

Petitioner's Demonstratives

***Guardant Health, Inc. v. University of Washington
Through Its Center for Commercialization***

IPR2022-00817

U.S. Patent No. 10,760,127

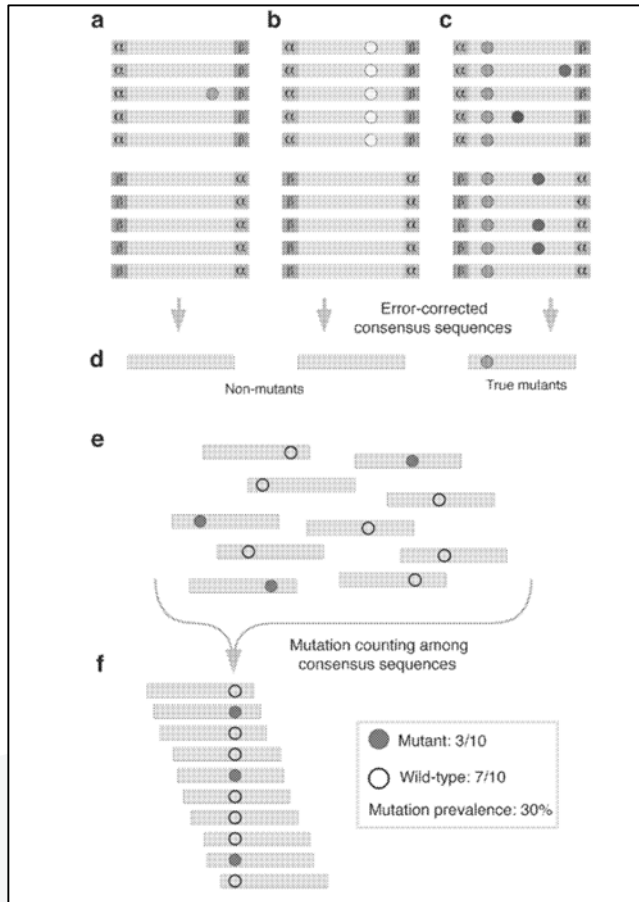
Instituted Grounds

Claims	35 U.S.C. §	Prior Art
1-10, 12, 15-28	103	Travers '075 and Travers 2010
11	103	Travers '075, Travers 2010, and NEB Expressions
13, 14, 29, 30	103	Travers '075, Travers 2010, and McCloskey

The Disclosed Invention & the Claims

The '127 Patent & Duplex Consensus Sequencing (DCS)

Figure 3



(57)

ABSTRACT

Next Generation DNA sequencing promises to revolutionize clinical medicine and basic research. However, while this technology has the capacity to generate hundreds of billions of nucleotides of DNA sequence in a single experiment, the error rate of approximately 1% results in hundreds of millions of sequencing mistakes. These scattered errors can be tolerated in some applications but become extremely problematic when “deep sequencing” genetically heterogeneous mixtures, such as tumors or mixed microbial populations. To overcome limitations in sequencing accuracy, a method Duplex Consensus Sequencing (DCS) is provided. This approach greatly reduces errors by independently tagging and sequencing each of the two strands of a DNA duplex. As the two strands are complementary, true mutations are found at the same position in both strands. In contrast, PCR or sequencing errors will result in errors in

16:19-28

The method of generating an error corrected double-stranded consensus sequence further comprises the second stance that is termed DCS. The DCS stage includes steps of comparing the sequence of the two single strand consensus sequences arising from a single duplex DNA molecule, and further reducing sequencing or PCR errors by considering only sites at which the sequences of both single-stranded DNA molecules are in agreement. The method that includes the first stage and the second stage termed Duplex Consensus Sequencing (DCS).

Pet. 1, 9; Reply 25

Claim 1 – No Consensus Sequencing

1. A method of sequencing DNA comprising:

- 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; and
- d) for at least some of the adapter-DNA molecules comprising barcodes—
 - confirming the presence of at least one sequence read derived from each of the original first and second strands of the adapter-DNA molecules;
 - comparing at least one of the confirmed first and second strand sequence reads to a reference sequence; and
 - analyzing one or more correspondences between the at least one of the confirmed first and second strand sequence reads and the reference sequence to identify a sequence variation.

Reply 25; EX1037, 80:7-10

Claim 22 – No Duplex Consensus Sequencing

22. A method of sequencing DNA comprising:

- a) attaching partially single-stranded adapters comprising barcodes selected from a plurality of distinct barcode sequences to double-stranded DNA fragments obtained from a bodily sample, wherein attachment of the adapters to double-stranded DNA fragments generates a library of tagged double-stranded adapter-DNA molecules;
- 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; and
- 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;
 - collapsing a plurality of strand sequence reads within the families to provide a consensus sequence for each of the at least some of the double-stranded DNA molecules in the library;
 - comparing the consensus sequence to a reference sequence; and
 - analyzing one or more correspondences between the consensus sequence and the reference sequence to identify a sequence variation.

Reply 25; EX1037, 83:9-17

The '127 Patent Specification – “attaching adapters”

EX1001, 10:61-11:17

ers. 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.

In some aspects of some embodiments, deliberate ligation of “U-shaped” adaptors or hairpin linkers containing 1) a double-stranded n-mer (or other form of degenerate or semi-degenerate double-stranded tag as enumerated above) plus 2) primer binding sites to both ends of a captured fragment may be desirable. Producing closed circles of captured material may help facilitate removal of non-captured DNA by exonuclease digestion given that circularized DNA will be protected from digestion by such enzymes. Additionally, closed circles may be pre-amplified using rolling circle amplification or serve as the substrate for continuous loop sequencing [49]. Recognition sites for restriction endonuclease digestion could be engineered into these adaptors to render closed loops open once again if more convenient for subsequent steps.

EX1001, claim 13

13. The method of claim 1, wherein the adapters comprise a Y-shape or a U-shape, and wherein the barcodes comprise a degenerate or semi-degenerate sequence containing between 3 and 20 nucleotide bases.

Institution Decision, p. 8

Indeed, citing Travers, the '127 patent discloses that “ligation and amplification with circularizing ‘linker’ (i.e. hairpin linkers affixed to both ends of a fragment) has been demonstrated as a step in the Pacific Biosciences sample preparation workflow.” Ex. 1001, 10:63–66. According to the '127 patent, “the published linker sequences” of Travers “would be adequate for use” in its method. *Id.* at 11:1–2.

Pet. 14-15, 17-19; Reply 1

Hairpin Adapters & Asymmetrical Adapter-DNA Molecules

Petition:

- Under plain meaning, the claims “encompass adapter-DNA molecules generated by attachment of Y-shaped or U-shaped adapters comprising barcodes to each end of double-stranded DNA molecules.”

Board:

- “Patent Owner does not dispute these assertions.”
- “Based on the current record, we agree with Petitioner the claims encompass Y-shaped adapters and hairpin adapters, provided that they comprise ‘barcodes selected from a plurality of distinct barcode sequences,’ which is also required by the claims.”

POR:

- No dispute

The '127 Patent Specification – “amplifying” & “sequencing”

EX1001, 10:61-11:17

ers. 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.

In some aspects of some embodiments, deliberate ligation of “U-shaped” adaptors or hairpin linkers containing 1) a double-stranded n-mer (or other form of degenerate or semi-degenerate double-stranded tag as enumerated above) plus 2) primer binding sites to both ends of a captured fragment may be desirable. Producing closed circles of captured material may help facilitate removal of non-captured DNA by exonuclease digestion given that circularized DNA will be protected from digestion by such enzymes. Additionally, closed circles may be pre-amplified using rolling circle amplification or serve as the substrate for continuous loop sequencing [49]. Recognition sites for restriction endonuclease digestion could be engineered into these adaptors to render closed loops open once again if more convenient for subsequent steps.

EX1001, 15:29-39

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 using any suitable method known in the art including, but not limited to, the Illumina sequencing platform, ABI SOLiD sequencing platform, Pacific Biosciences sequencing platform, 454 Life Sciences sequencing platform, Ion Torrent sequencing platform, Helicos sequencing platform, and nanopore sequencing technology.

10. The method of claim 1, wherein amplifying original strands comprises amplifying original strands via PCR amplification, cluster amplification, rolling circle amplification or a combination thereof.

Pet. 17-19; Reply, 4; DI, 8

Satija Confirms Broad Scope of Claims

Dr. Satija:



EX1037, 26:9-17

Q. And is your opinion that the claims of the '127 patent are limited to that example illustrated in Figure 1?

ATTORNEY POWERS: Objection, asked and answered.

A. So 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.

EX1037, 76:10-14, 77:14-17

Q. Do you understand the '127 patent claims as being limited to PCR-based amplification?

(Pause.)

A. No, I do not.

Q. And do you understand the claims of the '127 patent to be limited to sequencing on the Illumina sequencing platform?

A. No.

Reply 7

The Travers Publications

Travers Publications

- **No dispute that the Travers Publications describe the same SMRT sequencing commercial product**
- **No dispute that a POSA would reasonably look to both Travers Publications when contemplating SMRT sequencing**
- **No modification of Travers required – all limitations expressly disclosed**

For an obviousness analysis, a reference is prior art for all that it teaches. *Beckman Instruments, Inc. v. LKB Produkter AB*, 892 F.2d 1547, 1551 (Fed. Cir. 1989). The fact that an embodiment is preferred “is not controlling, since all disclosures of the prior art, including unpreferred embodiments, must be considered.” *Merck & Co., Inc. v. Biocraft Labs., Inc.*, 874 F.2d 804, 807 (Fed. Cir. 1989).

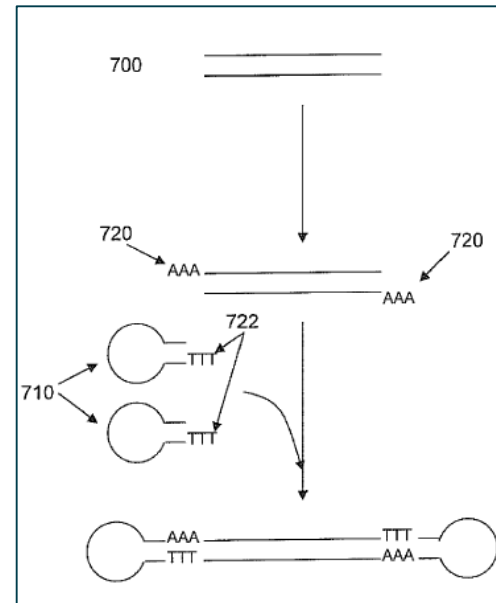
Institution Decision,
pp. 18-19

Patent Owner contends that Petitioner fails to show how to modify the preferred SMRT sequencing methods in the Travers references. Prelim. Resp. 27. Based on the current record, we do not see a need for modification. Travers '075's paragraph 122 is merely directed to an alternative use of the circular molecules (comprised of the DNA fragment of interest linked by the hairpin linkers), such as SMRTbell templates. See Ex. 1018 ¶ 122 (discussing “the constructs of the invention” and “the structurally circular nucleic acid segments”). As we understand it, these

Pet. 19-20; Reply 1-6; DI 14-19

Travers '075: attaching hairpin adapters comprising barcodes to DNA

[0051] In a typical sequencing process, a double stranded target nucleic acid, e.g., a nucleic acid for which sequence information is desired, is converted to a template configuration of the invention. Typically, this involves fragmentation of a population of a larger double stranded nucleic acid, e.g., a genome, plasmid, or portion thereof, or the like, into pools of smaller, overlapping double stranded target nucleic acids or nucleic acid fragments. The double stranded fragments are then processed to provide the linking oligonucleotides described above and in greater detail, below. A number of methods, as set forth herein, may be employed to provide the linking oligonucleotides connecting the sense and antisense strand. In one simple example, exogenous hairpin adapter sequences are used and simply ligated to the end(s) of the double stranded fragments to form such linking oligonucleotides. Such exogenous hairpin adapters will typically include two complementary nucleic acid sequence segments at opposing ends of a longer sequence, where they are separated by another stretch of non-complementary nucleotides. The result is a structure that includes a double stranded stem structure coupled to the single stranded loop. Examples of



[0100] As alluded to above, however, the connecting or linking oligonucleotide may be selected or generated to include sequence with identifiable sequence characteristics to facilitate its identification, both with respect to the contiguous template sequence with which it is associated, and also with respect to other template sequences that may exist in the same sample mixture. 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

Pet. 16-17, 21-24; Reply 1

Travers '075: amplification prior to sequencing

[0122] 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, which is incorporated herein by reference in its entirety for all purposes). Likewise, duplication processes may be employed to

Pet. 16-17, 24-25; Reply 3-6

Travers '075: generating sequence reads

[0054] By way of example, with respect to a partially contiguous template shown in FIG. 2A, obtaining the entire sequence, e.g., that of segments 202, 204 and 206 provides a measure of consensus sequence determination by virtue of having sequenced both the sense strand, e.g., segment 202, and the antisense strand, e.g., segment 204. In addition to providing sense and antisense sequence reads from a single template molecule that can be sequenced in one integrated process, the presence of linking segment 206 also provides an opportunity to provide a registration sequence that permits the identification of when one segment, e.g., 202, is completed and the other begins, e.g., 204. Such registration sequences provide a basis for alignment sequence data from multiple sequence reads from the same template sequences, e.g., the same molecule, or identical molecules in a template population. The progress of sequencing processes is sche-

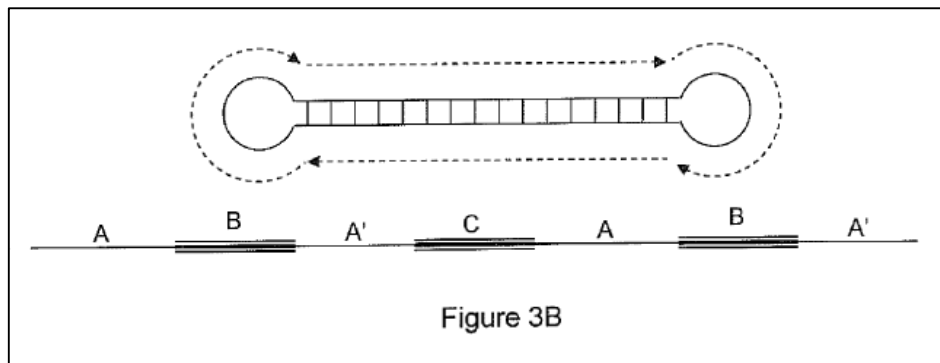
Travers '075: grouping sequence reads

[0058] Although referred to herein as comparing or assembling the sequence data from multiple reads of a given sequence, and/or from the sense and antisense strands of the sequence, it will be appreciated that any method of assigning a consensus determination to a particular base call from multiple reads of that position of sequence, and/or to a provide an overall consensus sequence for that segment, will be envisioned and encompassed by the term “compare”. Such methods include actual side-by-side comparisons, scoring methods whereby calls of iterative reads or from complementary sequences are scored by the number of occurrences, and optionally or alternatively additional signal/base calling metrics, to complex algorithms for assigning a base call from multiple indications of the base at a given position.

[0102] The different samples are then pooled for sequencing in a unified sequencing reaction. As the sequencing output from each run is based upon individual molecules, the sequencing data is then parsed out according to its origin by virtue of the barcode sequence that is integrated into the template molecules. In particular, because each sequence read is derived from a single molecule, the sequence of the insert portion of the template can be unambiguously linked to the sequence of its attached adapter sequence that includes the bar code sequence within that adapter sequence. Accordingly, each template sequence is then traced back to its origin, e.g., a particular sample, patient, etc.

Pet. 33-34; Reply 9-11

Travers '075: analyzing sequence reads & consensus



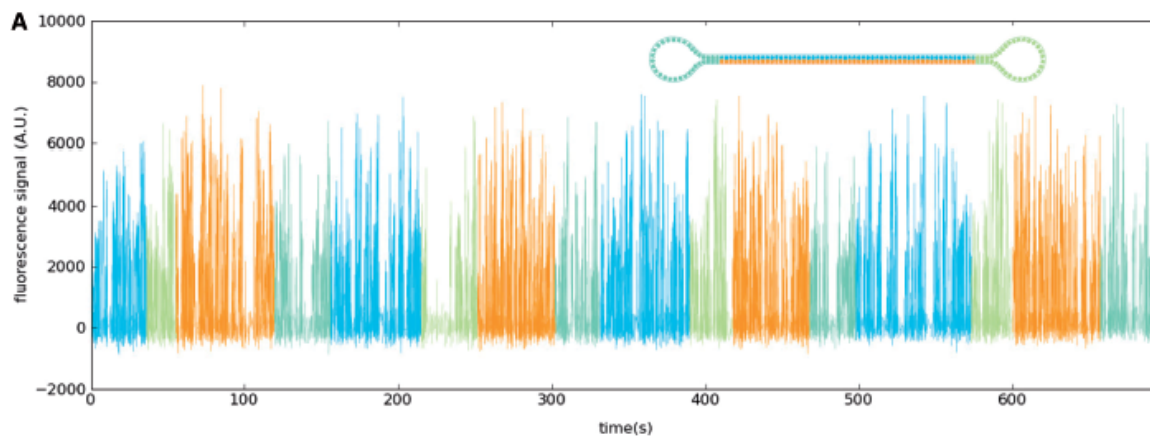
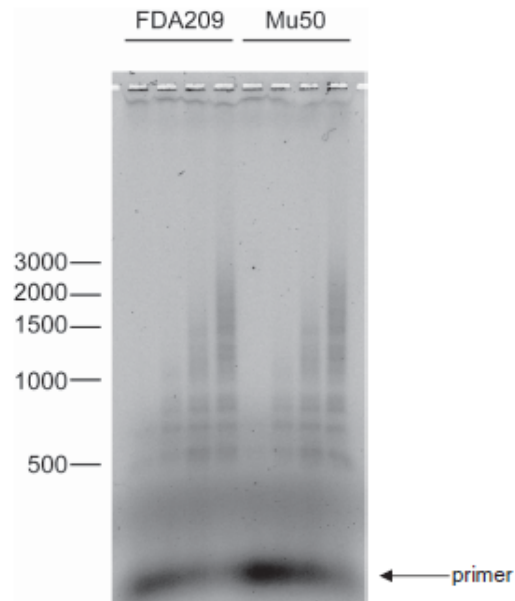
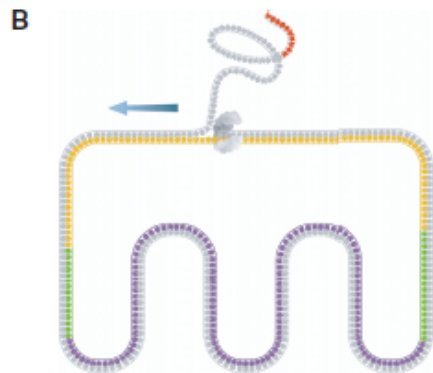
[0061] In another method for combination of data, a consensus is first established for one sequence read, e.g., a sense strand, and another consensus sequence is established for another read, e.g., the antisense strand. These sequences can be established by methods known in the art including heuristic methods for multiple-sequence alignment, optimal methods for multiple sequence alignment, or hidden Markov models in combination with a consensus determination algorithm such as Plurality, Quality-weighted Plurality, Bayesian methods, or machine learning approaches (neural networks, self-organizing maps). These consensus sequences can then be associated and combined via algorithms such as heuristic methods for multiple-sequence alignment, optimal methods for multiple sequence alignment, or hidden Markov models in combination with a consensus determination algorithm such

[0146]

for each ZMW chip for generating sequencing data as previously described. The sequenced fragments were aligned to the K12 MG1665 reference sequence.

Pet. 26-29, 34-37; Reply 2-3

Travers 2010



PO and Dr. Satija: Not Limited to Travers

POR:

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-

Dr. Satija:



214. Dr. Quackenbush cites the '127 patent's specification and a claimed embodiment in claim 10 to support his opinion that Travers '075's rolling circle replication embodiment works within the SMRT sequencing workflow of Travers '075 and Travers 2010. EX1002, ¶61, 151-154. However, the '127 patent's claimed embodiment in claim 10 is not limited to the Travers '075's rolling circle replication embodiment.

Reply, 3

Disputed Issues

PO's Response Arguments

- ***Subreads are not "sequence reads" (Claims 1-21)***
- ***"Grouping" requires molecular barcodes (Claims 22-30)***
- ***Travers' adapters are not asymmetric (Claim 19)***
- ***Travers' amplification embodiment is inoperable***
- ***McCloskey's barcodes are only for PCR (Claims 13, 14, 29 & 30)***

PO's "Subreads" Argument

POR 8-9:

“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). . . . Travers-2010 (EX1021) refers to the sense and antisense target DNA sequences as subreads, . . .”

But see WesternGeco LLC v. ION Geophysical Corp., 889 F.3d 1308, 1323-24 (Fed. Cir. 2018) (“It is well established that claims are not limited to preferred embodiments, unless the specification clearly indicates otherwise.”).

EX1001, 15:29-39

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 using any suitable method known in the art including, but not limited to, the Illumina sequencing platform, ABI SOLiD sequencing platform, Pacific Biosciences sequencing platform, 454 Life Sciences sequencing platform, Ion Torrent sequencing platform, Helicos sequencing platform, and nanopore sequencing technology.

EX1037, 76:10-14, 77:14-17

Q. Do you understand the '127 patent claims as being limited to PCR-based amplification?

(Pause.)

A. No, I do not.

Q. And do you understand the claims of the '127 patent to be limited to sequencing on the Illumina sequencing platform?

A. No.

Reply 6-9, 13

PO's "Subreads" Argument

- PO's linguistic distinction is of no moment:
 - Dr. Satija: "a 'sequence read' refers to a contiguous set of DNA nucleotides that correspond to the output of a DNA sequencing reaction..."
 - Travers meets Dr. Satija's definition
 - PO never addresses that Travers '075 expressly describes producing sense and antisense "sequence reads":

[0054] By way of example, with respect to a partially contiguous template shown in FIG. 2A, obtaining the entire sequence, e.g., that of segments 202, 204 and 206 provides a measure of consensus sequence determination by virtue of having sequenced both the sense strand, e.g., segment 202, and the antisense strand, e.g., segment 204. In addition to providing sense and antisense sequence reads from a single template molecule that can be sequenced in one integrated process, the presence of linking segment 206 also provides an opportunity to provide a registration sequence that permits the identification of when one segment, e.g., 202, is completed and the other begins, e.g., 204. Such registration sequences provide a basis for alignment sequence data from multiple sequence reads from the same template sequences, e.g., the same molecule, or identical molecules in a template population. The progress of sequencing processes is sche-

Reply 6-9, 13

PO's grouping argument

Satija ¶86:

A POSA would have understood that the subreads in Travers '075 and Travers 2010 are only "grouped" because they are physically connected to one another. Second, the sample barcodes disclosed in Travers '075 must be identical for all template molecules within a sample, and therefore are not fragment-specific by definition.

Claim 22:

...d) for at least some of the double-stranded adapter-DNA molecules in the library—

grouping the strand sequence reads into families based on i) the barcode sequence, and ii) DNA fragment-specific information;...

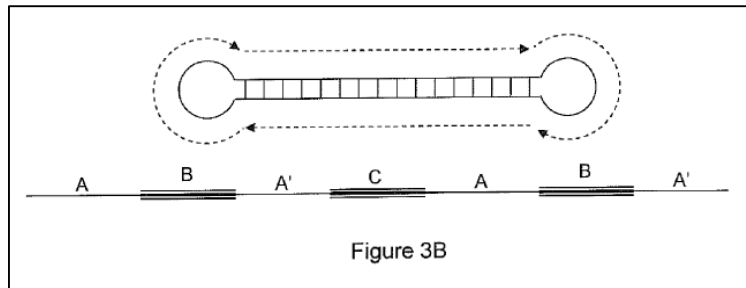
- *The claims generically recite "barcode" - there is no requirement for fragment-specific or molecular barcodes*

Reply 9-11

PO's "asymmetry" argument

POR 16:

*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.*



- *PO does not dispute the claims encompass Travers' adapter-DNA molecules.*
- *PO's argument ignores the language of claim 19*

*19. The method of claim 1, wherein 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**.*

Reply 11-13

PO's Inoperability Arguments

POR:

- Travers would allegedly suffer from several problems rendering the steps of its expressly disclosed embodiment “incompatible”

Legal Standard:

- *Ex Parte Coyle* (P.T.A.B. April 29, 2021) (“[W]here the evidence or arguments presented assert that features discussed in the reference relied upon are inoperative, *the claims* ... must distinguish from the alleged inoperative subject matter in the reference’s disclosure.”) (citing *In re Crosby*, 157 F.2d 198 (C.C.P.A. 1946))
- *SRI Int’l, Inc. v. Internet Sec. Sys.*, 511 F.3d 1186, 1193 (Fed. Cir. 2008) (“[I]f the specification of the [] patent was sufficient to enable the claims of that patent, so, too, is the description of [the prior art].”); *see also In re Paulsen*, 30 F.3d 1475, 1481 n.9 (Fed. Cir. 1994); *In re Epstein*, 32 F.3d 1559, 1568 (Fed. Cir. 1994)

’127 Patent:

- Claim 10 “... amplifying original strands comprises ... rolling circle amplification ...”
- Claim 13 “...the adapters comprise a Y-shape or a U-shape...”
- Support for U-shaped adapters and rolling circle amplification is Travers

Reply 3-6, 14-23

PO fails to distinguish Travers

Satija ¶215: Indeed, 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. Thus, the templates of the '127 patent could be further processed, not sequenced directly after rolling circle replication, as suggested by Dr. Quackenbush. However, Travers '075 and Travers 2010 do not disclose any of these downstream modification steps disclosed in the '127 patent.

EX1001

In some aspects of some embodiments, deliberate ligation of “U-shaped” adaptors or hairpin linkers containing 1) a double-stranded n-mer (or other form of degenerate or semi-degenerate double-stranded tag as enumerated above) plus 2) primer binding sites to both ends of a captured fragment may be desirable. Producing closed circles of captured material may help facilitate removal of non-captured DNA by exonuclease digestion given that circularized DNA will be protected from digestion by such enzymes. Additionally, closed circles may be pre-amplified using rolling circle amplification or serve as the substrate for continuous loop sequencing [49]. Recognition sites for restriction endonuclease digestion could be engineered into these adaptors to render closed loops open once again if more convenient for subsequent steps.

EX1018

[0080] In some cases, it may be desirable to provide endonuclease recognition sites within the linking oligonucleotide, which can allow for a mechanism to release a given template sequence from a synthesis reaction, i.e., by linearizing it, and allowing the polymerase to run off the linear template, and/or to expose the template to exonuclease activity, and thus terminate synthesis through removal of the template. Such sites could additionally be exploited as control sequences by pro-

Reply 5-6

PO fails to distinguish Travers

Dr. Satija:



EX1037, 95:10-100:8

Q. So at the top of the page you are citing to recognition sites for restriction endonuclease digestion; correct?

A. That's correct.

Q. A few lines down you state, Travers '075 and Travers 2010 do not disclose any of these downstream modification steps.

A. That's correct.

Q. Can you turn to paragraph 80 of Travers 2010 and let me know when you're there.

(Pause.)

A. Okay.

Q. Do you want to repeat the whole paragraph?

A. Sure. Do you want me to start from paragraph 80? In some cases it may be desirable to provide endonuclease recognition sites within the linking oligonucleotide which can allow for a mechanism to release a given template sequence from a synthesis reaction, i.e., by linearizing it and allowing the polymerase to run off the linear template and/or to expose the template to endonuclease activity and thus terminate synthesis through removal of the template.

Such sites could additionally be exploited as control sequences by providing specific binding locations for endonucleases engineered to lack cleavage activity but retain sequence-specific binding.

Reply 5-6

POSAs Don't Sabotage

In re Michalek, 162 F.2d 229, 232 (C.C.P.A. 1947) (“the failures of experimenters who have no interest in succeeding should not be accorded great weight . . .”)

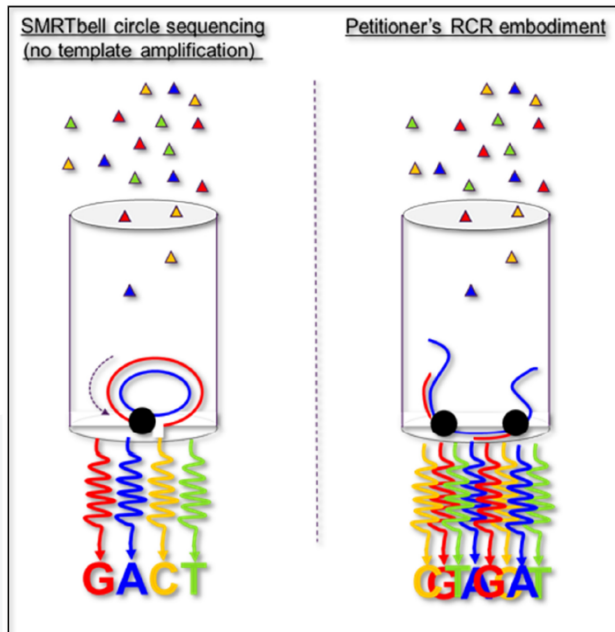
In re Weber, 405 F.2d 1403, 1407 (C.C.P.A. 1969) (“We do not think that appellants' mere showing that it is possible to operate within [the prior art] disclosure without obtaining [its] results is sufficient to overcome the strong presumption that the process of a patent if used by one skilled in the art will produce the results alleged by the patentee.”)

Larrea ¶28: 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.

Reply 3-6, 14-23; Paper 36, 7-10

PO's "Problem" 1

Satija ¶135: As shown below in the figure, a POA would have understood that if **two or more polymerases** generate products in a single ZMW well, the multiple fluorescent pulses from the different polymerases would be indistinguishable and produce **unusable data**.



EX1018

[0017] The invention is also directed to compositions useful in the foregoing methods. In one aspect, the invention provides compositions that comprise a template nucleic acid having a double stranded nucleic acid segment comprising a first strand segment and a second strand segment substantially complementary to the first strand segment, and at least a first linking oligonucleotide segment joining a 3' end of the first strand segment to the 5' end of the second strand segment. 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.

EX1035, 34

Ratio of Polymerase to Template for Binding

The stoichiometric optimum for the polymerase:template ratio is 2 polymerases bound to each template molecule (one to each hairpin adapter). To maximize loading efficiency, binding ratios per library size have been optimized and appear as the default setting in the Binding Calculator. The ratio can be modified in the Optional section of the Calculator, however, it is highly recommended that the default parameters be used. See the *Pacific Biosciences Binding Calculator Parameters Quick Reference Card* for a summary of the recommended ratios of polymerases to templates for each library.

Reply 15-18

PO's "Problems" 2 & 3

Satija ¶157: In the case of Travers '075's rolling circle replication embodiment, a POSA would have understood that the majority of that 30% occupancy would be filled by the smaller templates rather than the larger templates due to basic diffusion principles. As a result, a POSA would have expected these smaller templates to not provide useful sequencing data because they lack sufficient sequencing redundancy.

EX1035, 35

Loading of SMRTbell templates into ZMWs is size dependent. Small inserts load better than large inserts. This is particularly important when sequencing different PCR amplicon sizes. 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.

Reply 18-21

POSAs knew about standard library prep methods

Dr. Satija:



EX1037, 37:13-33

Q. And part of the reason you don't think it's necessary is because you brought your experience as a person of ordinary skill to bear in interpreting this; is that accurate?

ATTORNEY POWERS: Object to form, foundation.

A. So certainly when reading this sentence I can read it through the lens of a person of ordinary skill in the art in 2012. And standard library preparation methods were well known in the art as of March 2012.

EX1037, 40:2-5

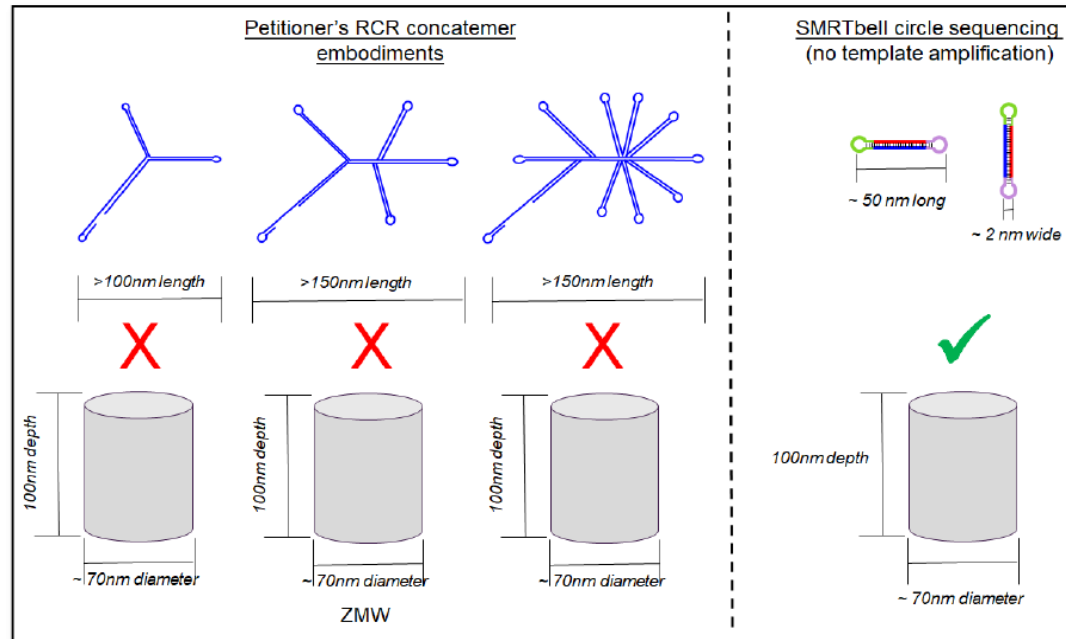
Q. In 2012 were any DNA fragment size selection methods known in the art?

A. Yes, there were size selection methods that were known in the art.

Reply 18-21

PO's "Problem" 4

Satija, ¶188



EX1021, 1

the insert and hairpins, respectively). In principle, the insert sequence can be of any length. In practice, we have created templates with inserts as short as 40bp, and as large as 25000bp, and we currently see no evidence of an intrinsic limit in the size of template that can be created. The hairpins can be constructed to include a wide variety of sequences, including different lengths of sequence, limited only by the thermodynamics of loop formation and primer binding.

EX1018, ¶83

could be tolerated. For purposes of certain redundant sequencing applications, a template that is between about 50 and about 500 bases may be desired. In other applications, where longer readlengths are obtained, or in non-redundant applications, templates that are from about 200 to about 50,000 bases in length may be used. Although described in terms of specific lengths, it will be appreciated that a variety of different template sizes may be employed for a variety of different specific applications.

Reply 21-23

Satija concedes DNA is not rigid

Dr. Satija:



EX1037, 51:22-52:9

Q. I think you say this in your declaration, but the SMRTbell that's shown in the figure here on page 108 is a two-dimensional schematic; correct?

A. That's correct.

Q. But DNA in solution doesn't assume a rigid conformation like a piece of lumber; correct?

A. That's correct.

Reply 21-23

PO's McCloskey argument

Satija:

A POSA would have understood that McCloskey deals *exclusively* with PCR amplification, which a POSA would understand to involve adding barcodes to track and identify separate *PCR products* that are amplification products of the same original template molecule.

McCloskey:

"In a first aspect, the present invention provides methods for authenticating a nucleic acid molecule and its sequence with a molecular barcode and batch-stamp. ..."

A second example of a specific use for the present invention concerns methylation patterns. ... A third example concerns ancient DNA, where samples generally have limited amounts of template... A further example concerns mosaics in human disease. ... The authentication of the mosaicism, and the quantification of the degree of mosaicism, are now possible using the present invention.

Reply, 14

No Nexus to Alleged Objective Indicia

No Nexus to Claims

POR:

- Cites Kirsch, GEN Edge as allegedly showing objective indicia

Reply:

- PO's references discuss DCS as described in Schmitt 2012, not the '127 patent
- DCS/Schmitt 2012 are not commensurate with claims; DCS is not recited
- No novelty over Travers & Kinde ascribed for any recited claim element

Sur-reply:

- Relies on GEN Edge as “not exclusively linked to ‘DCS of the Schmitt reference’”

GEN Edge expressly focused on DCS of the Schmitt reference:

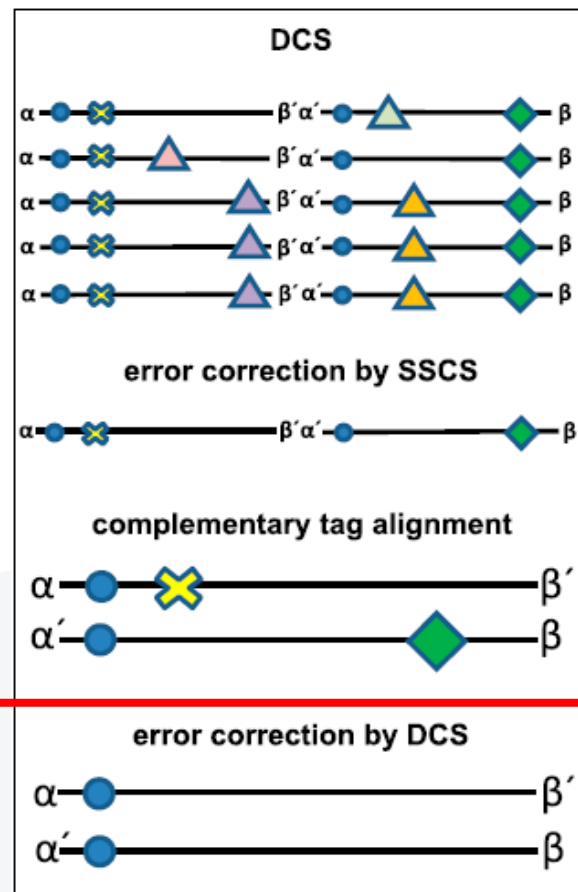
TwinStrand is young, with 25 employees. Although their website is sparse, their publication history is extensive. The duplex sequencing technology was first published in a 2012 *Proceedings of the National Academy of Sciences* paper entitled, “Detection of ultra-rare mutations by next-generation sequencing.” Since then, Salk and his team have published roughly a dozen other papers related to the technology.

Reply, 23-27

No Nexus: Schmitt 2012 & Unclaimed DCS

EX2007, 1

The authors do so by simply exploiting the redundant sequence information contained in the complementary DNA strand of double stranded genomic DNA molecule. They add a unique random yet complementary double-stranded nucleotide sequence to both strands of duplex DNA before amplification. 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). However, it also identifies the complementary DNA strand by 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 (Fig. 1).

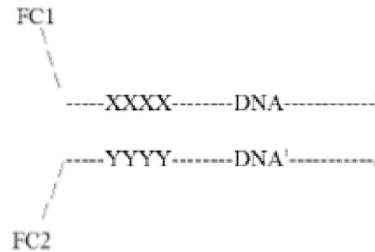


Reply, 23-27

PO Sur-Reply

PO argues claims are limited to “Product I”

Sur-Reply 2: “Columns 10-11 of the specification discuss “Product I” (a template molecule comprising a Y-shaped adapter on one end and a hairpin adapter on the other)”



- PO identifies no expert testimony in support of its new interpretation of columns 10-11 of the '127 patent
- PO's new interpretation conflicts with the specification and the claims
 - EX1001, 11:8-17 (“Producing **closed circles** of captured material Additionally, **closed circles** may be pre-amplified using rolling circle amplification or serve as the substrate for continuous loop sequencing [49].”)
 - Claim 10 “. . .wherein amplifying original strands comprises amplifying original strands via . . . , rolling circle amplification”
 - Claim 13 “. . .wherein the adapters comprise a Y-shape or a U-shape, ...”