

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

TWINSTRAND BIOSCIENCES, INC.,
Petitioner,

v.

GUARDANT HEALTH, INC.,
Patent Owner.

Case No. IPR2022-01400
Patent No. 11,149,306

PATENT OWNER RESPONSE

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I. INTRODUCTION

The petition challenges claims 1-29 of the '306 patent. The Board's Institution Decision ("ID," Paper 9), however, found the petition materials failed to demonstrate a reasonable likelihood to prevail regarding claims 4, 6, and 17-29. Petitioner's challenges to those claims still fails for the reasons identified in Patent Owner's prior briefing and confirmed in the Institution Decision. While the Institution Decision preliminarily found reason to institute regarding claims 1-3, 5, 7-16, Petitioner's challenge to those claims fails in view of a more developed record. As explained in detail herein, Petitioner fails to demonstrate claims 1-29 of the '306 patent are unpatentable as obvious.

As explained below, the petition materials fail to demonstrate the cited prior art discloses or renders obvious tagging cfDNA molecules with *duplex tags* comprising "molecular barcodes" as specifically recited in independent claim 1 and further required by claims 2-16 and 29. All witnesses now agree that to the extent Schmitt discloses "molecular barcodes," those are the SMIs. Regarding the asserted "hybrid embodiment," the SMI includes endogenous sequence not included in the duplex tags attached to the ends of the cfDNA molecule as specifically recited in claim 1. Mapping only the 3-mer portion of the hybrid SMI, as Petitioner does, to the claimed "molecular barcodes" is incorrect and

inconsistent with testimony of Petitioner's own witnesses, disclosure in Schmitt, and plain language of claim 1.

Additionally, the petition materials fail to demonstrate the cited prior art discloses or renders obvious reducing or tracking redundancy as claimed—in particular, “wherein reducing or tracking the redundancy of the plurality of sequence reads comprises mapping at least a subset of the plurality of sequence reads to the reference sequence” as required by claims 1-16 and 29 of the '306 patent. Petitioner cites to Schmitt for mapping consensus reads, rather than sequence reads as recited in the challenged claims. The petition materials also fail to identify prior art disclosure of the additional requirements of claims 3, 5, 7, and 14-16, all of which depend from claim 1.

Furthermore, the petition materials fail to establish motivation to combine the references as proposed with a reasonable expectation of success. Petitioner argues specifically that a POSA would be motivated to choose Schmitt's 3-mer hybrid approach in favor of Narayan for improved sensitivity in detecting cancer in a clinical setting with cfDNA but fails to substantiate any of this. Schmitt never reports detecting cancer in any sample and the petition materials offer no specific discussion or any calculations regarding sensitivity of the hypothetical 3-mer hybrid approach. This alone is reason to affirm the patentability of claims 1-29.

As the Board is well-aware, the Petitioner bears the unshifting burden to demonstrate obviousness including motivation to combine—patent owner is not required to prove no motivation. Nevertheless, record evidence undermines rather than supports the obviousness theory proposed here. The preponderance of evidence—including testimony from Dr. Quakenbush (EX2015), Dr. Hagemann (EX2016), and relevant scientific literature—demonstrates a POSA reviewing Schmitt would more reasonably view its DCS method as untested in the clinic (e.g., outside the academic laboratory environment), not particularly reliable or sensitive for detecting cancer mutations, and poorly designed for the clinical setting. As such, Petitioner provides no good reason why a POSA would abandon the verified “ultrasensitive” method of Narayan in favor of the prophetic and untested 3-mer hybrid SMI embodiment of Schmitt for clinical testing for cancer in cfDNA as proposed.

Accordingly, the petition challenges should be rejected and the '306 patent claims be found not-unpatentable.

II. GROUNDS REFERENCES

A. Narayan

“Ultrasensitive Measurement of Hotspot Mutations in Tumor DNA in Blood Using Error-Suppressed Multiplexed Deep Sequencing” by Narayan, et al.

(“Narayan,” EX1082) describes a method for sensitive and accurate detection of cancer mutations in clinical cfDNA specimens.

Here we present an approach that uses next-generation sequencing to quantify the small fraction of DNA molecules that contain tumor-specific mutations within a background of normal DNA in plasma. Using layers of sequence redundancy designed to distinguish true mutations from sequencer misreads and PCR misincorporations, we achieved a detection sensitivity of approximately 1 variant in 5,000 molecules.

EX1082, Abstract; *see also* EX2015, ¶31; EX2016, ¶15.

Narayan tested its method on minute quantities of cfDNA isolated from the plasma of cancer patients. EX1082, 3493 (“30 patients with stage I–IV non–small cell lung cancer ... DNA was extracted from 0.2 mL of each plasma sample”); EX1002, ¶251. According to Narayan, the disclosed method is not only sensitive but also “yields highly accurate base calls.” EX1082, Figure 1; *see also id.*, Figure 2 (“Suppression of spurious mutation counts to reveal low-abundance variants...”), Figure 3 (“high degree of accuracy and reproducibility”), 3495 (“Sensitive and accurate quantitation of mutant DNA”), 3497 (“The accuracy and sensitivity provided by the error suppression strategy enables quantitation of mutant ctDNA within a clinically informative range of concentrations.”); *see also* EX2016, ¶16.

B. Schmitt

Schmitt does not describe detection of cancer mutations in clinical settings. Nor does Schmitt describe improvements to such methods that include identifying and sorting paired and unpaired reads. Rather, Schmitt discloses Duplex Consensus Sequencing or “DCS” which is described as a method for reducing “artifactual” sequence errors (e.g., mistakes introduced during amplification or sequencing). *E.g.*, EX1083, ¶7, Abstract (“To overcome limitations in sequencing accuracy, a method Duplex Consensus Sequencing (DCS) is provided.”); *see also* EX2015, ¶32; EX2016, ¶¶17-19.

According to Schmitt, DCS makes use of “Single Molecule Identifiers” or “SMIs” to uniquely identify original sample molecules. DCS generally includes ligating adapters comprising SMI sequences to sheared DNA molecules obtained from cells, amplifying the SMI-tagged molecules using PCR, and sequencing the amplification progeny. The sequence reads for a single strand of an original double-stranded sample molecule are collapsed to form a “single strand consensus sequence” or “SSCS.” EX1083, ¶¶60, 63. SSCSs are then paired with a strand-mate SSCS to generate a duplex consensus sequence (“DCS”). *E.g.*, EX1083, ¶¶60 (“The consensus sequences were then paired with their strand-mate...”), 63. Schmitt exclusively describes the sample DNA molecules analyzed by its method

as randomly “sheared” and size selected cellular DNA. *E.g.*, EX1083, ¶¶56, 65; *see also* EX2015, ¶33.

Schmitt emphasizes the benefits of using SMI sequences which provide a high degree of diversity. EX1083, ¶¶16, 47, 49, 67, 75. Consistent with this teaching, Schmitt’s preferred embodiment involves ligating adapters comprising random 12-mer SMIs to both ends of sample DNA molecules. EX1083, ¶¶11, 60. Schmitt explains that use of 12-mer SMIs in this manner produces “up to 4^{24} (i.e., 2.8×10^{14}) distinct tag sequences.” EX1083, ¶16. Schmitt provides a single comment about a “hybrid” approach but offers no working examples or explanation of how it might be implemented. EX1083, ¶30; *see also* EX2015, ¶34.

III. PERSON OF ORDINARY SKILL IN THE ART

The ’306 patent describes improved methods for detecting rare genetic alterations, such as copy number variation, in polynucleotide samples. *E.g.*, EX1001, Abstract, 1:59-67, Figures 1 and 2, claims. EX2015, ¶¶35, 36. Given the nature of invention, a POSA would have had knowledge of the scientific literature concerning methods of DNA manipulation and analysis, including DNA sample preparation for sequencing, amplification, methods of DNA sequencing (including NGS and related sequencing methods), and bioinformatics methods for raw data analysis, as Dr. Spellman suggests. A POSA may have also worked as part of a multidisciplinary team and drawn upon not only his or her own skills, but also

taken advantage of certain specialized skills of others on the team, e.g., to solve a given problem. *See* EX1002, ¶¶27-30; EX2015, ¶29; EX2016, ¶12.

Dr. Quackenbush and Dr. Hagemann have applied Dr. Spellman’s definition of a POSA in their analyses. EX2015, ¶30. However, Dr. Spellman fails to observe his definition. In particular, a POSA would not ignore clinical considerations or the perspective of a clinician knowledgeable in the relevant technical area as Dr. Spellman does. Indeed, such a clinician would be an expected member of the “multidisciplinary team” referenced in Dr. Spellman’s POSA definition. EX2016, ¶¶13, 14.

IV. CLAIM CONSTRUCTION

In an *inter partes* review, a claim is given its ordinary and customary meaning in light of the specification. 37 C.F.R. §42.100(b); *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312 (Fed. Cir. 2005) (en banc). No specific construction is believed necessary here to determine that the recited terms are disclosed in the cited prior art. *Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co.*, 868 F.3d 1013, 1017 (Fed. Cir. 2017). The discussion below provides context regarding certain claim terms.

A. Tagging cfDNA molecules

“[C]laim construction must begin with the words of the claims themselves.”

Amgen Inc. v. Hoechst Marion Roussel, Inc., 457 F.3d 1293, 1301 (Fed. Cir. 2006).

Independent claim 1 requires tagging cfDNA with molecular barcodes.

Claim 1	(a) providing a population of cell-free deoxyribonucleic acid (cfDNA) molecules having first and second complementary strands; (b) tagging a plurality of the cfDNA molecules in the population with duplex tags comprising molecular barcodes to produce tagged parent polynucleotides, wherein the duplex tags are attached to both ends of a molecule of the plurality of the cfDNA molecules,
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As recited in claim 1, the duplex tags comprise the molecular barcodes, and language of claim 1 distinguishes the duplex tags from the cfDNA molecules. Step (a) of claim 1 recites providing a population of cfDNA molecules. Step (b) of claim 1 then recites tagging the cfDNA with duplex tags comprising molecular barcodes. Step (b) of claim 1 further expressly recites “wherein the duplex tags are attached to both ends of a molecule of the plurality of the cfDNA molecules.” As such, the language of claim 1 specifies that the duplex tags are attached to the cfDNA molecules and distinguishes the “cfDNA molecules” from the “duplex tags” with the molecular barcodes. EX2015, ¶¶38, 39.

B. Molecular barcode

The petition materials do not identify any special meaning to be applied to the term “molecular barcode” recited in claims 1-29. *See* Pet. 18-19; EX1002, ¶¶131-133; EX2017, 19:4-10. However, both Dr. Spellman and Dr. Quackenbush agree that to the extent Schmitt discloses “molecular barcodes,” those are the α and β SMI sequences in Schmitt. EX2015, ¶40; EX1002, ¶¶85, 87; *see also* EX2017, 12:20-25, 14:10-17; EX2028, 272 (“It is worth noting that the concept of a molecular barcode (also known as a unique molecular identifier (UMI), a single-molecule identifier (SMI) or simply a tag) is different from that of an index sequence.”).

With respect to Schmitt’s preferred embodiment, the α and β SMI sequences are provided by a 12-mer random SMI sequence present in the adapter molecule. EX1083, ¶12 (“every DNA fragment becomes labeled with two distinct SMI sequences (arbitrarily designated α and β ... [PCR duplicates] derived from one strand will have the α SMI sequence adjacent to flow-cell sequence 1 and the β SMI sequence adjacent to flow cell sequence 2.”), Figure 1; *see also* EX2015, ¶41.

With respect to Schmitt’s hybrid approach upon which the petition challenges rely, the α and β SMI sequences are the combination of exogenous n-mer and endogenous shear sequence. All experts, including Dr. Spellman, agree that the combination of n-mer and shear sequence (not the n-mer portion alone)

provides the “molecular barcode tag sequence ($\alpha\beta$ or $\beta\alpha$).” EX1002, ¶300 & n.18 (citing EX1083, ¶30); *see also* EX2015, ¶42.

V. GROUND 1: CLAIMS 1-3, 5, 7, 9-14 ARE NOT OBVIOUS OVER NARAYAN AND SCHMITT

A. Petition materials fail to establish tagging cfDNA molecules as recited in claim 1

The petition materials fail to establish the cited prior art discloses or teaches the tagging step of claim 1 of the '306 patent which recites:

(b) tagging a plurality of the cfDNA molecules in the population with *duplex tags comprising molecular barcodes* ... wherein the plurality of the cfDNA molecules are tagged with n different combinations of molecular barcodes, wherein n is at least 2 and no more than $100,000 * z$, wherein z is a mean of an expected number of duplicate molecules in the population of cfDNA molecules that map to identical start and stop positions on a reference sequence.

Petitioner addresses the tagging limitation at pages 27-29 where it relies exclusively on Schmitt's 3-mer hybrid SMI embodiment for the tagging requirement. The petition materials acknowledge SMIs as the “molecular barcodes” in Schmitt, and they acknowledge hybrid SMIs as the combination of shear sequence and exogenous n-mer sequence, but they improperly and inconsistently map only the exogenous 3-mer portion of the hybrid SMI in the discussion of the challenged claims. *E.g.*, Pet. 28-29; EX1002, ¶164 (“Schmitt's 3-mer hybrid tag, when ligated to each end of the target DNA molecules, provides

4,096 (64^2) possible ‘different combinations of molecular barcodes’ as recited in claim 1.”); *see also* EX2015, ¶¶17, 43, 44.

As explained in further detail below, the petition materials fail to demonstrate that Schmitt, including discussion of the hypothetical 3-mer hybrid SMI, describes tagging a plurality of cfDNA molecules in the population with duplex tags comprising molecular barcodes as specifically recited in claims 1-16 and 29. EX2015, ¶45.

1. There is no dispute the SMIs are the “molecular barcodes” in Schmitt

To the extent Schmitt discloses “molecular barcodes,” those are the α and β SMI sequences in Schmitt. *See* Section IV.A. Petitioner’s expert here, and its expert in a co-pending IPR, confirm that Schmitt’s α and β SMI are molecular barcodes. EX2015, ¶46.

Dr. Spellman repeatedly confirms that Schmitt’s α and β SMI are molecular barcodes. For example, Dr. Spellman states “[i]n Schmitt, these double-stranded nucleotide sequences are called ‘double stranded single molecule identifiers (SMI) sequence’ or molecular barcodes.” EX1002, ¶85. Referring specifically to Schmitt’s SMIs as the “molecular barcodes,” Dr. Spellman explains that in Schmitt “every DNA fragment becomes labeled with two distinct molecular barcodes that are ‘arbitrarily designed α and β in the single capture event shown.’” EX1002, ¶87 (original emphasis); *see also* EX2015, ¶47.

During his deposition, Dr. Spellman again testified that Schmitt uses the term “single molecule identifier” or “SMI” in referring to the α and β molecular barcodes:

Q. And does figure one depict molecular barcodes?

A. Yes, that would be alpha and beta.

Q. Okay. The term here is SMI, correct?

A. That is a term that Schmitt has used, yes.

Q. What does SMI stand for?

A. Single molecule identifier.

EX2017, 12:20-25; *see also id.*, 14:10-17.

Petitioner’s witness in a co-pending IPR matter, Dr. Satija, similarly testified that Schmitt (U.S. Pat. No. 10,760,127) uses the term “single molecule identifier” or “SMI” in referring to molecular barcodes:

Q. Is the SMI a barcode? (Pause.)

A. Yes, it is.

Q. You indicated that SMI stands for single-molecule identifier; is that right?

A. That’s correct.

Q. The term here is “identifier” not “barcode”; is that correct?

A. The term is “SMI,” which stands for single-molecule identifier.

Q. And do you consider an identifier the barcode?

A. Yes.

EX2018, 53:19-54:6.

We’ve also talked about how sometimes people use slightly different

words to refer to the same thing. For example, in my lab we often work with a construct known as a UMI, which is an abbreviation for unique molecular index. That term is used interchangeably with the term “molecular barcode.”

EX2018, 91:20-92:1.

So I would highlight that the term “SMI,” which is single-molecule identifier — “identifier” and “index” are also example of terms that are associated with barcode — appears many times in the specification.

EX2018, 92:13-17; *see generally id.*, 91:20-92:17; EX2015, ¶¶48, 49.

With regard to the relied upon “hybrid embodiment,” the α and β SMI (molecular barcodes) are the combination of exogenous n-mer and endogenous shear sequence—not just the exogenous n-mer portion alone. In fact, elsewhere in his declaration, Dr. Spellman plainly and repeatedly acknowledges that the combination of n-mer and shear sequence (not the n-mer portion alone) provides the “molecular barcode tag sequence ($\alpha\beta$ or $\beta\alpha$).” EX1002, ¶300 & n.18 (citing EX1083, ¶30); *see also* EX2015, ¶50.

This is also confirmed in Schmitt which states “[a] hybrid method using a combination of sheared ends and a shorter n-mer tag...in the adaptor may also serve as unique molecular identifiers.” EX1083, ¶30. Dr. Spellman cites to this same disclosure and repeatedly emphasizes that the combination of hybrid components forms the SMI (molecular barcode). *E.g.*, EX1002, ¶¶88 (“Schmitt discloses a ‘hybrid method’ of tagging, which uses a combination of the ends of

the DNA fragments and ‘a shorter n-mer tag...that, together with the sequence information within the target DNA, serve as ‘unique molecular identifiers.’”) (original emphasis), 89 (“Thus, when combined, they form unique molecular identifiers ‘X+L’ and ‘R+Y’ which can be used in Duplex Sequencing.”); *see also* EX2028, 275 (“Duplex consensus sequencing. ... Exogenous tags within each strand of the sequencing adapters and/or DNA fragment shear points serve as UMIs [“unique molecular identifiers”] that informatically relate reads from the two strands.”). EX2015, ¶51.

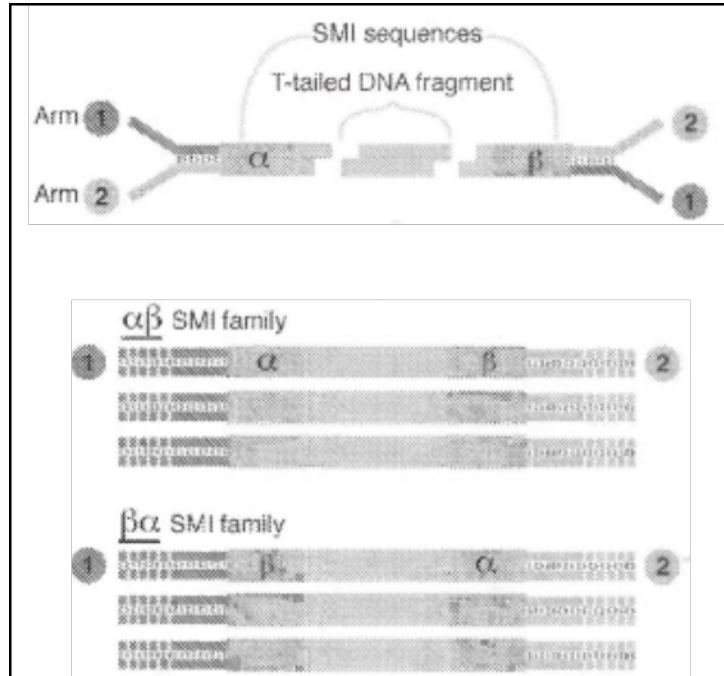
In all embodiments of Schmitt, the α and β SMIs (not a sub-portion of an SMI) serve as “molecular barcodes” to group reads and match SSCSs—critical steps in the DCS process. *See* EX1002, ¶39. For example, Figure 1 of Schmitt shows DNA molecules with SMIs (α and β) and grouping reads into SMI families. EX1083, ¶11 (“every DNA fragment becomes labeled with two distinct SMI sequences (arbitrarily designated α and β ... [PCR duplicates] derived from one strand will have the α SMI sequence adjacent to flow-cell sequence 1 and the β SMI sequence adjacent to flow cell sequence 2.”), Figure 1; *see also id.*, ¶¶60 (“Reads having common (i.e., identical) SMI sequences where grouped together, and were collapsed to generate a consensus read...The consensus sequences were then paired with their strand-mate by grouping each 24 nucleotide tag of the form AB in read 1 with its corresponding tag of form BA in read 2.”), 71 (“The first 10

nucleotides of each sequencing read pair, corresponding to the randomly sheared DNA ends, were combined, such that the first 10 nucleotides of read 1, referred to as A, was combined with the first 10 nucleotides of read 2, referred to as B, to yield an SMI tag of form AB.”), 72 (“the randomly sheared DNA ends were used as SMI’s”); Example 2 (“Demonstration of error-correction by DCS using randomly sheared DNA ends as Single Molecule Identifiers”); EX2017, 14:10-17; EX2015, ¶52.

Schmitt never describes only the n-mer portion of a hybrid SMI as an SMI or using only a portion of any SMI for grouping and matching in Schmitt’s DCS process. Just the opposite, Schmitt repeatedly emphasizes matching the entirety of the SMI sequences in its DCS process. *See, e.g.*, EX1083, ¶¶49 (“true PCR duplicates may be unambiguously identified by virtue of having a common (i.e., the same or identical) SMI sequence.”), 60 (“Reads having common (i.e., identical) SMI sequences were grouped together...Group together reads which have identical 24 nt SMIs.”), 63 (“members of the same PCR ‘family’ are then grouped together by virtue of having a common (i.e., the same) SMI tag sequence.”), 68 (same); EX2015, ¶53.

Petitioner does not argue otherwise. The example, the petition states at page 11 “Schmitt discloses that ‘sequence reads sharing a unique set of SMI tags are grouped into paired families with members having strand identifiers in either the

$\alpha\beta$ or $\beta\alpha$ orientation.” See also EX1002, ¶¶97, 176; EX1083, Figure 1 (reproduced in part below). Dr. Spellman plainly states that the combination of n-mer and shear sequence (not the n-mer portion alone) provides the “molecular barcode tag sequence ($\alpha\beta$ or $\beta\alpha$).” EX1002, ¶300 & n.18 (citing EX1083, ¶30); EX2015, ¶54.



Dr. Spellman indicates that in the hybrid embodiment the hybrid SMIs (i.e., X+L and R+Y), and not merely a sub-portion thereof, provide the α and β sequences for DCS.

Schmitt discloses, “[b]y virtue of the asymmetric nature of adapted fragments, two types of PCR products are produced from each capture event. Those derived from one strand will have the α SMI sequence adjacent to flow-cell sequence 1 and the β SMI sequence adjacent to flow cell sequence 2. PCR products originating from the complementary strand are labeled reciprocally.” For Schmitt’s 3-mer

hybrid tag embodiment depicted above, the “two types of PCR products” are (i) those derived from one strand with the *X + L identifier* adjacent to flowcell sequence 1 and the *R + Y identifier* adjacent to flowcell sequence 2;

EX1002, ¶98 (internal citations omitted); *see also* EX2015, ¶55.

Dr. Spellman asserts that, in general, “molecular barcodes, [] are used to label and track individual molecules.” EX1002, ¶41, n. 2. But Dr. Spellman never demonstrates, or even argues, that the 3-mer portion of a hybrid SMI alone is used to label and track individual molecules in Schmitt and, indeed, no such disclosure is found in Schmitt. To the contrary, as Dr. Spellman repeatedly acknowledges, the combination of the endogenous shear sequence and exogenous 3-mer sequence together provide the SMI used for grouping and matching in Schmitt’s DCS process. *E.g.*, EX1002, ¶¶89 (“Thus, when combined, they form unique molecular identifiers ‘X+L’ and ‘R+Y’ which can be used in Duplex Sequencing.”), 96 (“...grouping include the combination...”), 98 (“...Schmitt uses the combination...”); *see also* EX2015, ¶56.

Accordingly, to the extent Schmitt discloses “molecular barcodes,” those are the α and β SMIs in Schmitt. Schmitt never describes a sub-portion (3-mer portion) of a hybrid SMI as a molecular barcode and never describes using only the sub-portion (3-mer portion) of a hybrid SMI, or only a sub-portion of any SMI, for grouping and matching in Schmitt’s DCS process. To the extent Dr. Spellman cites

to only a 3-mer portion of a hybrid SMI as a “molecular barcode” that is incorrect and inconsistent with (i) his own testimony, (ii) Petitioner’s arguments and evidence in other cases (e.g., IPR2022-00817), and (iii) the disclosure provided in Schmitt. EX2015, ¶57.

2. The ’306 patent claims do not encompass Schmitt’s hybrid embodiment

Besides failing to address the content of Schmitt, the petition materials fail to demonstrate the cited references tag cfDNA molecules with duplex tags comprising molecular barcodes as specifically recited in claims 1-16 and 29. EX2015, ¶58.

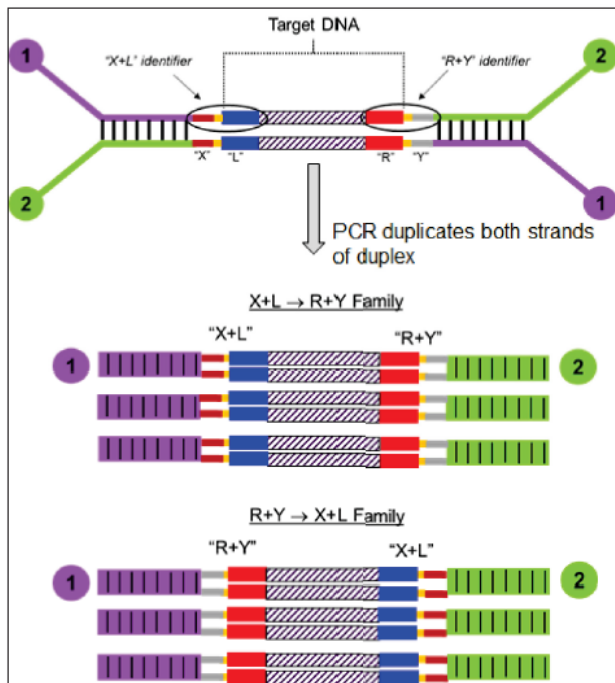
First, the cited hybrid SMI embodiment of Schmitt fails to provide “tagging a plurality of the cfDNA molecules in the population with duplex tags comprising molecular barcodes ...” as recited in claim 1. As explained below, even if the hybrid SMI sequences are viewed as molecular barcodes in Schmitt’s DCS process, those are not contained in adapter molecules or duplex tags that are attached to the DNA molecules. EX2015, ¶59.

As discussed above, the language of claim 1 specifies that the duplex tags are attached to the cfDNA molecules and distinguishes the “cfDNA molecules” from the “duplex tags” with the molecular barcodes. *See* Section IV.A; EX2015, ¶60.

The petition materials fail to adequately address the particular language of the claim 1. Pet. 27; EX1002, ¶¶161-162. Dr. Spellman repeatedly acknowledges the hybrid embodiment uses an amalgamation of sequences that together function as the SMI or molecular barcode for DCS. *E.g.*, EX1002, ¶161 (“Thus, the short n-mer tag combined with the end of the target DNA serve together to label the molecule.”). Dr. Spellman, however, improperly conflates the DNA molecule with the attached duplex tags. EX1002, ¶162 (“Schmitt’s hybrid tag embodiment comprises molecular barcodes *because the duplex tags comprise the end of the fragment DNA end sequences (‘sheared ends’)* and a ‘short n-mer tag’ to serve as ‘unique molecular identifiers.’”) (emphasis added). As discussed above, the language of claim 1 distinguishes between the “cfDNA molecules” and the attached “duplex tags comprising molecular barcodes,” whereas Petitioner’s claim mapping does not. EX2015, ¶61.

As illustrated in Dr. Spellman’s created graphic, the relied upon hybrid SMI sequences of Schmitt include sequence from the sample DNA molecule not contained in the adapter or the duplex tags. Pet. 12; EX1002, ¶98 (reproduced below). Dr. Spellman explains in his declaration that the hybrid SMI sequences (illustrated as X+L and R+Y) provide the α and β sequences for DCS. *E.g.*, EX1002, ¶98; *see also* Section V.A.1. The illustrated “X+L” and “R+Y” SMIs include sample DNA sequence separate from the adapter molecule or duplex tag

shown “attached to both ends” of a DNA molecule. Dr. Spellman confirms, consistent with Schmitt, sequence reads are grouped into families based on X+L and R+Y (i.e., SMI sequences)—not just “X” and “Y” portions—in either the $\alpha\beta$ or $\beta\alpha$ orientation. *E.g.*, EX1002, ¶¶89 (“Thus, when combined, they form unique molecular identifiers ‘X+L’ and ‘R+Y’ which can be used in Duplex Sequencing.”), 96 (“...grouping include the combination...”), 98 (“...Schmitt uses the combination...”); *see also* EX2015, ¶62.



Second, the petition materials fail to demonstrate the relied upon hybrid SMI embodiment results in “n is at least 2 and no more than 100,000*z...” different combinations of SMIs (molecular barcodes). Dr. Spellman testified that the hybrid SMI sequences (illustrated as X+L and R+Y) provide the α and β sequences for DCS. *E.g.*, EX1002, ¶98. But Dr. Spellman never calculates or otherwise identifies

the number of different SMI combinations in the proposed hybrid embodiment.

See, e.g., ¶¶164-167; *see also* EX2015, ¶63.

Dr. Spellman calculates that a 3-nucleotide n-mer provides 4,096 different combinations. *E.g.*, EX1002, ¶164. But Dr. Spellman's calculations do not take into account the shear sequences of the DNA molecule that he repeatedly acknowledges as included in the hybrid SMIs. *E.g.*, EX1002, ¶¶89, 96, 98. In fact, Dr. Spellman never explains (nor does Schmitt) how many nucleotides of shear sequence are included in the hypothetical 3-mer hybrid SMI. While Schmitt emphasizes that every DNA fragment is distinctly labeled with SMIs, the precise number of different SMI's is indeterminable in Dr. Spellman's incomplete hypothetical. *See, e.g.*, EX1083, ¶11 ("every DNA fragment becomes labeled with two distinct SMI sequences"); *see also* EX2015, ¶64.

Accordingly, the petition materials fail to demonstrate that Schmitt, including Dr. Spellman's hypothetical 3-mer hybrid embodiment, satisfies the limitations regarding tagging a plurality of cfDNA molecules in the population with duplex tags comprising molecular barcodes as specifically recited in claims 1-16 and 29. EX2015, ¶65.

B. The petition materials fail to establish disclosure of "mapping at least a subset of the plurality of sequence reads to the reference sequence"

The petition materials fail to establish the cited prior art discloses or teaches step (e) of claim 1 of the '306 patent which recites:

(e) reducing or tracking redundancy of a plurality of sequence reads from the set of sequence reads ..., wherein reducing or tracking the redundancy of the plurality of sequence reads comprises *mapping at least a subset of the plurality of sequence reads to the reference sequence*.

Petitioner addresses the wherein clause of step (e) at pages 31-32 where it relies exclusively on disclosure at ¶¶60, 66, and 68 of Schmitt. EX2015, ¶¶18, 66, 67.

While the petition materials acknowledge that consensus sequences are distinct from sequence reads, Petitioner's claim mapping confuses and conflates the terms. For example, Dr. Spellman opines that consensus sequences are generated from redundant sequence reads. *E.g.*, EX1002, ¶174 (“...reducing or tracking redundancy in the set of sequence reads based at least on the molecular barcode information, collapsing the redundant sequences into a plurality of consensus sequences that represent the original DNA molecules, ...”). But, with respect to mapping sequence reads, Petitioner points to disclosure of mapping consensus sequences—not sequence reads—to a reference sequence. *See* EX1002, ¶178; *see also* EX2015, ¶68.

Dr. Spellman points to ¶60 which describes Schmitt's multi-step data processing workflow as providing mapping sequence reads. However, Schmitt's workflow indicates that alignment to a reference sequence is the last step (Step

#10) of Schmitt's method and is performed after generating duplex consensus sequences. EX1083, ¶60 ("10. Align reads to the human genome."); *see also* EX2015, ¶69.

Paragraph 68 further discusses steps 9 and 10 of the same data processing workflow. Here, Schmitt describes pairing single strand consensus sequences to form a duplex consensus sequence (i.e., step 9). EX1083, ¶68 ("Finally, the complementary nature of the double-stranded SMI sequences was used to identify pairs of consensus groups that arose from complementary DNA strands."). Next, Schmitt discusses aligning duplex consensus sequences with the human genome (step 10) to determine mutation frequencies represented in the duplex consensus sequences relative to a reference sequence. EX1083, ¶68 ("Next, as above, analysis of mutation frequencies was restricted to sites with at least 10x coverage and at which fewer than 10% of reads disagreed from the hg19 reference sequence."); *see also id.*, ¶13, Figure 3; EX2015, ¶70.

The petition materials also identify ¶66 of Schmitt as providing relevant disclosure, but as Dr. Quackenbush explains this paragraph does not discuss DCS at all. This paragraph describes using "samtools rmdup"—an algorithm commonly used with Illumina sequencing—which filters out duplicate sequence reads using their shear points. EX1083, ¶66 ("PCR duplicates were filtered out with samtools rmdup, a standard tool which uses the shear points of DNA molecules to identify

PCR duplicates...”). Thus, to the extent Schmitt describes mapping sequence reads, it is in the context of a prior art method deemed inferior to consensus sequencing. EX2015, ¶¶71, 72.

Accordingly, the petition materials fail to demonstrate that Schmitt, satisfies mapping sequence reads to a reference sequence as specifically recited in claims 1-16 and 29. EX2015, ¶73.

C. The petition materials fail to establish motivation to combine Narayan and Schmitt with a reasonable expectation of success

Petitioner addresses motivation and expected success in the proposed combination of Narayan and Schmitt at pages 34-39. As discussed further below, Petitioner’s argument that a POSA would be motivated to choose Schmitt’s 3-mer hybrid approach in favor of Narayan for improved sensitivity in detecting cancer in a clinical setting with cfDNA is conclusory and unsubstantiated entirely. The preponderance of record evidence, in fact, instructs otherwise. Schmitt’s preferred exogenous 12-mer embodiment failed to detect any mutations in a laboratory setting and the hypothetical 3-mer hybrid approach would have been expected to be even more error-prone. EX2015, ¶¶20, 74; EX2016, ¶¶20-34. At bottom, Petitioner’s obviousness challenge is an unsubstantiated theory of what one hypothetically “could” do, not what a POSA reasonably *would* do with reasonable expectation of success.

1. Dr. Spellman's stated motivation is conclusory and unsubstantiated

Petitioner argues a POSA would have been motivated to use Schmitt's 3-mer hybrid method in place of Narayan for the purpose of detecting cancer. Pet. 34-35, 38; EX1002, ¶¶188 (“...for diagnostic or screening for cancer using cfDNA, such as in Narayan), 206 (“A POSA would have reasonably expected to successfully use Schmitt's Duplex Sequencing method with Narayan's cfDNA for cancer detection.”); EX2017, 16:24 (“That would be the motivation to combine with Narayan, yes.”). The petition materials assert specifically that a POSA would be motivated to turn to Schmitt's 3-mer hybrid approach in order to improve sensitivity of detection in the clinical setting. Pet. 35; EX1002, ¶¶189 (“Thus, a POSA would have been motivated to apply Schmitt's sequencing error correction to sequence cfDNA, as expressly disclosed in Narayan, to improve the sensitivity of sequence detection in clinical settings.”), 192 (“motivated to use Schmitt's 3-mer hybrid tagging approach”); *see also* EX2015, ¶75; EX2016, ¶¶20-26.

Despite specifically alleging improved sensitivity in clinical settings as reason to replace Narayan's “ultrasensitive” clinical assay, the petition materials provide no meaningful discussion of sensitivity of Schmitt's DCS method. Dr. Spellman never identifies or offers any explanation regarding sensitivity of Schmitt's 3-mer hybrid tag embodiment or addresses its application in clinical

settings. *See* EX1002, ¶¶188, 189. When asked about calculating Schmitt’s clinical sensitivity, Dr. Spellman said that his declaration did not provide such information.

Q. Do you provide anywhere in your declaration a calculation for the sensitivity of sequence detection in clinical settings for Schmitt’s DCS method?

A. Not to my recollection.

EX2017, 45:4-15; *see also* EX2015, ¶76.

The hypothetical 3-mer hybrid tag embodiment is not used in any working example in Schmitt. Its sensitivity in any setting, clinical or otherwise, is unknown and not addressed in the petition materials, including by Dr. Spellman. EX2015, ¶76; EX2016, ¶¶27, 28.

Regarding detecting cancer, Schmitt fails to provide a single example or embodiment where cancer mutations were detected in a clinical sample or clinical setting. As discussed further below, Schmitt reports in Example 1 that its preferred 12-mer exogenous SMI embodiment failed to detect any mutations in 3 µg of DNA. Schmitt provides no working example of a 3-mer hybrid method, let alone detecting cancer mutations in a clinical sample using such an approach. The petition materials present no evidence to the contrary. EX2015, ¶77; EX2016, ¶29.

Dr. Spellman’s conclusory assertions regarding cancer detection and improved sensitivity are neither explained in his declaration nor substantiated with evidence. *See* 37 C.F.R. 42.65 (“Expert testimony that does not disclose the

underlying facts or data on which the opinion is based is entitled to little or no weight.”); *In re NuVasive, Inc.*, 842 F.3d 1376, 1383 (Fed. Cir. 2016) (“[C]onclusory statements alone are insufficient and, instead, the finding must be supported by a reasoned explanation.”).

As Dr. Quackenbush and Dr. Hagemann explain, a POSA reviewing the content of Schmitt would not find credibility in these arguments. EX2015, ¶78; EX2016, ¶26.

2. Schmitt exemplified 12-mer exogenous SMI embodiment demonstrated poor sensitivity and did not detect mutations

The hypothetical 3-mer hybrid tag embodiment relied upon by Petitioner is not addressed in any experimental or working example in Schmitt. Schmitt’s Example 1 addresses the preferred 12 nucleotide exogenous SMI embodiment. *See* EX1083, ¶¶52-69. As explained by Dr. Quackenbush and Dr. Hagemann, a POSA would view Example 1 as evidence that Schmitt’s DCS method is not particularly sensitive and unreliable for detecting cancer mutations in clinical settings. EX2015, ¶79; EX2016, ¶¶27, 30, 31.

Regarding sensitivity in clinical settings, a sensitive test identifies true positives while avoiding false negatives. True positives (TP) are tests that come back positive when the patient has the disease. EX2019, 2 (“If a disease is proven present in a patient, the given diagnostic test also indicates the presence of disease, the result of the diagnostic test is considered true positive.”). False negatives (FN)

are tests that come back as negative when the patient has the disease. EX2019, 2; EX2017, 44:18-21 (“Q. What is a false negative in the context of medical diagnostic tests? A. A false negative would be a test that comes back as negative when it should have come back as positive.”). Sensitivity considers both true positives and false negatives. EX2019, 2 (“Sensitivity = TP/(TP + FN)”). EX2015, ¶80.

Schmitt’s preferred embodiment indicates insensitivity in the DCS method. In this example, Schmitt’s DCS method was applied to an exceptionally large amount of cellular DNA—3 micrograms. EX1083, ¶56. Yet, as Schmitt explains, “zero mutations” were identified. EX1083, ¶68; *see also* EX2017, 32:6-23 (confirming Schmitt’s failure to identify mutations); EX2015, ¶81

Schmitt’s preferred embodiment further demonstrates that Schmitt’s DCS method is not particularly sensitive at the DNA molecule level. Specifically, Example 1 starts with trillions of DNA molecules but produces duplex consensus sequences for only thousands of molecules (e.g., 29,409). EX1083, ¶68 (“29,409 SMI partner pairs were found”); EX2017, 11:2-3 (“The duplex consensus sequence is a method for determining the sequence of a DNA — a duplex DNA molecule.”). That is, Schmitt’s preferred embodiment produces duplex consensus sequences for about 2 molecules out of every billion DNA fragments (i.e., $29 \times 10^3 \div 15 \times 10^{12}$)

in the original library, while trillions of DNA molecules are lost to any meaningful analysis. EX2015, ¶82.

Dr. Spellman confirms this assessment of Example 1. Schmitt's 3 µg sample has about 1,000,000 haploid genome equivalents ($3 \mu\text{g} * 333,333 \text{ HGE}/\mu\text{g}$).

EX1083, ¶56 ("3 micrograms of DNA"); EX1002, ¶166, n. 12 ("1 µg of DNA would contain 333,333 HGEs"). Dr. Spellman testified that fragmenting a single haploid human genome produces millions (e.g., 15×10^6) DNA molecules.

EX2017, 30: 5-17 ("Q. Okay. So just in mathematic terms, three billion divided by 200 is 15 million, is that correct? A. Three times ten to the ninth, divided by two times ten to the second is 1.5 times ten to the seventh, which I agree is 15 million, yes."). Schmitt's 1,000,000 (10^6) haploid genome equivalent sample yields about 15 trillion (15×10^{12}) DNA molecules ($10^6 \text{ HGE} * 15 \times 10^6 \text{ molecules/HGE}$). *See* EX2017, 30:24-31:7 (explaining that a 333,333 HGE sample would have produced 5 trillion molecules). Yet, Schmitt reports that only a small fraction of the trillions of sample molecules are ultimately subjected to DCS. EX1083, ¶68 ("29,409 SMI partner pairs were found"); *see also* EX2015, ¶83.

Such massive loss in sample molecules is relevant in assessing detection reliability or sensitivity because mutations cannot be detected in sample molecules that are never analyzed. If a mutant molecule is not analyzed, it cannot generate a positive result and is effectively a false negative result. EX1083, ¶68 ("zero

mutations”). Given that Schmitt fails to analyze trillions of its sample molecules, a POSA would expect many false negatives. EX2019, 2 (“A test with high sensitivity tends to capture all possible positive conditions without missing anyone.”); EX2015, ¶84; EX2016, ¶¶31-33.

On the other hand, Narayan describes a method for “ultrasensitive” detection of mutations associated with cancer in clinical settings. *E.g.*, EX1082, Title, Abstract (“Using layers of sequence redundancy designed to distinguish true mutations from sequencer misreads and PCR misincorporations, we achieved a detection sensitivity of approximately 1 variant in 5,000 molecules.”); *see also* EX1002, ¶188. Narayan discusses detecting thousands of cancer mutations even with minute amounts of cfDNA. *E.g.*, EX1082, 3493 (“DNA was extracted from 0.2 mL of each plasma sample...”), Figure 4 (“Median genome equivalents per sample as determined by real-time PCR were 9602 (IQR: 5,412–11,513).”), 3497 (“The median concentration among samples with detectable mutations was 5,694 mutant KRAS molecules per mL (IQR: 2,655–25,123).”); *see also* EX1002, ¶251 (estimating Narayan’s samples had between 16.7 and 27.8 ng of cfDNA); EX2015, ¶85; EX2016, ¶¶15, 16.

As Dr. Quackenbush explains, concerns regarding Schmitt’s design and lack of good reason to turn to Schmitt’s DCS method for detection of cancer mutations in the clinical setting is further corroborated in the scientific literature. The

scientific literature demonstrates that skilled artisans viewed consensus sequencing methods like Schmitt's as perhaps an interesting laboratory protocol, but not designed for clinical settings. This is reflected in Narayan, for example, which discusses the consensus sequencing methods of Kinde (EX2004). Kinde and Schmitt bear the same fundamental design. Like Kinde, Schmitt amplifies molecules, redundantly sequences, and then collapses sequence reads into consensus sequences after grouping sequence reads into UID families. *See, e.g.*, EX1067, 102 (confirming same fundamental design of Kinde and Schmitt's DCS). Narayan describes "this approach [as] not designed to analyze multiple amplicons from samples containing limited DNA and was not tested on clinical specimens." EX1082, 3497; *see also* EX2004, 9534 ("Our endogenous UID approaches ... are not ideally suited for [clinical applications] because of the inevitable losses of template molecules during the ligation and other preparative steps."). Perakis similarly reports that skilled artisans did not view consensus sequence approaches like Schmitt's DCS method as designed or well suited for use in clinical settings. Perakis notes that as of 2017 Schmitt's DCS had not been used with cfDNA and that "[p]rospects of success are limited since the method is relatively inefficient when limited amounts of input DNA—as is most likely the case for cfDNA—are used." EX1067, 102. While Perakis was discussing the Schmitt 2012 paper (EX1064) and the preferred exogenous 12-mer embodiment, as discussed below,

the proposed 3-mer hybrid SMI approach would not mitigate these concerns but more likely make matters worse. EX2015, ¶86.

Accordingly, as Dr. Quackenbush and Dr. Hagemann explain, a POSA would view Schmitt's experimental data as reporting a failure to detect mutations in a an exceptionally large laboratory sample. And the large proportion of molecules not analyzed in Schmitt's DCS does not support Petitioner's argument of increased sensitivity. EX2015, ¶87; EX2016, ¶¶30, 34.

3. The 3-mer hybrid SMI embodiment would have been more error-prone compared to the 12-mer exogenous SMI

Sensitivity and ability to detect cancer mutations in a clinical setting using Schmitt's hypothetical 3-mer hybrid approach is unknown and unaddressed by the petition materials. As Dr. Quackenbush explains, various aspects of the 3-mer hybrid method would be made it even worse for detecting mutations relative to Schmitt's preferred exogenous 12-mer embodiment discussed above. This includes, for example, increased accumulation of errors in the SMI and increased errors caused by phasing problems on Illumina sequencing instruments, both of which are documented in Petitioner's own publications but ignored. These factors result in more error prone sequence information and fewer molecules for DCS error correction. EX2015, ¶88.

First, use of error-prone shear sequences provides an additional source of error in the SMI sequences in the proposed hybrid SMI embodiment. Schmitt,

among others, discloses that the sheared ends are “error prone.” EX1083, ¶60; *see also id.* (“The first 4 nucleotides located following the adaptor sequence were also removed due to the propensity for ligation and end-repair errors to result in an elevated error rate near the end of the DNA fragments.”); EX2004, SI2 (“We noticed a relatively high frequency of errors at the ends of the reads in the endogenous UID libraries prepared with the standard Illumina protocol, presumably introduced during shearing or end repair, so the first and last 3 bases of these tags were excluded from analysis.”). EX2015, ¶89.

Schmitt’s DCS critically relies on SMI sequence for grouping of sequence reads and matching SSCS. EX1083, ¶¶68 (“Thus filtering PCR duplicates using shear points resulted in discarding a large portion of the reads.”), 69 (“Sequence reads were considered only when the read data from each of the two strands is in perfect agreement.”); *see also* EX2013, 5; Paper 9, 35-36. Increased sequencing errors in the SMI means fewer molecules are available for DCS analysis.

Accordingly, as explained by Dr. Quackenbush, a POSA would have expected use of the error-prone SMIs of the hybrid method to result in even fewer consensus sequences for analysis and less error correction. EX2015, ¶89.

Second, the phasing requirements of the Illumina platform would result in more error-prone sequence information in the context of the proposed 3-mer hybrid approach. In short, phasing relates to the ability of the Illumina instrumentation to

properly synchronize sequence data with the appropriate nucleotide position.

Petitioner's own publications confirm that exogenous SMI sequences shorter than 12 nucleotides present problems regarding Illumina's phasing requirement.

Twelve random nucleotides per adapter (24 nt per final ligated molecule) significantly exceed the degeneracy needed to ensure unique labeling of every molecule in a library. However, a tag length of <12 is incompatible with the Illumina sequencer because of technical limitations of the platform's 'phasing' requirement and should be avoided.

EX2003, 2591; *see also* EX2015, ¶90.

With respect to phasing, Illumina uses intensity values from early sequencing cycles (e.g., cycles 2-12) to calibrate the instrument and account for loss of synchrony when calling bases. This calibration depends on having an equal representation of all four bases across the library. When sequencing cycles used for phasing calibration include fixed sequences, the instrument cannot properly synchronize the sequence data with the appropriate nucleotide position. EX2020, 11; EX2021, 1 ("Calculation of [phasing and prephasing] rates requires a balanced and random base composition in cycles 2–12."); *see also* EX2015, ¶¶91, 92.

When adapters comprising a 12-nucleotide random SMI sequence are used, the first 12 nucleotides of sequence satisfy the requirement for a balanced and random base composition in cycles 2-12. EX1083, ¶60 ("include a 12 nucleotide random sequence, followed by a 5 nucleotide fixed sequence"), (stating the "the

expected fixed sequence” occurs at read “positions 13-17”), Figure 2 (illustrating adapter used in Example 1). However, with the 3-mer hybrid SMI, only the first three nucleotides are balanced and random and are followed by a followed by a 5 nucleotide fixed sequence. Thus, libraries tagged with 3-mer hybrid SMI fail to properly synchronize sequence data with the appropriate nucleotide position.

EX2015, ¶93.

Failure to calibrate phasing generally increases the rate of sequencing errors. As a result, more errors would accumulate in the SMIs, leading to fewer reads grouped in families and fewer SSCS paired to yield DCS. As Dr. Quackenbush explains, a POSA would have also expected more disagreement between sequence reads leading to a decreased ability to generate consensus at any given position. Accordingly, the inability to control for phasing would have resulted in even fewer molecules generating a duplex consensus sequence than with the preferred embodiment. EX2015, ¶94.

Finally, the increased accumulation of errors in the SMI and increased errors caused by phasing problems on Illumina sequencing instruments discussed above would have been even more acute with cfDNA samples as proposed. The 3-mer tag would have reduced the already low rate at which molecules are analyzed with DCS (e.g., 2 in a billion) without the corresponding ability to increase the number of starting molecules that exists with laboratory samples. Accordingly, a POSA

would have been dissuaded from specifically using a 3-mer hybrid SMI, particularly with clinical cfDNA specimens. EX2015, ¶95.

4. The petition materials identify no good reason to select a 3-mer hybrid approach

Even if one were to contemplate application of Schmitt's DCS method to cfDNA (and as explained herein, a POSA would not be motivated to do so), the petition materials provide no good reason why a POSA would have specifically selected a 3-mer hybrid approach as proposed. As discussed above, a POSA would have recognized significant problems with a 3-mer hybrid approach that would lead to more error-prone sequence information and fewer molecules analyzed as compared to Schmitt's 12-mer embodiment. *See* Section V.C.3. The petition materials never grapple with any of the problems. *See* EX1002, ¶¶193-196; *see also* EX2015, ¶96.

Petitioner provides no reasoning as to why a POSA would specifically choose a 3-mer. Rather, all that is provided is generic comments about why shorter n-mers may offer benefits as compared to longer n-mers. *See* Pet. 35-36; EX1002, ¶¶193-196. But these arguments are not specific to choosing a 3-mer and would equally counsel for using a 12-mer as opposed to a 20-mer. For example, Dr. Spellman argues synthesizing longer oligonucleotides can be "problematic" and may result in "decreased synthesis yield and an increasing burden of truncated species as a contaminant." EX1002, ¶193. Dr. Spellman also argues that "longer n-

mer sequence increases the potential for hairpins to form within the n-mer.” *Id.* But Dr. Spellman never substantiates that synthesis of a 12-mer SMI was problematic or that hairpin formation was a concern with Schmitt’s preferred 12-mer embodiment. EX2015, ¶97.

Dr. Spellman also asserts that shorter n-mers would improve the efficiency of downstream PCR amplification. EX1002, ¶193. But lacking from Dr. Spellman’s analysis is any evidence that reducing the n-mer to 3 nucleotides versus 12 nucleotides would substantially improve amplification of library molecules. Indeed, it would seem implausible that such a small decrease would improve amplification efficiency given that library molecules themselves are much larger than any length n-mer described by Schmitt. EX1083, ¶¶16 (“Each n-mer sequence may be between approximately 4 to 20 nucleotides in length.”), 56 (“optimal range of ~200-500 bp”); *see also* EX2015, ¶98.

Dr. Spellman argues that “Schmitt’s 3-mer hybrid tags provides more value per sequencing read” on Illumina instruments and “still provide[] sufficient diversity.” EX1002, ¶¶194-195. These arguments fail to consider any of the numerous downsides to using a 3-mer hybrid approach—including the specific problems on Illumina instruments reported by the inventors. *See* Section V.C.3 (error-prone shear sequences, phasing problems and poor compatibility with Illumina sequencing instruments); EX1083, ¶60 (“error prone end repair”);

EX2003, 2591 (“However, a tag length of <12 is incompatible with the Illumina sequencer because of technical limitations of the platform’s ‘phasing’ requirement and should be avoided.”). These factors result in more error-prone sequence information and fewer molecules for DCS error correction. According to Dr. Quackenbush, a POSA would not ignore recognized downsides of using a 3-mer hybrid approach as Dr. Spellman does in his incomplete discussion regarding motivation. EX2015, ¶99.

5. Kukita is irrelevant to the obviousness theory advanced

Petitioner relies on citation to a sentence in a reference to Kukita (EX1008, 7) to support assertions of obviousness. Dr. Spellman states, for example, “a POSA would have appreciated that Schmitt’s Duplex Sequencing could be used to ‘enhance accuracy’ when sequencing cfDNA from cancer patients.” EX1002, ¶206 (citing EX1008, 7). In addressing motivation with a reasonable expectation of success, Dr. Spellman relies specifically on Kukita (EX1008). EX1002, ¶¶206-208. However, as Dr. Spellman acknowledged, Kukita is irrelevant to the stated theory of obviousness because it does not address the Schmitt PCT or the relied upon 3-mer hybrid approach. EX2015, ¶100.

Kukita does not discuss the Schmitt PCT publication that forms the basis of Petitioner’s obviousness theory. Instead, Kukita discusses a Schmitt 2012 PNAS paper (Ref. 19).

Our procedure is optimized for our objectives and social environment, but there is room for technical improvement. In addition to the paired-end method [9], methods to produce error-free sequences through the repeated sequencing of templates from a single molecule [18,19] might be applicable to enhance accuracy.

EX1008, 7; *see also* EX2015, ¶101.

Dr. Spellman's declaration states that there are significant differences between Schmitt (EX1083) and Schmitt 2012 PNAS (EX1068) that are critical to the proffered obviousness theory. For example, Dr. Spellman dismisses published literature critical of DCS on the basis that "Perakis did not cite to Schmitt, but instead to the Schmitt 2012 PNAS paper." EX1002, ¶121. Dr. Spellman goes on to indicate key differences between the references, stating that that Schmitt 2012 "focuses on using a 12-mer molecular barcode tag." *Id.* (citing EX1068, 14512, S1). Dr. Spellman also states that Schmitt includes discussion of the hybrid method, 3-nucleotide n-mers, and hybrid capture which are all absent from Schmitt 2012. EX1002, ¶122; EX2017, 38:12-39:3; *see also* EX2015, ¶102.

Like Perakis, Kukita does not discuss Schmitt (EX1083), it discusses only Schmitt 2012. EX1002, ¶105 ("Kukita then cited the Schmitt 2012 Duplex Sequencing paper"); EX2017, 41:21-42:10. By Dr. Spellman's admission, Kukita is not relevant to his theory of obviousness applying Schmitt's 3-mer hybrid embodiment to cfDNA. EX2015, ¶103.

Finally, even if considered, Kukita does not support Petitioner’s argument of motivation to replace Narayan’s approach with the 3-mer hybrid approach of Schmitt. Kukita specifically identifies Narayan (Ref. 9) in the context of enhancing accuracy. EX1008, 7 (“In addition to the paired-end method [9],...”); *see also* EX1067, 101 (“Narayan et al. described a deep sequencing algorithm that demands redundancy within each clonal sequence to produce extremely high quality base calls in short, mutation-prone regions of plasma DNA [176].”). Thus, if Kukita page 7 citations are taken as evidence of approaches that would be considered by a POSA, Kukita cites to Narayan but is silent regarding Schmitt’s 3-mer hybrid embodiment. EX2015, ¶104.

6. A POSA would the view larger blood volume requirements of Schmitt as a disadvantage

Petitioner argues that a POSA would have known that “they *could* obtain enough cfDNA from standard blood draws” to perform DCS. Pet. 37; EX1002, ¶211; *see also* Pet. 39. Regardless of whether a POSA “could” obtain enough cfDNA for DCS, Petitioner never identifies any reason why a POSA would have wanted to do so. EX2015, ¶105; This alone is reason to reject Petitioner’s assertions of obviousness. *Belden Inc. v. Berk-Tek LLC*, 805 F.3d 1064, 1073 (Fed. Cir. 2015) (“obviousness concerns whether a skilled artisan not only could have made but would have been motivated to make”).

While it is not Patent Owner's burden to prove no motivation, Dr. Hagemann explains that there were significant drawbacks to Petitioner's suggestion to dramatically increase the amount of blood taken from a patient to meet the requirements of Schmitt's hybrid method. EX2016, ¶¶106, 107. Petitioner never substantiates any benefits resulting from the proposed combination. Accordingly, the evidence weights in favor of no motivation. *See, e.g., Arctic Cat Inc. v. Polaris Indus.*, 795 F. App'x 827, 833 (Fed. Cir. 2019) ("The Board must weigh the benefits and drawbacks of the modification against each other, to determine whether there would be a motivation to combine."); *Henny Penny Corp. v. Frymaster LLC*, 938 F.3d 1324, 1332 (Fed. Cir. 2019) (affirming no motivation where the combination introduced "complexity"); *In re Schweickert*, 676 F. App'x 988, 995 (Fed. Cir. 2017) (affirming no motivation where the combination "add[ed] unwanted cost and complexity to the system").

The amount of blood Petitioner proposes using with Schmitt is far greater than what is used in Narayan's method. Narayan describes using just 0.2 mL of plasma which could be obtained from about 0.6 mL of blood. EX1082, 3493; EX1002, ¶71 ("ml of serum or plasma (3 ml of blood)."). In contrast, Dr. Spellman asserts that 7 to 12 mL plasma (21 to 36 mL of blood) would contain a sufficient amount of cfDNA for Schmitt's method. EX1002, ¶166; Paper 9, 28. That is, Dr. Spellman asserts that 35- to 60-fold more blood would be needed to perform

Schmitt's DCS method. However, Dr. Spellman¹ does not provide any reason why a POSA would have been motivated to abandon Narayan in favor of a method that requires so much more blood and plasma. EX2016, ¶¶26, 33, 35.

As Dr. Hagemann explains, a POSA would view Dr. Spellman's proposed modification of Schmitt to be undesirable and impractical for use in a clinical setting. As Dr. Hagemann explains, a POSA would be concerned by Dr. Spellman's proposal to use a blood draw roughly 50 times what Narayan would require, and would accordingly be discouraged from practicing Schmitt's method in the manner proposed. EX2016, ¶¶35, 36.

Generally, physicians prefer tests that draw as little blood as possible. Drawing significant quantities of blood is uncomfortable to the patient, difficult for laboratories to process, and puts a patient at risk for comorbidities. EX2016, ¶37.

As Dr. Hagemann explains, to minimize these costs in routine blood draws for DNA analysis, medical professionals draw substantially less blood for such a test than the roughly 30 mL quantity proposed by Dr. Spellman. Routine blood

¹ Dr. Spellman's testimony regarding clinical issues is entitled little, if any, weight. Dr. Spellman is a laboratory scientist, not a clinician. *See* EX1003. Moreover, as explained herein, his testimony regarding clinical issues is not consistent with evidence of record.

draws, such as those performed on patients during hospital stays, are generally a few mL; a draw of 30 mL would be out of the ordinary. The collection tubes used for phlebotomy in adults typically measure 7.5 mL, so collection of 30 mL would require multiple collection tubes. Most clinical laboratories would have no equipment to analyze such large quantities of blood. In light of these concerns and others, a POSA would consider Dr. Spellman's proposal dubious at best. Moreover, to draw a large amount of blood, only to discard the vast majority of the resulting sequence reads, requires a peculiar logic. EX2016, ¶38.

This preference towards analyzing only reasonable amounts of blood is borne out in the examples the petition materials identify as representative commercial blood analysis kits. For example, Dr. Spellman identifies the QIAamp DNA Micro Kit (Qiagen), the QIAamp DNA Blood Mini Kit, the Promega Wizard (Promega), and the NucleoSpin Plasma XS (Macherey-Nagel) as commercial products commonly used to extract cfDNA from plasma. EX1002, ¶¶78-79. Dr. Spellman further emphasizes that only the Qiagen extraction methods were found to be reliable in practice. EX1002, ¶78; *see also* EX2016, ¶39.

The Qiagen kits, however, are not designed to or even capable of handling the quantities of blood or plasma Petitioner proposes using. For example, the DNA Micro Kit is only designed to handle whole blood samples of up to 100 µL. EX2026, 7; EX1067, 95 (“highly recommended the use of extraction methods

specifically developed for cfDNA isolation”). The DNA Blood Mini Kit teaches using whole blood or plasma samples of up to 200 µL. EX2025, 17. Thus, the only kits Dr. Spellman identifies as reliably supporting DNA analysis use volumes much smaller than the 7 to 12 mL of plasma he proposes to use in DCS. EX2016, ¶40.

According to Dr. Hagemann, doctors favor using small quantities of blood to avoid negative patient outcomes. For example, patients in hospital settings will often be subjected to routine blood draws during their stays, and physicians have long recognized that the cumulative effect of such draws produces an increasing risk to patients. EX2029; EX2030, 1646-1647; EX2031. Additionally, large-volume peripheral blood draws may harm patients by causing injury to the vein from which blood is drawn. EX2016, ¶¶41-45; EX2029, 522; EX2030, 1646-1647; EX2031.

In light of the increased cost and difficulty of handling large quantities of blood, as well as negative patient outcomes, a POSA would consider the larger amounts of blood necessary to perform DCS on cfDNA according to Dr. Spellman’s proposal to be a significant drawback that would discourage pursuit of such a combination. EX2016, ¶46.

D. The petition materials fail to establish disclosure of the requirements of dependent claims

Dependent claims 2-16 and 29 depend from claim 1 and are patentable at least for the reasons discussed above. *See* Section V.A-C. Additional reasons why the petition materials fail to demonstrate obviousness of dependent claims 3, 5, 7, and 14 are discussed further below. EX2015, ¶¶, 19, 108.

1. Claim 3

3. The method of claim 1, wherein the population of cfDNA molecules comprises 1 nanogram (ng) to 100 ng of cfDNA molecules.

The petition materials fail to establish the cited prior art discloses the requirements of claim 3. Petitioner addresses claims 3 at pages 45-46 where it relies exclusively on Narayan. EX2015, ¶109.

The petition materials never address the difference between what is asserted as an amount of cfDNA sufficient for DCS (e.g., 1 µg) and the amount of cfDNA described by Narayan (16.7 to 27.8 ng). Dr. Spellman argues that smaller amounts of DNA may be accommodated by using additional rounds of PCR and hybrid capture. EX1002, ¶253. But Schmitt's Example 1 actually performed additional rounds of PCR and used hybrid capture and yet was only able to generate duplex consensus sequences for 29,409 molecules in a 3 µg sample. *Id.* (citing EX1083, ¶¶58, 59); *see also* Section V.C.2; EX2015, ¶110.

Even if it could be assumed (and it cannot be) that application of the hybrid 3-mer tag embodiment to 16.7 to 27.8 ng of cfDNA would have the same efficiency as the preferred embodiment of Example 1, a POSA would expect to generate duplex consensus sequences for as few as 164 cfDNA molecules (16.7 ng * 1 µg/1,000 ng * 29,409 DCS/3 µg). However, as Dr. Quackenbush explains, the 3-mer hybrid tag embodiment would be much less efficient, particularly when applied to minute amounts of cfDNA. *See* Section V.C.3; EX2015, ¶¶111, 112.

2. Claim 5

5. The method of claim 1, wherein the molecular barcodes are ligated to the plurality of the cfDNA molecules using more than an 80× excess of duplex tags as compared to the population of cfDNA molecules.

The petition materials fail to establish the cited prior art discloses the requirements of claim 5. Petitioner addresses this claim at pages 46-47 where it relies exclusively on Schmitt. The petition materials fail to identify disclosure of using an 80× excess of duplex tags specifically with cfDNA molecules or, for that matter, any DNA molecules. EX2015, ¶¶113, 114.

As an initial matter, Schmitt does not describe ligating molecular barcodes to cfDNA as required by the claims. Given the lack of disclosure, Petitioner relies solely on Schmitt's discussion of ligating adapters to 750 nanograms of randomly fragmented cellular DNA with lengths ranging from 200 to 500 bp. *See* EX1083,

¶56. This disclosure in Schmitt is deficient at least because it does not provide disclosure of ligating molecular barcodes to cfDNA. EX2015, ¶115.

The only mention of cfDNA in Petitioner's analysis of claim 5 is with respect to motivation in which he states "[a] POSA would have also understood that increasing adapter-DNA ligation efficiency was especially important when starting with lower amounts of DNA, like cfDNA." Pet. 47; EX1002, ¶261. Even if this unsupported argument is credited, it does not provide reason to use at least an 80× excess of duplex tags with cfDNA as required by claim 5. *See also* EX2003, 2591 ("the use of excess adapters can result in adapter dimers, which, owing to their small size, preferentially amplify during PCR"); EX2015, ¶116.

The petition materials rely on misleading molar excess calculations that are unrelated to the requirements of claim 5 or the theory of obviousness. For example, Dr. Spellman asserts that Schmitt discloses using a range of 41× to 103× excess of duplex tags based on randomly fragmented cellular DNA with lengths ranging from 200 to 500 bp. EX1002, ¶260. But Schmitt does not disclose a range of excess duplex tags. EX2015, ¶117. Accordingly, Petitioner's reliance on overlapping range case law is misplaced. Pet. 47 (citing *Ormco Corp. v. Align Technology, Inc.*, 463 F. 3d 1299, 1311 (Fed. Cir. 2006)).

Schmitt does not describe a range of excess of duplex tags but rather describes a ligation reaction performed using 250 pmol of adaptors and 750 ng of

DNA. EX1083, ¶56. Schmitt discloses size-selecting the DNA to an optimal range of 200-500 bp (*id.*), but Dr. Spellman errs when converting this size-selection to a teaching of using a range of 2.43-6.07 pmol of DNA. EX1002, ¶260. The selection does not produce a range of moles of DNA. Instead, Schmitt's size selection would produce one specific number of moles of DNA which would depend on the particular (but unknown) size distribution of the fragments in Schmitt's sample. At best, Dr. Spellman's calculation shows that this unknown number of moles of DNA would be somewhere between 2.43 and 6.07 pmol. But uncertainty regarding a value does not equate to disclosing a range of values. For similar reasons, the step of converting from a number of moles to molar ratio merely provides bounds to estimate the actual molar ratio in Schmitt's example. The actual molar ratio produced by Schmitt's example is a single, unknown value, not a range of values as suggested by Dr. Spellman. Dr. Spellman's own calculations produce a lower bound of 41× for this molar ratio value, which is outside of the range of 80× or more recited in claim 5. EX2015, ¶118.

Furthermore, Dr. Spellman misunderstands Schmitt's disclosure regarding size selection. In discussing ¶56 of Schmitt, Dr. Spellman suggests that Schmitt only removes fragments larger than 500 bp, not fragments smaller than 200 bp. EX1002, ¶260 n.16. This is incorrect. Schmitt describes its size-selection process as including the following steps immediately after removing fragments larger than

500 bp: “An additional 0.65 volumes of AMPure XP beads were added (this step allows fragments of approximately 200bp or greater to bind to the beads). The beads were washed and DNA eluted.” EX1083, ¶56. The step of binding fragments larger than 200 bp to beads and then eluting DNA upon washing the beads excludes fragments smaller than 200 bp, since the smaller fragments would not bind to the beads and would therefore be excluded from the eluted DNA. Thus, Schmitt’s size selection would exclude cfDNA, which typically ranges in length from 140 to 170 bp. EX1002, ¶260 n. 16; *see also* EX1001, 19:46-51 (disclosing the average length cfDNA molecule to be ~160 bp); EX2015, ¶119.

Moreover, Dr. Spellman bases his molar excess calculations on DNA lengths of 200 to 500 bp yet he acknowledges that cfDNA is 140 to 170 bp. If a range of lengths reflective of cfDNA fragments (e.g., ~140 to 170 bp), is used, Schmitt’s amount of adapters (250 pmol) is not within the claimed range. Dr. Spellman asserts that moles of DNA can be calculated using the following formula: moles dsDNA (mol) = mass of dsDNA (g)/((length of dsDNA (bp) x 617.96 g/mol/bp) + 36.04 g/mol). EX1002, ¶260 n.17. Applying this formula to 750 ng of cfDNA fragments with a length range of ~140 to 170 yields a molar amount between 7.14 to 8.66 pmol. Based on a molar amount between 7.14 to 8.66 pmol of cfDNA, the molar excess of adapters would be between 29× and 35×—that is, much less than half the claimed lower limit. EX2015, ¶120.

3. Claim 7

7. The method of claim 1, wherein z is between 2 and 8.

The petition materials fail to establish the cited prior art discloses the requirements of claim 7. Petitioner addresses claim 7 at pages 47-48 where it relies exclusively on Schmitt. EX2015, ¶121, 122.

Petitioner never addresses the requirements of claim 7, which is directed to a cfDNA sample having a z value between 2 and 8. As defined in claim 1, z is the mean of an expected number of duplicate molecules in the population of cfDNA molecules that map to identical start and stop positions on a reference sequence. Accordingly, claim 7 is directed to a cfDNA sample that is expected to have between 2 and 8 duplicate molecules at any given position on a reference sequence. EX2015, ¶123.

Petitioner asserts that “Schmitt’s 3-mer hybrid tag embodiment comprises 4,096 different combinations of molecular barcodes.” Even if the number of molecular barcodes is accepted (and it should not be for the reasons discussed above), it does not address the requirements of claim 7. Petitioner does not identify any disclosure in Schmitt of a cfDNA sample having a z value between 2 and 8 because there is no such disclosure. EX2015, ¶¶124, 125.

4. Claim 14

14. The method of claim 1, further comprising: (f) determining quantitative measures of (i) paired reads or (ii)

unpaired reads that map to a genomic locus of the reference sequence.

The petition materials fail to establish the cited prior art discloses or teaches determining quantitative measures of paired or unpaired reads that map to a genomic locus of the reference sequence as specifically recited in claim 14 of the '306 patent. Petitioner addresses claim 14 at pages 53-55 where it relies exclusively on disclosures in Schmitt. EX2015, ¶¶126, 127.

Dr. Spellman opines that a POSA would have understood claim 14 to require “calculating N1 (the paired reads) and N2 (the unpaired reads).” EX1002, ¶308 (citing EX1001, 32:43-49). Dr. Spellman further opines that, based on the '306 patent, a POSA would understand that N1 and N2 represent the number of paired and unpaired reads, respectively, that map to an arbitrary locus. *Id.* (citing EX1001, 32:43-49) (“The '306 patent discloses that for an arbitrary locus A, ‘there are N [total] amplified fragments,’ and that ‘we assign N1, N2, and N3 as the number of double strands, single-strands, and unseen fragments, respectively.’”). EX2015, ¶128.

While Dr. Spellman acknowledges that claim 14 requires quantifying paired or unpaired reads at a given genomic locus of a reference sequence, he fails to identify such disclosure in Schmitt (because there is none). At most, Dr. Spellman asserts that Schmitt discloses determining the total number of duplex consensus sequences obtained. *See* EX1002, ¶309 (“Thus, a POSA would understand that

Schmitt discloses calculating a quantitative measure of paired consensus sequences ('29,409 SMI partner pairs' and 'fewer than 1% of the tags')"). But such disclosure does not meet what Dr. Spellman acknowledges is required by claim 14. EX2015, ¶129.

VIII. GROUND 3: CLAIM 8 IS NOT OBVIOUS OVER NARAYAN, SCHMITT AND CRAIG

8. The method of claim 1, wherein the molecular barcodes have a length of 5 to 20 base pairs.

Claim 8 depends from claim 1 and is not obvious for the same reasons I discuss above. *See* Sections V.A-C. The petition materials fail to establish disclosure of (1) tagging cfDNA; and (2) mapping sequence reads in the manner required by claim 1. *See* Sections V.A & B. Furthermore, the petition materials fail to establish motivation to combine the relied upon references, including Narayan and Schmitt. EX2015, ¶130; *See* Section V.C.

Furthermore, the petition materials point to Craig as providing 6-mer sequences, but do not logically explain why a POSA would be motivated to combine Craig's disclosure with Schmitt with a reasonable expectation of success. And Dr. Spellman fails to explain how his proposal to make use of Craig's barcodes is compatible or consistent with the underlying obviousness theory based on Schmitt's hybrid embodiment. *See* EX1002, ¶¶355-362. For example, Dr. Spellman argues a POSA would be motivated to "mitigate barcode sequencing

errors,” but fails to grapple with disclosure in Schmitt indicating that the shear sequences used in the hybrid approach are error prone and increase sequencing errors. Swapping a 3-mer exogenous portion with a 6-mer from Craig does nothing to mitigate such errors in the SMI. EX2015, ¶131.

Additionally, Dr. Spellman argues motivation to achieve greater sequencing depth “to perform ‘deep sequencing’ to detect rare sequences in the cfDNA sample.” EX1002, ¶358. But this undermines the argument to replace Narayan with Schmitt in the first place. Narayan did detect rare mutations in a cfDNA sample, while Schmitt did not. Moreover, Narayan reports an average sequencing depth of over 100,000x. EX1082, 3494 (“median depth of 108,467 read pairs”). In contrast, Schmitt reports an average read depth of just 3x. EX1083, ¶68. Dr. Spellman does not explain how turning to Craig would cure the relatively poor sequencing depth reported in Schmitt and a POSA would not expect it to do so. EX2015, ¶132; EX2016, ¶32.

Accordingly, Dr. Spellman provides no good reason to replace Narayan with Schmitt, and further combine with teachings from Craig. The argument that is provided is little beyond an illogical attempt to map prior art references to claim elements and actually undermines his argument to combine Narayan and Schmitt in the first place. EX2015, ¶133.

IX. GROUND 4: CLAIMS 15 AND 16 ARE NOT OBVIOUS OVER NARAYAN, SCHMITT, AND KIVIOJA

15. The method of claim 14, further comprising: (g) estimating with a programmed computer processor a quantitative measure of tagged parent polynucleotides that map to the genomic locus based on the quantitative measures of the paired reads and the unpaired reads.

16. The method of claim 15, further comprising: (h) detecting copy number variation in the population of cfDNA molecules by determining a normalized quantitative measure determined in (g) at each of one or more genomic loci.

Claims 15 and 16 depend indirectly from claim 1 and are not obvious for the same reasons I discuss above. The petition materials fail to establish disclosure of (1) tagging cfDNA; and (2) mapping sequence reads in the manner required by claim 1. *See* Sections V.A & B. Finally, the petition materials fail to establish motivation to combine the relied upon references, including Narayan and Schmitt. *See* Section V.C. Claims 15 and 16 further depend from claim 14 and are not obvious for the same reasons I discuss above with respect to claim 14. *See* Section V.D.4. EX2015, ¶¶ 134, 135.

Petitioner addresses claims 15 and 16 at pages 63-69 where it relies on disclosures in Schmitt for determining quantitative measures of paired or unpaired reads that map to a genomic locus of the reference sequence and Kivioja for calculating the total number of molecules that map to a genetic locus. As explained herein, Schmitt does not disclose determining quantitative measures of paired or

unpaired reads that map to a genomic locus of the reference sequence as required by claims 15 and 16. *See* Section V.D.4. To the extent Kivioja discusses determining the total number of molecules at a genetic locus, it does not describe doing so using paired and unpaired reads. Petitioner does not argue to the contrary. *See* Pet. 64-65; *see also* EX2015, ¶¶136, 137.

Accordingly, claims 15 and 16 are not obvious for the additional reason that Kivioja fails to teach calculating the total number of molecules that map to a genetic locus in the manner claimed. EX2015, ¶138.

VI. GROUNDS CORRECTLY DEEMED DEFICIENT

The Board's Institution Decision found the petition materials failed to demonstrate a reasonable likelihood to prevail regarding claims 4, 6, and 17-29. The petition materials remain deficient as to these claims for at least the same reasons previously identified and addressed in the Board's institution decision. Paper 9, 30-41. The Board reached similar conclusions in another IPR. IPR2022-01152, Paper 11, 12-23. Additional discussion regarding claims 4, 6, and 17-29 is provided below. EX2015, ¶¶21, 139-141.

A. Ground 1: Claims 17-29

At institution, the Board found that Petitioner had not sufficiently explained how the cited references teach or suggest the sorting of reads into families of unpaired reads as recited in element 17(d) or determining distinct cfDNA

molecules based on unpaired reads as recited in claim 29. Paper 9, 30-37; *see also* IPR2022-01152, Paper 11, 19-22. As explained in detail below, the Board’s finding in this regard is correct and supported by record evidence. Moreover, claims 17-28 fail for additional reasons such as those discussed above regarding claim 1. In particular, claim 17 excludes the hybrid embodiment in its requirement for tagging cfDNA with molecular barcodes. *See* Section VI.A. Additionally, the petition materials fail to establish motivation with a reasonable expectation of success in combining Narayan and Schmitt. *See* Section VI.C. Finally, claim 29 depends from claim 1 and is patentable for all the reasons discussed above. *See* Sections VI.A-C.

As to the sorting requirement, claim 17 explicitly states what constitutes “paired” and “unpaired” reads.

...paired reads corresponding to sequence reads generated from a first tagged strand and a second tagged complementary strand derived from cfDNA molecules from among the tagged parent polynucleotides, ...
unpaired reads corresponding to sequence reads generated from a first tagged strand having no second tagged complementary strand derived from cfDNA molecules from among the tagged parent polynucleotides
EX1001, claim 17(d).

Petitioner’s challenge fails as to claims 17-29 at least because Schmitt does not disclose distinguishing or sorting sequence reads as “paired reads” or “unpaired reads” as those terms are specifically recited in the claims. Pet., 42 (“Claim 17(d)

and claim 1(e) both entail identifying the sequence reads as a paired read (one that has a complementary strand-mate) or an unpaired read (one that does not have a complementary strand-mate).”).

Schmitt does not mention unpaired reads as claimed and certainly does not disclose a method for identifying or sorting unpaired reads. To the contrary, Schmitt describes its method as an improvement over prior art error-correction methods that only analyzed one strand from an original duplex molecule. EX1083, ¶5. Schmitt explains that its method “capitalizes on the redundant information stored in complexed double-stranded DNA” and “reduces or eliminates artifactual mutations arising from DNA damage, PCR errors, and sequencing errors.” EX1083, ¶7. It is repeatedly explained in Schmitt, that DCS only generates consensus sequence for original molecules when the sequence information for **both strands** of the original duplex is in agreement. *E.g.*, EX1083, ¶¶10, 13, 14, 60, 63, 68.

Schmitt does not identify unpaired reads, as that term is used in the challenged claims, at any step of its method. Petitioner falsely asserts (p. 43) Schmitt’s grouping step sorts reads into paired and unpaired reads. But Schmitt’s grouping is agnostic to whether reads are paired or unpaired in the manner recited in the ’306 patent claims. Schmitt describes grouping as forming families of reads

that share a common SMI tag. *E.g.*, EX1083, ¶¶60. Schmitt’s disclosure, as well as its methods, are oblivious to unpaired reads as defined in the challenged claims.

The petition confuses and improperly conflates the consensus sequences (consensus reads) with PCR duplicate reads in Schmitt. *See* EX1001, claim 17. Petitioner states (p. 32) “Schmitt further discloses generating consensus sequences from ... unpaired sequence reads (meaning that a sequence read was generated for only the Watson strand or Crick strand).” Pet., 33. Schmitt discloses no such thing.

The portions of Schmitt’s provisional Petitioner identifies discuss selecting consensus sequences based on a stringent filtering step. EX1083, ¶¶60, 68. Specifically, after grouping, Schmitt describes collapsing reads to form a single-strand consensus sequence (“SSCS”). *E.g.*, EX1083, ¶¶60, 62-63. The SSCS are then paired with their strand-mate using the SMI sequences. *E.g.*, EX1083, ¶¶60-63, 67-68. That is, only such SSCS’s with matching SMI sequences (i.e., no sequencing error in the SMI tag) are utilized. *See* Paper 9, 35-36; IPR2022-01152, Paper 2 at 7-8.

Petitioner falsely asserts (p. 54) that “Schmitt discloses calculating...a quantitative measure of unpaired reads (more than 99%).” Schmitt never describes >99% of reads as unpaired as specifically recited in the challenged claims, but merely indicates that the vast majority of consensus sequences fail the stringent filtering step critical to performing the DCS analysis. *E.g.*, EX1083, ¶¶60, 68. That

fewer than 1% of tags could meet this stringent sequence matching is not evidence that >99% of tags are inherently or necessarily “unpaired” as that term is specifically used in claims 17-29. Schmitt only discloses that >99% of tags in consensus sequences did not meet the strict filtering criteria—i.e., “perfect agreement” between SMI sequences.

Sequences lack the requisite “perfect agreement” where a sequence error occurs in the SMI or barcode. The petition acknowledges that errors in barcodes commonly occur and prevent pairing of consensus sequences in Schmitt’s method. Petitioner, for example, argues “sequencing errors in the barcode itself ‘can cause one tag to appear identical to another (crossover) or sufficiently alter a sequence tag such that it is unrecognizable (loss) and untraceable to the source material.’” Pet., 62 (quoting EX1063, 2). Indeed, this problem was widely recognized in the scientific literature as specifically a problem in Schmitt’s DCS method and identified as a major cause of the data loss (e.g., >99% of tags) that Schmitt describes. For example, Stoler explains “sequencing errors within duplex tags” in Schmitt’s DCS is the likely cause of “the large number of families with only one read.” EX2013, 5. Stoler goes on to state “sequencing errors within tags [i]s one of the main causes of data loss” with Schmitt’s DCS method. EX2013, 6. Thus, the >99% of discarded consensus sequences in Schmitt are nothing more than what Schmitt indicates they are—i.e., consensus sequences did not meet the strict

filtering criteria (“perfect agreement”) between SMI sequences. *See also* IPR2022-01152, Paper 9 at 2-3 (conceding this characterization of Schmitt).

Petitioner asserts (pp. 33-34) that Table 1 describes paired and unpaired reads, but this fails for a number of reasons. First, as discussed above, the DCS reads of Table 1 are those that pass Schmitt’s strict filter which does not distinguish paired and unpaired reads. Second, while Table 1 uses the term “Initial reads per” SSCS read or DCS read, this does not qualify as the “sequence reads” required by the claims. In claim 17, each “sequence read,” is a series of nucleotides produced by sequencing a tagged strand derived from an original cfDNA molecule. Table 1, in contrast, provides information on the basis of gross nucleotide count. EX1009, ¶¶127-128. For example, Table 1 reports “initial reads per SSCS read” as a measure of the total number of nucleotides (“initial nucleotides”) divided by the number of total “SSCS nucleotides.” *Id.* In other words, for every nucleotide of SSCS obtained, the method required 75 nucleotides of initial data. While illustrating the inefficiencies of the method, Table 1 does not disclose identifying paired and unpaired reads as required by the claims.

Petitioner identifies no disclosure in Schmitt of “paired reads” and “unpaired reads” as recited in the challenged claims because there is none.

B. Ground 2: Claims 4 and 6

Petitioner alleges that claims 4 and 6, which depend from claim 1, would have been obvious over Narayan, Schmitt, and Meyer. *See Pet.*, 58-61. As explained above, the combination of Narayan and Schmitt does not teach or suggest each and every limitation of claim 1. Petitioner does not allege that Meyer teaches or suggests any of the limitations of claim 1. Accordingly, Ground 2 fails for the same reasons as discussed above with respect to claim 1. *See supra* §V.

Ground 2 also fails because, as the Board noted at institution and in other IPRs, tagging efficiency was not a simple function of molar excess, but also depends on factors including DNA quantity and quality. *See EX1015*, 4; *EX1031*, 5-6; Paper 9, 40-41. Yet Petitioner failed to address how the quality of cfDNA “compares to the quality of the genomic DNA samples of Meyer and Schmitt or how that would affect the expected tagging efficiency.” *IPR2022-00746*, Paper 14 at 14-16; *see also IPR2022-00747*, Paper 14 at 14-16; *IPR2022-01115*, Paper 14, 12–19; Paper 9, 39-40.

VII. CONCLUSION

For at least the reasons set forth above, petitioner has failed to meet its burden and the challenged claims should be found *not unpatentable*.

Respectfully submitted,

Date: May 24, 2023

/ Michael T. Rosato /
Michael T. Rosato, Lead Counsel
Reg. No. 52,182

CERTIFICATE OF COMPLIANCE

Pursuant to § 42.24(d), the undersigned certifies that this paper contains no more than 14,000 words, not including the portions of the paper exempted by § 42.24(b). According to the word-processing system used to prepare this paper, the paper contains 13,332 words.

Respectfully submitted,

Date: May 24, 2023

/ Michael T. Rosato /

Michael T. Rosato, Lead Counsel
Reg. No. 52,182

VIII. APPENDIX

EXHIBIT NO.	DESCRIPTION
2001-2002	Intentionally Left Blank
2003	Kennedy et al., “Detecting ultralow-frequency mutations by Duplex Sequencing,” <i>Nat. Protoc.</i> 9(11) (2014)
2004	Kinde et al., “Detection and quantification of rare mutations with massively parallel sequencing,” <i>PNAS</i> 108(23) (2011)
2005	Intentionally Left Blank
2006	Nix et al., “The stochastic nature of errors in next-generation sequencing of circulating cell-free DNA,” <i>PLOS ONE</i> (2020)
2007	Wang et al., “High efficiency error suppression for accurate detection of low-frequency variants,” <i>Nucleic Acids Research</i> 47(15) (2019)
2008	U.S. Patent No. 9,752,188 to Schmitt et al.
2009	U.S. Patent Application No. 16/277,724 File History Excerpts
2010	U.S. Patent Application No. 16/593,633 File History Excerpts
2011	U.S. Patent Application No. 14/712,754 File History Excerpts
2012	U.S. Patent No. 10,752,951 to Salk et al.
2013	Stoler et al., “Streamlined analysis of duplex sequencing data with Du Novo,” <i>Genome Biology</i> 17, 180 (2016)
2014	U.S. Patent Application No. 16/672,267 File History
2015	Declaration of Dr. John Quackenbush
2016	Declaration of Dr. Ian Hagemann
2017	Deposition Transcript of Dr. Paul Spellman
2018	Deposition Transcript of Dr. Rahul Satija

2019	Zhu et al., “Sensitivity, Specificity, Accuracy, Associated Confidence Interval and ROC Analysis with Practical SAS® Implementations” (2010)
2020	Kircher et al., “Addressing challenges in the production and analysis of Illumina sequencing data” (2011)
2021	Illumina Technical Note: Sequencing “Using a PhiX Control for HiSeq® Sequencing Runs” (2013)
2022	Dr. John Quackenbush CV
2023	Dr. Ian Hagemann CV
2024	Intentionally Left Blank
2025	QIAGEN Sample & Assay Technologies – “QIAamp DNA Blood Mini Kit” Third Edition (2012)
2026	QIAGEN Sample & Assay Technologies – “QIAamp DNA Micro Kit” Second Edition (2010)
2027	Intentionally Left Blank
2028	Salk et al., “Enhancing the accuracy of next-generation sequencing for detecting rare and subclonal mutations” <i>Nature Reviews / Genetics</i> (2018)
2029	Thavendiranathan et al., “Do Blood Tests Cause Anemia in Hospitalized Patients?” <i>Journal of General Internal Medicine</i> (2004)
2030	Salisbury et al., “Diagnostic Blood Loss From Phlebotomy and Hospital-Acquired Anemia During Acute Myocardial Infarction” <i>American Medical Association</i> (2011)
2031	M. Levi, “Twenty-five million liters of blood into the sewer” <i>Journal of Thrombosis and Haemostasis</i> (2014)

CERTIFICATE OF SERVICE

The undersigned certifies that the foregoing Patent Owner Response and accompanying Exhibits 2015-2023, 2025, 2026 and 2028-2031 were served on this 24th day of May, 2023, on the Petitioner at the following electronic service addresses:

Ralph Wilson Powers III
David H. Holman
Kristina C. Kelly
Christopher M. Gallo
Tyler C. Liu
STERNE, KESSLER, GOLDSTEIN & FOX
tpowers-PTAB@sternekessler.com
dholman-PTAB@sternekessler.com
kckelly-PTAB@sternekessler.com
cgallo-PTAB@sternekessler.com
tliu-PTAB@sternekessler.com
PTAB@sternekessler.com

Date: May 24, 2023

/ Michael T. Rosato /

Michael T. Rosato, Lead Counsel
Reg. No. 52,182