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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

GUARDANT HEALTH, INC.,
Petitioner,

v.

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

Case No. IPR2022-00450
Patent No. 10,689,699

PETITION FOR INTER PARTES REVIEW OF
U.S. PATENT NO. 10,689,699

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LISTING OF CHALLENGED CLAIMS

1. [**Preamble**] A method, comprising:

[**Element 1.1**] a) providing a population of circulating DNA molecules obtained from a bodily sample from a subject;

[**Element 1.2**] b) converting the population of circulating DNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a circulating DNA molecule of the population of circulating DNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes, such that each non-uniquely tagged parent polynucleotide is substantially unique with respect to other non-uniquely tagged parent polynucleotides in the population;

[**Element 1.3**] c) amplifying the population of non-uniquely tagged parent polynucleotides to produce a corresponding population of amplified progeny polynucleotides;

[**Element 1.4**] d) sequencing at least a portion of the population of amplified progeny polynucleotides to produce a set of sequence reads;

[**Element 1.5**] e) grouping the sequence reads into families, each of the families comprising sequence reads comprising the same identifier sequence and having the same start and stop positions, whereby each of the families comprises sequence reads amplified from the same non-uniquely tagged parent polynucleotide; and

[**Element 1.6**] f) collapsing sequence reads in each family to yield a base call for each family corresponding to one or more genetic loci.

2. The method of claim 1, further comprising detecting, at one or more loci, one or more of at least one single nucleotide variant and at least one copy number variant.

3. The method of claim 1, wherein converting comprises any of blunt-end ligation, sticky end ligation, PCR, ligation-based PCR, single strand ligation and circularization to a single strand.

4. The method of claim 1, further comprising generating a set of consensus sequences from the sequence reads, and detecting a presence of sequence variations in the set of consensus sequences compared with a reference sequence.

5. The method of claim 1, further comprising filtering out sequence reads that fail to meet a quality threshold.

6. The method of claim 1, further comprising selectively enriching regions from a genome or transcriptome of the subject prior to sequencing.

7. The method of claim 1, further comprising removing a subset of the sequence reads from further analysis prior to (e).

8. The method of claim 1, wherein the population of circulating DNA molecules includes a genetic variant having a variant frequency lower than about 1% or lower than about 0.01%.

9. The method of claim 1, wherein the population of circulating DNA molecules includes a genetic variant having a variant frequency as low as about 0.01% or as low as about 0.03%.

10. The method of claim 1, wherein an error rate of the base call of each family determined in step (f) is lower than about 1×10^{-6} or is as low as about 1.2×10^{-9}.

11. The method of claim 1, wherein an error rate of the base call of each family determined in step (f) is no more than about 1.5×10^{-4} or about 3.5×10^{-5}.

12. The method of claim 1, wherein the circulating DNA molecules are nucleic acid-based serum biomarkers.

13. The method of claim 1, wherein the identifier sequence comprises a polynucleotide barcode selected from about 2 to about 256 distinct barcode sequences.

14. The method of claim 1, wherein the identifier sequence comprises a polynucleotide barcode selected from about 256 to about 4,096 distinct barcode sequences.

15. The method of claim 1, wherein the polynucleotide barcodes are contained within a library generated from oligonucleotides comprising known sequences.

16. The method of claim 1, wherein said non-uniquely tagged parent polynucleotide can be differentiated from other non-uniquely tagged parent polynucleotides using a combination of at least a first non-unique polynucleotide barcode at a first end of said circulating DNA molecule and a second non-unique polynucleotide barcode at a second end of said circulating DNA molecule.

17. The method of claim 1, wherein the sequence reads are grouped into families based on i) the polynucleotide barcode and ii) at least one of: sequence information at a beginning of the sequence from the circulating DNA molecule and sequence information at an end of the sequence from the circulating DNA molecule.

18. The method of claim 1, wherein the population of circulating DNA molecules comprises double-stranded molecules, and wherein the identifier sequence further comprises a strand identifier, and wherein, for each family of sequence reads amplified from the same non-uniquely tagged parent polynucleotide grouped in step (e), the method further comprises determining if the family has at least one sequence read from each strand of the double-stranded molecule using the strand identifier.

19. The method of claim 1, wherein the circulating DNA molecules comprise double-stranded molecules, and wherein for each of a plurality of families, the method further comprises:

confirming the presence of at least one sequence read from each strand of the double-stranded molecule; and

comparing the at least one sequence read obtained from one strand to the at least one sequence read from the other strand to form a consensus sequence of the double-stranded molecule,

wherein the consensus sequence comprises only nucleotide bases at which the sequence of both strands of the double-stranded molecule are in agreement, such that a base call occurring at a particular position in the consensus sequence is identified as a true base call.

20. [**Preamble**] A method, comprising:

[**Element 20.1**] a) attaching a set of molecular tags to a population of circulating DNA molecules obtained from a bodily sample of a subject to produce a population of tagged original DNA molecules, wherein a plurality of the tagged original DNA molecules has identical molecular tags, and wherein each tagged original DNA molecule is substantially unique with respect to other tagged original DNA molecules in the population;

[**Element 20.2**] b) amplifying the population of tagged original DNA molecules to produce a corresponding population of DNA molecule amplicons;

[**Element 20.3**] c) sequencing at least a portion of the population of DNA molecule amplicons to produce a set of sequence reads;

[**Element 20.4**] d) grouping the sequence reads into families based on i) the molecular tag and ii) sequence information derived from the circulating DNA molecule, whereby each of the families comprises sequence reads amplified from the same tagged original DNA molecule; and

[**Element 20.5**] e) collapsing sequence reads in each family to provide an error-corrected consensus sequence read for each family corresponding to one or more at the genetic loci.

21. The method of claim 20, further comprising selectively enriching regions from the subject's genome or transcriptome prior to sequencing.

22. The method of claim 20, selectively enriching at least one of tagged original DNA molecules and DNA molecule amplicons for a subset of tagged polynucleotides that map to one or more genetic loci in a reference sequence.

23. The method of claim 20, further comprising detecting, at one or more loci, at least one single nucleotide variant or at least one copy number variant.

24. The method of claim 20, wherein at least a portion of the circulating DNA molecules are nucleic acid-based blood biomarkers.

25. The method of claim 20, wherein at least a portion of the circulating DNA molecules are derived from neoplastic cells.

26. The method of claim 20, wherein the set of molecular tags comprises about 2 to about 256 distinct molecular tags.

27. The method of claim 20, wherein the set of molecular tags comprises about 256 to about 4,096 distinct molecular tags.

Guardant Health, Inc., ("Petitioner") requests *inter partes* review of U.S. Patent No. 10,689,699 to Salk et al. ("the '699 patent," EX1001) which is currently assigned to The University of Washington ("Patent Owner"). This petition demonstrates by a preponderance of the evidence, that claims 1-27 of the '699 patent are unpatentable for failing to distinguish over prior art.

I. INTRODUCTION

There is a disconnect between what is discussed in the '699 specification and the claims that later issued. Analysis of both strands of a DNA duplex ("Duplex Consensus Sequencing" or "DCS") is identified in the '699 patent specification as the purported point of novelty and distinguishing feature from prior art methods, yet said feature is completely absent from both independent claims. *Compare* claims 1 and 20 *with*, Abstract ("This method uniquely capitalizes on the redundant information stored in double-stranded DNA, thus overcoming technical limitations of prior methods utilizing data from only one of the two strands.").[1] Citing Kinde specifically, the '699 patent acknowledges that molecular tagging and library amplification was a method known in the art for

[1] The '699 specification discusses only cellular DNA and "unique" labeling, yet the claims recite "non-unique" labeling and "circulating DNA"—terms found nowhere in the '699 specification.

reducing sequencing errors and increasing sensitivity. EX1001, 2:15-30. The background section states, however, that it "would be desirable to…***capitalize[] on the redundant information stored in complexed double-stranded DNA***." EX1001, 2:63-3:2.[2] But the independent claims of the '699 patent do not capitalize on the redundant information stored in double-stranded DNA. By stripping the alleged inventive feature from the claims during prosecution, the '699 patent claims were expanded to encompass the very prior art (*e.g.*, Kinde) that was admitted in the specification but now indistinguishable from what is now claimed. *See, e.g.*, EX1001, 2:16-30; 27:32-34, 33:8-11.[3] Accordingly, *inter partes* review should be instituted and the challenged claims canceled.

A. The '699 Patent

The '699 patent is entitled "Methods of lowering the error rate of massively parallel DNA sequencing using duplex consensus sequencing." EX1001, cover (54). The '699 patent specification proposes correcting sequencing errors with a tag-based method referred to as "Duplex Consensus Sequencing" or "DCS." *E.g.*,

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[2] All emphases are added unless otherwise indicated.

[3] While not acknowledged in the '699 specification, Kinde also discloses analysis of both strands of a DNA duplex in the "Supporting Information" section omitted from the Patent Owner's prior disclosure. *E.g.*, EX1039, S3 (left column).

EX1001, Abstract, 3:52-4:37. The ’699 patent exclusively applies its DCS method to analyzing cellular DNA molecules. *E.g.*, EX1001, 20:1-3, 25:14-15, 26:49-50, 28:23-25; *see also* EX1002, ¶21.

The ’699 patent’s DCS method makes use of “Single Molecule Identifiers” or “SMIs” which are used to identify original sample molecules. DCS generally includes assigning SMI sequences to sample molecules, amplifying the tagged molecules using PCR, and sequencing the amplification progeny. The resulting sequence reads are then grouped into families based on a common SMI sequence. EX1001, 3:17-39, Fig. 1, 3:52-4:3, 19:20-25. Following grouping, a “consensus sequence” is built from the grouped sequence reads and mutations are distinguished from sequencing errors. EX1001, 3:33-39, Fig. 3, 4:12-17; *see also* EX1002, ¶¶22, 23.

The ’699 specification describes using a sufficient diversity of “SMI tags” to promote labeling different DNA fragments in the sample with a different SMI tag—an aspect of the DCS method that the ’699 patent repeatedly describes as “unique” tagging or labeling. EX1001, 19:20-25 (“the use of SMI tags…allows every molecule to be ***uniquely*** labeled”); *see also id.*, 6:67-7:4, 16:11-15, 22:44-46, 23:2-3, 23:8-10, 28:51-55, 27:45-47; EX1002, ¶24.

The ’699 patent recognizes that unique molecular tagging was known in the art at the time. *See, e.g.*, EX1001, 2:15-19 (“[T]echniques whereby DNA

fragments to be sequenced are each uniquely tagged [34, 35] prior to amplification [36-41] have been reported."). The '699 patent attributes the purported novelty in the DCS method to analysis of both of the complementary strands of a DNA duplex. EX1001, 2:63-3:2, 17:62-18:8 ("The DCS approach overcomes the limitation of previous approaches by considering both DNA strands."); EX1002, ¶¶25, 26.

The '699 patent has 27 claims of which claims 1 and 20 are independent. Neither independent claim requires practicing the DCS method. These claims instead are directed to tagging DNA molecules such that a plurality have identical tags. Taking claim 20 as representative, it recites:

> 20. A method, comprising:
>
> a) attaching a set of molecular tags to a population of circulating DNA molecules obtained from a bodily sample of a subject to produce a population of tagged original DNA molecules, wherein a plurality of the tagged original DNA molecules has identical molecular tags, and wherein each tagged original DNA molecule is substantially unique with respect to other tagged original DNA molecules in the population;
>
> b) amplifying the population of tagged original DNA molecules to produce a corresponding population of DNA molecule amplicons;
>
> c) sequencing at least a portion of the population of DNA molecule amplicons to produce a set of sequence reads;

d) grouping the sequence reads into families based on i) the molecular tag and ii) sequence information derived from the circulating DNA molecule, whereby each of the families comprises sequence reads amplified from the same tagged original DNA molecule; and

e) collapsing sequence reads in each family to provide an error-corrected consensus sequence read for each family corresponding to one or more genetic loci.

Claim 1 is similar, but more generally recites "converting" the parent DNA molecules rather than "attaching," as required by claim 20. Claim 1 also recites the term "non-uniquely tagged" to encompass "wherein a plurality of the tagged original DNA molecules has identical molecular tags" as recited in claim 20. Claim 3 depends from claim 1 and recites that the "converting" includes PCR, in addition to attachment of molecular tags via ligation (e.g., sticky end ligation).

B. Prosecution History

The '699 patent was filed as U.S. Application No. 16/411,066 ("the '066 application," EX1006) on May 13, 2019, claiming priority to U.S. Provisional Patent Application Nos. 61/613,413, 61/625,623, and 61/625,319, filed Mar. 20, 2012; Apr. 17, 2012; and Apr. 17, 2012, respectively, via U.S. Application Nos. 15/660,785 and 14/386,800 (U.S. national stage of PCT/US2013/032665).

The issued claims of the ’699 patent bear no resemblance to those that were filed in the ’066 application. EX1006, 20; 112-119, 221-226. The claims that were prosecuted and later issued as claims 1-27 of the ’699 patent were substantially copied from U.S. Pat. No. 9,834,822 to Talasaz (EX1028), yet the claims lack the requisite supporting disclosure in any priority document. Petitioner demonstrates in a companion case (IPR2022-00449) that the ’699 patent is not entitled to a priority date earlier than the filing date of its underlying application of May 13, 2019. In this case, Petitioner demonstrates unpatentability of the challenged claims regardless of entitlement to the earliest claimed priority date (which is not conceded). *See* Section I.D.

C. State of the Art

This petition is supported by the Declaration of Dr. John Quackenbush, the Henry Picking Walcott Professor of Computational Biology and Bioinformatics and Chair of the Department of Biostatistics at Harvard University. EX1002; EX1003 (Dr. Quackenbush’s *curriculum vitae,* providing a summary of his education, training, and experience). As Dr. Quackenbush explains, molecular barcoding or tagging methods for consensus sequencing were known prior to the earliest claimed filing date of the ’699 patent. EX1002, ¶27. As discussed herein, the ’699 patent directly acknowledges that these techniques were known in the art.

Dr. Quackenbush explains that the scientific literature by March 2012 described molecular barcoding or tagging techniques for consensus sequencing and their use to improve the sensitivity of next-generation (also called "massively-parallel") sequencing. These techniques, while referred to in the art as "unique tagging," do not result in a different tag on literally every molecule of the sample. The Miner (reference 34, EX1037) and Shiroguchi (reference 40, EX1025) references, for example, are identified in the '699 patent as unique tagging techniques. EX1001, 2:16-19 ("[T]echniques whereby DNA fragments to be sequenced are each uniquely tagged [34, 35] prior to amplification [36-41] have been reported."). But both references describe their methods as producing some amount of identically tagged DNA fragments. Miner, for example, expressly describes unique tagging that includes a plurality of duplicate or identical exogenous tags in the population of tagged molecules. *E.g.*, EX1037, 2 ("in selecting 15 cloned PCR products from one DNA sample, the probability that two of these will be different genomic fragments labeled with identical 7 nt barcodes is 0.047[.]"). Shiroguchi similarly describes a molecular tagging technique where, on an individual transcript level, about 5% of the tagged population bears duplicate or identical tags. EX1025, 1349 ("…the required N_{eff} is ~100–400 for 95% unique labeling of all molecules (18)."), 1350-1351 ("… one could uniquely label nearly

every identical molecule in this system (with 95% unique labeling for even the most abundant transcript)."); *see also* EX1002, ¶28.

Dr. Quackenbush explains that consensus-based methods for producing error-corrected sequences also were known in the art. The '699 patent explains that molecular tagging techniques, as described above, had been used in conjunction with consensus sequencing methods. EX1001, 2:22-26 ("This approach has been used to improve counting accuracy of DNA [38, 39, 41] and RNA templates [37, 38, 40] and to correct base errors arising during PCR or sequencing [36, 37, 39]."). The Kinde reference (reference 36, EX1039) relies on molecular barcodes and, as the '699 patent confirms, discloses correcting errors in next-generation sequencing ("NGS") reads. *Id.*, 2:26-30; EX1039, 9531 ("Thus, Safe-SeqS decreased the presumptive sequencing errors by at least 70-fold."). Kinde cited Miner and another reference (Craig, EX1036) as examples supporting the performance of Kinde's Safe-SeqS approach with exogenous molecular barcodes. EX1039, 9530, 9535. Craig employed indexed DNA barcodes ligated to fragmented DNA. EX1036, Abstract. Craig employed TA ligation, a form of sticky-end ligation, to attach the molecular barcodes to the parent DNA molecules. EX1036, 887 (right column), 888 (left column); *see also* EX1002, ¶29.

D. Cited Prior art

Claims 1-27 of the ’699 patent are so broad that they encompass prior art methods, even assuming they are entitled to the earliest claimed priority date of March 20, 2012. Accordingly, as described below, Kinde, Miner, and Fan are prior art. *See also* EX1002, ¶30.

i. Kinde

Kinde et al. published “Detection and quantification of rare mutations with massively parallel sequencing” (“Kinde,” EX1039) in volume 108 of the *Proceedings of the National Academy of Sciences* on June 7, 2011. EX1039, 9530; *see also id*. (“This article contains supporting information online”). Accordingly, Kinde is prior art at least under 35 U.S.C. §102(a)(1) and pre-AIA 35 U.S.C. §102(a). *See also* EX1002, ¶31.

Kinde discloses “an approach that can substantially increase the sensitivity of massively parallel sequencing” to allow “confident identification of rare variants” that are “present in a small fraction of DNA templates.” EX1039, Abstract. Kinde refers to their approach to improving massively parallel sequencing as the “Safe-Sequencing System,” or “Safe-SeqS” for short. Kinde discloses that the keys to the Safe-SeqS approach are (i) assignment of a unique identifier (UID) to each template molecule; (ii) amplification of each uniquely tagged template molecule to create UID families; and (iii) redundant sequencing of

amplification products. EX1039, Abstract. Kinde discloses that mutations "not occurring in the original templates, such as those occurring during the amplification steps or through errors in base calling," are identified by labeling DNA molecules followed by amplification and using those labels to identify daughter molecules (amplification products) as part of a single family originating from the parent. EX1039, 9530 (Results). In other words, only genetic variations found in the vast majority (*e.g.*, 95%) of a given UID family represent the consensus sequence for the original nucleotide molecule. Using these tools, Kinde showed "how templates can be prepared and the sequencing data obtained from them more reliably interpreted, so that relatively rare mutations can be identified with commercially available instruments." EX1039, 9530 (right column); *see also* EX1002, ¶¶32-34.

Kinde discloses that "UIDs, sometimes called barcodes or indexes, can be assigned to nucleic acid fragments using a variety of methods," including "the introduction of exogenous sequences through PCR (40, 41) or ligation (42, 43)." EX1039, 9531 (left column) (citing EX1036, EX1037 for ligation). Kinde also discloses that "randomly sheared genomic DNA inherently contains UIDs consisting of the sequences of the two ends of each sheared fragment" and that "[p]aired-end sequencing of these fragments yields UID families that can be analyzed as described above." *Id.* Kinde provides examples of the Safe-SeqS

approach using exogenous and/or endogenous UIDs to determine the consensus sequence for the original parent molecules. *E.g.*, EX1039, 9531-9532, Figs. 1-3, Tables 1-2, 9535, S1. Kinde thereby reduced false positive mutations by 15- to 70-fold as compared to conventional analysis. *Id.* Kinde discuss these experiments in depth in the Supporting Information. EX1039, S1-S10; *see also* EX1002, ¶¶35-37.

ii. Miner

Miner et al. published "Molecular barcodes detect redundancy and contamination in hairpin-bisulfite PCR" ("Miner," EX1037) in volume 32 of *Nucleic Acids Research* in 2004. Accordingly, Miner is prior art at least under 35 U.S.C. §102(a)(1) and pre-AIA 35 U.S.C. §102(b). *See also* EX1002, ¶38.

Miner is cited as reference 43 in Kinde as providing a method for introducing exogenous UIDs using ligation for use in Kinde's Safe-SeqS approach. EX1039, 9531 (left column), 9335 (left column). The '699 patent subsequently cited Miner as a prior art example of a unique tagging techniques. EX1001, 2:16-19; *see also* EX1002, ¶¶39, 40.

Miner used "molecular barcoding to label each genomic DNA template with an individual sequence tag prior to PCR amplification." EX1037, Abstract. Miner used T4 ligase for sticky end ligation of the hairpin linker to the DNA. EX1037, 1-2 (Materials and Methods) & Fig. 1. Miner describes its tagging methods as producing some amount of identically tagged DNA fragments. For example, Miner

expressly describes its tagging approach as including a plurality of duplicate or identical exogenous tags in a population of tagged molecules. *E.g.*, EX1037, 2 ("in selecting 15 cloned PCR products from one DNA sample, the probability that two of these will be different genomic fragments labeled with identical 7 nt barcodes is 0.047[.]"); *see also* EX1025, 1349; Section I.C.; EX1002, ¶40.

iii. Fan

Fan et al. published "Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood" ("Fan," EX1021) in volume 105 of *Proceedings of the National Academy of Sciences* in 2008. Accordingly, Fan is prior art at least under 35 U.S.C. §102(a)(1) and pre-AIA 35 U.S.C. §102(b). *See also* EX1002, ¶41.

Fan is cited as reference 8 in Kinde as an existing application of massively-parallel sequencing (EX1039, 9531 (left column), 9335 (left column)), the sensitivity of which massively-parallel sequencing Kinde taught should be improved using the Safe-SeqS approach. The '699 patent similarly (and subsequently) cited Fan as an application of massively-parallel sequencing. EX1001, 1:30-46, 31:33-36; *see also* EX1002, ¶¶42, 43.

Fan discloses obtaining cell-free DNA ("cfDNA") from the plasma of pregnant women and sequencing it using high-throughput shotgun sequencing technology. EX1021, Abstract, 16266, 16270; *see also* EX1002, ¶44.

E. Level of Skill in the Art

As Dr. Quackenbush explains, a skilled artisan or person of ordinary skill in the art by March 2012 would typically have an advanced degree, such as a Ph.D., with research experience in genomics, molecular biology, bioinformatics, or a related field, or could have less education but significant professional experience in one or more of these fields. *See also* EX1002, ¶¶45-46; Section I.C. & D.

II. GROUNDS FOR STANDING

Petitioner certifies that, under 37 C.F.R. §42.104(a), the ’699 patent is available for *inter partes* review, and Petitioner is not barred or estopped from requesting *inter partes* review of the ’699 patent on the grounds identified.

III. MANDATORY NOTICES UNDER 37 C.F.R. §42.8

Real Party-in-Interest (37 C.F.R. §42.8(b)(1)): Guardant Health, Inc. is the real party-in-interest.

Related Matters (37 C.F.R. §42.8(b)(2)): Patent Owner has asserted the ’699 patent against Petitioner in the United States District Court in Delaware (1:21-cv-01126-LPS).

Lead and Back-Up Counsel (37 C.F.R. §42.8(b)(3))

Lead Counsel: Michael T. Rosato (Reg. No. 52,182)

Back-Up Counsel: Jad A Mills (Reg. No. 63,344); Sonja R. Gerrard (Reg. No. 72,802)

Service Information – 37 C.F.R. §42.8(b)(4). Petitioner hereby consents to electronic service.

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IV. STATEMENT OF THE PRECISE RELIEF REQUESTED FOR EACH CLAIM CHALLENGED

Petitioners request review of claims 1-27 of the '699 patent under 35 U.S.C. §311 and AIA §6. The grounds for relief are as follows:

Ground	Claims	Description
1	1-27	Unpatentable under 35 U.S.C. §103 as obvious over Kinde in view of Miner
2	1-27	Unpatentable under 35 U.S.C. §103 as obvious over Kinde and Miner in further view of Fan.

V. CLAIM CONSTRUCTION

No terms need be construed to find the claims unpatentable in view of the prior art. *Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co.*, 868 F.3d 1013, 1017 (Fed. Cir. 2017). In mapping the prior art to the claims, Petitioner has applied the "the meaning that [a] term would have to a person of ordinary skill in

the art in question at the time of the invention." *Phillips v. AWH Corp.*, 415 F.3d 1303, 1313 (Fed. Cir. 2005) (en banc).

While no construction is necessary, the discussion below provides context for understanding the scope of the term "circulating DNA molecules."

The term "circulating DNA molecules" is not used in the specification of the '699 patent. Instead, the '699 focuses its DCS method on analyzing cellular DNA molecules. *E.g.*, EX1001, 20:1-3, 25:14-15, 26:49-50, 28:23-25; *see also* EX1002, ¶47.

Statements made by Patent Owner during prosecution of a related case suggest the term "circulating DNA molecules" includes cfDNA obtained from the blood, such as the DNA obtained from blood plasma, which is DNA that was circulating in the blood stream without being encased within a cell. Specifically, Patent Owner argued that cfDNA and circulating microRNA are species of the related, though broader, term "circulating nucleic acid molecules." EX1005, 183-184 (citing Schwarzenbach (EX1020)). As Dr. Quackenbush explains, a POSA would understand from these statements that cfDNA obtained from blood is included within the meaning of the term "circulating DNA molecules." A POSA also would understand that the plain meaning of serum is plasma without the clotting factors or, in other words, blood with all cells and clotting factors removed. A POSA further would understand that cfDNA is found in blood plasma

and serum after removal of the cells (and also removal of clotting factors in the case of serum). EX1002, ¶48.

As explained by Dr. Quackenbush, a POSA would *not* understand "circulating DNA molecules" to include DNA extracted *ex vivo* from intact circulating cells (*e.g.*, blood cells, tumor cells). This is corroborated, for example, by the Schwarzenbach reference (cited in the '699 specification and again by Patent Owner during prosecution), which distinguishes between cell-free nucleic acid and nucleic acid obtained from intact cells. Schwarzenbach uses the term circulating cfNA (*i.e.*, cell-free nucleic acid) to refer exclusively to cell-free nucleic acids in the bloodstream. EX1020, 1-2 ("Biology of cfNA"); *see also* EX1023, 10515-16 (explaining that circulating microRNAs are not cell-associated). When discussing tumor cells that might also be present in the bloodstream, Schwarzenbach uses the different term: circulating tumor cells or "CTCs." EX1020, 8-9; EX1002, ¶49.

VI. DETAILED EXPLANATION FOR GROUNDS OF UNPATENTABILITY

A. [Ground 1] Claims 1-27 are unpatentable as obvious under 35 U.S.C. §103 over Kinde in view of Miner

Claims 1-27 of the '699 patent relate to using tagged parent polynucleotides that, as tagged, are substantially unique with respect to one another, to determine a consensus sequence for a family of amplification products generated from a given parent molecule. EX1001, claims. Claims 1 and 20 are independent, and involve:

- Attaching molecular tags to a population of circulating DNA molecules, or otherwise converting the circulating DNA molecules into tagged polynucleotides, the number of unique tags incorporated being smaller than the number of original parent DNA molecules in the population but sufficient to make the tagged molecules substantially unique in the population;
- Amplifying the tagged polynucleotides;
- Sequencing at least some of the amplified progeny;
- Grouping the sequenced reads that were amplified from the same tagged original parent molecules into families based on the tag and a sequence or position derived from the circulating DNA molecules; and
- Collapsing sequence reads in each family to yield a base call or provide an error-corrected consensus sequence for each family corresponding to one or more genetic loci.

Kinde discloses the same workflow discussed and claimed in the ’699 patent (e.g., attaching molecular tags, amplifying tagged polynucleotides, sequencing, grouping the sequence reads, and consensus sequence analysis). Kinde’s “Safe-SeqS” is a method for detecting and reducing sequencing errors in massively parallel sequencing applications. EX1039, Abstract, Title. Safe-SeqS uses

molecular barcodes called UIDs—comprising endogenous and/or exogenous nucleotide sequences—to identify the amplification progeny of original parent DNA molecule. *E.g.*, EX1039, 9531-9532, S1. Just like the '699 patent, DNA molecules in Kinde are provided with one or more UID, followed by amplification, sequencing, and grouping reads into UID families. A mutation at a given position as compared to a reference sequence is confidently identified when it represents the consensus base call (*i.e.*, the base present in 95% of reads within a family). EX1039, 9530. The '699 patent even acknowledges that consensus sequencing based on molecular barcoding or tagging was known, and further acknowledges the similarity of DCS to the methods of Kinde. EX1001, 2:16-30; 27:32-43, 33:8-11; *see also* EX1002, ¶50.

As discussed in detail herein, claims 1-27 are obvious over the prior art at the time, as illustrated by Kinde in view of Miner. *See also* EX1002, ¶¶51-141.

Kinde, particularly in view of the state of the art and admissions in the '699 patent, discloses all aspects of the challenged claims. It is well known that "anticipation is the epitome of obviousness." *In re McDaniel*, 293 F.3d 1379, 1385 (Fed. Cir. 2002). Moreover, a POSA would have had good reason to arrive at the claimed subject matter as a whole with a reasonable expectation of success despite any conceivable differences between the embodiments disclosed in Kinde and the claims of the '699 patent, particularly in view of prior art at the time as illustrated

by Miner. First, Kinde exemplifies its methodology on cellular DNA and discloses circulating DNA. Kinde provides at least as much disclosure regarding tagging circulating DNA as the priority documents for the ’699 patent. Second, Kinde acknowledges its tagging scheme generates a plurality of parent molecules having identical tags, but does not specifically disclose the rate of identical tags in its particular examples. Scientific literature at the time (cited in both the ’699 and Kinde), such as Miner, provides additional detail regarding the identical tagging that occurs in unique tagging approaches, thereby confirming Kinde’s disclosure. *See* Section I.D. Third, Kinde exemplifies adding exogenous barcodes to parent DNA molecules using PCR but also expressly teaches using ligation to do so, and specifically cites Miner to support this disclosure. Each of these subjects is discussed in further detail below. *See also* EX1002, ¶52.

Patent Owner cannot distinguish Kinde from the claims of the ’699 patent based on the application of molecular tagging to circulating DNA. Like the ’699 patent, Kinde exemplifies its tagging methodology exclusively with cellular DNA but Kinde references clinical applications which use circulating DNA. EX1039, 9531 (left column); EX1001, 1:45-48. In fact, as discussed in detail below, Kinde provides more disclosure supporting applying Safe-SeqS to circulating DNA (*e.g.*, DNA in plasma) than the ’699 patent does. To the extent the ’699 patent (or any of its priority documents) has any descriptive support for application of DCS to

circulating DNA, Kinde's earlier disclosure provides just as much, and more, description of circulating DNA so as to render the '699 patent claims obvious. *See Lockwood v. Am. Airlines, Inc.*, 107 F.3d 1565, 1572 (Fed. Cir. 1997) (written description requires possession and does not extend to undisclosed modifications); *Research Corp. Techs. v. Microsoft Corp.*, 627 F.3d 859, 870 (Fed. Cir. 2010) (obviousness requires a lower threshold than written description); *see also* EX1002, ¶53.

Nor can Patent Owner properly distinguish Kinde from the claims based on its tagging scheme. As discussed above in Section I.A, the focus of the '699 patent is unique tagging. To the extent the '699 patent discloses the tagging recited in Element 20.1 and 1.2, Kinde also discloses these elements. Kinde discloses that a known outcome of using its UIDs is that "two different original templates acquire the same UID[.]" EX1039, S1. Kinde explains that using a number of distinct UIDs that "greatly exceeds the number of original template molecules" will "minimize the probability that two different original templates acquire the same UID," but does not state that identical tagging of different original template DNA molecules is eliminated. *Id*. Instead, Kinde indicates that while duplicate tagging might be minimized, it occurs nonetheless. Moreover, Kinde cites Miner (reference 43) as an example of how to introduce "exogenous sequences through…ligation (42, 43)" for purposes of providing DNA molecules with UIDs. EX1039, 9531. As

discussed above, Miner reports a tagging scheme that includes a plurality of duplicate or identical exogenous tags in the population of tagged molecules. *E.g.*, EX1037, 2. The ’699 patent itself identifies Miner as exemplary prior art by which “DNA fragments to be sequenced are each uniquely tagged.” EX1001, 2:16-19; *see also* Section I.C.; EX1002, ¶54.

There is no meaningful distinction between the tagging methods disclosed by the ’699 patent and Kinde. For instance, Example 3 of the ’699 patent states plainly that it is using an endogenous tagging approach that had been previously described in Kinde. The ’699 patent explains that “DNA was randomly sheared,” ligated to Illumina sequencing adaptors using “standard library preparation methods,” and the “first 10 nucleotides of each sequencing read pair, corresponding to the randomly sheared DNA ends, were combined…to yield an SMI tag[.]” EX1001, 26:39-66. Reads were grouped into families according to these endogenous sequences and reaching consensus for the sequence required agreement among “at least 90% of family members.” *Id.* The ’699 patent expressly draws a comparison to the same approach reported in Kinde. EX1001, 27:4-43. If any distinction can be drawn between Kinde and the ’699 patent, it would be that the ’699 patent goes even further than Kinde in emphasizing the need for every template molecule to contain “a unique” tag. *E.g.*, EX1001, 3:55-61, 6:67-7:4, 19:20-25. The ’699 patent never mentions “non-unique tagging” or even the

presence of different parent polynucleotides with identical tags. Kinde, in contrast, acknowledges such duplicate tagging occurs in the context of this method, which is further confirmed by contemporaneous scientific literature, including Miner and Shiroguchi. *See* Section I.C.; *see also* EX1002, ¶55.

To the extent Patent Owner points to the so-called "hybrid" approach, that too is indistinguishable from what was previously disclosed in Kinde. *Compare* EX1001, 9:30-42, 27:53-61 *with*, EX1039, 9532 (left column), S1 (left column). Like Kinde did before it (EX1039, 9532 (left column)), the '699 patent acknowledges that when using shear points alone as SMI tags, "the limited number of shear points flanking any given nucleotide positions…limits the number of unique molecules that can be sequenced in a single experiment." EX1001, 27:53-61. As Kinde also did before it, the '699 patent proposes "[t]he use of shear points as SMIs" in conjunction with an exogenous SMI tag sequence. EX1001, 27:53-61; EX1039, S1 ("resulting DNA fragments contained UIDs composed of three sequences: 2 endogenous ones…plus the exogenous sequence"); *see also id.*, S1 ("12-exogenous sequences…increased the number of distinct UIDs by 12-fold over that obtained without exogenous UIDs."), 9531 & Table 1 (1,057 parent DNA molecules). Accordingly, the prior art at the time clearly discloses a molecular barcoding approach to consensus sequencing of DNA within the scope of both claims 20 and 1. *See also* EX1002, ¶56.

Regarding claim 20's requirement of "attaching" the tags to the original DNA molecules, Kinde expressly suggests using ligation to attach exogenous molecular barcodes to DNA molecules. EX1039, 9531 (left column). Kinde specifically identifies Craig and Miner, which disclose using known sticky-end ligation and TA-ligation approaches to attach exogenous molecular barcodes to polynucleotides, as supporting the ability to use ligation to attach the exogenous barcodes in the Safe-SeqS approach. *Id.* (citing EX1036; EX1037). In view of the foregoing discussion, a POSA would have been motivated to arrive at the claimed subject matter as a whole with a reasonable expectation of success. Accordingly, the challenged claims are rendered obvious in view of the prior art at the time, as illustrated by Kinde and Miner in view of the level of ordinary skill in the art. *See also* EX1002, ¶57.

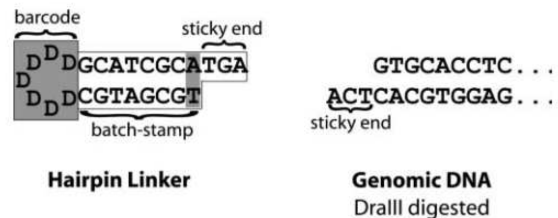
An element-by-element discussion of these claims, together with a discussion illustrating exemplary prior art disclosure and how each and every aspect of the challenged claims is found in the prior art, is provided below. *See also* EX1002, ¶¶58-141.

i. Claim 20

Preamble and Element 20.1

'699 patent	**Kinde**
"A method, comprising: a) attaching a set of molecular tags to a population of circulating DNA molecules obtained	"The keys to this approach…are (i) ***assignment of a unique identifier***

from a bodily sample of a subject to produce a population of tagged original DNA molecules, wherein a plurality of the tagged original DNA molecules has identical molecular tags, and wherein each tagged original DNA molecule is substantially unique with respect to other tagged original DNA molecules in the population”	***(UID) to each template molecule***….” EX1039, Abstract. “UIDs, sometimes called ***barcodes or indexes***, can be assigned to nucleic acid fragments using a variety of methods. These methods include the ***introduction of exogenous sequences through*** PCR (40, 41) or ***ligation (42, 43***). Even more simply, randomly sheared genomic DNA inherently contains ***UIDs consisting of the sequences of the two ends of each sheared fragment*** (Fig. 2 and Fig. S1).” EX1039, 9531 & Figs. 1-2. *see also id.*, 9535 (reference 43 is Miner (EX1037)); *see also id.*, 9532, Fig. 3 & Caption, 9635, S1 (describing using PCR to tag each strand of each template molecule with an exogenous UID). “In ***neoplastic diseases***,…the applications of ***rare mutant detection*** are manifold; they can be used to …follow the course of therapy when ***assessed in plasma***, and to identify patients with early, surgically curable disease when evaluated in…***plasma***, and other bodily fluids (9-11).” EX1039, 9530 (left column). “It is important that the number of distinct UIDs greatly exceeds the number of original template molecules to minimize the probability that ***two different original templates acquire the same UID***.” EX1039, S1; *see also id.*, 9531 & Table 1, S1.

	"[I]n selecting 15 cloned PCR products from one DNA sample, the probability that two of these will be ***different genomic fragments labeled with identical 7 nt barcodes*** is 0.047[.]" EX1037, 2. "***Ligation*** of the hairpin linker…to DraIII-cleaved genomic DNA…." EX1037, 1-2 & Fig. 1:  **Figure 1.** Schematic of barcoded and batch-stamped hairpin linker, designed for ligation to DraIII-cut genomic DNA of *FMR1*. The letter D represents a nucleotide randomly selected from A, G and T. *See also* EX1039, 9535 (left column); EX1002, ¶¶59-71.

Kinde teaches methods comprising "attaching a set of molecular tags to a population of DNA molecules obtained from a bodily sample of a subject to produce a population of tagged original DNA molecules." Kinde discloses using molecular barcodes—UIDs—comprising endogenous and/or exogenous sequences to uniquely identify original parent DNA molecules with their amplification progeny. *E.g.*, EX1039, 9531-9532 & Figs. 1-3, 9535, S1. Kinde uses PCR to add exogenous UIDs prior to library amplification and sequencing. *E.g.*, EX1039, 9531-9532, 9535, S1. Kinde applies the Safe-SeqS approach to either sheared or unsheared double-stranded DNA fragments obtained from a bodily sample in the

forms of human pancreas, lymphoblastoid cells, colonic mucosae, or blood lymphocytes. EX1039, 9531 (left column), 9532 (left column & Fig. 3 caption), S1 (left column). *See also* EX1002, ¶60.

In addition to disclosing the introduction of exogenous UIDs through PCR, Kinde also expressly suggests the use of ligation to attach exogenous UIDs to original DNA molecules, and specifically cites Craig (EX1036), and Miner (EX1037) for examples of how to do so. EX1039, 9531; *see also id.*, 9535. Craig and Miner each employ ligation to attach molecular barcode tags to DNA molecules. EX1037, 1-2 & Fig. 1 (sticky-end ligation); EX1036, 887 (TA ligation). As explained by Dr. Quackenbush, Kinde teaches or suggests using ligation to attach the set of molecular tags to the population of DNA molecules obtained from a bodily sample of a subject to produce a population of tagged original DNA molecules. *See also* EX1002, ¶61.

As discussed above, Patent Owner cannot distinguish Kinde from the claims of the ’699 patent based on the application of molecular tagging to circulating DNA. Like the ’699 patent, Kinde exemplifies its tagging methodology exclusively with cellular DNA but Kinde references clinical applications of massively parallel sequencing as including prenatal screening. EX1039, 9531 (left column); EX1001, 1:45-48. Like the ’699 patent, Kinde cites to the Chiu and Fan references which describe providing a population of circulating DNA molecules obtained from a

bodily sample in the form of DNA in maternal plasma. EX1039, 9530 (citing references 7-8), 9535 (reference titles). Kinde explains that massively parallel sequencing technology was useful for detecting rare mutants in blood plasma of individuals with malignant neoplastic disease (*i.e.*, cancer). EX1039, 9530 (left column). As explained above, a POSA would understand the term "circulating DNA molecules" to include cfDNA molecules circulating in the bloodstream, such as DNA obtained from a maternal plasma sample or tumor (neoplastic) DNA obtained from blood plasma. *See* Section V; *see also* EX1002, ¶¶62, 63.

Moreover, Kinde teaches that the Safe-SeqS approach is broadly applicable to improve the sensitivity of massively parallel sequencing and permits confident identification of rare variants appearing even at very low frequency. EX1039, Abstract. Kinde discloses the average size of the fragmented DNA was ~200 bp, but also expressly suggests applying Safe-SeqS to smaller fragments averaging 150 bp (range 125-175 bp). EX1039, 9535 (left column), 9532 (left column). Kinde concludes that Safe-SeqS can substantially improve the accuracy of massively parallel sequencing and "can be applied to virtually ***any sample preparation workflow*** or sequencing platform" and can "identify rare mutants in a population of DNA templates." EX1038, 9533 (Discussion); *see also* EX1002, ¶64.

To the extent the '699 patent demonstrates possession of applying tagging to circulating DNA, Kinde's earlier disclosure rendered obvious applying Safe-SeqS

to circulating DNA with a reasonable expectation of success before the earliest claimed priority date of the ’699 patent. *See Lockwood*, 107 F.3d at 1572; *Research Corp. Techs.*, 627 F.3d at 870.

Kinde teaches or suggests that “a plurality of the tagged original DNA molecules has identical molecular tags, and wherein each tagged original DNA molecule is substantially unique with respect to other tagged original DNA molecules in the population.” As discussed above, Kinde discloses that a known outcome of using UIDs is that “two different original templates acquire the same UID.” EX1039, S1 (right column). Kinde explains that using a number of distinct UIDs that “greatly exceeds the number of original template molecules” will “minimize the probability that two different original templates acquire the same UID,” but does not state that the presence of a plurality of the tagged original DNA molecules having identical UIDs is ever eliminated. Kinde instead indicates that one component of its method is that duplicate tagging occurs despite efforts to minimize it. *See also* EX1002, ¶65.

Moreover, Kinde specifically cites a reference that quantified the prevalence of duplicate tags. Kinde cites Miner (reference 43) as an example of how to introduce “exogenous sequences through…ligation (42, 43).” EX1039, 9531. As discussed above, Miner reports a tagging scheme that includes a plurality of duplicate or identical exogenous tags in the population of tagged molecules. *E.g.*,

EX1037, 2. Kinde also exemplified using fewer tags than the total number of tagged parent or original DNA molecules. In one example, Kinde used twelve exogenous tags for 1,057 DNA molecules. EX1039, 9531 & Table 1, S1. As explained by Dr. Quackenbush, a POSA reading the disclosure of Kinde would understand Kinde's discussion of duplicate tags as acknowledgement of employing a tagging scheme that includes a plurality of the tagged DNA molecules having identical molecular tags, as claimed. *See also* Section I.C. Kinde in view of Miner thus teaches or suggests a plurality of the tagged original DNA molecules having identical molecular tags. *See also* EX1002, ¶66.

Kinde teaches or suggests that the tagged original DNA molecules are substantially unique with respect to the other tagged original DNA molecules in the population. The '699 patent discloses that whether a molecule is "unique" is based on its sequence and that its DCS method distinguishes molecules (*e.g.*, family grouping) based on sequence identity (*i.e.*, SMI). *E.g.*, EX1001, 19:23-25, 22:41-44. While the specification does not recite the term "substantially unique" it presumably encompasses tagged DNA molecules that are sufficiently

distinguishable from other tagged DNA molecules in the population based on sequence.[4] *See also* EX1002, ¶¶67, 68.

As discussed below for Element 20.4, and just like the ’699 patent, Kinde discloses distinguishing tagged molecules based on sequence, including grouping of reads into “UID families.” EX1039, 9531-9532 & Tables 1-2, S1. The reads were grouped into families on based sharing a common UID. EX1039, 9535, S2. Kinde reports that the Safe-SeqS approach reduced the number of false positive mutations by 15-70-fold. EX1039, 9531, 9533 & Fig. 4, Tables 1-2. Kinde reported that Safe-SeqS, thus, “can substantially improve the accuracy of massively parallel sequencing (Tables 1 and 2).” EX1039, 9533. Kinde also suggests that the tagged DNA molecules are sufficiently distinguishable to an extent that even complementary strands from the same double-stranded DNA molecule can be separately identified and used to confirm the accuracy of consensus sequencing. *See, e.g.*, EX1039, S2-S3 (requiring at least two supermutants, one from each strand); *id.*, 9531 (describing ligation of “standard Illumina sequencing adapters to the ends of sheared DNA fragments to produce a standard sequencing library….[A] library prepared as described above”), Fig. 2

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[4] Two different tagged DNA molecules, even bearing identical SMIs, have different sequences by virtue of the molecules themselves. EX1002, ¶¶69.

caption ("One uniquely identifiable fragment is produced from each strand of the double-stranded template."). As Dr. Quackenbush explains, Kinde in view of Miner thus teaches or suggests a plurality of the tagged original DNA molecules has identical molecular tags, and wherein each tagged original DNA molecule is substantially unique with respect to other tagged original DNA molecules in the population. *See also* EX1002, ¶¶70, 71.

Element 20.2

'699 patent	**Kinde**
"b) amplifying the population of tagged original DNA molecules to produce a corresponding population of DNA molecule amplicons;"	"The keys to this approach…are… (ii) ***amplification*** of each uniquely tagged template molecule to create UID families" EX1039, Abstract; *see also id.*, 9531-9532 & Figs. 1-3 & Captions, S1 (left column). *See also* EX1002, ¶72, 73.

Kinde discloses amplifying the population of tagged original DNA molecules to produce a corresponding population of DNA molecule amplicons. EX1039, Abstract, 9531-9532, Figs. 1-3 & Captions, S1; EX1002, ¶73.

Element 20.3

'699 patent	**Kinde**
"c) sequencing at least a portion of the population of DNA molecule amplicons to produce a set of sequence reads;"	"The keys to this approach…are… (iii) ***redundant sequencing of the amplification products***." EX1039, Abstract; *see also id.*, 9531-9532 & Figs. 1-3 & Captions, S2.

	"**Sequencing**. Sequencing of all the libraries described above was ***performed using an Illumina GA IIx instrument*** as specified by the manufacturer." EX1039, 9535; *see also id.*, S2 (left column). *See also* EX1002, ¶¶74, 75.

Kinde discloses sequencing at least a portion of the population of DNA molecule amplicons to produce a set of sequence reads. EX1039, Abstract, 9531-9532 & Figs. 1-3, 9535, S2; EX1002, ¶75.

Element 20.4

'699 patent	Kinde
"d) grouping the sequence reads into families based on i) the molecular tag and ii) sequence information derived from the circulating DNA molecule, whereby each of the families comprises sequence reads amplified from the same tagged original DNA molecule; and"	"High quality reads were ***grouped in UID families on the basis of their endogenous or exogenous UIDs***." EX1039, 9535, S2. "[R]andomly sheared genomic DNA inherently contains ***UIDs consisting of the sequences of the two ends of each sheared fragment*** (Fig. 2 and Fig. S1)." EX1039, 9531. "**Endogenous UIDs**…. The resulting DNA fragments contained UIDs composed of ***three sequences: 2 endogenous ones, represented by the two ends of the original sheared fragments***, plus the ***exogenous sequence introduced during the indexing amplification***." EX1039, S1 (left column); *see also id.*, 9531 (1,057 "independent molecules").

	See also EX1002, ¶¶76-78.

Kinde discloses grouping the sequence reads into families based on the molecular tag and sequence information derived from the circulating DNA molecule, whereby each of the families comprises sequence reads amplified from the same tagged original DNA molecule. EX1039, Abstract, 9531-9533, 9535 Tables 1-2, S1-S2. For example, Kinde discloses grouping the sequence reads into UID families based on endogenous sequence UID tags. EX1039, 9531 & Table 1, 9535. Kinde also discloses grouping the sequence reads into UID families based on the exogenous tag and based on two endogenous sequences represented by the two ends ***of the original sheared fragments***. EX1039, 9535, S1-S2. As Dr. Quackenbush explains, a POSA thus would understand Kinde to teach or suggest grouping the sequence reads into families based on the molecular tag and sequence information derived from the circulating DNA molecule.; *see also* Section I.C.; EX1002, ¶77.

Moreover, Kinde teaches or suggests that each of the UID families comprises sequence reads amplified from the same tagged original DNA molecule. For example, Kinde expressly discloses that each UID family has two endogenous sequences represented by the two ends ***of the original sheared fragments***. EX1039, 9531, 9535, S1. Moreover, Kinde discloses that "PCR fragments with the same UID are considered mutants ('supermutants') only if ≥95% of them contain

the identical mutation." EX1039, Abstract. Kinde elaborates that "[m]utations not occurring in the ***original templates***, such as those occurring during the amplification steps or through errors in base calling, should not give rise to supermutants." EX1039, 9530. As Dr. Quackenbush explains, Kinde thus teaches or suggests that each of the UID families comprises sequence reads amplified from the same tagged original DNA molecule. EX1002, ¶78.

Element 20.5

'699 patent	Kinde
"e) collapsing sequence reads in each family to provide an error-corrected consensus sequence read for each family corresponding to one or more …genetic loci."	"PCR fragments with the same UID are considered mutant ("supermutants") ***only if ≥95% of them contain the identical mutation***." EX1039, Abstract. "A UID family in which ***at least 95% of family member have the identical mutation*** is called a "supermutant". ***Mutations not occurring in the original templates***…should not give rise to supermutants." EX1039, 9530. "[A]ll mutations observed with conventional analysis likely represented false positives (Table 1). With Safe-SeqS analysis of the same data, ***no supermutants were identified at any position***." EX1039, 9531 (right column); *see also* Table 1 (Conventional analysis mutations identified: 234,352), 9531-9532 & Tables 1-2 (Safe-SeqS ***reduced the***

	apparent frequency of mutation 15- to 70-fold). *See also* EX1002, ¶¶79-82.

Kinde discloses collapsing sequence reads in each family to provide an error-corrected consensus sequence read for each family corresponding to one or more genetic loci. EX1039, Abstract, 9530-9532. The '699 patent illustrates determining a consensus sequence by assessing sequence agreement between reads within the family. *E.g.*, EX1001, 21:35-38 ("Sequencing positions were discounted if the consensus group covering that position consisted of fewer than 3 members, or if fewer than 90% of the sequences at that position in the consensus group had the identical sequence."). Kinde discloses that requiring at least 95% sequence agreement within a particular family before reaching consensus that a mutation is present at a particular locus "can substantially improve the accuracy of massively parallel sequencing (Tables 1 and 2)." EX1039, 9533. As Dr. Quackenbush explains, Kinde thus teaches or suggests collapsing sequence reads in each family to provide an error-corrected consensus sequence read for each family corresponding to one or more genetic loci. EX1002, ¶¶80, 81; *see also* Section I.C.

Accordingly, Kinde in view of Miner teaches or suggests each and every element of claim 20 of the '699 patent and renders claim 20 obvious as a whole. EX1002, 82; *see also* Section I.C.

ii. Claim 1

Preamble and Element 1.1

Kinde in view of Miner renders obvious claim 1 for the same reasons explained in detail above for claim 20. Moreover, claim 1 is obvious regardless of whether PCR or ligation is used to convert the polynucleotides into tagged polynucleotides. Further detail and discussion is provided below. *See also* EX1002, ¶83.

’699 patent	Kinde
“A method comprising: a) providing a population of circulating DNA molecules obtained from a bodily sample from a subject;”	*See* Preamble and Element 20.1 above. *See also* EX1002, ¶¶84-87.

As discussed above in Section VI.A.i for the Preamble and Element 20.1, Kinde suggests providing a population of circulating DNA molecules obtained from a bodily sample from a subject in the form of DNA obtained from plasma. EX1039, 9530 (left column). As Dr. Quackenbush explains, Kinde thus teaches or suggests the Preamble and Element 1.1. EX1002, ¶¶85-87.

Element 1.2

’699 patent	Kinde
“b) converting the population of circulating DNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a circulating DNA	*See* Elements 20.1 and 20.2 above. *See also* EX1002, ¶¶88-92.

molecule of the population of circulating DNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes, such that each non-uniquely tagged parent polynucleotide is substantially unique with respect to other non-uniquely tagged parent polynucleotides in the population;"	

Element 1.2 is satisfied by the same disclosure of Kinde that satisfies Element 20.1. Element 1.2 more broadly recites "converting" the parent DNA molecules whereas Element 20.1 specifically requires that the conversion involve "attaching." *See also* EX1002, ¶89.

As discussed above for Element 20.1, Kinde discloses methods comprising attaching a set of molecular tags to a population of circulating DNA molecules to produce a population of tagged original parent DNA molecules, wherein a plurality of the tagged original DNA molecules has identical molecular tags, and wherein each tagged original DNA molecule is substantially unique with respect to other tagged original DNA molecules in the population. Kinde's UID approach satisfies Element 1.2 for the same reasons discussed above that it satisfies Elements 20.1. *See also* EX1002, ¶90.

As Dr. Quackenbush explains, Kinde's disclosure also satisfies the requirement that the tagged DNA molecules each comprise a sequence from a parent DNA molecule and an identifier sequence. Kinde's endogenous UID

represent the sequence of the two ends of the original sheared fragments. EX1039, 9531, S1. Kinde endogenous UIDs and its exogenous UIDs (*e.g.*, one of 12 index sequences) each constitute an "identifier sequence comprising one or more polynucleotide barcodes." EX1039, 9531, S1; *see also id*. ("The resulting DNA fragments contained UIDs composed of three sequences: 2 endogenous ones, represented by the two ends of the original sheared fragments, plus the exogenous sequence …."); EX1002, ¶91.

Accordingly, as Dr. Quackenbush explains, Kinde teaches or suggests every aspect of claim Element 1.2. EX1002, ¶92.

Element 1.3

’699 patent	Kinde
"c) amplifying the population of non-uniquely tagged parent polynucleotides to produce a corresponding population of amplified progeny polynucleotides;"	*See* Element 20.2 above. *See also* EX1002, ¶¶93, 94.

Kinde discloses amplifying the population of tagged parent DNA molecules to produce a corresponding population of amplified progeny. EX1039, Abstract, 9531-9532, Figs. 1-3 & Captions, S1; EX1002, ¶94.

Element 1.4

’699 patent	Kinde
"d) sequencing at least a portion the population of amplified progeny polynucleotides to produce a set of sequence reads;"	*See* Element 20.3 above. *See also* EX1002, ¶¶95, 96.

Kinde discloses sequencing at least a portion of the population of amplified progeny polynucleotides to produce a set of sequence reads. EX1039, Abstract, 9531-9532 & Figs. 1-3, 9535, S2; EX1002, ¶96.

Element 1.5

’699 patent	Kinde
“e) grouping the sequence reads into families, each of the families comprising sequence reads comprising the same identifier sequence and having the same start and stop positions, whereby each of the families comprises sequence reads amplified from the same non-uniquely tagged parent polynucleotide;”	*See* Element 20.4 above. *See also* EX1002, ¶¶97-100.

Element 1.5 is substantively identical to Element 20.4. Kinde teaches or suggests Element 1.5 for the same reasons discussed above that it teaches or suggests Element 20.4. As discussed above, Kinde discloses that each of the families comprises sequence reads comprising the same identifier sequence (e.g., endogenous or exogenous UID) and having the same start and stop positions. EX1039, S1. As Dr. Quackenbush explains, a POSA would thus understand from the disclosure of Kinde that each of the families comprises sequence reads comprising the same identifier sequence and having the same start and stop positions, whereby each of the families comprises sequence reads amplified from the same tagged parent polynucleotide. EX1002, ¶¶98-100; *see also* Section I.C.

Element 1.6

’699 patent	Kinde
“f) collapsing sequence reads in each family to yield a base call for each family corresponding to one or more genetic loci.”	*See* Element 20.5 above. *See also* EX1002, ¶¶101-103.

As discussed above in Section VI.A.i for Element 20.5, Kinde discloses collapsing sequence reads in each family to provide an error-corrected consensus sequence read for each family corresponding to one or more genetic loci. Kinde provides the error-corrected consensus sequence read for each family corresponding to one or more genetic locus by making a base call for genetic locus (*i.e.*, concluding the mutation is not present unless it is present in at least 95% of the progeny in the family). Kinde’s Safe-SeqS approach thus satisfies claim Element 1.6 for the same reasons discussed above that it satisfies claim Element 20.5. *See also* EX1002, ¶102.

Accordingly, as Dr. Quackenbush explains, Kinde discloses each and every element of claim 1 of the ’699 patent and renders it obvious as a whole. EX1002, ¶103; *see also* Section I.C.

iii. Claims 2 and 23

’699 patent	Kinde
2. [23.] The method of claim 1 [20], further comprising detecting, at one or more loci, one or more of at least one	*See* Sections VI.A.i-ii above. “***Single-base substitutions were identified*** by conventional and Safe-SeqS analysis.” EX1039, 9534 Fig. 4

single nucleotide variant and at least one copy number variant.	caption; *see also id.*, 9531 (left column: "***single-nucleotide polymorphisms***"), 9532 (right column: ***single-base substitutions***."), 9533 (right column: "***single-base substitutions***, although occasionally ***single-base deletions*** were also observed") "In our experiments with exogenous UIDs (Table 2), we required only one supermutant to ***identify a position as mutant***….we required two supermutants to ***identify a position as mutant*** in the experiments reported in Table 1…." EX1039, S3. *See also* EX1002, ¶¶104, 105.

As discussed above, Kinde in view of Miner renders each of claims 1 and 20 obvious. Kinde discloses that Safe-SeqS identified single-base substitutions and deletions and single-nucleotide polymorphisms. EX1039, 9534 Fig. 4 caption; *see also id.*, 9531 (left column), 9532 (right column); 9533 (right column). Kinde also disclosed that its Safe-SeqS methodology identified "a position as mutant." EX1039, S3. As Dr. Quackenbush explains, a POSA thus would understand Kinde to disclose the additional limitations of claims 2 and 23 and, in view of Miner, to render each of these claims obvious as a whole. EX1002, ¶105; *see also* Section I.C.

iv. Claim 3

’699 patent	Kinde
3. The method of claim 1, wherein converting comprises any of blunt-end ligation, sticky end ligation, PCR, ligation-based PCR, single strand ligation and circularization to a single strand.	*See* Elements 20.1 and 1.2 for disclosure of sticky end ligation and PCR from Kinde in view of Miner. *See also* EX1002, ¶¶106, 107.

As discussed above, Kinde in view of Miner renders claim 1 obvious. As discussed above for Elements 20.1 and 1.2, Kinde disclosed converting the original parent DNA molecules into tagged DNA molecules using PCR and suggested doing so using sticky-end ligation as disclosed in Miner and Craig. As Dr. Quackenbush explains, a POSA thus would understand Kinde in view of Miner to disclose the additional limitation of claim 3 and to render claim 3 obvious as a whole. EX1002, ¶107; *see also* Section I.C.

v. Claim 4

’699 patent	Kinde
4. The method of claim 1, further comprising generating a set of consensus sequences from the sequence reads, and detecting a presence of sequence variations in the set of consensus sequences compared with a reference sequence.	*See* Sections VI.A.i-ii above. “Apparent mutations[are] defined as any ***base call that varies from the expected base*** at a defined position…” EX1039, S2 (left column). “***Base calling and sequence alignment were performed with the Eland pipeline (Illumina)***. Only high-quality reads meeting the following criteria were used for subsequent analysis:…(iii) ≤3 mismatches to

	expected sequences." EX1039, S2 (left column). *See also* EX1002, ¶¶108-110.

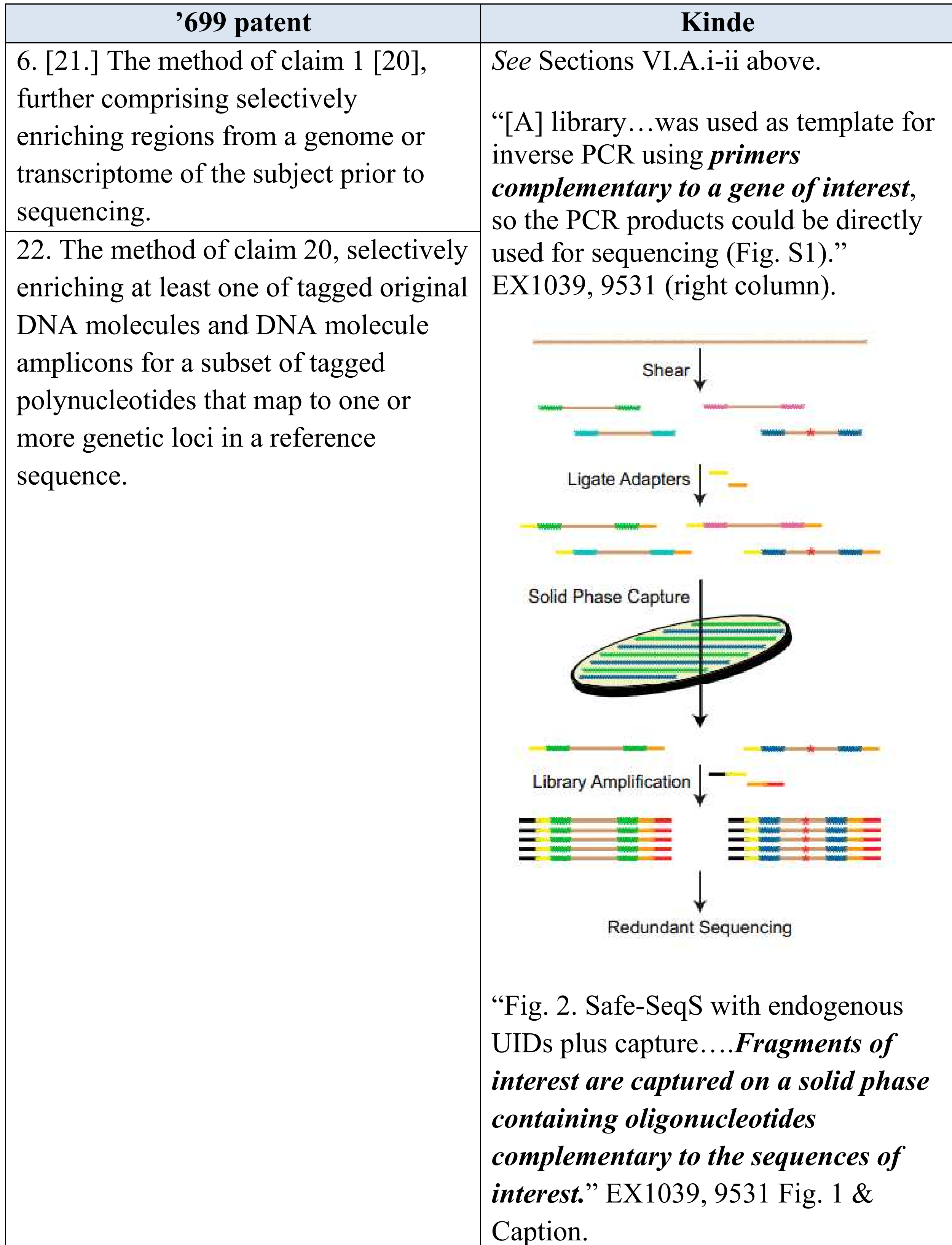
As discussed above, Kinde in view of Miner renders claim 1 obvious. As discussed above for Elements 20.5 and 1.6, Kinde discloses collapsing sequence reads in each family to provide an error-corrected consensus sequence read for each family corresponding to one or more …genetic loci by making a base call for each family corresponding to one or more genetic loci based on the presence of absence of a supermutant (at least 95% consensus). Kinde discloses using the Illumina Eland pipeline for "sequence alignment" and comparison to "expected sequences." EX1039, 9535, S2. As Dr. Quackenbush explains, a POSA thus would understand Kinde's disclosure of comparing to "expected sequences," as well as its disclosure in Tables 1-2 of mutations identified by "Conventional analysis" and by "Safe-SeqS analysis," to include generating a set of consensus sequences (Safe-SeqS) from the sequence reads (Conventional), and detecting a presence of sequence variations in the set of consensus sequences compared with a reference (expected based on alignment) sequence. EX1039, 9531-9532 & Tables 1-2. Accordingly, Kinde in view of Miner discloses the additional limitation of claim 4 and renders claim 4 obvious as a whole. *See also* Section I.C.; EX1002, ¶¶109, 110.

vi. Claims 5 and 7

’699 patent	Kinde
5. The method of claim 1, further comprising filtering out sequence reads that fail to meet a quality threshold.	*See* Sections VI.A.i-ii above. “**Sequencing**….***Only high-quality reads meeting the following criteria*** were used for subsequent analysis: (i) the first 25 bases passed the standard Illumina chastity filter; (ii) every base in the read had a quality score ≥20; and (iii) ≤3 mismatches to expected sequences. For the exogenous UID libraries, we additionally required the UIDs to have a quality score ≥30.” EX1039, S2. *See also* EX1002, ¶¶111-113.
7. The method of claim 1, further comprising removing a subset of the sequence reads from further analysis prior to (e).	

As discussed above, Kinde in view of Miner renders claim 1 obvious. Kinde discloses the Safe-SeqS approach includes filtering out sequence reads that fail to meet a quality threshold, including “the standard Illumina chastity filter,” every base in the read has a quality score of at least 20 or at least 30, and no more than 3 mismatches as compared to “expected sequences.” EX1039, S2. Only “high-quality” sequence reads satisfying these quality thresholds were permitted to proceed to grouping (step e), such that sequence reads not satisfying these quality thresholds were removed from further analysis prior to step e. EX1039, 9535 (right column), S2 (left column). Accordingly, Kinde in view of Miner discloses the additional limitations of claims 5 and 7 and renders each of claims 5 and 7 obvious as a whole. *See also* Section I.C.; EX1002, ¶¶112, 113.

vii. Claim 6, 21, and 22

’699 patent	Kinde
6. [21.] The method of claim 1 [20], further comprising selectively enriching regions from a genome or transcriptome of the subject prior to sequencing.	*See* Sections VI.A.i-ii above. “[A] library…was used as template for inverse PCR using ***primers complementary to a gene of interest***, so the PCR products could be directly used for sequencing (Fig. S1).” EX1039, 9531 (right column).  “Fig. 2. Safe-SeqS with endogenous UIDs plus capture….***Fragments of interest are captured on a solid phase containing oligonucleotides complementary to the sequences of interest.***” EX1039, 9531 Fig. 1 & Caption.
22. The method of claim 20, selectively enriching at least one of tagged original DNA molecules and DNA molecule amplicons for a subset of tagged polynucleotides that map to one or more genetic loci in a reference sequence.	

	See also EX1002, ¶¶114-116.

As discussed above, Kinde in view of Miner renders each of claims 1 and 20 obvious. Claims 6, 21, and 22 depend from claim 1 or 20 and further recite selectively enriching regions from a genome or transcriptome of the subject prior to sequencing or selectively enriching at least one of tagged original DNA molecules and DNA molecule amplicons for a subset of tagged polynucleotides that map to one or more genetic loci in a reference sequence. *See also* EX1002, ¶115.

Kinde discloses applying its Safe-SeqS methodology using PCR primers "complementary to a gene of interest, so the PCR products could be directly used for sequencing (Fig. S1)." EX1039, 9531 (right column). Kinde also discloses applying its Safe-SeqS methodology in combination with targeted sequence capture, whereby Kinde selectively amplifies captured, tagged polynucleotides that map to targeted regions of the genome prior to sequencing. EX1039, 9531, Fig. 2 & Caption. Kinde also discloses performing sequence alignment with the Illumina Eland pipeline and identifying mismatches as compared to "expected" (reference) sequences." EX1039, S2 (left column). As Dr. Quackenbush explains, a POSA thus would understand Kinde to disclose the additional limitations of claims 6, 21, and 22 and, in view of Miner, to render each of these claims obvious as a whole. EX1002, ¶116; *see also* Section I.C.

viii. Claims 8 and 9

’699 patent	Kinde
8. The method of claim 1, wherein the population of circulating DNA molecules includes a genetic variant having a variant frequency lower than about 1% or lower than about 0.01%. 9. The method of claim 1, wherein the population of circulating DNA molecules includes a genetic variant having a variant frequency as low as about 0.01% or as low as about 0.03%.	*See* Sections VI.A.i-ii above. “Detection and quantification of ***rare mutations*** with massively parallel sequencing.” EX1039, Title. “[I]nstrument-based errors are still limiting, particularly in clinical samples wherein the ***mutation prevalence can be ≤0.01%*** (11). In the work described herein, ***we show how*** templates can be prepared and the sequencing data obtained from them more reliably interpreted, so that ***relatively rare mutations can be identified*** with commercially available instruments.” EX1039, 9531 (right column). “The exogenous UID strategy (Fig. 3) was then used to determine the prevalence of ***rare mutations*** in a small region of the CTNNB1 gene…” EX1039, 9533 (right column). “As demonstrated here, the approach can ***easily be used to identify rare mutants in a population of DNA templates***….” EX1039, 9533 (right column). *See also* EX1002, ¶¶117, 118.

As discussed above, Kinde in view of Miner renders claim 1 obvious.

Claims 8 and 9 depend from claim 1 and specify that the population of circulating

DNA molecules includes a rare genetic variant having a variant frequency lower than about 1%, lower than about 0.01%, or as low as about 0.01%. The claims do not recite that such a variant is detected by the method, just that the population includes one. Such rare variants were well known in the art and their existence was disclosed by Kinde. *See, e.g.*, EX1039, 9531; *see also id.*, Title, Abstract, 9530-9531, 9533 (discussing confident identification of "rare variants" using Safe-SeqS approach to "substantially increase the sensitivity of massively parallel sequencing"). As Dr. Quackenbush explains, a POSA thus would understand Kinde to disclose the additional limitations of claims 8 and 9 and, in view of Miner, to render each of these claims obvious as a whole. EX1002, ¶118; *see also* Section I.C.

ix. Claims 10 and 11

'699 patent	Kinde
10. The method of claim 1, wherein an error rate of the base call of each family determined in step (f) is lower than about 1×10^{-6} or is as low as about 1.2×10^{-9}. 11. The method of claim 1, wherein an error rate of the base call of each family determined in step (f) is no more than about 1.5×10^{-4} or about 3.5×10^{-5}.	*See* Sections VI.A.i-ii above. "[S]pecificity can be increased by requiring more than one supermutant for mutation identification —the probability of introducing the same artifactual mutation twice or three times would be exceedingly low [***$(2 \times 10^{-6})^2$*** or ***$(2 \times 10^{-6})^3$***, respectively]." EX1039, 9534 (right column). *See also* EX1002, ¶¶119-122.

As discussed above, Kinde in view of Miner renders claim 1 obvious. Claims 10 and 11 depend from claim 1 and further specify various error rates. As an initial matter, Kinde discloses low error rates of 4×10^{-12} ($(2 \times 10^{-6})^2$) and 8×10^{-18} ($(2 \times 10^{-6})^3$) when more than one supermutant (e.g., two supermutants at complementary positions of complementary strands) is required to call the position. EX1039, 9534 (right column); *see also id.*, S2-S3 ("the specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation"). These error rates disclosed in Kinde certainly are within the scope of each of claims 10 and 11. *See also* EX1002, ¶120.

Moreover, the '699 patent admits that the error rates achieved with its methodology are comparable to those achieved in Kinde. Compared to a "state-of-the-art" Kinde embodiment where only one supermutant was required to make a base call, the '699 patent states that disregarding apparent mutations not appearing in the complementary strand "lowered the error rate of sequencing to a comparable degree[.]" EX1001, 27:37-43. Specifically, the '699 patent reports that relying on complementary strands to make base calls resulted in "an overall mutation frequency of 4.25×10^{-5}" when SMI families without a minimum of 3 members were filtered out and 5.33×10^{-5} when SMI families with a minimum member size of 1 were included. EX1001, 27:5-36. To the extent the claimed error rate refers to mutations/bp measures such as these, this too is disclosed by Kinde. Kinde

explains, for example, that "the error rate" determined for one conventional analysis was 2.4 x 10^{-4} mutations/bp. EX1039, 9531 (left column), Table 1; *see also id.*, Table 1 (2.4E-04, 2.3E-04 mutations/bp). In contrast, requiring detection of even a single supermutant reduced these error rates by 1-2 orders of magnitude. *See, e.g.*, EX1039, 9531, 9533 & Tables 1-2 ("Supermutants/bp…***3.5E-06***…0…***9.0E-06***…***1.4E-05***."). When evaluating error rates for specific polymerases, Kinde similarly reported an order of magnitude improvement using Safe-SeqS (4.5 x 10^{-7} vs. 9.1 x 10^{-6} errors/bp/PCR cycle, Safe-SeqS v. conventional), becoming "nearly identical" to the error rates measured in biological assays. EX1039, 9532 (right column). As Dr. Quackenbush explains, a POSA thus would understand Kinde to disclose the additional limitations of claims 10 and 11 and, in view of Miner, to render each of these claims obvious as a whole. EX1002, ¶¶121, 122; *see also* Section I.C.

x. Claims 12 and 24

'699 patent	Kinde
12. [24.] The method of claim 1 [20], wherein the circulating DNA molecules are nucleic acid-based serum biomarkers.	*See* Sections VI.A.i-ii above. "biomarkers" EX1039, 1039 (tag below abstract). "In ***neoplastic diseases***, which are driven by somatic mutations, the applications of ***rare mutant detection*** are manifold; they ***can be used*** to help identify residual disease at surgical margins or in lymph nodes, ***to follow***

	the course of therapy when assessed in plasma, and to identify patients with early, surgically curable disease when evaluated in stool, sputum, ***plasma, and other bodily fluids*** (9-11). ¶ These examples highlight the importance of identifying rare mutations for both basic and clinical research." EX1039, 9530 (left column). *See also* EX1002, ¶¶123-125.

As discussed above, Kinde in view of Miner renders each of claims 1 and 20 obvious. Claims 12 and 24 depend from claims 1 and 20, respectively, and specify that the circulating DNA molecules are nucleic acid-based serum biomarkers. A POSA would understand "nucleic acid-based serum biomarker" to include a nucleic acid that can be found in the serum and provide a measurable indicator of the severity or presence of some disease state. A skilled artisan would further understand DNA obtained from plasma of an individual with a neoplasm to constitute such a biomarker because such circulating DNA is present in the blood serum (i.e., which is defined as blood plasma without the clotting factors or blood with all cells and clotting factors removed) and may be used to determine whether a patient has a disease, such as cancer. *See also* Section V. Accordingly, claims 12 and 24 are directed to a characteristic of the DNA molecules, but the claims do not require that the method use the DNA molecules as biomarkers. *See also* EX1002, ¶124.

The claimed characteristic of cfDNA was well-known in the art and is disclosed by Kinde. For example, Kinde discloses that its approach is relevant to cancer diagnosis and "biomarkers." EX1039, 9530 (tag below Abstract). Kinde also discloses that the course of therapy for cancer (malignant neoplastic diseases) may be followed by assessing rare mutants "in plasma" (i.e., circulating DNA) and that patients may be diagnosed early with curable disease by evaluating rare mutants in "plasma and other bodily fluids." EX1039, 9530 (left column). As Dr. Quackenbush explains, a POSA thus would understand Kinde to disclose the additional limitations of claims 12 and 24 and, in view of Miner, to render each of these claims obvious as a whole. EX1002, ¶125; *see also* Section I.C.

xi. Claims 13, 14, 26, and 27

Claims 13, 14, 26 and 27 recite numerical ranges for distinct barcode sequences or molecular tags. Specifically claims 13 and 26 recite "about 2 to about 256" distinct barcode sequences or molecular tags, respectively. Claims 14 and 27 recite "about 256 to about 4096" distinct barcode sequences or molecular tags. As an initial matter, Patent Owner cannot distinguish the claimed ranges from the prior art based on any established criticality of those ranges. The '699 patent does not recite any such numerical ranges of barcodes/molecular tags, much less provide evidence of the criticality of the claimed ranges. *See also* EX1002, ¶126.

Should Patent Owner attempt to construct ranges from its specification to support claims 13, 14, 26, and 27, Kinde and Miner have substantially the same disclosure, if not more. For example, Kinde describes application of Safe-SeqS to a wide range of sample sizes. *E.g.*, EX1039, 9531 (1,057 DNA molecules), 9533 (300,000 oligonucleotide templates). As Dr. Quackenbush explains, a POSA would understand that tagging a small number of template molecules requires fewer exogenous sequences than a larger number of template molecules. A POSA would therefore choose a number of exogenous sequences appropriate to the sample at hand. Kinde discloses one example which uses "12 exogenous sequences" and mentions that "[t]his number could easily be increased." EX1039, S1 (left column); *see also* EX1002, ¶127.

Kinde's description of using 12 exogenous sequences is within the range "about 2 to about 256" as recited in claims 13 and 26. With respect to claims 14 and 27, Kinde explains that increasing the number of barcodes is beneficial to "minimize the probability that two different original templates acquire the same UID," even though such duplicates are not eliminated. EX1039, S1 (right column). Indeed, Miner discloses using 2,187 exogenous sequences which is within the range of "about 256 to about 4,096" and consistent with Kinde's teachings. EX1037, 2; *see also* EX1002, ¶128.

Given the lack of relevant disclosure in the ’699 patent, the combination of Kinde and Miner renders obvious claims 13, 14, 26, and 27. *See also* EX1002, ¶129.

xii. Claim 15

’699 patent	Kinde
15. The method of claim 1, wherein the polynucleotide barcodes are contained within a library generated from oligonucleotides comprising known sequences.	*See* Sections VI.A.i-ii above. “The ligated DNA…was amplified with a unique forward ***primer containing one of 12 index sequences*** at its 5’ end plus a standard reverse primer….” EX1039, S1 (left column). “An equivalent way to assign UIDs to fragments…would employ 10,000 forward primers and 10,000 reverse primers synthesized on a microarray. Each…would have gene-specific primers at their 3’ ends and ***one of 10,000 specific, predetermined, nonoverlapping UID sequences*** at their 5’ ends[.]” EX1039, 9532 (left column). *See also* EX1002, ¶¶130, 131.

As discussed above, Kinde in view of Miner renders claim 1 obvious. Claim 15 depends from claim 1 and further recites the polynucleotide barcodes are contained within a library generated from oligonucleotides comprising known sequences. Kinde discloses an embodiment in which the polynucleotide barcodes

are contained within a library generated from oligonucleotides comprising known sequences. EX1039, S1; *see also id.*, 9532. As Dr. Quackenbush explains, a POSA thus would understand Kinde to disclose the additional limitations of claim 15 and, in view of Miner, to render claim 15 obvious as a whole. EX1002, ¶131; *see also* Section I.C.

xiii. Claim 16 and 17

’699 patent	Kinde
16. The method of claim 1, wherein said non-uniquely tagged parent polynucleotide can be differentiated from other non-uniquely tagged parent polynucleotides using a combination of at least a first non-unique polynucleotide barcode at a first end of said circulating DNA molecule and a second non-unique polynucleotide barcode at a second end of said circulating DNA molecule. 17. The method of claim 1, wherein the sequence reads are grouped into families based on i) the polynucleotide barcode and ii) at least one of: sequence information at a beginning of the sequence from the circulating DNA molecule and sequence information at an end of the sequence from the circulating DNA molecule.	*See* Sections VI.A.i-ii above. “High quality reads were ***grouped in UID families on the basis of their endogenous or exogenous UIDs***.” EX1039, 9535, S2. “***UIDs consisting of the sequences of the two ends of each sheared fragment*** (Fig. 2 and Fig. S1).” EX1039, 9531 (left column); *see also* Fig. 2 & caption. “The resulting DNA fragments contained UIDs composed of three sequences: ***2 endogenous ones, represented by the two ends of the original sheared fragments*, *plus the exogenous sequence introduced during the indexing amplification.***” EX1039, S1 (left column). *See also* EX1002, ¶¶132, 133.

Claims 16 and 17 depend from claim 1. As discussed above, Kinde in view of Miner renders claim 1 obvious. Kinde also discloses the additional limitations of claims 16 and 17. As discussed above with respect to Elements 20.5 and 1.5, Kinde discloses grouping the sequence reads into families based on endogenous polynucleotide barcodes at first and second ends of the DNA molecule. For example, Kinde discloses grouping the families based on endogenous sequences at "the two ends of the original sheared fragments, plus the exogenous sequence introduced during the indexing amplification." EX1039, S1 (left column). Other Kinde examples also grouped the sequence reads into families based on endogenous polynucleotide barcodes at first and second ends of the DNA molecule (i.e., the barcode at first and second ends (and beginning and ending of sequence) of the DNA molecule also is sequence information derived from the parent DNA molecule). *See, e.g.*, EX1039, 9531, 9535, S2. As Dr. Quackenbush explains, a POSA thus would understand Kinde to disclose the barcodes at first and second ends of the DNA molecule, as recited in claim 16, and grouping the families based on sequence information at the beginning and the end of the sequence from the DNA molecule, as recited in claim 17 and, in view of Miner, renders each of claims 16 and 17 obvious as a whole. EX1002, ¶133; *see also* Section I.C.

xiv. Claim 25

’699 patent	Kinde
25. The method of claim 20, wherein at least a portion of the circulating DNA molecules are derived from neoplastic cells.	*See* Sections VI.A.i above. “In ***neoplastic diseases***, which are driven by somatic mutations, the applications of ***rare mutant detection*** are manifold; they ***can be used*** to help identify residual disease at surgical margins or in lymph nodes, ***to follow the course of therapy when assessed in plasma***, and to identify patients with early, surgically curable disease when evaluated in stool, sputum, ***plasma, and other bodily fluids*** (9-11). ¶ These examples highlight the importance of identifying rare mutations for both basic and clinical research.” EX1039, 9530 (left column). *See also* EX1002, ¶¶134, 135.

As discussed above, Kinde in view of Miner renders claim 20 obvious. Claim 25 depends from claim 20 and specifies that at least a portion of the circulating DNA molecules are derived from neoplastic cells. Kinde discloses that its approach is relevant to cancer diagnosis. EX1039, 9530 (tag below Abstract). Kinde also discloses that the course of therapy for cancer (malignant neoplastic diseases) may be followed by assessing rare mutants “in plasma” (i.e., circulating DNA) and that patients may be diagnosed early with curable disease by evaluating rare mutants in “plasma and other bodily fluids.” EX1039, 9530 (left column). As

Dr. Quackenbush explains, a POSA thus would understand Kinde to disclose the additional limitation of claim 25 and, in view of Miner, to render claim 25 obvious as a whole. EX1002, ¶135; *see also* Section I.C.

xv. Claims 18 and 19

’699 patent	Kinde
18. The method of claim 1, wherein the population of circulating DNA molecules comprises double-stranded molecules, and wherein the identifier sequence further comprises a strand identifier, and wherein, for each family of sequence reads amplified from the same non-uniquely tagged parent polynucleotide grouped in step (e), the method further comprises determining if the family has at least one sequence read from each strand of the double-stranded molecule using the strand identifier.	*See* Sections VI.A.i-ii above. “Fig. 1. Essential elements of Safe-Seqs. In the first step, ***each fragment*** to be analyzed is assigned a unique identification (UID) DNA sequence (green or blue bars).” EX1039, 9531 Fig. 1 Caption; *see also* Fig. 2 Caption (“One ***uniquely identifiable fragment*** is produced ***from each strand*** of the ***double-stranded template***; only one strand is shown.”) “Two UID assignment cycles produce two fragments—each with a different UID—from each ***double-stranded template molecule***, as shown.” EX1039, 9532 Fig. 3 Caption. “Mutations present in the template DNA…are ***expected to be present on both strands*** of the relevant templates.” EX1039, S2 “The errors from error-generating process viii can be reduced by ***requiring at least two supermutants to identify a position as mutant***. This requirement is reasonable because ***every preexisting mutation in a double-stranded DNA template should***
19. The method of claim 1, wherein the circulating DNA molecules comprise double-stranded molecules and wherein for each of a plurality of families, the method further comprises: confirming the presence of at least one sequence read from each strand of the double-stranded molecule and comparing the at least one sequence read obtained from one strand to the at least one sequence read from the other strand to form a consensus sequence of the double-stranded molecule, wherein the consensus sequence comprises only nucleotide bases at which the sequence of both strands of the double-stranded molecule are in agreement, such that a	

base call occurring at a particular position in the consensus sequence is identified as a true base call.	***give rise to two supermutants, one from each strand***." EX1039, S2 "When requiring multiple supermutants, the specificity can be further increased by ***requiring that each strand of the original double-stranded template contain the mutation*** or, when libraries are amplified using multiple wells, that rare mutations share an introduced sequence that identities the well in which the ***two mutations (i.e., one from each strand***) were amplified. EX1039, S3. *See also* EX1002, ¶¶136-140.

Claims 18 and 19 depend from claim 1. As discussed above, Kinde in view of Miner renders claim 1 obvious. Kinde also discloses the population of DNA molecules comprises double-stranded molecules and the identifier sequence further comprises a strand identifier: "Essential elements of Safe-SeqS. In the first step, each fragment to be analyzed is assigned a unique identification (UID) DNA sequence (green or blue bars)." EX1039, 9531 Fig. 1 Caption; *see also* Fig. 2 Caption ("One uniquely identifiable fragment is produced from each strand of the double-stranded template[.]"). *See also* EX1002, ¶¶137, 138.

Kinde also teaches or suggests grouping together the complementary strands of the original double-stranded molecule and comparing sequence reads from each strand using the strand identifier to provide a consensus sequence of the double-

stranded molecule, wherein the consensus sequence comprises only nucleotide bases at which the sequence of both strands of the double-stranded molecule are in agreement, such that a base call occurring at a particular position in the consensus sequence is identified as a true base call. For example, Kinde discloses that "Mutations present in the template DNA…are expected to be present on both strands of the relevant templates." EX1039, S2. Kinde thus teaches that true mutations should be confirmed by using the consensus sequence from each individual strand to determine a consensus sequence for the double-stranded molecule. *See, e.g.*, EX1039, S2-S3 ("specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation"); *see also* EX1039, 9535, S1 (describing use of Y-shaped Illumina adapters); EX1001, 26:49-54; 27:32-43 (disclosing that Kinde's Safe-SeqS method and DCS method may performed with standard Y-shaped Illumina adapters). As Dr. Quackenbush explains, a POSA thus would understand Kinde to disclose the additional limitations of claims 18 and 19 and, in view of Miner, to render these claims each obvious as a whole. EX1002, ¶¶139, 140; *see also* Section I.C.

As discussed above, Kinde in view of Miner renders obvious each of claims 1-27 as a whole. EX1002, ¶141.

B. [Ground 2] Claims 1-27 are unpatentable as obvious under 35 U.S.C. §103 over Kinde and Miner in further view of Fan.

As discussed above in Section VI.A, Kinde and Miner together render obvious each of claims 1-27 as a whole. As discussed above in Sections VI.A.i and VI.A.ii, Kinde suggests providing a population of circulating DNA molecules obtained from a bodily sample from a subject in the form of DNA obtained from plasma. EX1038, 9530 (citing references 7-8 for maternal plasma and 9-11 for cancer), 9535 (full citations). As also explained in Sections VI.A.i and VI.A.ii, Kinde specifically identifies Fan as obtaining DNA molecules from plasma. As discussed above, Kinde provides at least as much disclosure of applying molecular tagging to circulating DNA as Patent Owner contends was sufficient to show the priority document of the ’699 patent demonstrates possession of the same. Moreover, the Board has found that the sonication step described in Patent Owner’s specification is not a barrier to determining that application of DCS to cfDNA is obvious. IPR2019-00652, Paper 47 at 40-41; *see also* EX1037, 1 (describing preparing sample DNA using restriction endonuclease). To the extent the ’699 patent demonstrates possession of applying tagging to circulating DNA, Kinde’s earlier disclosure similarly taught applying Safe-SeqS to circulating DNA with a reasonable expectation of success before the earliest claimed priority date of the ’699 patent. *See Lockwood*, 107 F.3d at 1572; *Research Corp. Techs.*, 627 F.3d at 870; *see also* EX1002, ¶¶142, 143.

In view of Kinde's specific citation of Chiu and Fan for providing circulating DNA from maternal plasma, a POSA would have good reason to look to each of these references with a reasonable expectation of success for details on techniques for providing circulating DNA from maternal plasma. Fan, for example, describes extracting cfDNA from blood plasma. EX1021, 16266, 16270. Fan specifically extracted between 1.2 and 8 ng of cfDNA from blood plasma samples ranging from 1.3 to 3.2 ml. EX1021, SI7 (Table S1). Accordingly, Fan provides additional disclosure and support for providing a population of circulating DNA molecules. Accordingly, for the reasons stated here and in Ground 1, Kinde and Miner in further view of Fan render each of claims 1-27 obvious as a whole. EX1002, ¶144.

VII. SECTION 325(D) PRESENTS NO BAR TO INSTITUTION

Discretionary denial of institution under 35 U.S.C. §325(d) is not appropriate here where incomplete references were merely included in an IDS with nearly 100 references and no apparent substantive prior art analysis was applied by the Examiner. The materials presented here demonstrate material error in that the '699 patent should never have issued.

Section 325(d) should not be applied to deny institution in such circumstances even where references in a ground were listed on the IDS or even discussed in the body of the patent. *See, e.g.*, *Vudu, Inc. v. IndeaHub, Inc.*,

IPR2020-01689, Paper 16 at 18-20 (April 19, 2021) (§325(d) not applied where asserted references were cited repeatedly in the body of the patent and considered in IDS but never applied in a rejection); *Dish Network L.L.C., v. Sound View Innovations, LLC*, IPR2020-01041, Paper 13 at 16-22 (Jan. 19, 2021) (similar); *Cellco P'ship D/B/A Verizon Wireless v. Huawei Device Co., Ltd.*, IPR2020-01117, Paper 10 at 12-15 (Feb. 3, 2021) (§325(d) not applied where several ground references were listed on an IDS but not substantively addressed during prosecution); *Draftkings Inc. v. Interactive Games LLC*, IPR2020-01107, Paper 10 at 15-16 (Jan. 6, 2021) (examiner erred by overlooking relevant teachings in reference listed on IDS); *Honeywell Int'l, Inc. v. 3G Licensing S.A.*, IPR2021-00908, Paper 23 at 30-31 (Nov. 15, 2021) (same). Moreover, the IDS listing for the Kinde article did not reference the 10 pages of Kinde Supporting Information; its citation to Kinde mentions only the 6 pages of the main article. *See Mylan Pharms. Inc. v. Regeneron Pharms., Inc.*, IPR2021-00880, Paper 21, at 11-13 (Nov. 10, 2021) (§325(d) not applied where IDS submission included only a portion of the disclosure of the asserted reference).

The fact the Examiner never applied any prior art rejection indicates the prior examination was cursory at best. The Examiner certainly never made comment, let alone any rejection, based on Kinde, Miner, or Fan, or any combination thereof. *Advanced Bionics, LLC v. MED-EL Elekromedizinische*

Geräte GmbH, IPR2019-01469, Paper 6 at 10 (Feb. 13, 2020) (precedential) (explaining that "if the record of the Office's previous consideration of the art is not well developed or silent, then a petitioner may show the Office erred"). Moreover, where the '699 patent discusses Kinde and Miner, it makes comparisons, concessions and admissions that underscore Examiner error in the issuance of the '699 patent. *E.g.*, EX1001 2:16-38 (identifying Kinde and Miner as prior art tagging, error correction, and consensus sequencing), 27:32-43 (equating tagging scheme with that previously described in Kinde). To the extent any differences vis-à-vis Kinde exist, they are unclaimed here.

Moreover, where the '699 patent discusses Kinde and Miner, it fails to discuss pertinent disclosures of these references with respect to the claims that ultimately issued. The '699 patent ignores Kinde's teachings regarding using the complementary DNA strands for consensus sequencing, and Kinde and Miner's disclosures regarding duplicate tagging different DNA template molecules. *See, e.g.*, Sections VI.A.i-ii. The '699 patent also fails to acknowledge Kinde's disclosures regarding circulating DNA. The '699 patent never discloses performing any methodology on circulating DNA, whereas Kinde contains more disclosure regarding circulating DNA than the '699 patent, and did so years earlier. Furthermore, after issuance of the '699 patent, the Board concluded that Fan teaches providing a population of cell-free DNA molecules obtained from a bodily

sample from a subject and that it would have been obvious in 2012 to ligate molecular barcode tags to the ends of Fan's cfDNA (IPR2019-00652, Paper 47 at 3, 28-31). *See* Section VI.A.i. The Examiner did not have the benefit of the Board's decision (or the declaration of Dr. Quackenbush (EX1002) submitted with this petition) during prosecution.

The Examiner's failure to assert a rejection based on the combination of disclosures of Kinde and Miner relied upon in the petition, whether by themselves or in combination with Fan, represents substantive error. As there is no indication that the Examiner appreciated during prosecution the relevance of Kinde's full disclosure to the issued claims of the '699 patent, especially in view of Miner and Fan, Section 325(d) should not be applied to deny institution. *See Advanced Bionics* at 8-9 n.9.

VIII. THERE ARE NO *FINTIV* ISSUES

The present petition does not implicate the Board's discretion according to *Fintiv*. *Apple Inc., v. Fintiv, Inc.*, IPR2020-00019, Paper 11. Patent Owner filed its district court complaint August 3, 2021. Petitioner was diligent in preparing its petitions well in advance of the 12-month statutory deadline. The case is at a very early stage; the *Markman* hearing is currently scheduled for November 28, 2022. The trial is currently scheduled for November 2023 which, even if not postponed, would still take place after any final written decision here.

IX. CONCLUSION

For the reasons set forth above, claims 1-27 of the ’699 patent are unpatentable. Petitioners therefore request that a *inter partes* review of these claims be instituted.

Respectfully submitted,

Dated: January 31, 2022

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

X. CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. §42.24(d), the undersigned certifies that this Petition complies with the type-volume limitation of 37 C.F.R. §42.24(a). The word count application of the word processing program used to prepare this Petition indicates that the Petition contains 13,916 words, excluding the parts of the brief exempted by 37 C.F.R. §42.24(a).

Respectfully submitted,

Dated: January 31, 2022

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

XI. PAYMENT OF FEES UNDER 37 C.F.R. §§42.15(A) AND 42.103

The required fees are submitted herewith. If any additional fees are due at any time during this proceeding, the Office is authorized to charge such fees to Deposit Account No. 23-2415.

XII. Appendix – List of Exhibits

Exhibit No.	Description
1001	U.S. Patent No. 10,689,699 to Salk et al.
1002	Declaration of Dr. John Quackenbush
1003	*Curriculum Vitae* of John Quackenbush, Ph.D.
1004	Intentionally left blank
1005	U.S. Patent Application No. 16/411,045 File History
1006	U.S. Patent Application No. 16/411,066 File History
1007-1019	Intentionally left blank
1020	Schwarzenbach et al., "Cell-free nucleic acids as biomarkers in cancer patients," *Nature Reviews: Cancer* (May 12, 2011)
1021	Fan et al., "Noninvasive diagnosis of fetal aneuploidy by Shotgun sequencing DNA from maternal blood," *PNAS 105*(42) (October 21, 2008)
1022	Intentionally left blank
1023	Mitchell et al., "Circulating microRNAs as stable blood-based markers for cancer detection," *PNAS 105*(30) (July 29, 2008)
1024	Intentionally left blank
1025	Shiroguchi et al. "Digital RNA sequencing minimizes sequence-dependent bias and amplification noise with optimized single-molecule barcodes," *PNAS 109*(4) (January 24, 2012)
1026	Intentionally left blank
1027	Intentionally left blank
1028	U.S. Patent No. 9,834,822 to Talasaz
1029-1035	Intentionally left blank

1036	Craig et al., "Identification of genetic variants using bar-coded multiplexed sequencing," *Nature Methods* (October 2008)
1037	Miner et al., "Molecular barcodes detect redundancy and contamination in hairpin-bisulfite PCR," *Nucleic Acids Research* (2004)
1038	U.S. Patent Application No. 16/672,267 Notice of Allowance (July 21, 2021)
1039	Kinde et al., "Detection and quantification of rare mutations with massively parallel sequencing," *PNAS* (June 7, 2011)

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §§42.6(e) and 42.105(a), this is to certify that I caused to be served a true and correct copy of the foregoing Petition for Inter Partes Review of U.S. Patent No. 10,689,699 (and accompanying Exhibits 1001-1039) by overnight courier (Federal Express or UPS), on this 31st day of January, 2022, on the Patent Owner at the correspondence address of the Patent Owner as follows:

Perkins Coie LLP – University of Washington
PO Box 1247
Seattle WA 98111-1247

Universty of Washington Through Its Center For Commercialization
4311 11th Ave NE
Suite 500
Seattle, WA 98105

Respectfully submitted,

Dated: January 31, 2022

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