

Filed on behalf of: Guardant Health, Inc.

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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. IPR2022-00816
Patent No. 10,760,127

**PETITION FOR INTER PARTES REVIEW OF
U.S. PATENT NO. 10,760,127**

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LISTING OF CHALLENGED CLAIMS

1. [**Preamble**] A method of sequencing DNA comprising:

[**Element 1.1**] a) attaching adapters to double-stranded DNA fragments to generate a plurality of partially-complementary, asymmetrical double-stranded adapter-DNA molecules,

[**Element 1.2**] wherein the adapters comprise barcodes selected from a plurality of distinct barcode sequences;

[**Element 1.3**] b) amplifying original strands of at least a portion of the double-stranded adapter-DNA molecules to produce first and second strand copies;

[**Element 1.4**] c) sequencing a plurality of first and second strand copies to obtain first and second strand sequence reads for at least a portion of the adapter-DNA molecules; and

[**Element 1.5**] d) for at least some of the adapter-DNA molecules comprising barcodes—

confirming the presence of at least one sequence read derived from each of the original first and second strands of the adapter-DNA molecules;

[**Element 1.6**] comparing at least one of the confirmed first and second strand sequence reads to a reference sequence; and

[**Element 1.7**] analyzing one or more correspondences between at least one of the confirmed first and second strand sequence reads and the reference sequence to identify a sequence variation.

2. The method of claim 1, wherein the sequence variation is either (i) a processing error or a site of DNA damage, or (ii) a true variant, and wherein, for at least some of the adapter-DNA molecules, the method further comprises:

comparing the confirmed first strand sequence read and confirmed second strand sequence read;

identifying nucleotide bases that are not consistent between the confirmed first and second strand sequence reads; and

identifying nucleotide bases that are consistent between the confirmed first and second strand sequence reads,

wherein—

if the sequence variation is not a consistent nucleotide base, then categorizing the sequence variation as (i) a processing error or a site of DNA damage, and

if the sequence variation is a consistent nucleotide base, then categorizing the sequence variant as (ii) a true variant.

3. The method of claim 2, wherein prior to comparing the confirmed first strand sequence read and confirmed second strand sequence read, the method comprises associating the confirmed first strand sequence read with the confirmed second strand sequence read using one or more of an adapter sequence, a sequence length, sequence alignment information and original strand information.
4. The method of claim 1, further comprising generating a consensus sequence for a plurality of adapter-DNA molecules, wherein each consensus sequence comprises nucleotide bases at which both the confirmed first strand sequence read and the confirmed second strand sequence read are in agreement.
5. The method of claim 4, further comprising aligning the consensus sequence to the reference sequence and identifying a variant or a mutation occurring at a particular position or region in the consensus sequence as a true variant or mutation.
6. The method of claim 5, further comprising comparing the consensus sequence to the reference sequence and identifying a mutation type for each of the true mutations.
7. The method of claim 6, wherein the mutation type is selected from one or more of a transition, a transversion, a point mutation, a structural rearrangement, a single-nucleotide substitution, a single-nucleotide variation, and an insertion.
8. The method of claim 4, further comprising identifying a nucleotide sequence occurring at a particular position or region in the consensus sequence as a true nucleotide sequence.
9. The method of claim 1, further comprising determining nucleotide bases at which the confirmed first strand sequence read and the confirmed second strand

sequence read are not complementary, and identifying non-complementary bases as either an experimental error or a site of DNA damage.

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

11. The method of claim 1, wherein the double-stranded DNA fragments comprise at least one of a deaminated nucleic acid base and a nucleic acid base having oxidative damage, and wherein prior to or following step (a) the method further comprises chemically or enzymatically treating the double-stranded DNA fragments to remove or repair one or more damaged bases.

12. The method of claim 1, wherein following step (a), the method further comprises selectively enriching the adapter-DNA molecules based on size to provide enriched adapter-DNA molecules.

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

14. The method of claim 13, wherein the adapters comprise the barcodes in at least one of a double-stranded portion or a single-stranded portion of the adapter.

15. The method of claim 1, further comprising providing a sample comprising the double-stranded DNA fragments from a biological source, wherein the biological source comprises tissue.

16. The method of claim 1, wherein prior to the comparing step, the method comprises associating the confirmed first strand sequence read with the confirmed second strand sequence read using one or more of an adapter sequence and original strand information.

17. The method of claim 1, wherein the double-stranded DNA fragments were at least partially generated by nuclease cleavage.

18. The method of claim 1, wherein the first strand sequence reads derived from a particular original adapter-DNA molecule and the second strand sequence reads derived from the same particular original adapter-DNA molecule can be related based on a barcode sequence, a fragment-specific feature, or a combination thereof.

19. The method of claim 1, wherein the sequence reads derived from a particular original adapter-DNA molecule can be differentiated from the second strand sequence reads derived from the same particular original adapter-DNA molecule at least in part by an asymmetry of the asymmetrical double-stranded adapter-DNA molecules.

20. The method of claim 1, wherein prior to sequencing, the method further comprises selectively enriching adapter-DNA molecules or copies thereof for a subset of said adapter-DNA molecules that map to one or more genetic loci in a reference genome.

21. The method of claim 1, wherein prior to attaching adapters to double-stranded DNA fragments, the method further comprises selectively enriching double-stranded DNA fragments by size selection.

22. [**Preamble**] A method of sequencing DNA comprising:

[**Element 22.1**] a) attaching partially single-stranded adapters comprising barcodes selected from a plurality of distinct barcode sequences to double-stranded DNA fragments obtained from a bodily sample, wherein attachment of the adapters to double-stranded DNA fragments generates a library of tagged double-stranded adapter-DNA molecules;

[**Element 22.2**] b) amplifying strands from a plurality of the double-stranded adapter-DNA molecules in the library to produce strand copies;

[**Element 22.3**] c) sequencing a plurality of the strand copies to obtain strand sequence reads comprising one or more barcode sequences and DNA fragment-specific information; and

[**Element 22.4**] d) for at least some of the double-stranded adapter-DNA molecules in the library—

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

[**Element 22.5**] collapsing a plurality of strand sequence reads within the families to provide a consensus sequence for each of at least some of the double-stranded DNA molecules in the library;

[**Element 22.6**] comparing the consensus sequence to a reference sequence; and

analyzing one or more correspondences between the consensus sequence and the reference sequence to identify a sequence variation.

23. The method of claim 22, wherein the bodily sample comprises tissue obtained from a subject.

24. The method of claim 22, wherein at least some of the double-stranded DNA fragments are derived from a tumor or circulating neoplastic cells.

25. The method of claim 22, wherein the bodily sample is derived from a human subject having a tumor cell population, and wherein following step (d), the method further comprises identifying a genetic mutation conferring drug resistance present in one or more of the consensus sequences derived from the double-stranded DNA fragments obtained from the tumor cell population present in the bodily sample.

26. The method of claim 22, wherein the library comprises at least a subset of non-uniquely tagged double-stranded adapter-DNA molecules, and wherein non-uniquely tagged double-stranded adapter-DNA molecules are substantially identifiable with respect to other non-uniquely tagged double-stranded adapter-DNA molecules in the bodily sample using the one or more barcode sequences and DNA fragment-specific information.

27. The method of claim 22, further comprising selectively enriching double-stranded adapter-DNA molecules or copies thereof to enrich for a subset of DNA molecules that map to one or more genetic loci in the reference sequence.

28. The method of claim 22, wherein, prior to sequencing, double-stranded adapter-DNA molecules or copies thereof are selectively enriched using a hybridization capture method to provide target DNA molecules that map to one or more genetic loci in the reference sequence.

29. The method of claim 22, wherein the barcode sequences are 6 nucleotides in length.

30. The method of claim 22, wherein the barcode sequences are 3, 4, 5, 6, 7 or 8 nucleotides in length.

Guardant Health, Inc., (“Petitioner”) hereby requests review of United States Patent No. 10,689,127 to Jesse Salk et al. (hereinafter “the ’127 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 1-30 of the ’127 patent are unpatentable for failing to distinguish over prior art.

I. INTRODUCTION

The ’127 patent specification purports to have invented duplex consensus sequencing (“DCS”). But many claims do not even require consensus sequencing, much less duplex consensus sequencing, the purported point of novelty in the specification. The ’127 patent instead claims a far simpler concept—attaching partially-single-stranded adapters comprising barcodes to DNA fragments to detect sequence variants in the DNA fragments as compared to a reference sequence. The ’127 patent describes use of commercially available Y- or U-shaped adapters (*e.g.*, Illumina, Pacific Biosciences), and claims the use of Y- or U-shaped adapters with barcode sequences for otherwise standard sequencing processes. EX1001, 3:52-4:11, 6:4-41, 10:63-11:2, 18:49-57, 25:64-26:1.

As is established in this petition, individual tagging of both strands of duplex DNA using Y-shaped adapters was known in the art and described in numerous prior art references. For example, Kinde (EX1006) in view of Craig (EX1007)

renders obvious each of claims 1-10 and 12-30. Kinde discloses the same sequencing method of the claims. Kinde exemplifies the SafeSeqS consensus sequencing approach by using Y-shaped Illumina adapters, which generate partially single-stranded and partially-complementary, asymmetrical double-stranded adapter DNA molecules. Kinde discusses tagging the DNA molecules with exogenous barcodes, citing Craig specifically. Craig discloses employing barcodes in the complementary portion of partially single-stranded and partially-complementary Y-shaped Illumina adapters. Kinde also discusses comparing the sequences for both strands of a particular DNA molecule to identify and exclude artifacts of the amplification and sequencing process. Corroborating obviousness, Shiroguchi cites to Kinde, illustrates barcoded Y-shaped adapters, and expressly notes strand-distinguishing capability due to this adapter design. EX1010, 1347, Figure 1, Figure S5.

Kinde in view of Craig thus discloses or suggests performing consensus sequencing methods by attaching partially single-stranded and partially-complementary Y-shaped Illumina adapters containing barcodes to double-stranded DNA fragments in a manner that satisfies each of claims 1-10 and 12-30. Claim 11 is obvious in further view of NEB Expressions.

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 “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. EX1031, Abstract; EX1021, Abstract; EX1006, Abstract. Prior art consensus sequencing methods conventionally used barcodes to uniquely “label” original DNA molecules. With such labeling, methods could identify the amplification progeny (*i.e.*, copies) of original DNA molecules by virtue of inclusion of the same barcode also being copied during amplification. *E.g.*, EX1006, 9530 (describing copies as “UID” families); EX1031, 4 (“Identification of PCR duplicates ...”); EX1010, Figure 1. Prior art methods used the barcodes to group corresponding sequence reads into a family corresponding to a particular DNA molecule. *Id.* A consensus sequence could then

be generated for each family that 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, 3 (“...ability to read both strands on a single DNA molecule therefore enables correction for sequence context-dependent variation.”). There was nothing new about NGS, adapters comprising barcodes, or consensus sequencing and the ’127 patent does not assert otherwise. *See also* EX1002, ¶¶30-31.

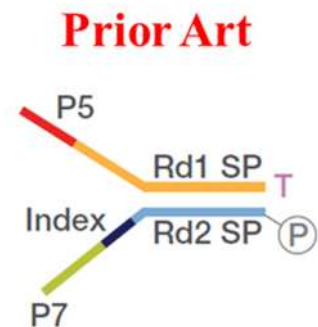
The purported novelty of the claimed sequencing methods relates to the use of partially-complementary adapters that, when ligated to double-stranded DNA, enable reads associated with the strands (*i.e.*, top and bottom) of double-stranded DNA molecules to be distinguished. Such partially single-stranded sequencing adapters were not only commercially available from companies like Illumina and Pacific Biosciences, but were routinely used to convert sample DNA into sequence-ready DNA molecules. EX1012, Figure 4¹; EX1013, Figure 1; EX1018,

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

Figure 7; EX1021, Figure 1. One such example is Illumina Y-shaped adapters. *See also* EX1002, ¶¶32-38; 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.

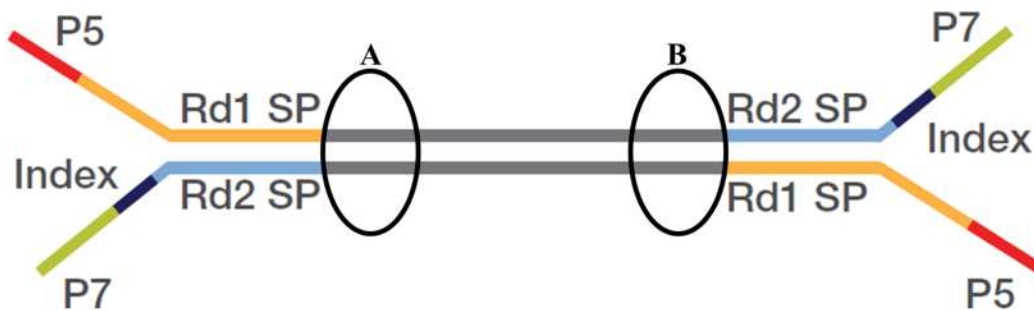
Illumina Y-shaped adapters are a type of prior art partially single-stranded, partially-complementary adapter relevant to the challenged claims. Indeed, the '127 patent describes its DCS method as using standard commercially available Illumina (*e.g.*, TruSeq) DNA sequencing adapters. EX1001, 25:52-26:1; *see also* 18:45-52. Illumina TruSeq adapters are Y-shaped adapters that include flow cell 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); *see also* EX1001, claim 14 (“wherein the adapters comprise the barcodes in at least one of ...a single-stranded portion of the adapter”).



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, 18:49-52 (“DCS does not require any significant deviations from the normal

workflow of sample preparation for Illumina DNA sequencing.”). 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 acknowledged in the ’127 patent. EX1001, 3:64-4:3, Figure 1; EX1002, ¶¶38-41.

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,

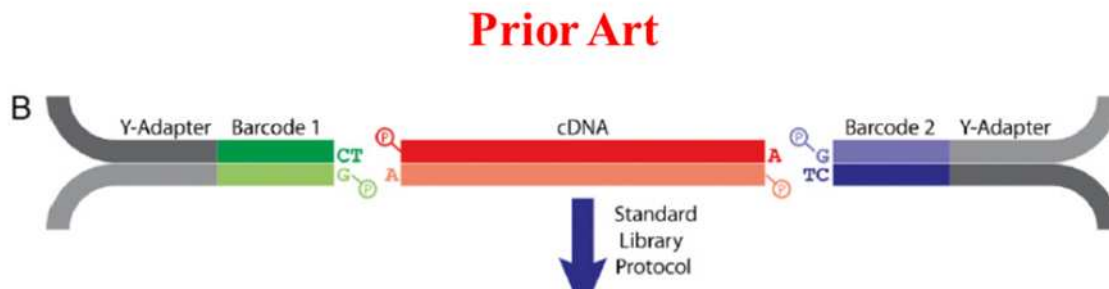
Figure 1, Figure S5; EX1029, 14:15-29, 19:34-64, Figures 1 and 4; EX1022, ¶¶42; EX1002, ¶¶42-50.

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 Safe-SeqS 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 adaptors 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, ¶¶43-45. Kinde specifically discloses that methods include “the introduction of exogenous sequences through” 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 ’127 patent. *Compare* EX1010, Figure 1B (reproduced below), *with*, EX1001, Figure 1, 3:64-4:3.



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.

In our approach, each cDNA molecule is attached to a unique barcode sequence from a large pool of barcodes before amplification (Fig. 1A)

(14). ... This concept has been applied recently...to improve the sensitivity of DNA mutation detection (16, 17)

EX1010, 1347 (citing Kinde (reference 17)).

Accordingly, prior to the '127 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, ¶¶46-50.

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. Kinde describes a barcode-based consensus sequencing method called Safe-SeqS.

...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 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).² EX1002, ¶¶51-53.

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

² Kinde is acknowledged as prior art in the specification of the ’127 patent. *E.g.*, EX1001, 2:26-32 (citing Kinde). As discussed herein, Kinde illustrates UIDs or barcodes on both ends of the target molecule (*e.g.*, Figure 2), ligates Y-shaped adapters to DNA fragments (*e.g.*, 9531, SI1), and cites to Craig that, in turn, discloses barcoded Y-shaped adapters.

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, ¶54.

iv. NEB Expressions

NEB Expressions was published by New England Biolabs in Spring 2007 and also distributed publicly through the U.S. Postal Service. EX1014, 1, 8. Its public availability is confirmed by an Internet Archive WayBack Machine capture of the publication in its entirety on the NEB website at least as early as November 19, 2008 and of the index page for the publication at least as early as March 21, 2008. EX1015; EX1016. Its prior art status also is confirmed by a citation of the publication in a review published in in September 2011. EX1017, 28, 31 (reference 20). NEB Expressions qualifies as a prior art under 35 U.S.C. §102(a)(1) and pre-AIA 35 U.S.C. §102(b). EX1002, ¶55.

B. The '127 Patent

The '127 patent is entitled “Methods of lowering the error rate of massively parallel DNA sequencing using duplex consensus sequencing.” EX1001, cover (54). The '127 patent specification proposes correcting sequencing errors with a tag-based method referred to as “Duplex Consensus Sequencing” or “DCS.” *E.g.*, EX1001, Abstract, 3:17-4:3.

As with prior art consensus sequencing methods, DCS generally includes tagging polynucleotides with identifier sequences (“SMI”), amplifying the SMI-tagged molecules using PCR, and sequencing the amplified products. EX1001, 2:15-30. The patent admits the prior art taught, for example, “techniques whereby DNA fragments to be sequenced are each uniquely tagged [34, 35] prior to amplification [36-41].”³ *Id.*, 2:16-19. Skilled artisans used SMI sequences so that “all amplicons derived from a particular starting molecule will bear its specific tag” and “any variation in the sequence or copy number of identically tagged sequencing reads can be discounted as technical error.” *Id.*, 2:19-23. As in Kinde, the sequenced tagged molecules are then grouped into families based on a common SMI sequence. *Id.*, 3:17-39, Figure 1, 3:52-4:3, 19:20-25. Like in Kinde, a “consensus” is determined from the grouped sequence reads which allows true mutations to be distinguished from sequencing errors. *Id.*, 3:33-39, Figure 3, 4:12-37; *see also* Section I.A.

The ’127 patent recognizes that barcode-based methods for generating error corrected consensus sequences, such as the Safe-SeqS method disclosed by Kinde, were known in the art at the time. *E.g.*, EX1001, 2:23-26 (“has been used to

³ Kinde (EX1006) is reference 36.

improve counting accuracy...and to *correct base errors arising during PCR or sequencing* [36, 37, 39].”), 2:26-30 (“Kinde et. al. reported a reduction in error frequency...”), 26:46-48 (to compare to Kinde, “reads were grouped into families by SMI tag”). EX1002, ¶¶21-29.

C. Prosecution History

The ’127 patent was filed as U.S. Application No. 16/503,382 (“the ’382 application”) on July 3, 2019, claiming priority, via U.S. Application Nos. 16/120,072, 15/660,785, 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 claims of the ’382 application were rejected as anticipated by Otwinowski (WO2013/181170) which Patent Owner overcame by arguing that the reference is not prior art. EX1004, 360-369, 407. In a subsequent telephonic interview, the Examiner and Patent Owner discussed “how the barcoded adapters label individual strands.” *Id.*, 422, 435. No further Office actions were issued.

The Examiner explained in the reasons for allowance that she did not understand the art as describing adapters that enable strand-specific labeling.

Further, as established in the interview, the method of the claims, as amended, provides the ability to label each strand of a target with asymmetric adaptors that enable strand specific labeling. With these adaptors, as depicted in Figure 1 and 3, the adaptors allow for specific

detection and identification of genuine mutations as opposed to sequence variations that are artifacts or side-effects of the sequencing process.

Further, a careful search of the prior art does not find any references which teach adapters of the format as claimed by Applicant.

EX1004, 432-433. As explained herein, adapters comprising barcodes — such as depicted in Figures 1 and 3 of the '127 patent — were known and well-documented in the prior art. *See* Section I.A. Accordingly, the Examiner's failure to identify such art and apply it to the claims represents material error.

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, ¶¶56-57.

II. GROUNDS FOR STANDING

Petitioner certifies that, under 37 C.F.R. §42.104(a), the '127 patent is available for *inter partes* review, and Petitioner is not barred or estopped from requesting *inter partes* review of the '127 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 '127 patent against Petitioner in the United States District Court in Delaware (1:21-cv-01126-LPS). Concurrently filed with this petition is another petition also requesting *inter partes* review of the '127 patent in IPR2022-00817.

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

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

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

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

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

Petitioners request review of claims 1-30 of the '127 patent under 35 U.S.C. §311 and AIA §6. The grounds for relief are as follows:

Ground	Claims	Description
1	1-10 and 12-30	Unpatentable under 35 U.S.C. §103 as obvious over Kinde in view of Craig
2	11	Unpatentable under 35 U.S.C. §103 as obvious over Kinde in view of Craig in further view of NEB Expressions

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 22 recites attaching “partially single-stranded adapters” to double-stranded DNA fragments to generate a library of tagged double-stranded adapter-DNA molecules. Independent claim 1 recites attaching adapters to the DNA fragments makes them “partially-complementary, asymmetrical double-stranded adapter-DNA molecules.” The claims also require that the adapters comprise barcodes selected from a plurality of distinct barcode sequences. Claim

13 depends from claim 1 and recites that the adapters comprise a Y-shape or a U-shape. In each case, the claims encompass the use of standard Y-shaped (e.g., Illumina) adapters (when those adapters comprise barcodes selected from a plurality of distinct barcode sequences). *E.g.*, EX1012, Figure 4.

As Dr. Quackenbush explains, and as explained above in Section I.A., standard Illumina Y-shaped adapters are partially single-stranded (the arms of the Y) and also are partially-complementary (the trunk of the Y). When the adapters are ligated to a double-stranded DNA molecule, the resulting adapter-DNA molecule is partially-complementary and asymmetric. 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; EX1002, ¶¶58-60. The same is true when using U-shaped hairpin adapters, which also are partially single-stranded and partially-complementary adapters. EX1002, ¶¶60-62. This understanding of the claims is consistent with the specification of the ’127 patent. *See, e.g.*, EX1001, 3:64-4:3, 6:4-41, 10:63-11:2, 18:45-57, 26:5-17, Figs. 1-2. Accordingly, the plain meaning of these claims is reasonably understood to encompass adapter-DNA molecules generated by attachment of Y-shaped or U-shaped adapters comprising barcodes to each end of double-stranded DNA molecules.

VI. DETAILED EXPLANATION FOR GROUNDS OF UNPATENTABILITY

A. [Ground 1] Claims 1-10 and 12-30 are unpatentable as obvious under 35 U.S.C. §103 over Kinde in view of Craig

As discussed in detail above and below, Kinde in view of Craig renders obvious each of claims 1-10 and 12-30. Kinde exemplifies consensus sequencing using Y-shaped Illumina adapters, which include index sequences and generate partially single-stranded and partially-complementary, asymmetrical double-stranded adapter DNA molecules. Kinde further cites Craig specifically for disclosing barcoded-adapters. Craig discloses employing barcodes selected from a plurality of distinct barcode sequences in the complementary portion of partially single-stranded and partially-complementary barcoded Y-shaped Illumina adapters. Kinde also expressly suggests comparing the consensus sequences for both strands of a particular DNA molecule to one another to identify and exclude artifacts of the amplification and sequencing process. Kinde in view of Craig thus describe performing consensus sequencing by attaching Y-shaped Illumina adapters having barcodes in a manner that satisfies each of claims 1-10 and 12-30. As discussed in further detail below, a skilled artisan would have had good reason to use barcoded adapters with redundant sequencing of DNA, such as described by Kinde and Craig, with a reasonable expectation of success of achieving what is claimed here. EX1002, ¶¶63-64.

There can be no novelty of the claimed invention residing in the requirement for partially-complementary, asymmetric adapter-DNA molecules (claim 1) or the requirement for determining a consensus sequence (claim 22). Section I.A. Each of these aspects was described in the prior art at the time. Each and every feature of claims 1-10 and 12-30—including any alleged points of novelty—is taught or suggested in the prior art, as illustrated by Kinde.

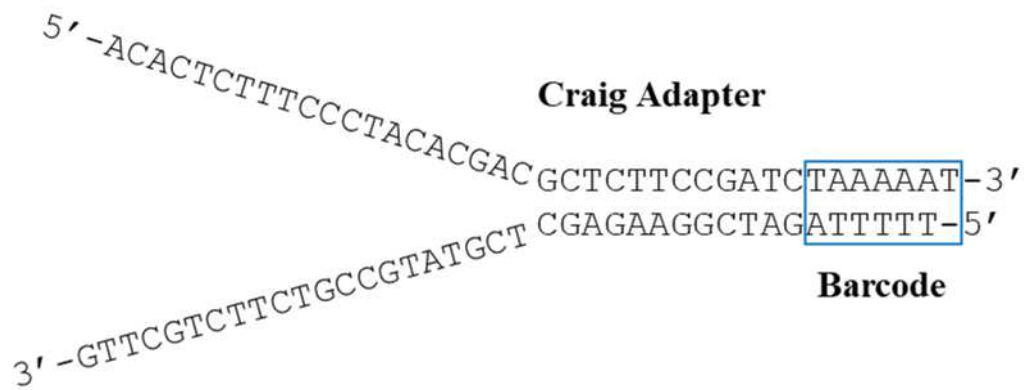
The '127 patent acknowledges that consensus sequencing based on barcoding or tagging was known, and further acknowledges similarity to the methods of Kinde. EX1001, 2:15-38; 26:19-57, 32:8-11. Indeed, Kinde discloses the same workflow discussed in the '127 patent (*e.g.*, attaching adapters comprising barcodes, amplifying tagged polynucleotides, sequencing, grouping the sequence reads, and generation of a consensus). EX1002, ¶¶65-67.

Kinde's "Safe-SeqS" is a method for reducing sequencing errors such that rare mutations may be detected. EX1006, Abstract, Title. Like DCS, Safe-SeqS 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 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 $\geq 95\%$ of reads within a

family) as compared to a reference sequence, which Kinde calls “supermutants.”
Id., 9530.

Kinde teaches performing consensus sequencing by ligating Y-shaped (*e.g.*, Illumina) adapters to double-stranded DNA molecules to produce partially-complementary, asymmetric adapter-DNA molecules. For example, Kinde describes an endogenous UID embodiment in which genomic DNA is randomly sheared and then ligated to Illumina sequencing adapters. EX1006, 9531 (“...ligated standard Illumina sequencing adapters to the ends of sheared DNA fragments...”), 9535 (DNA “...ligated to Y-shaped adapters...”). Kinde explains that this method produces “One uniquely identifiable fragment ...from each strand of the double-stranded template.” EX1006, Figure 2; Section I.A. *Compare* EX1001, Example 3; 25:64-26:1 (discussing “ligation of Illumina TruSeq DNA sequencing adaptors, all by standard library preparation methods.”).

Kinde also teaches that its methods include exogenous UIDs and specifically cites Craig which uses asymmetric Y-shaped sequencing adapters. Specifically, Kinde discloses that methods include “exogenous sequences [introduced] through ... ligation (42, 43).” EX1006, 9531. Reference 42 cited by Kinde is Craig (EX1007). Craig discloses Illumina Y-shaped adapters that include a barcode in the double-stranded portion. Below is an illustration from the Quackenbush Declaration of one of the 48 barcoded adapters disclosed by Craig.



EX1002, ¶70; EX1007, Supplementary Tables 3 and 4, Supplementary Methods. Craig describes ligating the disclosed adapters to target DNA. *Id.*; *see also* EX1002, ¶¶68-70.

A skilled artisan would have had good reason to combine Kinde and Craig to use Y-shaped adapters comprising barcode sequences, as disclosed in Craig, in order to arrive at the '127 patent claims. First of all, Kinde identifies Craig in expressly discussing exogenous barcodes with DNA molecules. To the extent Kinde's disclosure is not considered anticipatory, 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, Kinde describes sequencing and comparing the sequence of amplification progeny for each strand of the original DNA duplex to confirm the presence of apparent mutations in the original DNA duplex. Specifically, Kinde teaches that UIDs are assigned so that each strand of the DNA duplex is “uniquely

identifiable.” EX1006, Figure 2. Kinde explains that the specificity of sequencing may be increased “by requiring that each strand of the original double-stranded template contain the mutation.” EX1006, SI3; *id.*, SI2 (“Safe-SeqS Analysis”). Accordingly, a skilled artisan would have had good cause to utilize Y-shaped Illumina adapters and exogenous barcodes, as taught by Kinde in view of Craig, to determine whether apparent mutations were confirmed in both strands.

A skilled artisan also had a reasonable expectation of success in achieving what is claimed. Kinde expressly discusses the combined teachings. EX1002, ¶¶70-73. Moreover, Kinde and Craig both use Illumina Y-shaped adapters attached to each end of DNA molecules. *KSR*, 550 U.S. at 417. In a recent IPR filing, Patent Owner argued that Craig’s barcoded Y-shaped adapters are amenable to use in the claimed method. *Twinstrand Biosciences, Inc. v. Guardant Health, Inc.*, IPR2022-00746, Paper 2, 67 (“...POSA also would expect Craig’s barcodes to work in Schmitt’s⁴ methods...”).

⁴ Schmitt (Int. Pub. No. WO2013/142389) is the published version of PCT/US2013/032665 which is listed on the face of the ’127 patent as a priority document. EX1001, front page (63).

As Dr. Quackenbush explains, the scientific literature at the time further corroborates that a skilled artisan would have been motivated with reasonable expectation of success of arriving at what is claimed. Shiroguchi illustrates that barcoded Y-shaped adapters were well-known at the time, and further confirms such methods labeled each strand of a target with adapters that enable strand-specific labeling. Shiroguchi also discusses the use of such adapter molecules in consensus sequencing approaches as described by Kinde and others. EX1010, 1347 (“...to improve the sensitivity of DNA mutation detection (16, 17), ...”), 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, ¶74.

Moreover, there is no meaningful distinction between the tagging scheme employed in Kinde and the tagging scheme claimed in the '127 patent. To illustrate this, one may look to Example 3 of the '127 patent, which employs a tagging scheme relying on endogenous sequences where “DNA was randomly sheared” and then ligated to Illumina TruSeq sequencing adapters using “standard library preparation methods.” EX1001, 25:63-26:1; *see also* Section I.A. The '127 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, 25:60-26:20. Reads were grouped into families according to these endogenous sequences, and reaching consensus for the sequence required

agreement among “at least 90% of family members.” EX1001, 26:10-13. The ’127 patent even draws specific reference to Kinde in comparison. *Id.*, 26:46-48. Indeed, Kinde describes such an endogenous UID approach. EX1006, 9531 (...“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)...”); 9535, SII (“...ligated to Y-shaped adapters according to standard Illumina protocols...”); EX1002, ¶75.

The ’127 patent discloses adding double-stranded SMI sequences to sample DNA using modified Illumina adapters. EX1001, 27:50-55. The ’127 patent describes SMI adapters as “contain[ing] the standard sequences required for the Illumina HiSeq system ... with [the] addition of a double-stranded, complementary SMI sequence (or “tag”) of 12 random nucleotides” EX1001, 22:19-24.

Furthermore, Patent Owner filed a declaration in a related case that acknowledges SMI adapters are Illumina TruSeq adapters modified to include an SMI sequence in the double-stranded portion. EX1005, ¶5, 6 (“Sequencing both strands: Illumina TruSeq ...”). Kinde, similarly, discusses exogenous UID and identifies Craig as illustrative of barcoded Y-shaped adapters. EX1006, 9531 (citing Reference 42, Craig). Craig discloses Illumina adapters modified to include a barcode in the double-stranded portion. Craig further discloses that these adapters may be ligated to fragmented sample DNA prior to amplification. The tagging approaches

employed in the '127 patent are indistinguishable from those previously disclosed by Kinde, particularly in view of Craig (and further confirmed by Shiroguchi). Accordingly, each of claims 1-10 and 12-30 would have been obvious over Kinde in view of Craig. EX1002, ¶¶71-77.

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 sequencing DNA comprising:”

Kinde discusses DNA sequencing throughout its disclosure. Kinde discloses a method for “Detection and quantification of rare mutations with massively parallel sequencing.” EX1006, Title. Kinde discloses that Safe-SeqS can be applied to populations of DNA template for identification of mutations.

The results described above demonstrate that the Safe-SeqS approach can substantially improve the accuracy of massively parallel sequencing It can be implemented through either endogenous or exogenously introduced UIDs and can be applied to virtually any sample preparation workflow or sequencing platform. As demonstrated here, the approach can easily be used to identify rare mutants in a population of DNA templates.

EX1006, 9533. Craig also discloses sequencing DNA. EX1007, Title (“Identification of genetic variants using bar-coded multiplexed sequencing”), Abstract; EX1002, ¶¶78-81.

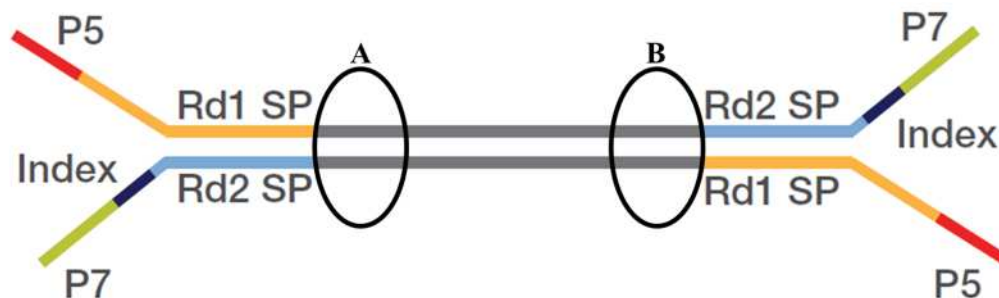
Element 1.1

“a) attaching adapters to double-stranded DNA fragments to generate a plurality of partially-complementary, asymmetrical double-stranded adapter-DNA molecules,”

Kinde and Craig disclose Element 1.1. Kinde discloses attaching Y-shaped adapters to double-stranded DNA fragments. EX1006, 9531 (“Endogenous UIDs”), Figure 2. Those double-stranded DNA fragments are “end-repaired,” and “A-tailed,” and then attached to “Y-shaped adapters” using ligation. EX1006, 9535, SI1. Kinde described these Y-shaped adapters as “standard Illumina sequencing adapters.” EX1006, 9531; EX1002, ¶¶82-83. 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.

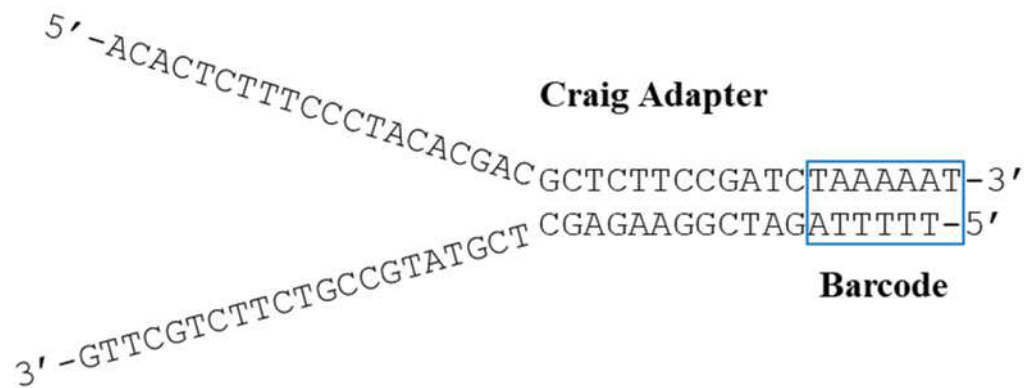
As explained above, Illumina adapters ligated to sample DNA produce partially-complementary, asymmetrical double-stranded adapter-DNA molecules in the Illumina workflow. Section I.A. By virtue of the asymmetric nature of the adapted fragments, two types of PCR products are produced from each capture 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 (modified to include annotations). The “asymmetry” discussed in the ’127 patent is no different. EX1001, 3:64-4:3, Figure 1.

The claimed asymmetric adapter-DNA molecules similarly result where barcodes are present in the double-stranded portion of Y-shaped adapters. Craig, specifically cited in Kinde, discloses Illumina adapters comprising barcodes. EX1007, 888, Supplementary Tables 3 and 4, Supplementary Methods. Craig’s adapter is a commercially available Illumina adapter like Kinde’s, but also has a barcode proximal to the ligation site in the double-stranded portion of the adapter. *Id.* Because this barcode is an extension of the complementary portion, the Y-shaped adapter remains partially-complementary and produces asymmetric PCR progeny. Craig ligates its modified Illumina adapters to both ends of the DNA fragment. EX1007, Figure 1; EX1002, ¶¶84-85.



Regardless of whether off-the-shelf Illumina adapters or Craig’s type of adapters are used, the result is asymmetrical double-stranded adapter-DNA molecules. This is confirmed by both Shiroguchi and the ’127 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, 3:64-4:3, 26:5-17; Section I.A.; EX1002, ¶¶86-87.

Element 1.2

“wherein the adapters comprise barcodes selected from a plurality of distinct barcode sequences;”

As an initial matter, while claims 1-21 require attachment of adapters comprising barcodes to double-stranded DNA, none of these claims require the barcode be used for any purpose. Accordingly, Patent Owner cannot distinguish over the prior art based on the utility of the barcode. *WesternGeco LLC v. ION Geophysical Corp.*, 889 F.3d 1308, 1323-24 (Fed. Cir. 2018) (citing *Comaper Corp. v. Antec, Inc.*, 596 F.3d 1343, 1348 (Fed. Cir. 2010)) (“It is well established

that claims are not limited to preferred embodiments, unless the specification clearly indicates otherwise.”).

As discussed above, commercially available Illumina adapters comprise an index (*i.e.*, barcode), EX1012, Figure 4, and Craig discloses barcodes in the double-stranded portion of the Y-shaped adaptors. Kinde discloses Y-shaped adapters and use of UIDs in its methods throughout its disclosure. EX1006, 9531 (“UIDs, sometimes called barcodes or indexes, can be assigned to nucleic acid fragments using a variety of methods...”); *see also id.* Abstract, 9530 (“Overview”), Figure 2, 9535, SI1 (“...ligated to Y-shaped adapters according to standard Illumina protocols.”).

In further regard to barcodes, the prior art teaches Y-shaped adapters comprising barcodes selected from a plurality of distinct barcode sequences. Craig discloses “48 different adapters,” which vary by index (*i.e.*, barcode). EX1007, 888, Supplementary Tables 3 and 4, Supplementary Methods. Craig’s DNA fragments were “ligated ... to one of the 46 indexed adapters.”⁵ EX1007, 888. A skilled artisan thus would have understood the prior art as teaching adapters

⁵ While Craig designed 48 different adapters, 46 were used experimentally.

comprising barcodes selected from a plurality of distinct barcode sequences. *See also* Section I.A.; EX1002, ¶¶88-91.

As discussed above, skilled artisans had good reason to use Y-shaped Illumina adapters comprising barcodes to arrive at the challenged claims in view of Kinde’s Safe-SeqS approach. For example, citing to Craig, Kinde discusses methods including assigning exogenous barcodes to nucleic acid fragments. EX1006, 9531. A POSA would have reasonably expected success in arriving at the challenged claims because this approach was widely used and documented in the prior art. *E.g.*, EX1010, Figure 1; EX1007, 887 (“Experimental design”); EX1009, ¶¶52-55; EX1012, Figure 4; EX1002, ¶¶92-95. The use of Y-shaped adapters comprising barcodes was a routine aspect of sequencing, including in methods directed to reduction of bias and error in the resulting reads. *E.g.*, EX1010, Figure 1; EX1009, ¶¶52-55; EX1006, 9531 (“...we ligated standard Illumina sequencing adapters...”).

Element 1.3

“b) amplifying original strands of at least a portion of the double-stranded adapter-DNA molecules to produce first and second strand copies;”

Kinde and Craig disclose Element 1.3. For example, Kinde discloses “amplification” of the tagged template molecules. EX1006, Abstract (“Our approach... The second is the amplification...”), Figure 2 (“amplified” by PCR),

SI1 (“PCR-mediated amplification”). 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”), SI1 (“...ligated to Y-shaped adapters...”); EX1002, ¶¶96-99; Section I.A.

Craig also teaches amplification of adapter-DNA molecules using PCR. EX1007, 887 (“After ligation, ..., enriched them by PCR amplification...”).

Element 1.4

“c) sequencing a plurality of first and second strand copies to obtain first and second strand sequence reads for at least a portion of the adapter-DNA molecules; and”

Kinde and Craig disclose Element 1.4. Kinde’s Safe-SeqS approach involves “redundant sequencing of the amplification products.” EX1006, Abstract; *see also id.*, Figure 2, 9535 (“Sequencing”). As discussed above for Element 1.2, Kinde’s adapters are ligated to both ends of the double-stranded fragments of sample DNA. Amplification of Kinde’s adapter-DNA molecules produces copies of the first and second strand. *Id.*, 9530 (“Overview”), Figure 2. A POSA would thus understand Kinde’s sequencing step includes sequencing the first and second strand copies to obtain first and second strand sequence reads for at least a portion of the adapter-DNA molecules. EX1002, ¶¶100-104. Indeed, Kinde expressly suggests comparing the sequences of complementary strands to one another to increase specificity. EX1006, SI3 (“...When requiring multiple supermutants, the specificity can be

further increased by requiring that each strand of the original double-stranded template contain the mutation. ...”).

Craig also discloses sequencing both strands. EX1007, 887 (“...sequenced them on the Illumina GA...”).

Element 1.5

“d) for at least some of the adapter-DNA molecules comprising barcodes—

confirming the presence of at least one sequence read derived from each of the original first and second strands of the adapter-DNA molecules;”

As Dr. Quackenbush explains, a POSA also would have understood that Kinde discloses Element 1.5. EX1002, ¶106. First, Kinde acknowledges that adapter-DNA molecules produce two distinct replication products: one for each of the original strands. EX1006, Figure 2 (“One uniquely identifiable fragment...from each strand of the double-stranded template.”); Section I.A. Second, Kinde teaches that the errors introduced by the methodology “can be reduced by requiring at least two supermutants to identify a position as a mutant,” or, in other words, requiring detection of “two supermutants, one from each strand” to conclude that the apparent mutation is a “preexisting mutation in a double-stranded DNA template[.]” *Id.*, SI2. 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 (“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, ¶¶106-108.

Element 1.6

“comparing at least one of the confirmed first and second strand sequence reads to a reference sequence; and”

Patent Owner has conflated comparing reads to a reference sequence as stated in Element 1.6 with comparing consensus sequence reads to a reference. The was directly addressed by the Examiner in a related continuation application. Specifically, the Examiner found that the specification (which is shared with the ’127 patent) only discusses the alignment of the consensus sequence. EX1025, 138.

Nevertheless, Kinde teaches Element 1.6. For example, Kinde discusses comparing reads with the reference sequence. EX1006, SI2 (“sequence alignment [was] performed with the Eland pipeline (Illumina)” only reads with “ ≤ 3 mismatches to expected sequences” are further analyzed with Safe-SeqS). Kinde specifically discloses using Eland for “alignment” of reads. A POSA would have understood that Eland is software marketed by Illumina that aligns reads to a

reference sequence. EX1011, Figure 1 (“Alignment to Reference Genome”)⁶. A skilled artisan would have understood that aligning reads to a reference sequence establishes the strand origin of a read — that is, whether the read is derived from the original first or second strand. EX1002, ¶¶109-12. That strands are differentiated through alignment is directly addressed in the corresponding patent application describing Safe-SeqS. EX1022, ¶42 (“The two strands of a double stranded template can be differentiated by the observed orientation of the sequences and the order in which they appear when sequence information is obtained from both ends.”); *see* Section I.A.

Element 1.7

analyzing one or more correspondences between at least one of the confirmed first and second strand sequence reads and the reference sequence to identify a sequence variation.”

As discussed above regarding Element 1.7, Kinde aligns reads with a reference sequence. Kinde further instructs that “a base [is identified] as mutant ... only if the same variant was identified in at least two members of at least one UID family.” EX1006, SI2. A skilled artisan would understand determining that a base

⁶ As demonstrated by EX1034 and its explanation of The Internet Archive, EX1011 was publicly available as of December 26, 2010.

is “mutant,” as Kinde instructs, requires analyzing one or more correspondences between a read and a reference sequence. EX1006, SI2 (“Sequencing”); *see also id.* (“Apparent mutations, defined as any base call that varies from the expected base at a defined position...”). That is, a position is mutant only if it is different in comparison to the expected base at the corresponding position of the reference sequence.

In summary, Kinde in view of Craig teaches or suggests Claim 1 as a whole. EX1002, ¶¶113-15.

ii. Claim 22

Preamble

“A method of sequencing DNA comprising:”

Kinde discloses a method for “Detection and quantification of rare mutations with massively parallel sequencing.” EX1006, Title. Kinde discloses that Safe-SeqS can be applied to populations of DNA template for identification of mutations. EX1006, 9533 (“...used to determine the prevalence of rare mutations...”). Craig also discloses sequencing DNA. EX1007, Title, Abstract; *see also* claim 1 Preamble above; EX1002, ¶¶116-18.

Element 22.1

“a) attaching partially single-stranded adapters comprising barcodes selected from a plurality of distinct barcode sequences to double-stranded DNA fragments obtained from a bodily

sample, wherein attachment of the adapters to double-stranded DNA fragments generates a library of tagged double-stranded adapter-DNA molecules;”

Kinde and Craig teach Element 22.1. As discussed above with respect to Element 1.1 and 1.2, Kinde in view of Craig teaches attaching adapters to double-stranded DNA fragments to generate a plurality of partially-complementary, asymmetrical double-stranded adapter-DNA molecules, wherein the adapters comprise barcodes selected from a plurality of distinct barcode sequences. *See* EX1006, Figure 2; EX1007, 887 (“Experimental design”), Figure 1, Supplementary Table 3. Kinde expressly refers to the adapter-ligated DNA fragments as a “sequencing library.” EX1006, 9531. The Y-shaped Illumina adapters employed in Kinde and relied upon for Element 1.1 are partially-single stranded adapters as recited in claim 22. *See* Section I.A.

Kinde also teaches obtaining DNA from a “bodily sample.” For example, Kinde teaches extracting DNA from “human pancreas” or “normal human colonic mucosae.” EX1006, SI1. Kinde further describes shearing the extracted DNA to produce fragments. EX1006, Figure 2 (“...fragment produced by random shearing...”), 9535 (“...acoustic shearing...”); EX1002, ¶¶119-22.

Element 22.2

“b) amplifying strands from a plurality of the double-stranded adapter-DNA molecules in the library to produce strand copies;”

Kinde and Craig disclose Element 22.2. Kinde discloses “amplification” of the tagged template molecules. EX1006, Abstract, Figure 2 (“amplified” by PCR), S11. Kinde specifically teaches amplification produces copies of both the first and second strands. *See, e.g.*, EX1006, Figure 2 (“One uniquely identifiable fragment is produced from each strand...”), S11 (“...ligated to Y-shaped adapters...”); EX1002, ¶¶123-26.

Craig also teaches amplification of adapter-DNA molecules using PCR. EX1007, 887 (“After ligation, ..., enriched them by PCR amplification...”). *See also* Element 1.3 above.

Element 22.3

“c) sequencing a plurality of the strand copies to obtain strand sequence reads comprising one or more barcode sequences and DNA fragment-specific information; and”

Kinde and Craig disclose Element 22.3. For example, Kinde discloses that sequencing of sheared DNA fragments “yields UID families” based on the endogenous sequences on each end of the sheared fragment. EX1006, 9531. Kinde further discloses sequencing reads comprising an exogenous barcode and DNA fragment-specific information. EX1006, S11 (“The resulting DNA fragments

contained UIDs composed of three sequences: 2 endogenous ones, represented by the two ends of the original sheared fragments, plus the exogenous sequence introduced during the indexing amplification.”).

Craig discloses obtaining strand sequence reads. EX1007, 887. Craig further discloses that the sequencing reads contained sequence of the DNA fragment (“Region Features”) and also an index (*i.e.*, barcode) that allowed the classification of the read into “different indexed pools.” EX1007, Supplementary Table 2; *see also* Element 1.4; EX1002, ¶¶127-30.

Element 22.4

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

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

Kinde in view of Craig teaches Element 22.4. Kinde discloses grouping reads “into UID families on the basis of their endogenous or exogenous UIDs.” EX1006, 9535. For endogenous barcodes, Kinde explains that “DNA inherently contains UIDs consisting of the sequences of the ends of each sheared fragment.” EX1006, 9531. Kinde’s endogenous UIDs, the randomly sheared ends of the fragments, are both barcode sequences and fragment-specific information. EX1006, 9531 (“...randomly sheared genomic DNA inherently contains UIDs...”); EX1002, ¶¶131-33. Kinde further discloses grouping reads based on

endogenous and exogenous UIDs. EX1006, SI1 (“The resulting DNA fragments contained UIDs composed of three sequences: 2 endogenous ones, represented by the two ends of the original sheared fragments, plus the exogenous sequence introduced during the indexing amplification.”).

Element 22.5

“collapsing a plurality of strand sequence reads within the families to provide a consensus sequence for each of at least some of the double-stranded DNA molecules in the library;”

Based on the '127 patent, a POSA would have understood “collapsing” to be the process by which consensus sequence information is drawn from redundant sequence reads derived from the same molecule. *E.g.*, EX1001, 20:53-55 (“Reads having common (i.e., identical) SMI sequences were grouped together, and were collapsed to generate a consensus read.”).

Kinde teaches Element 22.5. For example, Kinde assesses multiple reads and identifies a consensus among the multiple reads, Kinde distinguishes a true mutation, which Kinde called a “supermutant,” where “at least 95% of family members have the identical mutation.” EX1006, 9530. Indeed, distinguishing errors from true mutations by such consensus sequencing was well known in the art at the time. *See, e.g.*, EX1031, 4 (“To estimate error rates using [barcodes], we derived a consensus sequence for each [barcode] and called errors as non-reference positions”); EX1010, 1347 (“[Unique barcoding] has been applied recently ...

to improve the sensitivity of DNA mutation detection (16, 17) ...”) (reference 17 is Casbon, EX1031).

Furthermore, while analysis of both strands is not required by claim 22, Kinde discloses doing so. For example, Kinde suggests confirming the existence of a true mutation by determining whether the supermutant was found for each complementary strand of the original DNA molecule. EX1006, SI3 (“...the specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation ...”), SI2 (“These mutations are expected to be present in both strands of the relevant templates.”); EX1002, ¶¶134-38.

Element 22.6

“comparing the consensus sequence to a reference sequence;
and

analyzing one or more correspondences between the consensus
sequence and the reference sequence to identify a sequence
variation.”

Kinde discloses identifying sequence variants, also called mutations, by comparing the sequence reads to an expected sequence, EX1006, SI2 (“Sequencing”), (defining mutation as “base call that varies from the expected”). When performing Safe-SeqS, 95% agreement (or consensus) is required within a given 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). A POSA thus would have understood Kinde as disclosing Element 22.6. *See also* Element 1.7; EX1002, ¶139.

Accordingly, Kinde in view of Craig teaches or suggests every element of each of claim 22. EX1002, ¶¶139-42.

iii. Claim 2

2. The method of claim 1, wherein the sequence variation is either (i) a processing error or a site of DNA damage, or (ii) a true variant, and wherein, for at least some of the adapter-DNA molecules, the method further comprises:

comparing the confirmed first strand sequence read and confirmed second strand sequence read;

identifying nucleotide bases that are not consistent between the confirmed first and second strand sequence reads; and

identifying nucleotide bases that are consistent between the confirmed first and second strand sequence reads,

wherein—

if the sequence variation is not a consistent nucleotide base, then categorizing the sequence variation as (i) a processing error or a site of DNA damage, and

if the sequence variation is a consistent nucleotide base, then categorizing the sequence variant as (ii) a true variant.

As discussed above, Kinde in view of Craig renders claim 1 obvious. Kinde also discloses the subject matter recited in dependent claim 2. As discussed above with respect to Elements 22.6, Kinde teaches or suggests comparing the consensus

sequence of complementary strands of a DNA molecule to distinguish true mutations appearing in the source DNA from artifacts of processing. Kinde teaches that true mutations appearing in the source DNA should be confirmed in the consensus sequence of both complementary strands and teaches identifying non-complementary bases as either an experimental error or a site of DNA damage.

Kinde teaches that apparent mutations, meaning any base call that varies from the expected base at a defined position, can reflect actual “[m]utations present in the template DNA[.]” EX1006, SI2. Kinde teaches that “[t]hese mutations are expected to be present in both strands of the relevant templates.” *Id.* Kinde also teaches that apparent mutations may be attributed to “Error-generating processes.” *Id.* Kinde identifies such error-generating processes as including DNA damage in the forms of oxidized DNA or “damage” caused by shearing. *Id.* (items ii-iii). Kinde also identifies process errors as including error introduced during end repair of the sheared fragments, errors introduced by other enzymatic steps, through PCR amplification, during UID assignment, and errors in base calling. *Id.* (items iv-xi). Kinde thus teaches distinguishing true variants from processing/experimental errors by: (1) determining a consensus sequence for each UID family (supermutants representing at least 95% identity for that mutation within the UID family); and (2) concluding apparent mutations are true variants when they are found as consensus sequence supermutants on both complementary strands of a

given DNA molecule. EX1006, SI3 (“...the specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation ...”), SI2 (“These mutations are expected to be present in both strands of the relevant templates.”).

Accordingly, Kinde in view of Craig teaches or suggests every element of claim 2. EX1002, ¶¶143-45.

iv. Claims 3 and 16

3. The method of claim 2, wherein prior to comparing the confirmed first strand sequence read and confirmed second strand sequence read, the method comprises associating the confirmed first strand sequence read with the confirmed second strand sequence read using one or more of an adapter sequence, a sequence length, sequence alignment information and original strand information.

16. The method of claim 1, wherein prior to the comparing step, the method comprises associating the confirmed first strand sequence read with the confirmed second strand sequence read using one or more of an adapter sequence and original strand information.

As an initial matter, the specification of the ’127 patent does not describe using “sequence length” or “sequence alignment” to “associate” first and second strand reads. However, as explained herein, Kinde teaches substantially the same procedure as described in the ’127 patent for associating strands of an original DNA molecule for the purpose of forming a consensus sequence through

comparison of reads associated with amplification progeny. Accordingly, to the extent that the '127 patent has support for these limitations, so too does Kinde.

As explained for claim 2, Kinde teaches or suggests comparing the sequences of amplification progeny of the two complementary strands of an original DNA molecule to distinguish processing errors from true variants — that is, to form an error-corrected consensus sequence. *See also* EX1001, 2:23-30 (explaining that Kinde describes methods to “correct base errors arising during PCR or sequencing”).

Regarding claims 3 and 16, Kinde further teaches or suggests associating the confirmed first strand sequence read with the confirmed second strand sequence read using an adapter sequence and/or original strand information.

As discussed in detail above, Kinde discloses using UIDs (*i.e.*, barcodes) to group “high-quality reads” of the sequenced amplification progeny into families corresponding to the original DNA fragments. EX1006, 9530 (“Overview”), Tables 1 and 2. Kinde’s “endogenous UIDs” satisfy the requirement of “original strand information.” These endogenous UIDs illustrate “randomly sheared genomic DNA” that “inherently contains UIDs.” EX1006, 9531. These barcodes provide fragment-specific original strand information because they are part of the sample DNA molecule and were generated using random shearing. A POSA thus would have understood Kinde as teaching or suggesting associating the confirmed

first strand sequence read with the confirmed second strand sequence read using original strand information to facilitate the claimed comparing step. EX1002, ¶¶146-49.

Kinde further teaches using adapter sequences to associate the confirmed first strand sequence read with the confirmed second strand sequence read. Kinde discloses use of exogenous UIDs (*i.e.*, barcodes) to group “high-quality reads.” A skilled artisan would have understood adapters comprising barcodes selected from a plurality of distinct barcode sequences as within the scope of Safe-SeqS or, at minimum obvious, in view of Craig. EX1006, 9531 (“These methods include the introduction of exogenous sequences”), EX1010, 1347 (discussing Kinde). Kinde teaches grouping reads into families derived from the same original molecule based on the UID and explains that the UID may be exogenous or endogenous. EX1006, 9530 (“Overview”), 9531 (describing exogenous and endogenous UID). Accordingly, Kinde also teaches using adapter sequences, particularly UID, to associate first and second strand reads.

As Dr. Quackenbush explains, a POSA would have understood from Kinde’s disclosure and the background understanding in the art that associating the strands before performing the claimed comparing step facilitated accomplishing Kinde’s instruction for distinguishing true variants from process errors. EX1002, ¶¶150-51. Accordingly, Kinde in view of Craig renders claims 3 and 16 obvious.

v. Claims 4 and 8

4. The method of claim 1, further comprising generating a consensus sequence for a plurality of adapter-DNA molecules, wherein each consensus sequence comprises nucleotide bases at which both the confirmed first strand sequence read and the confirmed second strand sequence read are in agreement.

8. The method of claim 4, further comprising identifying a nucleotide sequence occurring at a particular position or region in the consensus sequence as a true nucleotide sequence.

Kinde in view of Craig teaches or suggests the additional limitations of claims 4 and 8 for the same reasons it teaches or suggests the additional limitations of claim 2. Kinde teaches or suggests comparing the consensus sequence of complementary strands of a DNA molecule to distinguish true mutations appearing in the source DNA from artifacts of processing. Kinde teaches that true mutations appearing in the source DNA should be found in the consensus sequence of both complementary strands and teaches identifying non-complementary bases as either an experimental error or a site of DNA damage. EX1006, SI2-SI3 (“Safe-SeqS Analysis”). Accordingly, a POSA would have understood that Kinde teaches or suggests the requirements of claim 4. EX1002, ¶¶152-53.

Kinde specifically teaches that true nucleotide sequences appearing in the source DNA molecule should be found in the consensus sequence of (*e.g.*, supermutants) both complementary strands of the DNA molecule and teaches identifying non-complementary bases at a particular position or region as either an

experimental error or a site of DNA damage as required by claim 8. EX1006, SI2-SI3 (“Safe-SeqS Analysis”).

Accordingly, Kinde in view of Craig renders claims 4 and 8 obvious. EX1002, ¶¶153-54.

vi. Claims 5-7

5. The method of claim 4, further comprising aligning the consensus sequence to the reference sequence and identifying a variant or a mutation occurring at a particular position or region in the consensus sequence as a true variant or mutation.

6. The method of claim 5, further comprising comparing the consensus sequence to the reference sequence and identifying a mutation type for each of the true mutations.

7. The method of claim 6, wherein the mutation type is selected from one or more of a transition, a transversion, a point mutation, a structural rearrangement, a single-nucleotide substitution, a single-nucleotide variation, and an insertion.

As discussed above, Kinde in view of Craig renders claims 1 and 4 obvious.

Kinde also teaches or suggests the additional subject matter recited in claims 5-7.

Kinde teaches or suggests comparing the consensus sequence of complementary strands of a DNA molecule to distinguish true mutations as required by claim 5. Kinde specifically teaches that true mutations appearing in the source DNA molecule should be found in the consensus sequence of (e.g., supermutants) both complementary strands of the DNA molecule and teaches identifying non-complementary bases at a particular position or region as either an

experimental error or a site of DNA damage. EX1006, SI2-SI3 (“Safe-SeqS Analysis”). Kinde discloses performing sequence alignment compared to the “expected sequence” and base calling using the “Eland pipeline (Illumina).” EX1006, SI2.

Kinde also discloses identifying a mutation type for each of the true mutations, as required by claim 6, stating that Kinde’s sequencing “allowed the type of mutation, rather than simply its presence, to be identified.” EX1006, 9530, 9533 (“that “[t]he vast majority (>99%) of supermutants were single-base substitutions, and that occasional single-base deletions were also observed....”); EX1002, ¶¶155-57.

With respect to claim 7, the ’127 patent does not describe identifying a structural rearrangement or an insertion using DCS. To the extent Patent Owner argues that these were art known types of variation or mutation that a POSA would have been motivated to detect using DCS — that is, it would have been obvious to apply DCS to detect such mutations — this fails to establish written description support. *Research Corp. Techs. v. Microsoft Corp.*, 627 F.3d 859, 870 (Fed. Cir. 2010) (“Entitlement to a filing date extends only to subject matter that is disclosed; not to that which is obvious.”). Moreover, this same rationale would apply to Kinde in view of Craig.

Moreover, Kinde discloses that “[t]he vast majority (>99%) of supermutants were *single-base substitutions*,” and that occasional single-base deletions were also observed....” EX1006, 9533; *see also id.*, Figure 4, Figure S2, Table S1.

Accordingly, a POSA would have understood that Kinde in view of Craig teaches or suggests the additional limitations recited in claims 5-7. EX1002, ¶¶158-59.

vii. Claim 9

9. The method of claim 1, further comprising determining nucleotide bases at which the confirmed first strand sequence read and the confirmed second strand sequence read are not complementary, and identifying non-complementary bases as either an experimental error or a site of DNA damage.

As discussed above with respect to claim 2, Kinde teaches that true mutations appearing in the source DNA should be confirmed in the consensus sequence of both complementary strands and teaches identifying non-complementary bases as either an experimental error or a site of DNA damage.

Kinde teaches that apparent mutations, meaning any base call that varies from the expected base at a defined position, can reflect actual “[m]utations present in the template DNA[.]” EX1006, SI2. Kinde teaches that “[t]hese mutations are expected to be present in both strands of the relevant templates.” *Id.* Kinde also teaches that apparent mutations may be attributed to “Error-generating processes.” *Id.* Kinde identifies such error-generating processes as including DNA damage in

the forms of oxidized DNA or “damage” caused by shearing. *Id.* (items ii-iii).

Kinde also identifies process errors as including error introduced during end repair of the sheared fragments, errors introduced by other enzymatic steps, through PCR amplification, during UID assignment, and errors in base calling. *Id.* (items iv-xi).

Kinde thus teaches distinguishing true variants from processing/experimental errors by: (1) determining a consensus sequence for each UID family (supermutants representing at least 95% identity for that mutation within the UID family); and (2) concluding apparent mutations are true variants when they are found as consensus sequence supermutants on both complementary strands of a given DNA molecule. EX1006, 9530 (“Overview”), EX1006, SI3 (“...the specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation ...”), SI2 (“These mutations are expected to be present in both strands of the relevant templates.”).

Accordingly, Kinde in view of Craig renders claim 9 obvious. EX1002, ¶¶160-62.

viii. Claim 10

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

As discussed above, Kinde renders claim 1 obvious. The '127 patent acknowledges that Kinde amplifies original molecules and identifies mutations associated with PCR amplification. EX1001, 2:16-26. Kinde also expressly discloses “amplifying original strands via PCR amplification.” EX1006, Abstract, Figure 2; EX1007, Figure 1; EX1002, ¶¶163-64.

ix. Claim 12 and 21

12. The method of claim 1, wherein following step (a), the method further comprises selectively enriching the adapter-DNA molecules based on size to provide enriched adapter-DNA molecules.

21. The method of claim 1, wherein prior to attaching adapters to double-stranded DNA fragments, the method further comprises selectively enriching double-stranded DNA fragments by size selection.

As discussed above, Kinde renders claim 1 obvious. Kinde discloses the limitations of claim 12. For example, Kinde discloses that, “After the second round of amplification,” “Fragments of the expected size were purified after agarose (mtDNA libraries) or polyacrylamide (all other libraries) gel electrophoresis.” EX1006, SII. As described above for Element 1.3, Kinde also teaches that “fragments are ligated to adapters ... so they can be subsequently be amplified by PCR.” EX1006, Figure 2. Thus, because amplification follows adapter ligation, Kinde’s teaching of selectively enriching the DNA molecules based on size also follows adapter ligation.

With respect to claim 21, Kinde suggests “selectively enriching double-stranded DNA fragments by size selection” prior to attaching adapters to double-stranded DNA fragments. According to Kinde, “fragments are sheared to an average size of 150 bp (range 125-175).” EX1006, 9532. Kinde subsequently attaches the Y-shaped Illumina adapters. *Id.*, 9531. Kinde further discloses that genomic DNA samples were prepared according to “standard Illumina protocols.” EX1006, 9535. As Dr. Quackenbush explains, standard Illumina protocols typically included a size selection step, following sonication and prior to attachment of adapters, to remove fragments less than about 100 nucleotides. EX1008, 7 (Illumina protocol describing use of a “QIAquick PCR Purification Kit”). Accordingly, Kinde suggests selectively enriching double-stranded DNA fragments by size selection whether this occurs before or after attaching the adapters to the double-stranded DNA fragments. EX1002, ¶¶165-168.

Accordingly, claims 12 and 21 would have been obvious.

x. Claims 13 and 14

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

14. The method of claim 13, wherein the adapters comprise the barcodes in at least one of a double-stranded portion or a single-stranded portion of the adapter.

As discussed above, Kinde renders claim 1 obvious. Kinde teaches an adapter comprising “a Y-shape” having barcodes and also discloses barcodes having “a degenerate or semi-degenerate sequence containing between 3 and 20 nucleotide bases” as required by claim 13. Craig discloses Y-shaped adaptors comprising 6 nucleotide barcodes. EX1007, 887 (“Experimental design”), Supplementary Tables 3 and 4.

As discussed above for Element 1.3, Kinde teaches using Y-shaped adapters comprising barcodes. Kinde exemplifies using barcodes having lengths of 12- or 14-nucleotide bases that are randomly generated. EX1006, 9532 (“Exogenous UID...there was a stretch of 12-14 random nucleotides...[t]he random nucleotides form the UIDs...”), Table S4; S11 (“...12- or 14-base UID...”). Prior art at the relevant time, as illustrated by Casbon, expressly discusses randomly generated barcodes on Y-shaped adaptors as a “degenerate base region” or “DBR.” EX1009, ¶54 (“DBR may thus include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more bases, including 15 or more, 20 or more, etc. In certain embodiments the DBR is from 3 to 10 bases in length. Moreover, each position in a DBR may have a different base composition.”). Moreover, Shiroguchi illustrates barcoded Y-shaped adaptors (e.g., Figure 1), cites both Kinde and Casbon regarding previous application of such adaptors, EX1010, 1347, and discusses experimentally evaluating “random barcode sequences” and “optimized barcode sequences.” EX1010, 1348 (“To

evaluate the difference between using random barcode sequences and our optimized barcode sequences ...”).

Claim 14 depends from claim 13 and requires the barcode to be located in either the single- or double-stranded portion of the adapter. As discussed above, commercially available Illumina adapters have a barcode in the single-stranded portion. EX1012, Figure 4; Section I.A. Craig discloses Y-shaped adaptors having barcodes in the double-stranded portion. EX1007, 887 (“Experimental design”), Supplementary Table 3. Shiroguchi confirms obviousness, citing to Kinde and illustrating barcodes in the double-stranded portion of asymmetric adapters. EX1010, 1347, Figure 1.

Accordingly, claims 13 and 14 and are obvious over Kinde and Craig, as further corroborated by knowledge in the art at the time. EX1002, ¶¶169-74.

xi. Claim 15

15. The method of claim 1, further comprising providing a sample comprising the double-stranded DNA fragments from a biological source, wherein the biological source comprises tissue.

Claim 15 specifies that the biological source or bodily sample of claim 1, comprises tissue. This is a feature of the source of the DNA sample used in the method, not the method itself. Such sources were well known in the art and are disclosed by Kinde. For example, Kinde extracted double-stranded DNA from a

bodily tissue sample in the forms of “human pancreas” and “normal human colonic mucosae.” EX1006, SI1. Kinde further describes shearing the extracted DNA to produce fragments. EX1006, Figure 2 (“...fragment produced by random shearing...”), 9535 (“...acoustic shearing...”). *See* Element 22.1. Moreover, Kinde explains that biological tissue sources include surgical tissue specimens. EX1006, 9530; EX1002, ¶¶175-76.

xii. Claim 17

17. The method of claim 1, wherein the double-stranded DNA fragments were at least partially generated by nuclease cleavage.

Claim 17 is obvious over Kinde and Craig. Random fragmentation is discussed in Kinde as a means for generating double-stranded DNA fragments. It was known that endonucleases cleave larger fragments to produce smaller DNA fragments suitable for ligation to adapters. This is described in Craig, which Kinde points to as providing disclosure relevant to barcoded Y-shaped adaptors. EX1006, 9531 (“introduction of exogenous sequences ...”). Craig discloses digesting sample DNA “to 200-300 bp fragments using DNase I enzyme (NEB).” EX1007, Supplementary Methods. Indeed, a corresponding Kinde patent application describing Safe-SeqS discloses that “nucleic acids may be obtained using a random fragment forming technique” and lists “endonucleases” as one of the optional methods. EX1022, ¶21; EX1002, ¶¶177-78.

xiii. Claim 18

18. The method of claim 1, wherein the first strand sequence reads derived from a particular original adapter-DNA molecule and the second strand sequence reads derived from the same particular original adapter-DNA molecule can be related based on a barcode sequence, a fragment-specific feature, or a combination thereof.

The specification of the '127 patent does not recite the term “fragment-specific feature.” As discussed above, both Kinde and Craig disclose barcode sequences. Kinde refers to UIDs as barcodes. EX1006, 9531 (“UIDs, sometimes called barcodes or indexes...”). Moreover, Kinde discloses using UIDs, including endogenous sequences, to group “high-quality reads” of the sequenced amplification progeny into UID families corresponding to the original DNA fragments. *Id.*, Tables 1 and 2, SI2 (“...Only high-quality reads...”). Kinde’s “UIDs” satisfy the requirement of “a barcode sequence, a fragment-specific feature, or a combination thereof.” EX1002, ¶¶179. Kinde’s endogenous UIDs are a feature of the sample DNA fragment as they involve “randomly sheared genomic DNA” that “inherently contains UIDs” at the termini. EX1006, 9531; *see also* SI1 (“The resulting DNA fragments contained UIDs composed of three sequences...”). EX1002, ¶¶179-80.

xiv. Claim 19

19. The method of claim 1, wherein the sequence reads derived from a particular original adapter-DNA molecule can be differentiated from the second strand sequence reads derived from the same particular original adapter-DNA molecule at least in part by an asymmetry of the asymmetrical double-stranded adapter-DNA molecules.

As discussed above, Y-shaped adapter-DNA molecules of the prior art provide the “asymmetrical” aspect discussed in the ’127 patent, and strand-specific sequence reads can, and were, differentiated. As discussed above, Kinde discloses Y-shaped Illumina adapters that, when ligated to double-stranded DNA molecules, result in asymmetric double-stranded adapter-DNA molecules. EX1006, 9531, 9535; EX1007, 888, Figure 1, Supplementary Methods. Craig also discloses Y-shaped adapter molecules. Kinde expressly discusses analysis of both strands in its method. EX1006, Figure 2, EX1006, SI3 (“...the specificity can be further increased by requiring that each strand of the original double-stranded template contain the mutation ...”), SI2 (“These mutations are expected to be present in both strands of the relevant templates.”). As explained above in Section I.A., asymmetric adapter-DNA molecules allow the first and second strands of the same original molecule to be differentiated. This means that sequence reads derived from a particular original adapter-DNA molecule can be differentiated from the second strand sequence reads derived from the same particular original adapter-

DNA molecule at least in part by an asymmetry of the asymmetrical double-stranded adapter-DNA molecules. *See also* EX1022, ¶42; EX1002, ¶¶181-82.

xv. Claims 20 and 27-28

20. The method of claim 1, wherein prior to sequencing, the method further comprises selectively enriching adapter-DNA molecules or copies thereof for a subset of said adapter-DNA molecules that map to one or more genetic loci in a reference genome.

27. The method of claim 22, further comprising selectively enriching double-stranded adapter-DNA molecules or copies thereof to enrich for a subset of DNA molecules that map to one or more genetic loci in the reference sequence.

28. The method of claim 22, wherein, prior to sequencing, double-stranded adapter-DNA molecules or copies thereof are selectively enriched using a hybridization capture method to provide target DNA molecules that map to one or more genetic loci in the reference sequence.

Patent Owner has conflated comparing reads to a reference sequence as stated in Element 1.6 with comparing consensus sequence reads to a reference. The was directly addressed by the Examiner in a related continuation application. Specifically, the Examiner found that the specification (which is shared with the '127 patent) only discusses the alignment of the consensus sequence. EX1025, 138.

As discussed above, Kinde and Craig render claims 1 and 22 obvious. Kinde also discloses the additional limitations of claims 20 and 27-28. For example, Kinde discloses selectively enriching sample DNA using hybridization capture

prior to sequencing. Figure 2 shows the use of a “Solid Phase Capture” that includes a “filter containing 2,594 nt corresponding to six cancer genes.” EX1006, Figure 2. Amplification follows capture, which results in selective enrichment. *Id.*; EX1002, ¶¶183-85. The filter is designed to catch DNA fragments corresponding to “six cancer genes” (i.e., targeting DNA fragments that map to one or more genetic loci in the reference sequence). EX1006, 9535, SII.

Accordingly, Kinde renders each of claims 20 and 27-28 obvious.

xvi. Claims 23-25

23. The method of claim 22, wherein the bodily sample comprises tissue obtained from a subject.

24. The method of claim 22, wherein at least some of the double-stranded DNA fragments are derived from a tumor or circulating neoplastic cells.

25. The method of claim 22, wherein the bodily sample is derived from a human subject having a tumor cell population, and wherein following step (d), the method further comprises identifying a genetic mutation conferring drug resistance present in one or more of the consensus sequences derived from the double-stranded DNA fragments obtained from the tumor cell population present in the bodily sample.

As discussed above, Kinde and Craig renders claim 22 obvious. Kinde further discloses the limitations of claim 23. For example, Kinde disclosed extracting DNA from “human pancreas” or “normal human colonic mucosae.” EX1006, SII. Kinde further describes shearing the extracted DNA to produce fragments. EX1006, Figure 2 (“...fragment produced by random shearing...”),

9535 (“...acoustic shearing...”). Moreover, Kinde explains that biological tissue sources include surgical tissue specimens. *Id.*, 9530 (“...surgical margins...”).

Kinde further suggests the limitations of claim 24. For example, Kinde discloses deriving DNA from “surgical margins” to identify the presence of “neoplastic diseases.” EX1006, 9530; *see also id.* (discussing “epigenetic changes in tumors”). Kinde suggests that skilled artisans “identify patients with early, surgically curable disease.” EX1006, 9530. Kinde expressly suggests that skilled artisans “Detect[] such mutations, particularly at a stage before they become dominant in the population.” EX1006, 9530; EX1002, ¶¶186-87.

Claim 25 requires the identification of “a genetic mutation conferring drug resistance.” The ’127 patent does not describe application of the DCS method to the identification of such mutations. To the extent Patent Owner argues that this was an art known type of mutations that a POSA would have been motivated to detect using DCS — that is, it would have been obvious to apply DCS to detect such mutations — this fails to establish written description support. *Research Corp.*, 627 F.3d at 870 (“Entitlement to a filing date extends only to subject matter that is disclosed; not to that which is obvious.”). Moreover, this same logic would apply to the application of the consensus sequencing method of Kinde.

Kinde also suggests identifying such mutations, Kinde cites References 32, titled “Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-

RAS upregulation.” EX1006, 9535. Kinde also notes that, in the context of disease, “Detection of such mutations, particularly at a stage before they become dominant in the population, will likely be essential to optimize therapy.” *Id.*, 9530. Kinde thereby suggests using its methods to detect identify mutations conferring drug resistance after using Kinde’s approach to confirm the presence of the mutations (*i.e.*, step d). EX1002, ¶¶188-189.

Accordingly, Kinde renders each of claims 23-25 obvious as a whole. EX1002, ¶¶186-89.

xvii. Claim 26

26. The method of claim 22, wherein the library comprises at least a subset of non-uniquely tagged double-stranded adapter-DNA molecules, and wherein non-uniquely tagged double-stranded adapter-DNA molecules are substantially identifiable with respect to other non-uniquely tagged double-stranded adapter-DNA molecules in the bodily sample using the one or more barcode sequences and DNA fragment-specific information.

Patent Owner cannot distinguish the prior art from the claims based on the recited “non-unique tagging” scheme. The ’127 patent never mentions “non-unique tagging.” The ’127 specification describes using a sufficient diversity of “SMI tags” to promote labeling different DNA fragments in the sample with a different SMI tag—an aspect of the DCS method that the ’127 patent repeatedly describes as “unique” tagging or labeling. EX1001, 18:37-42 (“the use of SMI tags...allows

every molecule to be *uniquely* labeled”); *see also id.*, 6:67-7:4, 15:29-33, 21:61-63, 22:19-20, 22:25-27, 27:63-67, 26:59-61; EX1002, ¶190.

Kinde discloses that a known outcome of using UIDs is that “two different original templates acquire the same UID.” EX1006, SI1. Kinde explains that using a number of distinct UIDs that “greatly exceeds the number of original template molecules” will “minimize the probability that two different original templates acquire the same UID,” but does not state that the presence of a plurality of the tagged original DNA molecules having identical UIDs is ever eliminated. Kinde instead indicates that one component of its method is that duplicate tagging occurs despite efforts to minimize it. *See also* EX1002, ¶191.

Kinde teaches or suggests that the tagged original DNA molecules are substantially identifiable with respect to the other tagged original DNA molecules in the population. The ’127 patent discloses that whether a molecule is “unique” is based on its sequence and that its DCS method identifies molecules (*e.g.*, family grouping) based on sequence identity (*i.e.*, SMI). *E.g.*, EX1001, 19:23-25, 22:41-44. While the specification does not recite the term “substantially identifiable” it presumably encompasses tagged DNA molecules that are sufficiently distinguishable from other tagged DNA molecules in the population based on

sequence.⁷ *See also* EX1002, ¶¶190-94.

xviii. Claims 29 and 30

29. The method of claim 22, wherein the barcode sequences are 6 nucleotides in length.

30. The method of claim 22, wherein the barcode sequences are 3, 4, 5, 6, 7 or 8 nucleotides in length.

Patent Owner cannot distinguish over Kinde based on any established criticality of the barcode lengths recited in claims 29 and 30. The specification of the '127 patent does not exemplify, or otherwise indicate, that the recited lengths of barcodes are preferable relative to the other disclosed lengths of barcodes. *E.g.*, EX1001, 7:4-10 (“Each n-mer sequence may be between approximately 3 to 20 nucleotides in length. Therefore, each n-mer sequence may be approximately 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 nucleotides in length. In one embodiment, the SMI sequence is a random degenerate nucleotide n-mer sequence which is 12 nucleotides in length.”).

In any event, Kinde in view of Craig teach a 6-nucleotide index sequence (*i.e.*, barcode). EX1007, 887 (“We used a six-base index with built-in redundancy

⁷ Two different tagged DNA molecules, even bearing identical SMIs, have different sequences by virtue of the molecules themselves.

for error correction ...”). Accordingly, Kinde in view of Craig renders obvious each of claims 29 and 30 as a whole. EX1002, ¶¶195-97.

B. [Ground 2] Claim 11 is unpatentable as obvious under 35 U.S.C. §103 over Kinde and Craig in further view of NEB Expressions

11. The method of claim 1, wherein the double-stranded DNA fragments comprise at least one of a deaminated nucleic acid base and a nucleic acid base having oxidative damage, and wherein prior to or following step (a) the method further comprises chemically or enzymatically treating the double-stranded DNA fragments to remove or repair one or more damaged bases.

As discussed above, Kinde and Craig render claim 1 obvious. The additional subject matter of claim 11 is disclosed by NEB Expressions.

NEB Expressions discloses that deaminated cytosine and oxidized bases (e.g., 8-oxo-guanine) are two common types of DNA damage that may be present in typical DNA samples. E.g., EX1014, 1-2 & Table 1 (listing deaminated cytosine and oxidized bases as types of damage typically found in DNA samples). NEB Expressions explains that the commercial product “PreCR™ Repair Mix” is “a cocktail of enzymes formulated to repair damaged DNA *in vitro* prior to PCR,” including specifically deaminated cytosine and oxidized guanine. EX1014, 1, 3 & Table 2 (listing deaminated cytosine and oxidized bases as types of damage enzymatically repaired by PreCR™ Repair Mix).

A POSA would have been motivated to use PreCR™ Repair Mix as described in NEB Expressions on the samples of Kinde or Craig because it allows

the “Unlock[ing of] genetic information that was previously inaccessible due to damaged DNA template.” EX1014, 1. As NEB Expressions explains, deaminated cytosine residues “may result in inhibition of PCR or [production of] mutagenic DNA products.” *Id.*, 2. NEB Expressions explains that “most DNA samples are susceptible to oxidation” and that oxidation of guanine to 8-oxo-guanine may occur *in vitro* (e.g., during the DNA extraction process) or *in vivo* (e.g., oxidation of mitochondrial DNA) and produces “mutagenic” DNA products. *Id.* Because deaminated and oxidized residues are mutagenic (e.g., cause base mispairing during replication), a POSA would have been motivated to repair these damaged bases using enzymes such as with the PreCR™ Repair Mix described in NEB Expressions. A POSA would have had a reasonable expectation of success because, as NEB Expressions explains, PCR™ Repair Mix is effective in repairing deaminated cytosines and oxidized guanine. *Id.*, 3.

Accordingly, Kinde in view of Craig in further view of NEB Expressions renders obvious claim 11 as a whole. EX1002, ¶¶198-201.

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. Furthermore, the materials presented here demonstrate material error in that the '127 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. The Examiner only applied the Otwinowski reference in a rejection, and the Examiner accepted Patent Owner's argument that Otwinowski is not prior art with no apparent critical evaluation of entitlement to the claimed priority date. 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 Elektromedizinische 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 '127 patent discusses Kinde, it makes comparisons, concessions and admissions that underscore Examiner error in the issuance of the '127 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.

Furthermore, the Examiner erred when she concluded that "the ability to label each strand of a target with asymmetric adaptors that enable strand specific

labeling” was not known in the art. EX1004, 432-433. As explained above, adapters that enable strand specific labeling were known in the art, widely used, and even commercially available as of the earliest claimed filing date of the ’127 patent. *See* Section I.A. That such adapters were commercially available and support DCS is directly stated in the ’127 patent. Specifically, Example 3 of the ’127 specification provides “In order to demonstrate the capability of DCS analysis to remove sequencing errors, a *sequencing library was prepared under standard conditions with commercially available sequencing adapters,*” EX1001, 26:19-23; *see also* 25:64-26:1 (describing ligation of Illumina TruSeq adapters). The ability to label each strand of a target with adapters that enable strand-specific labeling was known in the art and admitted by the inventors of the ’127 patent. Accordingly, the Examiner’s finding to the contrary is substantive error.

Moreover, while Kinde is discussed in the specification of the ’127 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 ’127 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. The ’127 patent similarly fails to discuss Kinde use of partially-complementary, asymmetric Y-shaped Illumina adapters. Accordingly, there is no indication the Examiner was aware of these

teachings in Kinde and the background knowledge in the art regarding asymmetric adapters. Issuance of the '127 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 (PTAB Mar. 20, 2020 (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 filed infringement contentions one week ago. 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 wasted upon the Board's institution. Also, despite Petitioner being the defendant in the litigation—something out of Petitioner's control—other circumstances weigh against discretionary denial. 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 1-30 of the '127 patent are unpatentable. Petitioners therefore request that a *inter partes* review of these claims be instituted.

Respectfully submitted,

Dated: April 8, 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 13,905 words, excluding the parts of the brief exempted by 37 C.F.R. §42.24(a).

Respectfully submitted,

Dated: April 8, 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. 10,760,127 to Salk et al.
1002	Declaration of Dr. John Quackenbush
1003	<i>Curriculum Vitae</i> of John Quackenbush, Ph.D.
1004	File History for Application No. 16/503,382
1005	Declaration of Michael Schmitt Under 37 C.F.R. § 1.131, United States Patent Application No. 14/386.800 (2017)
1006	Kinde et al., “Detection and quantification of rare mutations with massively parallel sequencing,” <i>PNAS</i> 108(23) (2011)
1007	Craig et al., “Identification of genetic variants using bar-coded multiplexed sequencing,” <i>Nature Methods</i> 5(10) (2008)
1008	Illumina, Preparing Samples for Sequencing Genomic DNA (2007)
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-molecule barcodes,” <i>PNAS</i> 109(4) (2012)
1011	Illumina, Complete Secondary Analysis Workflow for the Genome Analyzer, Technical Note: Illumina Systems and Software (2009)
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,” <i>Nature</i> 456 (2008)
1014	Evans et al., “NEB Expressions” New England BioLabs (2007)

1015	NEB Expressions, New England BioLabs, https://web.archive.org/web/20080321144426/http://www.neb.com/nebecomm/tech_reference/neb_transcripts.asp (Feb. 23, 2022)
1016	Affidavit of Nathaniel E. Frank-White
1017	Nisha et al., “Antioxidants and Their Protective Action Against DNA Damage,” <i>International Journal of Pharmacology and Pharmaceutical Sciences</i> 3 (2011)
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,” <i>Nucleic Acids Research</i> 38(15) (2010)
1022	International Publication No. WO 2012/142213 to Vogelstein et al.
1023	RESERVED
1024	RESERVED
1025	File History for Application No. 17/392,185
1026	RESERVED
1027	RESERVED
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 sequencing,” <i>Nucleic Acids Research</i> 39(12)(2011)

1032	RESERVED
1033	Affidavit of Nathaniel E. Frank-White
1034	Affidavit of Nathaniel E. Frank-White

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. 10,760,127 (and accompanying Exhibits 1001-1034) by overnight courier (Federal Express or UPS), on this 8th day of April, 2022, on the Patent Owner at the correspondence address of the Patent Owner as follows:

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

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

Respectfully submitted,

Dated: April 8, 2022

/ Michael T. Rosato /

Michael T. Rosato, Lead Counsel
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