

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

GUARDANT HEALTH, INC.,
Petitioner,

v.

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

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

DECLARATION OF DR. ANDRES LARREA

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I, Andres Larrea, declare as follows:

I. INTRODUCTION AND QUALIFICATIONS

1. I am over the age of eighteen (18) and otherwise competent to make this Declaration.

2. I have been retained on behalf of Guardant Health, Inc. by its counsel for the above-reference inter partes review (IPR) to provide my independent opinions. Guardant has agreed to compensate me for my time at a standard rate of \$545 per hour. My compensation does not depend in any way on the outcome of the IPR.

3. In a January 23, 2023, declaration (marked as EX2014, IPR2022-00817), Dr. Rahul Satija expresses the opinion that there are “*four* critical problems with Travers ’075’s rolling circle replication embodiment” that he says “would have rendered Travers ’075’s rolling circle replication embodiment unworkable and requiring undue experimentation,” such that “a POSA would have been dissuaded from considering such an embodiment.” *E.g.*, EX2014, ¶100. Dr. Satija enumerates these “problems” as follows: (1) “unreadable sequencing data” allegedly produced when multiple polymerases bind to multiple sequencing initiation sites on a single template in a single ZMW; (2) different-sized concatemer products from a rolling circle replication step would need to be separated and “small-sized replication products would not possess enough copies

of the target DNA required for effective SMRT sequencing”; (3) larger concatemer products would be “outcompeted by the smaller replication products for occupancy in the ZMWs for SMRT sequencing due to well-known DNA diffusion bias issues”; and (4) an alleged inability of large concatemers to fit into ZMW (zero-mode waveguides). *E.g.*, EX2014, ¶¶198-203, 123-126. I have been asked to evaluate Dr. Satija’s testimony about these four alleged problems.

4. My opinions expressed herein are based on my years of specialized education, knowledge, training, experience, and expertise, as well as any documents I may cite herein. When Dr. Satija refers to a “POSA,” he explains that this is an acronym for “a person of ordinary skill in the art,” who he says “is presumed to be aware of all pertinent art, thinks along conventional wisdom in the art and is a person of ordinary creativity.” EX2014, ¶28. Dr. Satija states that a POSA would have had knowledge of the scientific literature concerning methods of DNA manipulation and analysis, including DNA sample preparation for sequencing, amplification, method for DNA sequencing (including NGS and related sequencing methods) and bioinformatics methods for raw data analysis. EX2014, ¶29. Dr. Satija states that a POSA would have had an advanced degree, such as a Ph.D., in molecular biology, genomics, bioinformatics, or a related field, and have at least about two years of experience in the use and development of

sequencing technologies, or a lower level of formal education and more years of experience in one of these fields. EX2014, ¶30.

5. I understand that the earliest claimed priority date of the patent at issue is March 20, 2012. By March 2012, I had a Ph.D in Biochemistry and Molecular Biology and more than two years of experience in the use and development of sequencing technologies.

6. I received a B.S. in Chemistry from Florida State University in 2001 and a Ph.D in Biochemistry and Molecular Biology from the University of Miami Miller School of Medicine in 2008. Among other responsibilities, during my nearly two years as a Post-Doctoral Fellow from 2008-2010, I introduced next generation sequencing technologies to the lab to improve efficiency and cut costs. Beginning in May 2010, I worked as a Field Applications Specialist and Scientist at Pacific Biosciences for several years. Among other responsibilities, I provided expertise in third generation DNA sequencing technology and applications, including training others on sample preparation, experimental design, instrument operation, and data analysis.

7. In the intervening years, I have continued to work in this space, both in industry and as an outside consultant. Additional description regarding my education, training, experience, and expertise may be found in my C.V., which is submitted as EX1034.

II. ALLEGED PROBLEM 1: MULTIPLE POLYMERASE BINDING SITES

8. Dr. Satija first argues that rolling circle replication would result in multiple primer/polymerase binding sites throughout the concatemer (at least one start site for each copy of the SMRTbell template), permitting multiple polymerase enzymes to bind to a single template and allegedly preventing sequencing in a ZMW. EX2014, ¶128. Dr. Satija argues that more than one polymerase incorporating nucleotides in a ZMW will result in useless data. EX2014, ¶130. But Dr. Satija's opinion rests on a fundamental misapprehension of how SMRT sequencing operates which I will detangle below.

9. Dr. Satija's opinion erroneously assumes that in order for Single Molecule Real-Time (SMRT) sequencing to obtain data from a single polymerase bound to a template, binding of multiple polymerases to a template must be avoided. Dr. Satija gets the analysis exactly backwards. SMRT sequencing welcomes a relatively high ratio of polymerase to template to increase the number of templates complexed with polymerase (so they can be sequenced). However, even where multiple polymerases are bound, data is only obtained from the single polymerase that is immobilized at the bottom of the ZMW and not from any other polymerase that may be located elsewhere in the ZMW or in solution.

10. In fact, the presence of multiple polymerases complexed with a template molecule was not problematic in the least and was instead preferred. For

example, Pacific Biosciences' *Template Preparation and Sequencing Guide*

("Template Preparation Guide," EX1035) explains that binding a polymerase to both adapters of the template was optimal:

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

EX1035, 34; *see also* EX2014, ¶156. This recommendation in the Template Preparation Guide reflects a gain in efficiency realized from minimizing the number of templates that lack a bound polymerase. When every priming site of a template is complexed with a primer and polymerase, the potential for obtaining a sequence read for that template is maximized.

11. Dr. Satija correctly notes that the SMRT sequencing workflow involves forming primer:template:polymerase ternary complexes prior to ZMW loading. EX2014, ¶¶129-130 (citing EX1021, 2; EX2001, 3; EX1018, ¶43; EX2026, 2). Dr. Satija also correctly notes that the SMRT sequencing workflow promotes a distribution wherein one ternary complex is immobilized at the bottom of each ZMW. *Id.* But Dr. Satija is incorrect to the extent he asserts that the SMRT sequencing workflow requires that each complex have only a single polymerase. The key to the workflow, as explained by the very references Dr. Satija cites, is that ZMW were designed so that "only a single DNA polymerase is immobilized at the bottom of a ZMW." EX2014, ¶130 (citing EX2001, 3; EX2002, 4; EX2021, 4).

Although multiple polymerases may bind to the primer/polymerase bindings sites on the template and diffuse into the ZMW, each ZMW provides a window that enables real-time observation of only the single molecule of DNA polymerase that is immobilized at the bottom of the well as it synthesizes DNA. EX2021, 4. The presence of multiple primer/polymerase binding sites was no barrier to single molecule sequencing, but instead maximized the probability that a polymerase in a ternary complex attaches to the bottom of a ZMW to provide the desired sequencing data.

12. Dr. Satija misconstrues the Travers Publications (i.e., Travers 2010 and Travers '075) as teaching that the DNA template must have “one and only one sequencing primer binding site.” EX2014, ¶¶129 (citing EX1021, 2; EX2001, 3), 131 (asserting without citation to any support that a POSA would have recognized that the SMRTbell circularized template contains a single primer/polymerase binding site). This is incorrect. The Travers Publications each describe templates with more than one sequencing primer binding site.

13. For example, Travers '075 expressly provides for inclusion of one or more primer/polymerase binding sites in templates. EX1018, ¶¶14 (“In other preferred aspects, control sequences are provided within one or more of the li[n]king oligonucleotides or hairpin oligonucleotides described above, such as primer recognition sequences and the like.”), 17 (“The compositions also typically

include one or more of a primer sequence capable of hybridizing to at least a portion of the template nucleic acid and initiating polymerase mediated nucleic acid synthesis, and a polymerase enzyme.”), 67 (“By way of example, the single stranded linking oligonucleotides, not only can be selected to present a specific primer recognition sequence, they also provide the primer and polymerase binding site in a required single stranded conformation....”).

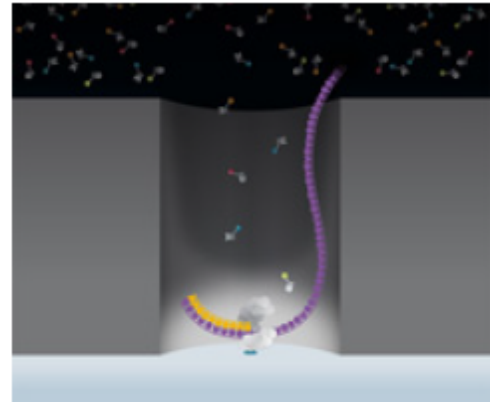
14. The Travers Publications further disclose ligating identical hairpin linkers, each of which includes a primer/polymerase binding site, to both ends of double-stranded nucleic acid fragments. EX1018, ¶¶103 (“For example, completely complementary double stranded nucleic acid fragments may be joined via blunt end ligation with hairpin adapter sequences. In such cases, given the reduced ability to control which adapter ligates to the different ends of the double stranded fragments, it may be desirable to employ a single type of adapter.”), 105 (“Complementary overhang sequences 722 are provided on the hairpin adapters, to provide for specific annealing and ligation of the hairpin adapters 710 to the double stranded fragments 700.”), 144 (“A single hairpin structure was used at both ends of the template.”), Figure 7; EX1021, 3 (describing blunt end ligation of the same hairpin linker to both ends of a DNA fragment); *see also* EX1036, 12-13 (describing ligating the same hairpin adapter comprising a sequencing primer binding site to both ends of genomic DNA fragments), (“We isolated genomic

DNA ...and sequenced it using previously described methods.¹⁵) (Reference 15 is Travers 2010).

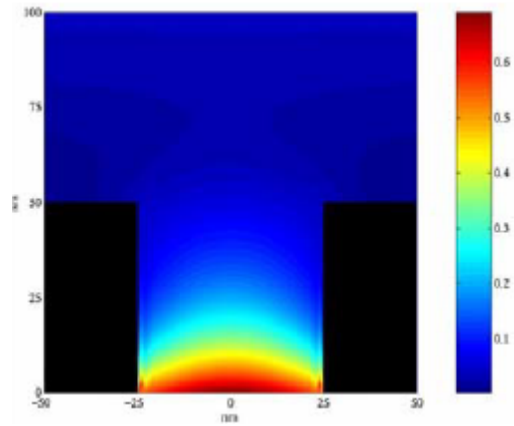
15. Accordingly, the Travers Publications confirm that “single molecule sequencing” routinely included sequencing of a single template molecule that had multiple primer/polymerase binding sites. Discussion in Travers of providing “a” site or “the” site for primer/polymerase binding do not imply that the template provides only one site for primer/polymerase binding, despite Dr. Satija’s unsupported and erroneous suggestion to the contrary. EX2014, ¶131 (citing EX1021, Abstract, 3, Fig. 1).

16. Similar to what the Travers Publications describe, the Template Preparation Guide describes the use of “universal hairpin adapters,” which include a sequencing primer binding site. EX1035, 3-4. The Template Preparation Guide describes that the universal hairpin adapter can be ligated to both ends of double-stranded DNA fragments. *See id.* (illustrating the SMRTbell hairpin adapters and describing how they are bound to and protect the ends of DNA fragments). Thus, consistent with the Travers Publications, the resulting templates described in the Template Preparation Guide have multiple polymerase binding sites.

17. Templates with multiple polymerases bound would routinely generate useable reads. This is because typically only one polymerase becomes bound to the bottom of the ZMW. EX2021, 1 (“The approach is based on eavesdropping on a single DNA polymerase molecule working in a continuous, processive manner.”), (“Within each chamber, a single DNA polymerase molecule is attached to the bottom surface such that it permanently resides within the detection volume.”), 4 (“A single DNA polymerase molecule is attached to the bottom of each waveguide. Biased immobilization of the enzyme, enhancing the sequencing yield of the SMRT Cell, is made possible through proprietary surface coatings that direct the protein attachment to the transparent floor of the ZMW.”), (“A single DNA polymerase molecule is attached to the bottom of the ZMW using a proprietary biased immobilization process.”); *see also id.*, Figure 6 (reproduced herein). It is this polymerase that is bound to the bottom of the ZMW whose activity is detected to provide sequencing data.



18. To the extent additional polymerases are bound to the template, these generally would not contribute signal. The excitation beam used for visualizing nucleotide incorporation penetrates only a short distance into the ZMW. EX2021, 4 (“Attenuated light from the excitation beam penetrates only the lower 20-30 nm of each waveguide, creating a detection volume of 20 zeptoliters (10^{-21} liters).”), Figure 4



(reproduced herein). Any polymerases that were outside the range of the excitation beam (i.e., those not bound to the bottom of the ZMW) would not contribute signal. Over time, additional polymerases would be released from the template, maintaining a single active polymerase at the bottom of the waveguide.

19. In contrast to multiple polymerases on a single template, which was perfectly acceptable, the technology does benefit from avoiding the loading of more than one template in a single ZMW. For example, the Template Preparation Guide cautions against loading “multiple polymerase-template complexes in the same ZMW.” EX1035, 39. That is, the Guide cautions against overloading such that multiple templates are loaded into a single ZMW. EX1035, 40 (Figure 13). Loading of multiple templates into a ZMW might not be preferred—but is easily

mitigated by either (1) choosing an appropriate loading concentration or; (2) bioinformatically trimming the read to only the high-quality region (HQR).

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

III. ALLEGED PROBLEMS 2 AND 3: DIFFERENT SIZED TEMPLATES

21. Alleged problems 2 and 3 each relate to diffusion bias favoring smaller template molecules for faster loading into ZMW. *E.g.*, EX2014, ¶145 (“The art was replete with references teaching that the small diameter of ZMW wells caused a bias towards preferentially sequencing smaller template molecules because smaller molecules diffused more easily into the ZMW.”). Dr. Satija argues “a POSA would have known that smaller DNA molecules have a much higher diffusion coefficient than the larger molecules” and that “smaller molecules would drastically outcompete the larger molecules” during the loading of ZMW. *E.g.*, EX2014, ¶¶145-146. Dr. Satija spends pages and pages of text to demonstrate that practitioners were well aware of diffusion bias and its implications. *E.g.*, EX2014, ¶¶139-163, 165-169. Curiously, he assumes without justification that POSAs were fully aware of diffusion bias but would ignore known and routine methods to address it. Dr. Satija is wrong.

22. Larger molecules diffuse slower than smaller molecules. To address this, it was routine to adjust the diffusion time depending on the size of the templates being distributed. *E.g.*, EX1035, 38 (“During a sequencing run, the required diffusion time is 30 minutes for SMRTbell templates up to 3 kb in size and 1 hour for SMRTbell templates greater than 3 kb.”).

23. It was also routine to perform size selection such that sequencing is targeted to templates of the desired size. For example, Pacific Biosciences expressly instructs users to perform a size selection step prior to template loading. EX1035, 27 (“it is desirable to increase the stringency of size selection”), 35 (“It is highly recommended to select amplicons of the same size (+/- 10%) to minimize loading bias.”), (“When sequencing large insert libraries (e.g. 20 kb library) for generating long read lengths, it is highly recommended to perform a size selection step to eliminate short SMRTbell templates that will preferentially load.”), 48 (“Because of an intrinsic loading bias that favors the immobilization of smaller DNA template sizes in the SMRT Cells, ... We strongly encourage performing at least a double-AMPure PB bead purification step ...”). A size-selection step permitted optimizing diffusion time for templates of a given size. As taught in the Travers Publications, a POSA would have also known that a variety of effective size selection means were readily—and even commercially—available. *E.g.*, EX1018, ¶¶145, 150 (“ChromaSpin 1000”); EX1021, Figure 3 (showing gel

electrophoresis of amplified concatemers); *see also* EX1035, 3 (“magnetic bead purification step with size-selective conditions”), 24 (“AMPure PB Size-Selection”); EX1036, 12 (“Sheared samples were then concentrated and size-selected ... by Solid Phase Reversible Immobilization (SPRI) paramagnetic bead based technology (AMPure XP™”).

24. Dr. Satija’s arguments relating to alleged Problems 2 and 3 assume that a POSA would forgo routine processing steps. This assumption is entirely unreasonable ignores the level of skill in the art. A POSA would have regarded size selection of template molecules to be a routine aspect of sequencing on the Pacific Biosciences platform described in the Travers publications. *E.g.*, EX1018, ¶¶145, 150; EX1035, 27, 35, 48; EX1036, 12.

25. Even if one accepts Dr. Satija’s unreasonable assumption that a POSA would forgo routine size selection processes or selecting an appropriate loading/diffusion time, his suggestion that this would preclude collection of sequencing data for informative molecules does not logically follow. First, loading bias is only a bias, it does not mean that larger templates are not loaded into any ZMW and sequenced. *E.g.*, EX1035, 35 (“Figure 11 ZMW Loading Bias for Various Insert Sizes”) (showing loading and sequencing of templates ranging from 160 bp to 4,251 bp). It certainly does not mean that only small, uninformative templates are exclusively loaded.

26. Second, Dr. Satija argues that certain smaller templates do “not provide useful sequencing data because they lack sufficient sequencing redundancy,” but he ignores that informative templates would be loaded and sequenced. EX2014, ¶¶157-158, 163, 169. For example, Dr. Satija specifically invokes “shorter, truncated products” lacking a complete copy of each strand. EX2014, ¶¶164. He also invokes template molecules he says are so small that they “would not provide enough target DNA copies to be able to resequence the target DNA sufficiently and achieve a desirable higher EQV[empirical quality value].” EX2014, ¶¶164. But Dr. Satija does not deny, nor could he reasonably deny, that valuable sequencing data would be obtained for informative templates.

27. Moreover, Travers itself cites a method for circularizing template, which increases the EQV of “short,” non-truncated concatemers. EX1018, ¶122 (incorporating U.S. Pat. No. 7,476,503 [EX1020] by reference); EX1020, 24:36-44 (“In another aspect, a similar result is accomplished by using a template-depend[en]t circular template bearing the sequence segment of interest. In particular, such synthesis product will typically include, in a single linear strand, multiple copies of the circular template, again, providing for iterative sequencing of the sequence segment of interest. Further, redundancy is additionally accomplished by circularizing this linear, multi-copy template and iteratively sequencing multiple copies, multiple times.”). In any case, Dr. Satija is wrong that

the sequencing data from long concatemers would be unfavorable unless each concatemer was read six different times. *E.g.*, EX2014, ¶121. Indeed, one read of a longer length (e.g., 6x) concatemer replicates the sequencing data from multiple reads of a shorter (e.g., circularized) strand. Dr. Satija’s hypothetical concerns about overly short templates that “not provide a sufficient number of copies,” EX2014, ¶169, has no practical bearing on the operability of the Pacific Biosciences sequencing platform described in the Travers Publications.

28. In sum, POSAs do not unreasonably ignore or forgo routine and commercially-available size selection protocols, particularly when specifically recommended in the manufacturer’s protocol. Even if one is to unreasonably underestimate the intelligence and capabilities of a POSA, as Dr. Satija does, Pacific Biosciences sequencing platforms would still generate useable sequence even if rolling circle amplification produced a range of concatemer sizes. Contrary to Dr. Satija’s assumptions, POSAs would not ignore simple, recognized techniques for accommodating diffusion rates where even Dr. Satija appears to concede that POSAs would recognize different diffusion rates. Accordingly, Dr. Satija’s alleged Problems 2 and 3 are not problems at all.

IV. ALLEGED PROBLEM 4: PHYSICAL SIZE OF ZMW

29. Dr. Satija argues that “physical or geometrical constraints on large concatemer products” would “impede their entry into the ZMW.” EX2014, ¶¶177-

178. Dr. Satija then constructs hypothetical concatemer products of 1,008, 1,680, and 3,360 nucleotides and argues that such templates “would have difficulties physically fitting within the narrow wells of Travers ’075 and Travers 2010.” EX2014, ¶188.

30. Dr. Satija’s argument that templates of 1,008, 1,680, and 3,360 nucleotides would not fit into a ZMW is simply wrong. The Travers Publications disclose that much larger (e.g., 50,000 nucleotide) templates would fit into a ZMW and even provide working examples of sequencing much larger (e.g., 5,120 nucleotide) templates. Moreover, the Template Preparation Guide instructs users that libraries containing much larger templates (e.g., 40,000 nucleotides) may be constructed and sequenced. In each case, these templates are much larger than the concatemer products Dr. Satija argues would not fit in a ZMW.

31. For example, Travers ’075 explains “templates that are from about 200 to about 50,000 bases in length may be used.” EX1018, ¶83; *see also id.*, ¶¶85. Travers ’075 further provides examples in which templates containing inserts in the range of 500–2,560 bp were sequenced. EX1018, ¶¶144 (“The genomic DNA was sheared using a nebulizer to generate fragments ranging from 500-1500 bp in size.”), 150 (“A portion of the genome sequence of Escherichia coli strain MG 1655 that contains a 2.56 kb exact repeat was targeted for sequencing”). Converting the templates of the examples into nucleotide length (i.e., # base pairs *

2 nucleotides/base pair = # nucleotides) yields templates in the range of 1,000-5,120 nucleotides. Travers '075 thus directly contradicts Dr. Satija's argument.

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

33. The Template Preparation Guide similarly undermines Dr. Satija's argument. It describes "sequencing large insert libraries (e.g. 20 kb library) for generating long read lengths." EX1035, 35; *see also id.*, 6 ("Assaying high-molecular weight DNA is critically important for constructing large insert libraries (e.g. 20 kb libraries)."). Converting the exemplary large insert library to nucleotides yields templates of 40,000 nucleotides. The Template Preparation Guide is thus consistent with the Travers Publications in contradicting Dr. Satija's argument.

34. Dr. Satija's argument appears to be based on a false premise that the templates assume rigid conformations that prevent them from entering ZMW.

EX2014, ¶¶170-195. But even Dr. Satija agrees that the template conformation would “fluctuate in solution.” EX2014, ¶188. Dr. Satija’s hypothetical conformations present no bar to SMRT sequencing in practice and his hypotheticals fail to account for the reality of how these molecules fluctuate in solution. Accordingly, Dr. Satija’s alleged Problem 4 is no problem at all.

V. CONCLUDING STATEMENTS

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

36. I declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Dated: April 13, 2023

By: / Andres Larrea /
Andres Larrea