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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. IPR2022-00935
Patent No. 10,287,631

PETITION FOR INTER PARTES REVIEW OF U.S. PATENT NO. 10,287,631

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LISTING OF CHALLENGED CLAIMS

1. [**Preamble**] A method of generating high accuracy sequence reads of a population of double-stranded target nucleic acid molecules, comprising:

[**Element 1.1**] ligating each of the double-stranded target nucleic acid molecules to at least one adapter molecule, to form a population of adapter-target nucleic acid complexes, wherein each of the adapter molecules comprises—

[**Element 1.2**] (a) a degenerate or semi-degenerate single molecule identifier (SMI) sequence that alone or in combination with the target nucleic acid fragment ends uniquely labels each ligated double-stranded target nucleic acid molecule such that each ligated double-stranded target nucleic acid molecule is distinguishable from other ligated double-stranded target nucleic acid molecules in the population, and

[**Element 1.3**] (b) a strand-distinguishing nucleotide sequence that, following the ligation step, provides a region of non-complementarity between a first strand of each adapter-target nucleic acid complex and a second strand of the same adapter-target nucleic acid complex;

[**Element 1.4**] for each of the adapter-target nucleic acid complexes—

amplifying each strand of the adapter-target nucleic acid complex to produce a plurality of first strand adapter-target nucleic acid complex amplicons and a plurality of second strand adapter-target nucleic acid complex amplicons;

[**Element 1.5**] sequencing the adapter-target nucleic acid complex amplicons to produce a plurality of first strand sequence reads and plurality of second strand sequence reads;

[**Element 1.6**] grouping the first strand sequence reads and the second strand sequence reads into a family of first and second strand sequence reads based on the degenerate or semi-degenerate SMI sequence alone or in combination with the target nucleic acid fragment ends;

[**Element 1.7**] separating the first and second strand sequence reads into a set of first strand sequence reads and a set of second strand sequence reads based on the region of non-complementarity between the first strand and the second strand of the adapter-target nucleic acid complex;

[**Element 1.8**] confirming the presence of at least one first strand sequence read and at least one second strand sequence read;

[**Element 1.9**] comparing the at least one first strand sequence read with the at least one second strand sequence read;

[**Element 1.10**] identifying nucleotide positions where the compared first and second strand sequence reads are non-complementary;

identifying nucleotide positions where the compared first and second strand sequence reads are complementary; and

generating a high accuracy consensus sequence read for each of the double-stranded target nucleic acid molecules in the population that includes only the nucleotide positions where the compared first and second strand sequence reads are complementary.

2. The method of claim 1, wherein the confirming step includes confirming the presence of at least 2 first strand sequence reads and at least 2 second strand sequence reads.

3. The method of claim 1, further comprising identifying one or more nucleotide positions that disagree between the at least one first strand sequence read and the at least one second strand sequence read.

4. The method of claim 1, wherein for any double-stranded target nucleic acid molecule in the population, the method further comprises:

comparing the high accuracy consensus sequence read to a respective reference sequence; and

identifying one or more nucleotide sequence variations present in the high accuracy consensus sequence read not present in the reference sequence.

5. The method of claim 4, further comprising identifying a variation occurring at a particular position in the high accuracy consensus sequence read as a true mutation.

6. The method of claim 4, further comprising identifying a variation that occurs at a particular position in only one of the first strand sequence read or the second strand sequence read as a potential artifact.

7. The method of claim 1, wherein for any double-stranded target nucleic acid molecule in the population, the method further comprises:

comparing the high accuracy consensus sequence read to a reference sequence;

identifying one or more variations present in the high accuracy consensus sequence read not present in the reference sequence; and

identifying at least one of a cancer, a cancer risk, a cancer metabolic state, a mutator phenotype, a carcinogen exposure, a chronic inflammation exposure, an age, a neurodegenerative disease, or a combination thereof in an organism from which the double-stranded target nucleic acid molecule is derived by the one or more variations present in the high accuracy consensus sequence read.

8. The method of claim 1, wherein the population of double-stranded target nucleic acid molecules includes double-stranded DNA or other nucleic acid fragments.

9. The method of claim 1, wherein each of the adapter-target nucleic acid complexes comprises at least two primer binding sites.

10. The method of claim 1, wherein the adapter molecule ligated to any particular double-stranded target nucleic acid molecule comprises a Y-shape, a U-shape, or a combination thereof.

11. The method of claim 1, wherein the population of adapter-target nucleic acid complexes comprise an SMI sequence in each of its strands.

12. The method of claim 1, wherein each adapter molecule comprises a double-stranded SMI sequence, and wherein the double-stranded SMI sequence comprises a first degenerate or semi-degenerate sequence and a second degenerate or semi-degenerate sequence.

13. The method of claim 12, wherein the first and second degenerate or semi-degenerate sequences are at least partially complementary.

14. The method of claim 1, wherein the population of adapter-target nucleic acid complexes comprise an SMI sequence at each terminus.

15. The method of claim 1, wherein the degenerate or semi-degenerate SMI sequence comprises from about 3 to about 20 nucleotides.

16. [**Preamble**] A method of generating high accuracy sequence reads of a population of double-stranded target nucleic acid molecules, wherein each individual double-stranded target nucleic acid molecule comprises one or more fragment features that distinguish the individual double-stranded target nucleic acid molecules from other double-stranded target nucleic acid molecules in the population, the method comprising:

[**Element 16.1**] ligating each of the double-stranded target nucleic acid molecules to at least one double-stranded adapter molecule, to form an adapter-target nucleic acid complex, wherein—

the at least one double-stranded adapter molecule comprises a region of non-complementarity between a first adapter strand of the double-stranded adapter molecule and a second adapter strand of the double-stranded adapter molecule, and

[**Element 16.2**] following the ligation step, the region of non-complementarity provides a strand-distinguishing nucleotide sequence such that a first strand of the adapter-target nucleic acid complex has a distinctly identifiable nucleotide sequence relative to its complementary second strand;

[**Element 16.3**] for each of the adapter-target nucleic acid complexes—

amplifying each strand of the adapter-target nucleic acid complex to produce a plurality of first strand adapter-target nucleic acid complex amplicons and a plurality of second strand adapter-target nucleic acid complex amplicons;

[**Element 16.4**] sequencing the adapter-target nucleic acid complex amplicons to produce a plurality of first strand sequence reads and plurality of second strand sequence reads;

[**Element 16.5**] grouping the first and second strand sequence reads into a family of sequence reads by identifying the one or more distinguishing fragment features shared by each strand of the double-stranded target nucleic acid molecule;

[**Element 16.6**] separating the first and second sequence reads from each family into a set of first strand sequence reads and a set of second strand sequence reads based on the strand-distinguishing nucleotide sequence;

[**Element 16.7**] confirming the presence of at least one first strand sequence read and at least one second strand sequence read;

[**Element 16.8**] comparing the at least one first strand sequence read with the at least one second strand sequence read; and

[**Element 16.9**] generating a high accuracy consensus sequence read for each of the double-stranded target nucleic acid molecules in the population that includes only the nucleotide positions where the compared first and second strand sequence reads are complementary.

17. The method of claim 16, wherein the double-stranded target nucleic acid molecule is a double-stranded DNA or other nucleic acid fragment.

18. The method of claim 16, wherein the one or more fragment features includes a shear point or other fragment region, or a combination thereof.

19. The method of claim 16, wherein the adapter-target nucleic acid complex comprises at least two primer binding sites.

20. The method of claim 16, wherein the adapter-target nucleic acid complex comprises a Y-shape, a U-shape, or a combination thereof.

21. The method of claim 16, wherein generating a high accuracy consensus sequence further comprises identifying nucleotide positions where the compared first and second strand sequence reads are non-complementary and scoring the identified non-complementary nucleotide positions as potential artifacts.

22. The method of claim 1, wherein each adapter molecule comprises a single stranded SMI sequence, and wherein the method further comprises converting the single-stranded SMI sequence to a double-stranded SMI sequence by polymerase extension.

23. The method of claim 1, wherein generating a high accuracy consensus sequence further comprises marking the identified nucleotide positions where the compared first and second strand sequence reads are non-complementary and scoring the identified non-complementary nucleotide positions as potential artifacts.

Guardant Health, Inc., ("Petitioner") hereby requests review of United States Patent No. 10,287,631 to Jesse Salk et al. (hereinafter "the '631 patent," EX1001), which is currently assigned to The University of Washington Through Its Center for Commercialization ("Patent Owner"). This petition demonstrates by a preponderance of the evidence, that claims 1-23 of the '631 patent are unpatentable for failing to distinguish over prior art.

I. INTRODUCTION

The '631 patent specification purports to have invented duplex consensus sequencing ("DCS"), which is essentially a poorly defined marketing term related to redundantly sequencing both strands of an original DNA duplex molecule to generate a consensus sequence read. *E.g.*, Claim 16. The '631 patent describes use of commercially available Y- or U-shaped adapters (*e.g.*, Illumina, Pacific Biosciences), and claims their use for otherwise standard sequencing processes. EX1001, 3:42-4:3, 5:63-6:33, 10:58-64, 18:54-62, 26:1-9. But sequencing multiple copies (*e.g.*, amplification progeny) of a molecule to determine sequence information by "consensus" was well-known at the time and described throughout the scientific literature, including processes that used Y-shaped or U-shaped adapters.

Kinde (EX1006), for example, renders obvious each of claims 16-21. Kinde discloses the same sequencing method of the claims. Kinde exemplifies consensus

sequencing using commercially available Y-shaped adapters that meet the limitations of the claims. Kinde also discusses comparing sequence for both strands of a particular DNA molecule to identify and exclude from the consensus sequence artifacts of the amplification and sequencing process to obtain high accuracy sequence reads.

Claims 1-15, 22, and 23 are obvious over Kinde in view of Craig (EX1007). Kinde discusses tagging the DNA molecules with exogenous barcodes, citing Craig specifically. Craig discloses employing barcodes in the double-stranded portion of Y-shape adapter molecules.

Further, corroborating obviousness of all claims, the Travers Publications (EX1018; EX1021) further demonstrates knowledge in the art regarding the use of "asymmetric" adapter molecules comprising a region of non-complementarity to compare the sequences of the first and second strands to achieve a high accuracy consensus sequence.

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

A. 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. As Dr. Quackenbush explains and as addressed in further detail below, the involved claims would not have been considered new or non-obvious to a person of ordinary skill in the art ("POSA") at the relevant time.

i. Consensus sequencing and strand-distinguishing ("asymmetric") adapters

Before March 20, 2012, skilled artisans were aware that sequencing errors may arise as artifacts of next-generation sequencing ("NGS"), and that such errors were mitigated by redundantly sequencing copies of a molecule and evaluating resulting sequence data for consensus. EX1021, Abstract; EX1006, Abstract; EX1031, Abstract; EX1006, 9530 (describing copies as "UID" families); EX1031, 4 ("Identification of PCR duplicates …"); EX1010, Figure 1. Consensus of sequence information determined based on multiple reads (i.e., sequenced copies) of a target molecule better represented the "true" sequence of the original molecule because the sporadic errors found in individual reads would not be represented in the consensus. EX1006, 9530 ("…at least 95% of family members …"); EX1031, 4 ("…derived a consensus sequence…"); EX1018, ¶15; EX1020, 24:9-20, 33:24-37; EX1021, Abstract ("…this consensus sequence is obtained from both the sense and antisense strands…"). There was nothing new about NGS, barcodes, or consensus sequencing and the '631 patent does not assert otherwise. *See also* EX1002, ¶27.

Prior to the ’631 patent, nucleic acid sequencing techniques conventionally used partially-complementary adapters that, when ligated to double-stranded DNA enable reads associated with each strand (i.e., top and bottom) of double-stranded DNA molecules to be distinguished. Such adapters were not only commercially available from Illumina and Pacific Biosciences (“PacBio”), but were routinely used to convert sample DNA into sequence-ready DNA molecules. EX1012, Figure 4[1]; EX1013, Figure 1; EX1018, Figure 7; EX1021, Figure 1. One such example is Illumina Y-shaped adapters. EX1029, Figure 1, Figure 4, 14:15-29, 19:17-64, 20:24-32, 25:1-5, 26:13-24. 31:8-18; EX1009, Abstract, ¶¶15-16, 52, 55; *see also* EX1002, ¶¶28-33.

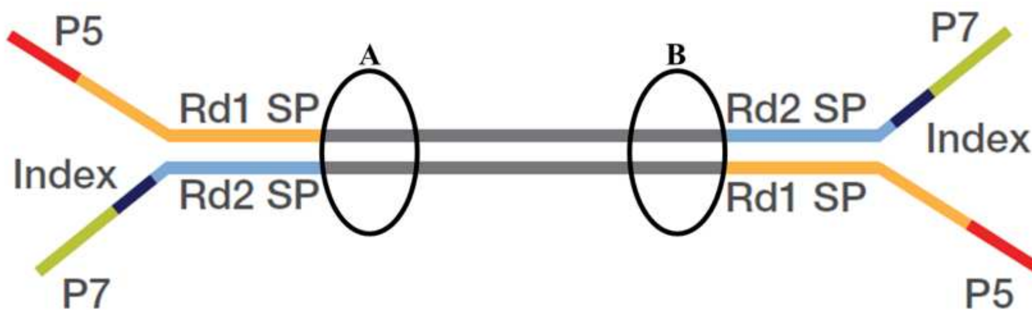
Illumina Y-shaped adapters are a type of prior art partially single-stranded (*e.g.*, region of non-complementarity), partially-complementary adapter relevant to the challenged claims. Indeed, the ’631 patent describes its DCS method as using standard commercially available Illumina (*e.g.*, TruSeq) DNA sequencing adapters. EX1001, 25:59-26:9; *see also id.*, 18:50-57. Illumina TruSeq adapters are Y-shaped adapters that include flow cell

[1] As demonstrated by EX1033 and its explanation of The Internet Archive, EX1012 was publicly available as of December 14, 2010.

sequences (*e.g.*, P5 and P7), sequencing primer binding sites (*e.g.*, Rd1 SP and Rd2 SP), and an index (*i.e.*, barcode). EX1012, Figure 4 (shown).

Typical Illumina sequencing workflows include ligation of Y-shaped adapters to sample DNA prior to PCR amplification. EX1013, Figure 1 ("DNA fragments are…joined to a pair of oligonucleotides in a forked adaptor configuration. The ligated products are amplified using two oligonucleotide primers, resulting in double-stranded blunt-ended material with a different adaptor sequence on either end."); EX1012, Figure 4; *see also* EX1001, 18:50-67 ("…the DCS approach can be generalized to nearly any sequencing platform …").

As shown below, by virtue of the asymmetric nature of the adapted fragments, two types of PCR products are produced from each capture event. Those derived from one strand will have the A sequence adjacent to flow-cell sequence P5 and the B sequence adjacent to flow cell sequence P7. PCR products originating from the complementary strand are labeled reciprocally (*i.e.*, P7 adjacent to A and P5 adjacent to B).



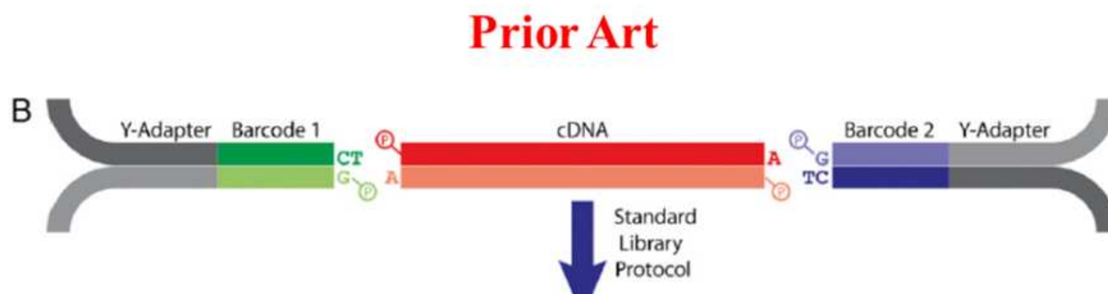
EX1012, Figure 4 (shown with annotations); *see also* EX1022, ¶42. This "asymmetry" is no different than what is discussed in the '631 patent. EX1001, 3:56-62, Figure 1; EX1002, ¶¶34-37.

Skilled artisans recognized the asymmetry of both Y- and U-shaped adapter molecules, including Illumina adapter-DNA molecules, and utilized the adapter structure to identify reads associated with each strand of double-stranded DNA molecules. As Dr. Quackenbush explains, this was corroborated in the scientific literature at the time. *E.g.*, EX1006, Figure 2; EX1009, ¶¶15-17, 55; EX1010, Figure 1, Figure S5; EX1018, ¶¶54, 76, 98; EX1021, Figure 4; EX1022, ¶42; EX1029, 14:15-29, 19:34-64, Figures 1 and 4; EX1002, ¶¶38-39.

Kinde, for example, discloses "ligat[ion of] standard Illumina sequencing adapters to the ends of sheared DNA fragments to produce a standard sequencing library." EX1006, 9531. Kinde explains that adapter-DNA molecules produced in this manner generate "One uniquely identifiable fragment … from each strand of the double-stranded template." EX1006, Figure 2. According to Kinde, the ability to individually label both strands of DNA molecules with Y-shaped adapters can be harnessed to improve the specificity of consensus sequencing for detection of rare mutations. EX1006, SI3 ("…specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation ….").

Y-shaped adapters conventionally included barcode sequences. As shown above, Illumina TruSeq Y-shaped adapters comprise a barcode or index. Prior art Y-shaped adapters further included a barcode in the double-stranded portion of the adaptor. EX1007, 888, Supplementary Methods; EX1002, ¶¶40-42. Kinde specifically discloses that methods include "the introduction of exogenous sequences" citing to Craig. EX1006, 9531.

Shiroguchi further illustrates that barcoded Y-shaped adapters were well-known at the time, and further confirms such methods enabled strand-specific labeling. Specifically, Shiroguchi describes Y-shaped adapters that include a barcode in the double-stranded portion and produce adapter-DNA molecules that are "asymmetric" in the manner discussed in the '631 patent. *Compare* EX1010, Figure 1B (reproduced below) *with*, EX1001, Figure 1, 3:56-62.



According to Shiroguchi, the adapters were "designed in the same Y-shaped construct as the conventional Illumina paired-end adapter with a 22- to 25-bp extension that contained the barcode and a T-overhang (Fig. 1B)." EX1010, SI3.

Shiroguchi confirms its strand-specific labeling enabled distinction of different strands of the DNA molecule. *See, e.g.*, EX1010, Figure S5 ("We are able to distinguish the different strands of an original cDNA molecule because of the design of the paired-end sequencing adapters (Fig. 1B)."), Figure 1. Moreover, Shiroguchi corroborates that POSAs at the time contemplated utilizing such adapter molecules in consensus sequencing approaches as described by Kinde and others. EX1010, 1347 (citing Casbon (reference 16) and Kinde (reference 17)).

Accordingly, prior to the '631 patent, skilled artisans knew about strand-specific labeling with Y-shaped adapters, consensus sequencing, and adapter-DNA "asymmetry" that enabled distinguishing sequence reads from different strands of double-stranded DNA molecules. EX1002, ¶¶43-46.

ii. Kinde

"Detection and quantification of rare mutations with massively parallel sequencing" by Kinde, et al. ("Kinde," EX1006) was published in *Proceedings of the National Academy of Sciences* and includes a manuscript and supporting information. According to Kinde's methods, DNA template molecules are assigned barcodes ("UIDs"), amplified and sequenced, and grouped into families for consensus sequence analysis:

> …Safe-Sequencing System ("Safe-SeqS"), involves two basic steps (Fig. 1). The first is the assignment of a unique identifier (UID) to each DNA template molecule to be analyzed. The second is the

> amplification of each uniquely tagged template, so that many daughter molecules with the identical sequence are generated (defined as a UID family). If a mutation preexisted in the template molecule used for amplification, that mutation should be present in every daughter molecule containing that UID (barring any subsequent replication or sequencing errors). A UID family in which at least 95% of family members have the identical mutation is called a "supermutant." Mutations not occurring in the original templates, such as those occurring during the amplification steps or through errors in base calling, should not give rise to supermutants.

EX1006, 9530. As discussed above and elsewhere herein, Kinde discloses ligating Illumina sequencing adapters, uniquely identifying each strand of the double-stranded template, and consensus sequencing and comparison of both strands. *E.g.*, EX1006, 9531, Figure 2, SI3. As recited on the front page, Kinde was published June 7, 2011 and qualifies as a prior art under 35 U.S.C. §102(a)(1) and pre-AIA 35 U.S.C. §102(a). EX1002, ¶47.

iii. 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 describes "multiplexed resequencing … on the Illumina Genome Analyzer using degenerate indexed DNA bar codes ligated to fragmented DNA before sequencing." EX1007, 887. Craig's

adapters are derived from standard Illumina adapters and include an "index in the forward and reverse directions respectively." *Id.*, Supplementary Methods. 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, ¶48.

iv. Travers

This petition relies on a patent publication and a paper describing sample preparation and sequencing methods developed at PacBio. These publications are collectively referred to as "the Travers Publications" and are described below.

Travers '075 (EX1018): U.S. Patent Publication No. 2009/0298075 to Travers, et al. is entitled "Compositions and Methods for Nucleic Acid Sequencing" and is assigned on its face to PacBio. Travers '075 was published on December 3, 2009 and qualifies as prior art under 35 U.S.C. §102(a)(1) and pre-AIA 35 U.S.C. §102(b). EX1002, ¶¶49-50.

The Travers Paper (EX1021): "A flexible and efficient template format for circular consensus sequencing and SNP detection," by Travers, et al. was published in *Nucleic Acids Research*. The Travers Paper is authored by scientists employed by PacBio (including inventors of Travers '075) and describes applications of the sequencing method described in Travers '075. *E.g.*, EX1021, Abstract, 7 ("All the authors are employees of Pacific Biosciences."), Figure 1.

The Travers Paper was published online on June 22, 2010 and qualifies as a prior art printed publication under 35 U.S.C. §102(a)(1) and pre-AIA 35 U.S.C. §102(b).

As discussed herein, the Travers Publications provide disclosure relevant to all aspects of the challenged claims. The Travers Publications disclose "asymmetric" U-shaped or hairpin adapters used for redundant sequencing of a target molecule. EX1018, Abstract (discussing "redundant sequence determination" and "iteratively sequencing the entire construct multiple times"), ¶57, Figure 3B; EX1021, Abstract (determining "consensus sequence" from "multiple passes on a single molecule"), Figure 1. The Travers method includes collectively analyzing (grouping) multiple reads of the target molecule and determining consensus sequence of the target molecule based on the multiple reads. EX1018, ¶58 ("assembling the sequence data from multiple reads of a given sequence…to provide an overall consensus sequence for that segment"). This has all been admitted by Patent Owner. EX1001, 10:58-11:12; EX1024, 550-551. Moreover, the Travers Publications disclose use of barcodes together with its U-shaped or hairpin adapters in determining a consensus sequence. EX1018, ¶100 ("template constructs that include sequence markers like a barcode"); *see also* EX1018, ¶¶15, 54, 101, 102, 122; EX1021, 3 ("ability to read both strands"), Figure 4 ("generating 4-fold coverage"); EX1002, ¶¶49-52.

B. The ’631 Patent

The ’631 patent is entitled “Methods of lowering the error rate of massively parallel DNA sequencing using duplex consensus sequencing.” EX1001, cover (54). The ’631 patent specification proposes correcting sequencing errors with a tag-based method referred to as “Duplex Consensus Sequencing” or “DCS.” *E.g.*, EX1001, Abstract, 3:10-62.

As with prior art consensus sequencing methods, DCS generally includes tagging polynucleotides with identifier sequences (“SMI,” barcode), amplifying the SMI-tagged molecules using PCR, and sequencing the amplified products. EX1001, 2:11-26, 3:10-40. As in Kinde, the sequenced tagged molecules are grouped into families based on a common SMI sequence. *Id*., 3:10-31, 3:44-62, 20:56-58, 26:51-53 (to compare to Kinde, “reads were grouped into families by SMI tag”), Figure 1. Like in Kinde and elsewhere, “consensus” is determined from the grouped sequence reads. *Id*., 3:26-40, 4:4-29, Figure 3; *see also* Section I.A.

Regarding “adapter molecules” recited in the current claims, the ’631 patent describes using prior art Y- or U-shaped adapter molecules. EX1001, 5:63-64 (“The SMI adaptor molecule may form a “Y-shape” or a “hairpin shape.”), 10:56-64 (“…As the sequence of the linker itself does not matter in the workflow, the published linker sequences from [Travers] would be adequate for use in the assay.”), 18:54-57 (“…use of DCS does not require any significant deviations from

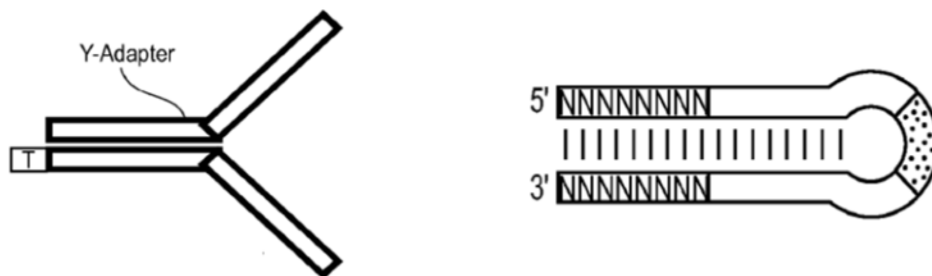
the normal workflow of sample preparation for Illumina DNA sequencing.), 22:51-52 ("Standard Illumina library preparation protocols…"), 26:1-9 ("…ligation of Illumina TruSeq DNA sequencing adaptors, all by standard library preparation methods…"); *compare* EX1010, Figure 1B (reproduced above) *with*, EX1001, Figure 1, 3:44-62; *see also Twinstrand Biosciences, Inc. v. Guardant Health, Inc.*, IPR2022-00746, Paper 2, 67 (Patent Owner conceding intent to encompass Craig's barcoded Y-shaped adapters); *see also* EX1002, ¶¶21-26.

C. Prosecution History

The '631 patent was filed as U.S. Application No. 15/660,785 ("the '785 application") on July 26, 2017, claiming priority, via U.S. Application Nos. 14/386,800 and PCT/US2013/032665, 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. The '785 application received disjointed examination due to replacement of assigned Examiners during *ex parte* prosecution.

The first Examiner rejected claims of the '785 application, *inter alia*, as anticipated by Steemers (WO2012/061832, EX1035). EX1004, 140-149. The first Examiner found the "SMI" limitation was met by disclosure in Steemers of using "pool[s] of random oligos" to "tag[] molecules uniquely so each individual molecule can be tracked and identified." EX1004, 144-145 (citing EX1035, 41-42); *see also id.*, 137 ("Using broadest reasonable interpretation art that teaches use

of random barcodes to uniquely tag molecules would read upon SMI sequence.”). The first Examiner also correctly found that conventional Y- or U-shaped adapters (shown below) satisfied the claim requirements as illustrated by Steemers.[2] EX1004, 141, 147 (citing EX1035, Figures 15 and 16).



In response, Patent Owner argued (incorrectly) that Steemers is “silent” regarding consensus sequencing. EX1004, 290 (“Steemers is silent with respect to generating high accuracy sequence reads...”). Consensus sequencing, however, is expressly disclosed by Steemers. EX1035, 46:31-47:2 (“Sequencing duplicate nucleic acid fragments is advantageous in methods that include creating a

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[2] During prosecution of a later filed continuation application, which issued as U.S. Pat. No. 10,760,127 (“the ’127 patent”), Patent Owner convinced a different Examiner that Y- and U-shaped adapter labeling of a target molecule was a point of novelty. EX1036, 432-433. The Examiner’s uncontested findings during prosecution of the ’631 patent, confirm that issuance of the ’127 patent represents error.

consensus sequence for duplicate fragments. Such methods can ***increase the accuracy*** for providing a consensus sequence for a template nucleic acid and/or library of template nucleic acids.").

After the Office Action response, the case was transferred to a different Examiner who immediately allowed the case. The Examiner largely just recited the claim language in the reasons for allowance. EX1004, 304.

D. Level of Skill in the Art

As Dr. Quackenbush explains, a person of ordinary skill in the art by March 20, 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, ¶¶53-54.

II. GROUNDS FOR STANDING

Petitioner certifies that, under 37 C.F.R. §42.104(a), the '631 patent is available for *inter partes* review, and Petitioner is not barred or estopped from requesting *inter partes* review of the '631 patent on the grounds identified.

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

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

Related Matters (37 C.F.R. §42.8(b)(2)): Patent Owner has asserted the '631 patent against Petitioner in the United States District Court in Delaware (1:21-cv-01126-LPS). Petitioner filed petitions for *inter partes* review against related U.S. Patent Nos.10,689,699 (IPR2022-00449 and IPR2022-00450) and 10,760,127 (IPR2022-00816 and IPR2022-00817). Additionally, TwinStrand Biosciences, Inc. filed petitions for *inter partes* review against related U.S. Patent Nos.10,801,063 (IPR2022-00746) and 10,889,858 (IPR2022-00747).

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

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

Petitioners request review of claims 1-23 of the '631 patent under 35 U.S.C. §311 and AIA §6. The grounds for relief are as follows:

Ground	Claims	Description
1	16-21	Unpatentable under 35 U.S.C. §103 as obvious over Kinde
2	1-15, 22, and 23	Unpatentable under 35 U.S.C. §103 as obvious over Kinde and Craig
3	1-23	Unpatentable under 35 U.S.C. §103 as obvious over Kinde in view of Craig and The Travers Publications

V. CLAIM CONSTRUCTION

No terms need be construed to find the claims unpatentable in view of the prior art. *Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co.*, 868 F.3d 1013, 1017 (Fed. Cir. 2017). In mapping the prior art to the claims, Petitioner has applied the "the meaning that [a] term would have to a person of ordinary skill in the art in question at the time of the invention." *Phillips v. AWH Corp.*, 415 F.3d 1303, 1313 (Fed. Cir. 2005) (en banc).

Independent claim 1 recites "a ***strand-distinguishing nucleotide sequence*** that, following the ligation step, ***provides a region of non-complementarity*** between a first strand of each adapter-target nucleic acid complex and a second strand of the same adapter-target nucleic acid complex." Independent claim 16 recites "an adapter-target nucleic acid complex" wherein ***"the region of non-complementarity provides a strand-distinguishing nucleotide sequence*** such that a first strand of the adapter-target nucleic acid complex has a distinctly identifiable nucleotide sequence relative to its complementary second strand." Claims 10 and

20, which depend from claims 1 and 16, respectively, recite that the adapters comprise a Y-shape or a U-shape.

As Dr. Quackenbush explains, and as explained above in Section I.A, standard Illumina Y-shaped adapters are double-stranded adapter molecules having a region of non-complementarity (the arms of the Y) between the first adapter strand and the second adapter strand, and provide a strand-distinguishing nucleotide sequence. When the adapters are ligated to a double-stranded DNA molecule, the resulting adapter-DNA molecule is partially-complementary, has a region of non-complementarity, and is "asymmetric," enabling distinction between reads from the different strands of the original DNA molecule. EX1010, Figure S5 ("We are able to distinguish the different strands of an original cDNA molecule because of the design of the paired-end sequencing adapters (Fig. 1B)."); EX1009, ¶¶14-16, 55. The same is true of U-shaped hairpin adapters. EX1002, ¶¶55-58. This understanding of the claims is consistent with the language of the claims (discussed above) and the specification of the '631 patent. *E.g.*, EX1001, Figures 1-3, 3:44-62, 4:4-9, 10:55-64, 18:54-59, 22:22-27.

Accordingly, the plain meaning of these claims is reasonably understood to encompass adapter-target nucleic acid complexes generated by attachment of Y-shaped or U-shaped adapters to each end of double-stranded DNA molecules.

VI. DETAILED EXPLANATION FOR GROUNDS OF UNPATENTABILITY

A. [Ground 1] Claims 16-21 are unpatentable as obvious under 35 U.S.C. §103 over Kinde

As discussed in detail below, Kinde discloses or renders obvious each of claims 16-21. Kinde discloses consensus sequencing methods that reduce errors associated with library preparation and sequencing to improve sequence accuracy. Kinde discloses its high-accuracy consensus sequencing methods are useful for detection of rare mutations in sample DNA. Kinde discloses that the specificity of the method may be further increased by excluding apparent mutations from the consensus sequence when they are not confirmed to be present in both strands of the original DNA fragment. EX1002, ¶59.

As explained in detail herein, a POSA would have had good reason to arrive at the claimed subject matter with a reasonable expectation of success. The obviousness of the claims is confirmed by the substantial identity between the '631 patent and the art at the time including Kinde. *In re McDaniel*, 293 F.3d 1379, 1385 (Fed. Cir. 2002) ("anticipation is the epitome of obviousness"). To the extent any differences vis-à-vis Kinde exist, they are unclaimed here or readily apparent in view of knowledge in the art as corroborated by the scientific literature at the time. *Intelligent Bio-Systems, Inc. v. Illumina Cambridge*, Ltd., 821 F.3d 1359, 1367 (Fed. Cir. 2016) ("The reasonable expectation of success requirement refers to the likelihood of success in combining references to meet the limitations of the

claimed invention…"); *Randall Mfg. v. Rea*, 733 F.3d 1355, 1362 (Fed. Cir. 2013) ("[T]he knowledge of [a skilled] artisan is part of the store of public knowledge that must be consulted when considering whether a claimed invention would have been obvious.").

Like the DCS method described in the '631 patent, Kinde uses barcodes—called unique identifiers or UIDs—to uniquely identify target DNA molecules and their amplification progeny. *E.g.*, EX1006, Figure 2. According to Kinde, target DNA molecules comprising a UID are amplified, sequenced, and then grouped into UID families. Base calls (*i.e.*, sequence) are evaluated based on consensus among multiple reads, and mutations are identified when they represent the consensus base call (*i.e.*, the base present in ≥95% of reads within a family) as compared to a reference sequence, which Kinde calls "supermutants." *Id*., 9530.

Kinde describes performing consensus sequencing by ligating Y-shaped (*e.g.*, Illumina) adapters to randomly sheared DNA molecules to produce adapter-DNA molecules that have a region of non-complementarity that provides a strand-distinguishing sequence. In Kinde's endogenous UID embodiment, the random shear sequence generated at the ends of the DNA molecules is the UID. EX1006, 9531 ("…DNA inherently contains UIDs consisting of the sequences of the two ends of each sheared fragment…"), ("…ligated standard Illumina sequencing adapters to the ends of sheared DNA fragments…"). Kinde explains that, from its

method of random shearing and ligating adapters, "One uniquely identifiable fragment is produced from each strand of the double-stranded template." EX1006, Figure 2; Section I.A. Kinde further discloses the specificity of its method is increased by analyzing both strands of the original double-stranded DNA and excluding sequence positions from the consensus sequence unless they are confirmed to be present in both complementary strands. EX1006, SI3 ("the specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation.").

Kinde discloses that the endogenous UID embodiment produces high-accuracy sequence analysis. For example, Kinde discloses that in one experimental example, the raw sequence reads identified "25,563 apparent mutations," but after applying Kinde's methods, there were "only eight supermutants." EX1006, 9531 & Table 1 (mutations/bp reduced ~98%). Kinde discloses "Safe-SeqS decreased the presumptive sequencing errors by at least 70-fold." *Id*.

The '631 patent acknowledges that consensus sequencing based on barcoding or tagging was known, and further acknowledges similarity to the methods of Kinde. EX1001, 2:11-33; 26:23-62, 32:33-36. Indeed, Kinde discloses the same workflow discussed in the '631 patent—attaching adapters (*e.g.*, Illumina Y-shaped adapters), amplifying tagged polynucleotides, sequencing, grouping the sequence reads, analyzing sequence reads for each strand of the original double-

stranded template, and generating consensus from redundant reads. EX1002, ¶¶60-63.

There is no meaningful distinction between the consensus sequencing method described by Kinde and that claimed by the '631 patent. To illustrate this, one may look to Example 3 of the '631 patent, which employs an indistinguishable process of randomly shearing DNA and then ligating it to Illumina TruSeq adapters using "standard library preparation methods." EX1001, 25:59-26:9; *see also* Section I.A. The '631 patent further explains that the "first 10 nucleotides of each sequencing read pair, corresponding to the randomly sheared DNA ends, were combined…to yield an SMI tag[.]" EX1001, 26:10-14. Reads were grouped into families according to these endogenous sequences, and consensus required agreement among "at least 90% of family members." EX1001, 26:15-18.

Kinde describes the same method. Kinde discloses randomly shearing genomic DNA then ligating it to Illumina adapters. EX1006, 9531 ("…we ligated standard Illumina sequencing adapters to the ends of sheared DNA fragments to produce a standard sequencing library …"). Kinde discloses that the randomly sheared ends provide a unique identification sequence. *Id*. ("randomly sheared genomic DNA inherently contains UIDs consisting of the sequences of the two ends of each sheared fragment …"). Kinde discloses grouping reads into "UID families" according to these endogenous sequences with consensus requiring

agreement among "95% of family members." EX1006, 9530, Figure 2. Kinde discloses "the specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation." EX1006, SI3; *see also id*., SI2; EX1002, ¶¶64-65.

To the extent that Kinde does not expressly detail precisely how it distinguishes the first and second strand sequence reads, a POSA would have understood that Kinde's sequence reads from the different strands were distinguishable based on the Y-shaped adapter design. As explained in detail above, the asymmetry of a Y-shaped adapter, such as a standard Illumina adapter, enables the two strands of a double-stranded nucleotide molecule to be distinguished. *See* Section I.A above. This is corroborated by both Shiroguchi and the '631 patent. EX1010, Figure 1, Figure S5 ("We are able to distinguish the different strands of an original cDNA molecule because of the design of the paired-end sequencing adapters (Fig. 1B)."); EX1001, Figure 1, 3:56-62, 26:10-22; EX1002, ¶¶66-67. As such, documented knowledge in the art confirms obviousness of the '631 patent claims in view of Kinde. *Randall*, 733 F.3d at 1362.

An element-by-element discussion of the obviousness of the claims, together with 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.

i. Claim 16

Preamble

> A method of generating high accuracy sequence reads of a population of double-stranded target nucleic acid molecules, wherein each individual double-stranded target nucleic acid molecule comprises one or more fragment features that distinguish the individual double-stranded target nucleic acid molecules from other double-stranded target nucleic acid molecules in the population, the method comprising:

To the extent the Preamble is limiting, Kinde discloses generating high-accuracy sequence reads wherein double-stranded nucleic acids are distinguished from other double-stranded nucleic acids in the population. As explained above, Kinde discloses a method for reducing errors in sequencing reads using substantially the same steps as DCS (*e.g.*, assigning UID, amplifying, sequencing, consensus formation). Kinde further describes generating high-accuracy sequence reads for populations of double-stranded target nucleic acid molecules. EX1006, 9533 ("Safe-SeqS approach can substantially improve the accuracy of massively parallel sequencing"), 9531 Figure 2 ("double-stranded template"); *see also* EX1001, 26:56-62 (describing DCS and Safe-SeqS as yielding a "comparable degree" of error correction). Kinde explains that its methods "substantially improve the accuracy of massively parallel sequencing" such that "rare mutants" may be identified in "population[s] of DNA templates." EX1006, 9533, 9530. Kinde further discloses increasing the specificity of the method for detecting rare

mutations "by requiring that each strand of the original double-stranded template contain the mutation." EX1006, SI3.

Kinde discloses distinguishing double-stranded nucleic acids within a population based on UIDs in a manner that addresses the claimed "fragment features." As an initial matter, the term "fragment features" is not used in the specification nor was it discussed during prosecution. Claim 18 depends from claim 16 and specifies that the fragment feature "includes a shear point or other fragment region." EX1001, 40:35-37. As discussed above, the '631 patent specification discloses randomly shearing genomic DNA and ligating Illumina TruSeq sequencing adapters, and repeatedly describes the endogenous sequence of the sheared molecule ends as an "SMI sequence." EX1001, Example 3 ("…Randomly Sheared DNA Ends as Single Molecule Identifiers"), 25:59-27:14; *see also* Section I.A.

Kinde's disclosure regarding "UIDs" is comparable to the '631 patent discussion of SMIs. For example, Kinde discloses assigning "unique identifier (UID)" sequences to the double-stranded nucleic acids in the population. EX1006, 9530; *see also id.*, Figure 1 ("each fragment to be analyzed is assigned a unique identification (UID) DNA sequence"), Figure 2; EX1001, 2:11-15 (citing Kinde as an example in which "DNA fragments to be sequenced are each uniquely tagged prior to amplification"). Kinde explains that UIDs may comprise endogenous

and/or exogenous sequences. EX1006, 9531 ("UIDs, sometimes called barcodes or indexes, can be assigned to nucleic acid fragments using a variety of methods. These methods include the introduction of exogenous sequences through PCR (40, 41) or ligation (42, 43). 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).").

Accordingly, Kinde discloses each aspect of the Preamble. EX1002, ¶¶68-72; *see also* Section I.A above.

Element 16.1

> ligating each of the double-stranded target nucleic acid molecules to at least one double-stranded adapter molecule, to form an adapter-target nucleic acid complex, wherein—
>
> the at least one double-stranded adapter molecule comprises a region of non-complementarity between a first adapter strand of the double-stranded adapter molecule and a second adapter strand of the double-stranded adapter molecule, and

Kinde discloses Element 16.1. Kinde discloses ligating Y-shaped adapters (*e.g.*, standard Illumina adapters) to double-stranded DNA molecules to form adapter-DNA complexes. EX1006, 9531 ("…we ligated ***standard Illumina sequencing*** adapters to the ends of sheared DNA fragments"), Figure 2. Double-stranded DNA fragments were sheared, "end-repaired," and "A-tailed," then ligated to "Y-shaped adapters."

EX1006, 9535, SI1. As explained herein, prior art Y-shaped adapters have a region of non-complementarity (*i.e.*, the arms of the Y) between the first and second strands of the double-stranded adapter molecule. EX1012, Figure 4 (shown); Section I.A*; see also* EX1002, ¶¶73-75.

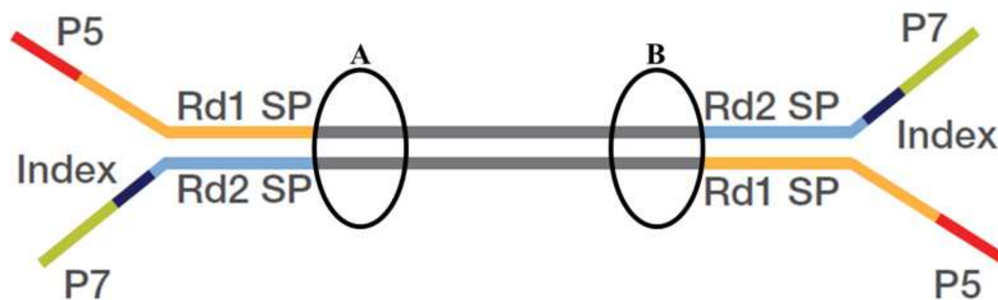
Element 16.2

> following the ligation step, the region of non-complementarity provides a strand-distinguishing nucleotide sequence such that a first strand of the adapter-target nucleic acid complex has a distinctly identifiable nucleotide sequence relative to its complementary second strand;

Kinde discloses and certainly renders obvious, Element 16.2. This entire element is simply reciting well-known and art-recognized properties of prior art, asymmetric, sequencing adapters (*e.g.*, standard Y-shaped Illumina adapters) that would have been recognized by a POSA at the time and are documented in the literature. *See* Section I.A.

As discussed above for Element 16.1, Kinde discloses ligating Y-shaped "standard Illumina sequencing adapters" to double-stranded DNA fragments "according to standard Illumina protocols." EX1006, 9531 ("Endogenous UIDs"), Figure 2, SI1. Kinde discloses that, ultimately, "[o]ne uniquely identifiable fragment is produced from each strand of the double-stranded template." EX1006, Figure 2; *see also* Section I.A; EX1002, ¶¶76-77.

As explained above, standard Illumina adapters ligated to sample DNA produce partially-complementary, asymmetrical double-stranded adapter-DNA molecules. Section I.A. By virtue of the non-complementary region of the adapted fragments, two types of PCR products are produced from each capture event. Those derived from one strand will have the A sequence adjacent to flow-cell sequence P5 and the B sequence adjacent to flow cell sequence P7. PCR products originating from the complementary strand are labeled reciprocally (*i.e.*, P7 adjacent to A and P5 adjacent to B).



EX1012, Figure 4 (modified to include annotations). The "asymmetry" discussed in the '631 patent is no different. EX1001, 3:56-62, Figure 1.

The region of non-complementarity provides a strand-distinguishing nucleotide sequence such that a first strand of the adapter-target nucleic acid complex has a distinctly identifiable nucleotide sequence relative to its complementary second strand. This is confirmed by both Shiroguchi and the '631 patent. EX1010, Figure 1, Figure S5 ("We are able to distinguish the different strands of an original cDNA molecule because of the design of the paired-end

sequencing adapters (Fig. 1B).”); EX1001, Figure 1, 3:56-62, 26:10-22; Section I.A; EX1002, ¶¶78-80.

Accordingly, it was well-known that, following ligation, prior art Y-shaped adapters (*e.g.*, Illumina adapters) provide an adapter-DNA complex where the top strand of the duplex is distinguishable from the bottom strand based on a nucleotide sequence in the region of non-complementarity. *See* Section I.A above; EX1013, Figure 1 (“double-stranded blunt-ended material with a different adaptor sequence on either end.”); EX1012, Figure 4; *see also* EX1001, 18:50-59 (“DCS does not require any significant deviations from the normal workflow of sample preparation for Illumina DNA sequencing.”), 22:22-27 (“…adaptors which contain the standard sequences required for the Illumina HiSeq system were synthesized, but with addition of a double-stranded, complementary SMI sequence.”); EX1002, ¶¶76-82.

Element 16.3

> for each of the adapter-target nucleic acid complexes—
>
> amplifying each strand of the adapter-target nucleic acid complex to produce a plurality of first strand adapter-target nucleic acid complex amplicons and a plurality of second strand adapter-target nucleic acid complex amplicons;

Kinde discloses Element 16.3. For example, Kinde discloses “amplification” of each strand of adapter-target nucleic acid complexes. EX1006, Abstract, Figure 2 (“PCR amplification”), SI1 (“PCR-mediated amplification”), (“18 cycles of PCR

”). Kinde specifically discloses that amplification produces copies of both the first and second strands. *E.g.*, EX1006, Figure 2 (“One uniquely identifiable fragment is produced from each strand…”), SI3 (“specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation”). Kinde thereby produced a plurality of first strand adapter-target nucleic acid complex amplicons and a plurality of second strand adapter-target nucleic acid complex amplicons. *E.g.*, EX1006, 9530 (“the amplification of each uniquely tagged template, so that many daughter molecules with the identical sequence are generated”), 9531 (“…69,505 UID families, with an average of 40 members per family…”); EX1002, ¶¶83-85; Section I.A.

Element 16.4

> sequencing the adapter-target nucleic acid complex amplicons to produce a plurality of first strand sequence reads and plurality of second strand sequence reads;

Kinde discloses Element 16.4. Kinde discusses sequencing throughout its disclosure. For example, Kinde’s approach involves “redundant sequencing of the amplification products.” EX1006, Abstract; *see also id.*, Figure 2, 9535 (“Sequencing”). As discussed above for Element 16.3, amplification of Kinde’s adapter-DNA molecules produces first and second strand amplicons. *Id.*, 9530 (“Overview”), Figure 2. Kinde further describes producing a plurality of first strand sequence reads and plurality of second strand sequence reads. Specifically,

Kinde discloses that UID families must have a plurality of reads (*i.e.*, members). EX1006, 9531 ("average of 40 members per family"). Kinde further discloses obtaining reads from both first and second strands of adapter-target nucleic acid complex amplicons. *E.g.*, EX1006, Figure 2 ("One uniquely identifiable fragment is produced from each strand…."). Indeed, Kinde expressly suggests comparing the sequences of complementary strands to one another to increase specificity. EX1006, SI3 ("specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation"); EX1002, ¶¶86-88.

Element 16.5

> grouping the first and second strand sequence reads into a family of sequence reads by identifying the one or more distinguishing fragment features shared by each strand of the double-stranded target nucleic acid molecule;

Kinde teaches or suggests Element 16.5. Kinde even expressly recites "[h]igh-quality reads were grouped into UID families on the basis of their endogenous or exogenous UIDs." EX1006, SI2; *see also id*., 9535 (same). Kinde also describes distinguishing sequence reads associated with each strand of the template. EX1006, 9531 Figure 2 ("One uniquely identifiable fragment is produced from each strand of the double-stranded template"), SI3 ("specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation").

The '631 patent specification does not recite the term "fragment features,"

but describes grouping based on SMIs. *See* Preamble. The ’631 patent describes “Randomly Sheared DNA Ends as Single Molecule Identifiers,” the “fragment features” recited in the claims. EX1001, 25:59-61. Kinde provides similar disclosure of using randomly sheared DNA fragment ends as its UIDs. EX1006, 9531 (“DNA inherently contains UIDs consisting of the sequences of the ends of each sheared fragment.”); *compare* EX1006, SI1 (“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.”) *with,* EX1001, 26:3-7 (“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 techniques.”), 26:64-65 (“using sheared DNA ends as unique molecular identifiers”). Kinde’s disclosure of using randomly sheared DNA ends as UIDs is indistinguishable from the ’631 patent specification’s description of using randomly sheared DNA ends as SMIs, or fragment features as required by the claims.

Accordingly, Kinde teaches or suggests grouping as recited in Element 16.5. EX1002, ¶¶89-92.

Element 16.6

> separating the first and second sequence reads from each family into a set of first strand sequence reads and a set of second strand sequence reads based on the strand-distinguishing nucleotide sequence;

Kinde discloses Element 16.6. As explained herein, Kinde discloses separately identifying the amplification progeny of each original strand and comparing their sequence reads. First, Kinde acknowledges that adapter-DNA molecules produce two distinct replication products: one "from each strand of the double-stranded template." EX1006, Figure 2. Second, Kinde instructs that evaluating the two strands further confirms the presence of a true variation in the original duplex. EX1006, SI3 ("the specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation"), SI2 (true "mutations are expected to be present in both strands of the relevant templates.").

While Kinde does not expressly detail how it separately identifies the amplification progeny of each original strand to compare the sequence reads, as explained above, a POSA would have appreciated that ligation of standard prior art Y-shaped adapters (*e.g.*, Illumina Y-shaped adapters) resulted in adapter-DNA complexes with strand-distinguishing sequences. *See* Sections I.A, V, VI.A.i (Elements 16.1-16.2). As discussed in detail above, the asymmetry described in the '631 patent is no different than what is provided by standard Y-shaped adapters as

described in the scientific literature at the time. *See* Section I.A; EX1010, Figure 1, Figure S5.

Accordingly, it would have been obvious to separate the first and second sequence reads as recited in Element 16.6 in view of Kinde and knowledge in the art at the relevant time. *See* Section I.A; EX1002, ¶¶93-96.

Element 16.7

> confirming the presence of at least one first strand sequence read and at least one second strand sequence read;

Kinde discloses Element 16.7. As discussed above for Element 16.6, Kinde discloses separately identifying the amplification progeny of each original strand and comparing the sequence reads from each strand to determine a consensus sequence. First, Kinde acknowledges that adapter-DNA molecules produce two distinct replication products: one "from each strand of the double-stranded template." EX1006, Figure 2. Second, Kinde instructs evaluating the two strands to confirm the presence of a true variation in the original duplex. EX1006, SI3 ("…the specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation …"); *see also id.*, SI2 ("These mutations are expected to be present in both strands of the relevant templates."). Reads for the first and second strand are confirmed in Kinde by obtaining such reads and evaluating the reads as Kinde instructs. *See* Section I.A; EX1002, ¶¶97-99.

Element 16.8

> comparing the at least one first strand sequence read with the at least one second strand sequence read; and

Kinde discloses Element 16.8. As discussed above for Element 16.6, Kinde discloses separately identifying the amplification progeny of each original strand. EX1006, Figure 2 ("One uniquely identifiable fragment…from each strand of the double-stranded template."). Kinde further discloses comparing the sequence reads from the first and second strands to determine an overall consensus on whether identified sequence information is present in both strands. EX1006, SI3 ("…the specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation …"), SI2 (true "mutations are expected to be present in both strands of the relevant templates."); *see also* EX1022, ¶9 ("Nucleotide sequences of the plurality of members of the family are compared."). The '631 patent claims are so broad as to encompass comparing at least one sequence read from the first strand with at least one sequence reads from the second strand. *See also* EX1018, ¶58; EX1002, ¶¶189-240. Accordingly, Kinde's teachings of distinguishing and analyzing reads corresponding to both strands of the template in order to improve the specificity of sequence determination discloses, or at a minimum, renders obvious Element 16.8. EX1002, ¶¶100-102.

Element 16.9

> generating a high accuracy consensus sequence read for each of the double-stranded target nucleic acid molecules in the population that includes only the nucleotide positions where the compared first and second strand sequence reads are complementary.

Kinde teaches or suggests Element 16.9. For example, Kinde generated high-accuracy consensus reads for the double-stranded target nucleic acid molecules in the population by (1) assigning UIDs (e.g., endogenous sequences on the ends of fragmented DNA); (2) ligating Y-shaped Illumina adapters to the DNA molecules; (3) amplifying the adapter-ligated DNA molecules; (4) redundantly sequencing the amplification products; and (5) excluding apparent mutations that do not represent the consensus of at least 95% of the PCR fragments with the same UID. EX1006, Abstract, 9531 (Endogenous UIDs); *see also id.*, SI2 ("UID families with <20 members had to be 100% identical at the mutant position…"); EX1002, ¶¶103-104.

Kinde confirms that its sequencing consensus analysis achieved high accuracy. For example, Kinde discloses an experiment wherein, before processing the raw sequence reads, there were "25,563 apparent mutations" but after application of Kinde's methods, there were "only eight supermutants." EX1006, 9531 & Table 1 (mutations/bp reduced ~98%). Kinde discloses that "Safe-SeqS decreased the presumptive sequencing errors by at least 70-fold." *Id*. As noted

above, the ’631 patent characterized its experimental results as providing a “comparable” reduction in error rates to that demonstrated by Kinde. EX1001, 26:51-62 (describing Kinde as “state-of-the-art,” asserting that DCS reduced the mutation/bp rate by 98%); *see also* EX1022, ¶20 (“This approach can be employed for any purpose where a very high level of accuracy and sensitivity is required from sequence data.”); EX1021, 1 (“high-accuracy consensus sequence”). Accordingly, Kinde’s method generated a high-accuracy consensus sequence read.

Kinde also teaches that consensus was determined by comparing sequence reads from the first and second strands and confirming that a given sequence read is found on both strands. *E.g.*, EX1006, SI3 (“specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation”). In other words, Kinde’s overall consensus determination included only nucleotide positions where the compared first and second strand sequence reads are complementary.

Given the complementary structure of a DNA duplex, Kinde teaches that mutations “are expected to be present in both strands of the relevant templates.” EX1006, SI2. Kinde explains that excluding from the consensus sequence nucleotide positions that do not agree in “each strand” will address errors introduced by oxidized templates, shearing, end repair, enzymatic steps, PCR, or even UID assignment, and will thereby better identify “preexisting mutation[s] in a

double-stranded DNA template [that] should give rise to two supermutants, one from each strand." EX1006, SI2. Thus, Kinde's processes teach or suggest that high-accuracy consensus sequence reads include only the nucleotide positions where the compared first and second strand sequence reads are complementary. EX1002, ¶¶105-107.

Both the Patent Owner and the '631 patent confirm that the claimed method is intended to encompass deriving consensus sequences for the top and bottom strand followed by a comparison of the two consensus sequences. *See, e.g.*, *Twinstrand*, IPR2022-00746, Paper 2, 52 ("Schmitt discloses that under optimal conditions, each DCS read arises from exactly two SSCS [single strand consensus sequence] reads (one from each strand of the initial duplex DNA molecule)"); Footnote 3, *supra*; EX1001, 16:9-22 ("Each DNA duplex should result in two single strand consensus sequences" preceding "a second stage of duplex consensus sequencing (DCS)").

Moreover, even if the claims further require excluding non-complementary base positions prior to analyzing consensus for each strand, Kinde renders this obvious. Kinde discloses setting various tolerance criteria, including tolerance criteria for excluding data based on sequence mismatches. For example, Kinde discloses excluding data that have more than 3 mismatches to expected sequences and nucleotide positions that are especially prone to sequencing errors. EX1006,

SI2. Kinde further discloses that nucleotide positions where the two strands of the DNA duplex do not match are likely errors. EX1006, SI2-SI3. Kinde thereby teaches or suggests including only the nucleotide positions where the compared first and second strand sequence reads are complementary even prior to analyzing consensus for each strand. EX1002, ¶¶108-10.

Accordingly, Kinde teaches or suggests each and every element of claim 16. EX1002, ¶¶103-10.

ii. Claim 17

> 17. The method of claim 16, wherein the double-stranded target nucleic acid molecule is a double-stranded DNA or other nucleic acid fragment.

Claim 16 is obvious as discussed above. Kinde discloses applying its methodologies to double-stranded DNA or other nucleic acid fragments. *E.g.*, EX1006, Abstract ("DNA templates"), 9530 (Results: "each DNA template"), 9531 (Endogenous UIDs: "sheared DNA"), Fig. 2 ("each strand of the double-stranded template."). Accordingly, Kinde teaches or suggests each and every element of claim 17. EX1002, ¶¶111-12.

iii. Claim 18

> 18. The method of claim 16, wherein the one or more fragment features includes a shear point or other fragment region, or a combination thereof.

Claim 16 is obvious as discussed above. Kinde discloses using shear points to group the first and second strand sequence reads into a family of sequence reads.

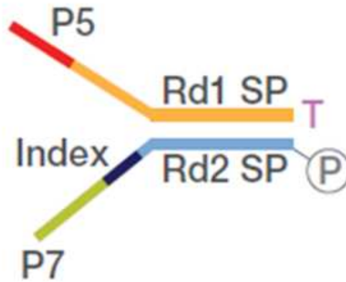
For example, Kinde discloses that “randomly sheared genomic DNA inherently contains UIDs consisting of the sequences of the two ends of each sheared fragment (Fig. 2 and Fig. S1).” EX1006, 9531; *see also id.*, Figure 2 (“The sequences of the ends of each fragment produced by random shearing (variously colored bars) serve as the unique identifiers (UIDs).”), SI1 (“The ends of each template molecule provide endogenous UIDs …”). Kinde further discloses using the sequences of the two ends of each sheared fragment to group reads derived from “original template molecules” into families. EX1006, 9531; *see also id.*, SI2 (“High-quality reads were grouped into UID families on the basis of their endogenous … UIDs…”).

Accordingly, Kinde teaches or suggests each and every element of claim 18. EX1002, ¶¶113-14.

iv. Claim 19

> 19. The method of claim 16, wherein the adapter-target nucleic acid complex comprises at least two primer binding sites.

Claim 16 is obvious as discussed above. As explained above, Kinde used Illumina Y-shaped adapters. Standard Illumina Y-shaped adapters comprise at least two primer binding sites. EX1002, ¶¶115-17; EX1012, Figure 4 (shown below); *see also* Section I.A.



The adapters exemplified by Kinde also include at least two primer binding sites. Specifically, Kinde discloses forward and reverse amplification primers that bind to sequences present in the non-complementary portion of adapter-target nucleic acid complexes. EX1006, SI1 ("…amplification of the libraries with primer sequences within the adapters…"); Table S4 ("Whole-genome amplification, for," "Whole-genome amplification, rev," "Postcapture amplification, for," "Postcapture amplification, rev"). Kinde also discloses that Illumina sequencing primers bind to sequences within the adapter. *Id.*, Table S4.

Accordingly, Kinde teaches or suggests each and every element of claim 19. EX1002, ¶¶115-17.

v. Claim 20

> 20. The method of claim 16, wherein the adapter-target nucleic acid complex comprises a Y-shape, a U-shape, or a combination thereof.

Claim 16 is obvious as discussed above. As discussed in regard to Element 16.1, Kinde discloses using standard Y-shaped Illumina adapters and exemplifies

using Y-shaped adapters. Accordingly, Kinde teaches or suggests each and every element of claim 20. EX1002, ¶118.

vi. Claim 21

> 21. The method of claim 16, wherein generating a high accuracy consensus sequence further comprises identifying nucleotide positions where the compared first and second strand sequence reads are non-complementary and scoring the identified non-complementary nucleotide positions as potential artifacts.

Claim 16 is obvious as discussed above. Kinde teaches generating a high-accuracy consensus sequence by scoring identified non-complementary nucleotide positions (compared between the first and second strand sequence reads) as potential artifacts. For example, Kinde discloses that actual mutations "are expected to be present in both strands of the relevant templates." EX1006, SI2-SI3. Kinde further discloses that apparent mutations detected in only one strand of the template may be attributed to "Error-generating processes." *Id*. Kinde thus teaches distinguishing true variants from processing/experimental errors (*i.e.*, potential artifacts) and excluding variations from the consensus sequence when they are not found on both complementary strands of a given DNA molecule. Accordingly, Kinde teaches or suggests each and every element of claim 21. EX1002, ¶¶119-20.

B. [Ground 2] Claims 1-15, 22, and 23 are unpatentable as obvious under 35 U.S.C. §103 over Kinde and Craig

Claim 1 is similar in scope to claim 16, but additionally requires "a degenerate or semi-degenerate single molecule identifier (SMI) sequence" on the adapter molecule that "uniquely labels each ligated double-stranded target nucleic acid molecule." During prosecution, the Examiner determined that "claim [1] is a species of claim [16]." EX1004, 140. Kinde in view of Craig also teaches the adapters of claim 1 and further teaches the use of the claimed adapters for consensus sequencing. As such, the combination of Kinde and Craig renders obvious each of claims 1-15, 22, and 23.

As discussed in Ground 1, Kinde exemplifies using Y-shaped Illumina adapters, which include a non-complementary portion that provides a strand-distinguishing sequence. *See* Sections I.A, VI.A. Kinde further cites Craig, which discloses barcoded-adapters and ligating exogenous barcodes to the template DNA. EX1006, 9531 (reference 42). Craig discloses Y-shaped Illumina adapters with a non-complementary portion that serves as a strand-distinguishing sequence and a barcode in the complementary portion. Kinde discloses that random nucleotide sequences generate large numbers of barcodes, which may be used to uniquely label sample molecules. EX1006, SI1 ("UIDs constituted 12 or 14 random nucleotide sequences…can generate 16.8 and 268 million distinct UIDs, respectively…"), ("the number of distinct UIDs greatly exceeds the number of

original template molecules to minimize the probability that two different original templates acquire the same UID"); *see also* EX1022, ¶¶22, 23.

A skilled artisan would have had good reason to arrive at the claimed invention in view of Kinde and Craig. First of all, Kinde identifies Craig regarding adding exogenous barcodes to DNA molecules. Specifically, Kinde discloses that methods include "exogenous sequences [introduced] through … ligation (42, 43)." EX1006, 9531; *see also* EX1022, ¶¶22, 23. Reference 42 cited by Kinde is Craig (EX1007). Craig discloses Illumina Y-shaped adapters that include a barcode in the double-stranded portion. EX1007, 888, Supplementary Tables 3 and 4, Supplementary Methods. Craig describes ligating the barcoded adapters to target DNA. *Id.*; *see also* EX1002, ¶¶121-24. 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).

Second, both Kinde and Craig disclose using Illumina Y-shaped adapters. *See* Sections I.A, VI above; EX1007, 888, Supplementary Tables 3 and 4, Supplementary Methods. In a recent IPR filing, Patent Owner argued that Craig's barcoded Y-shaped adapters are amenable to use in the DCS method of the '631 patent. *Twinstrand*, IPR2022-00746, Paper 2, 67 ("…POSA also would expect

Craig's barcodes to work in Schmitt's[3] methods…"). Accordingly, a skilled artisan would have had good reason with a reasonable expectation of success to use Y-shaped Illumina adapters with random barcodes, as taught by Kinde in view of Craig, to determine whether sequence variations could be detected in both strands. EX1002, ¶125.

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 adapters individually labeled each strand of a target DNA molecule. Shiroguchi also discusses the use of such adapter molecules in consensus sequencing approaches as described by Kinde and others. EX1010, 1347 ("to improve the sensitivity of DNA mutation detection ([citing Kinde])"), Figure 1, Figure S5; Section I.A. Accordingly, a skilled artisan had good reason with a reasonable expectation of success in achieving what is claimed. EX1002, ¶¶124-27.

[3] Schmitt (Int. Pub. No. WO2013/142389) is the published version of PCT/US2013/032665 which is listed on the face of the '631 patent as a priority document. EX1001, front page (63).

An element-by-element discussion of the claims, together with 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.

i. Claim 1

Preamble

> "A method of generating high accuracy sequence reads of a population of double-stranded target nucleic acid molecules, comprising:"

To the extent the preamble is limited, Kinde discloses it. *See* Claim 16 preamble discussion (Section VI.A.i).

Craig also discloses sequencing double-stranded DNA molecules. EX1007, Title ("Identification of genetic variants using bar-coded multiplexed sequencing"), Abstract; *see also* EX1002, ¶¶128-30.

Element 1.1

> "ligating each of the double-stranded target nucleic acid molecules to at least one adapter molecule, to form a population of adapter-target nucleic acid complexes, wherein each of the adapter molecules comprises—"

Kinde and Craig disclose Element 1.1. *See* Element 16.1 discussion above (Section VI.A.i).

Craig also ligates its Y-shaped adapters to the DNA fragment. EX1007, Figure 1; EX1002, ¶¶131-33.

Element 1.2

> "(a) a degenerate or semi-degenerate single molecule identifier (SMI) sequence that alone or in combination with the target nucleic acid fragment ends uniquely labels each ligated double-stranded target nucleic acid molecule such that each ligated double-stranded target nucleic acid molecule is distinguishable from other ligated double-stranded target nucleic acid molecules in the population, and"

As discussed above for Elements 16.1-16.2, Kinde discloses ligating Y-shaped adapters to form a population of adapter-target nucleic acid complexes. *See* Section VI.A.i above. Kinde's UIDs are also discussed above. *See, e.g.*, Claims 16 Preamble and Element 16.5 (Section VI.A.i discussing UIDs or "fragment features"). Element 1.2 further requires that the adapter molecule comprises single molecule identifier that are "degenerate or semi-degenerate."

Kinde in view of Craig teaches using conventional Y-shaped Illumina adapters having a random (*i.e.*, degenerate) barcode sequence in the double-stranded portion of the adapter that uniquely labels the ligated molecules so that they are distinguishable from one another. For example, Kinde exemplifies using barcodes having lengths of 12- or 14-nucleotide bases that are randomly generated (i.e., degenerate). EX1006, 9532 ("Exogenous UID…there was a stretch of 12-14 random nucleotides…[t]he random nucleotides form the UIDs…"), ("producing a uniquely tagged, double-stranded DNA fragment"), SI1 ("12- or 14-base UID"). Kinde discloses generating a large number of degenerate barcodes to facilitate

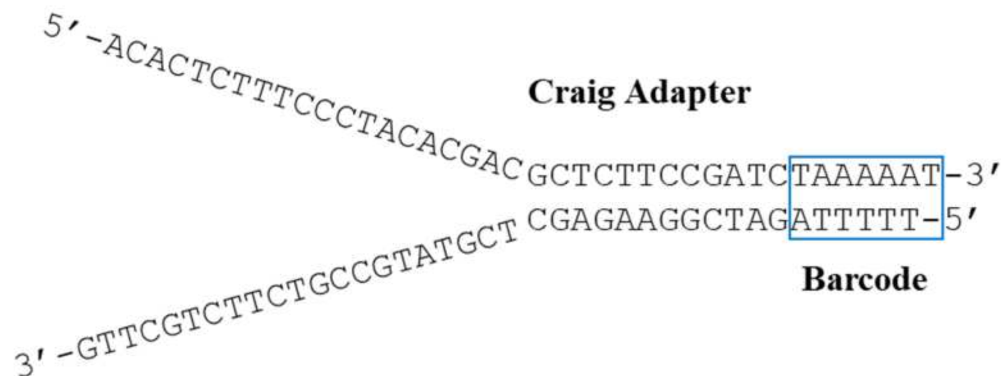
unique labeling and teaches that such unique labeling is desirable. EX1006, SI1 ("These primers can generate 16.8 and 268 million distinct UIDs, respectively. It is important that the number of distinct UIDs greatly exceeds the number of original template molecules…"), Table S4; *see also* EX1010, 1347-48 (discussing random barcode sequences). During *ex parte* prosecution, Patent Owner did not dispute that randomly generated barcodes qualify as degenerate single molecule identifier (SMI) sequences. *See* EX1004, 137 ("Using broadest reasonable interpretation art that teaches use of random barcodes to uniquely tag molecules would read upon SMI sequence.").

As discussed above, in view of Kinde and Craig, a POSA would have had good reason with a reasonable expectation of success to arrive at the subject matter claimed. Kinde discloses using Y-shaped adapters and expressly cites to Craig's teachings regarding adding exogenous UIDs using ligation. Craig illustrates that Y-shaped adapters comprising barcodes in the double-stranded portion of the adapter were well known in the art at the time. EX1007, 887 ("Experimental design"), Supplementary Tables 3 and 4. Accordingly, the recited aspects of Element 1.2 were known and obvious in the prior art at the time as illustrated by Kinde and Craig. EX1002, ¶¶134-38.

Element 1.3

> "(b) a strand-distinguishing nucleotide sequence that, following the ligation step, provides a region of non-complementarity between a first strand of each adapter-target nucleic acid complex and a second strand of the same adapter-target nucleic acid complex;"

Kinde and Craig disclose Element 1.3. The strand-distinguishing functionality of Y shaped adapters is discussed above. As also discussed in detail above, when Y-shaped adapters are ligated to a double-stranded nucleotide molecule, they present a region of non-complementarity (the arms of the Y) that provide a strand-distinguishing nucleotide sequence. *See* Element 16.2 discussion above (Section VI.A.i); Section I.A. As discussed above in regard to Element 1.1, Craig also describes and exemplifies ligation of Illumina Y-shaped adapters to double-stranded DNA molecules. In addition to employing barcodes in the double-stranded portion of Y-shaped adapters, Craig's Y-shaped adapter retains the strand-distinguishing sequence that provides a region of non-complementarity between the first and second strands of the adapter-target nucleic acid complex. EX1007, Figure 1; EX1002, ¶¶139-41.



Regardless of whether off-the-shelf Illumina adapters or Craig's type of adapters are used, the result is a strand-distinguishing nucleotide sequence in a region of non-complementarity. This is confirmed by both Shiroguchi and the '631 patent. EX1010, Figure 1, Figure S5 ("We are able to distinguish the different strands of an original cDNA molecule because of the design of the paired-end sequencing adapters (Fig. 1B)."); EX1001, Figure 1, 3:56-62, 26:9-22; Section I.A; EX1002, ¶¶139-43.

Element 1.4

> "for each of the adapter-target nucleic acid complexes—
>
> amplifying each strand of the adapter-target nucleic acid complex to produce a plurality of first strand adapter-target nucleic acid complex amplicons and a plurality of second strand adapter-target nucleic acid complex amplicons;"

Kinde discloses Element 1.4. *See* Element 16.3 discussion above (Section VI.A.i); *see also* EX1006, Abstract, 9531-9532 & Figs. 2-3 (Amplification), SI1; Section I.A; EX1002, ¶¶83-85.

Craig also teaches amplification of adapter-target nucleic acid complexes using PCR. EX1007, 887 ("After ligation, …, enriched them by PCR amplification…"). EX1002, ¶¶144-49

Element 1.5

> "sequencing the adapter-target nucleic acid complex amplicons to produce a plurality of first strand sequence reads and plurality of second strand sequence reads;"

Kinde discloses Element 1.5. *See* Element 16.4 discussion above (Section VI.A.i); *see also* EX1006, Abstract, 9535 (Sequencing), SI2 (Sequencing); Section I.A; EX1002, ¶¶86-88.

Craig also discloses sequencing both strands. EX1007, 887 ("…sequenced them on the Illumina GA…"). EX1002, ¶¶150-53.

Element 1.6

> "grouping the first strand sequence reads and the second strand sequence reads into a family of first and second strand sequence reads based on the degenerate or semi-degenerate SMI sequence alone or in combination with the target nucleic acid fragment ends;"

Kinde teaches or suggests Element 1.6. As discussed above in Element 16.5, Kinde teaches or suggests grouping the first and second strand sequence reads into a family of sequence reads by identifying the one or more distinguishing fragment features shared by each strand of the double-stranded target nucleic acid molecule. *See* Section VI.A.i above. Kinde further discloses "grouping" reads into UID families on the basis of "exogenous UIDs." EX1006, SI2; *see also id.*, 9535

(same). As discussed above for Element 1.2, Kinde discloses exogenous UIDS that are randomly generated (*i.e.*, degenerate). EX1006, 9532 & Fig. 3, SI1, Table S4. In discussing the introduction of exogenous sequences, Kinde cites specifically to Craig. EX1006, 9531 (Reference 42). As discussed above, Craig disclosed barcoded Y-shaped adapters. As such, Element 1.6 is disclosed or obvious in view of the prior art as illustrated by Kinde and Craig. EX1002, ¶¶154-56; Section I.A.

Element 1.7

> "separating the first and second strand sequence reads into a set of first strand sequence reads and a set of second strand sequence reads based on the region of non-complementarity between the first strand and the second strand of the adapter-target nucleic acid complex;"

Kinde discloses Element 1.7. *See* Element 16.6 discussion above (Section VI.A.i); *see also* Element 1.3 discussion above regarding a strand-distinguishing nucleotide sequence that, following the ligation step, provides a region of non-complementarity between the first strand and the second strand of the adapter-target nucleic acid complex; Section I.A; EX1002, ¶¶157-58.

Element 1.8

> "confirming the presence of at least one first strand sequence read and at least one second strand sequence read;"

Kinde discloses Element 1.8. *See* Element 16.7 discussion above (Section VI.A.i); *see also* Element 1.3 discussion above regarding a strand-distinguishing nucleotide sequence; Section I.A; EX1002, ¶¶159-60.

Element 1.9

> "comparing the at least one first strand sequence read with the at least one second strand sequence read;"

Kinde discloses Element 1.9. *See* Element 16.8 discussion above (Section VI.A.i); *see also* Element 1.3 discussion above regarding a strand-distinguishing nucleotide sequence; Section I.A; EX1002, ¶¶161-62.

Element 1.10

> "identifying nucleotide positions where the compared first and second strand sequence reads are non-complementary;
>
> identifying nucleotide positions where the compared first and second strand sequence reads are complementary; and
>
> generating a high accuracy consensus sequence read for each of the double-stranded target nucleic acid molecules in the population that includes only the nucleotide positions where the compared first and second strand sequence reads are complementary."

Kinde teaches or suggests Element 1.10. As discussed above for Element 16.9, Kinde discloses generating a high accuracy consensus sequence read for the double-stranded target nucleic acid molecules in the population. *See* Section VI.A.i; *see also* Element 1.3 discussion above regarding a strand-distinguishing nucleotide sequence. As also discussed therein, Kinde teaches achieving consensus by comparing sequence reads from the first and second strands and including in the consensus read only the nucleotide positions where the reads from both strands are complementary.

Element 1.10 further recites identifying nucleotide positions where the compared first and second strand sequence reads are non-complementary and positions where they are complementary. Kinde expressly teaches increasing specificity by making use of the complementary strands of a DNA duplex to identify processing errors (non-complementary base positions) and confirming true base calls (complementary base positions). *E.g.*, EX1006, SI3 ("specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation"), SI2 (mutations presented in the double-stranded DNA template "are expected to be present in both strands of the relevant templates"). Moreover, Kinde discloses setting various tolerance criteria, including tolerance criteria for excluding data based on sequence mismatches and that nucleotide positions where the two strands of the DNA duplex do not match are prone to error. EX1006, SI2-SI3. Kinde thereby again teaches or suggests Element 1.10. EX1002, ¶¶163-65; Section I.A.

Accordingly, Kinde and Craig teach or suggest each and every element of claim 1. EX1002, ¶¶163-67.

ii. Claim 2

> 2. The method of claim 1, wherein the confirming step includes confirming the presence of at least 2 first strand sequence reads and at least 2 second strand sequence reads.

Claim 1 is obvious as discussed above. Kinde at a minimum renders claim 2 obvious. A POSA would understand that Kinde indicates a preference for comparing a plurality of sequence read for each strand. For example, Kinde discloses performing repeated amplification cycles to increase the likelihood of having a greater number of sequence reads ("40 members per family", on average) and required the sequence reads to be identical to make a consensus call if there were not 20 or more sequence reads." EX1006, 9531, SI1 ("18 cycles of PCR"; "25 cycles of PCR"), SI2 ("UID families with <20 members had to be 100% identical"). Kinde also teaches requiring at least 2 sequence reads for each UID family. EX1006, SI2 ("Only UID families with two or more members were considered."). EX1002, ¶¶168-69; Section I.A. Accordingly, Kinde in view of Craig renders claim 2 obvious.

iii. Claims 4-5

> 4. The method of claim 1, wherein for any double-stranded target nucleic acid molecule in the population, the method further comprises:
>
> comparing the high accuracy consensus sequence read to a respective reference sequence; and

> identifying one or more nucleotide sequence variations present in the high accuracy consensus sequence read not present in the reference sequence.
>
> 5. The method of claim 4, further comprising identifying a variation occurring at a particular position in the high accuracy consensus sequence read as a true mutation.

Claim 1 is obvious as discussed above. EX1002, ¶¶128-67. Kinde discloses the conventional sequencing practices recited in claims 4-5. As discussed above for claim 1, Kinde discloses generating a high-accuracy consensus sequence for the purpose of determining whether variations between the consensus sequence and the expected sequence are true mutations. For example, Kinde discloses identifying sequence variants, also called mutations, by aligning the sequence reads using the Illumina Eland pipeline and comparing the reads to an expected (reference) sequence. EX1006, SI2 ("Sequencing"), (defining mutation as "base call that varies from the expected sequence"). When performing Kinde's method, 95% agreement (consensus) is required within a UID family before concluding that variation as compared to the reference genome is an actual mutation ("a supermutant"). EX1006, Abstract, 9530 ("Overview"), Figure 1, Tables 1-2 (listing supermutants), SI2 (Safe-SeqS Analysis). Kinde's identification of a supermutant is thus identification of a nucleotide sequence variation present in the high-accuracy consensus sequence read but not present in the reference sequence. When the supermutant is found on both strands of the DNA duplex (EX1006, SI2-3),

Kinde interprets this as a "mutation present in the template DNA," or a true mutation. EX1002, ¶170. Kinde illustrates that it was conventional to use reference sequences as a basis of comparison for sequence information to identify sequence variations and true mutations. *Id.* Accordingly, Kinde in view of Craig renders claims 4-5 obvious. *Id.*

iv. Claims 3, 6, and 23

> 3. The method of claim 1, further comprising identifying one or more nucleotide positions that disagree between the at least one first strand sequence read and the at least one second strand sequence read.
>
> 6. The method of claim 4, further comprising identifying a variation that occurs at a particular position in only one of the first strand sequence read or the second strand sequence read as a potential artifact.
>
> 23. The method of claim 1, wherein generating a high accuracy consensus sequence further comprises marking the identified nucleotide positions where the compared first and second strand sequence reads are non-complementary and scoring the identified non-complementary nucleotide positions as potential artifacts.

Claim 1 is obvious as discussed above. As discussed above for Elements 16.9 and 1.10 and for claim 21, Kinde teaches or suggests identifying one or more nucleotide positions that disagree between the at least one first strand sequence read and the at least one second strand sequence read because such reads likely reflect artifacts of "Error-generating processes" that should be excluded from the consensus sequence. *See* Sections VI.A.i, VI.A.vi, and VI.B.i above; EX1002,

¶¶103-109, 163-66; EX1006, SI2-SI3; EX1002, ¶171; Section I.A. Claim 4 is obvious for the reasons discussed in Section VI.B.iii above. Accordingly, Kinde in view of Craig renders each of claims 3, 6, and 23 obvious. EX1002, ¶¶171-72.

v. Claim 7

> 7. The method of claim 1, wherein for any double-stranded target nucleic acid molecule in the population, the method further comprises:
>
> comparing the high accuracy consensus sequence read to a reference sequence;
>
> identifying one or more variations present in the high accuracy consensus sequence read not present in the reference sequence; and
>
> identifying at least one of a cancer, a cancer risk, a cancer metabolic state, a mutator phenotype, a carcinogen exposure, a chronic inflammation exposure, an age, a neurodegenerative disease, or a combination thereof in an organism from which the double-stranded target nucleic acid molecule is derived by the one or more variations present in the high accuracy consensus sequence read.

Claim 1 is obvious as discussed above. As discussed regarding claim 4, Kinde discloses identifying sequence variants, also called mutations, by comparing the consensus to an expected sequence as recited in claim 7. *See* Section VI.B.iii above. Claim 7 further recites various conditions associated with variations present in the high-accuracy consensus sequence read. Kinde discloses identifying mutations associated with conditions such as "neoplastic diseases" or cancer.

EX1006, 9530; *see also id*. (discussing "epigenetic changes in tumors").

Accordingly, Kinde in view of Craig renders claim 7 obvious. EX1002, ¶¶173-74.

vi. Claim 8

> 8. The method of claim 1, wherein the population of double-stranded target nucleic acid molecules includes double-stranded DNA or other nucleic acid fragments.

Claim 1 is obvious as discussed above. The same additional limitations as in claim 8 are discussed above. *See* Section VI.A.ii; EX1002, ¶¶128-67; *see also* EX1006, 9532 & Fig. 3 (Exogenous UIDs: "DNA"). Accordingly, Kinde in view of Craig renders claim 8 obvious. EX1002, ¶¶175-76.

vii. Claim 9

> 9. The method of claim 1, wherein each of the adapter-target nucleic acid complexes comprises at least two primer binding sites.

Claim 1 is obvious as discussed above. As discussed above for claim 19, Kinde used Illumina Y-shaped adapters, which comprise at least two primer binding sites. *See* Section VI.A.iv; EX1002, ¶¶115-17; EX1006, SI1, Table S4; EX1012, Figure 4; *see also* Section I.A. Accordingly, Kinde in view of Craig renders claim 9 obvious. EX1002, ¶¶177-78.

viii. Claim 10

> 10. The method of claim 1, wherein the adapter molecule ligated to any particular double-stranded target nucleic acid molecule comprises a Y-shape, a U-shape, or a combination thereof.

Claim 1 is obvious as discussed above. The same additional limitations as in claim 10 are discussed above. *See* Section VI.A.v; EX1002, ¶¶128-67; Accordingly, Kinde in view of Craig renders claim 10 obvious. EX1002, ¶¶179-80.

ix. Claims 11 and 14

> 11. The method of claim 1, wherein the population of adapter-target nucleic acid complexes comprise an SMI sequence in each of its strands.
>
> 14. The method of claim 1, wherein the population of adapter-target nucleic acid complexes comprise an SMI sequence at each terminus.

Claim 1 is obvious as discussed above. Kinde in view of Craig teach the limitations of claims 11 and 14. As explained above, both Kinde and Crag describe ligating Y-shaped adapters to both ends (*i.e.*, termini) of sample DNA fragments. *See* Element 1.1. Because Kinde in view of Craig teaches ligating a barcoded adapter to both ends of sample DNA, a POSA would have understood that both strands of the adapter-target nucleic acid complexes comprise an SMI sequence, as required by claim 11. Because the barcoded adapters are ligated to both termini, the adapter-target nucleic acid complexes comprise an SMI sequence at each

terminus, as required by claim 14. Accordingly, Kinde in view of Craig renders claims 11 and 14 obvious. EX1002, ¶¶181-82.

x. Claims 12 and 13

> 12. The method of claim 1, wherein each adapter molecule comprises a double-stranded SMI sequence, and wherein the double-stranded SMI sequence comprises a first degenerate or semi-degenerate sequence and a second degenerate or semi-degenerate sequence.
>
> 13. The method of claim 12, wherein the first and second degenerate or semi-degenerate sequences are at least partially complementary.

Claim 1 is obvious as discussed above. As discussed above regarding Element 1.2, Kinde in view of Craig teaches using conventional Y-shaped Illumina adapters having a random (*i.e.*, degenerate) barcode sequence in the double-stranded portion of the adapter. Such adapters meet the additional requirements of claim 12. Moreover, because the "first and second degenerate" sequences are double-stranded, they are "at least partially complementary," as required by claim 13.

Accordingly, Kinde in view of Craig renders claims 12 and 13 obvious. EX1002, ¶¶183-84.

xi. Claim 15

> 15. The method of claim 1, wherein the degenerate or semi-degenerate SMI sequence comprises from about 3 to about 20 nucleotides.

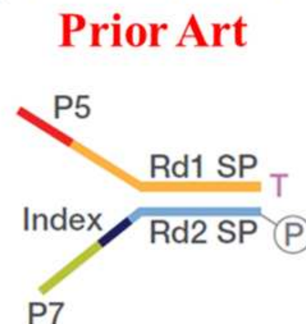
Claim 1 is obvious as discussed above. As discussed in regard to Element 1.2, Craig discloses Y-shaped adapters comprising 6 nucleotide barcodes. EX1007, 887 (“Experimental design”), Supplementary Tables 3 and 4. Kinde exemplifies using barcodes having lengths of 12- or 14-nucleotide bases that are randomly generated (i.e., degenerate). EX1006, 9532, Table S4; SI1; *see also* EX1009, ¶54 (calling randomly generated barcodes of 3-20 nucleotides on Y-shaped adaptors a “degenerate base region”); EX1010, 1347-48 (“random barcode sequences”).

Accordingly, Kinde in view of Craig renders claim 15 obvious. EX1002, ¶¶185-86.

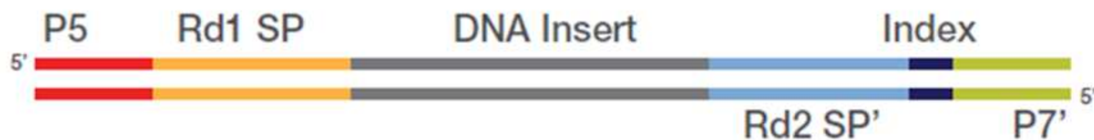
xii. Claim 22

> 22. The method of claim 1, wherein each adapter molecule comprises a single stranded SMI sequence, and wherein the method further comprises converting the single-stranded SMI sequence to a double-stranded SMI sequence by polymerase extension.

Claim 1 is obvious as discussed above. As explained above, Kinde in view of Craig teaches using standard Y-shaped Illumina adapters having a random barcode sequence in the double-stranded portion of the adapter. *See* Element 1.2 above. Standard commercially available Illumina (*e.g.*, TruSeq) adapters have a barcode in the single-stranded portion of the adapter. Section I.A. Polymerase extension from a primer binding to P7, converts the



single-stranded barcode (*i.e.*, index) into a double-stranded barcode during adapter synthesis.



EX1012, Figure 4.

To the extent Patent Owner argues that claim 22 is directed to adapter synthesis, synthesis of adapters using primer extension was known in the art. *E.g.*, EX1035, Figure 16 (illustrating conversion of a single-stranded barcode to a double-stranded sequence by polymerase extension); *see also* EX1002, ¶¶187-88.

C. [Ground 3] Claims 1-23 are unpatentable as obvious under 35 U.S.C. §103 over Kinde, Craig and the Travers Publications

As discussed in detail herein, Kinde, Craig and the Travers Publications render the claims obvious. Kinde is applied to claims 16-21 as set forth above in Ground 1. Kinde and Craig are applied to claims 1-15, 22 and 23 as set forth above in Ground 2. *See also* Section I.A.

The Travers Publications describe methods including attaching adapters, amplifying, and sequencing from both strands of an original double-stranded nucleic acid molecule to generate what they describe as "high-quality consensus" or "high-accuracy consensus" sequence. EX1021, Abstract, 1. The Travers Publications disclose consensus sequencing following the same essential workflow

of the ’631 patent (a workflow described throughout the scientific literature at the time). The ’631 patent even borrows the same terminology found in the Travers Publications (*e.g.*, “high accuracy consensus”) and expressly indicates its method is meant to capture Travers’ U-shaped (“hairpin”) adapters. *E.g.*, EX1001, 10:58-64 (“As the sequence of the linker itself does not matter in the workflow, the published linker sequences from [the Travers Paper] would be adequate for use in the assay.”), claim 10, 20 (Y-shape, U-shape). As such, the Travers Publications further corroborate that any aspects of amplification, redundant sequencing, adapter use, and consensus analysis of sequence reads recited in the ’631 patent claims were well-known in the art at the time. The Travers publications, particularly viewed in combination with other relevant art at the time, like Kinde and Craig, confirm the obviousness of claims 1-23. EX1002, ¶¶189-90.

The Travers Publications disclose a method for generating “high-accuracy” consensus sequences from both strands of an original DNA duplex molecule. According to Travers, adapters (green) are attached to both ends of double-stranded DNA to produce adapter-target nucleic acid complexes (“SMRTbell™ templates”) comprising a region of non-complementary that provides a strand-distinguishing nucleotide sequence. *See* Section I.A.

EX1021, Figure 1; *see also id*. ("Production of SMRTbell™ templates"); EX1018, Figures 2, 3, 7, ¶¶16, 51-55, 100-102 (describing adapters comprising barcodes). The SMRTbell™ templates are amplified by primer extension with a strand displacing polymerase to produce a concatemer comprising repeating copies of the sense and antisense strands separated by a copy of the adapter. *E.g.*, EX1018, ¶122; EX1021, Figures 1 and 4. Use of the SMRTbell allows for generation of a consensus sequence based on redundant reads of the sense and antisense strands of the original molecule. EX1021, Abstract; *see also* EX1021, 1 (multiple observations can then be used to generate high-accuracy consensus sequence from single molecules."), Figure 4; EX1018, ¶¶53, 54, 122; EX1002, ¶¶190-92. During *ex parte* prosecution of a related case, Patent Owner acknowledged that virtually every aspect of the claimed subject matter is disclosed by Travers '075. EX1024, 551; *see also* EX1001, 10:58-11:12, 15:33-43.

Given the breadth of the claims and the teachings in the cited references, a person of ordinary skill in the art would have good reason to look to the combined teachings of the cited references with a reasonable expectation of successfully arriving at the claimed subject matter. *In re Keller*, 642 F.2d 413, 425 (C.C.P.A. 1981) ("The test for obviousness is not whether the features of a secondary reference may be bodily incorporated into the structure of the primary reference…. Rather, the test is what the combined teachings of the references would have

suggested to those of ordinary skill in the art"); *see also In re Mouttet*, 686 F.3d 1322, 1332 (Fed. Cir. 2012); *In re Etter*, 756 F.2d 852, 859 (Fed. Cir. 1985).

As described in detail herein, both Kinde and the Travers Publications disclose methods of determining sequencing consensus based on amplification, redundant sequencing, and analysis of sequence reads of both strands of a DNA duplex. Moreover, both references utilized commercially available sequencing adapters that enable distinguishing of different duplex strands. Kinde discloses using prior art Y-shaped adapters, including those commercially available from Illumina at the time. Kinde cites to Craig, which further illustrates prior art Y-shaped adapters. The Travers publications discuss consensus sequencing using U-shaped ("hairpin") adapters that were commercially available from PacBio at the time. A POSA contemplating consensus sequence analysis would certainly look to prior art descriptions of consensus sequencing utilizing well-known and commercially available sequencing technologies. EX1002, ¶¶192-96. In fact, the authors of the '631 patent did just that. The '631 patent specification mirrors the disclosure of Kinde, borrows graphics and terminology from the prior art literature, and expressly describes its methods of encompassing prior art and commercially available Y- and U-shaped adapters—including Illumina Y-shaped adapters and PacBio U-shape ("hairpin") adapters. EX1001, 3:56-62, 26:9-22, claim 10, 20 (Y-shape, U-shape). *See Teva Pharms. USA, Inc. v. Corcept Therapeutics, Inc.*, 18

F.4th 1377, 1380-81 (Fed. Cir. 2021) ("The reasonable-expectation-of-success analysis must be tied to the scope of the claimed invention."); *Intelligent Bio-Systems*, 821 F.3d at 1367.

Accordingly, the Travers Publications disclose or teach virtually every aspect of the challenged claims, and certainly confirm the obviousness of the claims in view of Kinde and Craig. Relevant to claims 1-15, 22, and 23, the Travers Publications further discuss inclusion of barcodes with the adapter molecules. *See* element-by-element discussion in Grounds 1 & 2; *see also* EX1002, ¶¶197-240.

VII. THE SAME OR SUBSTANTIALLY THE SAME ARGUMENTS HAVE NOT BEEN PREVIOUSLY PRESENTED

Discretionary denial of institution under 35 U.S.C. §325(d) is not appropriate here. Craig was not presented in an IDS and there is no indication in the file history that its teachings were considered or previously presented to the Office. Only an incomplete version of Kinde was included in an IDS together with over a hundred references, and no apparent substantive prior art analysis of even the incomplete version of Kinde was applied by the Examiner. The Travers Publications were cited in an IDS but drew no substantive action. Furthermore, the materials presented here demonstrate material error in that the '631 patent should never have issued.

Section 325(d) should not be applied to deny institution in such circumstances even where references in a ground were listed on the IDS or even discussed in the body of the patent. *See, e.g.*, *Vudu, Inc. v. IndeaHub, Inc.*, IPR2020-01689, Paper 16, 18-20 (§325(d) not applied where asserted references were cited repeatedly in the body of the patent and considered in IDS but never applied in a rejection); *Dish Network L.L.C., v. Sound View Innovations, LLC*, IPR2020-01041, Paper 13, 16-22 (similar); *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); *Honeywell Int'l, Inc. v. 3G Licensing S.A.*, IPR2021-00908, Paper 23, 30-31 (same). Moreover, the IDS listing for the Kinde article did not reference the 10 pages of Kinde Supporting Information; its citation to Kinde mentions only the 6 pages of the main article. *See Mylan Pharms. Inc. v. Regeneron Pharms., Inc.*, IPR2021-00880, Paper 21, 11-13 (§325(d) not applied where IDS submission included only a portion of the disclosure of the asserted reference).

Limited activity during *ex parte* prosecution indicates the prior examination was cursory at best. While the first Examiner applied the Steemers reference in a

rejection, a second Examiner took over and promptly allowed the case. To the extent the second Examiner even considered Patent Owner's false assertion that Steemers fails to teach consensus sequencing, the second Examiner erred in not citing Steemers express disclosure of consensus sequencing. EX1035, 46:31-47:2. Furthermore, the Examiner never made comment, let alone any rejection, based on Kinde or a comparable consensus sequencing reference. *Advanced Bionics, LLC v. MED-EL Elekromedizinische Geräte GmbH*, IPR2019-01469, Paper 6, 10 (precedential) (explaining "if the record of the Office's previous consideration of the art is not well developed or silent, then a petitioner may show the Office erred"). Moreover, where the '631 patent discusses Kinde, it makes comparisons, concessions and admissions that underscore Examiner error in the issuance of the '631 patent. *E.g.*, EX1001 2:16-38 (identifying Kinde as prior art tagging, error correction, and consensus sequencing), 27:32-43 (equating tagging scheme with that previously described in Kinde). To the extent any differences vis-à-vis Kinde exist, they are unclaimed here.

Moreover, while Kinde is discussed in the specification of the '631 patent, it makes no mention of Kinde's teachings about using the complementary strands of the DNA molecule to confirm true genetic variants and identify process errors. For example, the '631 patent ignores Kinde's express suggestion that "specificity can be further increased by requiring that each strand of the original double-stranded

template contain the mutation." EX1006, SI3. Issuance of the '631 patent without accounting for these teachings thus represents substantive error.

VIII. THERE ARE NO *FINTIV* ISSUES

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 (IPR2022-00746, -00747) 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 a very early stage and is not currently assigned to a judge.

Indeed, the "Fintiv factors" favor institution here. The potential of a stay exists in the district court case if this proceeding is instituted, as Petitioner intends to request a stay if the IPR is instituted. The co-pending district court litigation is in its early stages. The trial date is uncertain, and the proximity to the projected statutory deadline for a final written decision is highly speculative. The parties only recently filed infringement contentions. The parties have not exchanged invalidity contentions, have not briefed claim construction, have not filed dispositive motions, and have not begun expert discovery. Expert reports and dispositive motions will not have come due by the time an institution decision has been rendered. Furthermore, Petitioner has diligently prepared the IPR petitions well in advance of the 12-month statutory deadline. Thus, there will be no

significant investment of resources expended upon the Board's institution. The present challenges are strong on the merits and there is a significant public interest against "leaving bad patents enforceable." *Thryv, Inc v. Click-To-Call Techs., LP*, 140 S. Ct. 1367, 1374 (2020). Moreover, Petitioner is the sole party to challenge this patent before the Board.

IX. CONCLUSION

For the reasons set forth above, claims 1-23 of the '631 patent are unpatentable. Petitioners therefore request that a *inter partes* review of these claims be instituted.

Respectfully submitted,

Dated: May 6, 2022

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

X. CERTIFICATE OF COMPLIANCE

Pursuant to 37 C.F.R. §42.24(d), the undersigned certifies that this Petition complies with the type-volume limitation of 37 C.F.R. §42.24(a). The word count application of the word processing program used to prepare this Petition indicates that the Petition contains 13,857 words, excluding the parts of the brief exempted by 37 C.F.R. §42.24(a).

Respectfully submitted,

Dated: May 6, 2022

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

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

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

XII. APPENDIX – LIST OF EXHIBITS

Exhibit No.	Description
1001	U.S. Patent No. 10,287,631 to Salk et al.
1002	Declaration of Dr. John Quackenbush
1003	*Curriculum Vitae* of John Quackenbush, Ph.D.
1004	File History for Application No. 15/660,785
1005	RESERVED
1006	Kinde e al., "Detection and quantification of rare mutations with massively parallel sequencing," *PNAS 108*(23) (2011)
1007	Craig et al., "Identification of genetic variants using bar-coded multiplexed sequencing," *Nature Methods 5*(10) (2008)
1008	RESERVED
1009	U.S. Patent Publication No. 2012/0071331 to Casbon et al.
1010	Shiroguchi et al., "Digital RNA sequencing minimizes sequence-dependent bias and amplification noise with optimized single-molcule barcodes," *PNAS 109*(4) (2012)
1011	RESERVED
1012	Illumina, TruSeq RNA and DNA Sample Preparation Kits, Data Sheet: Illumina Sequencing (2010)
1013	Bentley et al., "Accurate whole human genome sequencing using reversible terminator chemistry," *Nature 456* (2008)
1014-1017	RESERVED
1018	U.S. Patent Publication No. 2009/0298075 to Travers et al.
1019	RESERVED

1020	U.S. Patent No. 7,476,503 to Turner et al.
1021	Travers et al., "A flexible and efficient template format for circular consensus sequencing and SNP detection," *Nucleic Acids Research 38*(15) (2010)
1022	International Publication No. WO 2012/142213 to Vogelstein et al.
1023	RESERVED
1024	File History for Application No. 16/118,306
1025-1028	RESERVED
1029	U.S. Patent No. 8,029,993 to Mikawa
1030	RESERVED
1031	Casbon et al., "A method for counting PCR template molecules with application to next-generation seuencing," *Nucleic Acids Research 39*(12)(2011)
1032	RESERVED
1033	Affidavit of Nathaniel E. Frank-White
1034	RESERVED
1035	International Publication No. WO 2012/061832 to Steemers et al.
1036	File History for Application No. 16/503,382

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. 10,287,631 (and accompanying Exhibits 1001 through 1036) by overnight courier (Federal Express or UPS), on this 6th day of May, 2022, on the Patent Owner at the correspondence address of the Patent Owner as follows:

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Respectfully submitted,

Dated: May 6, 2022

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