

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

TEMPUS AI, INC.,

Petitioner,

v.

GUARDANT HEALTH, INC.,

Patent Owner.

Case IPR2025-01435

U.S. Patent 10,793,916

**PETITION FOR *INTER PARTES* REVIEW
OF CLAIMS 13-30 OF U.S. PATENT 10,793,916**

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TABLE OF AUTHORITIES

Cases

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Statutes

35 U.S.C. § 102.....18, 20, 21, 22, 24, 25

35 U.S.C § 112.....17

Other Authorities

37 C.F.R. § 1.102(e).....13

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EXHIBIT LIST

Exhibit	Description
1001	U.S. Patent No. 10,793,916 (“the ’916 patent”)
1002	Prosecution history for the ’916 patent
1003	Declaration of Michael Metzker, Ph.D.
1004	Curriculum Vitae of Michael Metzker, Ph.D.
1005	U.S. Patent No. 9,752,188 (“Schmitt”)
1006	PCT Publication No. WO 2012/142213 A2 (“Vogelstein”)
1007	U.S. Patent Application Publication No. 2011/0160078 (“Fodor”)
1008	PCT Publication No. WO 2012/099832 A2 (“Hendricks”)
1009	U.S. Patent Application Publication No. 2014/0296081 (“Diehn”)
1010	T. Forshew et al., <i>Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA</i> , SCIENCE TRANSLATIONAL MEDICINE, Vol. 4 Issue 136 (May 30, 2012) (“Forshew”)
1011	U.S. Patent No. 9,404,156 (“Hicks”)
1012	K. Shiroguchi et al., <i>Digital RNA Sequencing Minimizes Sequence-Dependent Bias And Amplification Noise With Optimized Single-Molecule Barcodes</i> , PNAS Vol. 109, No. 4 (Jan. 24, 2012) (“Shiroguchi”)
1013	U.S. Patent Publication No. 2012/0316074 A1 (“Saxonov”)
1014	Schwarzenbach et al., <i>Cell-free nucleic acids as biomarkers in cancer patients</i> , NATURE REVIEWS CANCER 11:426–437 (2011) (“Schwarzenbach”).

1015	Human genome variation, fact sheet, National Human Genome Research Institute (NHGRI)
1016	<i>Guardant Health, Inc. v. Foundation Medicine</i> , IPR2017-01448, Paper 2 – Petition for Inter Partes Review of U.S. Patent No. 9,340,830
1017	Metzker, <i>Sequencing technologies — the next generation</i> , NATURE REVIEWS GENETICS 11:31 –46 (2010) (“Metzker2010”)
1018	<i>Guardant Health, Inc. v. University of Washington</i> , IPR2022-00816, Paper 3 – Petition for Inter Partes Review of U.S. Patent No. 10,760,127
1019	Metzker & Caskey, <i>Polymerase Chain Reaction</i> , In ENCYCLOPEDIA OF MEDICAL DEVICES AND INSTRUMENTATION, Second Edition, Volume 5 (2006) (“Metzker2006”).
1020	Mamanova <i>et al.</i> , <i>Target-enrichment strategies for next-generation sequencing</i> , NATURE METHODS 7:111–118 (2010) (“Mamanova”)
1021	<i>Guardant Health, Inc. v. University of Washington</i> , IPR2022-00450, Paper 3 – Petition for Inter Partes Review of U.S. Patent No. 10,689,699
1022	<i>Guardant Health, Inc. v. University of Washington</i> , IPR2022-01388, Paper 2 – Petition for Inter Partes Review of U.S. Patent No. 10,689,699
1023	Intentionally Left Blank
1024	<i>Guardant Health, Inc. v. University of Washington</i> , IPR2022-00935, Paper 2 – Petition for Inter Partes Review of U.S. Patent No. 10,287,631
1025	U.S. Provisional Patent Application No. 61/600535 (“Diehn Provisional”)

1026	<i>Twinstrand Biosciences, Inc. v. Guardant Health, Inc.</i> , IPR2022-01400, Paper 2 – Petition for Inter Partes Review of U.S. Patent No. 11,149,306
1027	<i>Twinstrand Biosciences, Inc. v. Guardant Health, Inc.</i> , IPR2022-01400, Paper 41 – Final Written Decision
1028	<i>Twinstrand Biosciences, Inc. v. Guardant Health, Inc.</i> , IPR2022-01400, Paper 9 – Institution Decision
1029	Li et al., <i>Structure-independent and quantitative ligation of single-stranded DNA</i> , Analytical Biochemistry (2005)
1030	U.S. Patent No. 9,085,798 (“Chee”)
1031	Thomas et al., <i>Sensitive mutation detection in heterogeneous cancer specimens by massively parallel picoliter reactor sequencing</i> , NATURE MEDICINE 12:852–855 (2006)
1032	Buckingham, <i>Chromosomal structure and chromosomal mutations</i> , In MOLECULAR DIAGNOSTIC FUNDAMENTAL, METHODS, & CLINICAL APPLICATIONS, Eds. Buckingham & Flaws, F.A. Davis Company, Chapter 8, pp. 155–172 (2007)
1033	Gemayel et al., <i>Variable tandem repeats accelerate evolution of coding and regulatory sequences</i> , ANNUALS REVIEW OF GENETICS 44:445–477 (2010)
1034	Tóth et al., <i>Microsatellites in different eukaryotic genomes: Survey and analysis</i> , GENOME RESEARCH 10:967–981 (2000)
1035	Laghi et al., <i>Differences and evolution of the methods for the assessment of microsatellite instability</i> , ONCOGENE 27:6313–6321 (2008)
1036	Richard & Pâques, <i>Mini- and microsatellite expansions: the recombination connection</i> , EMBO REPORTS 1:122–126 (2000)
1037	Hastings et al., <i>Mechanisms of change in gene copy number</i> , NATURE REVIEWS GENETICS 10:551–564 (2009)

1038	Hiatt <i>et al.</i> , <i>Single molecule molecular inversion probes for targeted, high-accuracy detection of low-frequency variation</i> , GENOME RESEARCH 23:843–854 (2013)
1039	Somatic & germline mutations, https://my.clevelandclinic.org/health/body/23067-somatic--germline-mutations (last visited July 30, 2025)
1040	Gene changes and cancer, https://www.cancer.org/cancer/understanding-cancer/genes-and-cancer/gene-changes.html (last visited July 30, 2025)
1041	International Human Genome Sequencing Consortium, <i>Initial sequencing and analysis of the human genome</i> , NATURE 409:860–921 (2001)
1042	International Human Genome Sequencing Consortium, <i>Finishing the euchromatic sequence of the human genome</i> , NATURE 431:931–945 (2004)
1043	Forbes, et al., <i>COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer</i> , NUCLEIC ACIDS RESEARCH 39:D945–D950 (2011)
1044	<i>Genomic Data Commons Data Portal</i> , https://portal.gdc.cancer.gov/ ((last visited July 30, 2025)
1045	Metzker, <i>Emerging technologies in DNA sequencing</i> , GENOME RESEARCH 15:1767–1776 (2005)
1046	Rothberg <i>et al.</i> , <i>An integrated semiconductor device enabling non-optical genome sequencing</i> , NATURE 475:348-352 (2011)
1047	Jain <i>et al.</i> , <i>Improved data analysis for the MinION nanopore sequencer</i> , NATURE METHODS 12:351-356 (2015)
1048	Kinde et al., <i>Detection and quantification of rare mutations with massively parallel sequencing</i> , PNAS (June 7, 2011) (“Kinde”)

1049	Turner <i>et al.</i> , <i>Methods for genomic partitioning</i> , ANNUAL REVIEW OF GENOMICS AND HUMAN GENETICS 10:263-284 (2009)
1050	Edwards & Gibbs, <i>Multiplex PCR: Advantages, development, and applications</i> , GENOME RESEARCH 3:S65-S75 (1994)
1051	Sharma <i>et al.</i> , <i>(TG/CA)_n repeats in human gene families: abundance and selective patterns of distribution according to function and gene length</i> , BMC GENOMICS 6:83 pp. 1–12 (2005) (“Sharma (2005)”)
1052	Gnirke <i>et al.</i> , <i>Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing</i> , NATURE BIOTECHNOLOGY 27:182–189 (2009) (“Gnirke (2009)”).
1053	Meyerson <i>et al.</i> , <i>Advances in understanding cancer genomes through second-generation sequencing</i> , NATURE REVIEWS GENETICS 11:685–696 (2010) (“Meyerson (2010)”)
1054	Preston <i>et al.</i> , <i>Innovation at Illumina: The road to the \$600 human genome</i> , NATURE PORTFOLIO (2023) at https://www.nature.com/articles/d42473-021-00030-9 (“Preston (2023)”).
1055	Bentley <i>et al.</i> , <i>Accurate whole human genome sequencing using reversible terminator chemistry</i> , NATURE 456:53–59 (2008) (“Bentley (2008)”)
1056	Kircher <i>et al.</i> , <i>Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform</i> , NUCLEIC ACIDS RESEARCH 40:e3 pp. 1–8 (2012) (“Kircher 2012”)
1057	Pierce <i>et al.</i> , <i>A unique and universal molecular barcode array</i> , NATURE METHODS 3:601–603 (2006) (“Pierce (2006)”)
1058	Schmitt <i>et al.</i> , <i>Detection of ultra-rare mutations by next-generation sequencing</i> , PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES 109: 14508-14513 (2012) (“Schmitt (2012)”)

1059	Glenn, <i>Field guide to next-generation DNA sequencers</i> , MOLECULAR ECOLOGY RESOURCES 11:759-769 (2011) (“Glenn (2011)”)
1060	Cock <i>et al.</i> , <i>The Sanger FASTQ file format for sequences with quality scores, and the Solexa/Illumina FASTQ variants</i> , NUCLEIC ACIDS RESEARCH 38:1767–1771 (2010) (“Cock (2010)”)
1061	CASAVA 1.8: enhanced variant calling in whole-genome resequencing data (2011) (“CASAVA User Guide”)
1062	Li <i>et al.</i> , Mapping short DNA sequencing reads and calling variants using mapping quality scores, Genome Research 18:1851–1858 (2008) (“Li (2008)”)
1063	Li & Durbin, Fast and accurate short read alignment with Burrows–Wheeler transform, Bioinformatics 25:1754–1760 (2009) (“Li & Durbin (2009)”)
1064	Li & Durbin, Fast and accurate long-read alignment with Burrows–Wheeler transform, Bioinformatics 26: 589–595 (2010) (“Li & Durbin 2010”)
1065	Langmead <i>et al.</i> , Ultrafast and memory-efficient alignment of short DNA sequences to the human genome, Genome Biology 10:R25.1–R25.10 (2009) (“Langmead (2009)”)
1066	Li <i>et al.</i> , <i>The sequence alignment/map format and SAMtools</i> , BIOINFORMATICS 25:2078–2079 (2009) (“Li (2009)”)
1067	DePristo <i>et al.</i> , <i>A framework for variation discovery and genotyping using next-generation DNA sequencing data</i> , NATURE GENETICS 43:491–498 (2011) and Online Methods (collectively, “DePristo (2011)”)
1068	McKenna <i>et al.</i> , <i>The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data</i> , GENOME RESEARCH 20:1297-1303 (2010) (“McKenna (2010)”)

1069	https://github.com/broadinstitute/gatk/releases (last visited Aug 11, 2025) (“GATK Updates”)
1070	Koboldt <i>et al.</i> , <i>VarScan: variant detection in massively parallel sequencing of individual and pooled samples</i> , <i>BIOINFORMATICS</i> 25:2283–2285 (2009) (“Koboldt (2009)”)
1071	Koboldt <i>et al.</i> , <i>VarScan 2: Somatic mutation and copy number alteration discovery in cancer by exome sequencing</i> , <i>GENOME RESEARCH</i> 22:568–576 (2012) (“Koboldt (2012)”)
1072	Cibulskis <i>et al.</i> , <i>Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples</i> , <i>NATURE BIOTECHNOLOGY</i> 31:213–219 (2013) (“Cibulskis (2013)”)
1073	U.S. Patent No. 9,840,743
1074	U.S. Patent No. 9,834,822
1075	U.S. Patent Application Publication No. 2012/0165202 A1 (“Porreca”)
1076	U.S. Patent Application Publication No. 2010/0264331 (“Sacko”)
1077	M. van Lier, <i>et al.</i> , <i>A review on the molecular diagnostics of Lynch syndrome: a central role for the pathology laboratory</i> , <i>J. CELL. MOL. MED.</i> Vol. 13, No. 1-2 (2010) (“van Lier”)
1078	C. Boland and A. Goel, <i>Microsatellite Instability in Colorectal Cancer</i> , <i>GASTROENTEROLOGY</i> , 138(6) (June 2010) (“Boland 2010”)
1079	C. Boland, <i>et al.</i> , <i>A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer</i> , <i>CANCER RES</i> , 58(22) (Nov. 15, 1998) (“Boland 1998”)
1080	F. Sinicrope and D. Sargent, <i>Clinical implications of microsatellite instability in sporadic colon cancers - PMC</i> , <i>CURR OPIN ONCOL.</i> , 21(4) (July 2009) (“Sinicrope”)

1081	G. Li, <i>Mechanisms and functions of DNA mismatch repair</i> , CELL RESEARCH 18 (2008)
1082	U.S. Provisional Application 61/613,413 (“Schmitt '413 provisional”)
1083	Foundation Medicine, Inc. v. Guardant Health, Inc., IPR2019-00652, Paper 47 – Final Written Decision (“822FWD”)
1084	Foundation Medicine, Inc. v. Guardant Health, Inc., IPR2019-00652, Paper 54 – Termination
1085	Foundation Medicine, Inc. v. Guardant Health, Inc., IPR2019-00652, Paper 12 – Decision Granting Institution of <i>Inter Partes Review</i>
1086	Declaration of Sylvia D. Hall-Ellis, Ph.D.

LIST OF CHALLENGED CLAIMS

Claim No.	Claim Language
Claim 13	
13[pre]	A method for detecting a genetic variation in one or more microsatellite regions in a sample of cell-free nucleic acid molecules from a subject having a cancer, the method comprising:
13[a]	(a) ligating molecular barcodes from a set of molecular barcodes having 2 to 1,000,000 different molecular barcode sequences to a plurality of the cell-free nucleic acid molecules from the sample to produce tagged parent polynucleotides
13[b]	(b) amplifying a plurality of the tagged parent polynucleotides to produce amplified tagged progeny polynucleotides;
13[c]	(c) sequencing a plurality of the amplified tagged progeny polynucleotides to produce a set of sequencing reads; and
13[d]	(d) determining, from among a plurality of sequencing reads in the set of sequencing reads, a quantitative measure of polymorphic forms comprising microsatellite changes in the one or more microsatellite regions based at least on sequence information of the molecular barcodes, thereby detecting the genetic variation in the one or more microsatellite regions.
Claim 14	
14	The method of claim 13, wherein the genetic variation in the one or more microsatellite regions comprises an insertion or deletion (indel).
Claim 15	
15	The method of claim 13, wherein the sample is a bodily fluid sample selected from the group consisting of blood, plasma, and serum.
Claim 16	
16	The method of claim 13, wherein a molecular barcode from the set of molecular barcodes is attached on both ends of a molecule of the plurality of the cell-free nucleic acid molecules.
Claim 17	

17	The method of claim 13, wherein the molecular barcodes from the set of molecular barcodes have 2 to 1,000 different molecular barcode sequences.
Claim 18	
18	The method of claim 13, wherein the molecular barcodes are ligated to the plurality of the cell-free nucleic acid molecules by blunt-end ligation or sticky-end ligation.
Claim 19	
19	The method of claim 13, further comprising selectively enriching at least a portion of the amplified tagged progeny polynucleotides for target regions associated with cancer prior to the sequencing.
Claim 20	
20	The method of claim 13, further comprising filtering out sequencing reads from among the set of sequencing reads that fail to meet a quality threshold.
Claim 21	
21	The method of claim 13, further comprising mapping a plurality of sequencing reads from the set of sequencing reads to a reference sequence.
Claim 22	
22	The method of claim 21, further comprising grouping a subset of sequencing reads into families based on sequence information of the molecular barcodes and (1) a start base position of a given sequencing read from among the subset of sequencing reads at which the given sequencing read is determined to start mapping to the reference sequence or (2) a stop base position of the given sequencing read at which the given sequencing read is determined to stop mapping to the reference sequence.
Claim 23	
23	The method of claim 22, further comprising, for a plurality of the families, collapsing sequencing reads within a given family of the plurality of the families to generate consensus sequences.
Claim 24	

24	The method of claim 23, further comprising identifying one or more consensus sequence having the genetic variation as compared to the reference sequence.
Claim 25	
25	The method of claim 13, wherein the sample of cell-free nucleic acid molecules comprises 1 nanogram (ng) to 100 ng of cell-free nucleic acid molecules.
Claim 26	
26	The method of claim 13, wherein the cell-free nucleic acid molecules comprise cell-free deoxyribonucleic acid (cfDNA) molecules.
Claim 27	
27	The method of claim 13, further comprising determining a confidence score based on the genetic variation detected in the one or more microsatellite regions.
Claim 28	
28	The method of claim 13, further comprising determining a treatment regimen for the subject based on the quantitative measure of polymorphic forms comprising microsatellite changes in the one or more microsatellite regions.
Claim 29	
29	The method of claim 28, further comprising administering the treatment regimen to the subject.
Claim 30	
30[pre]	A method for detecting a genetic variation in one or more microsatellite regions in a sample of cell-free deoxyribonucleic acid (cfDNA) molecules from a subject having a cancer, the method comprising:
30[a]	(a) tagging a plurality of the cfDNA molecules from the sample with molecular barcodes from a set of molecular barcodes to produce tagged parent polynucleotides, wherein a molecular barcode from the set of molecular barcodes is attached to both ends of a molecule of the plurality of the cfDNA molecules, and

	wherein a plurality of the tagged parent polynucleotides has identical molecular barcode sequences;
30[b]	(b) amplifying the tagged parent polynucleotides to produce amplified tagged progeny polynucleotides;
30[c]	(c) sequencing a plurality of the amplified tagged progeny polynucleotides to produce a set of sequencing reads;
30[d]	(d) identifying, from among a plurality of sequencing reads in the set of sequencing reads, a plurality of unique sequencing reads based at least on sequence information of the molecular barcodes, wherein a unique sequencing, read corresponds to a nucleic acid sequence of a cfDNA molecule from among the tagged parent polynucleotides; and
30[e]	(e) determining, from among the plurality of unique sequencing reads, a quantitative measure of polymorphic forms comprising microsatellite changes in the one or more microsatellite regions, thereby detecting the genetic variation in the one or more microsatellite regions.

I. INTRODUCTION

U.S. Patent No. 10,793,916 (the “’916 patent”) is part of a family of Guardant patents reciting methods of detecting well-understood genetic variations using standard, well-known detection methods. Guardant itself has admitted in numerous proceedings that the steps of these methods were well-known and conventional before the ’916 patent’s earliest claimed priority date, including (1) tagging nucleic acid molecules with barcodes by ligation; (2) amplifying the tagged molecules; (3) sequencing the tagged molecules to produce sequence reads; and (4) determining genetic variants based on the sequence reads. The Board previously found one patent from the same family as the ’916 patent, U.S. Patent No. 9,834,822 (“the ’822 patent”), invalid based on, among other references, Schmitt and Forshew, both asserted here. *See* EX1083 (“822FWD”), p.67.¹

The ’822 and ’916 patents recite extremely similar methods of detecting genetic variants. The primary difference is that the ’822 claims were directed to identifying variants in *any region* of the genome, while the ’916 claims are restricted to identifying variants in *specific regions* called microsatellite regions. A POSA,

¹ Guardant appealed the 822FWD, and the appeal was decided on May 5, 2023. *Guardant Health, Inc. v. Vidal*, 2023 WL 3262962 (Fed. Cir. May 5, 2023) (rejecting Guardant’s appeal on all but one issue—presumption of nexus—vacating and remanding for fact finding on that issue). After remand, the Board made no further decision on the 822FWD because the proceeding was terminated pursuant to a settlement. EX1084, p.2.

however, would have understood that focusing on variants in microsatellite regions, as opposed to “mutations” or “sequence variations” (*see, e.g.*, ’822 claims 6, 7), in the full genome, was obvious. Indeed, microsatellite regions are naturally occurring genetic regions, and mutations in those regions—referred to as microsatellite instabilities (“MSI’s”)—have been known since at least the 1990s to be associated with cancer.

There is nothing unique about focusing the same methods found invalid in the ’822 patent on variant detection in microsatellite regions. Indeed, during the ’916 prosecution, the Examiner found that applying the general detection methods of the ’916 patent family to the “species” of microsatellite regions in particular would have been obvious in view of the teachings in Patent Publication 2010/0264331 (“Sacko”), which teaches that cell-free nucleic acids from the blood of cancer patients are associated with microsatellite mutations. EX1002, 359-362. Patent Publication 2012/0165202 (“Porreca”) is another reference that teaches the well-known clinical relevance of MSIs in ctDNA.

In short, the same teachings of detecting genetic variants across the genome of Schmitt and Forshev found to invalidate the ’822 patent are equally applicable to detecting variants in microsatellite regions. While those references, standing alone, are sufficient to invalidate the challenged claims in light of the obviousness of detecting variants in microsatellite regions as compared to other genome portions,

the additional references cited herein combine to compel the same result. In particular, Schmitt and Forsheew, together with Sacko and/or Porreca, establish that those of ordinary skill were already detecting—in fact quantifying²—mutations in microsatellite regions for, among other reasons, cancer diagnosis, using well-known and established methods. Thus, Petitioner respectfully requests that the Board institute *inter partes* review and cancel claims 13-30.³

II. STANDING

Petitioner certifies that the '916 patent is available for *inter partes* review and it is not estopped.

III. TECHNICAL BACKGROUND

A. Cell-free DNA

Cell-free DNA (cfDNA) are short DNA fragments that circulate outside of cells in blood or other body fluids. EX1003, ¶45. cfDNA can come from healthy

² While the '916 claims refer to determining a “quantitative measure,” the patent teaches that this can include a “frequency” (EX1001, 35:32-35), which is what '822 patent claim 1 recites.

³ Petitioner notes that Guardant submitted the 822FWD via IDS during prosecution, and the '916 patent was still allowed. However, Guardant attached the FWD without explanation (after the '916 patent had already been allowed once), the 822FWD was one of over 700 prior art references submitted during the '916 prosecution, and the Examiner did not address it. Given the '916 patent's issuance in 2020, the Examiner could not have been aware of the Federal Circuit's 2023 findings that many of the Board's determinations regarding the '822 patent and the prior art asserted here (Schmitt) were supported by substantial evidence—and that Guardant waived challenging most of those determinations in that appeal.

cells and also includes circulating tumor DNA (“ctDNA”), shed from dying tumor cells. cfDNA—and that it “may contain genetic aberrations associated with” human disease—“has been known in the art for decades.” EX1001, 1:49-51.

B. Genomic Variants & Disease

Comparing one person’s genome to another can reveal differences that are sometimes called “mutations” or “variants.” Cataloging mutations associated with cancer and other diseases has been studied for decades. *See, e.g.*, EX1016, 7-8 (by 2010, POSAs “would have appreciated that tens of thousands of mutations associated with cancer were catalogued in publicly accessible databases”).⁴

Many types of genomic variants have been identified, including insertions and deletions (collectively, “indels”), which are the addition or loss of stretches of bases. *See id.*, 7-8 (by 2010, “[d]ifferent types of somatic mutations were known in the art”); EX1003, ¶¶47-49.

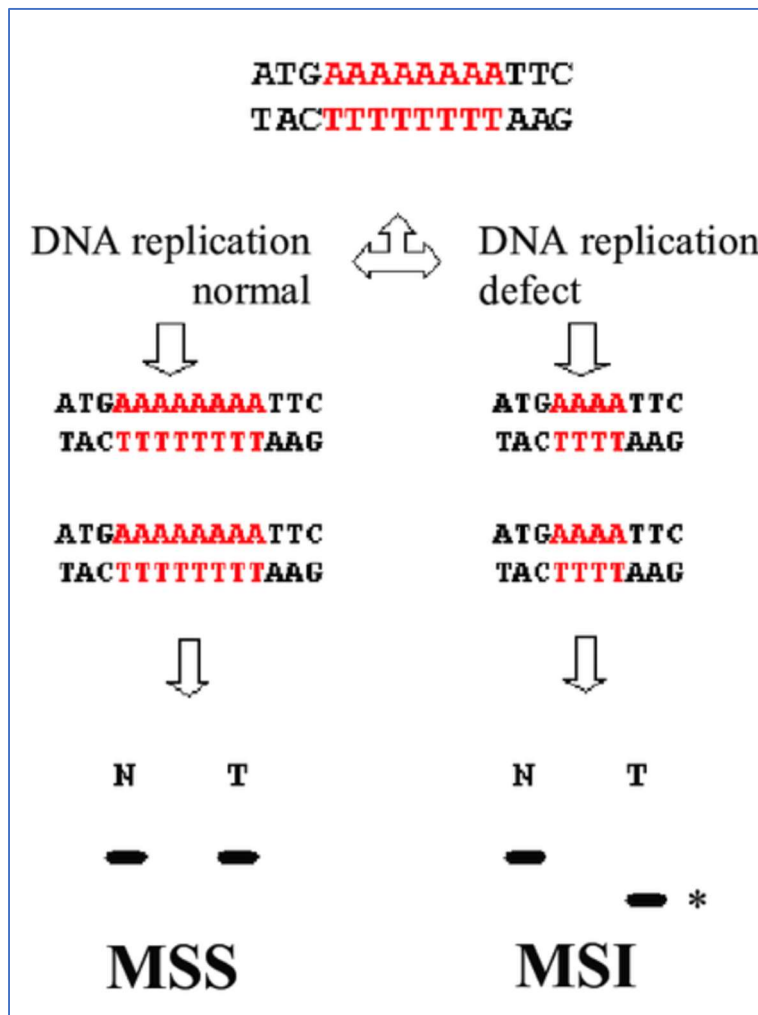
C. Microsatellite Regions and Instability

One particular type of indels relates to mutations in microsatellites—short DNA sequences (1-9 nucleotides) repeated anywhere between 5-50 times. EX1003,

⁴ Included in this section are Patent Owner’s admissions when challenging its adversaries’ patents (priority dates December 30, 2010, and March 20, 2012) in prior IPR proceedings. These admissions are binding statements against interest. *See Guardant Health, Inc. v. Univ. of Washington*, IPR2022-00450, Paper 13 at p.11 (Guardant’s admissions concerning the prior art in other IPR proceedings were admissions the Board should consider).

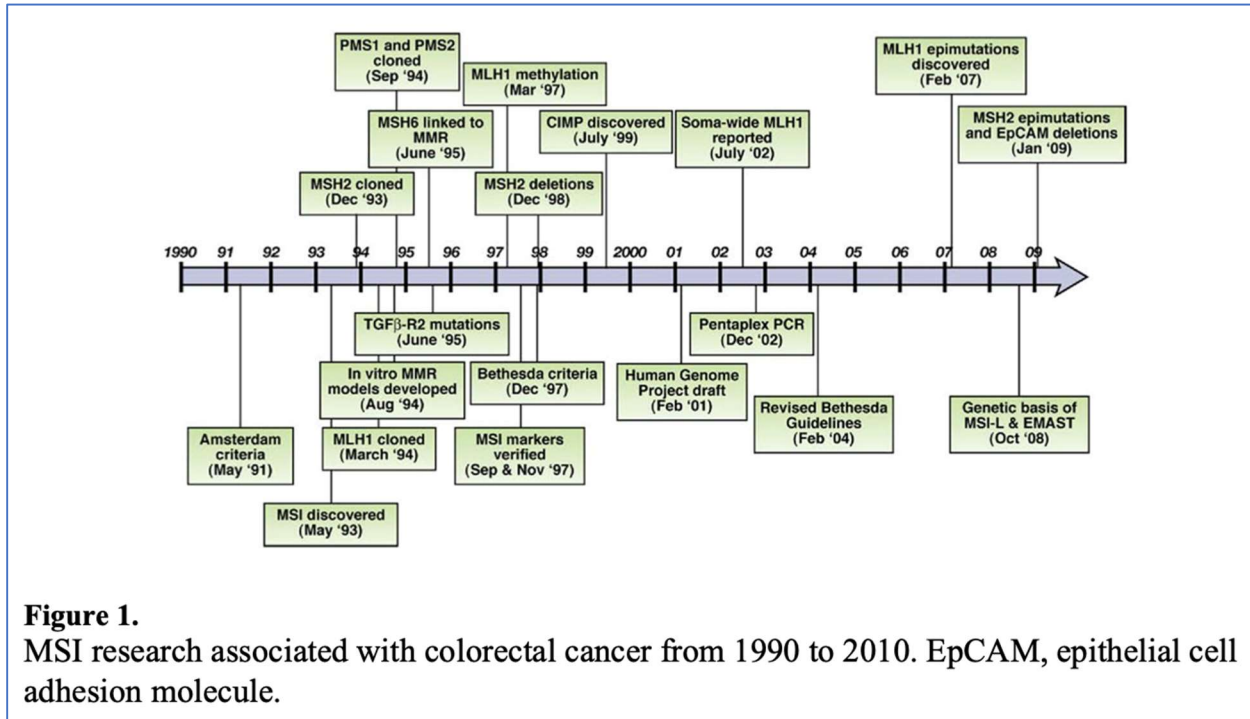
¶54. As known in the art, microsatellite instability (“MSI”) occurs when a subject’s natural DNA mismatch repair (MMR) is impaired such that during routine DNA replication (e.g., in cells), errors are introduced that do not get corrected. *Id.*, ¶¶54-55. The number of repeated bases in the replicated DNA is different from what it was in the original—either through additional repeats or omitted repeats. *Id.*

For example below, a normal microsatellite may have 8 repeating A-bases. If the MMR is defective, when the DNA is replicated, the size of the microsatellite can change, here becoming shorter by 4 nucleotides.



EX1077, 4; *see also* EX1003, ¶56.

The association of MSI with colorectal cancer has been studied for decades, as illustrated by the timeline below, which lists milestones in the identification of microsatellite regions associated with CRC across the 1990s and 2000s:



EX1078, p.2075; EX1003, ¶57.

Amidst ongoing research, in 1997, the National Cancer Institute developed MSI-related guidelines. EX1079 at 1 (“The form of genomic instability associated with defective DNA mismatch repair in tumors is to be called microsatellite instability (MSI).”). Thus, by 1997, microsatellite changes were well-known to have clinical relevance for cancer diagnosis and treatment, and it was known that

insertions/deletions in microsatellite regions could be caused by defective MMR. EX1003, ¶¶58-60.

Before 2012, detecting MSI from cfDNA in blood was being used as a biomarker in clinical testing for cancer. *See, e.g.*, EX1076, ¶[0005] (identifying MSI as “new potential marker, with considerable specificity for monitoring tumors” in 2010); EX1080, p.369 (“MSI is a molecular marker of defective DNA mismatch repair that is detected in approximately 15% of sporadic colon cancers”); *see also* EX1003, ¶61.

D. Next Generation Sequencing

The order of the four bases (A, C, T, G) in a DNA strand is their “sequence.” EX1003, ¶63. While DNA sequencing dates back to the 1970s, the technology most frequently employed for sequencing and identifying genetic variants by 2012 and still today is next-generation sequencing (“NGS”). EX1003, ¶¶64-66. The most widely-used NGS platform is commercially available from Illumina, which has been used since the mid-2000s. EX1003, ¶64. By 2010, Illumina workflows, including for sequencing cfDNA, were conventional and widely used. *See, e.g.*, EX1016, p.2 (NGS was a “well-known ‘off the shelf’ technolog[y]”); *id.*, p.7 (“[s]equencing using the Illumina platform [was] described throughout the [prior art]”).

E. Library Preparation, Including Ligating Adaptors

Before sequencing, the fragments to be sequenced are typically combined with short pieces of DNA called adaptors in a tagging step, which results in a library of molecules to be sequenced. EX1003, ¶75. Adaptors can be attached to the fragments through known methods, including ligation. *See, e.g.*, EX1021, p.23 (by March 2012, adaptors could be attached by “known” ligation approaches); EX1003, ¶75. When ligation is used, the tagging would be followed by amplification (e.g., by polymerase chain reaction (PCR)) to create multiple copies of each tagged molecule. *See, e.g.*, EX1018, p.5 (by March 2012 “[t]ypical Illumina sequencing workflows include[d] . . . PCR amplification”); *see also* EX1003, ¶¶67-73.

In Illumina sequencing libraries, these tagged adaptors include known primer sequences for sequencing and also frequently comprise identifier tags called “barcodes” which serve to distinguish sequence reads derived from one DNA fragment from reads of another. *See, e.g.*, EX1018, p.14 (by March 2012, “adapters comprising barcodes . . . were known and well-documented in the prior art”); EX1003, ¶76. Tagging DNA fragments with adaptors including barcodes was used, for example, to track samples (EX1013, ¶[24]), count sequencing reads (EX1012, p.1347), and generate consensus sequences (EX1006, ¶[42]). By March 2012 use of adaptors “comprising barcodes was a routine aspect of sequencing, including in

methods directed to reduction of bias and error in the resulting reads.” EX1018, p.30.

F. Sequence Reads

NGS typically outputs a large dataset of DNA sequences, referred to as “sequence reads.” EX1003, ¶79. Because the DNA fragment lengths vary, the sequence reads vary; in most applications they are short reads between 50 to a few hundred bases. *Id.*

G. Mapping/Alignment

After sequence reads are generated, when identifying mutations it is necessary to compare the reads to a reference sequence to know where in the genome the short sample DNA fragments originated from. EX1003, ¶80. This bioinformatic process, called mapping or alignment, has long been a “basic aspect of NGS.” EX1016, p.27; EX1003, ¶¶80-82.

Before 2012 “it was conventional to use reference sequences as a basis of comparison for sequence information to identify sequence variations and true mutations”—a process referred to as variant calling. 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.”). Indeed, by 2010, mapping/alignment could be done by “numerous ‘off the shelf’ software programs”

using publicly available reference sequences. EX1016, pp.2, 27 (“alignment programs [for aligning to a human genome were] known in the art”).

The comparison makes variant identification possible because a DNA fragment can be identified as containing a variant where its sequence differs from the reference at one or more nucleotide positions. EX1003, ¶82. However, because of sequencing errors mapping to a single sequence read alone (or even a handful of reads) was generally understood not to be sufficient to know whether a difference between a sequence read and a reference was a true mutation. *See, e.g.*, EX1018, pp.3-4 (by March 2012, “skilled artisans were aware that sequencing errors may arise as artifacts of [NGS], and that such errors were mitigated by redundantly sequencing copies of a molecule and evaluating resulting sequence data for consensus”).

H. Grouping/Collapsing to Generate Consensus Sequences

One well-known technique to improve variant-calling accuracy that relies on barcodes is known as consensus sequencing. *See, e.g.*, EX1024, p.1 (by March 2012 “[t]here was nothing new about NGS, barcodes, and consensus sequencing”); EX1003, ¶¶84-85. By 2012, consensus sequencing was “well-known [] and described throughout the scientific literature.” *See id.*; *see also, e.g.*, EX1021, p.8 (by March 2012, “consensus-based methods for producing error-corrected sequences also were known in the art”); EX1018, p.3-4 (by March 2012 “[p]rior art

methods” included generating consensus sequences “that better represented the ‘true’ sequence of the original molecule because the sporadic errors found in individual reads would not be represented in the consensus”).

Generating consensus sequences involves bioinformatically “grouping” together similarly-tagged sequence reads (those having the same barcode) into sets of reads called “families.” EX1003, ¶¶85-87. For each family, those grouped sequence reads are “collapsed” into an accepted consensus sequence based on the most frequent sequence. *Id.* The consensus sequence is expected to be more accurate because reads with sequencing errors at any given position will generally be present at a low frequency. *See, e.g.*, EX1021, p.18 (before March 2012, the 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 (*i.e.*, the base present in 95% of reads within a family)”); EX1003, ¶¶85-87.

IV. OVERVIEW OF THE '916 PATENT

A. Priority Date

The '916 patent purports to be a continuation of Application No. 14/425,189, the parent of U.S. Patent 9,840,743 (“’743 Patent”) and the '822 Patent. Petitioner assumes the claimed priority date of September 4, 2012, in this Petition only, without prejudice to its right to challenge that priority date elsewhere.

B. The '916 Patent Disclosure

The two challenged independent '916 claims, claims 13 and 30, recite well-known sequencing techniques and rudimentary analysis. Generally, they involve tagging cell-free nucleic acid molecules from a sample with barcodes (by ligation); amplifying the tagged molecules; sequencing the tagged, amplified molecules; and then analyzing the sequence reads for microsatellite changes in certain regions to detect genetic variation. The barcodes are used in the traditional manner to group and then collapse sequence reads into consensus sequences.

The '916 specification does not purport to invent any new tagging, amplifying, sequencing, grouping, or collapsing techniques, and in fact acknowledges the techniques to implement the claimed methods were well-known and routine in the art. Examples include (EX1001):

- 36:10-15 (“cell free polynucleotides may be isolated and extracted using a variety of techniques known in the art” including by “commercially available kits”);
- 38:31–36 (“assignment of unique or non-unique identifiers, or molecular barcodes in reactions of this disclosure may follow methods and systems described by, for example,” prior-art patents and patent applications);
- 38:56–59 (“PCR for sequencing may be performed using any means, including but not limited to use of commercial kits [list]”);
- 30:22–25 (“subsequent sequencing of cell free polynucleotides” may be done “by techniques known in the art”);
- 49:26-27 (“sequencing may be performed using any nucleic acid sequencing platforms known in the art”);

- 35:37–40 (as to mapping/aligning sequences, “sequences can be interrogated using the genome browser available” online); and
- 46:8–10 (“[c]onsensus sequences can be generated from families of sequence reads by any method known in the art.”).

The final detection step involves determining “a quantitative measure of polymorphic forms comprising microsatellite changes in the one or more microsatellite regions.” The ’916 teaches that “polymorphic forms include but are not limited to” numerous known genetic variations, though the Challenged Claims are limited to one—“microsatellite changes.” EX1001, 9:19-24. The ’916 patent broadly describes the recited “quantitative measure” as including, “for example, number, count, frequency (whether relative, inferred or absolute).” *Id.*, 35:32-34. The patent does not purport to be inventing any new quantitative measurement technique.

To the extent the ’916 specification does not expressly acknowledge this, and as discussed in Section III *supra*, each of the claimed steps was well-known in the art and obvious to apply for MSI detection. As discussed below, the dependent claims add only routine sequencing steps and quantitative analysis.

C. Prosecution History

The ’916 application was filed September 18, 2019, and its examination was accelerated under 37 C.F.R. § 1.102(e).

On October 22, 2019, the applicant filed a Notice of Concurrent Proceedings for Inter Partes Review Petitions of the '743 and '822 patents (IPR2019-00634 and IPR2019-00652), both of which claim priority to the same parent application as the '916 patent. EX1002, 222; EX1073; EX1074. On February 14, 2020, the Examiner issued an Office Action (OA), rejecting all pending claims based on nonstatutory obviousness-type double patenting over the claims of numerous other Guardant patents, including U.S. Patents 9,902,992 and 10,494,678, and claims of five other co-pending applications, all in view of Sacko. EX1002, 355-363. For each rejection, the Examiner stated:

The [patented/copending] claims are generic with respect to the instant claims . . . while the instant claims require the species 'microsatellite changes'.

Sacko et al. discloses that cell-free nucleic acids from blood of cancer patients are associated with microsatellite mutations and instabilities (see paragraph 0005).

One of ordinary skill in the art considering the [patented/copending] methods would have been motivated to modify them by applying them to microsatellite changes because Sacko et al. disclosed that microsatellite changes were known to occur in cell-free nucleic acids from blood of cancer patients.

Id., 359-362.

Rather than attempt to distinguish the '916 patent claims over its family members, the applicant filed a Terminal Disclaimer on May 4, 2020. *Id.*, 458. The applicant did not dispute the Examiner's findings. While the applicant made

amendments, those were not to overcome the obviousness-type rejection—only to “better clarify the claimed subject matter and/or correct for antecedent basis or dependency purposes.” *Id.*, 468-473. The Examiner issued an initial Notice of Allowability (NOA) on June 29, 2020. *Id.*, 1232-1233.

Following this NOA, the applicant made no amendments or arguments to distinguish the '916 claims. On July 13, 2020, the applicant again submitted amendments “for clarity and/or antecedent basis purposes,” which was followed by an August 19, 2020 issue notification. *Id.*, 1249-1254, 1274.

On August 28, 2020, the applicant filed a petition to withdraw application from issue under 37 CFR 1.313(c)(2) with an IDS citing the IPR2019-00634 and 2019-00652 (822FWD) Final Written Decisions.⁵ *Id.*, 1281-1282, 1455-1456.

⁵ Guardant’s 822FWD appeal was heard on February 8, 2023, and decided on May 5, 2023, after the '916 patent issued. *Guardant Health, Inc. v. Vidal*, 2023 WL 3262962, at *2-3 (Fed. Cir. May 5, 2023). Guardant appealed on four issues: (1) the Board’s construction of “converting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides”; (2) the Board’s finding that Schmitt (EX1005) teaches non-uniquely tagged parent polynucleotides under that construction; (3) the Board’s finding that POSA would have had a reasonable expectation of success in combining Schmitt’s hybrid DCS method with cfDNA; and (4) the Board’s assessment of evidence relating to presumption of nexus in the context of objective indicia of non-obviousness. *Id.* at *2-3. The Federal Circuit agreed with the Board on (1)-(3). *Id.* at *2-4. It further found the Board erred with respect to (4), and thus vacated the obviousness determination and remanded for further fact-finding. *Id.* at *5. The parties settled before the Board had opportunity to act further.

Guardant submitted no explanation, remaining silent on the decisions and their relevance. The petition was granted that same day. *Id.*, 1451.

On September 3, 2020, the Examiner issued a corrected NOA, which did not address in substance either cited FWD. *Id.*, 1457-1458.

The '916 patent issued on October 6, 2020—years before the 822FWD appeal was decided. EX1001, 1.

V. GROUNDS

Petitioner presents the following grounds of invalidity:

Ground	References	Basis	Claims
1	Schmitt in view of Forshew and Porreca	§ 103	13-30
2	Schmitt in view of Forshew and Sacko	§ 103	13-30

VI. LEVEL OF ORDINARY SKILL

A POSA at the time of the '916 patent 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 had knowledge of DNA sequencing, including NGS and related sequencing methods, and related sample preparation techniques; bioinformatics methods for analyzing sequence reads and mapping sequence reads onto genomes; and methods for identifying genetic variants in a sample. *Id.*

VII. CLAIM CONSTRUCTION⁶

Rules 42.104(b)(3) and (4) require a petition to identify, for each challenged claim, “[h]ow the challenged claim is to be construed” and “[h]ow the construed claim is unpatentable” under that construction. 37 C.F.R. §§ 42.104(b)(3), (4).

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.

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 terms meet those requirements.

VIII. PRIOR ART OVERVIEW

A. Schmitt (EX1005)

Schmitt is entitled “Methods of Lowering the Error Rate of Massively Parallel DNA Sequencing Using Duplex Consensus Sequencing.” Schmitt issued from Application 14/386,800, a national stage application of PCT/US2013/032665 filed March 15, 2013, and claiming the benefit of Provisional 61/613,413 filed March 20,

⁶ Petitioners reserve 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 ’916 patent. *See Samsung Elecs. Am., Inc. v. Prisia Eng’g Corp.*, 948 F.3d 1342, 1350 (Fed. Cir. 2020) (“[T]he Board may not cancel claims for indefiniteness in an IPR proceeding.”).

2012 (EX1082) (“’413 Provisional”). Application 14/386,800 validly claims priority to the ’413 Provisional and both name the same inventors. The Board previously found “at least one claim in Schmitt is entitled to the priority of the ’413 provisional.” EX1085, 16. Patent Owner did not dispute Schmitt’s March 20, 2012 priority date thereafter. Schmitt is prior art under 35 U.S.C. § 102(a)(2).⁷

Schmitt teaches an error-correction method for sequencing DNA referred to as Duplex Consensus Sequencing (“DCS”). EX1005, Abstract, 3:10-40, 16:4-57, 19:8-24:21. The DCS method reduces sequencing errors by independently tagging and sequencing each of the two strands of a target DNA fragment. *Id.* Because both complementary strands are sequenced, “true mutations” can be identified by comparing the same position in both individual strands. *Id.*, 16:37-57. If the same alteration appears in both, then the mutation is likely “true,” and not a sequencing or amplification error which typically appears in one strand. *Id.*

Schmitt’s DCS methods teach tagging sample DNA fragments with both unique and non-unique molecular barcodes to identify individual molecules. Figure 1 shows the tagging process (ligation followed by PCR). *Id.* Fig. 1. In particular, Schmitt teaches using “a single molecule identifier (SMI) adaptor molecule for use in sequencing a double-stranded target nucleic acid molecule.” *Id.*, 2:66–3:1. The

⁷ The ’916 patent was examined under the first-to-file provisions of the AIA. *See* EX1002, 358.

SMI adaptor molecule comprises (1) an SMI sequence or “tag” (a degenerate or semi-degenerate n-mer sequence⁸) and (2) an SMI ligation adaptor (which allows the SMI adaptor molecule to be ligated to the target DNA fragment). *Id.* at 3:1–9; *see also id.* at 5:57–59, 6:46–47. The DCS method involves the steps of ligating adaptors, amplifying, and then sequencing. *Id.*, 3:12–20.

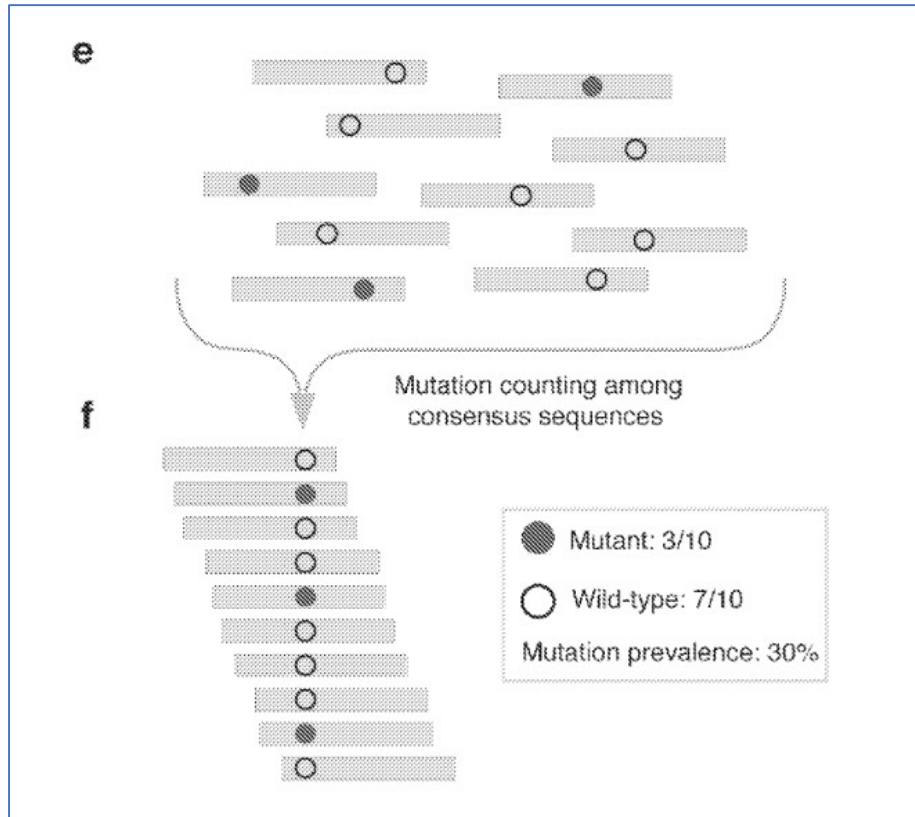
In one embodiment, the SMI sequence is “a unique, double-stranded, complementary n-mer random tag,” such that every DNA fragment is expected to become uniquely labeled with two distinct SMI sequences. *Id.* 3:47–53. The “nucleotide n-mer sequences may be any suitable length to produce a sufficiently large number of unique tags to label a set of sheared DNA fragments from a segment of DNA.” *Id.* 6:59–63. Schmitt provides an example: “nucleotide n-mer sequence which is 12 nucleotides in length” that, once ligated to each end of the target DNA fragment, “results in the generation of up to 4^{24} (i.e., 2.8×10^{14}) distinct tag sequences.” *Id.* 6:66–7:5.

Another embodiment is referred to as the “hybrid method.” Here, the SMI sequence comprises “a shorter n-mer tag (such as 1 or 2 or 3 or 4 or more degenerate or semi-degenerate bases).” *Id.* 9:9–13. While not unique labels themselves, these SMI adaptor molecules “serve as unique molecular identifiers” by combining

⁸ As known in the art, an “n-mer” refers to a short nucleotide sequence comprising “n” number of nucleotides.

information from the sheared ends of the target DNA fragment together with the short n-mer tag. *Id.*

For error correction through DCS, Schmitt teaches that, after sequencing, sequence reads sharing a unique set of SMI tags are grouped into paired families, each pair reflecting a consensus sequence of one original double-stranded DNA fragment. *Id.*, 4:4–10. Figure 3 illustrates this process, including the determination of mutations in the consensus sequences.



EX1005, Figure 3.

Because it involves identifying mutations (e.g., single-nucleotide variants in Figure 3), this process necessarily involves comparison to a reference sequence

showing the wild type (i.e., unmutated) sequence. Apparent “mutations” present in only one or a few family members, or mutations occurring in only one of the two strands, represent sequencing mistakes or PCR-introduced errors. *Id.*, 4:10–18. By contrast, “true mutations” are present on both strands and appear in all members of a family pair. *Id.*, 4:18–20. Schmitt explains that DCS “allows for a quantitative detection of sites of DNA damage” and teaches that “the ability to indirectly infer that damage is present on the DNA could be useful biomarker” including “for cancer risk, cancer metabolic state, mutator phenotype related to defective damage repair.” *Id.*, 15:42-51.

Schmitt was the primary reference relied on by the Board in the 822FWD. EX1083, 67.

B. Forshew (EX1010)

Forshew was a printed publication that was publicly available as of May 30, 2012, such that a POSA, exercising reasonable diligence, could have located it. EX1010 is a true and authentic copy as it existed on such date. Ex. 1086. 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, tagging with sample-specific barcodes, sequencing the fragments, and then aligning

the sequences to a reference sequence to identify mutations. *Id.*, Fig. 1, Supplement 1-4. Forshew refers to its method as tagged-amplicon deep sequencing—“TAm-Seq.” *Id.*, 1-2. TAm-Seq can be used to monitor mutation frequencies over time using samples from a patient and to compare those mutation frequencies to a reference sample. *Id.*, 8-9, Fig. 4; *see also id.*, 9 (disclosing experiments using TAm-Seq to identify mutations in specific cancer-associated genes in cfDNA).

The Board relied on Forshew as one of the references combined with Schmitt in the 822FWD. EX1083, 67.

C. Porreca (EX1075)

Porreca is a published patent application entitled “Methods and compositions for evaluating genetic markers.” Porreca was published June 28, 2012 and filed as Application No. 13/266,862 on April 30, 2010. It is prior art under 35 U.S.C. § 102(a)(1) and (2).

Porreca is directed to ways of improving “multiplex analysis of genomic loci” from patient samples. EX1075, Abstract; *id.*, ¶[0002]. Porreca’s methods apply to “any application where reduction of bias, e.g., associated with genomic isolation, amplification, sequencing, is important.” *Id.*, ¶[0025]. This includes analysis of cfDNA. *See, e.g. id.* (“detection of mutations in maternally-circulating fetal DNA”).

Porreca teaches “methods for detecting nucleic acid deletions or insertions in regions containing nucleic acid sequence repeats.” *Id.*, ¶[0157]. Porreca contemplates using NGS methods for quantitative measurements of genomic variation. *Id.*, ¶[0136] (“to make quantitative measurements (including genotype calling), these methods utilize the counts of sequencing reads of a given genomic locus as a proxy for the representation of that sequence in the original sample of nucleic acids.”); *see also id.*, ¶[0175] (counting based on “the number of different barcodes that are present”).

For microsatellites in particular, Porreca describes “[g]enomic regions that contain nucleic acid sequence repeats are often the site of genetic instability due to the amplification or contraction of the number of sequence repeats (e.g., the insertion or deletion of one or more units of the repeated sequence).” *Id.*, ¶[0158]. One result of genetic instability is “cancer, which has been associated with microsatellite instability (MSI) involving an increase or decrease in the genomic copy number of nucleic acid repeats at one or more microsatellite loci.” *Id.*, ¶[0160].

Porreca further teaches using the detected genetic variation to aid patients: “a diagnosis, prognosis, or disease risk assessment is provided to a subject based on a genotype determined for that subject at one or more genetic loci (e.g., based on the analysis of a biological sample obtained from that subject).” *Id.*, ¶[0017]. The

“genetic information from a tumor or circulating tumor cells is used to determine prognosis and guide selection of appropriate drugs/treatments.” *Id.*

D. Sacko (EX1076)

Sacko is a published patent application entitled “Method for assaying nucleic acids by fluorescence” and published Oct. 21, 2010. It is prior art under 35 U.S.C. § 102(a)(1).

During ’916 prosecution, the Examiner relied on Sacko to reject the pending claims for obviousness-type double-patenting over other Guardant patents and applications in the same family. The Examiner found that:

One of ordinary skill in the art considering the patented methods would have been motivated to modify them by applying them to microsatellite changes because Sacko et al. disclosed that microsatellite changes were known to occur in cell-free nucleic acids from blood of cancer patients.

EX1002, 359.

Sacko teaches “DNA extracted from the plasma of cancer patients usually has tumoral DNA characteristics[], such as strand instability, the presence of specific oncogenes, tumor suppressor genes and microsatellite alterations.” EX1076, ¶[0005]; *see also id.*, ¶[0003] (stating that “[the existence of cell free nucleic circulating in the blood has been known for a number of years,” and citing 1947 and 1966 references). Specifically, “microsatellite mutations and instabilities detected in the free genomic DNA of the serum suggest that it could be a new potential

marker, with considerable specificity for monitoring tumors.” *Id.* Sacko teaches, when used as a biomarker, MSIs could “complement the analysis of markers commonly used to monitor patients suffering from cancers or undergoing chemotherapy.” *Id.*, ¶[0006].

E. The Petition’s Combinations Were Not Previously Considered by the Examiner

Although Schmitt, Forshew, and Porreca were cited in Patent Owner’s IDSes (among hundreds of references), the Examiner did not expressly discuss those references or their combinations with other references as set forth herein, or have the benefits of the arguments in this Petition.

Indeed, Guardant buried Schmitt, Forshew, and Porreca amongst over 700 other references in an IDS. EX1002, 127-214. Schmitt, Forshew, and Porreca were not asserted in any Office Actions. Instead, the Examiner rejected Guardant’s claims for obviousness-type double patenting in view of patents and applications related to the ’916 (and ’822) in view of Sacko, but later withdrew the rejection based on a terminal disclaimer. EX1002, 355-363, 1232-1233. There is no suggestion that the Examiner substantively considered the arguments about Schmitt and Forshew that the Board relied on (or the subsequent Federal Circuit record) in light of the Examiner’s own findings about Sacko’s teachings of MSIs.

IX. GROUND 1: CLAIMS 13-30 ARE OBVIOUS OVER SCHMITT IN VIEW OF FORSHEW AND PORRECA

A. Motivation To Combine

A POSA would have been motivated to combine Schmitt, Forshew and Porreca. EX1003 ¶¶135-143.

A POSA would have been prompted to combine Schmitt’s teachings of the DCS method with Forshew’s teachings of sequencing and analysis of cfDNA with a reasonable expectation of success. The Board previously found that Schmitt and Forshew would have been combined by a POSA. *See* EX1083, p.49.

Schmitt teaches techniques for analyzing genetic alterations in DNA, including methods compatible with NGS and digital analysis platforms that enable detection of low-frequency variants. EX1005, Abstract, 1:28-55, 3:10-40, 16:4-57; 19:8-25:51; EX1003 ¶136. Schmitt explains that DCS “allows for a quantitative detection of sites of DNA damage” and teaches that “the ability to indirectly infer that damage is present on the DNA could be useful biomarker” including “for cancer risk, cancer metabolic state, mutator phenotype related to defective damage repair.” EX1005, 15:41-51. Schmitt explicitly teaches methods suitable for analyzing cfDNA (e.g., as taught by Forshew)⁹ using NGS and digital quantification

⁹ The Board found that Forshew “teach[es] the extraction and analysis of cfDNA.” EX1083 at 31.

approaches, which are well-suited for resolving length alterations in repetitive DNA regions such as microsatellites. EX1005, 3:44-62; 4:4-29; EX1003 ¶136.

MMR was a well-known mechanism to repair DNA damage—and it was also well-known that a defective MMR results in an increase in the frequency of insertions/deletions in microsatellite regions, i.e., MSIs. EX1081, 9 (“defects in MMR confer a mutator phenotype”). Schmitt explains that DCS “allows for a quantitative detection of sites of DNA damage” and teaches that “the ability to indirectly infer that damage is present on the DNA could be useful biomarker” including “for cancer risk, cancer metabolic state, *mutator phenotype related to defective damage repair.*” EX1005, 15:42-51.¹⁰

A POSA would have understood that Schmitt’s disclosure of “defective damage repair” includes defective MMR and observation of increased insertions and deletions in microsatellite regions. A POSA would have also known that Schmitt’s reference to using DCS to quantify sites of DNA damage and to infer a “mutator phenotype related to defective damage repair,” teaches the detection and quantification of MSIs. EX1003 ¶137.

Forsheew is analogous art to Schmitt and the ’916 patent because Forsheew is also directed to detecting genetic mutations in nucleic acid samples. *Id.*, ¶138. Similar to Schmitt, Forsheew teaches a deep-sequencing method that employs NGS

¹⁰ All emphasis added unless otherwise noted.

technology to detect “both abundant and rare mutations in circulating DNA from blood plasma.” EX1010, 1-2. Forshew teaches that its method is advantageous in part because it can be used to track mutation frequencies during the course of treatment by repeatedly sequencing cfDNA in plasma samples from the same patient. EX1010, 9. Forshew acknowledges the challenge posed by amplification errors, explaining that its method includes “[d]uplicate sequencing of each sample . . . to avoid false positives stemming from PCR errors.” EX1010, 9.

A POSA screening cfDNA as taught by Forshew, would have also looked to Schmitt’s DCS method for the same reasons. EX1010, Abstract, 1–2; EX1005, Abstract, 1:28–5; EX1003 ¶¶ 139-140. In addition to discussing DCS’s application to prenatal screening for fetal aneuploidy (an application relying on a type of cfDNA—fetal cfDNA), Schmitt also refers to “early detection of cancer.” EX1005, 1:42–45. A POSA screening for cancer mutations in cfDNA from plasma, according to Forshew, would have been prompted to look to Schmitt’s DCS method to reduce amplification/sequencing errors and to improve detection of rare genetic mutations. EX1010, 1 (“[s]ensitive methods for detecting cancer mutations in plasma may find use in early detection screening, prognosis, monitoring tumor dynamics over time, or detection of minimal residual disease”); *see also* EX1003 ¶140.

A POSA wishing to detect cfDNA genetic mutations with high accuracy and sensitivity as discussed in Forshew, would have been motivated to use Schmitt’s

improved DCS method because the DCS method “reduces or eliminates artifactual mutations arising from DNA damage, PCR errors, and sequencing errors,” and “allows rare variants in heterogeneous populations to be detected with unprecedented sensitivity.” EX1005, 2:56–60. Indeed, the Board previously found that a POSA “reading Schmitt would have understood that Schmitt’s DCS method was a reasonable substitute for the deep-sequencing and error-correction techniques used in the prior art—such as the [NGS] techniques [] Forshew used for prenatal cfDNA screening and for tumor cfDNA screening, respectively.” EX1083, p.55.

A POSA would have been additionally motivated to combine the teachings of Schmitt and Forshew with Porreca. EX1003 ¶¶ 141-143. Porreca is analogous art to Schmitt, Forshew, and the ’916 patent because it is also directed to detecting genetic mutations in nucleic acid samples. Porreca contemplates NGS methods (like Schmitt and Forshew), and using such methods to do quantitative measurements in the multiplex analysis of genetic loci in patients. EX1075, Abstract, ¶[0002], ¶[0017], ¶[0136], ¶[0175] (referring to NGS of amplicons comprising barcodes). Like Schmitt (EX1005, 15:42-51), Porreca teaches “methods for detecting nucleic acid deletions or insertions in regions containing nucleic acid sequence repeats[,]” (EX1075, ¶[0157], with the aim of adding in patient diagnostic and treatment, (*id.*, ¶[0017])).

As to microsatellites, Porreca teaches that cancer is associated with microsatellite instability (MSI). EX1075, ¶[0160]. A POSA applying the high sensitivity cfDNA analysis framework taught by Schmitt and Forshew would have been motivated to use these improved techniques for MSI detection as taught by Porreca because such a combination would improve the detection and quantification of cancer-associated genomic instability in a minimally invasive sample type such as cfDNA. EX1003 ¶141.

This combination would have been further motivated by the known clinical significance of MSI as a biomarker for cancer diagnosis, prognosis, and therapy selection. EX1005, 15:42-51; EX1003 ¶142. As of the relevant date, cfDNA for non-invasive cancer detection was well-known, and a POSA would have understood that applying MSI detection techniques to cfDNA could provide a powerful diagnostic tool for detecting disease, including cancer, including as taught by Porreca. EX1003 ¶142. A POSA would have reasonably expected that combining these teachings would yield a reliable and quantitative measure of microsatellite changes in cfDNA without requiring undue experimentation. *Id.*

Moreover, the proposed combination would have involved a predictable use of known techniques to address a recognized problem using conventional tools. Both Porreca and Schmitt rely on routine molecular biology and sequencing methods that were widely used in the art at the time. EX1003 ¶143. Schmitt provides the

necessary sample preparation, sequencing, and computational tools to analyze cfDNA (EX1005, 7:1–45; 11:8–30), while Porreca provides the rationale and methodology for interpreting microsatellite variations, (EX1003 ¶143). A POSA would have appreciated that applying Porreca’s MSI detection teachings to cfDNA using Schmitt’s analytical techniques would yield not only qualitative MSI status but also a quantitative measure of microsatellite change, thereby offering improved clinical resolution. EX1003 ¶143. Accordingly, the combination would have been obvious and well within the capabilities of a skilled artisan.

B. Reasonable Expectation of Success

A POSA would have a reasonable expectation of success combining Schmitt with Forsheu to identify rare mutations in cfDNA to arrive at the claimed inventions. EX1003 ¶¶144-147.

Indeed, the Board found a POSA “would have had a reasonable expectation of success in using Schmitt’s DCS method to detect genetic mutations in cfDNA.” EX1083, p.56 (“all necessary techniques for carrying out the claimed method steps of the [related] ‘822 patent were known and required only ordinary skill to perform”). As discussed, the ’916 claims too recite only well-known library preparation, sequencing, and analysis steps—all also disclosed by Schmitt.

Schmitt teaches “the DCS approach can be generalized to nearly any sequencing platform” and emphasizes “compatibility of DCS with existing

sequencing workflows.” EX1005, 18:44–61. As stated by the Board in the related ’822 Patent FWD:

Schmitt suggests to the skilled artisan to use the DCS method as a general technique for next generation sequencing and error correction, and the ’822 patent evinces that the technical details for isolating, barcoding, amplifying, sequencing, and analyzing cfDNA molecules were known to be routine in the art. These factors persuade us that an ordinarily skilled artisan would have had a reasonable expectation of success.

EX1083 at 56.

As with the ’822, a POSA would have had a reasonable expectation of success in combining the teachings of Schmitt and Forshew with Porreca to arrive at the ’916 claims, including to determine a quantitative measure of microsatellite changes in a cfDNA sample. EX1003 ¶146. As of the relevant date, the use of cfDNA with well-known NGS and bioinformatics techniques for detecting genetic alterations was well-established and taught by Schmitt (EX1005, 6:25-45; 10:1-15), including in combination with Forshew. Likewise, the methodology and motivation for identifying alterations in regions containing nucleic acid repeats, *i.e.*, MSIs, was well-known and described by, among other references, Porreca. EX1075, ¶¶ [157-160]; EX1003 ¶146. Both references—Schmitt and Forshew—rely on standard molecular biology tools, and neither requires novel chemistry or unconventional instrumentation.

A POSA would have understood that microsatellite loci—being short, repetitive DNA sequences—are readily detectable using the sequencing and analysis methods taught by Schmitt, and that alterations in regions containing nucleic acid repeats, as described by Porreca, could be quantitatively assessed through the same DCS techniques. EX1003 ¶147. Because the combination involves applying established detection principles to a known analyte (cfDNA) and a well-characterized target (microsatellite regions), a POSA would have had a high degree of confidence in achieving the desired outcome without undue experimentation.

C. Claim-by-Claim Analysis

1. Claim 13

- (a) **13[pre]: “A method for detecting a genetic variation in one or more microsatellite regions in a sample of cell-free nucleic acid molecules from a subject having a cancer, the method comprising:”**

To the extent the preamble is limiting, Schmitt with Forsheew and Porreca teaches 13[pre].

Schmitt teaches a tag-based method of error correction called “Duplex Consensus Sequencing (DCS),” that reduces amplification and sequencing errors, and “allow[s] rare variants . . . to be detected with unprecedented sensitivity.” EX1005, 2:56-62; *see also id.*, 16:4-57. DCS “allows for a quantitative detection of sites of DNA damage” and teaches that “the ability to indirectly infer that damage is present on the DNA could be useful biomarker” including “for cancer risk, cancer

metabolic state, mutator phenotype related to defective damage repair.” EX1005, 15:42-51. A POSA would have understood that Schmitt’s disclosure of “defective damage repair” includes defective MMR and the observation of increased insertions and deletions in microsatellite regions. EX1003 ¶149. Thus, Schmitt alone teaches detecting a quantitative measure of microsatellite changes. *Id.*

It would have been obvious to a POSA to combine Schmitt with Forshew to use the DCS method for sequencing and detecting mutations in cfDNA. *Id.*, ¶150. Forshew describes using “deep sequencing” of cfDNA to screen bases for low-frequency mutations, and that such methods “may find use in early detection screening, prognosis, monitoring tumor dynamics over time, or detection of minimal residual disease.” EX1010, 1. Forshew describes extracting cfDNA from cancer patient plasma samples to identify mutations. *Id.*, 1, 10. Forshew discloses using this cfDNA approach to directly identify genetic variants from plasma of cancer patients. *Id.*, 4, Tables 1-2.

To the extent Schmitt with Forshew alone does not expressly disclose “detecting a genetic variation in one or more microsatellite regions in a sample of cell-free nucleic acid molecules from a subject having a cancer,” a POSA would have found it obvious to do so based on Porreca. EX1003 ¶151. Porreca teaches “methods for detecting nucleic acid deletions or insertions in regions containing nucleic acid sequence repeats,” EX1075, ¶[0157] including “cancer, which has been

associated with microsatellite instability (MSI) involving an increase or decrease in the genomic copy number of nucleic acid repeats at one or more microsatellite loci (e.g., BAT-25 and/or BAT-26).” *Id.* ¶[0160].

(b) 13[a]: “ligating molecular barcodes from a set of molecular barcodes having 2 to 1,000,000 different molecular barcode sequences to a plurality of the cell-free nucleic acid molecules from the sample to produce tagged parent polynucleotides;”

Schmitt either alone or with Forsheew teaches 13[a]. EX1003 ¶¶153-157. Indeed, the Board found in the 822FWD that Schmitt with Forsheew teaches “converting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides,” and “each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.” EX1083, pp.29-31.

The ’916 patent acknowledges that attaching barcodes to DNA (*i.e.*, tagging) had been described as early as 2000. EX1001, 38:31-36. Schmitt teaches attaching barcodes that are part of “SMI-containing adaptors” to DNA fragments. EX1005, 2:66-3:9-20, Figs. 1, 4. Schmitt teaches that, in the DCS method, SMI adaptor molecules comprising an “SMI sequence (or ‘tag’) of nucleotides” and an SMI ligation adaptor are ligated to the ends of target parent polynucleotides. *See* EX1005, 3:1–9, 5:57–59, 6:46–51, 7:38-41. Schmitt discloses that the SMI tags “may be

between approximately 3 to 20 nucleotides in length.” EX1005, 6:63-64. The number of possible sequences in a set of barcodes is equal to $4^{(\text{length of the barcode})}$. For example, Schmitt expressly discloses examples of 4-mer tags. *See* EX1005, 4:30–54; *id.* at 9:9–13. Where the barcode sequence is a 4-mer, there are up to 4^4 (i.e., 256) different sequences possible. EX1003, ¶155.

Schmitt also discloses a DCS “hybrid method” tagging target DNA polynucleotides that uses “a combination of sheared ends and a shorter n-mer tag (such as 1 or 2 or 3 or 4 or more degenerate or semi-degenerate bases) in the adaptor.” EX1005, 9:9–13. Each of these shorter n-mer tags would result in a set of barcodes having between 2 and 1,000,000 barcode sequences.

Schmitt thus teaches ligating molecular barcodes from a set of molecular barcodes having 2 to 1,000,000 different molecular barcode sequences to a plurality of the cell-free nucleic acid molecules from the sample to produce tagged parent polynucleotides. Further, to the extent Patent Owner contends that Schmitt does not expressly teach the recited polynucleotide is cfDNA, Forshev teaches the extraction and analysis of cfDNA as described immediately above. EX1003, ¶157.

- (c) **13[b]: “amplifying a plurality of the tagged parent polynucleotides to produce amplified tagged progeny polynucleotides;”**
- (d) **13[c]: “sequencing a plurality of the amplified tagged progeny polynucleotides to produce a set of sequencing reads; and”**

Schmitt teaches 13[b] and [c]. EX1003, ¶¶158-160. The Board previously found that Schmitt “discloses [] amplifying the tagged polynucleotides and sequencing the amplified progeny.” EX1083, pp.31-32, *citing* EX1005, 3:10–20 and 21:55–57.

Schmitt’s DCS method includes the “steps of ligating a double-stranded target nucleic acid molecule to at least one SMI adaptor molecule to form a double-stranded SMI-target nucleic acid complex; amplifying the double-stranded SMI-target nucleic acid complex, resulting in a set of amplified SMI-target nucleic acid products; and sequencing the amplified SMI-target nucleic acid products.” EX1005, 3:10-20; *see also id.*, 21:55-57.

- (e) **13[d]: “determining, from among a plurality of sequencing reads in the set of sequencing reads, a quantitative measure of polymorphic forms comprising microsatellite changes in the one or more microsatellite regions based at least on sequence information of the molecular barcodes, thereby detecting the genetic variation in the one or more microsatellite regions.”**

Schmitt alone or with Forshew and Porreca teaches this limitation. EX1003, ¶¶161-170.

Schmitt discloses determining a quantitative measure of all types of mutations, including microsatellite changes, based at least on sequence information of the barcode, and thus detecting genetic variation in the one or more microsatellite regions. Schmitt’s DCS process “confirms the presence of a ‘true’ mutation by

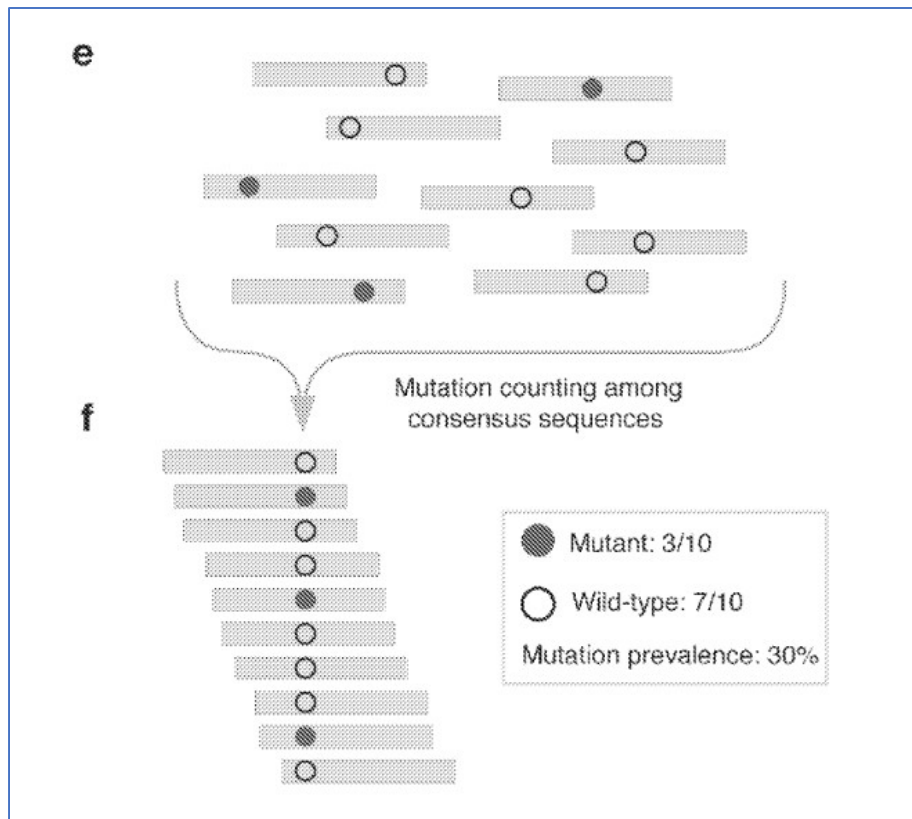
(i) identifying a mutation present in the paired target nucleic acid strands having one or more nucleotide positions that disagree; (ii) comparing the mutation present in the paired target nucleic acid strands to the error corrected double-stranded consensus sequence; and (iii) confirming the presence of a true mutation when the mutation is present on both of the target nucleic acid strands and appears in all members of a paired target nucleic acid family.” EX1005, 3:31-40; *see also id.*, 16:37-57; EX1003, ¶162.

Schmitt discloses that identification of mutations—including in microsatellite regions—in the DCS process is based at least on the sequence information of the barcodes because the barcodes are used to generate consensus sequences. Schmitt explains that, as part of its DCS method “a family of molecules is obtained that arose from a single DNA molecule; members of the same PCR ‘family’ are then grouped together by virtue of having a common (i.e., the same) SMI tag sequence.” EX1005, 21:55-61; *see also id.*, 20:39-64 (“[r]eads having common (i.e., identical) SMI sequences were grouped together, and were collapsed to generate a consensus read”).

As to “quantitative measure,” Schmitt teaches that the DCS method allows quantification of true mutations by detecting “the presence of a true mutation (as opposed to a PCR error or other artifactual mutation) in a target nucleic acid sequence.” EX1005, 16:43–47. The Board agreed that Schmitt teaches “determining

the *frequency* of bases called at the locus from among the families” and “further comprising detecting, at one or more loci, at least one single nucleotide variant, at least one gene fusion, and at least one copy number variant.” EX1083, pp.31-32 and 43, *citing* EX1005, 4:25-29, Figure 3, 62:49-51. As the '916 patent explains, measuring the frequency is a “quantitative measure.” EX1001, 35:32-34.

Schmitt’s Figure 3 shows how its SMI sequences are used to identify the total number of consensus sequences (10 total) and in doing so quantify both mutant and wild-type sequences (3 and 7, respectively). This process is referred to as “[m]utation counting”:



EX1005, Figure 3.

A POSA would have understood that Schmitt's mutation counting of "true mutations" included quantification of such mutations in one or more microsatellite regions, both because microsatellite changes are just another type of "mutation" and because Schmitt explicitly taught a POSA to look for mutations in microsatellite regions. EX1003, ¶166. As explained, microsatellite regions are naturally occurring in every genome and a POSA would have expected DNA fragments from microsatellite regions to be in any DNA sample, including those tested in Schmitt. *Id.* Indeed, there is nothing unique about the process of determining indel mutations in a microsatellite region (*id.*) and Schmitt does not preclude using the DCS method for evaluating DNA fragments from microsatellite regions.

In fact, as discussed for limitation 13[pre], Schmitt taught a POSA to detect mutations in microsatellite regions. EX1005, 15:42-51 (teaching "quantitative detection of sites of DNA damage"). A POSA would understand that Schmitt's disclosure of "defective damage repair" includes defective MMR and the observation of increased insertions/deletions in microsatellite regions. EX1003, ¶167. Thus, Schmitt alone teaches determining a quantitative measure of microsatellite changes in microsatellite regions, thereby detecting genetic variation in those regions.

To the extent Schmitt is found not to teach quantifying microsatellite changes in microsatellite regions, it would have been obvious to use Schmitt's DCS methods

to quantify mutations in microsatellite regions based on Forshew and/or Porreca, and thereby detect variation in those regions. Forshew teaches a method for identifying “cancer mutations present in circulating [cell-free] DNA” by targeting DNA fragments in regions of interest. EX1010, Abstract; EX1003, ¶168. A POSA would have understood that microsatellite regions were such “regions of interest” because, at the relevant time, it was well-known that MSIs were mutations associated with cancer. EX1003, ¶168.

Similarly, Porreca teaches “methods for detecting nucleic acid deletions or insertions in regions containing nucleic acid sequence repeats,” including in microsatellite regions which are associated with cancer. EX1075. ¶[0157]. As taught by Porreca, detecting MSI is quantitative as it involves “an increase or decrease in genomic copy number of nucleic acid repeats at one or more microsatellite loci.” *Id.*, ¶[0160]; *see also id.* ¶[0136] (“quantitative measurements” made by “utiliz[ing] the counts of sequencing reads of a given genomic locus as a proxy for the representation of that sequence in the original sample of nucleic acids”). Furthermore, based on Porreca’s teachings, a POSA would have known cancer mutations of particular interest to apply to Schmitt’s DCS methods include MSIs found in ctDNA. EX1003, ¶169.

Thus, based on Forshew and Porreca, a POSA would have been motivated to use Schmitt’s DCS method of quantifying mutations to focus on microsatellite areas

and determine a quantitative measure of microsatellite changes, thereby detecting genetic variation in the one or more microsatellite regions.

2. Claim 14: “The method of claim 13, wherein the genetic variation in the one or more microsatellite regions comprises an insertion or deletion (indel).”

Schmitt teaches Claim 14. EX1003, ¶¶171-173. As described in Section III, the presence of insertions/deletions in microsatellite regions, known as MSIs, was long-known at the relevant time. Schmitt teaches that the DCS method allows for the confirmation of “true mutations,” (EX1005, 16:43–47), and further explains that DCS “allows for a quantitative detection of sites of DNA damage” including “for cancer risk, cancer metabolic state, mutator phenotype related to defective damage repair,” (*id.*, 15:42-51).

A POSA would understand that Schmitt’s reference to “mutator phenotype related to defective damage repair” teaches the increase of insertion/deletion mutations in microsatellite regions associated with defective MMR. EX1003, ¶172. Schmitt’s approach could be readily combined with Forshe’s cfDNA analysis methods.

In addition, it would have been obvious to combine Schmitt’s DCS method with Porreca’s “methods for detecting nucleic acid deletions or insertions in regions containing nucleic acid sequence repeats.” EX1075, ¶[0157]; EX1003, ¶173. Porreca teaches that an “increase or decrease in the genomic copy number of nucleic

acid repeats at one or more microsatellite loci” is relevant to cancer. *Id.* ¶[0160]. Based on Porreca, a POSA would have known cancer mutations of particular interest to apply to the DCS methods of Schmitt include MSIs (genetic variation of insertions/deletions in the one or more microsatellite regions) found in ctDNA.

3. Claim 15: “The method of claim 13, wherein the sample is a bodily fluid sample selected from the group consisting of blood, plasma, and serum.”

Schmitt alone or with Forshew teaches Claim 15. EX1003, ¶¶174-176.

A POSA would understand that Schmitt teaches methods for analyzing bodily fluid samples that are blood, plasma, or serum samples that include ctDNA. EX1003, ¶175. For instance, Schmitt explains that the use of “deep sequencing” was well-known for clinical setting applications, including for “prenatal screening for fetal aneuploidy [9, 10], early detection of cancer [11] and monitoring its response to therapy [12, 13] with nucleic acid-based serum biomarkers, are rapidly being developed.” EX1005, 1:42-45.

Further, it would have been obvious to combine Schmitt’s DCS method with Forshew. EX1003, ¶176. Forshew teaches extracting cfDNA from plasma samples to identify mutations in cancer patients. *See* EX1010, 1 (stating that “[p]lasma of cancer patients contains cell-free tumor DNA”), 10 (stating that “[c]irculating DNA was extracted from between 0.85 and 2.2 ml of plasma”).

The Board agreed that Forshew teaches “providing a population of cell-free DNA (‘cfDNA’) molecules obtained from a bodily sample from a subject.” See EX1083, p.29. A POSA would know that a “bodily sample” containing cfDNA would be blood, plasma, or serum. EX1003, ¶176.

4. Claim 16: “The method of claim 13, wherein a molecular barcode from the set of molecular barcodes is attached on both ends of a molecule of the plurality of the cell-free nucleic acid molecules.”

Schmitt, alone or with Forshew, teaches Claim 16. EX1003, ¶¶177-179.

Schmitt describes embodiments where “the SMI adaptor molecules are ligated to both ends of a target nucleic acid molecule, and then this complex is used according to the methods described below.” EX1005, 7:38-42.¹¹ Accordingly, it would have been obvious to a POSA, with Schmitt to attach a molecular barcode from the set of molecular barcodes on both ends of a molecule of the plurality of the cfDNA molecules. EX1003, ¶178.

Further, to the extent Schmitt does not alone teach this limitation, it would have been obvious to combine with Porreca’s teachings: “The skilled artisan will appreciate that as part of a MIP library preparation process, adapters may be ligated onto the ends of the molecules of interest.” EX1075, ¶[0149].

¹¹ 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.

5. Claim 17: “The method of claim 13, wherein the molecular barcodes from the set of molecular barcodes have 2 to 1,000 different molecular barcode sequences.”

Schmitt teaches Claim 17. EX1003, ¶¶180-181. *See* limitation 13[a] (Section IX.C.1.b), *supra*; EX1003, ¶¶153-157.

As explained above, Schmitt expressly discloses examples of 4-mer tags which result in a set of 256 different molecular barcodes. *See* EX1005, 4:30–54; *id.* at 9:9–13; EX1003, ¶181. Schmitt’s “hybrid method” using shorter n-mer tag (such as 1 or 2 or 3 or 4 or more degenerate or semi-degenerate bases) in the adaptor” (EX1005, 9:9–11) would result in a set of barcodes having between 2 and 1,000 barcode sequences.

6. Claim 18: “ The method of claim 13, wherein the molecular barcodes are ligated to the plurality of the cell-free nucleic acid molecules by blunt-end ligation or sticky-end ligation.”

Schmitt teaches Claim 18. EX1003, ¶¶182-183.

Schmitt explains that the SMI adaptor comprising the SMI sequence (*i.e.*, barcode) is attached to the target nucleic acid sequence by ligation, and adaptors can be attached to both ends of the cfDNA molecules. EX1005, 3:1-6. Schmitt further explains: “[t]he SMI ligation adaptor may be any suitable ligation adaptor that is complementary to a ligation adaptor added to a double-stranded target nucleic acid sequence including, but not limited to a T-overhang, an A-overhang, a CG overhang

[i.e., sticky ends], *a blunt end*, or any other ligatable sequence.” *Id.*, 7:58-62. Schmitt thus teaches tagging using sticky-end or blunt-end ligation. EX1003, ¶183.

7. Claim 19: “The method of claim 13, further comprising selectively enriching at least a portion of the amplified tagged progeny polynucleotides for target regions associated with cancer prior to the sequencing.”

Schmitt, alone or with Forshew, teaches Claim 19. EX1003, ¶184-187.

The Board agreed that Schmitt with Forshew teaches “selectively enriching regions from a genome or transcriptome of the subject prior to sequencing.” See EX1083, p.29. A POSA would have understood “selectively enriching” to refer to a well-known technique of isolating particular genomic regions of interest for analysis, such as by sequencing. EX1003, ¶185.

As discussed above, Schmitt teaches producing amplified tagged progeny polynucleotides. Schmitt Example 1 explains that, before sequencing, “[t]arget capture was performed with the Agilent SureSelect system.” EX1005, 20:23-28, 22:50-67. The target capture here utilized 120 nucleotide “capture baits” designed in view of the 758 kb genomic region reference sequence. *Id.*, 20:23-29. In this example, “target capture” refers to hybrid capture, a known technique for preferentially isolating sequences of interest. EX1003, ¶186. Schmitt explains “selection and capture may be accomplished by any selection by hybridization method[.]” EX1005, 22:60-61; EX1003, ¶186.

It would have been obvious to combine Schmitt's selective enrichment of the amplified tagged progeny with Forshew to target regions associated with cancer. EX1003, ¶187. Forshew teaches that “[p]rimers were designed to amplify regions of interest in overlapping short amplicons (table S1).” EX1010, 2, 3 (“We designed a set of 48 primer pairs to amplify 5995 bases of genomic sequence covering coding regions (exons and exon junctions) of TP53 and PTEN, and selected regions in EGFR, BRAF, KRAS, and PIK3CA (table S1) by overlapping short amplicons (Fig. 1A).”). A POSA would know that these are target regions in genes associated with cancer. EX1003, ¶187. Forshew used this approach to directly identify mutations in plasma of cancer patients. EX1010, 4, Tables 1-2.

8. Claim 20: “The method of claim 13, further comprising filtering out sequencing reads from among the set of sequencing reads that fail to meet a quality threshold.”

Schmitt teaches Claim 20. EX1003, ¶188-192.

Schmitt discloses using “[a] standard sequencing approach with quality filtering for a Phred score of 30” EX1005, 24:66-67. A POSA would have understood that a Phred score is used to indicate the measure of base quality in DNA sequencing. EX1003 ¶190.

9. Claim 21: “The method of claim 13, further comprising mapping a plurality of sequencing reads from the set of sequencing reads to a reference sequence.

Schmitt, alone or with Forshew, teaches Claim 21. EX1003 ¶193-196.

The Board agreed that Schmitt teaches “mapping the sequence reads to a reference sequence.” EX1083, p.32, citing EX1005, 20:39-64, 23:10-14, 24:33-37. According to Schmitt, “[r]eads were aligned to the human genome with the Burrows Wheeler Aligner (BWA).” EX1005, 20:39-64; *see also id.*, 23:10-14 (referring to the well-known “hg19” human reference genome). A POSA would have understood that Schmitt’s aligning sequence reads to the human genome constitutes mapping of the sequence reads to a reference sequence. EX1003 ¶194.

Forsheew likewise teaches that sequence reads are mapped to a reference sequence before grouping. EX1010, SI 19 (mapping to “the hg19 reference genome using ELAND (Illumina) . . . to identify somatic mutations”); *id.*, SI 4 (reads aligned “to the hg19 reference genome using bwa-short in the single-end mode”). As discussed previously, it would have been obvious to combine the teachings of Forsheew with Schmitt’s DCS methods. EX1003 ¶195.

- 10. Claim 22: “The method of claim 21, further comprising grouping a subset of sequencing reads into families based on sequence information of the molecular barcodes and (1) a start base position of a given sequencing read from among the subset of sequencing reads at which the given sequencing read is determined to start mapping to the reference sequence or (2) a stop base position of the given sequencing read at which the given sequencing read is determined to stop mapping to the reference sequence.”**

Schmitt teaches Claim 22. EX1003, ¶¶197-201.

The Board previously found Schmitt teaches “grouping the sequence reads into families, each of the families comprising sequence reads comprising the same identifier sequence and having the same start and stop positions, whereby each of the families comprises sequence reads amplified from the same tagged parent polynucleotide.” EX1083, p.32.

In particular, Schmitt teaches that in its “hybrid” DCS method, sequences can be grouped by “[c]ombining information regarding the shear points of DNA with the SMI tag sequence”—i.e., the barcode. EX1005, 17:61-18:2; *see also id.*, 9:9-14, 17:41-61; *see also* EX1083, p.39 (referring to Schmitt’s disclosure of combining “sequence information from the sheared ends with the short n-mer tag” to achieve a “unique molecular identifier”). A POSA would have understood: (1) the “shear points” to refer to the start and stop positions of the fragment—its ends¹²—and (2) grouping based on start and stop positions provides an extra level of information in establishing families of amplicons arising from the same tagged parent polynucleotide, beyond just the identifier sequence. EX1003, ¶199.

Thus, a POSA would have understood that Schmitt’s “hybrid” method describes grouping reads into families wherein each family comprises sequence reads having the same identifier sequence (barcode) *and* the same sequences at both

¹² The Board found “library generation, shearing (or not shearing), and ligating adaptors to DNA fragments were well known and routine steps in the art before the earliest priority data for the ’822 patent.” EX1083, 41.

ends (start, stop positions) of the sequence derived from the original fragment. *Id.*, ¶¶199-200.

11. Claim 23: “The method of claim 22, further comprising, for a plurality of the families, collapsing sequencing reads within a given family of the plurality of the families to generate consensus sequences.”

Schmitt teaches Claim 23. EX1003, ¶¶202-203.

The Board previously found Schmitt teaches, “at each genetic locus of a plurality of genetic loci in the one or more reference sequences, collapsing sequence reads in each family to yield a base call for each family at the genetic locus.” EX1083, p.32. A POSA would have understood that each consensus sequence provides a consensus base call for each family at a position with sufficient coverage and agreement to form a consensus. EX1003, ¶203.

Schmitt teaches that each family of reads is collapsed to generate a consensus sequence. EX1005, 20:50-52 (“Reads having common (i.e., identical) SMI sequences were grouped together, and were collapsed to generate a consensus read.”). A POSA would have understood that a consensus read refers to a consensus sequence. EX1003, ¶204. Schmitt explains that to generate a consensus sequence, “[s]equencing positions were discounted if the consensus group covering that position consisted of fewer than 3 members or if fewer than 90% of the sequences at that position in the consensus group had the identical sequence.” EX1005, 20:53-56.

12. Claim 24: “The method of claim 23, further comprising identifying one or more consensus sequence having the genetic variation as compared to the reference sequence.”

Schmitt with Forshew teaches Claim 24. EX1003, ¶¶204-208.

Schmitt teaches that the DCS method allows quantification of true mutations by detecting “the presence of a true mutation (as opposed to a PCR error or other artifactual mutation) in a target nucleic acid sequence.” EX1005, 16:43–47. Schmitt teaches that the ability to detect a true mutation further confers “the ability to indirectly infer that damage is present on the DNA could be useful biomarker” including “for cancer risk, cancer metabolic state, mutator phenotype related to defective damage repair.” EX1005, 15:42-51. A POSA would understand that Schmitt’s reference to “damage” as a marker of “cancer risk,” teaches identifying a consensus sequence having a genetic variation compared to a reference sequence. EX1003, ¶205.

In addition, Forshew describes using “deep sequencing” of ctDNA to screen bases for low-frequency mutations to directly identify mutations in plasma of cancer patients. EX1010, 4, Tables 1-2. Forshew specifically teaches the identification of mutations compared to a reference sequence. *Id.*, SI 4, SI 19. Further, Porreca teaches the detection of genetic variations in microsatellite regions based on comparisons to a reference sequence. EX1075, ¶[0047]. Claim 24 is thus obvious in light of Schmitt’s DCS combined with Forshew and Porreca. EX1003, ¶208.

13. Claim 25: “The method of claim 13, wherein the sample of cell-free nucleic acid molecules comprises 1 nanogram (ng) to 100 ng of cell-free nucleic acid molecules.”

Schmitt with Forshew teaches Claim 25. EX1003, ¶¶209-212.

Forshew extracted cfDNA from between 0.85 and 2.2 ml of plasma. EX1010, 10. Table S6 lists the estimated amount of cell-free DNA sequenced from a number of patients, which ranged from 0.9 ng to 19.7 ng. *Id.*, Table S6.

14. Claim 26: “The method of claim 13, wherein the cell-free nucleic acid molecules comprise cell-free deoxyribonucleic acid (cfDNA) molecules.”

Schmitt with Forshew teaches Claim 26. EX1003, ¶¶213-215. *See* 13[pre] (Section IX.C.1.a).

The Board previously found that Forshew teaches “providing a population of cell-free (‘cfDNA’) molecules obtained from a bodily sample from a subject.” EX1083, p.29.

15. Claim 27: “The method of claim 13, further comprising determining a confidence score based on the genetic variation detected in the one or more microsatellite regions.”

Schmitt alone or with Porreca teaches Claim 27. EX1003, ¶¶216-219. The ’916 patent admits there were “known statistics of typical variances at reported positions in non-disease reference sequences,” which can be used to determine a confidence score for a detected mutation. EX1001, 51:5-8. Thus, it would have

been obvious to determine a confidence score based on a genetic variation detected in a microsatellite region. EX1003, ¶217.

Schmitt teaches that the DCS method allows quantification of true mutations by detecting “the presence of a true mutation (as opposed to a PCR error or other artifactual mutation) in a target nucleic acid sequence.” EX1005, 16:43–47. Given Schmitt’s teachings of the importance that the detection of the mutation be “true,” a POSA would have been motivated to determine a confidence score for a mutation call based on DCS. EX1003, ¶218. A confidence score would allow a POSA to evaluate the reliability of the mutation call. *Id.* For example, Schmitt teaches that the background error of DCS may be calculated: “the background (artifactual) error frequency of DCS may be calculated as: (probability of error on one strand)*(probability of error on other strand)*(probability that both errors are complementary).” A POSA would know that a confidence score can be computed by comparing the observed signal to the expected background error. *Id.*

To the extent Schmitt alone does not teach “determining a confidence score” based on the detected variation, it would have been obvious to combine Schmitt’s DCS methods for detecting mutations with Porreca’s teaching “determining a statistical confidence for the genotype based on the number of unique combinations of target nucleic acid and differentiator tag sequences.” EX1075, Claim 16. A

POSA would be motivated to do so because a confidence score allows them to evaluate the reliability or certainty of the detected genetic variation. EX1003, ¶219.

16. Claim 28: “The method of claim 13, further comprising determining a treatment regimen for the subject based on the quantitative measure of polymorphic forms comprising microsatellite changes in the one or more microsatellite regions.”

Schmitt with Porreca teach Claim 28. EX1003, ¶220-223. The ’916 patent admits it was well-known genetic alterations in cfDNA could be used for early detection and monitoring of cancer, including for treatment and disease management. EX1001, 1:39-51.

Schmitt teaches DCS methods provide “the ability to indirectly infer that damage is present on the DNA could be useful biomarker” including “for cancer risk, cancer metabolic state, mutator phenotype related to defective damage repair.” EX1005, 15:42-51. It was known that “MSI testing can provide useful prognostic information in CRC patients and inform decision-making with regard to adjuvant therapy,” including for treatment selection. EX1080, 3.

Further, Porreca teaches “methods for detecting nucleic acid deletions or insertions in regions containing nucleic acid sequence repeats,” EX1075, ¶[0157] including “cancer, which has been associated with microsatellite instability (MSI).” *Id.* ¶[0160]. Porreca also teaches that the detection of insertions and deletions, such

as MSIs, can be used for treatment selection. *Id.*, ¶ [0017] (“diagnosis, prognosis, or disease risk assessment”).

It would have been obvious to combine Schmitt with Porreca to determine a treatment regime for the subject based on the quantitative measure of polymorphic forms comprising microsatellite changes in the one or more microsatellite regions. EX1003, ¶223.

17. Claim 29: “The method of claim 28, further comprising administering the treatment regimen to the subject.”

Schmitt with Porreca teach Claim 29. EX1003, ¶224. As explained above, Schmitt with Porreca teach determining a treatment regimen. *See* Claim 28 (Section IX.C.16). Porreca teaches using detected genetic variation to aid patient treatment: “genetic information from [] circulating tumor cells is used to determine prognosis and *guide selection of appropriate drugs/treatments.*” EX1075 at ¶[0017] (emphasis added). Porreca’s selection of appropriate treatments teaches administering the treatment to the subject. EX1003, ¶224.

18. Claim 30

(a) 30[pre]

See 13[pre] (Section IX.C.1.a), Claim 26 (Section IX.C.14) *supra*; EX1003, ¶225.

(b) 30[a]

See 13[a] (Section IX.C.1.b), Claim 16 (Section IX.C.4) *supra*; EX1003, ¶226.

(c) 30[b]

See 13[b] (Section IX.C.1.c) *supra*; EX1003, ¶227.

(d) 30[c]

See 13[c] (Section IX.C.1.d) *supra*; EX1003, ¶228.

(e) 30[d]

See 22-23 (Section IX.C.10-11) *supra*; EX1003, ¶229.

(f) 30[e]

See 13[d] (Section IX.C.1.e) *supra*; EX1003, ¶230.

X. GROUND 2: CLAIMS 13-30 ARE OBVIOUS OVER SCHMITT IN VIEW OF FORSHEW AND SACKO

A. Motivation To Combine

A POSA would have been motivated to combine Schmitt and Forshew. *See* Section IX.A, *supra*. In addition, POSA would have been motivated to combine Schmitt and Forshew with Sacko. EX1003, ¶¶234-237.

Schmitt teaches detection and quantification of MSIs using its DCS method. EX1005, 15:42-51; *see* Section IX.A, *supra*.

To the extent Schmitt alone does not disclose teach detection and quantification of MSIs, Sacko teaches “DNA extracted from the plasma of cancer patients usually has tumoral DNA characteristics [] such as . . . microsatellite

alterations.” EX1076, ¶[0005]. A POSA, seeking to improve the detection and quantification of genomic instability in a minimally-invasive sample type such as cfDNA (e.g., for diagnostic or therapeutic testing purposes), would have found it obvious to apply Sacko’s teachings about the clinical utility of MSIs in ctDNA in blood plasma within the high sensitivity cfDNA analysis framework taught by Schmitt and Forshew, which are each capable of detecting low prevalence mutations. EX1003 ¶236. A POSA would be motivated to combine these teachings—especially in view of Sacko’s discussions of the importance of sensitivity for cfDNA measurements, (*see* EX1076, ¶¶[0014]-[0017], [0258]-[0259])—to yield a reliable and quantitative measure of microsatellite changes in ctDNA. EX1003 ¶237.

This combination would have been further motivated by the increasing awareness and use of MSI as an informative biomarker for cancer diagnosis and therapy selection, as described by, among other references, Sacko. EX1076, ¶[0006]; EX1005, 1:26–35; EX1003 ¶237. The use of cfDNA for non-invasive cancer detection and analysis was well-known, and a POSA would have understood that applying MSI detection techniques to cfDNA, either on alone or in combination with detecting other types of variants, could provide a powerful and accurate clinical tool. EX1003 ¶237. Sacko is analogous art to Schmitt, Forshew, and the ’916 patent (as recognized by the Examiner during prosecution) because Sacko is also directed to identifying genetic mutations indicative of health issues, including cancer. *Id.*

B. Reasonable Expectation of Success

A POSA would have had a reasonable expectation of success in combining Schmitt and ForsheW for the reasons above. *See* Section IX.B, *supra*.

In addition, a POSA would have had a reasonable expectation of success in combining Schmitt and ForsheW with Sacko to determine a quantitative measure of microsatellite changes in a cfDNA sample. EX1003, ¶239. The use of cfDNA with well-known NGS and bioinformatics techniques for detecting genetic alterations was known and specifically taught by Schmitt and ForsheW. The presence of microsatellite alterations in ctDNA in blood plasma was well-known and described by Sacko. EX1076, ¶[0005]; EX1003, ¶239.

A POSA understood that microsatellite loci are readily detectable using the sequencing and analysis methods taught by Schmitt, and that the presence of MSIs in cfDNA, as described by Sacko, could be quantitatively assessed through those same NGS-based techniques. EX1003, ¶239. Because the combination involves applying established detection principles to a known analyte (cfDNA) and a well-characterized target (microsatellite regions), a POSA would have had a high degree of confidence in achieving the desired outcome without undue experimentation.

The proposed combination would have involved a predictable use of known techniques to address a recognized problem using conventional tools. Sacko, ForsheW, and Schmitt rely on routine molecular biology and sequencing methods

that were widely used in the art. EX1003, ¶240. Schmitt provides the necessary sample preparation, sequencing, and computational tools to analyze cfDNA (EX1005, 7:1–45; 11:8–30), while Sacko provides the rationale and methodology for interpreting microsatellite variations in cfDNA. EX1003, ¶241. POSA would have appreciated that Sacko’s teaching of MSI presence in cfDNA could be quantified using Schmitt’s analytical techniques (combined with Forshew, as discussed) to yield not only qualitative MSI status but also a quantitative measure of microsatellite change, thereby offering improved clinical resolution. EX1003, ¶241.

Accordingly, the combination would have been obvious and well-within the capabilities of a POSA without undue experimentation.

C. Claim-by-Claim Analysis

1. Claim 13

(a) 13[pre]

To the extent this preamble is limiting, it is taught by Schmitt with Forshew and Sacko. *See* 13[pre] (Section IX.C.1.a), Claim 26 (Section IX.C.14) *supra*; EX1003, ¶¶242-243.

To the extent Schmitt with Forshew does not teach “detecting a genetic variation in one or more microsatellite regions,” a POSA would have found it obvious based on Sacko. *See, e.g.*, EX1076, ¶[0005]. The clinical relevance of evaluating microsatellite changes from a subject having cancer was well-known.

EX1003, ¶243. Schmitt teaches that quantification of DNA defects allows for MSI detection. *Id.*

Given Sacko's teachings that cfDNA could be used to screen for "microsatellite mutations and instabilities," it would have been obvious to combine Sacko with Schmitt and Forsheo to practice a method for detecting a genetic variation in one or more microsatellite regions in a sample of cfDNA from a subject having cancer. *Id.*

(b) 13[d]

Schmitt alone or with Forsheo and Sacko teaches this limitation. *See* 13[d] (Section IX.C.1.e) *supra*; EX1003, ¶¶244-245.

To the extent Schmitt alone or with Forsheo does not teach this limitation, it would have been obvious to combine with Sacko. From Sacko, a POSA knew that cancer mutations of particular interest include MSIs found in ctDNA. EX1076, ¶[0005] ("microsatellite mutations and instabilities detected in the free genomic DNA of the serum suggest that it could be a new potential marker, with considerable specificity for monitoring tumors"); EX1003, ¶245. A POSA would have known based on Sacko that cancer mutations of particular interest to apply to Schmitt's DCS methods include MSIs found in ctDNA.

2. Claim 26

Schmitt with Forshew and Sacko teach Claim 26. *See* Claim 26 (Section IX.C.14) *supra*; EX1003, ¶¶246-247.

Sacko discloses “DNA extracted from the plasma of cancer patients usually has tumoral DNA characteristics [citing Anker P. et al., 1999] such as strand instability. . . and microsatellite alterations.” EX1076, ¶[0005]. Moreover, Sacko explains that MSIs in “free genomic DNA of the serum” could be a “new potential marker,” including for monitoring tumors, (*id.*), and the application for these markers such as “complement the analysis of markers commonly used to monitor patients suffering from cancers or undergoing chemotherapy,” (*id.*, ¶[0006]).

3. Claim 28

Schmitt with Forshew and Sacko teach Claim 28. EX1003, ¶¶248-249. *See* Claim 28 (Section IX.C.16) *supra*; EX1003, ¶¶248-249.

Sacko explains that MSIs could be a “new potential marker” (EX1076, ¶[0005]), that could “complement the analysis of markers commonly used to monitor patients suffering from cancers or undergoing chemotherapy,” (*id.* at ¶[0006]). It would have been obvious to combine these teachings with Schmitt and Forshew to determine a treatment regime for the subject based on the quantitative measure of polymorphic forms comprising microsatellite changes in the one or more microsatellite regions. EX1003, ¶249.

4. Claim 29

Schmitt with Sacko teach Claim 29. EX1003, ¶250. As explained above, Schmitt with Sacko teach determining a treatment regimen. *See* Claim 28 (Section X.C.3). Sacko teaches MSIs could “complement the analysis of markers commonly used to monitor patients suffering from cancers or undergoing chemotherapy.” EX1076, ¶[0006]. Sacko’s referencing MSI markers to monitor ongoing chemotherapy therefore teaches administering a determined treatment. EX1003, ¶250.

5. Claim 30

(a) 30[pre]

See 13[pre] (Section X.C.1.a), 26 (Section X.C.2) *supra*; EX1003, ¶¶242-245.

(b) 30[e]

See 13[d] (Section X.C.b) *supra*; EX1003, ¶¶244-245.

6. Remaining Limitations and Claims

The remaining limitations of claim 13, and claims 14-25, 27, and 30, are rendered obvious by this ground for the same reasons discussed above for Ground 1. EX1003, ¶251.

XI. 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 '916 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”), 11,149,306 (the “'306 patent”), and 11,643,693 (the “'693 patent”).

Petitioner is unaware of other related matters before the Patent Office with respect to the '916 Patent. However, contemporaneous with this Petition, Petitioner is also seeking institution of IPRs against the '306 patent.

Related patents have previously been challenged in Inter Partes Review: IPR2019-00634 (U.S. Patent 9,840,743) and IPR2019-00652 (U.S. Patent 9,834,822). Both petitions were instituted and the challenged claims were at least partially cancelled by the Board.

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 for *Inter Partes* Review to Deposit Account No. 50-5708. Any additional fees that might be due are also authorized.

XII. 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 12,827 words, which is within the word limit allowed under 37 C.F.R. § 42.24(a)(i).

Date: August 15, 2025

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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 '916 patent as shown in USPTO PAIR:

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