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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. IPR2022-01388
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

The ’699 patent specification purports to have invented duplex consensus sequencing (“DCS”). But it is not clear any claim of the ’699 patent requires duplex consensus sequencing, the purported point of novelty in the specification. The ’699 patent instead claims a far simpler concept—tagging sample DNA molecules, amplifying the tagged molecules using PCR, sequencing the amplification progeny, and grouping reads into families based on a common tag sequence. To the extent consensus is even required by the broadest claims, it is limited to “single-strand” consensus which the ’699 patent acknowledges is described in the prior art, including by Kinde. EX1001, 2:26-30.

Moreover, it is now clear the challenged claims broadly encompass circulating cellular DNA (e.g., blood cells), which the prior art indisputably addresses. Prior IPR2022-00449 and -00450 were denied stating that the claims of the ’699 patent excluded circulating cellular DNA and Kinde lacks of disclosure of cell-free DNA. -450 Decision, 7-8 (claims “do not include cellular DNA

molecules”), 21 (“the asserted references do not teach applying their tagging methods to circulating cfDNA”). Several days prior to the Board’s decisions in those IPRs, however, Patent Owner offered its Opening Claim-Construction Brief in the co-pending district court case acknowledging the claims of the ’699 patent encompass circulating cellular DNA. EX1040, 15-16. There is now no dispute the challenged claims encompass circulating cellular DNA. A material change in circumstances warrants review of the present challenge.

As is established in this petition, the claimed methods are indistinguishable from the prior art applied to cellular DNA. Kinde in view of prior art at the time discloses and renders obvious the same tagging method and the same workflow as the ’699 patent. It is indisputable that Kinde describes application of its method to cellular DNA, including cells obtained from blood. Kinde discloses an approach using Illumina Y-shaped adapters and endogenous “barcodes” that is encompassed by many of the ’699 patent claims. Kinde also cites to Craig, which discloses employing barcodes in Illumina Y-shaped adapters. Utilizing such barcoded Y-shaped adapters consistent with Kinde’s workflow and standard Illumina protocols (as acknowledged in the ’699 patent) additionally reaches all challenged claims. Corroborating obviousness, additional prior art at the time (e.g., Shiroguchi) illustrates barcoded Y-shaped adapters and expressly cites to Kinde in discussing prior application of such barcoding “to improve the sensitivity of DNA mutation

detection." EX1025, 1347. The '699 patent even borrows its illustration of barcoded Y-shaped adapters from Shiroguchi. *Compare* EX1001, Fig. 1 *with* EX1025, Fig. 1B.

The prior art cannot be distinguished on the basis of the claimed non-unique tagging method—a term not found in the '699 patent specification. Patent Owner has represented that the plain language of the claims indicates they broadly encompass tagging DNA such that a plurality of the tagged parent polynucleotides has identical barcodes. EX1040, 5. The prior art, including Kinde, expressly discloses that a known outcome of prior art tagging methods is that a plurality different original templates will acquire the same barcode. This is stated in Kinde and further corroborated by additional prior art including Miner and Shiroguchi, both of which report and quantify tagged parent polynucleotides with identical barcodes.

Accordingly, Petitioner respectfully requests institution as to all challenged claims and cancellation of the claims as unpatentable.

A. The '699 Patent

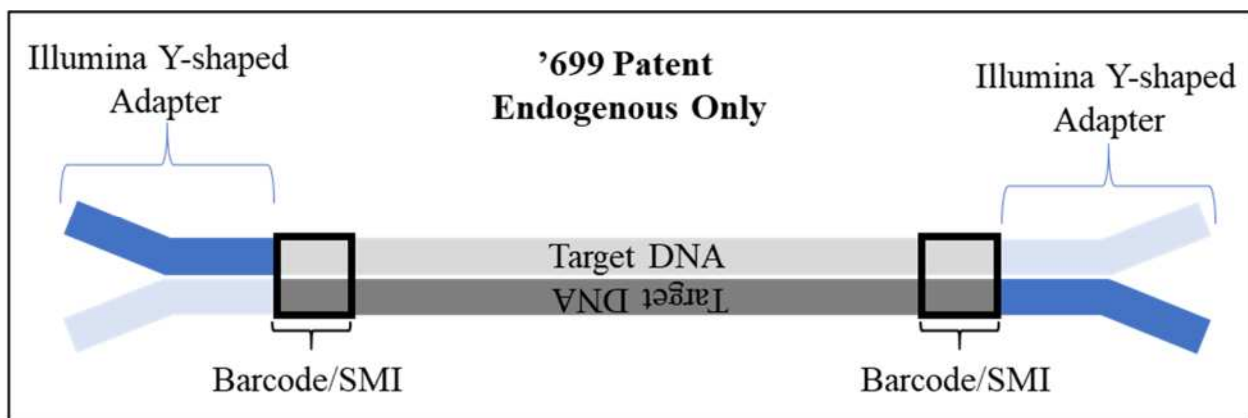
The '699 patent is entitled "Methods of lowering the error rate of massively parallel DNA sequencing using duplex consensus sequencing." EX1001, cover (54). The '699 patent specification proposes correcting sequencing errors with a

tag-based method referred to as "Duplex Consensus Sequencing" or "DCS." *E.g.*, EX1001, Abstract, 3:52-4:37; *see also* EX1002, ¶21.

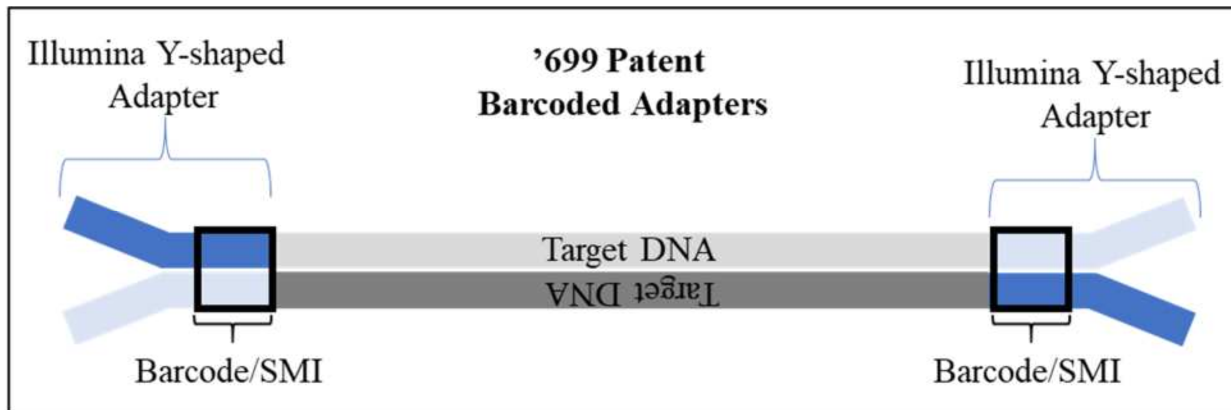
The '699 patent's DCS method makes use of identifiers ("single molecule identifiers") or tags—also recited as "barcodes" in some claims—to identify original sample molecules. DCS generally includes assigning SMI sequences to sample molecules, amplifying the tagged molecules using PCR, and sequencing 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.

Endogenous Only Method: The '699 patent instructs that DCS may be performed using barcodes consisting solely of endogenous sequences (i.e., sheared ends of target molecules). EX1001, 26:39-41 ("Example 3: Demonstration of Error-Correction by DCS Using Randomly Sheared DNA Ends as Single Molecule Identifiers"). The endogenous only embodiment is compared to one tagging approach in the Kinde reference. EX1001, 27: 32-43 ("To compare this result to the method of Kinde …"). With the endogenous only embodiment, sample DNA is randomly sheared then ligated to Illumina Y-shaped adapters. EX1001, 26: 50-54 ("The DNA was randomly sheared …, followed by end-repair, A-tailing, and

ligation of Illumina TruSeq DNA sequencing adaptors, all by standard library preparation methods."). As explained in the '699 patent, the 10 nucleotides "corresponding to the randomly sheared DNA ends" serve as "an SMI tag." Below is an illustration from the Quackenbush Declaration showing a target DNA molecule tagged according to the endogenous only embodiment of the '699 patent. EX1002, ¶¶23-25.



Barcoded Adapter Method: The '699 patent also instructs that DCS may be performed by ligation of "Y-shaped SMI adaptor molecule[s]" comprising barcodes to both ends of sheared DNA. EX1001, 6:4-30; *see also id.*, Figures 1-2, 19:32-35. Below is an illustration from the Quackenbush Declaration showing a target DNA molecule tagged with a barcoded Y-shaped adapter according the '699 patent. EX1002, ¶26.



B. Background in the Art

This petition is supported by the Declaration of Dr. John Quackenbush, the Henry Pickering 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, barcoding or tagging methods for consensus sequencing were known prior to the earliest claimed filing date of the '699 patent. EX1002, ¶¶27-47. As discussed herein, the '699 patent directly acknowledges that these techniques were known in the art.

i. Barcoding techniques and consensus sequencing

Dr. Quackenbush explains that the scientific literature by March 2012 described barcoding or tagging techniques and their use in DNA sequencing applications. Certain techniques were referred to in the art as "unique tagging," however they did 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. 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 its unique tagging as generating identically 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[.]”). In the co-pending district court case, Patent Owner has acknowledged that in one instance, Miner reports tagging a population of polynucleotides with a highly diverse set of tags such that 0.31% (~ 3 of every 1000) of the population of polynucleotides were tagged with the same molecular sequence. EX1040, 6; *see also* EX1037, 2. Shiroguchi similarly describes a tagging method 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).”). Consistent with the state of the art at the time, Kinde acknowledges “that two different original templates acquire the same UID” in its

tagging and discusses techniques to minimize (but not eliminate) such duplicate tagging. EX1039, SI1; *see also* EX1002, ¶¶27-31.

The ’699 patent explains that tagging techniques, as described above, had been used in conjunction with consensus sequencing methods. EX1001, 2:16-26. The Kinde reference cited in the ’699 patent (reference 36, EX1039) relies on tagging DNA molecules with barcodes and, as the ’699 patent confirms, discloses correcting errors in next-generation sequencing (“NGS”) reads. *E.g.*, EX1039, Abstract; *see also* EX1002, ¶32.

ii. Kinde

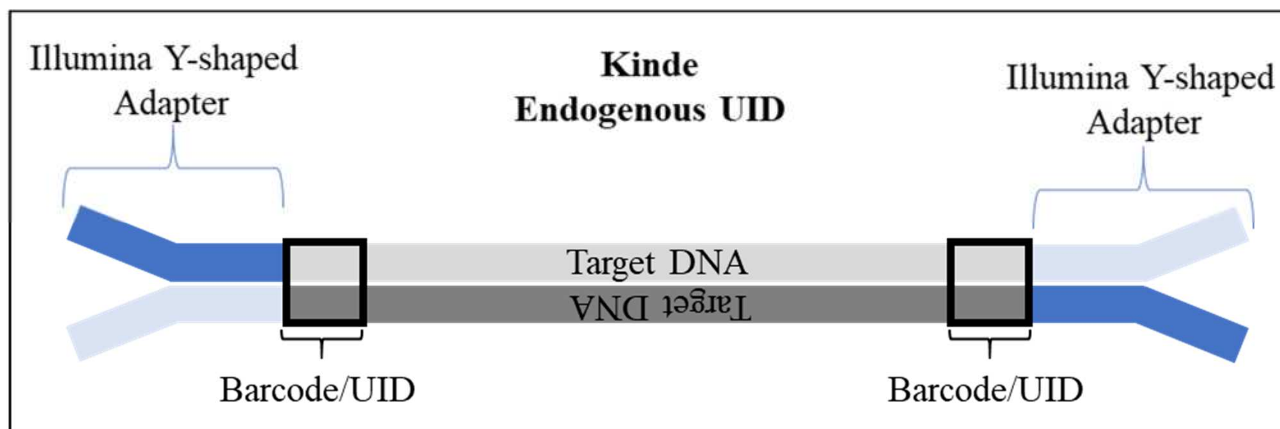
Kinde et al. published “Detection and quantification of rare mutations with massively parallel sequencing” (“Kinde,” EX1039) in volume 108 of the *Proceedings of the National Academy of Sciences* on June 7, 2011. EX1039, 9530; *see also id*. (“This article contains supporting information online”). Accordingly, 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, ¶33.

Kinde refers to the disclosed consensus sequencing method as “Safe-Sequencing System,” or “Safe-SeqS.” Kinde’s method uses UIDs, which are also referred to as unique identifiers and barcodes, to tag original sample molecules. *E.g.*, EX1039, 9530 (“a unique identifier (UID)”), 9531 (“UIDs, sometimes called barcodes or indexes, can be assigned to nucleic acid fragments using a variety of

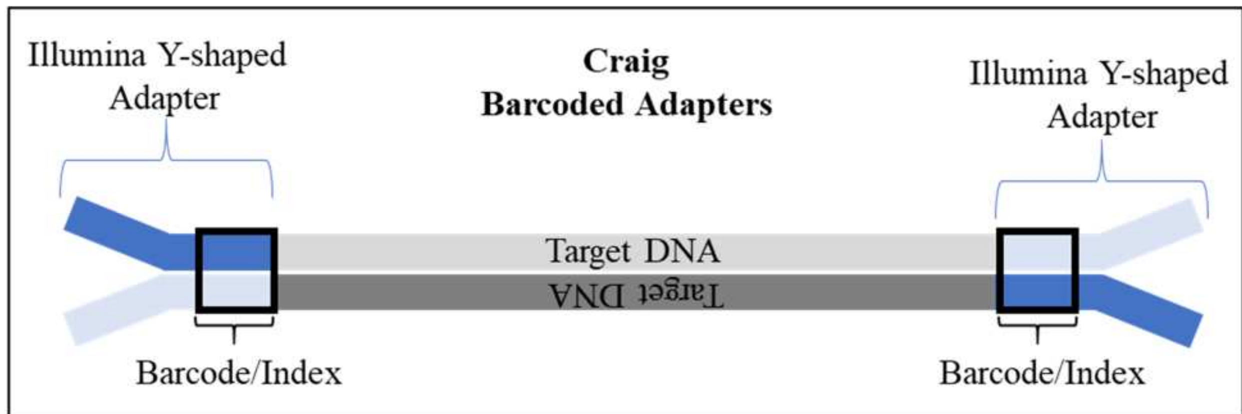
methods.”). Kinde describes its method as including assigning UID sequences to sample molecules, amplifying the tagged molecules using PCR, and sequencing the amplification progeny. EX1039, Abstract, 9531 (“Overview”). The resulting sequence reads are then grouped into families based on a common UID sequence. *Id*. Following grouping, a consensus sequence is built from the grouped sequence reads and mutations are distinguished from sequencing errors. *Id*.; *see also* EX1002, ¶34.

Endogenous UID Method: Kinde discloses that “randomly sheared genomic DNA inherently contains UIDs consisting of the sequences of the two ends of each sheared fragment.” EX1039, 9531. With respect to the endogenous UID embodiment, Kinde explains that sample DNA is randomly sheared then ligated to Illumina Y-shaped adapters. EX1039, 9531 (“we ligated standard Illumina sequencing adapters to the ends of sheared DNA fragments to produce a standard sequencing library”), 9535 (“To expose endogenous UIDs, DNA was fragmented to an average size of ~200 bp by acoustic shearing (Covaris) and then end-repaired, A-tailed, and ligated to Y-shaped adapters according to standard Illumina protocols.”). Kinde states “The sequences of the ends of each fragment produced by random shearing … serve as the unique identifiers (UIDs).” EX1039, Figure 2; *see also* EX1002, ¶¶35-36. Below is an illustration from the

Quackenbush Declaration showing a target DNA molecule tagged according to the endogenous UID embodiment of Kinde.



<u>Barcoded Adapter Method ("Craig Variation")</u>: The section titled "Endogenous UIDs" of Kinde cites to Craig, which discloses ligation of Y-shaped adapters comprising barcodes. Kinde states that "UIDs, sometimes called barcodes or indexes, can be assigned to nucleic acid fragments using a variety of methods" and "[t]hese methods include…ligation (42, 43)." EX1039, 9531. As discussed further herein, Reference 42, Craig, describes a set of barcoded Y-shaped adapters which, just like the endogenous UID method, are ligated to fragmented sample DNA. EX1036, Supplementary Methods (describing design of 48 barcoded Illumina Y-shaped adapters). While Craig is expressly discussed, Kinde itself does not exemplify utilizing Craig's barcoded Y-shaped adapters. Below is an illustration from the Quackenbush Declaration showing a target DNA molecule tagged according to the Craig Variation. *See also* EX1002, ¶37.



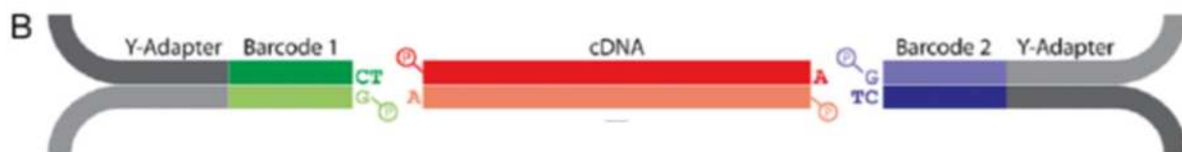
Kinde discloses other consensus sequencing embodiments, including an embodiment where target DNA molecules are tagged using PCR. This challenge, however, does not rely on PCR-based tagging. Nor does the present challenge rely on application of Kinde's method to cell-free DNA. *See also* EX1002, ¶38.

iii. Shiroguchi

"Digital RNA sequencing minimizes sequence-dependent bias and amplification noise with optimized single-molecule barcodes" by Shiroguchi, et al. ("Shiroguchi," EX1025) was published in *Proceedings of the National Academy of Sciences* and includes a manuscript and supporting information. As recited on the front page, Shiroguchi was published January 24, 2012, and qualifies as a prior art under 35 U.S.C. §102(a)(1) and pre-AIA 35 U.S.C. §102(a). EX1002, ¶39. Shiroguchi further provides evidence of the state of the art and evidence of how a POSA would understand the teachings of the prior art at the time.

Shiroguchi (Reference 40, EX1025) was acknowledged in the '699 patent as prior art describing a method tagging DNA fragments prior to amplification.

EX1001, 2:16-19 ("For example techniques whereby DNA fragments to be sequenced are each uniquely tagged [34, 35] ***prior to amplification*** [36-41] have been reported."). Shiroguchi's tagging method mirrors the barcoded adapter method of the '699 patent. Figure 1B (shown below) depicts Shiroguchi's tagging method which uses barcoded Illumina Y-shaped adapters. EX1025, Figure 1, SI3 ("In-Depth Design and Preparation of Adapter"), SI4-SI5 ("Sample-Adapter Ligation, Sequencing Sample Preparation, and Sequencing"). EX1002, ¶40.



Like the Miner reference discussed below, Shiroguchi explains that its tagging method does not result in a different barcode on every target DNA molecule. EX1025, 1349 ("even for the most abundant cDNA fragments in our sample, the required Neff is ~100–400 for 95% unique labeling of all molecules"), 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, ¶41.

Shiroguchi also expressly corroborates that POSAs at the time were aware of utilizing barcoded adapter molecules in consensus sequencing approaches as described by Kinde and others.

> In our approach, each cDNA molecule is attached to a unique barcode sequence from a large pool of barcodes before amplification (Fig. 1A) (14). … This concept has been applied recently…to improve the sensitivity of DNA mutation detection (16, 17) ….

EX1025, 1347 (citing Kinde (reference 17)); *see also* EX1002, ¶42.

iv. Miner

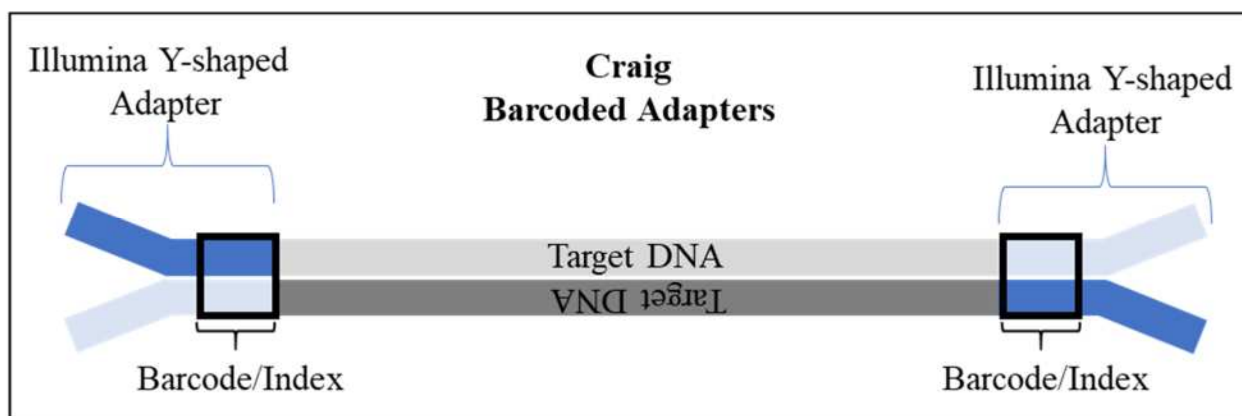
Miner et al. published "Molecular barcodes detect redundancy and contamination in hairpin-bisulfite PCR" ("Miner," EX1037) in volume 32 of *Nucleic Acids Research* in 2004. Accordingly, Miner is prior art at least under 35 U.S.C. §102(a)(1) and pre-AIA 35 U.S.C. §102(b). *See also* EX1002, ¶43.

The '699 patent acknowledges Miner as a prior art example of a tagging technique. EX1001, 2:16-19. Miner discloses "barcoding to label each genomic DNA template with an individual sequence tag prior to PCR amplification." EX1037, Abstract. Miner describes its tagging methods as producing some amount of identically tagged DNA fragments. For example, Miner expressly describes its tagging approach as including identically 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; EX1002, ¶44.

v. Craig

"Identification of genetic variants using bar-coded multiplexed sequencing" by Craig, et al. ("Craig," EX1007) was published in *Nature Methods* and includes a manuscript and supporting information. Craig was published October 2008 and qualifies as a prior art under 35 U.S.C. §102(a)(1) and pre-AIA 35 U.S.C. §102(b). EX1002, ¶45.

Craig discloses Illumina Y-shaped adapters comprising barcodes. EX1036, 888, Supplementary Tables 3 and 4. Below is an illustration from the Quackenbush Declaration showing a target DNA molecule tagged according to Craig.



Craig exemplifies ligating its barcoded adapters to fragmented PCR amplicons, *id*., 888, but states "it is straightforward to envision using other sample preparation methods." *Id*., 892. Craig explains "[o]ne could replace the pooled amplicons from our experimental strategy with total genomic DNA." *Id*.; *see also* EX1002, ¶¶46-47. In a recent IPR filing, Patent Owner argued that prior art Y-shaped adapters of Craig are amenable to use in the DCS method of the '699 patent. IPR2022-00746,

Paper 2, 67 ("…POSA also would expect Craig's barcodes to work in Schmitt's methods…").

C. 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), and then broadened significantly. Petitioner does not concede supporting disclosure exists or entitlement to the earliest claimed priority date. However, Patent Owner's interpretation of the claims of the '699 patent in this forum and others, renders the claims unpatentable regardless of priority date.

Only incomplete versions of the Kinde and Shiroguchi references were cited in an IDS, and never applied by the Examiner in any rejection. Craig was not before the Examiner during *ex parte* prosecution. Patent Owner's district court arguments, including its arguments regarding the scope of the claims (e.g.,

"circulating DNA molecules" including cellular DNA) and concessions of prior art duplicate tagging (e.g., Miner) were not previously considered and applied by the Office. As described in further detail herein, the claims of the '699 patent are the result of material error by the Office.

D. 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, ¶¶48-49.

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. EX1046.

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). Guardant filed petitions for *inter partes* review of the ’699 patent (IPR2022-00449 and IPR2022-00450). Guardant has also filed petitions for *inter partes* review against related U.S. Patent Nos. 10,760,127 (IPR2022-00816 and IPR2022-00817); 10,287,631 (IPR2022-00935); and 10,752,951 (IPR2022-01158 and IPR2022-01159). Additionally, TwinStrand Biosciences, Inc. filed petitions for *inter partes* review against related U.S. Patent Nos. 10,801,063 (IPR2022-00746 and IPR2022-01115); 10,889,858 (IPR2022-00747 and IPR2022-01116); and 11,118,221 (IPR2022-01152).

It is not believed these cases affect or will be affected by the present case, but out of an abundance of caution, Guardant calls attention to previous challenges to Guardant patents by a different third party: *Foundation Medicine, Inc. et al v. Guardant Health, Inc.*, Case No. IPR2019-00130 re: U.S. 9,598,731; *Foundation Medicine, Inc. et al v. Guardant Health, Inc.*, Case No. IPR2019-00634 re: U.S. 9,840,743; *Foundation Medicine, Inc. et al v. Guardant Health, Inc.*, Case Nos. IPR2019-00636 and IPR2019-00637 re: U.S. 9,902,992; *Foundation Medicine, Inc. et al v. Guardant Health, Inc.*, Case Nos. IPR2019-00652 and IPR2019-00653 re: U.S. 9,834,822; *Guardant Health, Inc. v. Vidal*, Case No. 21-1103 (CAFC) re: U.S. 9,840,743; *Guardant Health, Inc. v. Vidal*, Case No. 21-1104 (CAFC) re: U.S. 9,834,822; and Opposition in European Patent No. 2,893,040.

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); Patrick M. Medley (Reg. No. 74,735)

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

V. CLAIM CONSTRUCTION

Claim terms are given their plain and ordinary meaning in view of the specification and file history. 37 C.F.R. §42.100(b)[1]; *Phillips v. AWH Corp.*, 415 F.3d 1303, 1313 (Fed. Cir. 2005) (en banc). Consolidated Trial Practice Guide ("CTPG"), 45 ("The Office …applies the same claim construction standard that would be used to construe the claim in a civil action….").

While the scope of the challenged claims is discussed throughout the petition, additional discussion for certain terms is provided below.

A. "circulating DNA molecules"

The term "circulating DNA molecules" is not used in the specification of the '699 patent. 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, ¶52.

Consistent with discussion provided in recently filed IPR2022-00449 and -00450, the term "circulating DNA molecules" was initially understood as

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[1] While certain '699 patent claim verbiage was borrowed from claims of patents not owned by Patent Owner, the '699 patent claims are construed in view of the '699 patent specification. The claims of different patents are construed in view of their different specifications.

intended to encompass circulating cfDNA. After the filing of those petitions, however, Patent Owner argued that the term "circulating DNA molecule" more broadly encompasses both ***cells*** and ***cfDNA*** molecules in the bloodstream. *E.g.*, IPR2022-00449, Paper 8, 26 ("the specification describes circulating DNA molecules, both within cells and cell-free"), 27 ("including circulating cells"), 30 ("circulating tumor cells"). Guardant did not dispute Patent Owner's broader construction of "circulating DNA molecules" in the -449 and -450 IPRs and does not dispute it here.

In IPR2022-001158 and -001159, Guardant similarly stated it did not oppose the broader interpretation for the term "double-stranded circulating nucleic acid molecule" in those IPRs or IPRs against related patents. *See, e.g.*, -1159IPR Pet. 16-17 ("Petitioner does not oppose Patent Owner's interpretation of the term for prior art unpatentability analysis in these IPRs against the '951 patent and its related patents."). For example, a more expansive construction underscores the obviousness of the '951 patent claims as Kinde discloses obtaining double-stranded DNA from blood lymphocytes. EX1039, SI1 ("Genomic DNA from …blood lymphocytes…")").

In the corresponding district court case, Patent Owner argued the term broadly includes cellular DNA (e.g., blood cells) within the circulatory system. EX1040, 16 ("The parties have also agreed that the scope of the claim terms

include, ***among other DNA***, cell-free circulating within the circulatory system.”). Patent Owner’s Opening Claim-Construction Brief identifies the following construction for “circulating DNA molecule(s)” as agreeable: *DNA molecules that circulate within the circulatory system, which can include cell-free DNA and cellular DNA*. EX1040, 16.

Accordingly, the term “circulating DNA molecules” is construed herein as including circulating cellular DNA, as Patent Owner has argued to the Board and at district court, and is unopposed by Petitioner. CTPG, 45 (“The Office …applies the same claim construction standard that would be used to construe the claim in a civil action….”); *see also* EX1002, ¶53.

B. “tagged parent polynucleotide”

Claim 1 recites the term “tagged parent polynucleotide.” Claim 1 would reasonably be understood, in view of the specification, as inclusive of both endogenous only and barcoded adapter approaches as discussed herein. *See* Section I.A.; *see also* EX1002, ¶¶54-58.

According to the claims of the ’699 patent, a tagged parent polynucleotide is inclusive of “a sequence” from a circulating DNA molecule and “an identifier sequence.”

> … 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 …
>
> EX1001, claim 1.

As to the first element, claim 1 recites "a sequence" but not the entire sequence from the circulating DNA molecule. The specification describes that, in some instances, endogenous sequence of the parent DNA molecule makes up a different portion of the "tagged parent polynucleotide" recited in the claims—i.e., the identifier sequence. *E.g.*, EX1001, 9:38-42 ("A hybrid method using a combination of sheared ends and a shorter n-mer tag…may also serve as unique molecular identifiers."), 26:39-27:61 (Example 3) (describing "…Using Randomly Sheared DNA Ends as Single Molecule Identifiers."). Thus, "a sequence" as recited in the claims need not be the entire sequence of the parent DNA molecule. *See also* EX1002, ¶56.

As to the "identifier sequence" element, the claims recite inclusion of one or more barcodes. EX1001, claim 1. The '699 specification explains that the randomly sheared ends of cellular DNA alone may serve as an identifier sequence or tag to uniquely identify parent polynucleotides. EX1001, 26:39-27:61 ("Example 3: Demonstration of Error-Correction by DCS Using Randomly Sheared DNA Ends as Single Molecule Identifiers"). The specification in this regard repeatedly refers to the randomly sheared ends both as identifiers ("single

molecule identifiers") and tags. *See, e.g.*, EX1001, 26:58-62 ("The first 10 nucleotides of each sequencing read pair, corresponding to the randomly sheared DNA ends, …yield an SMI tag of form AB."), 27:8-9 ("… the randomly sheared DNA ends were used as SMI's."), 27:15-17 ("The data was then subjected to DCS analysis with the SMI tags…"), 27:32-34 ("To compare this result to the method of Kinde et al. [36], reads were grouped into families by SMI tag..."), 27:45-47 ("… DCS analysis, using sheared DNA ends as unique molecular identifiers..."); *see also* EX1002, ¶57.

The discussion in the specification regarding "barcodes" further indicates they comprise endogenous sequence from the circulating DNA molecule. As Dr. Quackenbush explains, barcodes are nucleic acid sequences used for identification of molecules. While different researchers might use the term differently, a barcode is often viewed as including exogenous and/or endogenous sequences. EX1002, ¶58. The '699 patent recites the term "barcode" in an instance where an exogenous sequence is used to identify sample origin for multiplexing (i.e., analysis of multiple different samples). EX1001, 21:13-20. Elsewhere, however, the specification cites and incorporates by reference Kinde et al. (EX1039) which describes barcodes as inclusive of endogenous or exogenous sequences.

> UIDs ["Unique Sequence Identifiers"], 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; *see also* EX1001, citing Kinde at 2:16-38, 17:62-64, 24:9-14, 27:32-43, and incorporating by reference at 19:53-55.

C. "non-uniquely tagged"

The term "non-uniquely tagged" is not recited in the '699 patent specification and Patent Owner has advanced various arguments regarding the metes and bounds of the term. But Patent Owner has identified an outer boundary of "non-uniquely tagged" for purposes of prior art analysis—"non-uniquely tagged" is satisfied where a plurality of the tagged parent polynucleotides has identical barcodes. For example, claim 1 recites "non-uniquely tagged" and independent claim 20 recites "wherein a plurality of the tagged original DNA molecules has identical molecular tags." Patent Owner has equated these limitations. *See, e.g.*, -00450IPR, POPR, 5 ("similarly recite[d] in" in claims 1 & 20 with "different phraseology"). At district court, Patent Owner argued "[a] 'non-unique' tag is just as it sounds, a tag that has a nucleotide sequence that is identical

to other tags that are attached to multiple parent polynucleotide molecules in the same sample."[2] EX1040, 5; *see also* EX1002, ¶59.

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

Kinde discloses the same workflow discussed and claimed in the '699 patent (e.g., converting DNA into tagged polynucleotides, amplifying tagged polynucleotides, sequencing, grouping the sequence reads, and consensus sequence analysis). Kinde uses barcodes called UIDs to identify the amplification progeny of original parent DNA molecules. *E.g.*, EX1039, 9531, SI1. Just like the '699 patent, Kinde describes assigning UID to sample molecules, followed by amplification, sequencing, and grouping reads into UID families. *E.g.*, EX1039, Abstract; EX1001, 3:17-27; *see also id*., 2:16-19 ("For example techniques whereby DNA fragments to be sequenced are each uniquely tagged [34, 35] prior to amplification [36-41] have been reported.") (Reference 36 is Kinde). 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

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[2] Different Guardant patents expressly define "non-uniquely tagged." Neither party has argued said express definition is applicable to the '699 patent.

sequencing based on 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, ¶¶60-61.

As discussed in detail herein, claims 1-27 are obvious over the prior art at the time, as illustrated by Kinde further corroborated by prior art at the time, including Miner. *See also* EX1002, ¶¶_. As explained in further detail below, independent claim 1 and its dependent claims, encompass Kinde's endogenous method in which sheared cellular DNA is converted into tagged parent polynucleotides by ligation of Illumina Y-shaped adapters. *See* Section I.B.ii. In addition, all claims encompass the obvious variation of Kinde's method utilizing barcoded adapters (i.e., the Craig Variation) in which sheared cellular DNA is converted into tagged parent polynucleotides using barcoded Illumina Y-shaped adapters.[3] *See* Section I.B.ii. Kinde discloses other consensus sequencing embodiments, including an embodiment where target DNA molecules are tagged using PCR. This challenge however, does not rely on PCR-based tagging.

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[3] Craig is sufficiently addressed throughout the petition, regardless of whether it is considered as a "grounds reference." *Randall Mfg. v. Rea*, 733 F.3d 1355, 1363 (Fed. Cir. 2013) (explaining that "evidence of the state of the art" must be considered).

Moreover, because the challenged claims encompass circulating cellular DNA the present challenges do not rely on application of Kinde to cell-free DNA. *See also* Section V.A; EX1002, ¶62-63.

Kinde, particularly in view of the state of the art and admissions in the ’699 patent and by Patent Owner, discloses and render obvious all aspects of the challenged claims. As discussed, many of the ’699 patent claims encompass Kinde’s endogenous UID approach applied to circulating cellular DNA. 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 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, including Miner. First, the term “circulating DNA molecules” includes cellular DNA (e.g., blood cells) within the circulatory system. Section V.A. Kinde discloses cellular DNA obtained from blood samples and “blood lymphocytes” and exemplifies applying its endogenous UID embodiment to “cultured lymphoblastoid cells.” EX1039, 9530, SI1. 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. EX1039, SI1. Scientific literature at the time (cited in both the ’699 and Kinde), such as

Miner (and Shiroguchi), provides additional detail regarding the identical tagging that occurs in unique tagging approaches, thereby confirming Kinde's disclosure.[4] *See* Sections I.B.iii & I.B.iv. Third, Kinde teaches that combining parent polynucleotides with Illumina Y-shaped adapters and tags located at the sheared ends and further cites prior art disclosing barcoded Illumina Y-shaped adapters. Each of these subjects is discussed in further detail below. *See also* EX1002, ¶64.

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 that it is using an endogenous tagging approach that had been previously described by Kinde. EX1001, 27:32-34 ("To compare this result to the method of Kinde et al. [36] …"). 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; EX1039, 9531 ("…randomly sheared genomic DNA inherently contains UIDs consisting of the sequences of the two ends of each sheared fragment…").

[4] As discussed in more detail below, Patent Owner concedes that Miner describes identically tagging a plurality of molecules. EX1040, 6; *see also* discussion of Elements 1.2 and 20.1 below.

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." EX1001, 26:39-66; EX1039, SI2 ("≥95% of members"). The '699 patent expressly draws a comparison to the same approach reported in Kinde. EX1001, 27:4-43; s*ee also* Sections I.A & I.B.ii; EX1002, ¶65.

The '699 patent and the prior art also similarly disclose tagging molecules using barcoded adapters. The '699 patent instructs that DCS may be performed by ligation of "Y-shaped SMI adaptor molecule[s]" comprising barcodes to both ends of sheared DNA. EX1001, 6:4-30; *see also id*., Figures 1 and 2. Kinde in view of the prior art also renders obvious attaching Y-shaped adapters comprising barcodes. Kinde explains that UIDs may be introduced "through …ligation (42, 43)." EX1039, 9531. Reference 42, Craig, describes a set of barcoded Y-shaped adapters which, just like the endogenous UID method, are ligated to fragmented sample DNA. EX1036, Supplementary Methods (describing design of 48 barcoded Y-shaped Illumina adapters); *see also* Section I.B.v. Craig, like Kinde, expressly discusses ligation of Illumina Y-shaped adapters to fragmented sample DNA prior to amplification. EX1036, 892 ("…One could replace the pooled amplicons from our experimental strategy with total genomic DNA."); *see also* EX1002, ¶66.

Further regarding barcoded Y-shaped adapters, a skilled artisan would have had good reason to to use Y-shaped adapters comprising barcode sequences, as

disclosed in Craig, in a workflow as in Kinde in order to arrive at the ’699 patent claims. As discussed, Kinde identifies Craig in expressly discussing use of barcoded Y-shaped adapters with DNA molecules. EX1002, ¶67. Shiroguchi also discusses barcoded Y-shaped adapters and cites to Kinde. EX1025, 1347, Fig. 1. An express suggestion in the literature, while not required to demonstrate obviousness, strongly supports a conclusion of obviousness. *KSR Int’l Co. v. Teleflex Inc.*, 550 U.S. 398 (2007).

Moreover, a skilled artisan also had good reason with a reasonable expectation of success in achieving what is claimed. The prior art, including both Kinde and Shiroguchi, at a minimum suggests the combined teachings. EX1002, ¶68. Further, Kinde and Craig both use Illumina Y-shaped adapters attached to each end of DNA molecules, illustrating barcoding techniques and prior art adapters that were well-known, utilized consistent with what is claimed, and documented in the scientific literature. *KSR*, 550 U.S. at 417. In a recent IPR filing, Patent Owner argued that Craig’s barcoded Y-shaped adapters are amenable to use in the claimed method. *Twinstrand Biosciences, Inc. v. Guardant Health, Inc.*,

IPR2022-00746, Paper 2, 67 ("…POSA also would expect Craig's barcodes to work in Schmitt's[5] methods…").

As Dr. Quackenbush explains, the scientific literature at the time further corroborates that a skilled artisan would have been motivated with reasonable expectation of success of arriving at what is claimed. Shiroguchi illustrates that barcoded Y-shaped adapters were well-known at the time, and further confirms such methods labeled each strand of a target with adapters that enable strand-specific labeling. Shiroguchi also discusses the use of such adapter molecules in consensus sequencing approaches as described by Kinde and others. EX1025, 1347 ("…to improve the sensitivity of DNA mutation detection (16, 17), …"), Figure 1; Section I.B.iii. Accordingly, a skilled artisan had good reason with a reasonable expectation of success in achieving what is claimed. EX1002, ¶69.

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. But no such distinction is claimed here. The '699 patent never

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[5] Schmitt (Int. Pub. No. WO2013/142389) is the published version of PCT/US2013/032665 which is listed on the face of the '699 patent as a priority document.

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* Sections I.B.iii & I.B.iv; *see also* EX1002, ¶70. The method claimed in the ’699 patent is indistinguishable from those previously disclosed by prior art such as Kinde, particularly in view of Kinde’s discussion of Craig, and further confirmed by Minor and Shiroguchi.

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, ¶71.

i. Claim 1

Preamble and Element 1.1

> A method, comprising:
>
> a) providing a population of circulating DNA molecules obtained from a bodily sample from a subject;

Kinde renders obvious the Preamble and Element 1.1. There can be no dispute Kinde discloses application of its methods to cellular DNA. A blood sample is an abundant source of cellular DNA, as Patent Owner’s own publications acknowledge. EX1045, 6 (reporting 3.5 µg of cellular DNA obtained from a blood

sample). As explained above, the term “circulating DNA molecules” includes ***cellular DNA*** (e.g., blood cells) within the circulatory system. Section V.A. Kinde discloses that cellular DNA may be obtained from blood and “blood lymphocytes” and exemplifies applying its endogenous UID embodiment to “cultured lymphoblastoid cells.” EX1039, 9530, SI1. Kinde further discloses that its consensus sequencing method is compatible with virtually any sample preparation workflow.

> It can be implemented through either endogenous or exogenously introduced UIDs and can be applied to virtually any sample preparation workflow or sequencing platform. As demonstrated here, the approach can easily be used to identify rare mutants in a population of DNA templates.

EX1039, 9533; *see also* EX1002, ¶¶72-74.

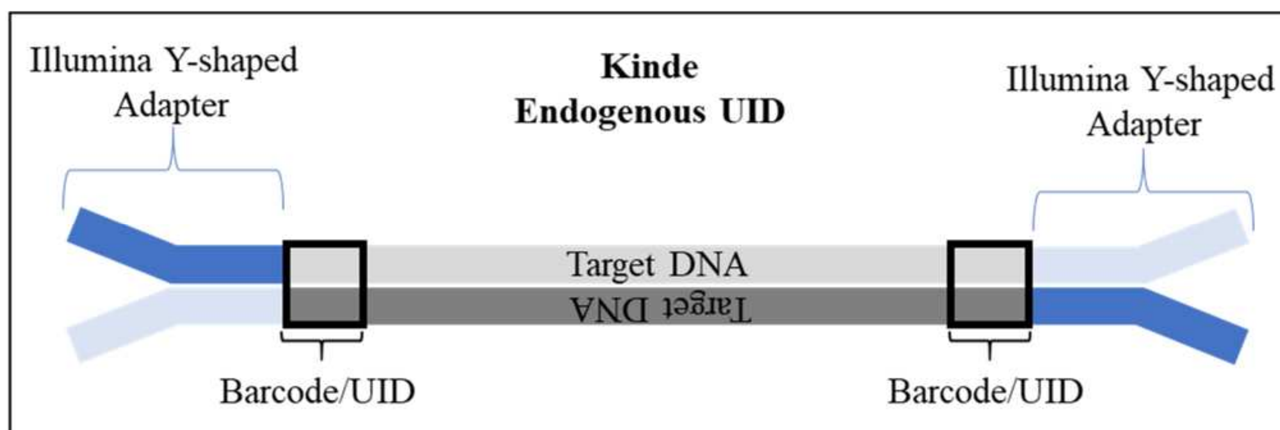
Element 1.2

> 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;

Kinde discloses or teaches Element 1.2. As explained above, in Section V.B, claim 1 is reasonably understood in view of the ’699 patent specification as

encompassing both Kinde's endogenous UID approach and use of barcoded Y-shaped adapters (e.g., the Craig variation). Either embodiment is sufficient to render claim 1 obvious. *See also* Section I.A; EX1002, ¶¶75-76.

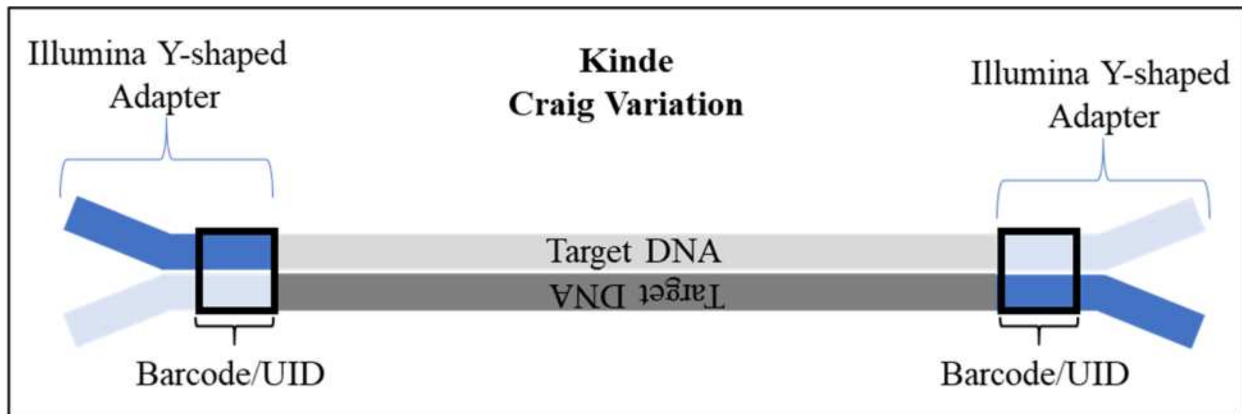
Kinde discloses that sheared DNA molecules may be converted into tagged parent polynucleotides comprising a sequence from a circulating DNA molecule and an identifier sequence. As disclosed in Kinde, sheared cellular DNA (including DNA obtained from blood lymphocytes) "inherently contains UIDs consisting of the sequences of the two ends of each sheared fragment." EX1039, 9531; *see also* Element 1.1. Kinde discloses ligating "standard Illumina sequencing adapters to the ends of sheared DNA fragments," *id*., to produce tagged parent polynucleotides as shown below. *See also* EX1002, ¶77.



The tagged parent polynucleotides produced according to the endogenous UID embodiment of Kinde are within the scope of claim 1 because these molecules include a sequence from a circulating DNA molecule (e.g., DNA from blood and

blood lymphocytes) and the sheared ends provide an identifier sequence (i.e., a unique identifier or UID). *See* Sections I.A, I.B, V.B; *see also* EX1002, ¶78.

The Craig Variation is also within the scope of claim 1. With the Craig Variation, Kinde teaches that Illumina Y-shaped adapters may be utilized with sheared DNA molecules and also cites Craig, which discloses barcoded Illumina Y-shaped adapters. EX1039, 9531 (UIDs may be introduced "through …ligation (42, 43)."); *see also* EX1025, 1347 ("In our approach, each cDNA molecule is attached to a unique barcode sequence … This concept has been applied recently…to improve the sensitivity of DNA mutation detection (16, 17) ….”). Use of barcoded Illumina Y-shaped adapters would not require any significant deviation from Kinde's workflow or standard Illumina protocols, as acknowledged in the '699 patent itself. EX1001, 11:28-31 ("the sequence of the linker itself does not matter in the workflow"); 18:56-59 ("no altered steps in library preparation"); 19:32-35 ("DCS does not require any significant deviations from the normal workflow of sample preparation for Illumina DNA sequencing."); *see also* EX1039, 9533 ("can be applied to virtually any sample preparation workflow or sequencing platform"), EX1036, Abstract ("We developed a generalized framework …"), EX1025, 1350 ("…uses standard commercial protocols for sample preparation…") EX1002, ¶79.



The tagged parent polynucleotides produced according to the Craig Variation are within the scope of claim 1 because these molecules include a sequence from a circulating DNA molecule (e.g., DNA from blood and blood lymphocytes) and an identifier sequence provided by the barcoded adapter. *See also* EX1002, ¶80.

Patent Owner cannot distinguish the prior art from the claims based on the recited "non-unique tagging" scheme, particularly given the scope of the term here. *See* Section V.C; EX1002, ¶81.

Kinde discloses that a known outcome of using UIDs is that "two different original templates acquire the same UID." EX1039, SI1. Kinde further explains that "number of different molecules that can be examined using endogenous UIDs is limited." EX1039, 9532. 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," *id.*, SI1, 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, ¶82.

Prior art confirms that duplicate tagging occurs in tagging methods such as Kinde. For example, Miner reports that its tagging method produces a plurality of duplicate or identically 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* Section I.B.iii. Patent Owner has recently conceded that Miner's tagging method results in at least 3 out of 1000 tagged target molecules bearing non-distinct tags, thereby producing populations of tagged molecules within the scope of "non-uniquely tagged." EX1040, 6 ("Miner reports tagging a population of polynucleotide molecules with a highly diverse set of tags, such that the probability of two genomic fragments being tagged with the same molecular sequence was exceedingly improbable—a .31% chance in one instance."). Kinde expressly acknowledges such non-distinct tagging and a comparable rate applied to Kinde illustrates that prior art tagging clearly falls within the scope of what is claimed. For example, Kinde reports analyzing "69,505 original template molecules" with the endogenous UID method. EX1039, 9531. If 0.31% of these template molecules were identically tagged, there would be 215 (i.e., 69,505 x 0.0031) identically

tagged molecules.[6] Larger populations of original template molecules would be expected for a larger cellular DNA sample (e.g., blood sample). Not only are identically tagged molecules expressly acknowledged in Kinde as present, but additional prior art at the time confirms that identically tagged molecules are both present and abundant (relative to the scope of the challenged claims) in prior art tagging methods such as Kinde. *See also* EX1002, ¶¶83-84.

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 identifies molecules (e.g., family grouping) based on sequence identity (i.e., SMI). *E.g.*, EX1001, 19:23-25, 22:41-44. Two different tagged DNA molecules, even bearing identical SMIs, have different sequences by virtue of the molecules themselves. While the specification does not recite the term "substantially unique" it presumably encompasses tagged DNA molecules that are sufficiently distinguishable from other tagged DNA

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[6] If Shiroguchi's higher rate of identically tagged molecules (i.e., 5%) were applied to Kinde's disclosure of 69,505 original template molecules, there would be 3,475 (i.e., 69,505 x 0.05) identically tagged molecules. *See* Section I.B.iii.; EX1002, ¶85.

molecules in the population based on sequence. *See also* EX1002, ¶86.

To the extent required by these claims, the prior art also discloses tagging sample DNA. In Kinde's endogenous UID approach, for example, Y-shaped adapters are attached to fragmented genomic DNA. EX1039, 9535; *see also* Section I.B.ii. Craig discloses barcoded Illumina adapters, and Craig discusses that these adapters may be attached to fragmented sample DNA prior to amplification. EX1036, 892; *see also* Section I.B.v. Illumina's product documentation illustrates that typical Illumina sequencing workflows include attachment of Y-shaped adapters to sample DNA prior to PCR amplification. EX1042, Figure 4; *see also* EX1002, ¶¶87.

Element 1.3

> c) amplifying the population of non-uniquely tagged parent polynucleotides to produce a corresponding population of amplified progeny polynucleotides;

Kinde discloses Element 1.3. For example, Kinde discloses "amplification" of the tagged template molecules. EX1039, Abstract ("Our approach… The second is the amplification…"), Figure 2 ("amplified" by PCR), SI1 ("PCR-mediated amplification"); *see also* EX1001, 2:16-19 ("techniques whereby DNA fragments to be sequenced are each uniquely tagged [34, 35] prior to amplification [36-41] have been reported."); EX1002, ¶¶88-89.

Element 1.4

> d) sequencing at least a portion of the population of amplified progeny polynucleotides to produce a set of sequence reads;

Kinde discloses Element 1.4. Kinde discusses sequencing throughout its disclosure. For example, Kinde's approach involves "redundant sequencing" of the tagged strands and of their amplification products. EX1039, Abstract; *see also id*., 9530-9531, Figs. 1-2, 9535 ("Sequencing"); *see also* EX1002, ¶¶90-91.

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

Kinde teaches Element 1.5. As an initial matter, claim 1 recites grouping and certain characteristics but does not recite grouping based on those characteristics. The '699 patent certainly does not describe grouping sequence reads into families based on start and stop position. Regardless, Kinde's method of grouping reads is indistinguishable from that described in the '699 patent. Like the '699 patent, Kinde discloses that reads derived from the same original molecule may be grouped "into UID families on the basis of their …UIDs." EX1039, 9535; *see also id*., 9530 ("If a mutation preexisted in the template molecule used for amplification, that mutation should be present in every daughter molecule containing that UID…"); EX1001, 2:19-23 ("Because all amplicons derived from a

particular starting molecule will bear its specific tag, any variation in the sequence …of identically tagged sequencing reads can be discounted as technical error."), 26:63 ("Reads were grouped according to SMI sequence."). *see also* EX1002, ¶¶92-94.

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.

Based on the '699 patent, a POSA would have understood "collapsing" to be the process by which consensus sequence information is drawn from redundant sequence reads derived from the same molecule. *E.g.*, EX1001, 21:32-34 ("Reads having common (i.e., identical) SMI sequences were grouped together, and were collapsed to generate a consensus read."); *see also* EX1002, ¶¶95-96.

Kinde discloses or teaches Element 1.6. For example, Kinde assesses multiple reads and identifies a consensus among the multiple reads, Kinde distinguishes a true mutation, which Kinde called a "supermutant," where "at least 95% of family members have the identical mutation." EX1039, 9530. Indeed, distinguishing errors from true mutations by such consensus sequencing was well known in the art at the time. *See, e.g.*, EX1025, 1347 ("[Unique barcoding] has been applied recently … to improve the sensitivity of DNA mutation detection (16, 17) …"); EX1001, 2:23-26 ("This [unique barcoding] approach has been used to

… correct base errors arising during PCR or sequencing [36, 37, 39].”); *see also* EX1002, ¶97.

Accordingly, claim 1 rendered obvious by the prior art at the time. EX1002, ¶¶72-98.

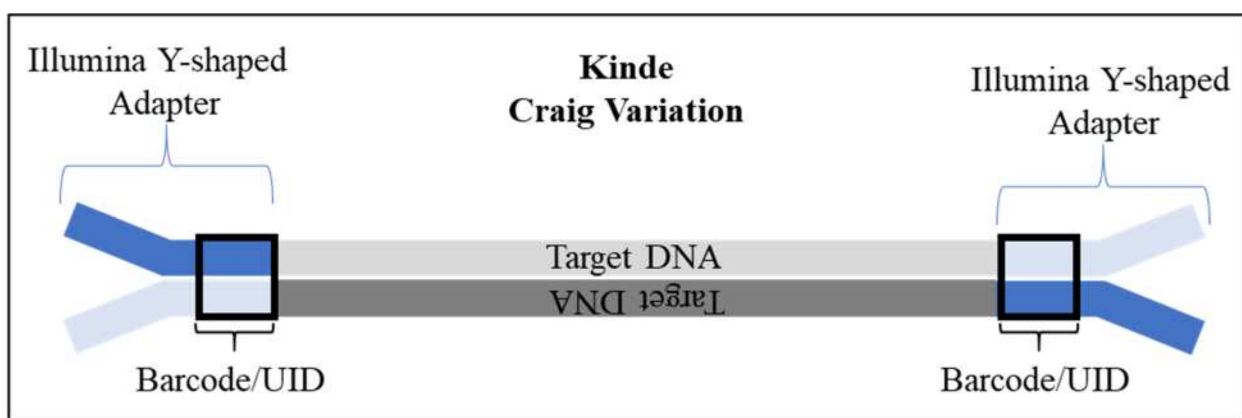
ii. Claim 20

Preamble and Element 20.1

> 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;

Discussion above regarding claim 1 is relevant to claim 20. Kinde discloses “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.” As explained above with respect to Element 1.1, the term “circulating DNA molecules” includes ***cellular DNA*** (e.g., blood cells) within the circulatory system and Kinde discloses that cellular DNA may be obtained from blood and “blood lymphocytes.” EX1039, 9530, SI1. Tagging cellular DNA obtained from a blood sample (e.g., blood lymphocytes) according to the Craig Variation is within the scope of claim 20. The Craig Variation includes attaching

barcoded Illumina Y-shaped adapters to sheared DNA molecules. EX1039, 9531 (UIDs may be introduced "through …ligation (42, 43)."); *see also* EX1025, 1347 ("In our approach, each cDNA molecule is attached to a unique barcode sequence … This concept has been applied recently…to improve the sensitivity of DNA mutation detection (16, 17) …."). Sheared DNA tagged according to the Craig Variation is shown below. *See also* EX1002, ¶¶99-100.



The tagged parent polynucleotides produced according to the Craig Variation are within the scope of claim 20 because, in this method, barcodes are attached to circulating DNA molecule (e.g., cellular DNA from blood) to produce a population of tagged original DNA molecules. *See also* EX1002, ¶101.

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, SI1. 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, ¶102.

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. Two different tagged DNA molecules, even bearing identical SMIs, have different sequences by virtue of the molecules themselves. *See also* EX1002, ¶¶103-104.

Element 20.2

> b) amplifying the population of tagged original DNA molecules to produce a corresponding population of DNA molecule amplicons;

Kinde discloses Element 20.2 for the same reasons it discloses Element 1.3. Kinde discloses "amplification" of the tagged template molecules. EX1039, Abstract, Figure 2, SI1; *see also* EX1001, 2:16-19; EX1002, ¶¶105-106.

Element 20.3

> c) sequencing at least a portion of the population of DNA molecule amplicons to produce a set of sequence reads;

Kinde discloses Element 20.3 for the same reasons that it discloses Element 1.4. Kinde discusses sequencing throughout its disclosure. For example, Kinde's approach involves "redundant sequencing" of the tagged strands and of their amplification products. EX1039, Abstract; *see also id.*, 9530-9531, Figs. 1-2, 9535 ("Sequencing"); EX1002, ¶¶107-108.

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

As discussed above regarding Element 1.5, Kinde discloses that reads derived from the same original molecule may be grouped "into UID families on the basis of their …UIDs." EX1039, 9535; *see also id.*, 9530 ("If a mutation preexisted

in the template molecule used for amplification, that mutation should be present in every daughter molecule containing that UID…"), Abstract ("amplification of each uniquely tagged template molecule to create UID families"), 9530 (defining "daughter molecules with the identical sequence" as a "UID family"), 9531 (explaining that UIDs may be endogenous or exogenous). *See also* EX1002, ¶¶109-110.

Element 20.4 also broadly recites "sequence information derived from the circulating DNA molecule." To the extent determinable, Kinde satisfies this limitation. Kinde discloses that its analysis including UID family grouping makes use of sequence information from the template DNA molecule. EX1039, 9530 (defining "daughter molecules with the identical sequence" as a "UID family"). Kinde also discloses alignment of sequence reads to a reference. EX1039, SI2 ("…alignment w[as] performed with the Eland pipeline (Illumina)…"). Kinde also discloses that reads are removed from analysis where sequence information is of insufficient quality and where sequence information exceeds a threshold level of base mismatches against a reference sequence. *Id*. ("Only high-quality reads meeting the following criteria were used for subsequent analysis: … (ii) every base in the read had a quality score ≥20; and (iii) ≤3 mismatches to expected sequences."); *see also* EX1002, ¶¶109-111.

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.

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, 9530-9531. 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." 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, ¶¶112-113.

Accordingly, claim 20 is rendered obvious by the prior art at the time. EX1002, ¶¶99-114.

iii. Claims 2 and 23

> 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.

> 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.

Kinde discloses "the approach can easily be used to identify rare mutants in a population of DNA templates." EX1039, 9533; *see also id*., 9534 (describing detection of "variant bases"), SI3 (describing application to "Mutation prevalences in normal human tissues"). As Dr. Quackenbush explains, a POSA would understand the rare mutants and variant bases described by Kinde to include single nucleotide variants. Accordingly, claims 2 and 23 are rendered obvious by the prior art at the time. EX1002, ¶¶115-116.

iv. Claim 3

> 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.

As discussed, both Kinde and Craig utilize Illumina adapters according to prior art protocols. Kinde, for example, disclosed converting the original parent DNA molecules into tagged DNA molecules using sticky-end ligation. EX1039, 9535 ("DNA was fragmented to an average size of ~200 bp by acoustic shearing (Covaris) and then end-repaired, A-tailed, and ligated to Y-shaped adapters

according to standard Illumina protocols."); *see also* EX1001, 26:50-54 ("The DNA was randomly sheared by the Covaris AFA system, followed by end-repair, A-tailing, and ligation of Illumina TruSeq DNA sequencing adaptors, all by standard library preparation methods."). Accordingly, claim 3 is rendered obvious by the prior art at the time. EX1002, ¶¶117-118.

v. Claim 4

> 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.

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, SI2. As Dr. Quackenbush explains, a POSA thus would understand Kinde's disclosure of comparing to "expected sequences" to include 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 sequence. EX1039, 9531. Accordingly, the prior art renders claim 4 obvious. EX1002, ¶¶119-121.

vi. Claims 5 and 7

> 5. The method of claim 1, further comprising filtering out sequence reads that fail to meet a quality threshold.
>
> 7. The method of claim 1, further comprising removing a subset of the sequence reads from further analysis prior to (e).

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, and no more than 3 mismatches as compared to "expected sequences." EX1039, SI2. Only "high-quality" sequence reads satisfying these quality thresholds were included in final grouping (step e), such that sequence reads not satisfying these quality thresholds were removed from further analysis. *Id*. Accordingly, claims 5 and 7 are rendered obvious by the prior art at the time. EX1002, ¶¶122-123.

vii. Claim 6, 21, and 22

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

Kinde discloses applying its methodology in combination with targeted sequence capture, whereby "genes of interest [are captured] on a solid phase" prior to sequencing. EX1039, 9531, Figure 2. Kinde also discloses performing sequence alignment with the Illumina Eland pipeline and identifying mismatches as compared to "expected" (reference) sequences." EX1039, SI2. Accordingly, claims 6, 21, and 22 are rendered obvious by the prior art at the time. EX1002, ¶¶124-125.

viii. Claims 8 and 9

> 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%.

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. *E.g.*, EX1039, 9531; *see also id.*, Title, Abstract, 9530, 9533 (discussing identification of "rare variants" using the disclosed approach). Accordingly, claims 8 and 9are rendered obvious by the prior art at the time. EX1002, ¶¶126-127.

ix. Claims 10 and 11

> 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 $1x10^{-6}$ or is as low as about $1.2x10^{-9}$.
>
> 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 $1.5x10^{-4}$ or about $3.5x10^{-5}$.

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.*, SI2-SI3 ("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, ¶¶128-129.

Moreover, the '699 patent admits that the error rates achieved with its methodology are comparable to those achieved in Kinde. EX1001, 27:32-43. Specifically, the '699 patent reports that relying on complementary strands to make base calls resulted in "an overall mutation frequency of $4.25x10^{-5}$" 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.

Accordingly, claims 10 and 11 are rendered obvious by the prior art at the time. EX1002, ¶¶130.

x. Claims 12 and 24

> 12. The method of claim 1, wherein the circulating DNA molecules are nucleic acid-based serum biomarkers.
>
> 24. The method of claim 20, wherein at least a portion of the circulating DNA molecules are nucleic acid-based blood biomarkers.

Patent Owner has argued analysis of a whole blood sample is inclusive of nucleic acid-based biomarkers. IPR2022-00746, Paper 2, 22 (arguing that application of Duplex Sequencing to DNA obtained from whole blood is evidence of application of the method to cell-free DNA). While Petitioner does not necessarily agree, Kinde teaches or suggests applying its method to a blood sample, including "blood lymphocytes." EX1039, SI1. Kinde also suggests detecting "donor DNA in the ***blood*** of organ transplant patients." *Id*., 9530. Kinde further explains that its method may be applied with "any sample preparation workflow." *Id*., 9533. Accordingly, claims 12 and 24 are rendered obvious by the prior art at the time.EX1002, ¶¶131-132.

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, ¶133-134.

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, the Craig Variation uses barcoded Illumina Y-shaped adapters. EX1039, 9531; *see also* Section I.B.ii. Furthermore, Craig describes 48 barcoded adapters which is within the range of “about 2 to about 256.” EX1036, 888. 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, ¶135.

Given the lack of relevant disclosure in the ’699 patent, the prior art renders obvious claims 13, 14, 26, and 27. *See also* EX1002, ¶136.

xii. Claim 15

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

Claim 15 depends from claim 1 and further recites the polynucleotide barcodes are contained within a library generated from oligonucleotides comprising known sequences. For example, the Craig Variation uses barcoded Illumina Y-shaped adapters. EX1039, 9531. Furthermore, Craig describes 48 barcoded adapters comprising known sequences. EX1036, Supplementary Tables 3 and 4 (listing barcode sequences). Accordingly, claim 15 is rendered obvious by the prior art at the time. EX1002, ¶¶137-138.

xiii. Claim 16 and 17

> 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.

As discussed above with respect to Element 1.2, Kinde teaches or suggests that non-uniquely tagged parent polynucleotides are substantially unique with

respect to the other non-uniquely tagged parent polynucleotides in the population. Kinde further discloses using UIDs, within the adapter or the sheared ends, to differentiate tagged molecules. *E.g.*, EX1039, Abstract ("amplification of each uniquely tagged template molecule to create UID families"), (defining "daughter molecules with the identical sequence" as a "UID family"), 9531 (explaining that UIDs may be endogenous or exogenous). Kinde further teaches that 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, SI1. Accordingly, claims 16 and 17 are rendered obvious by the prior art at the time. *See also* EX1002, ¶¶139-140.

xiv. Claim 25

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

As discussed above, Kinde discusses cellular DNA sources, including blood cells. Kinde discloses that its approach is relevant to cancer diagnosis. *E.g.*, EX1039, 9530 ("counting genetic or epigenetic changes in tumors can inform fundamental issues in cancer biology"). Moreover, as described above with respect to claims 12 and 24, it would have been obvious to apply Kinde's method to cellular DNA from blood. Accordingly, claim 25 is rendered obvious by the prior art at the time. EX1002, ¶¶141-142.

xv. Claims 18 and 19

> 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.

Kinde 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)." EX1039, Figure 1; *see also* Figure 2 ("One uniquely identifiable fragment is produced from each strand of the double-stranded template[.]"); EX1002, ¶¶143-144.

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, SI2. 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. *E.g.*, EX1039, SI3 ("specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation"); *see also* EX1039, 9535, SI1 (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). Accordingly, claims 18 and 19 are rendered obvious by the prior art at the time. EX1002, ¶145.

VII. DISCRETIONARY DENIAL IS NOT APPROPRIATE HERE

A. Discretionary Denial is Not Appropriate Under Section 325(d)

The Board's §325(d) analysis applies a two-part framework guided by several non-exclusive *Becton, Dickinson* factors. *Advanced Bionics, LLC v. Med-El Elektromedizinische Geräte GMBH*, IPR2019-01469, Paper 6, 9-10 (precedential); *Becton, Dickinson & Co. v. B. Braun Melsungen AG*, IPR2017-01586, Paper 8, 17-18 (precedential, §III.C.5 ¶1). First, the Board considers whether "the same or substantially the same" art or arguments were previously presented to the Office. *Advanced Bionics*, Paper 6, 8. If so, the Board considers whether the petition demonstrates that the Office "erred in a manner material to the patentability of challenged claims." *Id.* Denial of institution is not warranted here because the petition does not present "the same or substantially the same prior art or arguments" previously considered times by the Office. Moreover, to the extent the second prong is reached, the petition establishes that the Office materially erred in issuing the '699 patent.

First, the references relied upon to establish unpatentability of the claims, were not presented in their entirety or were not presented at all. Craig was neither disclosed in an IDS nor applied by the Examiner in a prior art rejection. The Miner reference was disclosed in an IDS but not applied by the examiner in a prior art rejection. *Cellco P'ship D/B/A Verizon Wireless v. Huawei Device Co., Ltd.*,

IPR2020-01117, Paper 10, 12-15 (§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, 15-16 (examiner erred by overlooking relevant teachings in reference listed on IDS). Indeed, the Examiner never applied a prior art rejection. While Kinde and Shiroguchi were listed in an IDS, the Supporting Information published along with these references (which is relied on in the petition) was absent. *See Mylan Pharms. Inc. v. Regeneron Pharms., Inc.*, IPR2021-00880, Paper 21, 11-13 (§325(d) not applied where IDS submission included only a portion of the disclosure of the asserted reference). Finally, Patent Owner's opening claim construction brief was served July 28 and not before the Examiner during *ex parte* prosecution. EX1040, EX1041. Accordingly, the Examiner could not have been aware of Patent Owner's broad interpretation of the claims.

Second, even if prong two is reached, the current petition establishes that allowance of the claims was material error. The claims broadly encompass circulating cellular DNA and tagging wherein a plurality of the tagged parent polynucleotides has identical barcodes. There can be no dispute that Kinde describes a method for detecting rare mutation in genomic DNA as the '699 patent acknowledges. EX1001, 2:14-32. Furthermore, Kinde expressly describes its method as applicable to blood and blood lymphocytes. EX1039, 9530, SI1. As to

the claimed tagging scheme, Kinde discloses producing a plurality of tagged parent polynucleotides with identical barcodes. EX1039, SI1. This disclosure is confirmed by additional prior art references, including Miner, which Patent Owner now concedes generates identically tagged parent polynucleotides at a rate of at least 3 in every 1000 molecules. EX1040, 6.

B. Discretionary Denial is Not Appropriate in View of Previous IPRs

Regardless of the statutory institution standard based on the likelihood of success on the merits, the Board will, at times, "exercise the Director's discretion to decline to institute review" when non-institution promotes "the integrity of the patent system, the efficient administration of the Office, and the ability of the Office to timely complete proceedings[.]" CTPG, 55-56. Among other things, the Board will take into account whether "repeated attacks on patents" constitute an "abuse of the review process." *Id.* There is, however, "no *per se* rule precluding the filing of follow-on petitions." *Id.* 58 (quoting *General Plastic Co., Ltd. v. Canon Kabushiki Kaisha*, IPR2016-01357, Paper 19, 15 (precedential). Instead, the Board is tasked with assessing "the potential impacts on both the efficiency of the *inter partes* review process and the fundamental fairness of the process for all parties with the aid of seven non-exclusive factors. *Id.,* 56-58. Indeed, care must be taken not to privilege a formalistic analysis of these factors over the overarching fairness and efficiency concerns to which they are directed because "[t]he General Plastic

factors, alone or in combination, are not dispositive, but part of a balanced assessment of all relevant circumstances in the case, including the merits." *Id.,* 58.

It is appropriate for the Board to institute the present petition in view of the Board's decisions denying institution in IPR2022-00449 and IPR2022-00450. A prior petition by the same petitioner against the same patent claims presents no bar to institution under *General Plastic* when the Board never reached the merits of patentability under the prior petition and the petitioner derived few if any benefits from seeing the decision denying institution and the patent owner preliminary response. *See, e.g.*, *Regeneron Pharms., Inc. v. Novartis Pharma AG*, IPR2021-00816, Paper 13, 17-20 (instituting subsequent petition where prior decision denying institution under *Fintiv* did not reach the patentability merits and Patent Owner disturbed the basis for non-institution by terminating the parallel trial); *Wells Fargo Bank, N.A. v. USAA*, IPR2019-01081, Paper 9, 12-20 (instituting subsequent petition where prior CBM non-institution "did not reach the merits of the prior art ground of unpatentability," and the subsequent IPR petition presented argument that could not have been raised in the prior petition). Institution is appropriate here because the decisions denying institution did not reach the patentability merits of the petitions on various key issues and because Patent Owner's post-petition claim construction fundamentally altered the patentability

landscape, affording petitioner with a patentability argument that even Patent Owner argued could not have been raised in the prior petitions.

When Guardant filed the petitions in IPR Nos. 2022-00449, -00450, Guardant explained that the claims of the ’699 patent were substantially copied from U.S. Patent No. 9,384,822 to Talasaz and lack the requisite supporting disclosure in any priority document that pre-dates Talasaz, making Talasaz anticipatory prior art. IPR2022-00450, Paper 2, 1. Guardant also explained that because the ’699 patent and Kinde provided substantially identical (though minimal) discussion of cfDNA, any argument by Patent Owner that the claims of the ’699 patent are entitled to an earlier priority date would only underscore a lack of distinction of the claims over Kinde and vice versa. *Id.*, 1, 3. Guardant presented the same claim construction of circulating DNA molecules in the two petitions, and requested both written description and obviousness of the claims be evaluated on the same level playing field. That never happened.

After Guardant had filed its petitions in IPR Nos. 2022-00449, -00450, Patent Owner argued two different interpretations for the same term in the same patent. In IPR2022-00449, Patent Owner argued that the term “circulating DNA molecule” broadly encompasses both circulating cfDNA molecules and circulating cellular DNA. *E.g.*, IPR2022-00449, Paper 8, 26 (“the specification describes circulating DNA molecules, both within cells and cell-free”), 26 (“*circulating*

neoplastic *cells*”), 27 (“circulating DNA molecules are obtained from…circulating cells”), 30 (“circulating tumor cells….”). In IPR2022-00450, Patent Owner ignored cellular DNA entirely and only argued Kinde’s applicability to cfDNA. IPR2022-00450, Paper 8, 4. Patent Owner specifically argued that the petition should be denied because of Guardant’s previous responses in a different case contested Kinde’s disclosure of cfDNA. *See, e.g.*, *id.*, 14. Guardant did not dispute Patent Owner’s broader construction of “circulating DNA molecules” and argued that Kinde plainly discusses application of its method to blood cells, thereby satisfying Patent Owner’s different construction. *See, e.g.*, IPR2022-00450, Paper 11, 1. Guardant also argued that expanding the claimed genus to include cellular DNA only exacerbates the ’699 patent’s lack of priority. *Id.* Patent Owner responded that any argument its claims encompassed cellular DNA should be ignored because the petitions proffered a claim construction that “excluded cellular DNA from its arguments.” *See, e.g.*, IPR2022-00450, Paper 12, 1, 3 (“This is false.”).

The Board’s decisions denying institution did not address Patent Owner’s broader interpretation of its claims or any argument the ’699 patent claims encompassed application of Kinde’s method to circulating cellular DNA. IPR2022-00449, Paper 13, 7; IPR2022-00450, Paper 13, 7-8. The Board denied institution in both petitions without ever reaching the merits of unpatentability on the circulating cellular DNA issue, and certainly not in IPR2022-00450. Specifically, the Board

denied the priority challenge under §325(d) based on its conclusion that an examiner had considered in a sibling application descriptive support for the even broader term "circulating nucleic acid molecules." IPR2022-00449, Paper 13, 9-18. The Board then denied the other petition on the basis that the claims encompassed only circulating cfDNA (and not cellular DNA) and Guardant had previously argued that Kinde did not appreciate the challenges which are presented when working with cfDNA. IPR2022-00450, Paper 13, 10-20. The Board acknowledged the petition's reliance on Kinde's citation to two references for teaching attaching Y-shaped adapters containing UIDs, but disregarded this argument because "those reference, like Kinde, only discuss analysis of cellular DNA, and not cfDNA." *Id.*, 19-20. The Board thus never addressed the merits of Petitioner's patentability arguments in the petitions that Kinde provides at least as much support for tagging cfDNA as the priority documents for the '699 patent. Nor did the Board address petitioner's argument that Kinde at least discloses tagging circulating DNA molecules under Patent Owner's interpretation of the term which includes circulating cellular DNA.

Four days before the Board issued those decisions, Patent Owner's counsel in the parallel district court case served its opening claim construction brief directly contradicting the Board's interpretation of the '699 patent claims as stated in IPR2022-00450. EX1040; EX1041. Patent Owner specifically agreed that

circulating DNA molecules is not limited to circulating cfDNA. EX1040, 16 ("The parties have also agreed that the scope of the claim terms include, ***among other DNA***, cell-free DNA circulating within the circulatory system."). Patent Owner specifically agreed that the term includes cellular DNA (e.g., blood cells) within the circulatory system. EX1040, 15-16 (identifying an agreeable construction for "circulating DNA molecule(s)" as DNA molecules that circulate within the circulatory system, which can include cell-free DNA and ***cellular DNA***.)

Patent Owner's construction of circulating DNA molecules just four days before the institution decisions were issued provides ample justification for Guardant to file, and the Board to institute, the instant petition. *See Regeneron Pharms., Inc*, IPR2021-00816, Paper 13, 17-20; *Wells Fargo Bank, N.A.*, IPR2019-01081, Paper 9, 12-20.

General Plastic factors 2-5 support institution because the Board never reached the merits of the petitions' patentability arguments under Patent Owner's interpretation (and now agreed construction at district court) and the current petition was necessitated by Patent Owner's district court actions—which Patent Owner did not disclose to the Board. Guardant could not have included Patent Owner's claim construction in the prior petitions because of the way Patent Owner timed its disclosure of its claim construction positions to obtain strategic advantage. Patent Owner's recent claim construction revelation was material.

Indeed, Patent Owner's turnabout disturbed the very bases for non-institution by making Kinde's lack of actual disclosure of tagging of cfDNA molecules and Guardant's prior statements on that issue non-dispositive and even irrelevant. Guardant filed this petition immediately after realizing the Board had relied on claim construction representations in the pre-institution sur-replies that Patent Owner later abandoned just days before the decisions were issued without informing the Board. Guardant thus files this petition within two weeks of the Board's decisions. Moreover, though the circumstances of the challenge have changed remarkably in view of Patent Owner's about-face on claim construction, Guardant's underlying unpatentability theory has not changed remarkably. Because the Board never reached the merits of the current petition's patentability arguments and the current petition was necessitated by Patent Owner's actions, there is no significant, undue prejudice from Guardant having been served with the preliminary responses or the Board's institution decisions. *General Plastic* factors 2-5 therefore favor institution here. *See Regeneron Pharms., Inc*, IPR2021-00816, Paper 13, 17-20; *Wells Fargo Bank, N.A.*, IPR2019-01081, Paper 9, 12-20.

Moreover, there is no instituted trial against the '699 patent and no reason the Board cannot issue a decision within the statutory deadline (factors 6-7). Accordingly, each of factors 2-7 favor institution and the General Plastic factors as a whole favor institution. *Id.* Should the Board institute this petition, Guardant will

withdraw its rehearing requests in IPR2022-00449, -00450, providing an efficient means to resolve the merits of the unpatentability of the '699 patent and thereby promote the integrity of the patent system. Because institution will serve the interests of efficiency and patent system integrity that are foundational to the A.I.A., institution should be granted. CTPG, 55-56.

Patent Owner's claim construction gamesmanship also means that discretionary denial would not be appropriate under §325(d). The term "circulating DNA molecules" is construed herein as including (not excluding) circulating cellular DNA molecules, consistent with the parties' claim construction positions at district court. CTPG, 45 ("The Office …applies the same claim construction standard that would be used to construe the claim in a civil action…."). As discussed above, at least the IPR2022-00450 petition was denied on the erroneous bases that 1) "circulating DNA molecules" included circulating cfDNA but excluded circulating cellular DNA; and 2) that "Patent Owner does not dispute" that interpretation. Paper 13, 7. The Board therefore never reached Kinde's disclosure of tagging circulating cellular DNA and focused instead on prior statements by Guardant regarding whether Kinde specifically discloses tagging ***cfDNA*** obtained from the blood. Patent Owner's alternate position on claim construction renders the petition case non-cumulative and also amounts to new evidence supporting the unpatentability of the claims. Under Patent Owner's

district court claim construction there can be no dispute that Kinde discloses tagging circulating DNA molecules. Accordingly, institution should be granted. *See Advanced Bionics, LLC v. Med-El Elektromedizinische Geräte GMBH*, IPR2019-01469, Paper 6, 8-9 (precedential).

C. Discretionary Denial is Not Appropriate in View of the District Court Proceeding

The parties appear to be in agreement that the present petition does not implicate the Board's discretion according to *Apple Inc. v. Fintiv, Inc.*, IPR2020-00019, Paper 11 (precedential). Patent Owner recently filed two petitions (IPR2022-01115, -01116) challenging patents at issue in the same district court proceeding in which it argued that there are no *Fintiv* issues. The corresponding district court case is at an early stage and is still not currently assigned to a judge. Should circumstances change or it become necessary, Guardant is willing to file the requisite stipulation in accordance with the recent USPTO Guidelines regarding *Fintiv*.

VIII. 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: August 10, 2022

/ Michael T. Rosato /

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

IX. 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 13,752 words, excluding the parts of the brief exempted by 37 C.F.R. §42.24(a).

Respectfully submitted,

Dated: August 10, 2022

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

X. 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.

XI. 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	Intentionally left blank
1006	U.S. Patent Application No. 16/411,066 File History
1007-1024	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	Intentionally left blank
1039	Kinde et al., "Detection and quantification of rare mutations with massively parallel sequencing," *PNAS* (June 7, 2011)

1040	Plaintiff's Opening Claim-Construction Brief, *Twinstrand Biosciences, Inc. et al. v. Guardant Health, Inc.*, Case No. 21-1126-VAC-SRF (July 27, 2022)
1041	Notice of Service, *Twinstrand Biosciences, Inc. et al. v. Guardant Health, Inc.*, Case No. 21-1126-VAC-SRF (July 28, 2022)
1042	Data Sheet: Illumina Sequencing - TruSeq RNA and DNA Sample Preparation Kits, Illumina (2010)
1043	European Patent No. EP 2 893 040 to Talasaz et al.
1044	Response to Notice of Opposition, European Patent No. 2 893 040 (May 2020)
1045	Krimmel et al., "Ultra-deep sequencing detects ovarian cancer cells in peritoneal fluid and reveals somatic *TP53* mutations in noncancerous tissues," *PNAS 113*(21) (May 24, 2016)
1046	Summons In a Civil Action, *Twinstrand Biosciences, Inc. et al. v. Guardant Health, Inc.*, Case No. 21-1126-VAC-SRF (August 11, 2021)

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 1001-1046) by overnight courier (Federal Express or UPS), on this 10th day of August, 2022, on the Patent Owner at the correspondence address of the Patent Owner as follows:

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4311 11th Ave NE
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Respectfully submitted,

Dated: August 10, 2022

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