 15-28) contain *no discussion* of any reasonable expectation of successfully practicing these claimed embodiments. *Id.*, 37-58. Petitioner's scant analysis cannot meet its burden to articulate why a POSA would have had a reasonable expectation of success in implementing a RCR step after ligating adapters but before sequencing. As the Board held in *Qiagen*, "[s]uch conclusory assertions, lacking factual substantiation, are insufficient for evaluating reasonable expectation of success as part of an obviousness determination." *Qiagen* at 37

(citing *Wasica Fin. GmbH v. Cont'l Auto. Sys., Inc.*, 853 F.3d 1272, 1286 (Fed. Cir. 2017)); *Magnum Oil*, 829 F.3d at 1380. Moreover, as discussed below, the record evidence contradicts Petitioner's conclusory assertions and shows that a POSA would *not* have had a reasonable expectation of successfully practicing Petitioner's RCR embodiment. EX2014, ¶¶211-213.

A. A POSA would have known that Petitioner's rolling circle replication embodiment was fraught with technical obstacles.

As discussed above, Petitioner's proposed RCR embodiment introduces many technical problems with no articulated benefit whatsoever. Moreover, these technical problems would have destroyed any reasonable expectation a POSA may have had of successfully implementing Petitioner's combination. *See* Sections V(C)(1)-(3) (discussing Problems 1-4).

Especially pertinent here, in order to even attempt to "obtain first and second strand reads sequence reads⁶" from any template molecules, a POSA would have appreciated that the RCR replicon would need to represent at least one full revolution around the circular molecule. EX2014, ¶212. But with no way to

⁶ Claim 1 specifically recites "obtain[ing] first and second strand sequence reads." As noted above, Petitioner has not properly construed "first and second strand sequence reads." Regardless, the technical problems discussed throughout this paper apply even under Petitioner's apparent construction.

precisely control the RCR process, essentially all such replicons would proceed at least slightly further than exactly one full circle around the circular molecule. *Id.*; EX1021, Fig. 3. And when such products contain even slightly more than exactly one copy of the circular template, a POSA would appreciate they then contain multiple primer/polymerase binding sites. EX2014, ¶212. As discussed above (Section V(C)(1), such templates would be essentially worthless in ZMW-based sequencing given their likelihood of binding and initiating sequencing by synthesis reactions with two or more polymerases. EX2014, ¶¶128-137, 212.

Taken with the other technical problems with Petitioner's RCR embodiment discussed above, a POSA would have had no reasonable expectation of success in implementing Petitioner's RCR embodiment into the sequencing applications of Travers-2010. *Id.*

B. The '127 patent's claimed rolling circle replication embodiment does not support Petitioner's embodiment.

Attempting to portray its RCR embodiment as somehow workable with SMRT sequencing, the Petition cites the '127 patent's disclosure of rolling circle replication and a claimed embodiment in claim 10. *See e.g.*, Pet., 19, 48. Unlike the Petition, however, the '127 patent's embodiment in claim 10 is not limited to Petitioner's specific RCR embodiment using PacBio sequencing. EX2014, ¶¶214-216. When discussing hairpin adapters and circular templates, the '127 patent discloses that "[r]ecognition sites for restriction endonuclease digestion could be

engineered into these adapters ... if more convenient for *subsequent steps*.”

EX1001, 11:15-18; EX2014, ¶215. Petitioner’s RCR-based petition, however, includes no such downstream modification steps. And critically, the ’127 patent also discloses that amplified products are sequenced “using any *suitable* method known in the art.” *Id.*, 15:33-39. A POSA reading the ’127 patent would have known which sequencing methods were suitable for certain template preparation methods and unsuitable for others. EX2014, ¶215. And unlike Petitioner’s obviousness theory, claim 10’s embodiment is not limited to SMRT sequencing and ZMW arrays. *Id.*, ¶216.

Petitioner alleges that Patent Owner “acknowledged” that Travers-’075 describes “amplification” as recited in claims 1 and 22 of the ’127 patent during prosecution of a family member. Pet., 24, 31-32. This is simply not true. A proper reading the statement in context shows that Patent Owner *disagreed* with the Examiner’s assertion that Travers-’075 discloses using PCR products (i.e., using DNA that had been amplified) as a template for generating sequence reads.

EX1024, 551. As Patent Owner explained in its Reply to Office Action:

The Office Action alleges that Travers discloses this feature by teaching that “template constructs may be prepared from PCR products to provide a population of identical molecules from which to generate sequence reads”.... *Applicant respectfully disagrees.*

EX1024, 551.

Further, a review of the record shows that Patent Owner’s prosecution statements referred to ¶[0083]⁷ of Travers-’075. As already discussed above, this paragraph in Travers-’075 refers explicitly to *sequencing by synthesis* (“polymerase mediated sequencing process”)—which is *not* the same as the claimed template amplification step. EX1024, 551; EX1018, ¶[0083]; EX2014, ¶96. Petitioner’s arguments here have no merit.

VII. A POSA WOULD NOT HAVE HAD ANY REASON TO COMBINE MCCLOSKEY WITH TRAVERS-’075 AND TRAVERS 2010 (CLAIMS 13-14, 29-30).

Petitioner also fails to demonstrate by a preponderance of the evidence in its challenge to claims 13-14 and 29-30 (Ground 3) that a POSA would have combined McCloskey’s purported disclosure of random barcodes with the Travers publications. EX2014, ¶¶217-224. Claim 13 recites, “the barcodes comprise a degenerate or semi-degenerate sequence containing between 3 and 20 nucleotide bases.” EX1001, 39:16-19. Claim 14 depends from claim 13, and recites certain structural arrangements of the degenerate or semi-degenerate barcodes within the

⁷ Patent Owner’s Reply to Office Action cites Travers-’075 ¶[0081]. EX1024, 551. This was a clerical error, with the intended citation to ¶[0083], evident from the quoted language. EX1024, 551; EX1018, ¶[0083].

adapters. *Id.*, 39:20-22. Claims 29 and 30 recite barcodes of certain lengths. *Id.*, 40:55-58. Recognizing that the combination of Travers-2010 and Travers-'075 fails to disclose all the elements of claim 13-14 and 29-30, Petitioner uses hindsight to select disclosures in McCloskey (EX1023). *See e.g.*, Pet., 60-65; EX1002, ¶¶191-204.

Petitioner cherry-picked McCloskey for Ground 3 based on McCloskey's use of terms such as "random barcode" and "hairpin adapter," but failed to appreciate that no POSA would have combined McCloskey with Petitioner's RCR embodiment. McCloskey relates *exclusively* to PCR amplification—i.e., adding barcodes to track and identify *PCR products* after amplification, because PCR products are *separate molecules*. EX2014, ¶219. McCloskey discloses that "[t]he *present invention* relates to the field of *polymerase chain reaction (PCR) amplification* of nucleic acid templates ... particularly to the use of barcodes and batch-stamps for verifying the *authenticity of PCR products* and other sequence information." EX1023, ¶[0002]. This central teaching in McCloskey is consistent throughout its working examples. *See e.g.*, EX1023, ¶¶[0048]-[0049], [0054], [0065], [0073], [0074], [0078], [0084]-[0085], [0092]-[0098]; EX2014, ¶220.

Indeed, a POSA would not have had any reason to use McCloskey's random barcodes—designed and used exclusively for amplicon products that are separate molecules (e.g., PCR products)—in Petitioner's RCR embodiment, because

Petitioner's RCR concatemers would have contained all the target DNA copies *physically linked together* as a single molecule. EX2014, ¶221. There would have been no need for such random barcodes. Dr. Quackenbush's only proffered rationale for using McCloskey's random barcodes in Petitioner's RCR embodiment is that Travers-'075 discloses using *index barcodes* and McCloskey discloses using random barcodes for sample identification. EX1002, ¶198 (citing EX1018, ¶[0100] and EX1023, ¶[0021]).

Additionally, Petitioner fails to appreciate that claims 29 and 30 each depends from claim 22, and thus each requires grouping the strand sequence reads into families based on the "barcode sequence" *and* "DNA fragment-specific information." EX1001, 40:14-16; EX2014, ¶223. Dr. Quackenbush focuses exclusively on a 6-mer barcode purportedly disclosed in McCloskey, arguing that a POSA would select a 6-mer from McCloskey "*if that length of barcode was sufficient to assign a unique identity to each target nucleic acid molecule in the sample.*" EX1002, ¶203. Petitioner and Dr. Quackenbush completely ignore the requirement in claims 29-30 that the grouping step requires using the barcode sequence *and DNA fragment-specific information. Id.* This is yet another failure of proof in the Petition.

It is not enough for Petitioner to argue that McCloskey's barcodes *could* be used in a RCR concatemer, Petitioner must prove that McCloskey's barcodes

would be used. *Personal Web*, 848 F.3d at 994; *Gordon*, 733 F.2d at 902.

Petitioner fails to make any such showing here, and thus fails to meet its burden of persuasion in its challenge to claims 13-14 and 29-30 (Ground 3).

VIII. OBJECTIVE INDICIA OF NONOBVIOUSNESS FURTHER SUPPORTS A CONCLUSION OF PATENTABILITY

An obviousness analysis requires assessing all four *Graham* factors. *In re Cyclobenzaprine Hydrochloride Extended-Release Capsule Patent Litig.*, 676 F.3d 1063, 1075 (Fed. Cir. 2012). Petitioner completely ignored objective indicia in its obviousness analysis—another failure of proof. *Graham v. John Deere*, 383 U.S. 1 at 17-18 (1996); *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 1538 (Fed. Cir. 1983).

At the heart of the '127 patent's groundbreaking duplex sequencing technology is its method of *relating* sequence reads based on shared SMI tags and also *distinguishing* first and second strand sequence reads using the asymmetrical nature of the SMI adapter-DNA molecules. As the '127 patent discloses, “*each strand* in a DNA duplex pair generates a *distinct, yet related* population of PCR duplicates after amplification *owing to the complementary nature of the SMIs* on the two strands of the duplex.” EX1001, 21:46-49. This distinction is achieved in part “[b]y virtue of *the asymmetric nature* of adapted fragment.” *Id.*, 3:64-65.

Shown below, objective indicia of nonobviousness for the claimed duplex

sequencing methods further support the patentability of the challenged claims.

EX2014, ¶¶225-244.

A. Industry praise

Kirsch (EX2007) praises the '127 patent's duplex sequencing methods, speaking directly to the method's advances over the prior art: "Based on the data presented in the article by Schmitt et al., the Gordian knot in sequencing accuracy has apparently been cut by the duplex sequencing approach." EX2007, 1. Kirsch raved that duplex sequencing may "pave the way to explore the full power of NGS" and may ultimately "change the history of NGS." *Id.*; EX2014, ¶¶226-235. As Kirsch further noted, the "enthusiasm [over duplex sequencing] is warranted because duplex sequencing reportedly reduces the error rate of NGS up to 10 million-fold." EX2007, 1. Similarly, in 2020, GEN Edge hailed the '127 patent's duplex sequencing method as a "one in (ten) million" technology thought to be a "game changer" in applications where finding low frequency mutations is critically important, such as "tumor evolution, liquid biopsies, prenatal diagnosis, [and] fetal genetic health." EX2065, 1-2.

"In order to accord substantial weight to secondary considerations in an obviousness analysis, the evidence of secondary considerations must have a nexus to the claims...." *Fox Factory, Inc. v. SRAM, LLC*, 944 F.3d 1366, 1373 (Fed. Cir. 2019) (internal quotations omitted). Dr. Satija shows that the foregoing evidence of

industry praise indeed has a nexus to the challenged claims, such as at least claims 2-9, 16, 18, and 19. EX2014, ¶¶226-235. For example, claim 16 recites, “*associating the confirmed first strand sequence read with the confirmed second strand sequence reads using one or more of an adapter sequence and original strand information.*” EX1001, 39:27-31. Claim 18 recites that the “*first strand sequence reads*” and the “*second strand sequence reads*” can be “*related based on a barcode sequence, a fragment-specific feature, or a combination thereof.*” *Id.*, 39:35-40. And claim 19 recites, that the “*first strand sequence reads ... can be differentiated from the second strand sequence reads ... at least in part by an asymmetry of the asymmetrical double-stranded adapter-DNA molecules.*” *Id.*, 39:41-46. Kirsch praises the ’127 patent’s duplex sequencing method for these same claimed features, stating that the methods “*exploit[] the redundant sequence information contained in the complementary DNA strand of double stranded genomic DNA molecule*” (i.e., first and second strands, as claimed) by “*add[ing] a unique random yet complementary double-stranded nucleotide sequence to both strands of duplex DNA before amplification*” (i.e., adapter and barcode sequences, as claimed). EX2007, 1; EX2014, ¶233. GEN Edge further recognized that, in duplex sequencing, “*an asymmetric portion of the adapters ... distinguishes the strands so that each can be recognized as the original reference or anti-reference strand of the duplex.*” EX2065, 1-2; EX2014, ¶233.

Evidence of industry praise also has a nexus to at least claims 2-9. Claim 2 recites “*comparing* the confirmed *first strand* sequence read and the confirmed *second strand* sequence read,” “*identifying* nucleotide bases” that are or are not consistent between the two strands, and “*categorizing*” sequence variations as a “processing error,” “a site of DNA damage,” or “a true variant.” EX1001, 37:53-38:32. Claim 3 recites “*associating*” the *first strand* sequence read with the *second strand* sequence read “using one or more of an *adapter sequence*, a sequence length, sequence alignment information and original strand information.” *Id.*, 38:33-39. Claims 4-8 recite methods of generating a “*consensus sequence*” (claim 4), “*aligning* the consensus sequence to a reference sequence” (claim 5), “*comparing* the consensus sequence with the reference sequence” (claim 6), “*identifying* a mutation type” (claims 6-7), and “*identifying* a nucleotide sequence as a true nucleotide sequence” (claim 8). *Id.*, 38:40-61. And claim 9 recites “*determining* nucleotide bases” at which the *first strand* and *second strand* are not complementary and “*identifying*” the non-complementary bases as “an experimental error or a site of DNA damage.” *Id.*, 38:62-67. Kirsch discloses that the method first “*identif[ies]* families of DNA molecules that share the *same tag sequence* on a *single strand*,” and further “*identifies* the *complementary DNA strand* by searching for the *complementary tag* sequences.” EX2007, 1; EX2014, ¶¶229-232. Likewise, GEN Edge discloses, “the technique *independently tracks*

both strands of individual DNA molecules and *compares* the results to *identify and eliminate errors*.” EX2065, 1; EX2014, ¶¶229-232. Thus, these objective indicia have a nexus to at least claims 2-9, 16, 18, and 19. *Id.*

B. Long-felt need

The '127 patent's duplex sequencing methods also satisfied a long-felt, but unmet need for the ability to accurately detect low-frequency mutations without invasive procedures. *Rambus Inc. v. Rea*, 731 F.3d 1248, 1257 (Fed. Cir. 2013). Before the invention of the '127 patent, clinicians performed invasive bone marrow biopsies to obtain a sufficient cell sample size for DNA analysis with NGS. EX2051, 2. Coupled with the “inherently high sequencing error rate of 1%, which results in hundreds of millions of sequencing mistakes,” NGS applications for clinical diagnostics were limited. EX2007, 1; EX2051, 2. The inventors of the '127 patent solved these long-standing problems by “develop[ing] *duplex sequencing* technology to improve sequencing accuracy, making it possible to find low-frequency variants at a level of detection that *has not been achievable before*.” EX2052, 4 (noting a “particular *unmet need*”); *see also* EX2053, 1 (“It is *the first* NGS technology able to directly measure ultra-rare mutations.”). This evidence also has a nexus to at least claims 2-9, 16, 18, and 19. EX2014, ¶¶236-239.

C. Unexpectedly superior results

Evidence of unexpectedly superior results support nonobviousness when “there is a difference between the results obtained and those of the closest prior art,” and that difference “would not have been expected” by a POSA. *Bristol-Myers Squibb v. Teva Pharmaceuticals USA*, 752 F.3d 967, 977 (Fed. Cir. 2014). Here, the ’127 patent’s duplex sequencing methods demonstrated unexpectedly superior results over the allegedly closest prior art (Travers-2010’s SMRT sequencing). Travers-2010 discloses that SMRT sequencing provides its highest empirical quality value (EQV) at 6× sequencing depth which provides an error detection of about one in 10^4 (0.0001). EX1021, Fig. 7; EX2014, ¶¶240-244; EX2066. In contrast, embodiments of the ’127 patent’s duplex sequencing methods have a “theoretical background error rate of less than one artifactual error per 10^9 nucleotides sequenced,” with “the error frequency of DCS ... approximated as... 3.8×10^{-10} .” EX2050, 5; EX2014, ¶242. These surprising and superior results are *over 6 orders of magnitude* greater in sensitivity than the allegedly closest prior art—far more than “a mere difference in degree.” EX2014, ¶242; *BMS*, 752 F.3d at 977. Indeed, this astonishingly low error rate allows for measuring “mutation frequencies as low as 5×10^{-8} in human nuclear DNA.” EX2062, 3; EX2014, ¶242. This evidence, too, has a nexus to the claimed duplex sequencing methods. *See e.g.*, EX1001, claims 2-9, 16, 18, and 19; EX2014, ¶¶240-244.

This objective evidence—ignored by Petitioner—further demonstrates the nonobviousness of the claimed methods.

IX. CONCLUSION

Petitioner has failed to demonstrate by a preponderance of evidence that any of the challenged claims are unpatentable over the asserted art.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

/R. Wilson Powers III/

Date: January 13, 2023
1100 New York Avenue, NW
Washington, DC 20005
(202) 371-2600

Ralph Wilson Powers III, Ph.D.
Registration No. 63,504
Lead Attorney for Patent Owner

**CERTIFICATE OF COMPLIANCE WITH TYPE-VOLUME LIMITATION,
TYPEFACE REQUIREMENTS, AND TYPE STYLE REQUIREMENTS**

1. This Patent Owner Response complies with the type-volume limitation of 14,000 words, comprising 13,999 words, excluding the parts exempted by 37 C.F.R. § 42.24.
2. This Patent Owner Response complies with the general format requirements of 37 C.F.R. § 42.6 and has been prepared using Microsoft® Word 2016 in 14-point Times New Roman.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

/R. Wilson Powers III/

Date: January 13, 2023
1100 New York Avenue, NW
Washington, DC 20005
(202) 371-2600

Ralph Wilson Powers III, Ph.D.
Registration No. 63,504
Lead Attorney for Patent Owner

CERTIFICATION OF SERVICE (37 C.F.R. §§ 42.6(e))

The undersigned hereby certifies January 13, 2023, true and correct copies of the foregoing **PATENT OWNER RESPONSE UNDER 37 C.F.R. § 42.120** and **EXHIBITS 2014-2071** were served electronically via e-mail in their entirety on the following counsel for Petitioner:

Michael T. Rosato
Jad A. Mills
Sonja R. Gerrard
Patrick M. Medley
WILSON SONSINI GOODRICH & ROSATI
mrosato@wsgr.com
jmills@wsgr.com
sgerrard@wsgr.com
pmedley@wsgr.com

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

/R. Wilson Powers III/

Dated: January 13, 2023
1100 New York Avenue, NW
Washington, DC 20005
(202) 371-2600

Ralph Wilson Powers III, Ph.D.
Registration No. 63,504
Lead Attorney for Patent Owner