UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

TEMPUS AI, INC.,

Petitioner,

v.

GUARDANT HEALTH, INC.,

Patent Owner.

Case IPR2025-01434

U.S. Patent 11,149,306

PETITION FOR *INTER PARTES* REVIEW OF CLAIMS 1-29 OF U.S. PATENT 11,149,306

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EXHIBIT LIST

Exhibit	Description
1001	U.S. Patent No. 11,149,306 ("the '306 patent")
1002	Prosecution history for the '306 patent
1003	Declaration of Michael Metzker, Ph.D.
1004	Curriculum Vitae of Michael Metzker, Ph.D.
1005	PCT Publication No. WO 2013/123442 A1 ("Bielas")
1006	PCT Publication No. WO 2012/142213 A2 ("Vogelstein")
1007	U.S. Patent Application Publication No. 2011/0160078 ("Fodor")
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1011	U.S. Patent No. 9,404,156 ("Hicks")
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1023	Declaration of Sylvia D. Hall-Ellis, Ph.D
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1027	*Twinstrand Biosciences, Inc. v. Guardant Health, Inc.*, IPR2022-01400, Paper 41 – Final Written Decision
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LIST OF CHALLENGED CLAIMS

Claim No.	Claim Language
	Claim 1
[1pre]	A method, comprising:
[1a]	(a) providing a population of cell-free deoxyribonucleic acid (cfDNA) molecules having first and second complementary strands;
[1b1]	(b) tagging a plurality of the cfDNA molecules in the population with duplex tags comprising molecular barcodes to produce tagged parent polynucleotides, wherein the duplex tags are attached to both ends of a molecule of the plurality of the cfDNA molecules,
[1b2]	wherein the plurality of the cfDNA molecules are tagged with n different combinations of molecular barcodes, wherein n is at least 2 and no more than 100,000*z, wherein z is a mean of an expected number of duplicate molecules in the population of cfDNA molecules that map to identical start and stop positions on a reference sequence;
[1c]	(c) amplifying a plurality of the tagged parent polynucleotides to produce amplified progeny polynucleotides;
[1d]	(d) sequencing at least a subset of the amplified progeny polynucleotides to produce a set of sequence reads; and
[1e]	(e) reducing or tracking redundancy of a plurality of sequence reads from the set of sequence reads using at least sequencing information from the molecular barcodes of the duplex tags to determine distinct cfDNA molecules from among the tagged parent polynucleotides, wherein the distinct cfDNA molecules are determined based on (i) paired reads corresponding to sequence reads generated from a first tagged strand and a second tagged complementary strand derived from cfDNA molecules from among the tagged parent polynucleotides, or (ii) unpaired reads corresponding to sequence reads generated from a first tagged strand having no second tagged complementary strand derived from cfDNA molecules from among the tagged parent polynucleotides,

[1f]	wherein reducing or tracking the redundancy of the plurality of sequence reads comprises mapping at least a subset of the plurality of sequence reads to the reference sequence.
	Claim 2
2	The method of claim 1, wherein the population of cfDNA molecules is obtained or derived from a subject having cancer.
	Claim 3
3	The method of claim 1, wherein the population of cfDNA molecules comprises 1 nanogram (ng) to 100 ng of cfDNA molecules.
	Claim 4
4	The method of claim 1, wherein the molecular barcodes are ligated to the plurality of the cfDNA molecules using more than a 10× excess of duplex tags as compared to the population of cfDNA molecules, wherein at least 20% of the cfDNA molecules from the population are tagged with the duplex tags.
	Claim 5
5	The method of claim 1, wherein the molecular barcodes are ligated to the plurality of the cfDNA molecules using more than an 80× excess of duplex tags as compared to the population of cfDNA molecules.
	Claim 6
6	The method of claim 5, wherein at least 40% of the cfDNA molecules from the population are tagged with the duplex tags.
	Claim 7
7	The method of claim 1, wherein z is between 2 and 8.
	Claim 8
8	The method of claim 1, wherein the molecular barcodes have a length of 5 to 20 base pairs.
	Claim 9
9	The method of claim 1, further comprising, prior to the sequencing, enriching at least a subset of the amplified progeny

	polynucleotides for target regions of interest to produce enriched progeny polynucleotides.
	Claim 10
10	The method of claim 9, wherein the target regions of interest comprise genetic sequences of a plurality of genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF 1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1.
	Claim 11
11	The method of claim 10, further comprising, prior to the sequencing, amplifying a plurality of the enriched progeny polynucleotides.
	Claim 12
12	The method of claim 1, wherein the duplex tags are part of sequencing adapters.
	Claim 13
13	The method of claim 1, wherein reducing or tracking the redundancy of the plurality of sequence reads comprises grouping the paired reads or the unpaired reads into families based at least in part on (i) the molecular barcodes associated with the paired reads or the unpaired reads and (ii) sequence information at the start and stop positions of the paired reads or the unpaired reads.
	Claim 14
14	The method of claim 1, further comprising: (f) determining quantitative measures of (i) paired reads or (ii) unpaired reads that map to a genomic locus of the reference sequence.

	Claim 15
15	The method of claim 14, further comprising: (g) estimating with a programmed computer processor a quantitative measure of tagged parent polynucleotides that map to the genomic locus based on the quantitative measures of the paired reads and the unpaired reads.
	Claim 16
16	The method of claim 15, further comprising: (h) detecting copy number variation in the population of cfDNA molecules by determining a normalized quantitative measure determined in (g) at each of one or more genomic loci.
	Claim 17
[17pre]	A method, comprising:
[17a]	(a) tagging a population of double-stranded cell-free deoxyribonucleic acid (cfDNA) molecules obtained or derived from a sample of a subject with a set of tags comprising molecular barcodes to produce tagged parent polynucleotides;
[17b]	(b) amplifying a plurality of the tagged parent polynucleotides to produce amplified progeny polynucleotides;
[17c]	(c) sequencing at least a subset of the amplified progeny polynucleotides to produce a set of sequence reads; and
[17d]	(d) sorting a plurality of sequence reads from the set of sequence reads into (i) families comprising paired reads corresponding to sequence reads generated from a first tagged strand and a second tagged complementary strand derived from double-stranded cfDNA molecules from among the tagged parent polynucleotides, and (ii) families comprising unpaired reads corresponding to sequence reads generated from a first tagged strand having no second tagged complementary strand derived from double-stranded cfDNA molecules from among the tagged parent polynucleotides.
	Claim 18
18	The method of claim 17, wherein the sample is blood, plasma, or serum.

	Claim 19
19	The method of claim 17, wherein the population of double-stranded cfDNA molecules comprises 1 nanogram (ng) to 100 ng of double-stranded cfDNA molecules.
	Claim 20
20	The method of claim 17, wherein the tagging comprises ligating the molecular barcodes to double-stranded cfDNA molecules.
	Claim 21
21	The method of claim 17, wherein the set of tags comprises 2 to 10,000 different molecular barcode sequences.
	Claim 22
22	The method of claim 17, further comprising, prior to the sequencing, enriching at least a subset of the amplified progeny polynucleotides for target regions of interest to produce enriched progeny polynucleotides.
	Claim 23
23	The method of claim 22, wherein the target regions of interest comprise genetic sequences of a plurality of genes selected from the group consisting of ALK, APC, BRAF, CDKN2A, EGFR, ERBB2, FBXW7, KRAS, MYC, NOTCH1, NRAS, PIK3CA, PTEN, RB1, TP53, MET, AR, ABL1, AKT1, ATM, CDH1, CSF1R, CTNNB1, ERBB4, EZH2, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, MLH1, MPL, NPM1, PDGFRA, PROC, PTPN11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, VHL, TERT, CCND1, CDK4, CDKN2B, RAF1, BRCA1, CCND2, CDK6, NF1, TP53, ARID1A, BRCA2, CCNE1, ESR1, RIT1, GATA3, MAP2K1, RHEB, ROS1, ARAF, MAP2K2, NFE2L2, RHOA, and NTRK1.
	Claim 24
24	The method of claim 17, wherein (d) further comprises reducing or tracking redundancy of a plurality of sequence reads from the set of sequencing reads, wherein the reducing or tracking comprises mapping at least a subset of the plurality of sequence reads to a reference sequence, and the reducing or tracking is based on (i) the

	molecular barcodes associated the paired reads or the unpaired reads and (ii) sequence information at the start and stop positions of the paired reads or the unpaired reads.
	Claim 25
25	The method of claim 24, wherein reducing or tracking the redundancy of the plurality of sequence reads comprises determining a base call at one or more genomic loci for a plurality of sequence reads that map to the one or more genomic loci on the reference sequence.
	Claim 26
26	The method of claim 24, wherein reducing or tracking the redundancy of the plurality of sequence reads comprises collapsing the plurality of sequence reads to produce consensus sequences representative of a sequence of the original double-stranded cfDNA molecules from among the tagged parent polynucleotides.
	Claim 27
27	The method of claim 24, further comprising determining quantitative measures of at least two of (i) paired reads that map to one or more genomic loci, (ii) unpaired reads that map to one or more genomic loci, (iii) a read depth of the paired reads, and (iv) a read depth of the unpaired reads.
	Claim 28
28	The method of claim 27, further comprising estimating with a programmed computer processor a quantitative measure of tagged parent polynucleotides that map to a genomic locus of the reference sequence based on the quantitative measures of at least two of (i) paired reads, (ii) unpaired reads, (iii) a read depth of the paired reads, and (iv) a read depth of the unpaired reads at the genomic locus.
	Claim 29
29	The method of claim 1, wherein the distinct cfDNA molecules in (e) are determined based on (i) the paired reads and (ii) the unpaired reads.

I. INTRODUCTION

U.S. Patent No. 11,149,306 ("'306 patent") claims methods for detecting genetic variants. The claimed methods involve certain well-known steps: tagging polynucleotides with duplex tags comprising molecular barcodes, amplifying the tagged polynucleotides, sequencing the resulting polynucleotides, and grouping sequence reads into families of paired and/or unpaired reads. Each of these steps were conventional by 2013 (the earliest priority date), a fact confirmed by the prior art and Guardant's own admissions in other proceedings.

During prosecution of the '306 patent, Guardant overcame multiple rejections by amending the "tagging" step of claim 1 to recite a range of the potential number of "different combinations of molecular barcodes" that could be used. Under that element, the number of combinations can be between "2 and 100,000*z, wherein z is a mean of an expected number of duplicate molecules." The '306 specification states that "z is expected to be between 2 and 8." EX1001, 20:49-50. But Guardant's addition of this numerical range does not make the claims patentable. The prior art – including the Bielas and Vogelstein references relied on in each Ground – is replete with examples of using combinations of barcodes within this range.[1] *See, e.g.*, EX1005 (Bielas), 16:3-5 ("*e.g.,* pools of 4,094, 16,384 or 65,536 unique cyphers,"

[1] Although Bielas and Vogelstein were cited during prosecution, along with over 700 other references, they were never discussed or relied on in any claim rejection. *See* Section VIII.G.

also referred to as barcodes); EX1006 (Vogelstein), ¶[0028] (barcodes with "at least 4, at least 16, at least 64, at least 256, at least 1,024, at least 4,096, at least 16,384 … different sequences"). Accordingly, this claim element was not a reason to overcome the earlier rejections and is not a basis to uphold patentability of the challenged claims.

Similarly, although the '306 patent was subject to a prior *inter partes* review by Twinstrand Biosciences ("Twinstrand IPR"), resulting in claim affirmance (EX1027), the limitations the Board relied on to distinguish the art in that proceeding are not novel or non-obvious. Specifically, for claim 1 the Board found the references relied on did not disclose duplex tags that are "attached to both ends of a molecule." But Bielas, which was not considered during the Twinstrand IPR, discloses attaching a barcode to both ends of a molecule. *See, e.g.*, EX1005, 16:12-13 ("[E]ach target nucleic acid molecule … has identical cyphers ***on each end of one or more target nucleic acid molecules***.").

For claim 17, the Board found that the Twinstrand IPR references did not disclose sorting sequence reads into families of paired and unpaired reads. But it was well-known that sequence reads could be sorted into either paired or unpaired reads, and a POSA would have found using both obvious in view of the prior art cited herein. EX1005, 4:11-15 ("sequencing reads having identical cypher pairs, along with their reverse complements, were grouped into families"); EX1006, ¶[61]

("reads [are] grouped into UID-families based on their endogenous or exogenous UIDs"); EX1003, ¶¶112, 134. The Board should re-evaluate the '306 patent in light of previously overlooked art that establishes the challenged claims are invalid.

II. STANDING

Petitioner certifies the '306 patent is available for *inter partes* review and it is not estopped.

III. TECHNICAL BACKGROUND

A. DNA

DNA has four nucleotides: adenine (A), cytosine (C), guanine (G), and thymine (T). The order of these in a DNA strand is their "sequence." EX1003, ¶¶40-43.

Cell-free DNA (cfDNA) are short DNA fragments that circulate outside of cells in blood or other body fluids. cfDNA can include DNA from healthy cells and also circulating tumor DNA (ctDNA) shed from dying tumor cells. That cfDNA "may contain genetic aberrations associated with a particular disease" was well-known by December 2013. EX1001, 1:41-42; EX1014; EX1003, ¶¶45-53.

B. Genetic Variants & Disease

Comparing one person's genome to another can reveal differences called "mutations" or "variants." EX1015; EX1003, ¶46. Cataloging mutations associated with cancer has been an area of study for decades. EX1003, ¶¶46-53; EX1016 at 7-

8.[2] By December 2010, "[d]ifferent types of somatic mutations were known in the art." EX1016 at 7-8.

C. Next Generation Sequencing

Sequencing includes methods used to determine the order of nucleotide bases in DNA fragments. EX1003, ¶¶54-55. While DNA sequencing dates to the 1970s, the technology most frequently employed for sequencing and identifying genetic variants by 2013—and still today—is called next-generation sequencing ("NGS").

The most widely used NGS platform, commercially available from Illumina, has been in use since the mid-2000s. EX1017, p.34; EX1003, ¶¶56-60. Before 2013, Illumina workflows, including for sequencing cfDNA, were conventional and widely used. EX1016, p.2 (by December 2010, NGS was a "well-known 'off the shelf' technolog[y]"); *id.*, p.7.

D. Amplification and Target Enrichment

Before sequencing, collected DNA fragments are amplified to create multiple copies. EX1003, ¶¶61-63; EX1007, ¶[0128], [0181]. A well-known amplification technique is polymerase chain reaction ("PCR"). EX1018, p.6 (by March 2012

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[2] Admissions made by Patent Owner when challenging its adversaries' patents (priority dates December 30, 2010, and March 20, 2012) in IPR proceedings, cited herein, are binding statements against interest. *See Guardant Health, Inc. v. Univ. of Washington*, IPR2022-00450, Paper 13 at p. 11 (finding that Guardant's admissions concerning prior art in other IPR proceedings were admissions the Board should consider).

"[t]ypical Illumina sequencing workflows include[d] … PCR amplification"); EX1030, 13:40-45. PCR uses short primer molecules and a cyclical process to amplify sample molecules, generating thousands to millions of DNA copies in a matter of hours. EX1003, ¶¶61-63; EX1019, pp.380-387.

Amplification can be non-specific—amplifying all molecules in a sample population equally—or targeted—amplifying only those of interest. The latter approach is referred to as targeted enrichment. EX1020, p.111; EX1007, ¶¶[0058]-[0059], [0121]; EX1030, 12:63-13:4; EX1003, ¶¶64-67. Several hybridization-based capture methods have been developed to target sequences of interest. EX1003, ¶¶68-69.

E. Library Preparation, Including Tagging With Barcodes

Before sequencing, fragments are typically combined with short sequences of nucleotides called adaptors by tagging, which results in a library of molecules. Adaptors can be added to fragments through amplification (*e.g.*, PCR) or direct attachment (*e.g.*, ligation). EX1003, ¶¶70-71; EX1021, p.23 (by March 2012, adaptors could be attached "using known sticky-end ligation and TA-ligation approaches"); EX1007, ¶¶[0127], [0190].

Adaptors also frequently include identifiers called "barcodes," which distinguish reads derived from different DNA fragments. EX1003, ¶¶71-73; EX1022, p.14 (by March 2012, "adapters comprising barcodes … were known and

well-documented in the prior art"). Tagging DNA fragments with barcodes was used, for example, to count sequencing reads (EX1012, p.1347), and reduce redundancy by generating consensus sequences (EX1006, ¶[42]; EX1030, 14:39-50). *See also* EX1023, p.30.

Tagging can be of one DNA strand (at one or both ends), or both complementary strands of double-stranded DNA fragments (at one or both ends). This latter approach, called "duplex tagging," was well-known before 2013. EX1003, ¶73; EX1007, ¶¶[0184], [0204], [0233]; EX1018, p.5; *see also id.*, p.9 (duplex tagging was known to "enable[] distinguishing sequence reads from different strands of double-stranded DNA molecules"). Use of wide number of different barcodes was known, as were techniques for crafting smaller sets of barcodes that, *e.g.*, did not repeat nucleotides. *See, e.g.*, EX1012. It was also routine for researchers to use a large molar excess of tags to increase tagging efficiency. *See, e.g.*, EX1007, ¶¶[0121], [0179]; EX1029, p.244.

F. Sequence Reads

NGS typically outputs a large dataset of DNA sequences, referred to as "sequence reads." EX1003, ¶74; EX1007, ¶[0249]. Each read represents the order of the nucleotides detected by the sequencer. EX1003, ¶74.

G. Mapping/Alignment

After generating sequence reads, it can be useful to compare reads to a reference sequence to know where in the genome the DNA fragments came from. EX1003, ¶75. This is called mapping or alignment and has long been a "basic aspect of NGS." EX1016, p.27; EX1024, p.56 (by March 2012 "conventional sequencing practices" included "identifying sequence variants, also called mutations, by aligning the sequence reads using the Illumina Eland pipeline and comparing the reads to an expected (reference) sequence."); EX1007, ¶[0190], [0249]; EX1055. By 2010, mapping/alignment could be done by "numerous 'off the shelf' software programs" using publicly available reference sequences. EX1016, pp.2, 27.

Because of sequencing errors,[3] mapping to a single sequence read alone (or even a handful of reads) was generally insufficient to know whether a difference between a sequence read and a reference was a true mutation. EX1003, ¶¶75-78; EX1018, pp.3-4.

H. Creating A Consensus Sequence

One technique to improve accuracy in variant calling uses barcodes to create a consensus sequence, which before 2013 "was well-known [] and described throughout the scientific literature." EX1024, p.1; *see also* EX1021, p.8; EX1030,

[3] Even at 99.9% accuracy, there will be an error—a base called incorrectly—at 1 out of every 1000 bases.

14:39-50; EX1003, ¶¶79-82. Creating a consensus sequence involves "grouping" together similarly-tagged sequence reads (those having the same barcode) into "families." For each family, grouped sequence reads are "collapsed" into a single consensus sequence. EX1003, ¶80; *see also* EX1021, p.18 (before March 2012, prior art taught that "[a] mutation at a given position as compared to a reference sequence is confidently identified when it represents the consensus base call").

IV. OVERVIEW OF THE '306 PATENT

A. Priority Date

Petitioner assumes the earliest claimed priority date of December 28, 2013, without prejudice to its right to challenge that date in other proceedings.

B. Disclosure of the '306 Patent

The '306 patent acknowledges that in the prior art, "methods have been developed for accurate copy number variation estimation," including "for cfDNA for many applications." EX1001, 1:46-55. The patent also admits "many of these [prior art] methods are able to reduce or combat the errors introduced by the sample preparation and sequencing processes for all molecules that are converted and sequenced," but claims the prior art methods were "not able to infer the counts of molecules that were converted but not sequenced." *Id.*, 1:59-63.

The '306 patent thus purports to disclose a method whereby "[t]he number of unseen molecules can be estimated." *Id.*, 2:17-18. But the claims do not include such a limitation. Instead, they recite sequencing methods that were well-known

prior to December 2013, involving tagging polynucleotides with molecular barcodes, amplifying tagged polynucleotides, sequencing amplified polynucleotides, and (1) reducing/tracking redundancy based on families of either paired or unpaired reads (claim 1) or (2) sorting reads into paired and unpaired reads (claim 17). These are conventional methods used to create a consensus sequence, which, as noted *supra*, Guardant has admitted to the Board were already known in the art.

Indeed, the '306 patent generally concedes that the recited steps of the claimed methods were already known:

1. Tagging with barcodes. The '306 patent states that "[t]agging disclosed herein can be performed ***using any method***" (*id.*, 17:35-36) and references prior art references disclosing tagging with unique or non-unique barcodes (*id.*, 22:19-25 (citing, *e.g.*, Fodor)[4]). The '306 patent describes that barcodes can range in number from "at least the same as the estimated number of molecules in the sample" to "at least two, three, four, five, six, seven, eight, nine, ten, one hundred or one thousand times as many as the estimated number of molecules in the sample." EX1001, 18:26-19:21, 22:34-66.

[4] Fodor discloses that tagging enables "[m]olecules that would otherwise be identical in information content [to] be labeled to create a separately detectable product that is unique or approximately unique." EX 1007 at Abstract.

2. Amplifying tagged parent polynucleotides. The ’306 patent states that amplification is done by routine methods, including “PCR-based amplification.” *Id.*, 29:66-30:2; 30:5-17 (listing known polymerases used for amplification).

3. Sequencing. The ’306 patent states that “[t]he sequencing method can be massively parallel sequencing” using any of several conventional methods listed (including Illumina sequencing) “or any other sequencing methods known in the art.” *Id.*, 30:51-67.

4. Reducing redundancy or sorting reads. The ’306 patent states that “[s]equencing of amplified polynucleotides can produce reads of the several amplification products from the same original polynucleotide, referred to as ‘redundant reads.’” *Id.*, 31:34-38. The patent describes known methods for identifying redundant reads, including “based on their distinct barcode,” “based on sequences at the beginning and end of a read,” or “combining information from a barcode with start/stop sequence.” *Id.*, 31:38-58.

The ’306 patent also states “[c]onsensus sequences can be generated from families of sequence reads by any of a variety of methods” to reduce or track redundancy, and identifies several known methods. *Id.*, 31:30-32:29.

As discussed in Section IV.C, the limitation added to claim 1 during prosecution to obtain allowance recites tagging molecules “with n different combinations of molecular barcodes, wherein n is at least 2 and no more than

100,000*z, wherein z is a mean of an expected number of duplicate molecules in the population of cfDNA molecules that map to identical start and stop positions on a reference sequence" (the "n different combinations element"). EX1002.684-689, 737-742. But this is merely a broad range of the number of potential combinations of barcodes used for tagging, which was well-known in the art.[5]

C. Prosecution History

The '306 patent issued October 19, 2021 from Application No. 16/945,124, filed July 31, 2020. On the filing date applicants filed multiple IDSs listing over 750 references and a Track One request, which was granted. EX1002.28-128, 226, 241.

On December 21, 2020, the Examiner rejected the claims as anticipated and obvious based on U.S. 10,752,951 to Salk. EX1002.542-547. In rejecting the dependent claims under §103 the Examiner found they "merely relate to routine optimization of known-important reaction parameters." EX1002.545.

Following an Interview (EX1002.550) applicants amended the claims (EX1002.551-556) and presented arguments to distinguish Salk (EX1002.656-660). On May 18, 2021, the Examiner maintained the rejections over Salk. EX1002.675-

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[5] In IPR2022-01400, the Board found that "claim 1 does not require a particular 'relationship' between the number of tags and the expected number of duplicate molecules (z), nor does it require 'tailoring' the recited method based on z. *See* Prelim. Resp. 19, 43. Rather, ***element 1(b) recites a range*** for 'n' the number of 'different combinations of molecular bar codes, wherein n is at least 2 and no more than 100,000*z.'" EX1028, p.23.

679. In response, applicants amended claim 1 to add that the "n different combinations element." EX1002.684-689. The Examiner subsequently allowed the claims without explanation. EX1002.737-742.

D. IPR2022-01400

On August 12, 2022, Twinstrand Biosciences, Inc. ("Twinstrand") filed IPR2022-01400 challenging the '306 patent. EX1026. Twinstrand asserted obviousness grounds based on Narayan, Schmitt, Meyer, Craig, and Kivioja. The Board instituted review and issued a final written decision ("FWD") affirming patentability of the claims. EX1027. In the FWD the Board found Twinstrand had failed to show Schmitt disclosed elements [1b1] and [17d] of the independent claims.

For element [1b1], the Board determined Twinstrand had contended Schmitt disclosed molecular barcodes consisting of a combination of a 3-mer tag ***and*** portions of the endogenous cfDNA sequence. EX1027, pp.22-26. Because the barcodes relied on by Twinstrand included portions of the endogenous sequence, the Board concluded they were not "'attached to both ends' of the cfDNA molecule" as recited. *Id.*, p.23.

For element [17d], the Board found Twinstrand had failed to show Schmitt disclosed sorting reads into families of both paired and unpaired reads. *Id.*, p.30.

V. GROUNDS

Petitioner presents the following grounds of invalidity:

Ground	Basis	References	Claims
1	§103	Bielas and Vogelstein	1-2, 7-8, 12-13, 17-18, 20-21, 24-26, and 29
2	§103	Bielas, Vogelstein and Forshew	3, 9, 10-11, 19, and 22-23
3	§103	Bielas, Vogelstein, Hendricks and/or Diehn	4-6
4	§103	Bielas, Vogelstein and Hicks	14-16 and 27-28

VI. LEVEL OF ORDINARY SKILL

A POSA as of the relevant date would have had a Ph.D. in bioinformatics, genetics, molecular biology or a related field, and at least five years of research in an academic or industry setting, including at least two to three years of research experience in the field of cancer genomics. EX1003, ¶36. The POSA would have knowledge of DNA sequencing, including NGS and related sequencing methods, and related sample preparation techniques; bioinformatics methods and tools for analyzing sequence reads and mapping sequence reads onto genomes; and methods for identifying genetic variants in a sample. *Id*.

VII. CLAIM CONSTRUCTION[6]

Petitioner does not believe any terms require construction to resolve the patentability disputes because the challenged claims are rendered unpatentable by the cited art under any reasonable interpretation of the claims.

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[6] Petitioner reserves the right to pursue different claim constructions, including that certain claim terms are indefinite, during this and related proceedings as well as in any district court litigation concerning the ’306 patent.

Petitioner takes no position in this Petition as to whether the claims meet the requirements of Section 35 U.S.C § 112, and no statements herein should be interpreted to mean that Patent Owner's interpretations are correct or that the claim terms meet those requirements.

VIII. THE PRIOR ART

A. Bielas (EX1005)

Bielas is a patent application filed February 15, 2013 and published August 22, 2013. It is prior art under at least §102(a)(1).

Bielas discloses methods for accurately detecting mutations in DNA sequences by tagging double-stranded nucleic acid molecules with duplex molecular barcode tags, called "cyphers" or "barcodes." EX1005, 7:23-27; 30:12-13. Bielas's methods can be used to analyze "circulating tumor mtDNA" (a known type of cfDNA) and "diagnose and stage cancer." *Id.*, 25:27-26:1; EX1003, ¶104.

Bielas recognizes that "massively parallel sequencing represents a particularly powerful form of digital PCR" but PCR amplification "suffers from the inherent error rate of polymerases employed for amplification." EX1005, 2:9-15. Bielas addresses these problems by ligating to target molecules "dual cyphers (*i.e.,* barcodes or identifier tags), one on each end, so that sequencing each complementary strand can be connected back to the original molecule." *Id.*, 7:23-27. The barcodes are used to differentiate between mutations in the original fragment (a "true

mutation") and those created by PCR (an "artifact mutation"). EX1005, 5:8-23; EX1003, ¶106.

Bielas explains barcodes can vary in length. EX1005, 3:4-6, 8:19-21, 14:3-16. The barcodes on each end of a target molecule can be the same or different. *Id.*, 3:9-12, 4:24-27, 16:3-26. After sequencing, combined Cypher-fragment sequences are used for de-duplication, as shown below, *e.g.*, in Figure 3B (annotated):

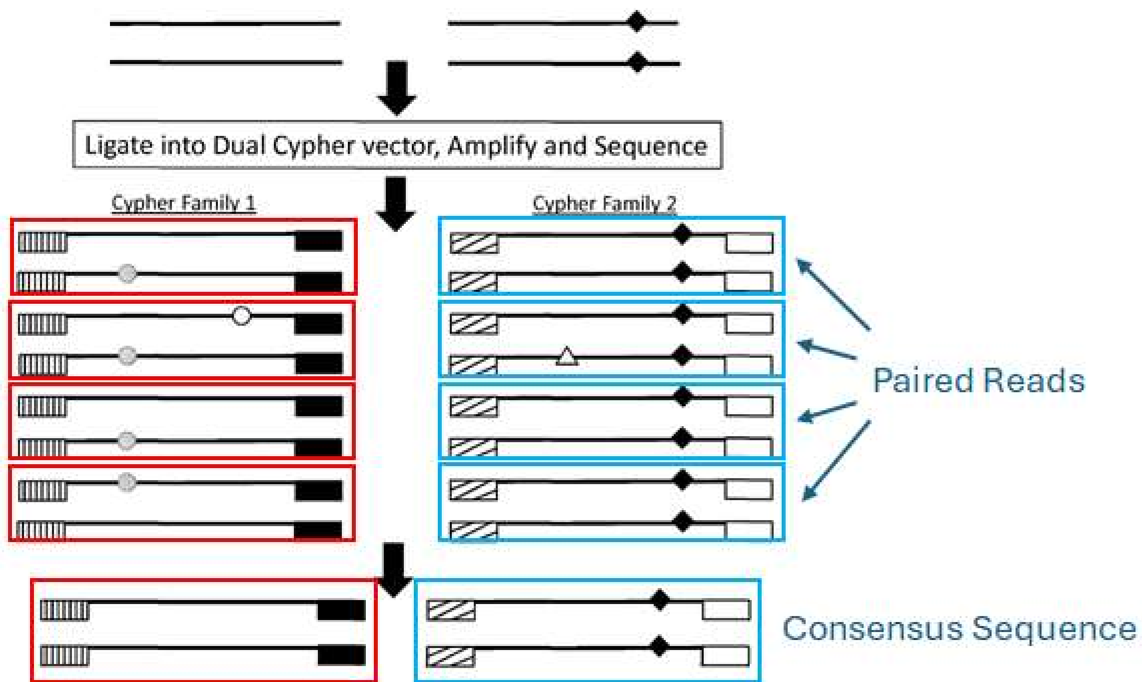

Fig. 3B

In Bielas, "cypher tags are used to computationally deconvolute the sequencing data and map all sequence reads to single molecules (*i.e.*, distinguish PCR and sequencing errors from real mutations)." *Id.*, 25:7-9. "All sequencing reads having identical cypher pairs, along with their reverse complements, were

grouped into families. Comparison of family sequences allowed for generation of a consensus sequence." *Id.*, 4:11-15.

B. Vogelstein (EX1006)

Vogelstein is a patent application filed April 12, 2012 and published October 18, 2012. It is prior art under at least §102(a)(1).

Like Bielas, Vogelstein discloses methods for reducing errors in parallel nucleic acid sequencing. EX1006, ¶¶[02], [05]. It teaches use of unique identifiers (UID), attached to one or both ends of nucleic acid fragments, that are from 2 to 4,000 bases in length. *Id.*, ¶[07]; ¶[22]. Tagged fragments are amplified to produce multiple "daughter" copies. *Id.*, ¶[19].

Vogelstein teaches that "[f]amily members are sequenced and compared to identify any divergencies within a family." *Id.*, ¶[28]. After sequencing, "[h]igh quality reads [are] grouped into UID-families based on their endogenous or exogenous UIDs." *Id.*, ¶[61]. "If a mutation pre-existed in the template molecule used for amplification, that mutation should be present in a certain proportion, or even all, of daughter molecules containing that UID." *Id.*, ¶[19].

C. Hendricks (EX1008)

Hendricks is a patent application filed January 17, 2012, and published July 26, 2012. Hendricks is prior art under at least 35 U.S.C. §102(a)(1).

Hendricks is directed to ligation methods, including for nucleic acid molecules. EX1008, ¶[0003]. Hendricks describes "small footprint ligase" ("SFL") that ligates short polynucleotides, such as primers and probes, to other longer polynucleotides. *Id.*, ¶[0011].

Hendricks discloses how to calculate the efficiency of ligation reactions, *id.*, ¶[0017], ¶[0076], as well as factors affecting ligation efficiency, *id.*, ¶¶[0007], [0079]. Hendricks further teaches that "[t]he ligation should produce a significant or detectable amount of ligation product," and teaches achieving "10%, 20%, 30%, 50%" or higher ligation efficiency. *Id.*, ¶[0076].

D. Diehn (EX1009)

Diehn is a U.S. Patent application filed March 13, 2014, and published October 2, 2014. It claims priority to provisional application no. 61/798,925 (EX1025, "Diehn Provisional"), filed March 15, 2013. Diehn is prior art under 35 U.S.C. §102(a)(2).[7]

Diehn is directed to methods for creating a library of mutated genomic regions and using the library to analyze cancer-specific genetic alterations. EX1009, ¶¶[0011]-[0015]. Libraries were prepared using "cfDNA and shorn tumor, germline,

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[7] The Diehn Provisional specification is substantially the same as Diehn's specification, the two applications have identical claim sets, and the Diehn Provisional provides §112 support for at least one claim in Diehn. *See* EX1009; EX1025; EX1003, ¶119; *Dynamic Drinkware, LLC v. Nat'l Graphics, Inc.*, 800 F.3d 1375 (Fed. Cir. 2015).

and cell line genomic DNA." EX1009, ¶[0142]. The DNA was washed and "eluted into 50 uL 1x ligation buffer with ligase and 100-fold molar excess of indexed Illumina TruSeq adapters." EX1009, ¶[0143]. Ligation was performed and the "ligated fragments were then amplified using 500 nM Illumina backbone oligonucleotides and a variable number of PCR cycles." EX1009, ¶[0143].

E. Forshew (EX1010)

Forshew is an article published on May 30, 2012. EX1023, ¶¶42-48. It is prior art under 35 U.S.C. §102(a)(1).

Forshew teaches a method of detecting rare "cancer mutations present in circulating [cell-free] DNA" using a "liquid biopsy" approach. EX1010, Abstract. Forshew teaches targeting DNA fragments from genomic regions, amplifying and sequencing the fragments, and aligning the sequences to a reference sequence to identify mutations. EX1010, Fig. 1, Supplement 1-4. Forshew employs a "two-step amplification process" where the second step is selective enrichment. EX1010, p.3.

F. Hicks (EX1011)

Hicks is a U.S. Patent filed on October 21, 2011 and issued on August 2, 2016. It is prior art under 35 U.S.C. §102(a)(2).

Hicks discloses methods "for obtaining from genomic material genomic copy number information unaffected by amplification distortion." EX1011, at Abstract. Hicks teaches tagging DNA molecules such that the molecules become unique based

on "the combination of the information in the tag and the information in the nucleic acid." EX1011 at 19:11-17. "[S]equences are then mapped to specific regions of the human genome and deconvoluted using the unique sample tag to determine which sequence reads originated from which tumor cells." *Id.*, 18:10-13. "After deconvoluting by sample tag, the number of different tagged nucleic acid molecules mapped to each location is counted." *Id.*, 19:36-38.

G. The Petition's Combinations Were Not Previously Considered By The Patent Office

The combinations of art and arguments in this Petition are different from those previously considered by the Office. Although Bielas, Vogelstein, Hendricks, Diehn, Forshew and Hicks were cited during prosecution with approximately 750 other references in multiple IDSs all filed on the same day (EX1002.27-128), the Examiner did not rely on any of the references as a basis for rejection and there is no evidence they were discussed or substantively considered. Moreover, the Examiner was not presented with the specific combinations of references as set forth in this Petition, and did not have the benefit of the arguments in this Petition. *Oticon Medical AB et al. v. Cochlear Limited*, IPR2019-00975, Paper 15, 9-20 (PTAB Oct. 16, 2019) (precedential) (finding the first prong of *Advanced Bionics* unsatisfied when the particular combinations in the petition were not considered by the examiner).

This Petition also includes new evidence such as admissions by Patent Owner from other IPR proceedings and testimony from Dr. Michael Metzker. EX1003. And as demonstrated in Section IX.B.1(d), the limitation added to independent claim 1 during prosecution to secure allowance is disclosed in both the Bielas and Vogelstein references relied on in this Petition.

These facts demonstrate the Examiner erred in overlooking the teachings of the references cited in the Grounds herein. *See Ecto World, LLC v. Rai Strategic Holdings, Inc.*, IPR2024-01280, Paper 13 at 5-7.

IX. GROUND 1: CLAIMS 1-2, 7-8, 12-13, 17-18, 20-21, 24-26, AND 29 ARE RENDERED OBVIOUS BY BIELAS IN VIEW OF VOGELSTEIN

A. Motivation To Combine

A POSA would have been motivated to combine the teachings of Bielas and Vogelstein. EX1003, ¶¶131-137.

Bielas and Vogelstein are analogous art directed to the field of nucleic acid sequencing. EX1005, 1:15-19; EX1006, ¶[02]. Both references recognize it was desirable to identify mutations present in small fractions of DNA using massively parallel sequencing, but that high error rates made this challenging. EX1005, 2:7-22; EX1006, ¶¶[04]-[05]. Both references disclose methods to address errors, improving accuracy, by attaching barcodes to target molecules prior to amplification.

A POSA would have been motivated to combine Bielas and Vogelstein for several reasons. EX1003, ¶¶131-137. Both references are in the same field of DNA sequencing, recognize the benefits of massively parallel sequencing and the associated problems from introduced errors, and seek to reduce errors by tagging target fragments with barcodes used to group families of sequence reads and generate consensus sequences. *See, e.g.*, EX1006, ¶¶[19], [23]. Indeed, Bielas cites an article by the inventors of the Vogelstein reference describing the Safe-SeqS methods disclosed in Vogelstein, demonstrating that a POSA would have considered Vogelstein's teachings in connection with Bielas. EX1005, 2:21-27 (citing EX1048).

Furthermore, a POSA would have been motivated to consider Bielas' teaching of using duplex tags to group families of paired sequence reads based on Vogelstein's recognition that using paired reads, as opposed to unpaired reads, could further reduce errors. In Vogelstein, fragments tagged with a UID and amplified are grouped into families of single-stranded fragments that each share the same UID. *Id.*, ¶¶[19], [27]-[28]. Vogelstein recognizes, however, that "an additional criteria for calling mutations could require that the mutation appears in only one or in both strands of the originally double stranded template." EX1006, ¶[42]; *see also id.* ¶[67]. Vogelstein thus teaches it could be beneficial to consider families of paired reads. EX1003, ¶133.

This disclosure would motivate a POSA to consider Bielas' teaching of attaching barcodes to each end of duplex strands of target molecules, amplifying and sequencing the molecules, and grouping sequencing reads with their complements into paired families. EX1005, 4:9-12. According to Bielas, "[c]omparison of family sequences allowed for generation of a consensus sequence wherein 'mutations' (errors) arising during library preparation [] and during sequencing [] were computationally eliminated." *Id.*, 4:12-15. Given Vogelstein's recognition of the potential benefits of comparing complementary strands, and Bielas' teaching of methods for comparing complementary strands, a POSA would have been motivated to consider Bielas in connection with Vogelstein in order to further reduce or eliminate errors in sequencing, and thus improve variant detection. EX1003, ¶¶133-135.

A POSA combining Bielas and Vogelstein would have had a reasonable expectation of success in achieving the claimed inventions. *Id.*, ¶¶136-137. The references both teach that their methods are general techniques for NGS and error correction, which can be used with standard and well-known methods for library preparation, tagging, amplification and sequencing, that are applicable to small fractions of DNA in a sample, such as cfDNA. *See, e.g.*, EX1005, pp.2, 9-11; EX1006, ¶¶[04]-[05], [20], [22], [27]-[28]; EX1003, ¶¶136-137. Both references rely on standard molecular biology tools, and neither requires novel chemistry or

unconventional instrumentation. As discussed in Section III, the sequencing methods in Bielas and Vogelstein were well-known in the art by December 2013. A POSA would have understood that the methods disclosed in Vogelstein could be used with those disclosed in Bielas. EX1003, ¶¶131-137.

In particular, a POSA would have understood that Bielas and Vogelstein's grouping into families, reducing redundancy, and mapping to a reference sequence could be successfully applied to amplification and sequencing of cfDNA to generate families of both paired reads and unpaired reads, as both references teach the advantages of generating such families using barcodes to reduce errors. EX1003, ¶135. Further, a POSA would have expected that Bielas' methods (applied to circulating tumor DNA) could be successfully used in conjunction with Vogelstein's methods (applied to DNA in blood and maternal plasma), as these types of DNA are all present in small amounts in natural samples. *Id.*, ¶137.

B. Claim-by-Claim Analysis

1. Claim 1

(a) Preamble [1pre]

To the extent the preamble is limiting, Bielas discloses methods for detecting mutations in DNA sequences. EX1005, 3:17-27, 7:23-27; EX1003, ¶¶138-139.

(b) Element [1a]

Bielas discloses "providing a population of cell-free deoxyribonucleic acid (cfDNA) molecules having first and second complementary strands." EX1003, ¶¶140-141.

Bielas discloses providing "a double-stranded nucleic acid molecule library that includes a plurality of target nucleic acid molecules." EX1005, 2:29-30, 4:24-5:3; *see also id.*, 8:29-31 ("'[T]arget nucleic acid molecules' and variants thereof refer to a plurality of double-stranded nucleic acid molecules that may be fragments or shorter molecules generated from longer nucleic acid molecules, including from natural samples."). Bielas discloses that its methods are "useful in detecting rare mutants against a large background signal, such as when … detecting ***circulating mutant DNA in blood***," and "to quantify circulating tumor cells (CTCs) and ***circulating tumor mtDNA (ctmtDNA)***." *Id.*, 23:12-14, 25:27-28.

Circulating tumor DNA (ctDNA) are fragments of DNA freely floating in the bloodstream outside of cancer cells and are a well-known type of cfDNA. EX1003, ¶141; *see also* Section III.A.

(c) Element [1b1]

Bielas discloses "tagging a plurality of the cfDNA molecules in the population with duplex tags comprising molecular barcodes to produce tagged parent

polynucleotides, wherein the duplex tags are attached to both ends of a molecule of the plurality of the cfDNA molecules."[8] EX1003, ¶¶142-144.

Duplex tags comprising molecular barcodes. Bielas discloses the use of "dual cyphers (*i.e.,* barcodes or origin identifier tags)" attached to the ends of double-stranded target DNA molecules. EX1005, 4:24-30; *see also id.*, 1:15-19, 7:23-27, 8:19-28. In Bielas, the terms "'random cypher' or 'cypher' or 'barcode' or 'identifier tag' and variants thereof are used interchangeably and refer to a nucleic acid molecule having a length ranging from about 5 to about 50 nucleotides." EX1005, 8:19-21; EX1003, ¶143. The dual cyphers (*i.e.*, molecular barcodes) in Bielas are "duplex tags" because they differentially label the complimentary strands of a double-stranded cfDNA molecule. Specifically, the dual cyphers are double-stranded nucleic acid molecules comprised of basepairs. *See, e.g.,* EX1005, 4:9-10 ("Target nucleic acid molecules were ligated into a cypher vector library containing previously catalogued dual, ***double-stranded cyphers***."), 7:25-26 ("[T]arget molecules include a single ***double-stranded cypher*** or dual cyphers (*i.e.*, barcodes or identifier tags), one on each end."), 12:11-21 ("double-stranded sequence of the [] cypher"), 27:27-28 ("A single [NGS] run on MiSeq® demonstrated optimal coverage and diversity at the upstream ***seven basepair cypher*** in the vector

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[8] The '306 patent discloses that "duplex tags" are "tags that differently label the complementary strands (*i.e.*, the 'Watson' and 'Crick' strands) of a double-stranded molecule." EX1001, 17:9-13.

library."); EX1003, ¶143. A POSA would understand that a "base pair" consists of two different, but complementary, nucleotide bases (*e.g.*, adenine and thymine) paired together. EX1003, ¶143. Because the nucleotide bases are complementary, a POSA would understand that the bases in the top strand are different than the bases on the bottom strand. Thus, the dual cyphers in Bielas are duplex tags comprising molecular barcodes. *Id.*, ¶143.

Tagging molecules to produce tagged parent polynucleotides. Bielas discloses methods for "tagging double stranded nucleic acid molecules" with the dual cyphers (*i.e.*, molecular barcodes). EX1005, 1:15-19, 4:24-27, 8:19-28; EX1003, ¶144. The nucleic acid molecules are tagged with the dual cyphers to produce a library of tagged parent polynucleotides "having a formula of … Xa_Y_Xb … or Xb_Y_Xa (in 5' to 3' order), wherein (a) Xa comprises a first random cypher, (b) Y comprises a target nucleic acid molecule, and (c) Xb comprises a second random cypher." EX1005, 2:29-3:4, 4:24-5:7, 12:11-21; *see also* Figs. 2, 3A, 3B; EX1003, ¶144. A POSA would understand the tagged polynucleotides are tagged ***parent*** polynucleotides because they are subsequently amplified and sequenced to generate progeny polynucleotides. EX1005, 3:17-22; EX1003, ¶144.

Tags attached to both ends. The tags in Bielas are attached to both ends of the target molecules.[9] EX1005, 16:11-13 ("[E]ach target nucleic acid molecule or a subset of target molecules has identical cyphers ***on each end*** of one or more target nucleic acid molecules."), 4:24-26 ("target nucleic acid molecules include dual cyphers … one on each end"), 8:26-28 ("Cyphers … may be incorporated directly ***onto target molecules of interest***."), 12:11-20, 15:12-15.[10]

(d) Element [1b2]

Bielas alone or in combination with Vogelstein discloses that "the plurality of the cfDNA molecules are tagged with n different combinations of molecular barcodes, wherein n is at least 2 and no more than 100,000*z, wherein z is a mean of an expected number of duplicate molecules in the population of cfDNA molecules that map to identical start and stop positions on a reference sequence." EX1003, ¶¶145-150.

Without taking a position on whether a POSA would have understood the "z" term, based on dependent claim 7 "z" must include 2 to 8. Under this interpretation,

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[9] In the district court litigation Guardant has taken the position that this element is met when at least one barcode is attached to each end of a molecule. Petitioner reserves the right to pursue a different claim construction for this term in the district court litigation, including that the limitation is indefinite.

[10] As discussed in Section IV.D, the Board found in IPR2022-01400 that this was absent from the prior art. But as demonstrated here, attaching molecular barcodes to both ends of a cfDNA molecule was known.

Bielas alone or in view of Vogelstein discloses the use of a number of different molecular barcode combinations within the claimed range.[11]

Combinations of molecular barcodes. Bielas discloses each end of a molecule is tagged with barcodes having "a length ranging from about 5 to about 50 nucleotides."[12] EX1005, 8:19-21. The barcodes at each end together form a combination of molecular barcodes. EX1005, 12:11-21, 15:20-22; EX1003, ¶147.

"n different combinations." Bielas discloses use of broad ranges of numbers of combinations of barcodes. *See, e.g.*, EX1005, 12:22-13:21, 14:3-28, 16:3-26. Bielas explains "[t]he number of nucleotides contained in each of the random cyphers or barcodes will govern the total number of possible barcodes available for use in a library." EX1005, 13:17-20. "For example, a barcode of 7 nucleotides would have a formula of 5'-NNNNNNN-3' … wherein N may be any naturally

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[11] Tempus expressly reserves the right to argue that this claim element is indefinite in the co-pending district court litigation.

[12] A POSA also would have understood that a length of "about 5" nucleotides includes a length of 4 nucleotides. EX1003, ¶147. At a minimum, it would have been obvious in view of Vogelstein (EX1006) to use barcodes of a length of 4 nucleotides. Such barcodes would yield a maximum of 256 different combinations if the same barcode were used on each end of the target molecule, or 65,536 different combinations (4^4 x 4^4 (or 256 x 256)) if a different barcode were used on each end. EX1003, ¶147. As taught by Bielas, however, it may be desirable to use barcodes that "exclud[e], for example, sequences in which all the nucleotides are identical (*e.g.,* all A or all T or all C or all G) or [] exclud[e] sequences in which three contiguous nucleotides are identical or [] exclud[e] both of these types of molecules." EX1005, 14:10-13. This would result in a smaller number of combinations also within the range of 2 to 100,000*z. EX1003, ¶147.

occurring nucleotide. The four naturally occurring nucleotides are A, T, C, and G, so the total number of possible random cyphers is 4^7, or 16,384." *Id.*, 14:3-7. Bielas further discloses that molecules may be tagged with the same barcode on both ends: "In certain embodiments, the double-stranded sequences of the Xa and Xb cyphers are the same (*e.g.*, Xa = Xb) for one or more target nucleic acid molecules." EX1005, 3:9-10; *see also id.*, 5:28-30. In such cases, the number of combinations of barcodes is simply 4^[barcode length]. EX1003, ¶148; *see also* EX1005, 14:3-8. For example, if the barcodes are 7 nucleotides in length, as one example that is disclosed in Bielas, there are 4^7 or 16,384 possible barcode combinations.[13]

Furthermore, Bielas discloses that it may be desirable not to use all possible combinations of barcodes. For example, Bielas discloses using barcodes having a length of "about 5 to about 50 nucleotides" where "at least two of those nucleotides are different in each cypher" or "each cypher does not contain three contiguous nucleotides that are identical." *Id.*, 14:17-28. Such methods for crafting a set of barcodes were known in the art. *See* Section III.E.

Thus, a POSA would understand that Bielas' barcodes of "about 5 to about 50 nucleotides" discloses tagging with a number of different combinations of molecular barcodes within the claimed range of "at least 2 and no more than 100,000*z"

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[13] Bielas also discloses "sequences from a particular pool of cyphers (*e.g.*, pools of 4,094, 16,384 or 65,536 unique cyphers) may be used more than once." EX1005, 16:3-5.

regardless of the value of z, and particularly if the value of z is equal to between 2 and 8. EX1003, ¶149.

In addition, Vogelstein likewise discloses that molecular barcodes—its "unique identifiers" or "UIDs"—"may be added to one or both ends of the fragments." EX1006, ¶¶[07], [22]. The barcodes "consist[] of 2 to 4,000 nucleotides" (*id.*, ¶[0022]) and may be "at least 4, at least 16, at least 64, at least 256 … different sequences" (*id.*, ¶[0028]). Using Vogelstein's UIDs on both ends of a cfDNA molecule, as disclosed in both Vogelstein and Bielas, discloses the "n different combinations element." EX1003, ¶150. For example, barcodes of 2 nucleotides would have 4^2 x 4^2 = 256 possible barcode combinations. EX1003, ¶150.

(e) Element [1c]

Bielas alone or in combination with Vogelstein discloses "amplifying a plurality of the tagged parent polynucleotides to produce amplified progeny polynucleotides." EX1003, ¶¶151-153.

Bielas discloses that after target molecules are ligated into a library containing the "dual, double-stranded cyphers," "[t]he target sequences were ***amplified*** and sequenced." EX1005, 4:10-11; *see also id.*, 3:17-22. Techniques for amplifying DNA molecules were well-known (*see* Section III.D) and Bielas discloses amplification using such known techniques. *See, e.g.*, EX1005, 5:30-6:3. The

amplification process produces amplified progeny polynucleotides. *See, e.g.*, EX1005, 7:8-9 ("[NGS] has opened the door to sequencing multiple copies of an amplified single nucleic acid molecule."); 6:11-13; EX1003, ¶152.

Vogelstein also discloses amplifying a plurality of the tagged parent polynucleotides to produce amplified progeny polynucleotides. EX1003, ¶153; EX1006, ¶[27] ("Amplification of fragments containing a UID can be performed according to known techniques to generate families of fragments," including PCR). A POSA would understand that the family of fragments are progeny polynucleotides. EX1003, ¶153.

(f) Element [1d]

Bielas alone or in combination with Vogelstein discloses "sequencing at least a subset of the amplified progeny polynucleotides to produce a set of sequence reads." EX1003, ¶¶154-156.

Bielas discloses "[e]ach strand of the double-stranded nucleic acid library (*e.g.*, genomic DNA, cDNA) can be amplified and ***sequenced*** using, for example, [NGS] technologies." EX1005, 5:30-6:3, 10:25-29. NGS techniques were well-known. Section III.C; EX1003, ¶155. Sequencing generates "sequence information from each complementary strand of a first double-stranded nucleic acid molecule" termed "sequence reads." EX1005, 5:30-6:6; EX1003, ¶155.

Vogelstein also discloses this element. EX1006, ¶[28] ("Family members are sequenced and compared to identify any divergencies within a family. Sequencing is preferably performed on a massively parallel sequencing platform, many of which are commercially available."); EX1003, ¶156.

(g) Element [1e]

Bielas alone or in combination with Vogelstein discloses "reducing or tracking redundancy of a plurality of sequence reads from the set of sequence reads using at least sequencing information from the molecular barcodes of the duplex tags to determine distinct cfDNA molecules from among the tagged parent polynucleotides, wherein the distinct cfDNA molecules are determined based on (i) paired reads corresponding to sequence reads generated from a first tagged strand and a second tagged complementary strand derived from cfDNA molecules from among the tagged parent polynucleotides, or (ii) unpaired reads corresponding to sequence reads generated from a first tagged strand having no second tagged complementary strand derived from cfDNA molecules from among the tagged parent polynucleotides."[14] EX1003, ¶¶157-161.

Reducing/tracking redundancy using at least sequencing information from the molecular barcodes. After tagged parent polynucleotides are amplified and

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[14] In IPR2022-01400, the Board determined that the plain language of this claim element requires use of paired ***or*** unpaired reads, but not both. EX1028, pp.24-25.

sequenced in Bielas, "[a]ll sequencing reads ***having identical cypher pairs***, along with their reverse complements, were grouped into families." EX1005, 4:10-12. Thus, redundant reads (*i.e.*, amplified copies of parent polynucleotides) are grouped into families based at least on sequence information from the barcodes because they are grouped based on "identical cypher pairs." EX1003, ¶158. As discussed below, the families of sequences are used to reduce and/or track redundancy by determining consensus sequences.

To determine distinct cfDNA molecules based on paired reads. Because reads in Bielas are grouped into families "along with their reverse complements," they are families of paired reads. EX1005, 4:10-12; EX1003, ¶158. Once grouped into families of paired reads, Bielas discloses that redundancy of reads is reduced and/or tracked to determine distinct cfDNA molecules through generation of consensus sequences – *i.e.*, a consensus double-stranded read from the family is determined.[15] EX1005, 4:12-15 ("Comparison of family sequences allowed for generation of a consensus sequence wherein 'mutations' (errors) arising during library preparation (open circle) and during sequencing (gray circle and triangle)

[15] Reducing and/or tracking redundancy must encompass generating consensus sequences because claim 26, which depends from claim 1, recites "reducing or tracking the redundancy of the plurality of sequence reads comprises collapsing the plurality of sequence reads to produce consensus sequences." *See also* EX1001, 9:42-46.

were computationally eliminated."); *see also id.*, 25:7-9. This is shown below in Figure 3B (annotated):

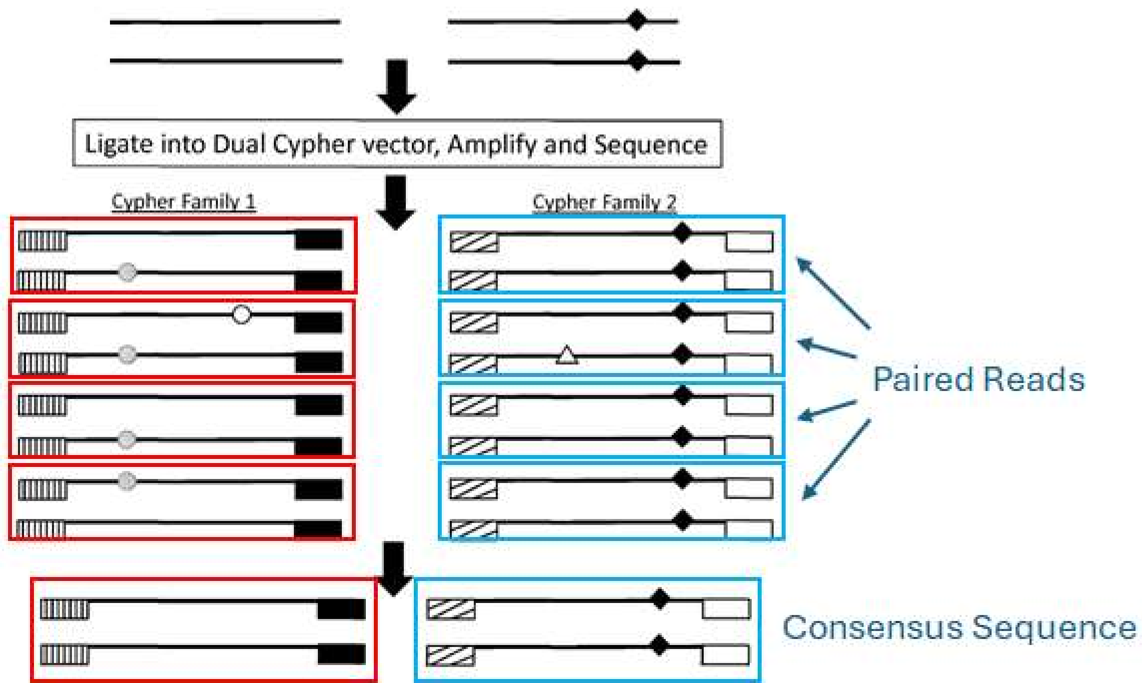

Fig. 3B

Thus, Bielas discloses using families of ***paired reads*** to reduce and/or track redundancy of a plurality of sequence reads by generating a consensus sequence that identifies a distinct cfDNA molecule. EX1005, 4:11-15, 23:6-11; EX1003, ¶158.

To the extent additional disclosure is required, Vogelstein discloses reducing and/or tracking redundancy based on families of ***unpaired reads***. EX1003, ¶159.

Reducing/tracking redundancy using at least sequencing information from the molecular barcodes. Vogelstein discloses "amplification of each uniquely tagged template, so that many daughter molecules with the identical sequence are generated." EX1006, ¶[19]. After sequencing, "reads [are] grouped into UID-

families ***based on their endogenous or exogenous UIDs***." *Id.*, ¶[61]. A POSA would understand these are families of unpaired reads. EX1003, ¶159.

To determine distinct cfDNA molecules based on unpaired reads. Vogelstein further teaches that "[a] nucleotide sequence is identified as accurately representing an analyte nucleic acid fragment when at least l% of members of the family contain the sequence." *Id.*, ¶[07]. Thus, a POSA would understand redundant reads are tracked and/or reduced to identify an "accurate[] … nucleic acid fragment." EX1003, ¶159. In addition, Vogelstein recognizes "various amplifications are not perfect, so every strand of every original template molecule is not recovered as a UID family." EX1006, ¶[66]. Only a fraction of the library fragments from the original library are used in the amplification step, resulting in some sequence reads having no second tagged complementary strand. EX1003, ¶¶59-60, 151-153. Thus, a POSA would understand that at least some of the families in Vogelstein consist of families of unpaired reads that have no second tagged complementary strand derived from among the tagged parent polynucleotides. EX1003, ¶160.

(h) Element [1f]

Bielas alone or in combination with Vogelstein discloses that "reducing or tracking the redundancy of the plurality of sequence reads comprises mapping at least a subset of the plurality of sequence reads to the reference sequence." EX1003, ¶¶162-164.

Bielas discloses that "using the methods of this disclosure, a person of ordinary skill in the art can associate each DNA sequence read to an original template DNA." EX1005, 23:6-7. In Bielas, "[b]ase calling and sequence alignment will be performed using, for example, the Eland pipeline (Illumina, San Diego, CA)." *Id.*, 25:9-10. A POSA would know the Eland pipeline is a computational tool used for processing raw sequencing data generated by Illumina's NGS platforms and mapping sequence reads to a reference sequence.[16] EX1003, ¶163; EX1055. Thus, base calling and sequence alignment involves mapping sequence reads to a reference sequence in connection with reducing redundancy by generating a consensus sequence. EX1003, ¶163.

To the extent additional disclosure is required, Vogelstein also discloses that after sequencing "[b]ase-calling and sequence alignment was performed with the Eland pipeline (Illumina)." EX1006, ¶[60]. Vogelstein discloses that after mapping sequence reads "[o]nly high quality reads … were used for subsequent analysis." *Id.* These "reads were grouped into UID-families based on their endogenous or exogenous UIDs." EX1006, ¶[61]. Thus, a POSA would understand that Vogelstein discloses mapping at least a subset of the plurality of sequence reads to the reference

[16] Guardant has admitted that "identifying sequence variants, also called mutations, by aligning the sequence reads using the Illumina Eland pipeline and comparing the reads to an expected (reference) sequence" was a "conventional sequencing practice[]" by March 2012. EX1024, p.56.

sequence as part of the process of reducing and/or tracking redundancy. EX1003, ¶164.

2. Claim 2

Bielas discloses "the population of cfDNA molecules is obtained or derived from a subject having cancer." EX1003, ¶¶165-166.

Bielas discloses "target nucleic acid molecules" that are "fragments or shorter molecules generated from longer nucleic acid molecules, including from natural samples (*e.g.*, a genome)…." EX1005, 8:29-9:1. Bielas further discloses its methods are "useful in detecting rare mutants against a large background signal, such as when … detecting circulating mutant DNA in blood." EX1005, 23:12-16. Bielas also discloses use of "circulating tumor mtDNA (ctmtDNA)," which can be quantified to "diagnose and stage cancer." EX1005, 25:15-26:12, 24:4-20, 26:6-9. A POSA would have understood that "circulating mutant DNA in blood" and "ctmtDNA" are cfDNA. EX1003, ¶166. Thus, Bielas discloses "cfDNA molecules [] obtained or derived from a subject having cancer." EX1003, ¶166.

3. Claim 7

Bielas alone or in combination with Vogelstein discloses "wherein z is between 2 and 8." EX1003, ¶¶167-169.

If z is between 2 and 8, there are no more than 200,000 to 800,000 different combinations of molecular barcodes. As discussed for Element [1b2], Section

IX.B.1(d), Bielas discloses use of numbers of different combinations of molecular barcodes that fall within this range. For example, Bielas discloses molecular barcodes "having a length ranging from about 5 to about 50 nucleotides" and that the molecules may be tagged with the same barcode on both ends. EX1005, 8:19-21, 3:9-10. If, for example, barcodes are 5 nucleotides in length, and the same barcode is used on each end, there are 1,024 possible barcode combinations, which falls within the claimed range. EX1003, ¶168. Likewise, Bielas discloses further reducing the number of combinations by excluding, *e.g.*, barcodes that contain three identical contiguous nucleotides.

Vogelstein also discloses wherein z is between 2 and 8. As discussed in connection with Element [1b2], Section IX.B.1(d), Vogelstein discloses molecular barcodes "consisting of 2 to 4,000 nucleotides" (EX1006, ¶[0022]) and a range of numbers of different combinations of molecular barcodes, including "at least 4, at least 16, at least 64, at least 256" (*id.*, ¶[0028]). A POSA would understand that barcodes of 2-5 nucleotides, or combinations of barcodes of "at least 4" to "at least 256" discloses the claimed range. *Ormco Corp. v. Align Tech.*, 463 F.3d 1299, 1311 (Fed. Cir. 2006); EX1003, ¶169.

4. Claim 8

Bielas alone or in combination with Vogelstein discloses that the "molecular barcodes have a length of 5 to 20 base pairs." EX1003, ¶¶170-172.

Bielas discloses molecular barcodes with "a length ranging from about 5 nucleotides to about 50 nucleotides (or about 5 nucleotides to … about 20 nucleotides)." EX1005, 3:4-8. The barcodes in Bielas are double-stranded, which a POSA would understand means they consist of base pairs of nucleotides. *Id.*, 4:9-10, 27:27-28; EX1003, ¶171. Thus, Bielas discloses the claimed range of "5 to 20 base pairs." EX1003, ¶171; *Ormco*, 463 F.3d at 1311.

Vogelstein also discloses molecular barcodes "consisting of 2 to 4,000 nucleotides." EX1006, ¶[0022]. A POSA would understand that Vogelstein's range of "2 to 4,000 nucleotides" entirely encompasses the claimed range of "5 to 20 base pairs." EX1003, ¶172; *Ormco*, 463 F.3d at 1311.

5. Claim 12

Bielas alone or in combination with Vogelstein discloses "the duplex tags are part of sequencing adapters." EX1003, ¶¶173-176.

Bielas discloses an exemplary figure of a "vector of the present disclosure, wherein adaptor sequences are included and are useful for, for example, bridge amplification methods before sequencing." EX1005, 4:1-3, 9:23-26.

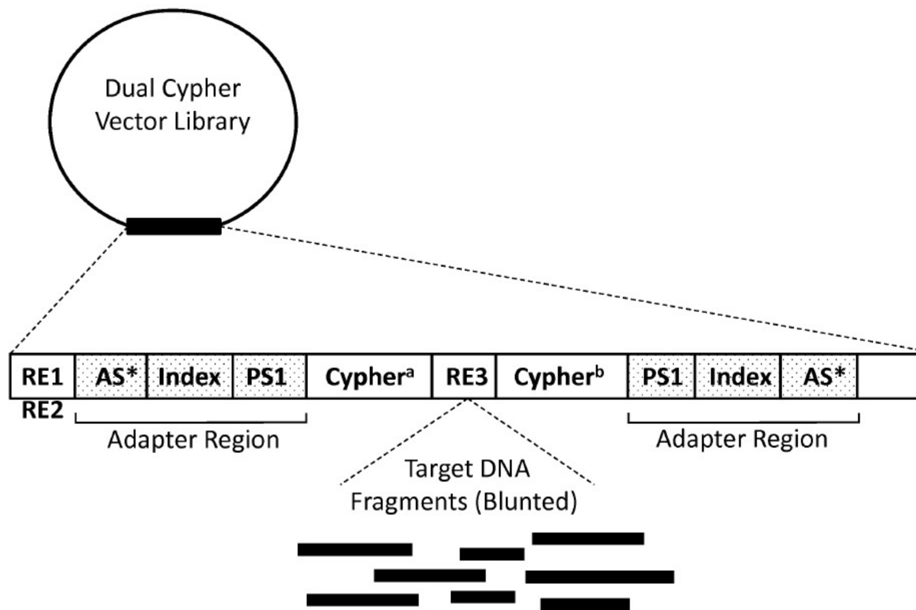

Fig. 2

Bielas discloses that the "DNA fragments are ligated into the *SmaI* site of the library of dual cypher vectors shown in Figure 2 to generate a target genomic library." EX1005, 24:23-24. A POSA would understand this disclosure to mean that the duplex tags, which include molecular barcodes, are contained within sequencing adapters. EX1003, ¶175; *see also* EX1005, claim 14 ("wherein the plurality of random cyphers further comprise at least one adapter sequence (AS)").

Vogelstein discloses that "DNA (sheared or unsheared) is amplified with a set of gene-specific primers. One of the primers has a random DNA sequence (*e.g.,* a set of 14 N's) that forms the unique identifier (UH) (variously shaded bars), located 5' to its gene-specific sequence, and both have sequences that permit universal amplification in the next step (earth hatched and cross hatched bars)." EX1006,

¶[14]. Vogelstein also discloses that "[a]ttachment of an exogeneous UID to an analyte nucleic acids fragment may be performed by any means known in the art, including enzymatic, chemical, or biologic. One means employs a polymerase chain reaction. Another means employs a ligase enzyme." *Id.*, ¶[22]. And Vogelstein explains that "[a] UID may be contained within a nucleic acid molecule that contains other regions for other intended functionality." *Id.* Thus, it would have been obvious to a POSA to attach the UIDs as part of sequencing adapters by a ligation reaction. EX1003, ¶176.

6. Claim 13

Bielas alone or in combination with Vogelstein discloses that "reducing or tracking the redundancy of the plurality of sequence reads comprises grouping the paired reads or the unpaired reads into families based at least in part on (i) the molecular barcodes associated with the paired reads or the unpaired reads and (ii) sequence information at the start and stop positions of the paired reads or the unpaired reads." EX1003, ¶¶177-180.

As discussed above regarding Element [1e], Bielas and Vogelstein disclose reducing and/or tracking the redundancy by grouping paired or unpaired reads into "families" based on the molecular barcodes associated with the paired or unpaired reads. *See* Section IX.B.1(g).

Bielas further discloses reducing and/or tracking the redundancy of sequence reads by grouping paired reads into families based on sequence information at the start and stop positions of the paired reads. EX1003, ¶179. Specifically, Bielas discloses "the first about 5 nucleotides to about 20 nucleotides of the target nucleic acid molecule sequence may be used as a further identifier tag together with the sequence of an associated random cypher." EX1005, 14:13-16. Bielas further discloses using the first about 5 nucleotides to about 20 nucleotides at both ends, *i.e.*, at the start and stop positions. EX1005, 15:5-11; EX1003, ¶179. Using sequence information at the start and stop positions, Bielas discloses reducing and/or tracking the redundancy of sequence reads. EX1005, 4:12-15.

Vogelstein also discloses reducing and/or tracking the redundancy of sequence reads by grouping paired reads or unpaired reads into "families" based on either exogenous barcodes, endogenous barcodes formed from the sequence information at the ends (*i.e.*, start and stop positions) of the target fragments, or a combination of the two. *See, e.g.*, EX1006, ¶¶[07], [09], [26]; *see also id.* ¶[56] ("The resulting DNA fragments contained UIDs composed of three sequences: two endogenous ones, represented by the two ends of the original sheared fragments plus the exogenous sequence introduced during the indexing amplification."). Thus, a POSA would understand from Vogelstein that sequence reads can be grouped into

families based on barcodes as well as sequence information at the start and stop positions of the reads. EX1003, ¶180.

7. Claim 17

(a) Preamble 17[pre]

To the extent the preamble is limiting, Bielas discloses this limitation. *See* Section IX.B.1(a), regarding Element 1[pre].

(b) Element [17a]

Element [17a] is substantially the same as Element [1b1]. As discussed above, Bielas discloses Element [1b1]. *See* Section IX.B.1(c).

Element [17a] specifically recites tagging "double-stranded" cfDNA molecules "from a sample of a subject with a set of tags comprising molecular barcodes." As discussed above regarding Element [1a], Bielas discloses the use of cell-free DNA (cfDNA) molecules having first and second complementary strands. *See* Section IX.B.1(b). And as discussed above regarding Claim 2, Bielas alone or in combination with Vogelstein discloses that the cfDNA molecules are obtained or derived "from a sample of a subject." *See* Section IX.B.2.

(c) Element [17b]

Bielas alone or in combination with Vogelstein discloses Element [17b] for the same reasons discussed above for Element [1c]. *See* Section IX.B.1(d).

(d) Element [17c]

Bielas alone or in combination with Vogelstein discloses Element [17c] for the same reasons discussed above for Element [1d]. *See* Section IX.B.1(e).

(e) Element [17d]

Bielas in combination with Vogelstein discloses "sorting a plurality of sequence reads from the set of sequence reads into (i) families comprising paired reads corresponding to sequence reads generated from a first tagged strand and a second tagged complementary strand derived from double-stranded cfDNA molecules from among the tagged parent polynucleotides, and (ii) families comprising unpaired reads corresponding to sequence reads generated from a first tagged strand having no second tagged complementary strand derived from double-stranded cfDNA molecules from among the tagged parent polynucleotides." EX1003, ¶¶187-194.

Sorting into families comprising paired reads. Bielas discloses sorting sequence reads from the set of sequence reads into families comprising paired reads corresponding to sequence reads generated from a first tagged strand and a second tagged complementary strand. EX1003, ¶188. Specifically, Bielas discloses that "[a]ll sequencing reads having identical cypher pairs, ***along with their reverse complements***, were grouped into families." EX1005, 4:11-15. This is shown below in Figure 3B (annotated):

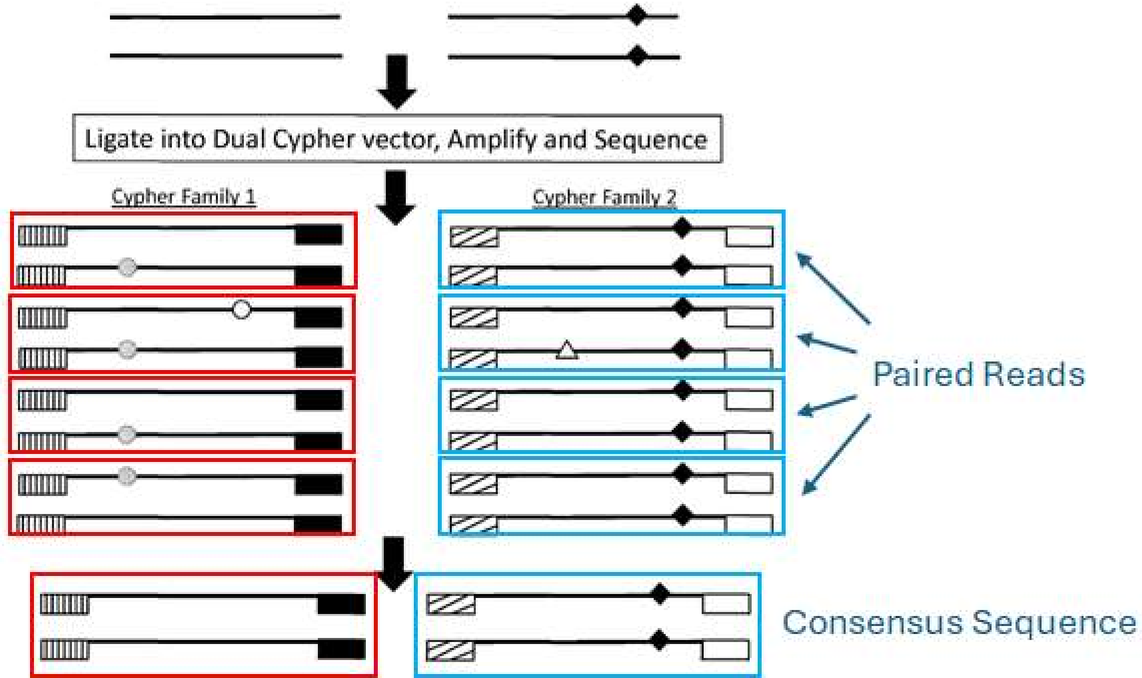

Fig. 3B

A POSA would understand that these families comprise "paired reads corresponding to sequence reads generated from a first tagged strand and a second tagged complementary strand" because Bielas uses "sequencing reads having identical cypher pairs, along with their reverse complements," thus indicating that these reads are paired reads. EX1003, ¶188.

Sorting into families comprising unpaired reads. Vogelstein discloses sorting sequence reads into families comprising unpaired reads. EX1003, ¶¶189-190. Vogelstein discloses that "amplification of each uniquely tagged template, so that many daughter molecules with the identical sequence are generated (defined as a UID family)." EX1006, ¶[19]. After sequencing, "[h]igh quality reads [are]

grouped into UID-families." *Id.*, ¶[61]; *see also id.*, ¶[09]. A POSA would understand that the UID families are unpaired reads. EX1003, ¶¶189-190.

Unpaired reads corresponding to sequence reads generated from a first tagged strand having no second tagged complementary strand. Vogelstein discloses that "various amplifications are not perfect, so every strand of every original template molecule is not recovered as a UID family." EX1006, ¶[66]. Only a fraction of the library fragments from the original library are used in the bridge amplification step, resulting in some sequence reads having no second tagged complementary strand. EX1003, ¶¶59-60, 190. Thus, a POSA would understand that at least some families in Vogelstein are families of unpaired reads that have no second tagged complementary strand derived from cfDNA molecules from among the tagged parent polynucleotides. EX1003, ¶190.

The fact that not all sequence reads would have a complementary strand would motivate a POSA to combine the teachings of Bielas and Vogelstein. EX1003, ¶¶191-194. Although Vogelstein primarily discloses grouping sequence reads into single-stranded families (EX1003, ¶¶189-190), it recognizes that "specificity can be even further increased by requiring that each strand of the original double stranded template contain the mutation." EX1006, ¶[67]; *see also id.*, ¶¶[33], [42]. Vogelstein thus suggests considering families of paired reads to further reduce

errors, and Bielas teaches how to use families of paired reads to reduce redundancy. EX1003, ¶¶191-194.

Given the recognition that not all sequencing reads will have counterparts, a POSA would be motivated to use Bielas' approach of sorting sequence reads into families of paired reads where possible, in order to obtain additional reduction in errors suggested by Bielas and Vogelstein. EX1003, ¶192. A POSA would, however, also continue to use Vogelstein's approach of sorting reads into families of unpaired reads for any reads that have no counterpart. EX1003, ¶192. For example, when considering the usefulness of the unpaired read approach, Vogelstein reported reducing sequencing errors from 15-fold to 70-fold, which is substantial and would have motivated one of ordinary skill in the art to use the unpaired approach in the absence of the paired approach. EX1006, ¶¶ [41], [52], [54]. In this way, Bielas and Vogelstein together provide dual benefits—further improved error reduction (from paired reads) and maximal use of sequencing data (by also considering unpaired reads). EX1003, ¶¶191-194.

At a minimum, using both paired and unpaired reads would have been obvious to try as one of three options for tracking or reducing redundancy—(1) paired reads only, (2) unpaired reads only, or (3) both. Using both paired and unpaired would avoid wasting resources (*e.g.*, discarding unpaired reads) and would be expected to

have predictable results given that methods of generating consensus sequences from both unpaired and paired reads were taught by the prior art. EX1003, ¶193.

8. Claim 18

Bielas alone or in combination with Vogelstein discloses that "the sample is blood, plasma, or serum." EX1003, ¶¶195-197.

Bielas describes using "circulating mutant DNA in blood" from a patient. EX1005, 23:14; *see also* 25:27-28.

Vogelstein also discloses that "[d]etection of donor DNA in the blood of organ transplant patients is an important indicator of graft rejection and detection of fetal DNA in maternal plasma can be used for prenatal diagnosis in a non-invasive fashion." EX1006, ¶[03].

9. Claim 20

Bielas alone or in combination with Vogelstein discloses that "the tagging comprises ligating the molecular barcodes to double-stranded cfDNA molecules." EX1003, ¶¶198-200.

Bielas discloses that "[t]arget nucleic acid molecules were ligated into a cypher vector library containing previously catalogued dual, double-stranded cyphers." EX1005, 4:9-10, 4:24-30.

Vogelstein also discloses "UIDs, sometimes called barcodes or indexes, can be assigned to nucleic acid fragments in many ways. These include the introduction

of exogenous sequences through PCR (40, 41) or ligation (42, 43)." EX1006, ¶[39]; *see also id.*, ¶[22].

10. Claim 21

Bielas alone or in combination with Vogelstein discloses that "the set of tags comprises 2 to 10,000 different molecular barcode sequences." EX1003, ¶¶201-203.

Bielas discloses molecular barcodes "having a length ranging from about 5 to about 50 nucleotides." EX1005, 8:19-21. Bielas discloses that the same barcode can be used on each end of the molecule (*id.*, 3:9-10, 5:28-30), meaning that barcodes of 5 or 6 nucleotides in length discloses the claimed range of "2 to 10,000 different molecular barcode sequences" (*i.e.*, 4^5 = 1,024 and 4^6 = 4,096). EX1003, ¶202.

Vogelstein's disclosure of UIDs with 2 to 4,000 nucleotides, as well as its disclosure of a broad range of number of different unique identifier sequences, also comprises the claimed "2 to 10,000 different molecular barcode sequences." EX1006, ¶¶[22], [30]; EX1003, ¶203. For example, UIDs with 2 nucleotides on each end of the DNA fragment provides 256 barcodes. EX1003, ¶203. Vogelstein further discloses a range of numbers of different combinations of molecular barcodes that includes "at least 4, at least 16, at least 64, at least 256, at least 1,024, at least 4,096 … different sequences." EX1006, ¶¶[28], [30].

11. Claim 24

Bielas alone or in combination with Vogelstein discloses "reducing or tracking redundancy of a plurality of sequence reads from the set of sequencing reads, wherein the reducing or tracking comprises mapping at least a subset of the plurality of sequence reads to a reference sequence, and the reducing or tracking is based on (i) the molecular barcodes associated the paired reads or the unpaired reads and (ii) sequence information at the start and stop positions of the paired reads or the unpaired reads." EX1003, ¶¶204-207.

Reducing redundancy based on mapping to reference sequence. As discussed above regarding Element [1g], Bielas alone or in combination with Vogelstein discloses reducing or tracking the redundancy of sequence reads by mapping at least a subset of the plurality of sequence reads to a reference sequence. *See* Section IX.B.1(h).

Reducing redundancy based on molecular barcodes. As discussed above regarding Element [1f] and Claim 13, Bielas alone or in combination with Vogelstein discloses reducing or tracking the redundancy of sequence reads based on the molecular barcodes associated with the paired reads or the unpaired reads. *See* Sections IX.B.1(h) and IX.B.6.

Reducing redundancy based on barcodes and start and stop information. As discussed above regarding Claim 13, Bielas alone or in combination with

Vogelstein discloses reducing or tracking the redundancy of sequence reads based on sequence information at the start and stop positions of the paired reads or the unpaired reads. *See* Section IX.B.6.

12. Claim 25

Bielas alone or in combination with Vogelstein discloses that "reducing or tracking the redundancy of the plurality of sequence reads comprises determining a base call at one or more genomic loci for a plurality of sequence reads that map to the one or more genomic loci on the reference sequence." EX1003, ¶¶208-210.

Bielas explains that "[b]ase calling and sequence alignment will be performed using, for example, the Eland pipeline (Illumina, San Diego, CA)."[17] EX1005, 25:9-10. The base calling and sequence alignment disclosed in Bielas is a step in which raw sequencing data is mapped to a reference sequence in connection with reducing redundancy by generating a consensus sequence. EX1003, ¶¶209.

Vogelstein also discloses that after sequencing, "[b]ase-calling and sequence alignment was performed with the Eland pipeline (Illumina)." EX1006, ¶[60]. Vogelstein discloses that after mapping the sequence reads "[o]nly high quality reads … were used for subsequent analysis." *Id.* Then, these "[h]igh quality reads were grouped into UID-families based on their endogenous or exogenous UIDs."

[17] A POSA would have known that "base calling" means determining the most likely nucleotide for that particular base position. EX1003, ¶209; EX1005, p.11; *see also* discussion of Eland pipeline in Section IX.B.1(h).

EX1006, ¶[61]. Thus, a POSA would understand Vogelstein discloses mapping at least a subset of the plurality of sequence reads to the reference sequence as part of the process of reducing or tracking the redundancy of the plurality of sequence reads. EX1003, ¶210.

13. Claim 26

Bielas discloses "reducing or tracking the redundancy of the plurality of sequence reads comprises collapsing the plurality of sequence reads to produce consensus sequences representative of a sequence of the original double-stranded cfDNA molecules from among the tagged parent polynucleotides." EX1003, ¶¶211-213.

Bielas discloses that "[a]ll sequencing reads having identical cypher pairs, along with their reverse complements, were grouped into families. Comparison of family sequences allowed for generation of a consensus sequence wherein 'mutations' (errors) arising during library preparation (open circle) and during sequencing (gray circle and triangle) were computationally eliminated." EX1005, p.4. This is shown in Figure 3B (annotated):

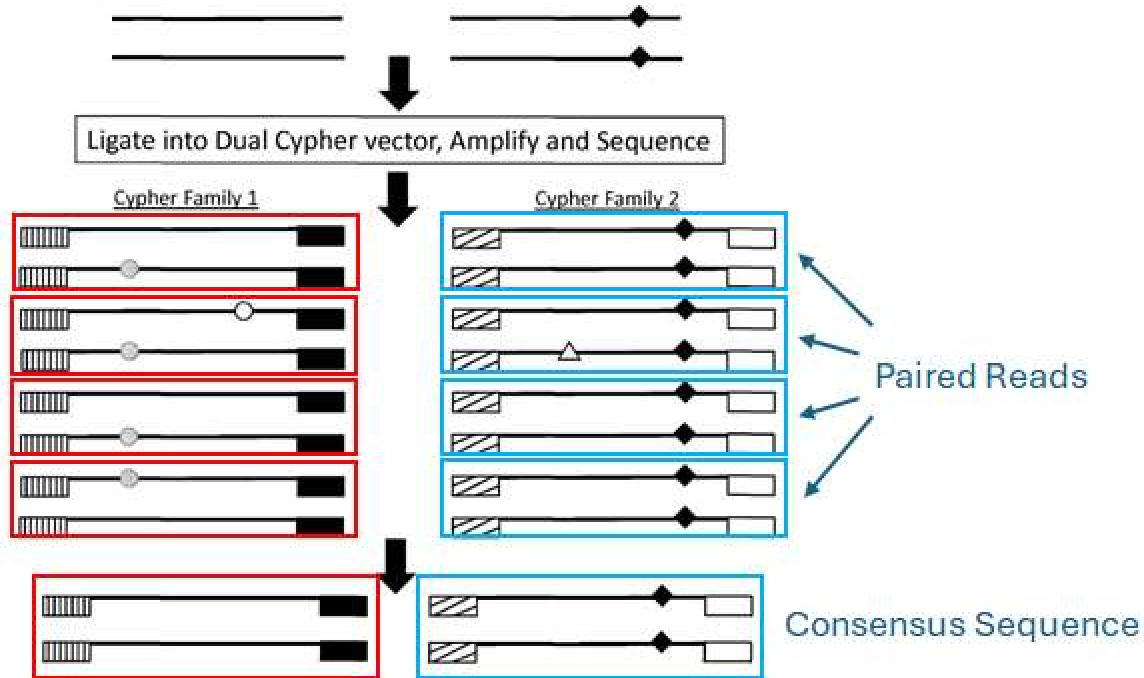

Fig. 3B

Vogelstein also discloses that after sequencing, "[h]igh quality reads [are] grouped into UID-families based on their endogenous or exogenous UIDs." *Id.*, ¶[61]. A POSA would understand that this is done to generate consensus sequences. EX1003, ¶213.

14. Claim 29

Bielas in combination with Vogelstein discloses that "the distinct cfDNA molecules in (e) are determined based on (i) the paired reads and (ii) the unpaired reads." EX1003, ¶¶214-217.

Paired reads. As discussed in connection with Element [1e], Bielas discloses determining distinct cfDNA molecules based on paired reads. *See* Section IX.B.1(g).

Unpaired reads. As discussed in connection with Element [1e], Vogelstein discloses determining distinct cfDNA molecules based on unpaired reads. *See* Section IX.B.1(g).

Paired and unpaired reads. As discussed in connection with Element [17d], it would have been obvious in view of Bielas and Vogelstein, and a POSA would have been motivated, to use Bielas' approach of reducing redundancy based on families of paired reads, in order to obtain the additional reduction in errors suggested by both Bielas and Vogelstein, but continue to use Vogelstein's approach of reducing redundancy based on families of unpaired reads for any sequencing reads that do not have a counterpart. *See* Section IX.B.7(e).

X. GROUND 2: CLAIMS 3, 9-11, 19, AND 22-23 ARE RENDERED OBVIOUS BY BIELAS IN VIEW OF VOGELSTEIN AND FORSHEW

A. Motivation To Combine

A POSA would have been motivated to combine Bielas and Vogelstein's teachings, as described above, with Forshew's teaching of using 0.9 ng to 19.7 ng of cfDNA molecules. EX1003, ¶¶219-224.

Forshew is analogous art to Bielas and Vogelstein in that it is directed to methods for identifying genetic mutations through nucleic acid sequencing using barcodes. EX1003, ¶220. And Forshew and Bielas both disclose sequencing "[c]irculating cell-free DNA extracted from plasma or other body fluids." EX1010, p.1; EX1005, 23:12-14, 25:27-28.

A POSA would have been motivated, in implementing Bielas and Vogelstein's teachings, to optimize the amount of cfDNA for sequencing. EX1003, ¶220. Bielas and Forshew recognize that the concentration of cfDNA is expected to be relatively low in natural samples. EX1010, p.1; EX1005, 1:21-2:6. Both reference disclose methods of sequencing using tagging that enable detection of low-levels of mutations in samples. A POSA would have been motivated to consider Forshew's examples of amounts of cfDNA molecules contained in different sample volumes sufficient for use in identifying mutations in cfDNA. EX1003, ¶220.

A POSA also would have been motivated to combine Bielas and Vogelstein's teachings with Forshew's teaching of selective enrichment and examples of cancer genes. EX1003, ¶221. As discussed in Section III.D, targeted enrichment was well-known in the art. Indeed, Forshew used a preamplification step employing multiplex PCR followed by singleplex PCR to amplified 11 target regions. EX1010, p.3, Fig. 1. Bielas and Vogelstein disclose methods for identifying mutations present in small fractions of DNA templates. *See, e.g.*, EX1006, Abstract; EX1005, 1:21-2:6. A POSA would have been motivated, in implementing Bielas and Vogelstein's teachings, to enrich sequences of known cancer-associated genes for more efficient sequencing. EX1003, ¶221. Vogelstein discloses use of a hybridization capture step to target genes of interest. EX1006, ¶[25]. Such an enrichment process is

particularly important when using cfDNA, which is present in relatively low amounts. EX1003, ¶221; EX1010, p.1.

A POSA would also be motivated to combine Bielas and Vogelstein with Forshew, as Bielas and Vogelstein disclose that their methods are useful for cancer diagnostics, and Forshew pinpoints certain (known) genes of interest such as TP53 and EGFR for that same purpose. EX1010, p.3; EX1011, p.21; EX1003, ¶222.

A POSA combining Bielas, Vogelstein, and Forshew would have had a reasonable expectation of success in arriving at the methods of Claims 3, 9-11, 19, 22-23. EX1003, ¶223. The prior art recognizes that cfDNA may be present in relatively low concentrations, as it teaches modifications to protocols for lower amounts of DNA, such as Bielas' "amplify[ing] the number of each molecule" through PCR and digital PCR and Vogelstein's increased number of PCR cycles to address low amounts of DNA molecules. EX1005, p.2; EX1006, ¶[55]. The prior art also disclosed numerous commercial kits for extracting DNA from blood. EX1005, p.28; EX1006, ¶[0057]. A POSA would have recognized that Forshew's teaching of 0.9 to 19.7 ng of cfDNA molecules could be used as a starting point for the methods disclosed in Bielas and Vogelstein. EX1003, ¶223.

A POSA would have further expected that Vogelstein's enrichment techniques could be used on the known cancer genes in Forshew. A POSA would have expected to successfully perform Forshew's second round of singleplex

amplification of the enriched target DNA because PCR amplification was a routine technique known in the field. EX1003, ¶224.

B. Claim-by-Claim Analysis

1. Claims 3 and 19

Bielas in combination with Vogelstein and Forshew discloses using 1 ng to 100 ng of double-stranded cfDNA molecules. EX1003, ¶¶225-226, 235-236. Forshew extracted a range of amounts of cfDNA from plasma samples for sequence library preparation, including between 0.9 and 19.7 ng as shown in Table S6. EX1010, pp.17-18. A POSA would understand that Forshew's range of sample volumes, which falls within the claimed range of 1 to 100 ng, could be used with the methods disclosed in Bielas and Vogelstein. EX1003, ¶¶225-226, 235-236; *Ormco*, 463 F.3d at 1311.

2. Claims 9 and 22

Bielas in combination with Vogelstein and Forshew discloses "prior to the sequencing, enriching at least a subset of the amplified progeny polynucleotides for target regions of interest to produce enriched progeny polynucleotides." EX1003, ¶¶227-229, 237.

Vogelstein discloses "[c]omplexity of the analyte fragments can be decreased by a capture step" that will "typically … employ hybridization to probes representing a gene or set of genes of interest." EX1006, ¶[25]. Hybridization capture is a targeted sequencing technique used to enrich specific DNA sequences in regions of

interest. EX1003, ¶228. Vogelstein provides an example where sequences of six genes of interest were selectively enriched. EX1006, ¶[40]. A POSA would understand that Vogelstein's "capture step" employing "hybridization" discloses claims 9 and 22. EX1003, ¶228.

Forshew also discloses enrichment of amplified progeny polynucleotides for target regions of interest. EX1003, ¶229. Forshew discloses amplifying the "selected regions" of certain cancer-associated genes. EX1010, p.3 (primer pairs designed for targeted amplification of regions of genes TP53, PTEN, EGFR, BRAF, KRAS, PIK3CA). Forshew explains that target regions are enriched by "amplif[ying] DNA from each sample in duplicate … producing mean read depth of 3200 above Q30 for each of the 9024 expected read groups (48 amplicons x 2 directions x 94 barcoded samples)." EX1010, p.3. A POSA would understand that a preliminary round of amplification can be useful to generate "amplified progeny polynucleotides" as a target enrichment step. EX1003, ¶229. A POSA would understand that Forshew's second round of singleplex amplification of "selected regions" "in duplicate" and at high accuracy (Q30) increases the proportion of those selected regions within the sample. EX1003, ¶229.

3. Claims 10 and 23

Bielas in combination with Vogelstein and Forshew discloses examples of cancer genes that include specific genes listed in Claims 10 and 23. EX1003, ¶¶230-231, 238.

Forshew discloses designing primer pairs to amplify "genomic sequence covering coding regions … of TP53 and PTEN, and selected regions in EGFR, BRAF, KRAS, and PIK3CA (table S1)." EX1010, p.3; Fig.1a. A POSA would have understood that Forshew's targeting of regions in TP53, EGFR, BRAF, and KRAS disclose or render obvious the claimed "plurality of genes selected from the group …" because the claimed genes are cancer genes expressly disclosed and analyzed by Forshew. EX1003, ¶231.

4. Claim 11

Bielas in combination with Vogelstein and Forshew discloses "prior to the sequencing, amplifying a plurality of the enriched progeny polynucleotides." EX1003, ¶¶232-234.

Vogelstein discloses the sequential steps of enrichment, amplification, and sequencing. Vogelstein describes an experiment where a first round of "[a]mplification of a fragment of human genomic DNA" was performed, resulting in enriched progeny polynucleotides; then, a second round of amplification of the enriched progeny polynucleotides occurred using "[f]ifty uL PCR reactions."

EX1006, ¶[59]. Then, "[t]he entire contents of each well were then used as templates for the exogenous UIDs strategy described above," including sequencing. EX1006, ¶[59]. A POSA would understand that Vogelstein discloses this limitation because Vogelstein's "Post-Capture Amplification" discloses post-enrichment amplification of enriched polynucleotides. EX1006, at 32; EX1003, ¶233.

Bielas also discloses amplifying a plurality of progeny polynucleotides prior to sequencing. *See* Section IX.B.1(e), regarding element [1c]. To the extent a POSA enriches at least a subset of the amplified progeny polynucleotides for target regions of interest using the methods of Vogelstein or Forshew, as discussed in Section X.B.2, with the methods disclosed by Bielas, it would have been obvious to amplify a plurality of enriched progeny polynucleotides prior to sequencing. EX1003, ¶234.

XI. GROUND 3: CLAIMS 4-6 ARE RENDERED OBVIOUS BY BIELAS IN VIEW OF VOGELSTEIN, HENDRICKS AND/OR DIEHN

A. Motivation To Combine

A POSA would have been motivated to combine Bielas and Vogelstein with Hendricks and/or Diehn. EX1003, ¶¶240-243.

Hendricks is analogous art to Bielas and Vogelstein in that it is directed to methods used for nucleic acid sequencing. EX1003, ¶242. Both Bielas and Vogelstein disclose ligation as a means of attaching barcodes to target nucleic acid molecules. EX1005, 4:9-10; EX1006, ¶[39]. Vogelstein teaches that the barcodes should be in excess of the target DNA molecules. EX1006, ¶[08]. A POSA would

have been motivated, in implementing Bielas and Vogelstein's teachings, to look to Hendricks' ligation methods to "produce a significant or detectable amount of ligation product" in order to maximize the sequenceable target DNA, and thus ensure high quality sequencing data, especially when starting with relatively low amounts of cfDNA. EX1008, ¶[0076]; EX1003, ¶242.

A POSA would also have been motivated to combine Bielas, Vogelstein, and Hendricks with Diehn. EX1003, ¶242. Like Bielas and Vogelstein, Diehn is analogous art directed to methods used for identifying genetic mutations using nucleic acid sequencing. EX1003, ¶242. Furthermore, both Diehn and Bielas disclose sequencing cfDNA. EX1009, ¶[0082]; EX1005, 23:12-14, 25:27-28. Diehn explains that "[i]ncreasing adapter concentration during ligation increases ligation efficiency and reporter recovery." EX1009 ¶[0130]. A POSA would have known this correlation and thus been motivated by Diehn's use of molar excess to obtain the Hendricks's range of ligation efficiency. EX1009, ¶¶[0130], [0157]; EX1008, ¶[0076]; EX1003, ¶242.

A POSA combining Bielas, Vogelstein, Hendricks, and Diehn would have had a reasonable expectation of success in arriving at the methods of Claims 4-6. EX1003, ¶243. A POSA would have expected that using a higher molar excess of tags relative to DNA molecules would achieve a higher ligation efficiency, thus increasing the number of tagged molecules and thus improving overall effectiveness

of the downstream steps. EX1009, ¶[157]; EX1003, ¶243. Indeed, Diehn expressly notes this correlation: "Increasing adapter concentration during ligation increases ligation efficiency and reporter recovery." EX1009, ¶[130]; EX1003, ¶243. A POSA would also have appreciated that increasing the adapter-DNA ligation efficiency was beneficial when using relatively low amounts of DNA, such as cfDNA. EX1003, ¶243; EX1010, p.1.

B. Claim-by-Claim Analysis

1. Claims 4-6

Bielas in combination with Vogelstein, Hendricks and/or Diehn discloses claim 4's requirement that "the molecular barcodes are ligated to the plurality of the cfDNA molecules using more than a 10× excess of duplex tags as compared to the population of cfDNA molecules, wherein at least 20% of the cfDNA molecules from the population are tagged with the duplex tags"; claim 5's requirement that "the molecular barcodes are ligated to the plurality of the cfDNA molecules using more than an 80× excess of duplex tags as compared to the population of cfDNA molecules"; and claim 6's requirement that "at least 40% of the cfDNA molecules from the population are tagged with the duplex tags." EX1003, ¶¶244-249.

Bielas and Vogelstein disclose ligation of barcodes to target nucleic acid molecules. EX1005, 4:9-10; EX1006, ¶[39]. Hendricks discloses a ligation process that, if used with the teachings of Bielas and Vogelstein, would result in at least 20%

or at least 40% of the cfDNA molecules being tagged with duplex tags. EX1003, ¶245. Specifically, Hendricks discloses ligation that "produce[s] a significant or detectable amount of ligation product. Optionally, the efficiency of ligation is at least … 20%, 30%, 50%, 60%, 70%, 75%, 80% …." EX1008, ¶[76]. Hendrick's range of ligation efficiency of "at least … 20%" or "50%," in addition to its disclosure of higher efficiencies, discloses this element. EX1003, ¶245.

Hendricks also discloses ligating adaptors using more than a 10× excess and more than an 80× excess of tags as compared to DNA molecules. EX1003, ¶246. Hendricks discloses "[l]igation reactions were performed in a 96 well plate using 2 nM template/primer, 2.0 μM ligase, 2-5 μM short oligonucleotide in a total volume of 50 μL." EX1008, ¶[138]. A POSA would understand that using the lower end of the range of concentrations of short oligonucleotide (2 μM) calculations comparing the short oligonucleotides to the ligase would show a 1000× molar excess, and using the higher end range of concentration (5 μM) results in a 2500× molar excess. EX1003, ¶246. This discloses the claimed "more than a 10× excess" and "more than an 80× excess." EX1003, ¶246.

To the extent additional disclosure is needed, Diehn discloses ligating adaptors to cfDNA molecules using more than an 10x excess or more than an 80x excess of tags as compared to molecules. EX1003, ¶247. Diehn discloses starting with "[c]ell-free DNA (cfDNA) … isolated from 1-5 mL plasma." EX1009,

¶¶[141], [42]. Before ligation, that DNA was added to "2 concentrations of Illumina adapters in the ligation reaction: 12 nM (10-fold molar excess to cfDNA fragments) and 120 nM (100-fold molar excess)." EX1009, ¶¶[159], [143]. A POSA would understand that the adapter ligation process in Diehn would be applicable to Bielas and Vogelstein, and that Diehn's 100-fold molar excess discloses the claimed ranges of "more than a 10× excess" and "more than an 80× excess." EX1003, ¶247; *Ormco*, 463 F.3d at 1311.

XII. GROUND 4: CLAIMS 14-16 AND 27-28 ARE RENDERED OBVIOUS BY BIELAS IN VIEW OF VOGELSTEIN AND HICKS

A. Motivation To Combine

A POSA would have been motivated to combine Bielas and Vogelstein's teachings, as described above, with Hicks teachings of quantitatively measuring sequencing reads and estimating tagged parent polynucleotides. EX1003, ¶¶251-253.

Hicks is analogous art to Bielas and Vogelstein as it is directed to methods used for nucleic acid sequencing. EX1003, ¶252. The methods of accurately detecting mutations in nucleic acid sequences disclosed in Bielas and Vogelstein (EX1005, 3:17-22; EX1006, Abstract) are important to enable determination or estimation of the frequency of mutations. EX1003, ¶252. This is recognized, for example, in Vogelstein's example where "[t]he exogenous UID strategy (Fig. 3) was then used to determine the prevalence of rare mutations." EX1006, ¶[52]; *see also*

id. ¶[31]; EX1005, 1:21-2:27. A POSA would have been motivated, in implementing Bielas and Vogelstein's teachings, to use the methods disclosed in Hicks to estimate the number of cfDNA molecules that map to particular loci because such data would be useful for calculating copy number variations, which in turn is useful for the sensitive diagnosis of genetic conditions. EX1003, ¶252.

A POSA combining Bielas, Vogelstein, and Hicks would have had a reasonable expectation of success in arriving at the methods of Claims 14-16 and 27-28. EX1003, ¶253. Like Bielas and Vogelstein, Hicks discloses using barcodes to track original target molecules. *See, e.g.*, EX1011, 19:11-17. A POSA would have expected the tagging methods in Bielas and Vogelstein could be used with the quantification methods in Hicks because in each reference the barcodes are used to link sequence reads back to original molecules. EX1005, 4:24-27; EX1006, ¶[19]; EX1011, 19:11-27. And in each reference the barcodes are formed by attaching barcodes to target molecules using known techniques. EX1005, 9:22-26; EX1006, ¶[22]; EX1011, 18:5-8.

B. Claim-by-Claim Analysis

1. Claims 14 and 27

Claim 14 depends from claim 1, and recites: "determining quantitative measures of (i) paired reads or (ii) unpaired reads that map to a genomic locus of the reference sequence." Claim 27 depends from claim 24, and recites determining

quantitative measures of "at least two of (i) paired reads that map to one or more genomic loci, (ii) unpaired reads that map to one or more genomic loci, (iii) a read depth of the paired reads, and (iv) a read depth of the unpaired reads."

Bielas in combination with Vogelstein and Hicks discloses determining quantitative measures of paired reads and unpaired reads that map to a genomic locus of the reference sequence. EX1003, ¶¶254-257, 264-267.

As discussed in Section IX.B.1(h), Vogelstein and Bielas disclose mapping at least a subset of the plurality of sequence reads to a reference sequence. Vogelstein also discloses that its methods "can be used to quantitate," including to compare "relative abundance of two analyte DNA fragments." EX1006, ¶[31]. For example, Vogelstein discloses examples where the number of families are quantified, as well as the number of mutations represented by the families. EX1006, ¶[52]; *see also id.*, ¶[61].

To the extent additional disclosure is required, Hicks discloses determining quantitative measures of sequence reads that map to a reference sequence. Hicks explains that "[a]fter deconvoluting by sample tag, the number of different tagged nucleic acid molecules mapped to each location is counted." EX1011, 19:36-39; *see also id.* 18:10-13. A POSA would understand that "deconvoluting by sample tags" reduces each family of polynucleotides to a single sequence, and the number of

sequences is then counted to determine the number of tagged nucleic acid molecules mapped to each location. EX1003, ¶¶256, 266.

If a POSA used the teachings of Bielas and Vogelstein to generate families of paired and unpaired reads, as discussed for claims 1 and 17, it would have been obvious to quantify the number of paired or unpaired reads that map to a genomic locus of the reference sequence using the techniques in Vogelstein and Hicks. EX1003, ¶¶257, 267.

2. Claims 15 and 28

Claim 15 depends from claim 14 and recites: "estimating with a programmed computer processor a quantitative measure of tagged parent polynucleotides that map to the genomic locus based on the quantitative measures of the paired reads and the unpaired reads." Claim 28 depends from claim 27, and recites "estimating with a programmed computer processor a quantitative measure of tagged parent polynucleotides that map to a genomic locus of the reference sequence based on the quantitative measures of at least two of (i) paired reads, (ii) unpaired reads, (iii) a read depth of the paired reads, and (iv) a read depth of the unpaired reads at the genomic locus."

Bielas in combination with Vogelstein and Hicks discloses estimating with a programmed computer processor a quantitative measure of tagged parent polynucleotides that map to the genomic locus based on quantitative measures of

paired reads and unpaired reads, which discloses both claims 15 and 28. EX1003, ¶¶258-261, 268-271.

Hicks discloses a quantitative measure of tagged parent polynucleotides can be estimated using quantitative measures of the number of families that map to a genomic locus. Specifically, Hicks explains that "[a]fter deconvoluting by sample tag, the number of different tagged nucleic acid molecules mapped to each location is counted." EX1011, 19:36-39, 18:10-13. Hicks discloses a counting method where

> for a given region comprising multiple locations, the maximum copy number of the region is not less than the maximum number of different tagged nucleic acid molecules mapped to any location in that region. The maximum number of different tagged nucleic acid molecules centered about a moving window of a fixed number of locations can be taken as the measurement of true copy number of the window. This will provide at worst an underestimate of the maximum true copy number for that window, and never an overestimate.

EX1011, 19:44-53.

260. Thus, one of ordinary skill in the art would have understood that in the '156 Patent (Hicks), the number of different families mapped to a location are used to count the tagged parent polynucleotides that map to the genomic locus. It would have been obvious to a POSA that the quantification method in Hicks could be used with the quantified families of paired and unpaired reads formed by the combined methods of Bielas and Vogelstein. EX1003, ¶¶260, 270.

Furthermore, it would have been obvious for the calculations performed in Hicks to be performed using a programmed computer processor. EX1003, ¶¶261,

271. Indeed, a POSA would understand that several of the Figures provided in Hicks were generated using a computer processor. EX1003, ¶¶261, 271; EX1011, Figs. 2-4, 6. Use of computer processors to perform such analysis was routine by December 2013. EX1003, ¶¶261, 271.

3. Claim 16

Bielas in combination with Vogelstein and Hicks discloses "detecting copy number variation in the population of cfDNA molecules by determining a normalized quantitative measure determined in (g) at each of one or more genomic loci." EX1003, ¶¶262-263.

Hicks discloses that the reference genome is "artificially divide[d] … into 'bins' of several kilobases…. Each bin contains a number of random genomic sequence reads from which a mean value is computed to determine genomic copy number at low sequence coverage." EX1011, 18:15-22. A POSA would understand that Hick's method using bins to calculate a mean value to determine genomic copy number discloses the claimed "detecting copy number variation in the population of cfDNA molecules by determining a normalized quantitative measure determined in (g) at each of one or more genomic loci." EX1003, ¶263.

XIII. MANDATORY NOTICES AND FEES

A. Real Party-in-Interest

Pursuant to 37 C.F.R. §42.8(b)(1), Tempus AI, Inc. is the real party-in-interest.

B. Related Matters

Pursuant to 37 C.F.R. §42.8(b)(2), Patent Owner has asserted the ’306 patent against Petitioner in *Guardant Health, Inc. v. Tempus AI, Inc.*, No. 1:24-cv-687 (D. Del.). In that action, Patent Owner also asserts U.S. Patent Nos. 9,902,992 (“the ’992 patent”), 10,793,916 (the “’916 patent”), and 11,643,693 (the “’693 patent”).

Petitioner also notes the petition previously filed by Twinstrand Biosciences, Inc. in case IPR2022-01400, which resulted in a final written decision affirming the patentability of the claims of the ’306 patent.

Petitioner is not aware of any other related matters before the Patent Office with respect to the ’306 Patent. However, contemporaneous with this Petition, Petitioner is also seeking institution of IPR against the ’916 patent.

C. Counsel and Service Information

Pursuant to 37 C.F.R. §42.8(b)(3) and (4), the designations of counsel and address for service are listed below. Petitioner consents to electronic service at the email addresses below.

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Pursuant to 37 C.F.R. § 42.10(b), a Power of Attorney has been filed herewith.

D. Payment of Fees

The undersigned authorizes the Office to charge the fee required for this Petition, and any additional fees that might be due, for *Inter Partes* Review to Deposit Account No. 50-5708.

XIV. CONCLUSION

For the reasons above, *inter partes* review is requested.

Date: August 15, 2025

Respectfully submitted,

/s/ James Glass

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CERTIFICATION UNDER 37 C.F.R. § 42.24

Under the provisions of 37 C.F.R. § 42.24, the undersigned hereby certifies that the word count for the foregoing Petition for *inter partes* review (excluding the table of contents, table of authorities, mandatory notices, certificate of service or word count, and appendix of exhibits or claim listing) totals 13,987 words, which is within the word limit allowed under 37 C.F.R. § 42.24(a)(i).

Date: August 15, 2025

/s/ James Glass
James M. Glass (Reg. No. 46729)

CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §§ 42.6(e), 42.105(a), the undersigned hereby certifies that true and correct copies of this Petition and its supporting exhibits were served via FedEx at the official correspondence address for the attorneys of record for the ’306 patent as shown in USPTO PAIR:

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