

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

GUARDANT HEALTH, INC.,
Petitioner,

v.

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

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

DECLARATION OF DR. JOHN QUACKENBUSH

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I, John Quackenbush, declare as follows:

I. SCOPE OF WORK

1. I have been asked to respond to certain opinions expressed by Dr. Rahul Satija in his declaration submitted in this case as Exhibit 2014. As explained in further detail below, Dr. Satija's arguments are inconsistent with the language of the claims and the '127 patent specification, often conflict with express disclosure in the prior art references, and his conclusions are undermined by various undisputed points and admissions in the '127 patent itself.

II. THE TRAVERS PUBLICATIONS ADMITTEDLY DISCLOSE AMPLIFICATION FOLLOWED BY SEQUENCING

2. Dr. Satija argues the '127 patent claims recite an amplification step that is followed by sequencing. EX2014, ¶¶50-59. Regardless of whether claims 1-30 are interpreted to require a separate amplification step, the Travers Publications expressly disclose an embodiment where adapters are attached to a template molecule, followed by amplification then sequencing. Patent Owner has repeatedly admitted this prior art disclosure. While Dr. Satija falsely suggests this embodiment is inoperable (discussed below), he too acknowledges the Travers Publications disclose such an embodiment and that same embodiment is encompassed by the '127 patent claims. EX2014, ¶214 (claims encompass, though are "not limited to the '075 rolling circle embodiment"); EX1037, 94:24-95:15 (confirming claims encompass but are not limited to Travers' amplification

embodiment); *see also* EX2014, ¶¶97-100, 122, 125, 127, 137, 138, 146, 165, 167, 169, 177, 179, 189, 190, 192-196, 198, 204-206, 208, 209, 211, 213, 214 (acknowledging “Travers ’075’s rolling circle replication embodiment”).

3. Nor could Dr. Satija dispute Travers’ disclosure—the ’127 patent acknowledges it and even critically relies on Travers. For example, the ’127 patent acknowledges that Travers 2010 discloses amplification followed by sequencing, and that such amplification was a recognized step in the Pacific Biosciences workflow. I agree.

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

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

EX1001, 10:63-11:18 (Reference 49 is Travers 2010); *Compare with* EX1018, ¶¶80, 122.

4. The Travers Publications disclose amplifying adapter-DNA molecules as part of sequencing. Travers 2010 describes amplifying DNA molecules having ligated U-shaped linkers. For example, Figure 3 shows the products (i.e., concatemers) resulting from rolling circle replication of a circular SMRTbell

template. EX1021, Figure 3; *see also id.*, 4 (“We assessed the ability of these templates to support the generation of long products such as would be seen in rolling-circle replication in a bulk extension assay.”). Additionally, Travers ’075 expressly states the following:

Although the constructs of the invention are described primarily, and preferably, for use directly as templates for, e.g., sequencing applications, it will be appreciated that these structures may also serve as intermediate structures in the preparation of templates that provide for sequence redundancy in line with that provided by such constructs. For example, the structurally circular nucleic acid segments described herein, may be used as templates in a rolling circle replication process to produce concatamer molecules that include repeating copies of both the sense and antisense strands of the originating double stranded segment included within the circular nucleic acid. These replicated products may then be employed directly as template molecules in a template dependent sequencing process, as described elsewhere herein (See also U.S. Pat. No. 7,476,503 [EX1020], which is incorporated herein by reference in its entirety for all purposes). Likewise, duplication processes may be employed to produce multiple copies of a prepared circular construct using methods previously described (See, e.g., U.S. Patent Application No. 61/072,160, previously incorporated herein by reference in its entirety for all purposes).

EX1018, ¶122; *see also* 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[s] 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.”).

5. Accordingly, there does not appear to be any dispute that the Travers Publications describe amplifying adapter-DNA molecules prior to sequencing. Dr. Satija also does not dispute that the '127 patent claims encompass Travers' U-shaped adapters, amplifying adapter-DNA molecules (e.g., rolling circle amplification), and sequencing on the Pacific Biosciences sequencing platform.

III. THE TRAVERS PUBLICATIONS DESCRIBE “FIRST AND SECOND STRAND SEQUENCE READS” AS REQUIRED BY CLAIMS 1-21

6. Dr. Satija appears to argue the Travers Publications do not teach first strand sequence reads and second strand sequence reads, as recited in claims 1-21. EX2014, ¶¶60-65, 75-80. Dr. Satija does not offer a similar argument with respect to claim 22. I disagree with Dr. Satija's argument regarding claims 1-21 for at least the reasons discussed below.

7. Dr. Satija's argument regarding “first and second strand sequence reads” is not commensurate with the language of the claims or guidance in the '127 patent specification. Claim 1 expressly encompasses use of U-shaped adapters and rolling circle amplification, and broadly encompasses sequencing on the Pacific Biosciences sequencing platform. *See* EX1001, claims 10, 13. The specification specifically instructs use of Travers adapters, amplification, and continuous loop sequencing as suitable for use with the disclosed methods. EX1001, 10:63-11:15; *see also id.*, 15:29-39 (“amplified by a method known in the art (e.g., a PCR or non-PCR method known in the art), ... sequenced using any suitable method

known in the art including, but not limited to, ..., Pacific Biosciences sequencing platform, ..."); EX2014, ¶214 ("the '127 patent's claimed embodiment in claim 10 is not limited to the Travers '075's rolling circle replication embodiment."); EX1037, 94:24-95:15 (confirming claims encompass but are not limited to Travers' amplification embodiment).

8. Dr. Satija argues claim 1 "indicates" (but does not require) "multiple, distinct sequencing reactions, each of which results in a sequence read..." EX2014, ¶61. However, Dr. Satija does not identify language in claim 1 reciting multiple, distinct sequencing reactions nor does he identify any definition in the specification expressly limiting a "sequence read" in such a manner. *Cf.* EX1001, 10:63-11:15;15:33-39 ("Pacific Biosciences sequencing platform").

9. Instead, Dr. Satija directs his discussion to non-limiting embodiments utilizing Y-shaped adapters, PCR amplification and sequencing on the Illumina sequencing platform. *E.g.*, EX2014, ¶¶60 ("such as the sequencing-by-synthesis process depicted in Figure 1."), 62 (citing EX1001, 4:12-17, 4:45-47, 9:5-13, Figures 1 and 3). But I understand Dr. Satija conceded the claims are not limited to Y-adapters, PCR amplification, or Illumina sequencing. EX1037, 26:9-17 ("Figure 1 is an overview of a duplex consensus sequencing method. It is a schematic figure. It is not a precise set of limitations by which to interpret the claims."), 76:10-14 ("Q. Do you understand the '127 patent claims as being limited to PCR-

based amplification? A. No, I do not.”), 77:14-17 (Q. And do you understand the claims of the ’127 patent to be limited to sequencing on the Illumina sequencing platform? 17 A. No.”).

10. Dr. Satija’s remaining argument amounts to the unremarkable observation that Travers is able to distinguish between a longer raw sequence read from the concatemer and discrete sequence reads corresponding to the sense and antisense strand. EX2014, ¶¶61, 63, 64, 76-78. This argument fails for at least two reasons.

11. First, the “subreads” of Travers 2010 meet Dr. Satija’s own definition of a sequence read. Specifically, Dr. Satija states “[a] POSA reading this claim [claim 1] would understand that a ‘sequence read’ refers to a contiguous set of DNA nucleotides that correspond to the output of a DNA sequencing reaction...” EX2014, ¶60. The so-called “subreads” of Travers 2010 are a contiguous set of DNA nucleotides” and clearly “correspond” to output of the sequencing process performed on the Pacific Biosciences sequencing platform. For example, Figure 4 of Travers 2010 (EX1021) depicts an example of sequence data generated for a template molecule. Read data is demarcated and colored to identify each respective contiguous set of DNA nucleotides corresponding to the sense and antisense strands, and such data corresponds to the output of a DNA sequencing reaction. Dr. Satija does not assert otherwise. EX1021, Figure 4, (“Demonstration of single-

molecule traces that include both sense and antisense strands...blue corresponding to the sense strand, orange corresponding to the antisense strand...”).

12. Second, Dr. Satija focuses on the use of the term “subread” in Travers 2010 but ignores that Travers ’075 refers to the sense and antisense reads as “sequence reads.” EX1018, ¶61 (“...a consensus is first established for one sequence read, e.g., a sense strand, and another consensus sequence is established for another read, e.g., the an[ti]sense strand. ... “); *see also id.*, ¶¶55 (“Because the template is circular, this process can continue to provided multiple repeated sequence reads from the one template, e.g., shown as providing a second round of the sequence data (A-B-A’-C-A-B-A’).”), 62 (“...the methods by which a consensus sequence is called from repeated sequence reads of a given segment (including reads of the sense and antisense strands), ...”). This further illustrates that a POSA would recognize the sense and antisense reads discussed in Travers 2010 and Travers ’075 are sequence reads as ordinarily understood by those in the art.

13. In summary, Dr. Satija’s argument regarding “first and second strand sequence reads” is inconsistent with the language of the claims and guidance in the specification. The Travers Publications demonstrate disclosure of sequence reads corresponding to the sense and antisense strands that meets the requirements of claims 1-21.

IV. THE TRAVERS PUBLICATIONS DESCRIBE GROUPING AS IN CLAIMS 22-30

14. Dr. Satija argues claims 22-30 distinguishes over Travers in that Travers does not describe “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, claim 22; EX2014, ¶¶81-87. Again, Dr. Satija does not dispute that the ’127 patent claims encompass the Travers U-shaped adapters or that those adapters contain barcodes as recited in either claims 1 or 22. According to Dr. Satija, the Travers Publications, while disclosing barcoded adapters, do not specifically utilize the barcodes for grouping as recited in claim 22. Dr. Satija’s argument is not commensurate with the language of the ’127 patent claims or the guidance in the specification, and I disagree for at least the reasons set forth below.

15. Claims 22-30 of the ’127 patent generically recite “barcode.” Dr. Satija, however, ignores the language of the claims and his argument revolves around drawing distinction between sample barcodes (also known as index barcodes or multiplex barcodes) and molecular barcodes. *E.g.*, EX2014, ¶¶84 (“Pooling of indexed barcoded samples is very different from molecular barcoding...”), 85 (“Guardant argued [in an unrelated case] that index barcodes are different from molecular barcodes. I agree.”), 86 (arguing Travers’ barcodes “are

not fragment-specific by definition”). There is no requirement in claims 22-30 for “molecular” or “fragment-specific” barcodes. The claims simply recite “barcode.”

16. At the relevant timeframe, various types of barcodes were routinely used in nucleic acid sequencing applications. For example, index barcodes—also referred to as sample barcodes or multiplexing barcodes—were commonly used to identify nucleic acid molecules belonging to a particular sample. *E.g.*, EX1018, ¶100; EX1012, Figure 4. In fact, the only usage of the term “barcode” in the specification of the ’127 patent is in connection with a multiplexing barcode. EX1001, 20:34-39 (“where XXXXXX indicates the position of a fixed multiplexing barcode sequence”).

17. The ’127 patent does not use the term “molecular barcode” and neither do the claims. Claim 22 generically recites “barcodes” and requires grouping based on “the barcode sequence.” Travers discloses such a grouping. For example, Travers describes including barcodes in template molecules such that sequence data may be pooled for analysis. EX1018, ¶100 (“...template constructs that include sequence markers like a barcode...Different template samples including distinguishable connecting oligonucleotide tags could then be pooled for analysis in a single sequencing process.”); *see also* EX1037, 86:4-10 (“The important thing is that those indexing barcodes enable material from different sources to be pooled together and then effectively indexed after the sequencing

data is collected so that each molecule can be returned back or grouped together with molecules from the same original source ...”).

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

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

V. THE TRAVERS PUBLICATIONS DESCRIBE USING THE ASYMMETRY OF ADAPTER-DNA MOLECULES AS REQUIRED BY CLAIM 19

20. Dr. Satija argues that the Travers Publications do not teach distinguishing first and second strand sequence reads as recited in claim 19 “using the asymmetric nature of the adapters.” EX2014, ¶89; *see generally id.*, ¶¶88-94.

As explained in further detail below, I disagree to the extent Dr. Satija argues the Travers Publications do not disclose what is recited in claim 19.

21. As an initial observation, Dr. Satija's argument regarding adapter asymmetry is not commensurate with the language of claim 19. Dr. Satija repeatedly directs argument only to the adapter. EX2014, ¶¶89 (“using the asymmetric nature of the adapters”), 90 (“asymmetry of adapters”), 91 (“asymmetry of the adapters”), 92 (“adapter asymmetry”), 93 (“asymmetric nature of adapters”), 94 (“asymmetric nature of the hairpin adapters”). But the plain language of claim 19 recites distinguishing first and second strand sequence reads based on the “asymmetry of the asymmetrical double-stranded adapter-DNA molecules.” Even the portion of the specification Dr. Satija identifies as supporting his arguments expressly states that it is the asymmetric nature of the adapter-DNA fragments that allows the strands to be distinguished. EX2014, ¶89 (quoting EX1001, 3:64-4:3) (“the asymmetric nature of adapted fragments”).

22. My previous declaration explained that the U-shaped adapters of Travers are asymmetric, are encompassed by the '127 patent claims, and generate asymmetric double-stranded adapter-DNA molecules. *E.g.*, EX1002, ¶¶49-52, 69. Dr. Satija does not dispute any of this, nor could he. EX1001, 10:63-11:2 (“As the sequence of the linker itself does not matter in the workflow, the published linker sequences from [Travers 2010] would be adequate for use in the assay.”).

23. Indeed, Dr. Satija affirmatively argues Travers 2010 describes asymmetry associated with its adapter-DNA molecules. For example, Dr. Satija points to disclosure in Travers 2010 of using two different hairpin adapters such that the “circular DNA template [has] one and only one sequencing primer binding site.” EX2014, ¶129; *see also* EX1021, 2 (providing sequence for “two hairpin-forming oligonucleotides”); EX1018, ¶92 (“In addition, asymmetric linking oligonucleotides, e.g., having different numbers of nucleotides joining the sense and antisense strands, may be used within a single template construct.”).

24. Nor does Dr. Satija dispute Travers’ use of the U-shaped linkers and corresponding asymmetric molecules in the sequencing process to distinguish sequence reads corresponding to the sense and antisense strands. Dr. Satija argues that Travers describes using “adapter sequences as landmarks or ‘registration sequences’ that mark the portion of the sequence read where one strand ends and the next strand begins.” EX2014, ¶91 (citing EX1018, ¶54); *see also id.*, ¶¶92, 93. This argument offers no distinction from what is claimed. That is, Dr. Satija acknowledges Travers describes using an asymmetry of the adapter-DNA molecule (e.g., position and sequence of the two adapters) to distinguish sequence reads for each of the two strands of an original DNA molecule. The disclosure of the Travers Publications meets the requirements of claim 19. Dr. Satija fails to demonstrate otherwise.

VI. INOPERABILITY ARGUMENTS ARE MERITLESS

25. Dr. Satija argues that a POSA would not have been motivated to use Travers' rolling circle replication embodiment with the Pacific Biosciences sequencing platform. EX2014, ¶¶18-25, 96-216. I disagree. The Travers Publications are from the same authors describing the same sequencing technology and sequencing platform, and admittedly expressly disclose amplification followed by sequencing—i.e., the “rolling circle replication embodiment” that Dr. Satija and the '127 patent inventors repeatedly acknowledge is discussed in the prior art. Dr. Satija's description of this disclosure as a “combination” is misleading and inaccurate. Dr. Satija's argument essentially boils down to a meritless charge of inoperability, which is contradicted by the '127 patent itself as well as the express content of the Travers' Publications.

26. As discussed above, there is no real dispute that the '127 patent claims encompass use of Travers' U-shaped linkers, amplifying adapter-DNA molecules using rolling circle amplification, followed by sequencing—including sequencing using Pacific Biosciences sequencing platform. EX1001, 10:59-11:18; claim 10, 13; *see also* EX2014, ¶214 (“the '127 patent's claimed embodiment in claim 10 is not limited to the Travers '075's rolling circle replication embodiment.”); EX1037, 94:24-95:15 (confirming claims encompass but are not limited to Travers' amplification embodiment). There is no meaningful distinction between the '127

patent discussion of this subject matter and what is found in the prior art—in fact, the '127 patent cites to and relies specifically on Travers. *Compare*, e.g., EX1001, 11:3-18 *with* EX1018, ¶¶80, 122.

27. Dr. Satija argues that Travers's embodiment that sequences a plurality of first and second strand copies generated by rolling circle amplification was “unworkable” because of four alleged problems: (1) multiple polymerase binding sites; (2) different sized templates; (3) diffusion bias; and (4) the physical size of ZMW. EX2014, ¶¶123-203. But Dr. Satija's discussion of each of these alleged problems is contradicted by disclosure in the Travers Publications. I have also reviewed the declaration of Dr. Andres Larrea (EX1033), who explains in detail that Dr. Satija's alleged problems posed no practical barrier to performing that Travers embodiment. I agree with Dr. Larrea's analysis.

28. Dr. Satija's opinion about multiple primer/polymerase binding sites (alleged Problem 1) is directly contradicted by the Travers Publications, which specifically teach templates with multiple binding sites. EX1018, ¶¶14, 17, 67, 103, 105, 144, Fig. 7; EX1021, 3. Moreover, the Travers Publications teach the very same “downstream” techniques Dr. Satija cited in the '127 patent as addressing these issues. EX1018, ¶¶80 (desirable to provide endonuclease recognition sites within the linking oligonucleotide), 78 (engineered pause point within the linking oligonucleotide region); EX1037, 94:15-99:16; EX2014, ¶¶214-

15; EX1001, 11:15-18. In this respect, the '127 patent merely incorporates the methodologies disclosed by Travers.

29. Regarding different sized templates and diffusion bias, Dr. Larrea aptly demonstrates that routine size selection techniques were available to resolve Dr. Satija's concerns. Dr. Satija's argument (alleged Problems 2 and 3) unreasonably assumes a POSA would be aware of generating molecules of different size but abandon routine loading time selection or size selection that even the manufacturer's Template Preparation Guide instructs utilizing.

30. Regarding the physical size of the ZMW (alleged problem 4), Dr. Satija's arguments are directly contradicted by the Travers Publications themselves. Travers '075 provides working examples in which templates containing inserts in the range of 500–2,560 bp were sequenced. EX1018, ¶¶83 (discussing templates of 200 to 50,000 bases in length, including examples with thousands of base pairs), 85, 144, 150; EX1021, 1 (40-25,000 bp with no intrinsic limit to template size), 5 (1,000 bp). Dr. Satija's argument incorrectly assumes a physical inflexibility of the template in the face of DNA polymerase activity that is completely unsupported. I agree with Dr. Larrea that Dr. Satija's ZMW dimension arguments are invalid.

31. Accordingly, Dr. Satija's arguments regarding inoperability are inconsistent with the '127 patent and the prior art, including the Travers Publications, and are wholly without merit.

VII. SECONDARY CONSIDERATIONS

32. Dr. Satija argues that praise, long-felt need, and unexpected results associated with "duplex sequencing" provides objective evidence of nonobviousness of claims 2-9, 16, 18, 19. *E.g.*, EX2014, ¶¶227-229, 237-238, 241-243. For example, Dr. Satija relies on the Kirsch article (EX2007) to establish praise of "the duplex sequencing approach." EX2014, ¶227. However, the Kirsch article is all about duplex consensus sequencing ("DCS") in a reference to Schmitt. EX1037, 127:15-129:18 ("[Schmitt] also identifies the complementary DNA strand by searching for the complementary tag sequence among the SSCS duplex consensus sequences, DCS...."). Even Dr. Satija characterizes the proffered evidence of secondary considerations as pertaining to DCS. EX2014, ¶229 ("Dr. Kirsch praises Patent Owner's duplex consensus sequencing method..."); *see also id.*, ¶¶227-228, 237-238, 241-243. None of claims 2-9, 16, 18, 19 require performance of duplex consensus sequencing. Indeed, the evidence presented by Dr. Satija relates to unclaimed features and features found in the prior art. Accordingly, it is my opinion that there is no link between the method of claims 2-

9, 16, 18, 19 (or any other claim of the '127 patent) and the alleged secondary considerations evidence put forward by Dr. Satija.

33. The '127 patent describes DCS as a two-stage process, where the second stage (termed “DCS”) includes matching (by SMI) previously constructed single-strand consensus sequences (SSCSs). *See, e.g.*, EX1001, 16:19-28. Kirsch similarly describes the DCS process discussed in the Schmitt reference. EX2007, 1 (“[Schmitt] also identifies the complementary DNA strand by searching for the complementary tag sequences among the SSCS reads [duplex consensus sequences (DCS)].”).

34. I have reviewed the claims of the '127 patent and none requires performing DCS as that method is described either in the specification or by Kirsch. Claims 2, 3, 9, 16, 18, and 19, all of which depend from claim 1, do not require generating a consensus sequence much less a duplex consensus sequence or DCS. EX1037, 80:7-10 (“Q. Does claim 1 recite the term ‘consensus sequence’? A. I don’t see the words ‘consensus sequence’ appearing in claim 1.”). Claims 4-8, which also depend from claim 1, recite “consensus sequence” but require neither a single-strand consensus sequence, matching SSCSs, nor a duplex consensus sequence.

35. The claims of the '127 patent are so broad as to encompass the prior art, including prior art methods described by the Travers Publications.

Furthermore, I understand that the Board has found that Kinde (EX1006) in view of Craig (EX1038) teaches each limitation of claim 1 of the '127 patent. *Guardant Health, Inc. v. University of Washington*, IPR2022-00816, DI, 13 (“we find Petitioner has shown sufficiently that the combination of Kinde and Craig teaches each limitation of claim 1”).

36. That the claims recite only features found in the prior art is further confirmed by Kirsch. Kirsch explains Kinde’s Safe-SeqS, like DCS, uses tags (i.e., UIDs) to group original strands into families and generate a single-strand consensus sequence (SSCS). EX2007, 1 (“This approach allows them to identify families of DNA molecules that share the same tag sequence on a single strand, called single-strand consensus sequence (SSCS), analogous to a recently published approach termed Safe-SeqS (6).”). Indeed, Kinde instructs “High-quality reads were grouped into UID families on the basis of their endogenous or exogenous UIDs.” EX1006, 9535; *see also id.*, 9531 (“the uniquely tagged fragments are amplified, producing UID families, each member of which has the same UID”); EX1037, 137:1-6 (“Q. Is it your understanding that Kinde does not disclose identifying families of DNA molecules that share the same tag sequence on a single strand? ... A. That’s not my precise understanding.”). Kinde further describes calling mutations when 95% of reads within a family have the same

mutation. EX1006, 9530 (“at least 95% of family members have the identical mutation”), 9531 (Figure 1).

37. Kirsch ascribes no novelty over Kinde with respect to tagging sample DNA fragments with adapters comprising barcodes and indeed there is none. There can be no dispute that barcoded adapters were known in the art and were even commercially available prior to the '127 patent. *E.g.*, EX1012, Figure 4 (Illumina brochure describing commercially available barcoded adapters). Indeed, Kinde identifies prior art barcoded Y-shaped adapters (e.g., Craig, EX1038) in its discussion of options for assigning UIDs to molecules. EX1006, 9531 (“UIDs, sometimes called barcodes or indexes, can be assigned to nucleic acid fragments using a variety of methods. These methods include the introduction of exogenous sequences through PCR (40, 41) or ligation (42, 43).”) (Reference 42 is Craig).

38. Vogelstein, describing Safe-SeqS, further elaborates on the addition of exogenous UIDs to sample DNA molecules.

Attachment of an exogenous UID to an analyte nucleic acids fragment may be performed by any means known in the art, including enzymatic, chemical, or biologic. One means employs a polymerase chain reaction. Another means employs a ligase enzyme. The enzyme may be mammalian or bacterial, for example. Ends of fragments may be repaired prior to joining using other enzymes such as Klenow Fragment of T4 DNA Polymerase. Other enzymes which may be used for attaching are other polymerase enzymes. An UID may be added to one or both ends of the fragments. A UID may be contained within a nucleic acid molecule that contains other regions for other intended functionality. For example, a universal priming site may be added to permit later amplification.

EX1022, ¶22.

39. Kirsch identifies the difference between DCS and one embodiment of Kinde as “searching for the complementary tag sequences among the SSCS reads [duplex consensus sequences (DCS)]. A sequence base at a given position is then kept only if the read data from each of the two strands match perfectly.” EX2007, 1. However, as I discuss above, the claims of the ’127 patent do not require DCS. Furthermore, searching for and comparing consensus sequences for both strands of an original DNA molecule was known in the art as confirmed by both Kinde and Vogelstein. For example, Kinde explains “the specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation.” EX1006, SI3. Vogelstein also describes identifying mutations on both strands of an original DNA molecules.

Safe-SeqS analysis can also determine which strand of a template is mutated, thus an additional criteria for calling mutations could require that the mutation appears in only one or in both strands of the originally double stranded template. Massively parallel sequencers are able to obtain sequence information from both ends of a template in two sequential reads. (This type of sequencing experiment is called a “paired end” run on the Illumina platform, but similar experiments can be done on other sequencing platforms where they may be called by another name.) The two strands of a double stranded template can be differentiated by the observed orientation of the sequences and the order in which they appear when sequence information is obtained from both ends. ...

EX1022, ¶42; *see also id.*, ¶62 (explaining mutations “present in the zygote, [or] occurred later during embryonic and adult development, ... are expected to be present in both strands of the relevant templates”).

VIII. CONCLUDING STATEMENTS

40. 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.

41. 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: / John Quackenbush /
John Quackenbush