UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

TWINSTRAND BIOSCIENCES, INC.
Petitioner,

v.

GUARDANT HEALTH, INC.
Patent Owner.

Case IPR2022-01400
U.S. Patent No. 11,149,306

PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 11,149,306

Mail Stop "PATENT BOARD"
Patent Trial and Appeal Board
U.S. Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

TABLE OF CONTENTS

I. Introduction 1

II. Statement of Precise Relief Requested and Reasons Therefor (37 C.F.R. §42.22(A)) 2

III. State of the art before December 2013 2

- A. Optimization techniques for DNA library preparation were well known. 2
- B. Cell-free DNA isolated from blood was widely used in NGS platforms. 5
  - 1. The presence of cell-free tumor DNA in human blood was well known. 5
  - 2. Isolating cfDNA from blood was routine with off-the-shelf kits. 6
- C. The prior art taught that Duplex Sequencing dramatically lowers NGS error rate. 7
- D. The prior art taught applying Duplex Sequencing to cfDNA. 13

IV. The '306 patent and its prosecution history 14

V. Person of ordinary skill in art 18

VI. Claim construction 18

VII. Identification of the challenge (37 C.F.R. §42.104(b)) 19

VIII. The facts and law weigh against discretionary denial of institution. 21

- A. This Petition satisfies 35 U.S.C. §325(d). 21
- B. The *Fintiv* factors do not support discretionary denial. 24

IX. Ground 1: claims 1-3, 5, 7, 9-14, 17-27, and 29 would have been obvious over Narayan and Schmitt 25

- A. Claim 1 26
  - 1. "A method, comprising: (a) providing a population of cell-free deoxyribonucleic acid (cfDNA) molecules having first and second complementary strands…" 26
  - 2. "(b) tagging a plurality of the cfDNA molecules in the population with duplex tags comprising molecular barcodes to produce tagged parent polynucleotides, wherein the duplex tags are attached to both ends of a molecule of the plurality of the cfDNA molecules,

wherein the plurality of the cfDNA molecules are tagged with n different combinations of molecular barcodes, wherein n is at least 2 and no more than 100,000*z, wherein z is a mean of an expected number of duplicate molecules in the population of cfDNA molecules that map to identical start and stop positions on a reference sequence…” 26

3. “(c) amplifying a plurality of the tagged parent polynucleotides to produce amplified progeny polynucleotides...” 29

4. “(d) sequencing at least a subset of the amplified progeny polynucleotides to produce a set of sequence reads…” 30

5. “(e) reducing or tracking redundancy of a plurality of sequence reads from the set of sequence reads using at least sequencing information from the molecular barcodes of the duplex tags to determine distinct cfDNA molecules from among the tagged parent polynucleotides, wherein the distinct cfDNA molecules are determined based on (i) paired reads corresponding to sequence reads generated from a first tagged strand and a second tagged complementary strand derived from cfDNA molecules from among the tagged parent polynucleotides, or (ii) unpaired reads corresponding to sequence reads generated from a first tagged strand having no second tagged complementary strand derived from cfDNA molecules from among the tagged parent polynucleotides, wherein reducing or tracking the redundancy of the plurality of sequence reads comprises mapping at least a subset of the plurality of sequence reads to the reference sequence.” 30

6. A POSA would have had a reason to combine Narayan and Schmitt. 34

7. A POSA would have had a reasonable expectation of success. 37

B. Claim 17 39

1. “A method, comprising: (a) tagging a population of double-stranded cell-free deoxyribonucleic acid (cfDNA) molecules obtained or derived from a sample

of a subject with a set of tags comprising molecular barcodes to produce tagged parent polynucleotides…” 40

2. “(b) amplifying a plurality of the tagged parent polynucleotides to produce amplified progeny polynucleotides…” 41

3. “(c) sequencing at least a subset of the amplified progeny polynucleotides to produce a set of sequence reads…” 41

4. “(d) sorting a plurality of sequence reads from the set of sequence reads into (i) families comprising paired reads corresponding to sequence reads generated from a first tagged strand and a second tagged complementary strand derived from double-stranded cfDNA molecules from among the tagged parent polynucleotides, and (ii) families comprising unpaired reads corresponding to sequence reads generated from a first tagged strand having no second tagged complementary strand derived from double-stranded cfDNA molecules from among the tagged parent polynucleotides.” 41

C. Claims 2 and 18 44
D. Claims 3 and 19 45
E. Claims 5 and 20 46
F. Claim 7 47
G. Claim 21 48
H. Claims 9-10 and 22-23 48
I. Claim 11 50
J. Claim 12 51
K. Claim 13 52
L. Claims 14 and 27 53
M. Claim 24 55
N. Claim 25 56
O. Claim 26 57
P. Claim 29 58

X. Ground 2: claims 4 and 6 would have been obvious over Narayan, Schmitt, and Meyer 58

XI. Ground 3: claim 8 would have been obvious over Narayan, Schmitt, and Craig 61

XII. Ground 4: claims 15, 16, and 28 would have been obvious over Narayan, Schmitt, and Kivioja. 63

A. Claims 15 and 28 63

B. Claim 16 67

XIII. Objective indicia do not support patentability. 69

XIV. Certification of standing and IPR eligibility (37 C.F.R. §42.104(a)) 69

XV. Mandatory Notices (37 C.F.R. §42.8(a)(1)) 69

XVI. Conclusion. 72

TABLE OF AUTHORITIES

Cases

Abbott Labs. v. Sandoz, Inc.,
544 F.3d 1341 (Fed. Cir. 2008) 62

Advanced Bionics, LLC v. MED-EL Elektromedizinische Geräte GmbH,
IPR2019-01469 (P.T.A.B., Feb, 13, 2020) 21

Amgen v. Hoffman-La Roche,
580 F.3d 1340 (Fed. Cir. 2009) 23

Apple Inc. v. Fintiv, Inc.,
IPR2020-0019, Paper 11 (P.T.A.B., March 20, 2020) 25, 27

Bayer Healthcare Pharms., Inc. v. Watson Pharms., Inc.,
713 F.3d 1369 (Fed. Cir. 2013) 68

Bristol-Myers Squibb Co. v. Teva Pharms. USA, Inc.,
752 F.3d 967 (Fed. Cir. 2014) 23

Dynamic Drinkware, LLC v. Nat'l Graphics, Inc.,
800 F.3d 1375 (Fed. Cir. 2015) 4, 21

Grunenthal GMBH v. Anticep Bioventures II LLC,
2020 WL 4342293 (P.T.A.B. July 28, 2020) 23

Hanwha Sols. Corp. v. Rec Solar PTE. Ltd.,
IPR2021-00988, Paper 12 (P.T.A.B. Dec. 13, 2021) 26

In re Koller,
613 F.2d 819 (CCPA 1980) 23

Medtronic, Inc. v. Niazi Licensing Corp.,
IPR2018-00609, Paper 8 (P.T.A.B., Aug. 20, 2018) 21

Ormco Corp. v. Align Technology, Inc.,
463 F. 3d 1299 (Fed. Cir. 2006) ....passim

OSI Pharms., LLC v. Apotex Inc.,
939 F.3d 1375 (Fed. Cir. 2019) 23

Renesas Elec. Corp. v. Lone Star Silicon Innov. LLC,
2019 WL 1373644 (P.T.A.B. March 25, 2019) 24

Velander v. Garner,
348 F.3d 1359 (Fed. Cir. 2003) 23

Yeda Research v. Mylan Pharms, Inc.,
906 F.3d 1031 (Fed. Cir. 2018) 23

Statutes

35 U.S.C. §102 20

35 U.S.C. §103 ....2, 20

35 U.S.C. §112 21

35 U.S.C. §325..21, 24

Rules

37 C.F.R. §42.10(b) 71

37 C.F.R. §42.100..18

37 C.F.R. §42.106(a) 71

37 C.F.R. §42.22.. 2

37 C.F.R. §42.63(e) 71

37 C.F.R. §42.8(b) 69, 70, 71

EXHIBIT LIST

Exhibit #	***Description***
1001	Talasaz, A. and Mortimer, S.A.W., “Methods And Systems For Detecting Genetic Variants,” U.S. Patent No. 11,149,306 (filed July 31, 2020; issued October 19, 2021)
1002	Declaration of Paul T. Spellman, Ph.D.
1003	*Curriculum Vitae* for Paul T. Spellman, Ph.D.
1004	Murtaza, M., *et al*., “Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA,” *Nature* 497: 108-112 (2013)
1005	Meyer, M., *et al*., “Parallel tagged sequencing on the 454 platform,” *Nature Protocols* 3(2): 267-278 (2008)
1006	Kivioja, T., *et al*., “Counting absolute numbers of molecules using unique molecular identifiers,” *Nature Methods* 9(1): 72-76 (2012)
1007	Craig, D.W., *et al*., “Identification Of Genetic Variants Using Barcoded Multiplexed Sequencing”, *Nature Methods* 5:887–893 (2008)
1008	Kukita, Y., *et al*., “Quantitative Identification of Mutant Alleles Derived from Lung Cancer in Plasma Cell-Free DNA via Anomaly Detection Using Deep Sequencing Data,” *PLOS One* 8(11): 1-31 (2013)
1009	Schmitt, M., *et al*., “Method of Lowering the Error Rate of Massively Parallel DNA Sequencing Using Duplex Consensus Sequencing,” International Publication Number WO2013/142389 (filed on March 15, 2003; published on September 26, 2013)
1010	Alberts, B., et al., *Eds*., “Chapter 4: DNA and Chromosomes,” and “Chapter 8: Manipulating Protein, DNA and RNA”, *Molecular Biology of the Cell*, pp. 191-234 and pp. 469-546, Fourth Edition, Garland Science, United States (2002)
1011	Metzker, M.L., “Sequencing technologies — the next generation,” *Nature Reviews* 11:31-46 (2010)
1012	Mardis, E.R., “Next-Generation Sequencing Platforms,” *Annu. Rev. Anal. Chem.* 6:287–303 (2013)
1013	Franca, L.T.C., *et al*., “A Review of DNA Sequencing techniques,” *Quarterly Reviews of Biophysics* 35(2): 169-200 (2002)
1014	Ong, J., *et al*., “Overview of the Agilent Technologies SureSelectTM Target Enrichment System,” *Journal of Biomolecular Techniques,* 22(Suppl.): S30 (2011)

Exhibit #	***Description***
1015	Technical Data Sheet, KAPA HTP Library Preparation Kit Illumina® platforms, KAPA Biosystems (July 2013)
1016	Rohland, N. and Reich, D., "Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture," *Genome Research* 22:939–946 (2012)
1017	Zheng, Z., "Titration-free 454 sequencing using Y adapters," *Nature Protocols* 6(9): 1367-1376 (2011)
1018	Glenn, T.C., "Field guide to next-generation DNA sequencers," *Molecular Ecology Resources* 11: 759–769 (2011)
1019	Neiman, M., *et al.*, "Library Preparation and Multiplex Capture for Massive Parallel Sequencing Applications Made Efficient and Easy," *PLOS ONE* 7(11): e48616 (2012)
1020	Blumenstiel, B., *et al.*, "Targeted Exon Sequencing by In-Solution Hybrid Selection," *Current Protocols in Human Genetics* 18.4.1-18.4.24 (2010)
1021	So, A.P., *et al.*, "Increasing the efficiency of SAGE adaptor ligation by directed ligation chemistry," *Nucleic Acids Research* 32(12): e96 (2004)
1022	van Nieuwerburgh, F., *et al.*, "Quantitative Bias in Illumina TruSeq and a Novel Post Amplification Barcoding Strategy for Multiplexed DNA and Small RNA Deep Sequencing," *PLoS ONE* 6(10): e26969 (2011)
1023	Vennison, S.J., "Chapter 5: Restriction Digestion and Ligation of DNA," in *Laboratory Manual for Genetic Engineering*, pp. 32-35, Eastern Economy Edition, PHI Learning Private Limited, New Delhi (2009)
1024	Meyer, M., and Kircher, M. "Illumina sequencing library preparation for highly multiplexed target capture and sequencing." *Cold Spring Harbor Protocols* 2010.6 (2010): pdb-prot5448.
1025	U.S. Provisional Appl. No. 61/798,925
1026	Diehn, M., *et al.*, "Identification And Use Of Circulating Tumor Markers," U.S. Patent Publication No. 2014/0296081 (filed March 13, 2014; published Oct. 2, 2014)
1027	Quail, M.A., "A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers," *BMC Genomics* 3:341- (2012)
1028	TruSeq™ DNA Sample Preparation Guide, Illumina (November 2010)

Exhibit #	***Description***
1029	Park, N., *et al*., "An improved approach to mate-paired library preparation for Illumina sequencing," *Methods in Next Generation Sequencing* 1(1): 10-20 (2013)
1030	Costa, G.L., *et al*., "Methods for Preparing Single-Stranded DNA Libraries," U.S. Patent Appl. Publ. No. 2004/0185484 A1 (filed December 13, 2019; published January 12, 2021)
1031	Fisher, S., *et al*., "A scalable, fully automated process for construction of sequence-ready human exome targeted capture libraries," *Genome Biology* 12:R1 (2011)
1032	Heitzer, E., *et al*., "Tumor-associated copy number changes in the circulation of patients with prostate cancer identified through whole-genome sequencing ," *Genome Medicine* 5:30-46 (2013)
1033	Mertes, F., *et al*., "Targeted enrichment of genomic DNA regions for next-generation sequencing," *Briefings In Functional Genomics* 10(6): 374 -386 (2011)
1034	Mamanova, L., *et al*., "Target-enrichment strategies for next generation Sequencing," *Nature Methods* 7(2): 111–118 (2010)
1035	Bentley, D.R., *et al*., "Accurate whole human genome sequencing using reversible terminator chemistry," *Nature* 456:53-359 (2008)
1036	Grada, A. and Weinbrecht, K., "Next-Generation Sequencing: Methodology and Application," *Journal of Investigative Dermatology* 133:1-4 (2013)
1037	Gogol-Doring, A. and Chan, W., "Chapter 16: An Overview of the Analysis of Next Generation Sequencing Data," in *Next Generation Microarray Bioinformatics Methods and Protocols*, Wang, J. ed., pp. 249-257, Humana Press United States (2012)
1038	Li, H. and Durbin, R., "Fast and accurate short read alignment with Burrows–Wheeler Transform," *Bioinformatics* 25(14): 1754–1760 (2009)
1039	Forshew, T., *et al*., "Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA," *Sci Transl Med* 4(136): 136ra68 (2012)
1040	Crowley, E., *et al*., "Liquid biopsy: monitoring cancer-genetics in the blood," *Nat. Rev. Clin. Oncol*. 10: 472-484 (2013)
1041	Benesova, L., *et al*., "Mutation-based detection and monitoring of cell-free tumor DNA in peripheral blood of cancer patients," *Analytical Biochemistry* 433: 227–234 (2013)

Exhibit #	***Description***
1042	Koffler, D., *et al*., "The Occurrence of Single-Stranded DNA in the Serum of Patients with Systemic Lupus Erythematosus and Other Diseases," *Journal of Clinical Investigation* 52: 198-204 (1973)
1043	Leon, S.A., *et al*., "Free DNA in the Serum of Cancer Patients and the Effect of Therapy," *Cancer Research* 37: 646-650 (1977)
1044	Goessl, C., "Diagnostic potential of circulating nucleic acids for oncology," *Expert Rev. Mol. Diagn.* 3(4), (2003)
1045	Diehl, F., *et al*., "Detection and quantification of mutations in the plasma of patients with colorectal tumors," *PNAS* 102(45): 16368–16373 (2005)
1046	Perkins, G., "Multi-Purpose Utility of Circulating Plasma DNA Testing in Patients with Advanced Cancers," *PLoS One* 7(11): 1-9 (2012)
1047	Jahr, S., *et al*., "DNA Fragments in the Blood Plasma of Cancer Patients: Quantitations and Evidence for Their Origin from Apoptotic and Necrotic Cells," *Cancer Research* 61: 1659–1665 (2001)
1048	Heitzer, E., *et al*., "Establishment of tumor-specific copy number alterations from plasma DNA of patients with cancer," *Int. J. Cancer* 133, 346–357 (2013)
1049	Blood Drawing For Human Subject Research, Duke University Health System (December 13, 2012), downloaded from https://irb.duhs.duke.edu/sites/irb.duhs.duke.edu/files/Blood_Collect_Policy_Statement_12-13-2012.pdf, last visited March 22, 2022
1050	Dawson, S-J., *et al*., "Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer," *N Engl J Med* 368(13): 1199-1209 (2013) with Supplementary Appendix
1051	Chan, K.C.A., *et al*., "Cancer Genome Scanning in Plasma: Detection of Tumor-Associated Copy Number Aberrations, Single-Nucleotide Variants, and Tumoral Heterogeneity by Massively Parallel Sequencing," *Clin. Chemistry* 59(1): 211–224 (2013)
1052	Leary, R.J., *et al.,* "Detection of Chromosomal Alterations in the Circulation of Cancer Patients with Whole-Genome Sequencing," *Sci Transl Med.* 4(162): 1-12 (2012)
1053	Fan, H.C., *et al*., "Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood," *PNAS* 105(42): 16266 –16271 (2008)

Exhibit #	***Description***
1054	Sehnert, A.J., *et al*., "Optimal Detection of Fetal Chromosomal Abnormalities by Massively Parallel DNA Sequencing of Cell-Free Fetal DNA from Maternal Blood," *Clinical Chemistry* 57:7 1042–1049 (2011)
1055	Chiu, R.W.K., *et al*., "Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study," *BMJ* 342:c7401 (2011)
1056	Mitchell, P.S., *et al*., "Circulating microRNAs as stable blood-based markers for cancer detection," *PNAS* 105(30): 10513–10518 (2008)
1057	Ehrich, M., *et al*., "Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting," *American Journal of Obstetrics & Gynecology* 205.e1-205.e11 (2011)
1058	Swanson, A., *et al*., "Non-invasive prenatal testing for fetal aneuploidy by massively parallel DNA sequencing of maternal plasma: the future has arrived today," Molecular-Genetic and Cytogenetic Diagnostics *J Lab Med* 36(5): 269–275 (2012)
1059	Fan, H.C., *et al*., "Analysis of the Size Distributions of Fetal and Maternal Cell-Free DNA by Paired-End Sequencing," *Clinical Chemistry* 56(8): 1279–1286 (2010)
1060	Kuang, Y., *et al*., "Noninvasive Detection of *EGFR* T790Min Gefitinib or Erlotinib Resistant Non-Small Cell Lung Cancer," *Clin Cancer Res* 15(8): 2530-2636 (2009)
1061	Schwartzenbach, H., *et al*., "Cell-free nucleic acids as biomarkers in cancer patients," *Nature* 11: 426-437 (2011)
1062	Fan, H.C., *et al*., "Non-invasive prenatal measurement of the fetal genome," *Nature* 487: 320–324 (2012)
1063	Faircloth, B.C. and Glenn, T.C., "Not All Sequence Tags Are Created Equal: Designing and Validating Sequence Identification Tags Robust to Indels," *PLOS ONE* 7(8): e42543 (2012)
1064	Schmitt, M.W., *et al*., "Detection of ultra-rare mutations by next-generation sequencing," *PNAS* 109(36): 14508–14513 (2012)
1065	Kennedy, S.R., *et al*., "Ultra-Sensitive Sequencing Reveals an Age-Related Increase in Somatic Mitochondrial Mutations That Are Inconsistent with Oxidative Damage," PLOS Genetics 9(9): e1003794 (2013)
1066	File History for U.S. Patent No. 11,149,306
1067	Perakis, S., *et al*., "Chapter Three - Advances in Circulating Tumor DNA Analysis," *Advances in Clinical Chemistry* 80: 73-157 (2017)

Exhibit #	***Description***
1068	Schmitt, M.W, *et al.*, "Sequencing small genomic targets with high efficiency and extreme accuracy," *Nature Methods* 12(5): 423-4226 with Suppl. Material (2015)
1069	Complaint, *Illumina, Inc. v. Guardant Health, Inc.; Helmy Eltoukhy; And Amirali Talasaz*, 22-cv-00334 (D.Del.) (March 17, 2022)
1070	Krimmel, J.D., *et al.*, "Ultra-deep sequencing detects ovarian cancer cells in peritoneal fluid and reveals somatic TP53 mutations in noncancerous tissues," *PNAS* 113(21): 6005-6010 (2016)
1071	Billett, H.H., "151, Hemoglobin and Hematocrit," Clinical Methods The History, Physical and Laboratory Examinations, pp. 718-719, Third Edition, Ed. Walker, H.K., et al., Butterworths, United States (1990)
1072	Brown, T.A., Ed., "Chapter 4: Studying DNA," *Genomes*, pp. 96-124, Second Edition, John Wiley & Sons, Inc., United States (2002)
1073	Stasinopoulos, D.M., *et al.*, "Generalized Additive Models for Location Scale and Shape (GAMLSS) in R," Journal of Statistical Software 23(7): 1-46 (2007)
1074	Final Written Decision *Foundation Medicine, Inc. v. Guardant Health, Inc.*, IPR2019-00652, Paper 47 (August 18, 2020)
1075	Dr. Spellman's Calculations for prior art disclosures of molar ratios of adapters relative to DNA fragments
1076	Kitzman, J.O., *et al.*, "Non-invasive whole genome sequencing of a human fetus," *Sci Transl Med.* 4(137): 137ra76 (2012)
1077	ThruPLEX™ DNA-seq Kit Instruction Manual, Rubicon Genomics
1078	Complaint, *Twinstrand Biosciences, Inc. & The University of Washington v. Guardant Health, Inc.*, 1-21-cv-01126 (D. Del.) (August 3, 2021)
1079	Decision Denying Institution for *Inter Partes* Review, *Foundation Medicine, Inc. v. Guardant Health, Inc.*, IPR2019-00636 and IPR2019-00637, Paper 10 (August 20, 2019)
1080	Pray, L.A., "Eukaryotic Genome Complexity," Nature Education 1(1):96 (2008)
1081	Semsarian, C. and Seidman, C.E., "Molecular medicine in the 21st century," *Internal Medicine Journal* 31: 53–59 (2001)
1082	Narayan *et al.*, "Ultrasensitive Measurement of Hotspot Mutations in Tumor DNA in Blood Using Error-Suppressed Multiplexed Deep Sequencing," *Cancer Research*, 72(14):3492-3498 (2012)
1083	U.S. Provisional Application No. 61/625,623, filed on April 17, 2012

Exhibit #	***Description***
1084	Ledergerber. C. and Dessimoz, C., "Base-calling for next-generation sequencing platforms," Briefings in Bioinformatics 12(5):489-497 (2011)
1085	Illumina, Inc.s Answering Brief In Opposition to Defendants' Motion to Transfer Under Sections 1404 and 1406, Illumina, Inc. & v. Guardant Health, Inc.; Helmy Eltoukhy; and Amirali Talasaz, Dkt.40, 1:22-cv-00334-VAC-CJB (D. Del.) (June 29, 2022)
1086	Illumina, Inc.s Answering Brief In Opposition to Defendants' Motion Motion to Dismiss, Illumina, Inc. & v. Guardant Health, Inc.; Helmy Eltoukhy; and Amirali Talasaz, Dkt. 41, 1:22-cv-00334-VAC-CJB (D. Del.) (June 29, 2022)
1087	United States District Courts – National Judicial Caseload Profile, 95 pages, available at https://www.uscourts.gov/statistics-reports/federal-court-management-statistics-march-2022, last accessed August 11, 2022
1088	Plaintiff's Motion to Sever and Stay Guardant Health's Counterclaims, TwinStrand Biosciences, Inc., & University of Washington v. Guardant Health, Inc., Dkt. 75, 1-21-cv-01126-VAC-SRF (D. Del) (June 17, 2022)
1089	Stolovitzky, G. and Cecchi, G., "Efficiency of DNA replication in the polymerase reaction," *PNAS USA* 93:12947-12952 (1996)
1090	Nelms, B. and Labosky, P., "A predicted hairpin cluster correlates with barriers to PCR, sequencing and possibly BAC recombineering," *Sci. Rep.*, 1:106 (2011)
1091	Hilbers *et al.*, "Hairpin formation in synthetic oligonucleotides," *Biochmie* 67:685-695 (1985)
1092	Integrated DNA Technologies, "Oligonucleotide Yield, Resuspension, and Storage" (August 29, 2013), available from https://web.archive.org/web/20130829004823/http:/www.idtdna.com/pages/docs/technical-reports/oligonucleotide-yield-resuspension-and-storage.pdf?sfvrsn=7

I. INTRODUCTION

Guardant's claimed methods were known in the prior art. As detailed below, Guardant's claimed steps of tagging, amplifying, sequencing, and reducing or tracking redundancy in the sequence reads based on paired and unpaired reads, are all straight out of Schmitt, with only trivial additions.

Further, Guardant misled the Office during prosecution in at least two significant ways. *First,* Guardant misrepresented the quantity of cell-free DNA (cfDNA) in a human blood sample, arguing that a skilled artisan would not have expected Schmitt's methods to be applicable to cfDNA because human blood (according to Guardant) contained insufficient quantities of cfDNA. Tellingly, Guardant never provided the Examiner with any prior art evidence to support its argument. It's no wonder. The prior art flatly contradicts Guardant's argument, showing that *ample* quantities of cfDNA are in human blood. In fact, the prior art expressly suggested benefits of using Duplex Sequencing (also called "Duplex Consensus Sequencing" or "DCS") with cfDNA. EX1008, 7.

Second¸ Guardant misled the Examiner by relying on a statement from a *2017 publication*—not prior art—as alleged evidence that a person of skill in the art *in 2013* would not have expected Schmitt's methods to work with cfDNA. But Guardant's position is contradicted by contemporaneous evidence—including Schmitt itself—that taught applying DCS methods to cfDNA.

The Board should institute trial and cancel the claims of the ’306 patent.

II. STATEMENT OF PRECISE RELIEF REQUESTED AND REASONS THEREFOR (37 C.F.R. §42.22(A))

TwinStrand Biosciences, Inc. (“TwinStrand”) petitions for IPR, requesting cancellation of claims 1-29 of U.S. Patent No 11,149,306 (“the ’306 patent”; EX1001), assigned to Guardant Health, Inc. This Petition is supported by the declaration of Paul Spellman, Ph.D. (EX1002), Professor of Molecular and Medical Genetics and an expert in genetics and genomics approaches to detecting and monitoring human diseases. This Petition demonstrates that claims 1-29 are unpatentable under 35 U.S.C. §103.

III. STATE OF THE ART BEFORE DECEMBER 2013

Before the ’306 patent’s earliest possible priority date (December 28, 2013)[1], next-generation sequencing (“NGS”) methods were well known. EX1011, 32-41; EX1002, ¶¶31-63.

A. Optimization techniques for DNA library preparation were well known.

Before December 2013, most NGS methods began with the preparation of a library of template DNA fragments. This was accomplished by fragmenting DNA or by using previously fragmented DNA, such as cfDNA (discussed below). EX1012, 291; EX1011, 32. Library preparation commonly involved “tagging” the

[1] TwinStrand does not concede the ’306 patent is entitled to this priority date.

DNA with "adapters" having an identifier sequence, which were added to the DNA fragment ends. *Id.* Adapters often contained "molecular barcodes" or "identifiers," which are nucleotide sequences that help to further identify and distinguish the sequenced DNA fragments from one another. EX1083, ¶¶[0005], [0008]-[0009], [0030]; EX1005, Fig. 1; EX1002, ¶¶38-39. A common technique for tagging was ligation. EX1083, ¶[0020]; EX1005, Fig. 1; EX1015, 4-5; EX1031, 11-12, Fig. 4; EX1002, ¶39.

Guardant's claims recite open-ended ranges of "more than a 10×" and "more than a 80×" excess of adapters relative to cfDNA molecules, and ligation efficiencies of "at least 20%" and "at least 40%." But long before December 2013, artisans already knew to use significant molar excesses of adapters to achieve high efficiency ligations. EX1020, 18.4.15; EX1031, 2. EX1083, ¶[0005]; EX1002, ¶¶40-51; EX1021, 5, Fig. 2; EX1026[2], ¶¶[0130], [0143], [0159]; EX1025,

---

[2] EX1026 ("Diehn") is the publication of U.S. 14/209,807 (filed March 13, 2014), which claims priority to U.S. 61/798,925 (filed March 15, 2013) (EX1025, "Diehn-925"). The Diehn-925 provisional specification is substantially the same as Diehn's specification, including identical claim sets. EX1025; EX1026. Thus, Diehn is prior art to the '306 patent under 35 U.S.C. §102(a)(2) because Diehn-925 provides §112 support for at least one claim in Diehn. EX1002, ¶¶49-51; *Dynamic*

¶¶[0106], [0119], [0135].

For example, So 2004 taught that the yield of adapter-ligated DNA depended on "the amount of … adaptor introduced into the ligation mixture, and *increased with increasing adaptor concentration*." EX1021, 5, Fig. 2; EX1002, ¶44. Diehn disclosed that "[i]ncreasing adapter concentration during ligation increases ligation efficiency and reporter recovery" and used adapter concentrations that were in "100-fold molar excess" compared to the DNA targets. EX1026, ¶¶[0130], [0143], [0159]; EX1025, ¶¶[0106], [0119], [0135]; EX1024, 4-10 (adapter:DNA molar ratio of 90:1); EX1005, 272-273 (adapter:DNA ratios from 790:1 to 7,509:1); EX1022, 1-2, S7-S8 (adapter:DNA molar ratios from 230:1 to 315:1); EX1023, 32; EX1002, ¶¶49-51. And unwanted ligation byproducts such as adapter-dimers were easily removed from the library through standard clean-up steps. *Id.*; EX1002, ¶52.

Using a high molar excess of adapters was known to increase ligation efficiency, and yield a more complex library to sequence deeply. EX1019, 1; EX1020, 18.4.15; EX1031, 2; EX1083, ¶[0005]; EX1002, ¶¶42-48. The art reported that 40% or more of input DNA could be recovered as adapter-ligated DNA when high molar ratios of adapters were employed. EX1031, 4; EX1005,

---

Drinkware, LLC v. Nat'l Graphics, Inc., 800 F.3d 1375 (Fed. Cir. 2015).

274; EX1002, ¶¶53-56. For example, the KAPA datasheet disclosed that "15-40% of input DNA is typically recovered as adapter-ligated molecules." EX1015, 4. Meyer disclosed that, "the expected overall recovery [after adapter-DNA ligation] … is between 40 and 60%." EX1005, 274; EX1002, ¶53. Fisher similarly reported a 47% yield of adapter-ligated DNA fragments. EX1031, Fig. 3; EX1002, ¶55. Thus, artisans knew how to achieve high-efficiency adapter-DNA ligation using excess adapters.

B. Cell-free DNA isolated from blood was widely used in NGS platforms.

By 2013, "cell-free" DNA ("cfDNA") was routinely analyzed in NGS applications, such as cancer screening and diagnostics. EX1002, ¶¶64-80; EX1082; EX1008.

1. The presence of cell-free tumor DNA in human blood was well known.

Before December 2013, artisans knew that cfDNA is "fragmented to an average length of 140 to 170 base pairs (bp)" when released into the bloodstream. EX1039, 1; EX1041, 228; EX1040, 473. Artisans understood that these cfDNA fragments were well-suited to serve as a DNA template library for NGS, which requires similarly short fragments. EX1039, 1; EX1051, 212; EX1053, 16271; EX1054, 1043; EX1032, 3; EX1002, ¶¶64-73.

Given cfDNA's presence in blood, artisans appreciated the prospects of

using routine blood draws for non-invasive cancer screening and diagnostic tests. EX1040, 472. For example, artisans understood that the median cfDNA concentration in plasma for cancer patients ranges from 83.5-139 ng/ml. EX1047, 1660, Table 1; EX1048, 347. A POSA desiring, e.g., 1 μg of DNA for NGS library preparation, would only need about 7-12 ml of plasma. EX1002, ¶¶68-71. And, much lower quantities of DNA could be used in NGS applications. EX1028, 22; EX1047, 1660, Table 1; EX1048, 347; EX1002, ¶70; EX1027, 9. Thus, artisans knew there was ample cfDNA in human blood for NGS applications. EX1047, 1660, Table 1; EX1048, 347; EX1002, ¶¶71-73; EX1040, 473; EX1050, 00015; EX1039, 10; EX1051, 212; EX1004, 109, Table S2; EX1008, 8; EX1032, 3; EX1049, 1; EX1082, 3493.

2. Isolating cfDNA from blood was routine with off-the-shelf kits.

Before December 2013, off-the-shelf kits for isolating cfDNA from blood were readily available. These kits were also known to provide sufficient quantities of DNA for NGS analysis. EX1060, 2631-2632; EX1002, ¶¶77-80.

Numerous reports detailing NGS methods for analyzing cfDNA from human blood used the same or similar cfDNA extraction kits. EX1082, S2; EX1008, 8; EX1032, 3; EX1046, 2; EX1050, 00016; EX1051, 212; EX1039, 10; EX1002, ¶¶78-80. Indeed, the prophetic examples in the ’306 patent depict a common commercial kit to extract cfDNA from blood. EX1001, 53:47-50.

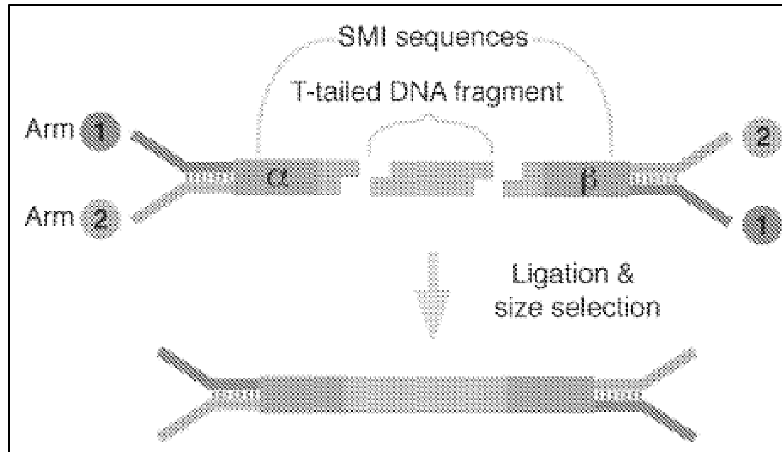
C. The prior art taught that Duplex Sequencing dramatically lowers NGS error rate.

Guardant's claims recite steps of reducing or tracking redundancy in sequence reads based on "paired reads" and "unpaired reads." These elements are the hallmarks of Schmitt's DCS methods. EX1002, ¶¶81-84.

DCS is an error correction method that allows tracking each strand of DNA throughout an NGS workflow. EX1083, Abstract. Schmitt teaches reducing or tracking redundancy of top (Watson) and bottom (Crick) strand sequence reads by sorting them into families and collapsing them into consensus sequences. *Id.*, Fig. 1. Schmitt uses this to generate "paired consensus sequences" to reduce sequence errors. *Id.*

Schmitt discloses that DCS "greatly reduces errors" by "overcoming technical limitations of prior methods utilizing data from only one of the two strands." *Id.*, Abstract. Schmitt's DCS methods are summarized below.

(1) Tagging DNA with adapters comprising molecular barcodes. Schmitt discloses ligating double-stranded adapters containing Single Molecule Identifiers ("SMIs"), or molecular barcodes, to the ends of each double-stranded DNA fragment in a DNA library, as shown below in Schmitt's Figure:

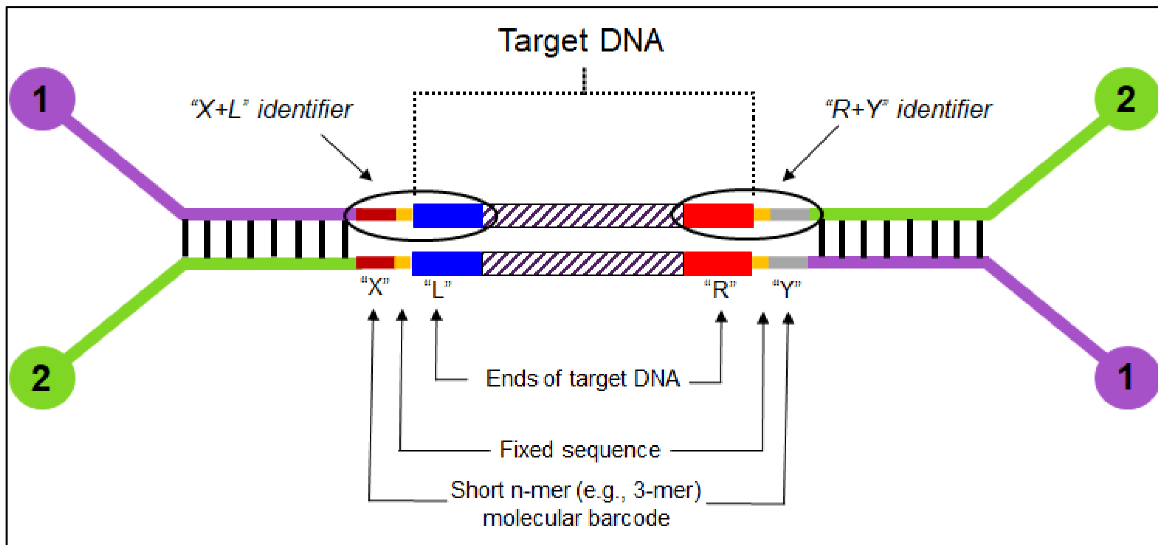


Id., ¶¶[0011], [0062], Fig. 1; EX1002, ¶¶85-90. This produces a library of adapter-tagged double-stranded DNA fragments. *Id.* In the diagram above, the two molecular barcode tags are represented as α and β.

Schmitt discloses embodiments of tagging essentially every DNA fragment in the sample with a different barcode (uniquely tagging). But like Guardant's later claims, Schmitt also discloses *non-uniquely tagging* the DNA fragments in a sample (i.e., some DNA fragments will have the same barcode). Here, Schmitt discloses a "hybrid method" of tagging, which uses a combination of the ends of the DNA fragments and "a shorter n-mer tag (such as 1 or 2 or 3 or 4 or more degenerate or semi-degenerate bases)." As such, the shorter n-mer tag, *together with the sequence information within the target DNA*, serve as "unique molecular identifiers." EX1083, ¶¶[0030], [0047], [0075].

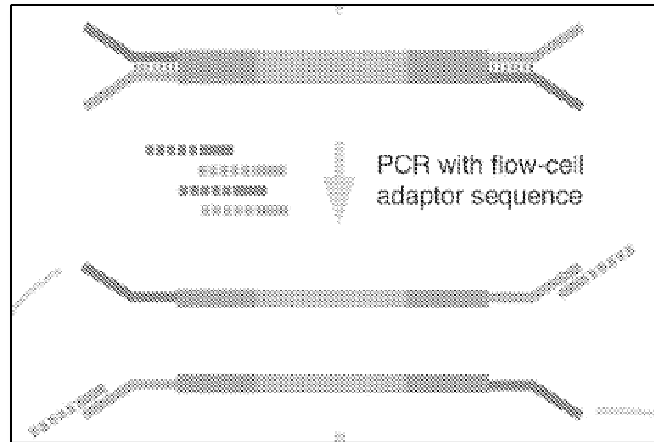
In Schmitt's hybrid method, the tagging is non-unique because the number of different molecular barcodes in a set of short n-mer tags is quite limited,

resulting in some DNA fragments being tagged with the same molecular barcode by design. EX1002, ¶¶88-89. Dr. Spellman's diagram of Schmitt's hybrid tagging method is depicted below.



EX1002, ¶89. In the figure above, the ends of the DNA fragment are designated "L" and "R," and the short 3-mer molecular barcodes are labeled "X" and "Y." *Id.* When combined, they form unique molecular identifiers "X+L" and "R+Y" which can be used in DCS. *Id.*; EX1083, ¶[0030].

(2) Amplification. After ligation, the adapter-tagged DNA molecules are amplified by, e.g., PCR. EX1083, ¶¶[0009], [0011]. This amplifies each strand of the double-stranded DNA fragment, as shown in Schmitt's Figure 1:



Id., ¶¶[0011], [0062], Fig. 1. In Schmitt's diagram above, the PCR amplification utilizes primers containing the Illumina flow-cell-compatible tails. *Id.*, ¶[0011]; EX1002, ¶89.

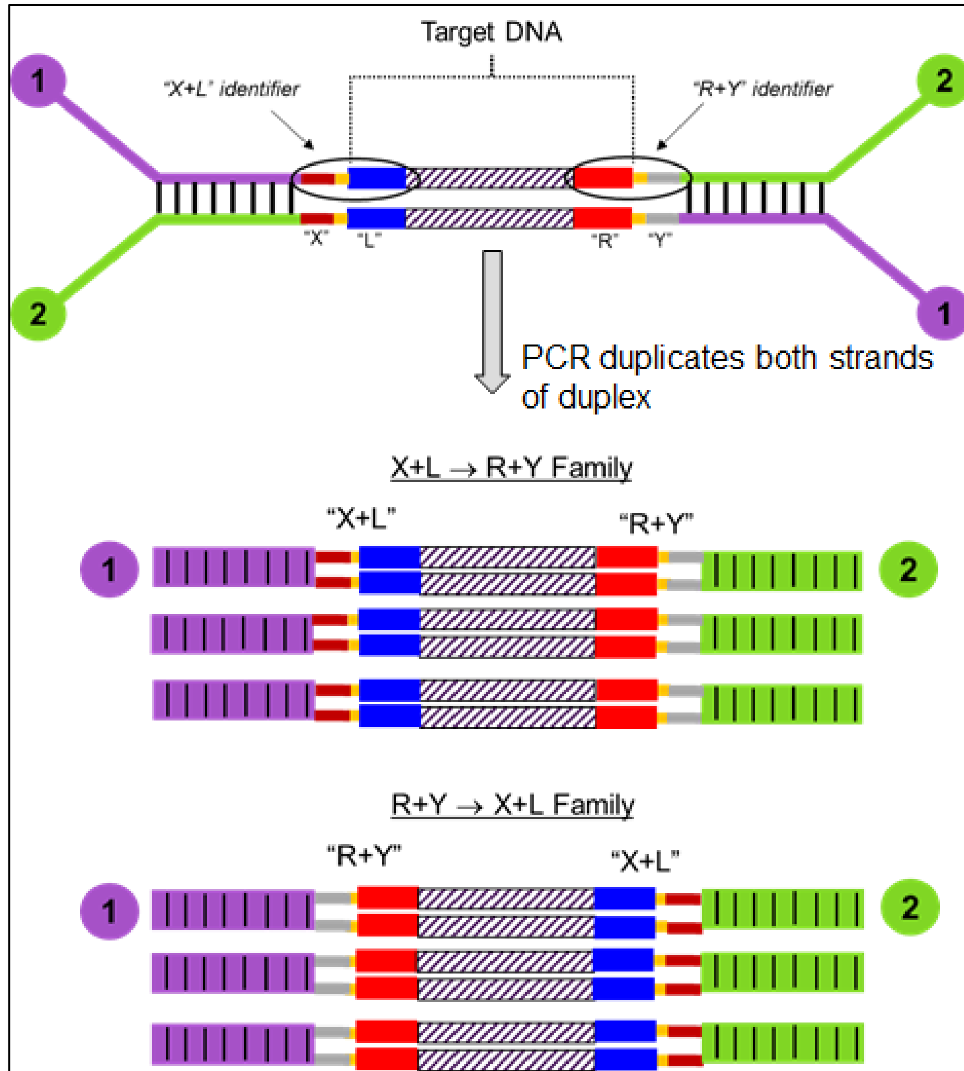
(3) Target enrichment. Schmitt discloses hybrid capture target enrichment to allow for greater sequencing depth at a defined region in a genome. EX1083, ¶[0065]; EX1002, ¶¶91-93. In an example embodiment, Schmitt discloses using a commercial kit for target enrichment, stating that "[t]he adaptor-ligated library was PCR amplified and subjected to SureSelect [hybrid] capture." EX1083, ¶[0065]. Schmitt discloses a further amplification step of the enriched adapter-tagged DNA fragments. EX1083, Fig. 1; EX1002, ¶93.

(4) Sequencing. Schmitt discloses that the amplified enriched adapter-tagged DNA fragments can be sequenced "using any suitable method known in the art," such as Illumina's methods. EX1083, ¶[0042]; *id.,* ¶[0009], Fig. 1; EX1002, ¶94. Schmitt's working examples use Illumina sequencing. EX1083, ¶¶[0062]-

[0075].

(5) Mapping the sequence reads to a reference sequence. After sequencing, Schmitt discloses that the sequences can be mapped to a known reference sequence. EX1083, ¶[0060]; EX1002, ¶95. For example, in one embodiment, Schmitt discloses that the "[r]eads were aligned to the human genome with the Burrows-Wheeler Aligner (BWA)." EX1083, ¶¶[0060], [0066]. Mapping sequence reads to a reference sequence was routine in NGS. EX1011, 40; EX1002, ¶95.

(6) Grouping the sequenced strands into paired families. Schmitt teaches grouping the sequenced strands into paired families with common pairing of molecular barcode tags. EX1083, ¶¶[0011], [0013], [0060], [0063]; EX1002, ¶¶96-98. As Schmitt explains in one embodiment, sequence reads sharing "a unique set of SMI tags are grouped into paired families with members having strand identifiers in either the αβ or βα orientation. Each family pair reflects one double-stranded DNA fragment." EX1083, ¶[0013], Fig. 3; *id.*, ¶¶[0011], [0042], [0062], Fig. 1; EX1002, ¶97. In Schmitt's hybrid-tag embodiment, the strands are grouped based on *the combination* of the short n-mer barcode and the end of the target DNA. EX1083, ¶[0030]. Dr. Spellman's diagram below depicts an embodiment of Schmitt's grouping using the hybrid tag:



EX1002, ¶98. Schmitt also discloses using the asymmetrical portions of the adapters in combination with the molecular barcodes to distinguish the individual strands. EX1083, ¶[0011]; EX1002, ¶98.

(7) Generating single-strand consensus sequences. After grouping, Schmitt discloses creating a single-strand consensus sequence ("SSCS") for each original strand. EX1083, ¶[0063]; EX1002, ¶99. Schmitt explains that "[t]he sequences of uniquely tagged PCR duplicates are subsequently compared in order

to create a PCR consensus sequence." EX1083, ¶[0063]; *id.*, ¶¶[0011], [0013]; EX1002, ¶99. Schmitt discloses that collapsing the redundant sequence reads into SSCSs reduces the redundancy of the sequence reads. EX1083, ¶¶[0007], [0044], [0060]; EX1002, ¶99.

(8) Generating Duplex Consensus Sequences. Next, each SSCS is compared with its complementary strand-mate SSCS, if present, to identify any mismatched bases. EX1083, Fig. 3, ¶¶[0013], [0060], [0063], [0068]; EX1002, ¶¶100-101. Schmitt discloses that the complementary strands "contain redundant sequencing information" and that tracking the redundant sequence information using DCS can eliminate sequencing errors. EX1083, ¶[0044]. As Schmitt explained, "[s]equence reads at a given position are kept only if the read data from each of the two paired strands is in agreement." *Id.*, ¶[0063]. Schmitt's DCS thus allowed the removal of artifactual errors and identification of true mutant sequences. *Id.*

D. The prior art taught applying Duplex Sequencing to cfDNA.

Artisans knew that cfDNA could be analyzed with Schmitt's DCS. First, Schmitt itself discloses applying DCS on fragmented double-stranded DNA, including *cfDNA*. EX1083, ¶¶[0003], [0011], [0041], [0048], [0065]; EX1002, ¶¶102-106. A POSA would have immediately understood that Schmitt contemplated using cfDNA, evident by Schmitt's express references to analyzing

"*nucleic acid-based serum biomarkers*"[3] for "early detection of cancer and monitoring its response to therapy." EX1083, ¶¶[0003], [0048] (citing cfDNA references EX1053, EX1055, EX1056, and EX1057); EX1002, ¶103.

Second, in November 2013, Kukita expressly disclosed the potential benefit of using DCS for screening cfDNA from blood of cancer patients. EX1008, 2, 7. Kukita reported a high incidence of false positive mutations in the art, and recognized that is "not acceptable for diagnostic applications." *Id.,* 7. Understanding that "there is room for technical improvement," Kukita then cited Schmitt 2012, stating that "[Schmitt's] methods to produce error-free sequences … might be applicable to enhance accuracy." *Id.*; EX1002, ¶105.

In sum, before December 2013, the art taught methods for sequencing cfDNA from blood using DCS to reduce errors in the sample preparation and sequencing processes. Guardant's '306 patent merely attempts to claim these established methods.

IV. THE '306 PATENT AND ITS PROSECUTION HISTORY

The '306 patent issued on October 19, 2021 from U.S. Appl. 16/945,124 filed July 31, 2020, and claims a December 28, 2013 priority date. [4] The '306

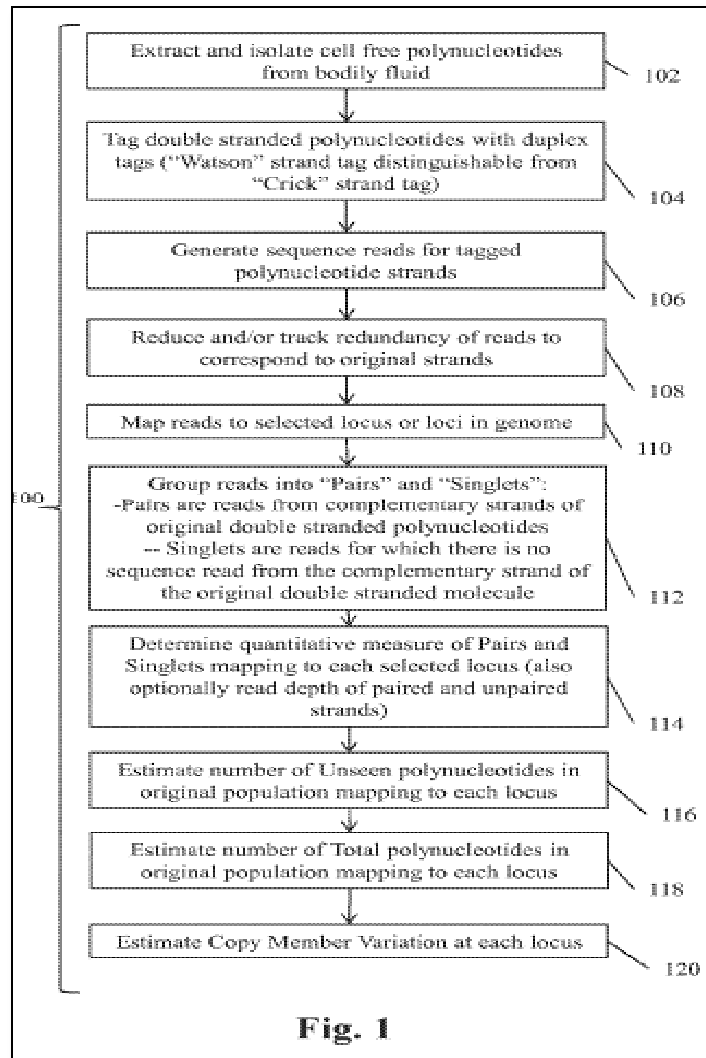
[3] Unless stated otherwise, emphasis is added throughout this Petition.

[4] *See supra* note 1.

patent discloses “methods for detecting genetic variants,” comprising the steps of

1. **Tagging** a library of double-stranded cfDNA molecules with “duplex tags,” EX1001, 17:8-29:25, Fig. 1.
2. **Enrichment and amplification** of the tagged cfDNA molecules. *Id.* 29:18-25, 29:44-30:32, 40:59-41:9, 54:27-32.
3. **Sequencing** the library of tagged cfDNA molecules. *Id.*, 29:26-31:29, Fig. 1.
4. **Mapping** the sequence reads to a “reference sequence.” *Id.*, 33:62-34:52, Figs. 1-3.
5. **Reducing or tracking redundancy in the sequence reads** to generate consensus sequence reads (e.g. “collapsing”) by comparing multiple sequence reads from a single original DNA fragment. *Id.*, 31:30-34, 31:59-33:61, Figs. 1, 4C. The ’306 patent discloses that reducing or tracking redundancy includes “comparing sequence reads having the same or similar molecular barcodes and the same or similar end of fragment sequences.” *Id.*, 41:17-23; 32:17-23.
6. **Sorting** the sequence reads into paired or unpaired reads. *Id.*, 35:7-40:8, Fig. 1.

The ’306 patent’s Figure 1 depicts these basic steps:



Id., Fig. 1.

The '306 patent has 29 claims, with claims 1 and 17 as independent claims. Claims 1 and 17 recite methods comprising "reducing or tracking redundancy of a plurality of sequence reads" (claim 1) and "sorting a plurality of sequence reads" (claim 17) from cfDNA, comprising steps of tagging, amplifying, sequencing, reducing or tracking redundancy (claim 1), and sorting (claim 17) the reads into families comprising paired and unpaired reads. *Id.*, 61:6-43, 62:45-65.

Dependent claims 2-16 and 18-29 add certain limitations, such as additional aspects of the cfDNA molecules, criteria for the duplex tags and molecular barcodes, specific gene targets, and simple quantitative steps. *Id.*, 61:44-62:44, 62:66-64:31; EX1002, ¶¶107-117.

During prosecution, the Examiner rejected Guardant's claims as anticipated by and obvious over USPN 10,752,951 ("Salk"). EX1066, 539-540.

In response, Guardant argued that Salk does not disclose "cfDNA" in its specification. *Id.*, 561-562. Guardant also argued that a skilled artisan would not have had a reasonable expectation of successfully using cfDNA with Schmitt's DCS, relying solely on a statement from a 2017 publication ("Perakis," EX1067). *Id.,* 562-563.

The Office maintained the rejections over Salk, noting that Salk discloses, *inter alia*, "grouping reads into families," "mapping reads to a reference sequence," and "sorting sequence reads into families." *Id.*, 672-674. In response, Guardant *amended the specification* and claims to recite language pertinent to claim 1 (e.g., "n different unique identifiers, wherein n is at least 2 and no more than 100,000*z"). *Id.*, 685-686. Guardant also argued that the Salk priority document does not disclose cfDNA or the new language of claim 1. *Id.*, 692-693.

The Office issued a Notice of Allowance on September 2, 2021, with no substantive comments regarding the prior art, Guardant's arguments, or Perakis.

Id., 724-742.

V. PERSON OF ORDINARY SKILL IN ART

A POSA in the ’306 patent’s technical field would have had knowledge of the scientific literature concerning methods of DNA manipulation and analysis, including DNA sample preparation for sequencing, amplification, methods of DNA sequencing (including NGS and related sequencing methods), and bioinformatics methods for raw data analysis. EX1002, ¶¶27-30.

A POSA typically would have had (i) a Ph.D. in molecular biology, genetics, bioinformatics, or a related field, and have at least about two years of experience in the use and development of sequencing technologies; or (ii) a Master's degree in one of the same fields with at least about five years of the same experience. A POSA may have worked as part of a multidisciplinary team and drawn upon not only her own skills, but of others on the team, e.g., to solve a given problem. For example, a chemist and a statistician may have been part of a team. *Id.*

VI. CLAIM CONSTRUCTION

Claim terms must be given their “ordinary and customary meaning … as understood by one of ordinary skill in the art” in view of the specification and prosecution history. 37 C.F.R. §42.100(b).

The ’306 patent does not expressly define the phrase “**wherein z is a mean**

of an expected number of duplicate molecules in the population of cfDNA molecules that map to identical start and stop positions on a reference sequence," as recited in claim 1. A POSA would understand that the population of cfDNA molecules that map to identical start and stop positions will vary, depending on the number of haploid genome equivalents (HGEs) in the sample. EX1002, ¶¶131-133. The '306 patent discloses that "[a] sample can comprise *various amount[s]* of nucleic acid that contains genome equivalents. For example, a sample of about 30 ng DNA can contain about 10,000 (10^4) haploid human genome equivalents and, in the case of cfDNA, about 200 billion (2×10^{11}) individual polynucleotide molecules." EX1001, 16:60-64. According to the '306 patent, "in a sample comprising about 10,000 haploid human genome equivalents of fragmented genomic DNA, e.g., cfDNA, z is expected to be between 2 and 8." *Id.*, 20:47-50. Thus, a POSA would understand that a sample with 10,000 HGEs has about 2 to 8 cfDNA molecules that map to identical start and stop positions. EX1002, ¶133.

Terms not specifically construed are given their plain and ordinary meaning in light of the claims and specification.

VII. IDENTIFICATION OF THE CHALLENGE (37 C.F.R. §42.104(B))

TwinStrand requests IPR based on the grounds below.

Ground	35 U.S.C. Section	Claims	References

	(AIA)		
1	§103	1-3, 5, 7, 9-14, 17-27, 29	Narayan and Schmitt
2	§103	4, 6	Narayan, Schmitt, and Meyer
3	§103	8	Narayan, Schmitt, and Craig
4	§103	15, 16, 28	Narayan, Schmitt, and Kivioja

The references are prior art for at least for the following reasons. **Narayan** (EX1082) published on July 15, 2012; **Meyer** (EX1005) published on January 31, 2008; **Craig** (EX1007) published on September 14, 2008; and **Kivioja** (EX1006) published on November 20, 2011. Each of these references published before December 28, 2013, and is prior art to the '306 patent under AIA 35 U.S.C. §102(a)(1). **Schmitt** (EX1009) was filed as PCT/US2013/032665 on March 15, 2013 and published as WO 2013/142389 on September 26, 2013. Schmitt is prior art under AIA 35 U.S.C. §102(a)(1) and §102(a)(2). Schmitt claims priority to, *inter alia*, Provisional Application No. 61/625,623 ("Schmitt-623," EX1083), filed on April 17, 2012. As Dr. Spellman confirms, the relied-upon disclosures in Schmitt cited in this petition were carried forward from Schmitt-623, as evident by this Petition's citations to Schmitt-623 throughout, Dr. Spellman's table showing the carried-forward disclosures, and Dr. Spellman's declaration citations. EX1002,

¶142. Additionally, Schmitt-623 provides §112 support for "at least one claim" in Schmitt. *Id*. For example, claims 15-21 in Schmitt are recited *verbatim* as claims 11-17 in Schmitt-623. EX1083, claims 11-17; EX1009, claims 15-21; EX1002, ¶143. *Dynamic Drinkware,* 800 F.3d at 1381-82; *Medtronic, Inc. v. Niazi Licensing Corp.,* IPR2018-00609, Paper 8 at 11 (P.T.A.B., Aug. 20, 2018). Thus, Schmitt is §102(a)(2) prior art to the '306 patent at least as of April 17, 2012.

VIII. THE FACTS AND LAW WEIGH AGAINST DISCRETIONARY DENIAL OF INSTITUTION.

A. This Petition satisfies 35 U.S.C. §325(d).

The *Advanced Bionic*s two-part test does not apply here because (i) the combinations of art and arguments in this Petition are different from those previously considered by the Office, and (ii) during prosecution, Guardant misled the Examiner into making errors that were material to the patentability of Guardant's claims. *Advanced Bionics, LLC v. MED-EL Elektromedizinische Geräte GmbH,* IPR2019-01469, Paper 6 (P.T.A.B., Feb, 13, 2020) (precedential).

Guardant buried Narayan, Schmitt, Craig, and Kivioja amongst nearly 750 other references in an IDS. EX1066, 122-221. Meyer was *never* cited in an IDS or disclosed to the Examiner in this patent family, despite clearly being material to the patentability of Guardant's claims. None of Narayan, Schmitt, Craig, or Kivioja were asserted in any Office Actions in this patent family. The Examiner rejected Guardant's claims as anticipated and obvious over Salk, but later withdrew the

rejection based on misleading and legally erroneous arguments by Guardant, addressed below. *Id.*, 561-564. Salk shares its specification with Schmitt, but the Examiner was never presented with the four combinations of art (Grounds 1-4) presented here. This Petition also includes new evidence such as declaration testimony from Dr. Paul Spellman. EX1002.

Guardant misled the Examiner into making two main errors in allowing the claims. *First,* Guardant made a legally erroneous argument by relying on a single post-filing publication (Perakis) when arguing that "a person of ordinary skill in the art would recognize that there was no reasonable expectation of success in applying the method of Schmitt et al. to cfDNA." EX1066, 562-563. But Perakis published in *2017*, and is *not* prior art, nor is it reflective of the state of the art in 2013 or even afterwards. *Id.*; EX1067; EX1002, ¶¶118-130; *Bristol-Myers Squibb Co. v. Teva Pharms. USA, Inc.*, 752 F.3d 967, 974 (Fed. Cir. 2014) (rejecting appellant's argument that there was no reasonable expectation of success because the argument was based on post-filing evidence, not prior art). The law has long held that while post-filing evidence may be used to support objective indicia of nonobviousness, a reasonable expectation of success must be measured from the *time of the invention*. *See In re Koller*, 613 F.2d 819, 824 (CCPA 1980); *Velander v. Garner*, 348 F.3d 1359, 1377 (Fed. Cir. 2003); *Amgen v. Hoffman-La Roche*, 580 F.3d 1340, 1362 (Fed. Cir. 2009); *Yeda Research v. Mylan Pharms, Inc.*, 906

F.3d 1031, 1042 (Fed. Cir. 2018); *OSI Pharms., LLC v. Apotex Inc.*, 939 F.3d 1375, 1385 (Fed. Cir. 2019); *see also*, *Grunenthal GMBH v. Anticep Bioventures II LLC*, 2020 WL 4342293, *7-8 (P.T.A.B. July 28, 2020); *Renesas Elec. Corp. v. Lone Star Silicon Innov. LLC*, 2019 WL 1373644, *13 (P.T.A.B. March 25, 2019).

Moreover, Perakis cites Schmitt 2012, *not Schmitt* (EX1009), and merely states that "*the original protocol* [from Schmitt 2012] has not yet been used with ctDNA." EX1067, 102 (citing EX1064 as reference 182). Schmitt contains additional disclosures not present in Schmitt 2012. EX1002, ¶¶122-123. For example, the "original protocol" disclosed in Schmitt 2012 did not utilize hybrid capture enrichment, which is expressly taught in Schmitt. EX1002, ¶122; EX1083, ¶¶[0058], [0065], Fig. 1; EX1064, Fig. 1B. A POSA would have appreciated that hybrid capture can enrich for target regions of interest and improve detection and efficiency. EX1083, ¶¶[0058], [0065], Fig. 1; EX1014, S30; EX1002, ¶122.

Moreover, Guardant withheld Kukita, actual prior art evidence *from 2013*, that expressly suggests applying Schmitt's DCS to cfDNA to "enhance accuracy." EX1008, 7; EX166, 122-221; EX1002, ¶125.

Second, Guardant misled the Examiner into making a technical error that the amount of cfDNA extracted from blood would be insufficient for DCS. EX1066, 563. The prior art was replete with evidence demonstrating that more than enough cfDNA for DCS could be extracted from routine blood draws using commercially

available kits. EX1002, ¶¶127-130; EX1048, 347; EX1047, 1660, Table 1; EX1046, Table 1.

Meanwhile, the ’306 patent discloses no special methods or reagents needed to isolate cfDNA or prepare it for sequencing. Example 1 discloses using as little as “2.5 ng DNA … as the starting material,” and the patent claims that the methods can be performed with as little as *1 nanogram* of cfDNA. EX1001, 54:8-10, 56:9-12, 61:47-49, 63:1-3. Indeed, the ’306 patent suggests using a routine commercial DNA extraction kit from the prior art. EX1001, 53:60-62; EX1002, ¶128. This evidence flatly contradicts Guardant’s representations to the Examiner.

Any argument this Petition should be denied under §325(d) is meritless.

B. The *Fintiv* factors do not support discretionary denial.

The *Fintiv* factors do not support discretionary denial of institution. *Apple Inc. v. Fintiv, Inc.*, IPR2020-0019, Paper 11, 6 (P.T.A.B., March 20, 2020) (precedential). Guardant delayed in filing its counterclaims until about six months after TwinStrand brought suit; asserting the ’306 patent against TwinStrand on January 11, 2022. TwinStrand was diligent in filing this Petition within weeks of the earliest possible allowed date. *Hanwha Sols. Corp. v. Rec Solar PTE. Ltd.*, IPR2021-00988, Paper 12 at 15 (P.T.A.B. Dec. 13, 2021) (IPR filed “two weeks after the earliest possible date it could have been filed by statute” showed diligence; “the strategic choice of filing for *inter partes* review, as opposed to post-

grant review, is a litigation decision that falls squarely within Petitioner's prerogative"). Additionally, the parallel district court litigation is still in the early stages. The median time to trial in the District of Delaware is 36.3 months (EX1087, 14), which would place the trial's estimated start date in late August 2024—approximately six months after a Final Written Decision would be expected in this IPR. And even that timeframe is uncertain as the case has yet to be assigned to a new judge after Judge Stark's departure.

Moreover, Guardant's standing to even assert its infringement counterclaims is in significant doubt because its patent portfolio is embroiled in a lawsuit brought by Illumina. Illumina asserts Guardant and its inventors misappropriated trade secrets and breached their employment contracts with Illumina. Illumina seeks a judgement that it is at least a co-owner of the Guardant patents, including the '306 patent and the others asserted by Guardant against TwinStrand. EX1069, 1-2, 28-30; EX1086, 16-25; EX1085. TwinStrand has thus moved to stay Guardant's counterclaims, adding further uncertainty to the time to trial. EX1088. Accordingly, *Fintiv* weighs in favor of institution. *Apple*, 5-6.

IX. GROUND 1: CLAIMS 1-3, 5, 7, 9-14, 17-27, AND 29 WOULD HAVE BEEN OBVIOUS OVER NARAYAN AND SCHMITT

The challenged claims recite nothing more than applying the known prior art methods of DCS to cfDNA. As shown below, Narayan and Schmitt disclosed all the elements of claims 1-3, 5, 7, 9-14, 17-27, and 29. EX1002, ¶¶140-146, 156-

335.

A. Claim 1

1. "A method, comprising: (a) providing a population of cell-free deoxyribonucleic acid (cfDNA) molecules having first and second complementary strands…"

Narayan discloses isolating cfDNA ("cell-free tumor DNA") from the blood of cancer patients, sequencing it, and analyzing the sequence reads. EX1082, Abstract; EX1002, ¶157. Schmitt teaches methods of reducing or tracking redundancy in sequence reads by classifying consensus sequences ("duplex consensus sequencing") in double-stranded DNA samples. EX1083, ¶¶[0009], [0048], [0053]; EX1002, ¶158. Schmitt discloses that DCS "capitalizes on the redundant information stored in complexed *double-stranded DNA*" (i.e., molecules having first and second complementary strands). EX1083, ¶[0007]. Schmitt also discusses and incorporates by reference Fan (EX1053), Chiu (EX1055), Mitchell (EX1056), and Ehrich (EX1057), which describe sequencing "nucleic acid-based serum biomarkers" (i.e., cfDNA) in prenatal screening and "early detection of cancer." EX1083, ¶¶[0003], [0048], pp.38-42; EX1002, ¶¶159-160.

2. "(b) tagging a plurality of the cfDNA molecules in the population with duplex tags comprising molecular barcodes to produce tagged parent polynucleotides, wherein the duplex tags are attached to both ends of a molecule of the plurality of the cfDNA molecules, wherein the plurality of the cfDNA molecules are tagged with n different combinations of molecular barcodes, wherein n is at least 2 and no more than 100,000*z, wherein z is a mean of an

expected number of duplicate molecules in the population of cfDNA molecules that map to identical start and stop positions on a reference sequence…"

Tagging a plurality of the cfDNA molecules. Schmitt discloses a "hybrid method" of tagging both ends of DNA molecules from a sample. EX1083, ¶[0030]; EX1002, ¶161. As discussed above in Section III(C), Schmitt's hybrid method uses both the fragment DNA end sequences *and* "a shorter n-mer tag" in the adapter together to "serve as unique molecular identifiers." *Id.* Thus, the added tag together with the end of the target DNA serve together to label the molecule. EX1083, ¶[0030].

Schmitt's hybrid tag embodiment comprises "duplex tags comprising molecular barcodes" that are "attached to both ends" of a cfDNA molecule. The '306 patent discloses that "duplex tags" are "tags that differently label the complementary strands (i.e., the 'Watson' and 'Crick' strands) of a double-stranded molecule." EX1001, 17:9-13. As discussed above in Section III(C) and shown in Dr. Spellman's figures, Schmitt's hybrid tag embodiment differently labels the complementary strands by tagging both ends of the molecule and relying on the asymmetrical nature of the duplex tags. EX1083, ¶¶[0011], [0030]; EX1002, ¶162. And Schmitt's hybrid tag embodiment comprises molecular barcodes because the duplex tags comprise both the fragment DNA end sequences and "a shorter n-mer tag" to serve as "unique molecular identifiers." EX1083, ¶[0030];

EX1002, ¶¶162-163.

Tagging with "n different combinations of molecular barcodes, wherein n is at least 2 and no more than 100,000*z …." Schmitt discloses using 3-mer barcodes in the hybrid tag embodiment. EX1083, ¶¶[[0014], [0030], Fig. 4. A POSA would have understood that Schmitt's 3-mer barcode would yield a set of 4^3 (i.e., 64) different molecular barcodes, or "identifiers," which provides 4,096 (i.e., 64^2) total "different combinations of molecular barcodes," considering the tagging occurs on both ends of the target molecule. EX1002, ¶164.

A POSA would also know that the number of cfDNA molecules in the sample that map to identical start and stop positions on a reference sequence depends on the number of HGEs in the sample. EX1002, ¶165. For example, a cfDNA sample with an average fragment length of 170 bp comprising about 10,000 HGEs may have over 176 billion total cfDNA molecules in the sample ((10,000 HGEs × 3 billion bp per genome)/170 bp per fragment), but according to the '306 patent, only about 2 to 8 cfDNA molecules map to identical start and stop positions. EX1002, ¶165; EX1001, 20:47-50. In this example, a POSA following Schmitt's 3-mer hybrid tag method would satisfy this claim element because the 4,096 total different combinations of molecular barcodes are more than 2 and no more than 200,000 to 800,000 (i.e., "100,000*z, wherein z is 2 to 8"). *Id.* Indeed, the '306 patent discloses that a sample of 10,000 HGEs "can be tagged with …

about *64 different identifiers*,” just like Schmitt’s 3-mer hybrid tag embodiment. *Id.*, 20:50-54; EX1083, ¶[0030]; EX1002, ¶166.

At bottom, a POSA would have understood that Schmitt’s 4,096 different combinations of 3-mer barcodes would fall within the claimed range of at least 2 and no more than 100,000*z, because the POSA would have been motivated to use routine amounts of plasma, such as 7-12 mL, isolated from a standard blood draw. EX1049, 1; EX1002, ¶166. A plasma sample of about 7-12 mL would provide about 1 μg of cfDNA (about 300,000 HGEs), in which z would be much higher than 2-8 for 10,000 HGEs as disclosed in the patent. EX1002, ¶166; EX1047, 1660, Table 1; EX1048, 347; EX1049, 1; EX1001, 20:47-50. Thus, whether z is 1, 10, 100, 800, etc., Schmitt’s 4,096 different combinations of barcodes would fall between 2 and 100,000*z. EX1002, ¶¶166-167; *Ormco Corp. v. Align Technology, Inc.*, 463 F. 3d 1299, 1311 (Fed. Cir. 2006). Accordingly, Schmitt discloses this element of claim 1.

3. “(c) amplifying a plurality of the tagged parent polynucleotides to produce amplified progeny polynucleotides...”

Schmitt discloses amplifying the tagged parent polynucleotides by PCR. EX1083, ¶¶[0009], [0042], [0062], [0063], [0070]-[0075], Fig. 1; EX1002, ¶¶168-169. For example, Schmitt discloses, that the “tagged strands are then PCR amplified.” EX1083, ¶[0062].

4. "(d) sequencing at least a subset of the amplified progeny polynucleotides to produce a set of sequence reads…"

Schmitt discloses this limitation. Schmitt discloses generating a set of sequence reads "using any suitable method known in the art" and provides working examples using Illumina's sequencing platform. EX1083, ¶¶[0042], [0059], Fig. 1; EX1002, ¶¶170-172.

5. "(e) reducing or tracking redundancy of a plurality of sequence reads from the set of sequence reads using at least sequencing information from the molecular barcodes of the duplex tags to determine distinct cfDNA molecules from among the tagged parent polynucleotides, wherein the distinct cfDNA molecules are determined based on (i) paired reads corresponding to sequence reads generated from a first tagged strand and a second tagged complementary strand derived from cfDNA molecules from among the tagged parent polynucleotides, or (ii) unpaired reads corresponding to sequence reads generated from a first tagged strand having no second tagged complementary strand derived from cfDNA molecules from among the tagged parent polynucleotides, wherein reducing or tracking the redundancy of the plurality of sequence reads comprises mapping at least a subset of the plurality of sequence reads to the reference sequence."

Schmitt discloses this limitation. EX1002, ¶¶173-185.

Reducing or tracking redundancy: Schmitt discloses that DCS "capitalizes on the *redundant information* stored in complexed double-stranded DNA." EX1083, ¶¶[0007], [0044]. A POSA would have understood that Schmitt discloses tracking redundancy in sequence reads because Schmitt discloses that, after tagging the DNA molecules and amplifying them via PCR, "a family of

molecules is obtained that arose from a single DNA molecule," and that "members of the same PCR 'family' are then grouped together by virtue of having a common (i.e., the same) SMI tag sequence." EX1083, ¶¶[0063], [0043]; EX1002, ¶¶174-177. Schmitt provides a schematic of family groupings in Figure 3, stating that "sequence reads … sharing a unique set of SMI tags are grouped into paired families with members having strand identifiers in either the αβ or βα orientation"—i.e., using "at least sequence information from the molecular barcodes," as recited in claim 1. EX1083, ¶[0013], Fig. 3; EX1002, ¶176.

The '306 patent discloses that "reducing redundancy in the set of sequence reads comprises collapsing sequence reads produced from amplified products of an original polynucleotide molecule in the sample back to the original polynucleotide molecule." EX1001, 9:42-46. This is taught in Schmitt. After grouping the reads into families, Schmitt teaches reducing the redundancy of the sequence reads by generating a consensus sequence for each family ("collapsing" the families into "single strand consensus sequences (SSCSs))." EX1083, ¶¶[0060], [0063]; EX1002, ¶177.

Reducing or tracking redundancy comprises mapping sequence reads to a reference sequence: Schmitt discloses that "[r]eads were aligned to the human genome with the Burrows-Wheeler Aligner." EX1083, ¶[0060]; *id.*, ¶¶[0066], [0068]; EX1002, ¶178. Schmitt also discloses mapping the raw sequence reads,

stating that "[m]utations were *initially scored* without consideration of the SMI sequences," and that "PCR duplicates were filtered out with *samtools rndup*...." EX1083, ¶[0066]. Both the BWA and SAMtools (***s***equence ***a***lignment/***m***ap tools) software disclosed in Schmitt were well known mapping tools. EX1038, 1757; EX1002, ¶179.

Determining distinct cfDNA molecules based on paired or unpaired reads: Schmitt discloses that "each strand in a DNA duplex pair generates a *distinct*, yet related population of PCR duplicates after amplification owing to the complementary nature of the SMI on the two strands of the duplex." EX1083, ¶[0062]; *id.,* ¶[0049] (describing using DCS to identify "distinct molecules" over "true PCR duplicates"); EX1002, ¶180. Schmitt further discloses generating consensus sequences from paired sequence reads (meaning that a sequence read was generated for both the Watson strand and Crick strand) and unpaired sequence reads (meaning that a sequence read was generated for only the Watson strand or Crick strand). EX1083, ¶¶[0060], [0068], Fig. 3; EX1002, ¶181.

Schmitt exemplifies this step schematically in, e.g., Figure 3 showing that sequence reads are "grouped into paired families" and that "[e]ach family pair reflects one double-stranded DNA fragment." EX1083, ¶[0013]; EX1002, ¶182. A POSA therefore would have known that Schmitt discloses determining distinct DNA molecules from sequence reads of a first tagged strand and a second tagged

complementary strand – i.e., paired reads, as recited in claim 1(e)(i). EX1002, ¶183. Indeed, Schmitt discloses in a working example that "consensus sequences were then *paired with their strand-mate*" and that the "complementary nature" of the double-stranded sequences "was used to *identify pairs of consensus groups* that arose from complementary DNA strands EX1083, ¶¶[0060], [0068]; EX1002, ¶183. By following this teaching in Schmitt, the POSA would have also determined distinct DNA molecules from sequence reads of a first tagged strand having no second tagged complementary strand – i.e., unpaired reads, as recited in claim 1(e)(ii). EX1002, ¶183; EX1083, ¶¶[0013], [0068]. The POSA would have appreciated that, while the unpaired reads provide less confidence regarding sequence accuracy compared with paired sequence reads, the unpaired reads nevertheless provide useful sequence information and would not be discarded. EX1002, ¶183.

In addition to the above, the Schmitt PCT discloses a separate working example of reducing or tracking redundancy of sequence reads. EX1009, ¶¶[00120]-[00137], Fig. 6; EX1002, ¶¶184-185. Schmitt discloses that, under optimal conditions, the ratio of SSCS reads to DCS reads is 2:1, and a SSCS:DCS ratio greater than 2 indicates that some SSCS reads could not be paired with a mate to generate a DCS. EX1009, ¶[00128]; EX1002, ¶184. Schmitt's example discloses a SSCS:DCS ratio of 4.23 which Schmitt rounds to 4 in Table 1 ("SSCS reads per

DCS read"). *Id.* Thus, a POSA would understand that in Schmitt's Example 4, more than half of the total SSCS reads remained unpaired, while the other reads were paired. *Id.*

6. A POSA would have had a reason to combine Narayan and Schmitt.

Schmitt alone discloses embodiments within the scope of claim 1, detailing all the steps of tagging, amplifying, sequencing, and reducing or tracking redundancy of sequencing reads, and further disclosing cfDNA. EX1083, ¶¶[0003], [0007], [0009], [0042], [0043], [0048], [0053], [0062], [0063], [0070]-[0075], Fig. 1; EX1002, ¶¶187-205. Moreover, a POSA would have had several reasons to combine Narayan's teachings with Schmitt's DCS. EX1002, ¶¶187-205.

Method for reducing or tracking redundancy in sequence reads: Narayan discloses using NGS for analyzing cfDNA from cancer patients, and Schmitt discloses methods of error correction for NGS by generating duplex consensus sequences (i.e., reducing or tracking redundancy in sequence reads). A POSA would have known that error correction is important when using NGS for diagnostic or screening applications as taught in Narayan, and would have been motivated to use cfDNA as disclosed in Narayan and in Schmitt's DCS methods. EX1002, ¶¶187-189. Indeed, Narayan reported "a limit of detection of approximately 1 variant in 5,000 molecules" and that "mutant counts would need to be several-fold above background to guide clinical decisions." EX1082, 3497;

EX1002, ¶188. A POSA would have understood that reducing or tracking sequence read redundancy using Schmitt's DCS would improve the sensitivity of sequence detection and thus been motivated to use it in in clinical settings as suggested by Narayan. *Id.*

Tagging the cfDNA molecules: A POSA would have been motivated to use Schmitt's 3-mer hybrid tagging approach for several reasons. First, the POSA would have appreciated that Schmitt's 3-mer hybrid tag embodiment offers advantages of simplified adapter synthesis, improved synthesis yield, and improved downstream PCR amplification, compared to longer barcodes such as Schmitt's 12-mer or 20-mer embodiments. EX1083, ¶¶[0047], [0062], Fig. 2; EX1092, 1-2; EX1090, 1; EX1091, 689-690; EX1089, 12951; EX1002, ¶¶192-194. A POSA would have known that incorporating a simpler 3-mer molecular barcode into the adapters can be done with a pre-made set of barcodes because there are a limited number of molecular barcode sequences (64) and different combinations (4,096). *Id.* This makes adapter synthesis easier and more efficient, while still enabling the POSA to perform DCS using the 3-mer barcodes and the ends of the DNA fragments together as unique identifiers. *Id.*

Second, the POSA also would have known that they could get more value per sequencing read when using Schmitt's 3-mer hybrid tags. For example, the POSA would have known that Illumina instruments can sequence 150-200 bp per

read. EX1012, 296; EX1018, 764; EX1002, ¶194. Using a longer barcode sequence such as a 20-mer on each end of the DNA fragment (40 total barcode nucleotides per fragment) would use up 40 bases of the sequence read. *Id.* Using a short n-mer, such as Schmitt's 3-mer[5] (6 total barcode nucleotides), would allow for more of the sequencing read to be dedicated to the target DNA instead of barcode nucleotides, while the 4,096 total different combinations of molecular barcodes in the set would still be diverse enough for tagging (when combined with the ends of the target DNA) to perform DCS. EX1002, ¶195. Indeed, Schmitt teaches that a shorter n-mer tag in the hybrid tag embodiment "minimiz[es] loss of sequencing capacity due to sequencing of the [barcode] itself." EX1083, ¶[0047]; EX1002, ¶¶194-196.

The number of different combinations of molecular barcodes: As discussed above, Schmitt's 3-mer hybrid tag embodiment comprises 4,096 different combinations of molecular barcodes, within the claimed range of at least 2 to no more than 100,000*z. EX1002, ¶197; EX1083, ¶[0030]; EX1047, 1660, Table 1; EX1048, 347; EX1049, 1; EX1001, 20:47-50. A POSA would have been motivated to use Schmitt's 3-mer hybrid tag method for the reasons discussed

---

[5] For these reasons, it would have also been obvious to a POSA to use Schmitt's 1-, 2-, or 4-mer tags in the hybrid tag embodiment. *Ormco*, 1311.

above for tagging.

Amplifying, Sequencing, Mapping, and Reducing or tracking redundancy through paired and unpaired reads: A POSA would have been motivated to incorporate these steps from Schmitt because Schmitt teaches each of these steps as part of DCS. EX1002, ¶¶198-205; Section III(C-D).

Guardant may argue that there was no reason to combine Schmitt with Narayan because of allegedly insufficient amounts of cfDNA in human blood for DCS. This argument fails because, as discussed above in Sections III(B) and (D), it is factually wrong and is contradicted by the prior art. A POSA would have known that sufficient amounts of cfDNA could be extracted from blood. EX1002, ¶190; Section III(B). And the '306 patent discloses nothing more than routine techniques (e.g., QIAamp kit) to extract cfDNA and further discloses using as little as 2.5 ng cfDNA as starting material. EX1001, 54:8-10; EX1002, ¶190. Moreover, Schmitt discloses using enrichment techniques such as hybrid capture to increase the relative concentration of sequences of interest. EX1083, ¶¶[0058], [0065]; EX1002, ¶191. And a POSA would have readily appreciated that more cfDNA, if needed, could be obtained by simply increasing the amount of plasma sample used for cfDNA extraction. EX1002, ¶191; EX1049, EX1071, 718.

7. A POSA would have had a reasonable expectation of success.

A POSA combining Narayan and Schmitt would have expected to

successfully arrive at the method of claim 1 for several reasons. EX1002, ¶¶206-219.

Method for reducing or tracking redundancy through paired and unpaired sequences: A POSA would have expected to successfully use cfDNA, as expressly disclosed in Narayan, in Schmitt's DCS because the prior art expressly taught that DCS could "enhance accuracy" when sequencing cfDNA from cancer patients. EX1008, 7; EX1002, ¶¶206-208. Schmitt also discloses tracking and reducing redundancy ("redundant information") in sequence reads by grouping the reads into families and collapsing the families into consensus sequences. EX1083, ¶¶[0007], [0014], [0044], [0060], Fig. 3. These express teachings *in the prior art* contradict Guardant's baseless prosecution arguments. EX1066, 562-563; Section VIII(A).

Tagging the cfDNA molecules: A POSA would have expected to successfully tag a plurality of the cfDNA molecules with duplex tags comprising molecular barcodes because Schmitt teaches that DCS can be successfully performed using the hybrid tagging method with 3-mer molecular barcodes. EX1083, ¶[0030]; EX1002, ¶216. The POSA would have known that this embodiment in Schmitt produces 4,096 different combinations of molecular barcodes ("n different combinations"), and would have expected it to work in DCS as Schmitt expressly teaches. *Id.* Moreover, the POSA would have also expected

success because library preparation in Schmitt's 3-mer hybrid method requires synthesis of a small, finite number of barcodes, making it simpler and more efficient than synthesizing sets of much longer random barcodes such as a 20-mer. EX1002, ¶217.

Sufficient cfDNA in blood samples: A POSA would have further expected to successfully modify Narayan with Schmitt's DCS because, as discussed above in Sections III(B) and (D), the POSA would have known that human blood contains ample quantities of cfDNA for use in DCS. EX1002, ¶¶209-215; Sections III(B) and (D). And the '306 patent admits that the method can work with as little as *2.5 nanograms* of cfDNA, without needing any special methods or reagents. EX1001, 54:8-10; EX1002, ¶213.

In sum, Narayan and Schmitt disclose all the elements of claim 1, and a POSA would have had a reason to combine the references with a reasonable expectation of success in so doing. Claim 1 was therefore obvious (notwithstanding any evidence of objective indicia of nonobviousness as discussed for all challenged claims in Section XIII, below).

B. Claim 17

Like claim 1, claim 17 recites nothing more than applying the known prior art methods of DCS to cfDNA. Narayan and Schmitt disclose all the elements of claim 17.

1. "A method, comprising: (a) tagging a population of double-stranded cell-free deoxyribonucleic acid (cfDNA) molecules obtained or derived from a sample of a subject with a set of tags comprising molecular barcodes to produce tagged parent polynucleotides…"

Claim 17(a) is substantially the same as claim 1(b), however claim 17(a) specifically recites tagging "double-stranded" cfDNA molecules "from a sample of a subject with a set of tags comprising molecular barcodes," whereas claim 1(b) recites tagging cfDNA molecules "with duplex tags comprising molecular barcodes" and further places limitations on the number of different combinations of molecular barcodes. EX1001, 61:10-21, 62:46-50. As discussed above, Narayan and Schmitt disclose all the elements of claim 1(b). Section IX(A)(2). Schmitt discloses that DCS "capitalizes on the redundant information stored in complexed *double-stranded DNA*" (i.e., double-stranded DNA molecules). EX1083, ¶[0007]; EX1002, ¶¶220-222. Schmitt also cites and incorporates by reference Fan (EX1053), Chiu (EX1055), Mitchell (EX1056), and Ehrich (EX1057), which each describe methods of sequencing double-stranded cfDNA molecules. EX1083, ¶¶[0003], [0048], pp. 38-42; EX1002, ¶222; Section IX(A)(1).

Also discussed above for claim 1, Schmitt discloses tagging DNA molecules from a sample of a subject. EX1083, ¶[0030]; EX1002, ¶223. And Schmitt's 3-mer hybrid tag embodiment comprises a "set of tags" as claimed in claim 17 because it comprises 64 different molecular barcodes – i.e., a "set." EX1083, ¶[0030];

EX1002, ¶224; Section IX(A)(2). Additionally, and independently, Schmitt discloses embodiments of *uniquely* tagging double-stranded cfDNA molecules that meet the elements of claim 17. EX1083, ¶[0016] (disclosing an embodiment using a "random degenerate nucleotide n-mer sequence which is 12 nucleotides in length" that "results in generation of up to 4^{24} (i.e., 2.8×10^{14}) distinct tag sequences."); EX1002, ¶225. Thus, just as Schmitt discloses all the elements of claim 1(b), Schmitt also discloses all the elements of the claim 17(a). EX1002, ¶¶220-226; Section IX(A)(2).

2. "(b) amplifying a plurality of the tagged parent polynucleotides to produce amplified progeny polynucleotides…"

Schmitt discloses all the elements of the amplifying step in claim 17(b) for the same reasons discussed above for the amplifying step in claim 1(c). EX1002, ¶¶227-228; Section IX(A)(3); EX1083, ¶¶[0009], [0042], [0062], [0063], [0070]-[0075].

3. "(c) sequencing at least a subset of the amplified progeny polynucleotides to produce a set of sequence reads…"

Schmitt discloses all the elements of the sequencing step in claim 17(c) for the reasons discussed above for the sequencing step in claim 1(d). EX1002, ¶¶229-230; Section IX(A)(4).

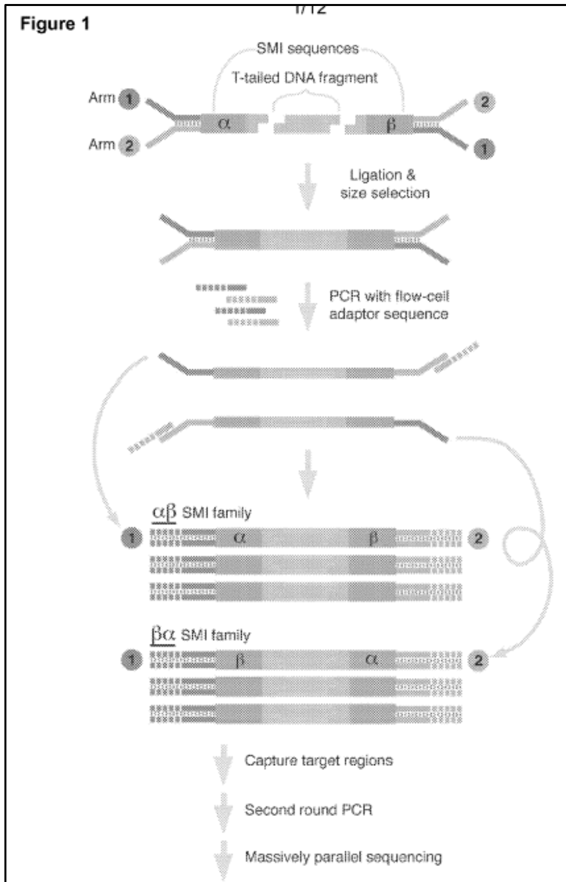
4. "(d) sorting a plurality of sequence reads from the set of sequence reads into (i) families comprising paired reads corresponding to sequence reads generated from a first

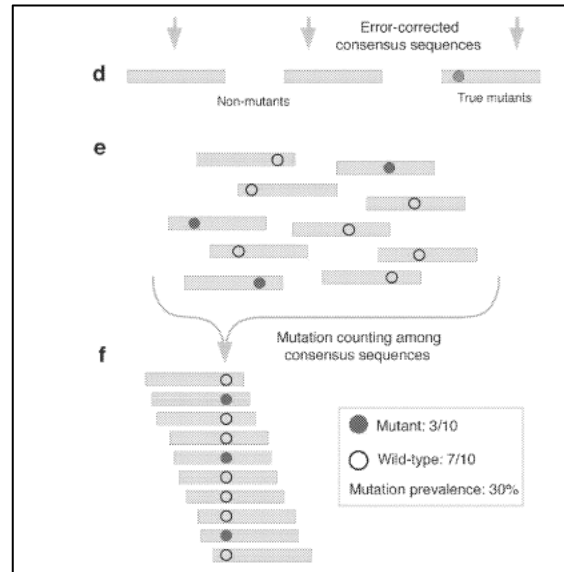
> **tagged strand and a second tagged complementary strand derived from double-stranded cfDNA molecules from among the tagged parent polynucleotides, and (ii) families comprising unpaired reads corresponding to sequence reads generated from a first tagged strand having no second tagged complementary strand derived from double-stranded cfDNA molecules from among the tagged parent polynucleotides."**

Claim 17(d) and claim 1(e) both entail identifying the sequence reads as a paired read (one that has a complementary strand-mate) or an unpaired read (one that does not have a complementary strand-mate). EX1001, 61:26-43, 62:55-65; EX1002, ¶231. As discussed above, Schmitt discloses claim 1(e); therefore, Schmitt discloses claim 17(d) for the same reasons. EX1083, ¶¶[0013], [0060], [0068], Fig. 3; EX1002, ¶232; Section IX(A)(5).

In addition, a POSA would have known that Schmitt discloses claim 17's "sorting" the reads into families comprising paired and unpaired reads, because Schmitt discloses generating duplex consensus sequence reads (paired reads), which requires "sorting" the reads into paired reads and unpaired reads. *Id.* This is depicted in, e.g., Schmitt's Figures 1 and 3. EX1083, Figs. 1, 3, ¶[0013].

Additionally, Schmitt's Figure 1 depicts the upstream processes of tagging the parent polynucleotide and amplifying it to produce amplified progeny polynucleotides before sequencing and sorting the sequence reads. EX1083, Fig 1; EX1002, ¶¶233-235.





EX1083, Figs. 1, 3, ¶¶[0043], [0063]; EX1002, ¶234.

Schmitt further discloses that, after tagging the DNA molecules and amplifying them via PCR, "a family of molecules is obtained that arose from a single DNA molecule," and that "members of the same PCR 'family' are then grouped together [i.e., sorted] by virtue of having a common (i.e., the same) SMI tag sequence." EX1083, ¶[0063]; *id.,* ¶[0043]; EX1002, ¶235. Schmitt provides a schematic of family groupings in Figure 3, explaining that "sequence reads …sharing a unique set of SMI tags are grouped [i.e., sorted] into *paired families* with members having strand identifiers in either the αβ or βα orientation."

EX1083, ¶[0013], Fig. 3; EX1002, ¶¶236-237.

In addition, as discussed above for claim 1(e), Schmitt's Example 4 discloses an additional example of sorting the sequence reads into families of paired and unpaired reads. EX1009, ¶¶[00120]-[00137], Fig. 6; EX1002, ¶237.

Reasons to combine. A POSA would have combined Narayan and Schmitt for the reasons discussed above for claim 1. EX1002, ¶238; Section IX(A)(6).

Reasonable expectation of success. A POSA reading Narayan and Schmitt would have had expected to successfully practice the method of claim 17 for the reasons discussed above for claim 1. EX1002, ¶239; Section IX(A)(7).

Thus, claim 17 was obvious. EX1002, ¶¶220-239.

C. Claims 2 and 18

Claims 2 and 18 depend from claims 1 and 17, respectively, and further recite that the population of cfDNA molecules is "obtained or derived from a sample from a subject having cancer" (claim 2) and the "sample is blood, plasma, or serum" (claim 18). EX1001, 61:44-46, 62:66-67; EX1002, ¶¶240-241. Narayan discloses sequencing cfDNA from "patients with stage I-IV *non-small cell lung cancer*," and that "DNA was extracted from 0.2 mL of each *plasma* sample." EX1082, 3493; EX1002, ¶242. Schmitt describes "clinical applications" such as prenatal or cancer screening using "*nucleic acid-based serum biomarkers*." EX1083, ¶[0003]; EX1002, ¶243. Schmitt also discloses and incorporates by

reference Fan (EX1053), Chiu (EX1055), Mitchell (EX1056), and Ehrich (EX1057), which each further discloses sequencing cfDNA from blood samples. EX1083, ¶¶[0003], [0048] and pp. 38-42; EX1002, ¶243. Accordingly, Narayan and Schmitt disclose the elements of claims 2 and 18.

The rationales for obviousness of claims 1 and 17 also apply to claims 2 and 18, respectively. EX1002, ¶244. Additionally, the POSA would have expected to successfully obtain a population of cfDNA molecules from a sample, such as a blood, serum, or plasma sample, from a subject having cancer as discussed in Section III(B). EX1002, ¶¶245-247. Thus, claims 2 and 18 were obvious.

D. Claims 3 and 19

Claims 3 and 19 depend from claims 1 and 17, respectively, and recite that the population of [double-stranded] cfDNA molecules "comprises 1 nanogram (ng) to 100 ng of [double-stranded] cfDNA molecules." EX1001, 61:47-49, 63:1-3.

Narayan discloses extracting cfDNA from "0.2 mL of each plasma sample," which a POSA would have known, based on the published concentration ranges of cfDNA in plasma, contains a median of 16.7 to 27.8 ng cfDNA—squarely within the claimed range. EX1082, 3493; EX1002, ¶¶248-251; EX1047, 1660, Table 1; EX1048, 347; *Ormco*, 1311. The rationales for obviousness of claims 1 and 17 also apply to claims 3 and 19, respectively. EX1002, ¶252.

Additionally, the POSA would have expected to readily optimize the amount

of cfDNA for use in DCS (i) by simply increasing the plasma sample volume; and (ii) because Schmitt teaches modifications to the protocol for lower amounts of DNA (like Narayan's cfDNA), such as increasing the PCR cycles and hybrid capture enrichment. EX1083, ¶¶[0058]-[0059], [0065], [0069]; EX1002, ¶253; EX1049, EX1071, 718. The art also disclosed numerous commercial kits for extracting cfDNA from human blood. EX1002, ¶¶254-255; Section III(B)(2). Thus, claims 3 and 19 were obvious.

E. Claims 5 and 20

Claim 5 depends from claim 1, and recites that "the molecular barcodes are ligated to the plurality of the cfDNA molecules using more than an 80× excess of duplex tags as compared to the population of cfDNA molecules." EX1001, 61:56-59. Claim 20 depends from claim 17, and recites that "the tagging comprises ligating the molecular barcodes to double-stranded cfDNA molecules." *Id.*, 63:4-6.

Schmitt discloses these limitations. Schmitt discloses that the adapters can contain a "T-overhang, an A-overhang, a CG overhang, blunt end or any other *ligatable* sequence." EX1083, ¶¶[0041], [0020]; EX1002, ¶¶256-259. Schmitt also discloses ligating with "more than an 80× excess" of duplex tags when disclosing ligating "250 pmol adaptors" to "750 ng" of size-selected DNA (~200-500 base pairs). EX1083, ¶[0056]. A POSA would have recognized that 750 ng of DNA for the selected DNA range of ~200-500 bp would be equivalent to 2.43-6.07 pmol of

DNA. EX1002, ¶260. In Schmitt's reaction comprising 250 pmol of adapters, the excess of adapters relative to the DNA molecules therefore ranges from approximately 41× to 103× molar excess (depending on the size range of the DNA molecules), overlapping with the claimed range of "more than an 80× excess." EX1002, ¶260.

"Where a claimed range overlaps with a range disclosed in the prior art, there is a presumption of obviousness." *Ormco*, 1311. Guardant provides no evidence in the '306 patent or its file history that the claimed range is critical or produces unexpected results. *Id.*

Moreover, a POSA would have been motivated by Schmitt to ligate adapters efficiently, especially when using cfDNA, to maximize sequenceable target. EX1002, ¶¶261-265; EX1083, ¶[0056]. As discussed above, there was a strong motivation in the art to perform adapter-DNA ligations with more than an 80× excess of adapters relative to DNA molecules to achieve a high ligation efficiency. EX1002, ¶¶261-267; Section III(A). And, a POSA would have appreciated that increasing the adapter-DNA ligation efficiency was especially important when starting with lower amounts of input DNA, like cfDNA. *Id.* Accordingly, claims 5 and 20 were obvious.

F. Claim 7

Claim 7 depends from claim 1 and recites that "z is between 2 and 8."

EX1001, 61:63. As discussed above for claim 1, Schmitt's 3-mer hybrid tag embodiment meets this limitation because Schmitt's 3-mer tag comprises 4,096 different combinations of molecular barcodes and thus falls within the claimed range of 2 to no more than 200,000 to 800,000 (100,000*z). EX1002, ¶¶268-272; EX1047, 1660, Table 1; EX1048, 347; EX1049, 1; EX1001, 20:47-50. The rationales for obviousness of claim 1—including a POSA's motivations for using Schmitt's 3-mer hybrid tag and reasons to expect success—also apply to claim 7. Accordingly, claim 7 was obvious.

G. Claim 21

Claim 21 depends from claim 17, and further recites that "the set of tags comprises 2 to 10,000 different molecular barcode sequences." EX1001, 63:7-9. Schmitt's 3-mer hybrid tag meets this limitation. As discussed above, a POSA would have known that Schmitt's 3-mer hybrid tag results in 64 different molecular barcodes, which is squarely within the claimed range of "2 to 10,000." EX1083, ¶[0030]; EX1002, ¶¶273-274. *Ormco*, 1311. The rationales for obviousness of claim 17—including a POSA's motivations for using Schmitt's 3-mer hybrid tag and reasons to expect success—also apply to claim 21. EX1002, ¶¶275-277. Accordingly, claim 21 was obvious.

H. Claims 9-10 and 22-23

Claims 9 and 22 depend from claims 1 and 17, respectively, and recite "prior

to the sequencing, enriching at least a subset of the amplified progeny polynucleotides for target regions of interest to produce enriched progeny polynucleotides." EX1001, 61:66-62:2, 63:10-13. Claims 10 and 23 depend from claims 9 and 22, respectively, and further recite that "the target regions of interest comprise genetic sequences of a plurality of genes selected from the group consisting of …BRAF,…EGFR,…KRAS…." *Id.*, 62:3-17, 63:14-27.

Narayan and Schmitt disclose all of these limitations. EX1002, ¶¶278-287. Regarding claims 9 and 22, Schmitt discloses *enriching* the PCR-amplified DNA for a target region of interest using a commercial hybrid capture kit. EX1002, ¶¶280-282; EX1014, S30. Schmitt states: "[t]he adapter-ligated library was PCR amplified and subjected to *SureSelect capture*," a hybrid capture enrichment protocol. EX1083, ¶¶[0065], [0057]-[0058]; EX1002, ¶281.

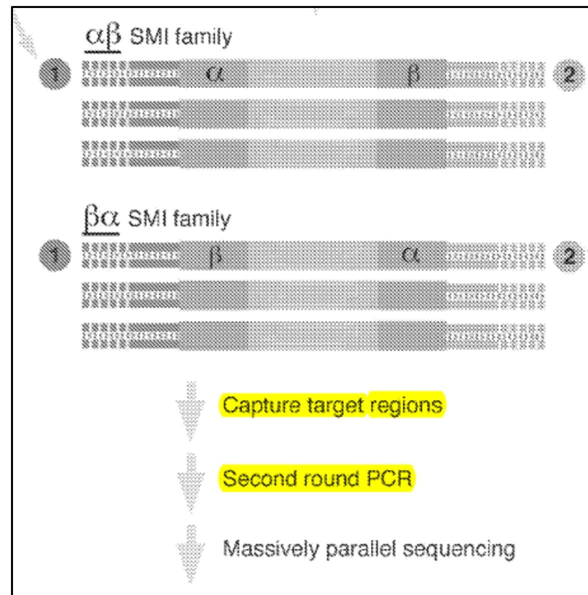
Schmitt also discusses using deep sequencing for the "detection of cancer." *Id.*, ¶[0003]. Narayan discloses target regions of interest recited in claims 10 and 23. Narayan discloses enriching "mutation hotspot regions" (genetic sequences of a plurality of genes) and detecting specific gene mutations, including e.g., *KRAS, EGFR,* and *BRAF*, as claimed in claims 10 and 23. EX1082, 3493, Figs. 3-4; EX1002, ¶283.

The rationales for obviousness of claims 1 and 17 also apply to claims 9-10 and 22-23. EX1002, ¶¶284-287. Additionally, the POSA would have been

motivated to enrich for genomic regions comprising sequences from known cancer-associated genes like *KRAS, EGFR,* and *BRAF* as disclosed in Narayan because Narayan refers to these genes as containing “mutation hotspot regions” and are thus relevant for cancer diagnostics and screening. EX1082, 3493; EX1002, ¶284. A POSA would have expected to successfully enrich for target regions of interest from a plurality of the same genes recited in claims 10 and 23 because they were known cancer-associated genes and Schmitt specifically indicates that hybrid capture enrichment is compatible with DCS. EX1083, ¶¶[0058], [0065]; EX1082, 3493; EX1014, S30; EX1034; EX1033; EX1002, ¶286. Accordingly, claims 9-10 and 22-23 were obvious.

I. Claim 11

Claim 11 depends from claim 10, and further recites “prior to the sequencing, amplifying a plurality of the enriched progeny polynucleotides.” EX1001, 62:18-20. Schmitt discloses this limitation because Schmitt discloses amplifying enriched polynucleotides, describing “[p]ost-capture [i.e., post-enrichment] amplification. Captured DNA was amplified with PCR primers….” EX1083, ¶[0059]. Schmitt’s Figure 1 also depicts post-enrichment amplification:



EX1083, Figure 1 (highlighting added); EX1002, ¶¶288-289.

The rationales for obviousness of claims 1, 9, and 10 also apply to claim 11. Additionally, the POSA would have been motivated to amplify the enriched target DNA before sequencing because Schmitt teaches performing a "Second round PCR" as part of DCS. EX1083 Figure 1, ¶[0059]; EX1002, ¶290. The POSA would have expected to successfully perform amplification of the enriched target DNA because PCR amplification after enrichment—as taught in Schmitt—was a routine technique in NGS. EX1002, ¶¶291-292; EX1083 Figure 1, ¶[0059].

J. Claim 12

Claim 12 depends from claim 1, and recites that "the duplex tags are part of sequencing adapters." EX1001, 62:21-22. Schmitt discloses all the limitations of claim 12. As discussed above for claim 1, Schmitt discloses duplex tags. EX1002,

¶¶293-294; Section IX(A)(2). Schmitt further discloses that the duplex tags "may also include at least two *sequencing primer binding sites*, each corresponding to a sequencing read." EX1083, ¶[0021]; EX1002, ¶294. A POSA would have understood, therefore, that the duplex tags were part of the sequencing adaptors. EX1002, ¶294.

The rationales for obviousness of claim 1 also apply to claim 12. Additionally, a POSA would have been motivated to include duplex tags in the sequencing adapters because doing so reduces the steps for library preparation EX1002, ¶¶295-296. Accordingly, claim 12 was obvious. EX1002, ¶¶293-297.

K. Claim 13

Claim 13 depends from claim 1, and recites that "reducing or tracking the redundancy of the plurality of sequence reads comprises grouping the paired reads or the unpaired reads into families based at least in part on (i) the molecular barcodes associated with the paired reads or the unpaired reads and (ii) sequence information at the start and stop positions of the paired reads or the unpaired reads." EX1001, 62:23-29. Schmitt's hybrid tag embodiment meets this limitation. As discussed above, Schmitt's hybrid tag embodiment uses "a *combination of sheared ends* ***and*** *a shorter n-mer tag*" to serve as unique molecular identifiers. EX1083, ¶[0030]; EX1002, ¶¶298-299; Section IX(A)(2). And Schmitt discloses grouping the paired and unpaired sequence reads into families based on the

combination of the start and stop positions ("sheared ends") and the molecular barcode sequences. EX1083, ¶¶[0013], [0030], [0043], [0063], Figs. 1, 3; EX1002, ¶300.

The rationales for obviousness of claim 1—including a POSA's motivations for using Schmitt's 3-mer hybrid tag and reasons to expect success—also apply to claim 13. EX1002, ¶¶302-304. Accordingly, claim 13 was obvious.

L. Claims 14 and 27

Claim 14 depends from claim 1, and further recites: "determining quantitative measures of (i) paired reads or (ii) unpaired reads that map to a genomic locus of the reference sequence." EX1001, 62:30-33. Claim 27 depends from claim 24, and recites determining quantitative measures of "at least two of (i) paired reads that map to one or more genomic loci, (ii) unpaired reads that map to one or more genomic loci, (iii) a read depth of the paired reads, and (iv) a read depth of the unpaired reads." EX1001, 64:16-21. Schmitt discloses these limitations.

The '306 patent discloses that for an arbitrary locus A, "there are N [total] amplified fragments," and that "we assign N1, N2, and N3 as the number of double strands, single-strands, and unseen fragments, respectively." *Id.*, 32:43-49. The '306 patent provides the simple formula, "N = N1 + N2 + N3 (N1 and N2 are known from the sequence readouts, and N and N3 are unknown)." *Id.*, 32:43-52,

35:13-28, Fig. 1; EX1002, ¶¶305-308. Claim 14 thus recites calculating N1 ("paired reads") and N2 ("unpaired reads"). Claim 27 reads on the same calculations.

Schmitt discloses determining quantitative measures of paired and unpaired reads in, e.g., Example 1. Schmitt discloses using the complementary, double-stranded SMI sequences "to *identify pairs of consensus groups* that arose from complementary DNA strands." EX1083, ¶[0068]. A POSA would have known that Schmitt's "consensus groups" are a measure of sequence reads because *consensuses are built from reads*. EX1002, ¶309. Schmitt further explains that, "[i]n a pilot experiment, after grouping of PCR duplicates as above, *29,409 SMI partner pairs* [i.e., paired reads] were found, indicative that fewer than 1% of tags had their corresponding partner tag present in the library." *Id.* Thus, Schmitt discloses calculating a quantitative measure of paired reads ("29,409 SMI partner pairs" and "fewer than 1% of the tags") and a quantitative measure of unpaired reads (more than 99%), as claimed. EX1002, ¶309. And Schmitt discloses mapping the reads to a one or more genomic loci of a reference sequence. EX1083, ¶¶[0060], [0066], [0068]; EX1002, ¶309. Schmitt also discloses determining a quantitative measure of the read depth at specific sites for unpaired reads ("20x coverage") and paired reads ("10x coverage"). EX1083, ¶¶[0067], [0068], [0072]; EX1002, ¶309. Accordingly, Schmitt discloses all the elements of claims 14 and

27. Also, as discussed above for claims 1(e) and 17(d), Schmitt's Example 4 provides an additional example of quantitating paired and unpaired reads. EX1009, ¶[0128], Table 1; EX1002, ¶¶310.

The rationales for obviousness of claims 1, 17, and 24 also apply to claims 14 and 27. EX1002, ¶¶311-314. In addition, a POSA would have been motivated to determine the quantitative measures of the paired and unpaired sequence reads (or read depths) that map to one or more genomic loci because doing so improves accuracy and reduces error rate sequencing. EX1083, ¶¶[0067]-[0068]; EX1002, ¶¶312-313. The POSA would have expected to successfully perform the calculations because Schmitt teaches how to quantitate SSCS and DCS reads. *Id.* Accordingly, claims 14 and 27 were obvious.

M. Claim 24

Claim 24 depends from claim 18, and further recites "reducing or tracking redundancy of a plurality of sequence reads from the set of sequencing reads, wherein the reducing or tracking comprises mapping at least a subset of the plurality of sequence reads to a reference sequence, and the reducing or tracking is based on (i) the molecular barcodes associated the paired reads or the unpaired reads and (ii) sequence information at the start and stop positions of the paired reads or the unpaired reads." EX1001, 63:28-64:4.

The elements of claim 24 are substantively the same as claim 1(e) (reducing

or tracking redundancy; mapping to a reference sequence) and claim 13 (reducing or tracking redundancy is based at least on molecular barcodes and sequence information from start and stop positions). EX1001, 61:26-43, 62:23-29. As discussed above for claims 1 and 13, Schmitt discloses these limitations. EX1002, ¶¶315-316; Sections IX(A)(5) and IX(K). The rationales for obviousness of claims 1, 13, and 18 also apply to claim 24. EX1002, ¶¶317-318. Accordingly, claim 24 was obvious.

N. Claim 25

Claim 25 depends from claim 24, and further recites "determining a base call at one or more genomic loci for a plurality of sequence reads that map to the one or more genomic loci on the reference sequence." EX1001, 64:5-9. Schmitt discloses this limitation.

A POSA would have known that "base calling" in NGS means determining the most likely nucleotide for that particular base position. EX1002, ¶¶319-320; EX1007, 888; EX1084, 489. Indeed, the '306 patent confirms that "base calling" is, "determining for each locus the most likely nucleotide." EX1001, 32:30-33. Schmitt discloses the limitation of clam 25 because, e.g., Schmitt teaches generating *sequence reads* (which comprise base calls) "using any suitable method known in the art." EX1083, ¶¶[0042], [0059], Fig. 1; EX1002, ¶320. Schmitt also discloses using the sequence reads (base calls) for sequence variant detection, e.g.,

when "group[ing] together PCR duplicates that arose from individual single-stranded DNA molecules and … creat[ing] a consensus sequence from the family of duplicates" (i.e., SSCS) and "when the read data from each of the two strands is in perfect agreement" (i.e., DCS). EX1083, ¶¶[0067]-[0068]; EX1002, ¶321. And as discussed above for claims 1(e) and 24, Schmitt discloses mapping sequence reads to one or more genomic loci on the reference sequence. EX1083, ¶¶[0060], [0066], [0068]; EX1002, ¶322; Sections IX(A)(5) and IX(M).

The rationales for obviousness of claims 1 and 24 also apply to claim 25. EX1002, ¶¶323-324. Accordingly, claim 25 was obvious.

O. Claim 26

Claim 26 depends from claim 24, and further recites "collapsing the plurality of sequence reads to produce consensus sequences representative of a sequence of the original double-stranded cfDNA molecules from among the tagged parent polynucleotides." EX1001, 64:10-15.

As discussed above for claims 13 and 17(d) (Sections IX(B)(4) and IX(K)), Schmitt discloses grouping and sorting the sequence reads into families and then reducing the redundancy of the reads by generating a plurality of consensus sequences for each family ("collapsing" the families). EX1083, ¶¶[0060], [0063], [0068]; EX1002, ¶¶325-326. Schmitt exemplifies this step schematically in, e.g., Figure 3 showing that sequence reads are "grouped into paired families" and that

"[e]ach family pair reflects one double-stranded DNA fragment." EX1083, ¶[0013], Fig. 3; EX1002, ¶326. Accordingly, Schmitt discloses all the elements of claim 26.

The rationales for obviousness of claims 13, 17, and 24 also apply to claim 26. EX1002, ¶¶327-329. Thus, claim 26 was obvious.

P. Claim 29

Claim 29 depends from claim 1, and recites that "the distinct cfDNA molecules in (e) are determined based on (i) the paired reads and (ii) the unpaired reads." EX1001, 64:29-31.

As discussed above for claim 1(e), Schmitt discloses identifying distinct molecules using paired reads *and* unpaired reads, as claimed in claim 29. EX1083, ¶¶[0007], [0013], [0043], [0044], [0060], [0063], Fig. 3; EX1002, ¶¶330-331. The rationales for obviousness of claim 1 also apply to claim 29. EX1002, ¶¶332-334. Accordingly, claim 29 was obvious.

X. GROUND 2: CLAIMS 4 AND 6 WOULD HAVE BEEN OBVIOUS OVER NARAYAN, SCHMITT, AND MEYER

Claim 4 depends from claim 1, and recites that "the molecular barcodes are ligated to the plurality of the cfDNA molecules using more than a 10× excess of duplex tags as compared to the population of cfDNA molecules, wherein at least 20% of the cfDNA molecules from the population are tagged with the duplex tags." EX1001, 61:5-55. Claim 6 depends from claim 5, and recites that "at least

40% of the cfDNA molecules from the population are tagged with the duplex tags." *Id.*, 61:60-62.

As discussed above for claim 5, Schmitt discloses using a range of approximately 41× to 103× molar excess of adapters to DNA molecules, overlapping with claim 5's range of "more than an 80× excess." EX1083, ¶¶[0020], [0041], [0056]; EX1002, ¶¶336-338. Thus, the same disclosure in Schmitt also overlaps with the claim 4's range of "more than a 10× excess." *Id.*; *Ormco*, 1311. Meyer also discloses this limitation. EX1005, 272-274, Fig. 1; EX1002, ¶339. In particular, Meyer discloses using 100-500 ng of nebulized (fragmented) DNA and 2 µl of adapters from a 500 µM stock solution, which would result in a range of approximately 790× to 7,509× molar excess of adapters to DNA molecules in the reaction.[6] EX1005, 272-274; EX1002, ¶339. Accordingly, both Schmitt and Meyer disclose ranges that overlap with the claimed "more than a 10× excess" in claim 4. *Ormco*, 1311.

Meyer also teaches tagging at least 40% of the DNA molecules. Meyer discloses that "the expected overall recovery [after adapter-DNA ligation] through this step is *between 40 and 60%*" falling within the claimed ranges of "at least

[6] For the same reason, Meyer also discloses the "more than an 80× excess" element of claim 5. EX1002, ¶339.

20%" (claim 4) and "at least 40%" (claim 6). EX1005, 274; *Ormco*, 1311; EX1002, ¶340. A POSA would have understood that Meyer discloses that 40-60% of the DNA molecules are tagged because (i) Meyer's Figure 1 depicts the DNA fragments tagged on both ends (Fig. 1), (ii) Meyer's adapter ligation quantification "step 18" would only detect adapter-ligated DNA fragments, and (iii) Meyer discloses successfully achieving 40-60% tagged DNA fragments. EX1002, ¶340; EX1005, 274, Fig. 1.

The rationales for obviousness of claims 1 and 5 also apply to claims 4 and 6. Additionally, given the motivation to optimize adapter-DNA ligation for high efficiency and the routine methods for doing so (*see* Sections III(A) and IX(E)), a POSA would have expected to successfully tag "at least 40%" of the DNA molecules. EX1002. ¶¶342-350. First, Meyer expressly discloses successfully tagging 40-60% of DNA molecules. EX1005, 274; EX1002, ¶347. Second, Meyer's disclosure of 40-60% tagged DNA molecules is consistent with other disclosures in the background art, such as the KAPA data sheet ("15-40% of input DNA is typically recovered as adapter-ligated molecules") and Fisher (discloses recovering 47% of the library as adapter-ligated DNA fragments). EX1015, 4; EX1031, Fig. 3; EX1005, 274; EX1002, ¶347. And third, the POSA would have expected Schmitt's sticky-end adapter-DNA ligation to be even more efficient than Meyer's blunt-end adapter-DNA ligation, which nevertheless achieved 40-60%

adapter-ligated DNA fragments. EX1002, ¶348; EX1005, 273; EX1083, ¶¶[0055]-[0056].

Moreover, a POSA would have understood that adapter-DNA tagging efficiency is a *result-effective variable* that only requires routine skill to optimize. EX1002, ¶348. Thus, Guardant's claims of having "at least 20%" and "at least 40%" of the cfDNA molecules tagged would have been obvious because it is nothing more than claiming an optimum value in a known process. *Abbott Labs. v. Sandoz, Inc.*, 544 F.3d 1341, 1379 (Fed. Cir. 2008). Accordingly, claims 4 and 6 were obvious.

XI. GROUND 3: CLAIM 8 WOULD HAVE BEEN OBVIOUS OVER NARAYAN, SCHMITT, AND CRAIG

Claim 8 depends from claim 1, and recites that "the molecular barcodes have a length of 5 to 20 base pairs." EX1001, 61:64-65. Craig and Schmitt disclose this limitation. EX1007, Table S4; EX1083, ¶[0030]; EX1002, ¶¶351-354.

Craig discloses methods of DNA sequencing using "DNA sequence barcodes ligated to fragmented DNA prior to sequencing." EX1007, Abstract. Craig discloses "[a] total of 48 different 6-mer index sequences" that were "appended to adapter sequence[s]" and used for Illumina sequencing. *Id.*, 888, Table S4; EX1002, ¶¶353-354. Thus, Craig discloses a set of 48 different barcodes, each of which is *six nucleotides long*, falling squarely within the claimed range of 5 to 20 base pairs. *Id.*

The rationales for obviousness of claim 1 also applies to claim 8. EX1002, ¶¶355-362. Additionally, a POSA reading Schmitt would have been motivated to use Craig's set of 48 6-mer barcodes in Schmitt's DCS because Craig's barcodes would further mitigate the risk of sequencing errors. The art taught that sequencing errors in the barcode itself "can cause one tag to appear identical to another (crossover) or sufficiently alter a sequence tag such that it is unrecognizable (loss) and untraceable to the source material." EX1063, 2; EX1002, ¶355.

Craig discloses that their 6-mer barcode design can "control, tolerate, and measure error during base-calling." EX1007, 888. Craig's 48 6-mer barcodes "were chosen so that 1, and in some cases 2, sequencing errors could be tolerated without [a barcode] being incorrectly identified as being a different valid [barcode]." *Id.* As Craig explained, "the 1st and 5th base [in the barcode] are identical and represent an XOR-based checksum of bases 2-4 so that the index sequence remains identifiable even with an uncalled or low-quality base." *Id*. Table S4. A POSA would have immediately drawn parallels between Craig's set of 48 barcodes and Schmitt's 3-mer barcodes containing 64 barcodes, and recognized the additional advantage of being able to easily identify a sequencing error if the barcode read is not one of Craig's pre-defined 48 6-mers. EX1002, ¶¶356-357.

A POSA implementing Schmitt's DCS with Narayan's cfDNA would have desired "deep sequencing" in order to detect rare sequences in the cfDNA sample,

and would have recognized that Schmitt teaches performing DCS at greater depth for greater sensitivity. EX1083, ¶¶[0003], [0068]-[0069]. However, a POSA also would have understood that the likelihood of a barcode error increases at greater sequencing depth. EX1002, ¶¶358-359. Therefore, a POSA would have been motivated to use Craig's 48 6-mer barcodes for sequencing at greater depth (providing the sensitivity needed for detecting rare sequences) while further mitigating the risk of barcode sequencing errors. EX1002, ¶¶359-360; EX1007, 2, Table S4. Although Craig uses the barcodes as indexes to identify samples, a POSA would have understood that the same barcodes would be useful as non-unique molecular barcodes in view of Schmitt's teachings. For example, Schmitt discloses that the n-mer molecular barcodes in the hybrid tag embodiment can be a 6-mer ("a shorter n-mer tag [] such as 1 or 2 or 3 or 4 *or more*" bases) and that the molecular barcodes can be "semi-degenerate" and "oligonucleotides of known sequence," just like Craig's. EX1083, ¶¶[0017], [0030]; EX1002, ¶¶360-362. Accordingly, claim 8 was obvious.

XII. GROUND 4: CLAIMS 15, 16, AND 28 WOULD HAVE BEEN OBVIOUS OVER NARAYAN, SCHMITT, AND KIVIOJA.

A. Claims 15 and 28

Claim 15 depends from claim 14, and further recites: "estimating with a programmed computer processor a quantitative measure of tagged parent polynucleotides that map to the genomic locus based on the quantitative measures

of the paired reads and the unpaired reads." EX1001, 62:35-39. Claim 28 depends from claim 27, and similarly recites "estimating with a programmed computer processor a quantitative measure of tagged parent polynucleotides that map to a genomic locus of the reference sequence based on the quantitative measures of at least two of (i) paired reads, (ii) unpaired reads, (iii) a read depth of the paired reads, and (iv) a read depth of the unpaired reads at the genomic locus." EX1001, 64:22-28.

As discussed above, Schmitt discloses determining quantitative measures of (i) – (iv) at one or more loci. EX1083, ¶¶[0060], [0066], [0068]; EX1002, ¶¶363-367; Section IX(L). Schmitt also discloses "methods of single-molecule counting for accurate determination of DNA or RNA copy number." EX1083, ¶[0048]. Schmitt cites and incorporates by reference Kivioja, which discloses methods of estimating quantitative measures of unseen ("N3" in the '306 patent) and total molecules ("N" in the '306 patent) of "cell-free DNA from the plasma." EX1006, 2; EX1083, ¶[0048], p.41; EX1001, 32:42-33:12, 35:13-28, Fig. 1; EX1002, ¶367. Kivioja discloses that "the original number of molecules in a sample can be estimated as *the sum of observed* [detected] *and unobserved* [unseen] UMIs." EX1006, 4-5. Kivioja discloses that, "[e]ven if some UMIs are not observed, the original number of molecules can be *estimated using count statistics*." *Id*., 72; EX1002, ¶367.

Kivioja discloses a method for estimating the number of unseen ("unobserved") molecules and total observed molecules, stating that "[t]he number of unobserved UMIs can be estimated based on the distribution of the copy numbers of the observed UMIs." EX1006, 5. Kivioja discloses using the estimated number of unseen molecules to calculate the total number of molecules "by fitting a zero-truncated Poisson distribution to the UMI copy number distribution using the generalized additive models for location, scale and shape (GAMLSS) R package and *adding the predicted number of unobserved UMIs to the observed UMI count*." *Id.*; EX1002, ¶¶368-369. Kivioja discloses estimating these quantitative measures using a "programmed computer processor," as claimed in claims 15 and 28, because Kivioja discloses estimating the total number of molecules using the "*(GAMLSS) R package*." EX1006, 5; EX1002, ¶369. A POSA would have known that the "GAMLSS R Package" is a software package implemented via programmed computer processor because Kivioja cites Stasinopoulos 2007 (EX1073), which describes the GAMLSS R package software. EX1006, 5; EX1073, 12-13; EX1002, ¶369. Additionally, Schmitt discloses using "Python code" for "carrying out the *pairing and scoring of partner strands*" (i.e., determining quantitative measures). EX1083, ¶¶[0060]-[0061], Appx. A; EX1002, ¶369.

A POSA would have known that Schmitt discloses quantifying the paired

(N1) and unpaired (N2) sequences for at one or more loci of a reference sequence (see claims 14 and 27)—which, together, account for the total detected molecules ("observed molecules," as disclosed in Kivioja). EX1002, ¶370. A POSA would have then used Kivioja's statistical modeling estimates to calculate the number of unseen molecules (N3). EX1002, ¶370. The POSA would have then added that number to the total observed molecules calculated from Schmitt (N1 + N2) to estimate the total number (N) of cfDNA molecules that map to each genetic locus of the reference sequence. EX1002, ¶370. Indeed, Kivioja discloses estimating the number of molecules for each locus by adding the "number of unobserved UMIs [N3] to the observed UMI count [N1+N2]." EX1006, 5. To estimate the total number of *tagged* molecules (claims 15 and 28), the POSA would have simply multiplied the total molecules "N" by the % tagging efficiency.[7] *Id.* Accordingly, Narayan, Schmitt, and Kivioja disclose all the elements of claims 15 and 28.

A POSA's reasons to combine Narayan and Schmitt are discussed above in Ground 1. A POSA would have further had a reason to combine Kivioja with Narayan and Schmitt because Schmitt expressly cites and incorporates Kivioja by reference. EX1083, ¶[0048], p.41; EX1002, ¶¶371-374. *Bayer Healthcare*

---

[7] A POSA would have known routine methods for determining tagging efficiency. EX1002, ¶370; EX1005, 274.

Pharms., Inc. v. Watson Pharms., Inc., 713 F.3d 1369, 1374-75 (Fed. Cir. 2013) (obvious to combine references when one "refers expressly" to the other). Moreover, a POSA would have been motivated to calculate the number of total and unseen cfDNA molecules that map to particular loci because such data would be useful for calculating copy number variations, e.g., for cancer genes. EX1006, 2; EX1083, ¶[0048]; EX1032, Abstract; EX1051, 212; EX1002, ¶373. Additionally, as Kivioja explained, counting the total number of molecules in the sample "can improve accuracy of almost any next-generation sequencing method." EX1006, Abstract; EX1002, ¶374. And determining the number of tagged molecules only further confirms the accuracy of sequencing. EX1002, ¶374.

A POSA reading Narayan, Schmitt, and Kivioja would have expected to successfully practice the method of claims 15 and 28 because calculating the total number of molecules and multiplying by the percentage tagging efficiency requires routine mathematical calculations after determining the quantities of absolute molecules, paired reads, and unpaired reads, as taught in Kivioja and Schmitt. EX1002, ¶¶375-376. Accordingly, claims 15 and 28 were obvious.

B. Claim 16

Claim 16 depends from claim 15, and recites: "detecting copy number variation in the population of cfDNA molecules by determining a normalized quantitative measure determined in (g) at each of one or more genomic loci."

EX1001, 62:40-44. This limitation is taught in Kivioja and Schmitt.

As discussed above for claim 15, Kivioja and Schmitt teach determining the quantitative measure of tagged polynucleotides. EX1002, ¶¶377-379. Schmitt discloses using its duplex tags "in methods of single-molecule counting for accurate determination of *DNA or RNA copy number*," and cites Kivioja. EX1083, ¶[0048] (citing reference [38], Kivioja, EX1006). Kivioja discloses detecting copy number by determining a normalized quantitative measure, stating that "the library can be amplified, *normalized* or otherwise processed without loss of information about the original molecule count because the number of UMIs in the library acts as a molecular memory of the number of molecules in the starting sample." EX1006, 1; EX1002, ¶379. Kivioja further discloses determining copy number variation at one or more genomic loci in a population of cfDNA molecules ("cell-free DNA from the plasma of pregnant women") because Kivioja discloses observing "*increased and decreased copy numbers* of all 5-megabase-pair intervals in *chromosomes 21 and X*, respectively." EX1006, 2; EX1002, ¶379.

The rationales for obviousness of claim 15 also apply to claim 16. EX1002 ¶¶380-383. Additionally, a POSA would have been motivated to determine copy number variations in a sample because Schmitt discloses that it is useful for "sensitive diagnosis of genetic conditions" and "quantification of circulating neoplastic cells." EX1083, ¶[0048]; EX1002, ¶381. A POSA would have expected

to successfully practice the method of claim 16 because Kivioja teaches straightforward methods for detecting copy number variation in a population of cfDNA molecules. EX1006, 1-2; EX1002, ¶382. Accordingly, claim 16 was obvious.

XIII. OBJECTIVE INDICIA DO NOT SUPPORT PATENTABILITY.

Guardant did not assert objective indicia during prosecution. Previously, during an IPR of a different Guardant patent, Guardant alleged that its commercial product satisfied a long-felt need and enjoyed commercial success. EX1074, 63. There, the PTAB held that Guardant failed to demonstrate a nexus between the challenged claims and the product. *Id.*, 64-65. The same is true here, as Guardant has not provided evidence showing any product has a nexus to the challenged claims. EX1002, ¶384. Should Guardant submit such evidence, TwinStrand requests the opportunity to rebut it.

XIV. CERTIFICATION OF STANDING AND IPR ELIGIBILITY (37 C.F.R. §42.104(A))

TwinStrand certifies that the ’306 patent is IPR-eligible and TwinStrand is not barred or estopped from requesting IPR of any ’306 patent claim.

XV. MANDATORY NOTICES (37 C.F.R. §42.8(A)(1))

Real party-in-interest 37 C.F.R. §42.8(b)(1): TwinStrand Biosciences, Inc. The University of Washington is noted as a potential real party-in-interest but is not believed to be a real party-in-interest under governing legal standards.

Related Matters (37 C.F.R. §42.8(b)(2)): The ’306 patent has been asserted in *TwinStrand Biosciences, Inc. et al v. Guardant Health, Inc*., 1-21-cv-01126 (D. Del.). The ’306 patent is also the subject of *Illumina, Inc. v. Guardant Health, Inc.; Helmy Eltoukhy; and Amirali Talasaz*, 22-cv-00334 (D.Del.) (March 17, 2022).

Petitioner has also filed a petition for IPR of Guardant’s related patents, U.S. Patent Nos. 10,801,063 (IPR2022-00746; IPR2022-01115), 10,889,858 (IPR2022-00747; IPR2022-01116), and 11,118,221 (IPR2022-01152).

Lead and back-up counsel (37 C.F.R. §42.8(b)(3)) for TwinStrand Biosciences, Inc. are:

Lead Counsel	Back-Up Counsel
Ralph Wilson Powers III, Ph.D. (Reg. No. 63,504) STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C. 1100 New York Avenue, NW Washington, DC 20005 202.772.8876 (telephone) 202.371.2540 (facsimile) tpowers-PTAB@sternekessler.com	David H. Holman, Ph.D. (Reg. No. 61,205) Kristina C. Kelly, Esq. (Reg. No. 58,164) Christopher M. Gallo, Ph.D. (Reg. No. 70,291) Tyler C. Liu, Esq. (Reg. No. 72,126) STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C. 1100 New York Avenue, NW Washington, DC 20005 202.371-2600 (telephone) 202.371.2540 (facsimile) dholman-PTAB@sternekessler.com kckelly-PTAB@sternekessler.com cgallo-PTAB@sternekessler.com tliu-PTAB@sternekessler.com

Notice of Service Information (37 C.F.R. §42.8(b)(4)): Please direct all correspondence regarding this Petition to counsel at the above addresses and PTAB@sternekessler.com. TwinStrand Biosciences, Inc. consents to service by email at the addresses above.

Procedural Statements: This Petition is filed in accordance with 37 C.F.R. §42.106(a). Concurrently filed herewith are a Power of Attorney and Exhibit List under 37 C.F.R. §42.10(b) and §42.63(e), respectively. The required fee is paid through Deposit Acct. No. 19-0036 (Customer ID No. 45324). The Office is

authorized to charge any fee deficiency, or credit any overpayment, to Deposit Acct. No. 19-0036 (Customer ID No. 45324).

XVI. CONCLUSION.

Claims 1-29 should be canceled.

Respectfully submitted,
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C

/R. Wilson Powers III/

Ralph Wilson Powers III, Ph.D.
Registration No. 63,504
Lead Attorney for Petitioner

Date: August 12, 2022
1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

CERTIFICATE OF WORD COUNT (37 C.F.R. § 42.24(d))

Petitioner certifies that this Petition is 13,998 words in length, as determined by Microsoft Word® word count feature, excluding any table of contents, mandatory notices under § 42.8, certificate of service or word count, or appendix of exhibits or claim listing.

Respectfully submitted,
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C

/R. Wilson Powers III/

Date: August 12, 2022

1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

Ralph Wilson Powers III, Ph.D.
Registration No. 63,504
Lead Attorney for Petitioner

CERTIFICATE OF SERVICE (37 C.F.R. § 42.6(e)), § 42.105(a))

I certify that the above-captioned **PETITION FOR *INTER PARTES* REVIEW FOR U.S. PATENT NO. 11,149,306**, and associated Exhibits 1001-1092 were served in their entireties upon the Patent Owner on August 12, 2022, via FedEx® at the following addresses:

Guardant Health / WSGR
650 Page Mill Road
Palo Alto CA 94304
Patent Owner's Correspondence Address of Record for U.S. Patent No. 11,149,306

Respectfully submitted,
STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C

/R. Wilson Powers III/

Date: August 12, 2022
1100 New York Avenue, N.W.
Washington, D.C. 20005-3934
(202) 371-2600

Ralph Wilson Powers III, Ph.D.
Registration No. 63,504
Lead Attorney for Petitioner

17939975.docx