

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

NEOGENOMICS LABORATORIES, INC.

Petitioner,

v.

NATERA, INC.

Patent Owner.

**EXPERT DECLARATION OF NIAL L LENNON, PH.D. IN SUPPORT OF
PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO.
11,319,596**

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I, Dr. Niall Lennon, make this declaration in connection with the proceeding identified above.

I. INTRODUCTION

1. I have been retained as an independent expert witness on behalf of NeoGenomics Laboratories, Inc. (“NeoGenomics”) in the above-captioned *inter partes* review (“IPR”) to provide my independent evaluation and opinions with respect to the patentability of claims 1, 2, 5, and 10–18 of U.S. Patent No. 11,319,596 (“the ’596 patent”). I understand that the patentability of these claims are being challenged by NeoGenomics in the IPR. I also understand that Natera, Inc. (“Patent Owner”) has asserted claims 1, 11, 12, and 18 against NeoGenomics in *Natera, Inc. v. NeoGenomics Labs, Inc.*, No. 23-cv-00629 (M.D.N.C.) (the “co-pending litigation”).

2. I am being compensated for my time at my standard consulting rate, which is \$600 per hour. My compensation does not depend in any way on the outcome of this proceeding or my opinions in this declaration.

II. SUMMARY OF OPINIONS

3. It is my opinion that the challenged claims of the ’596 patent are obvious and unpatentable. The following is a summary of my opinions:

4. Claims 1, 2, 5, 10, 12–14, and 16–18 are obvious in view of Diaz Jr. and Bardelli, “Liquid Biopsies: Genotyping Circulating Tumor DNA,” Journal

of Clinical Oncology, Vol. 32, Number 6, 579-586 (February 20, 2014) (“Diaz”) (Ex.1006) and Kinde *et al.*, “Evaluation of DNA from the Papanicolaou Test to Detect Ovarian and Endometrial Cancers,” Science Translational Medicine, Vol. 5, Issue 167 (January 9, 2013) (“Kinde 2013”) (Ex.1007).

5. Claims 1, 2, 5, 10–14, and 16–18 are obvious in view of Diaz, Kinde 2013, and U.S. Patent Publication No. 2012/0270212 (“Rabinowitz”) (Ex.1009).

6. Claim 15 is obvious in view of Diaz, Kinde 2013, and Fleischhacker and Schmidt, “Circulating nucleic acid (CNAs) and cancer—A survey,” Biochimica et Biophysica Acta, 1775:181-232 (October 7, 2006) (“Fleischhacker”) (Ex.1010).

7. Claims 1, 2 10–13, and 15–18 are obvious in view of Rabinowitz and Bettegowda *et al.*, Science Translational Medicine, Vol. 6, Issue 224 (February 19, 2014) (“Bettegowda”) (Ex.1008).

III. QUALIFICATIONS

8. I am currently part of the Institute Leadership team at the Broad Institute of MIT and Harvard, one of the largest genome sequencing centers in the world. I am also currently the Senior Director of the Genomics Platform, an Institute Scientist, and an Associate Director of the Gerstner Center for Cancer Diagnostics. Specifically, at the Gerstner Center, I evaluate new and upcoming

techniques with respect to liquid biopsies. As part of my role, I am responsible for the technology and clinical development strategy for the Genomics Platform at the Broad. I am directly responsible for the evaluation, optimization, and application of Next-Generation Sequencing (“NGS”) for clinical trials, research, and diagnostics. I also oversee custom method development for use on NGS platforms, such as Illumina’s line of sequencing instruments. I am also the founder, Chief Scientific Officer, and current Chairman of the Board of Broad Clinical Laboratories, a CLIA/CAP clinical laboratory that performs cancer and inherited disease molecular assays.

9. Prior to my current position, I was the Senior Director of Translational Genomics at the Broad. Before that I was the Director of Technology and Clinical Development at the Broad. Before that I was a Research Scientist for the Genome Sequencing Platform of the Broad. Prior to joining the Broad I was a Postdoctoral Research Fellow at Harvard Medical School. I have over 19 years of experience studying and researching molecular biotechnology, including nucleic acid sequencing and its application to cancer, germline, and viral genomics.

10. I have authored over 80 peer reviewed articles, including articles directed to genomic technologies and the application of sequencing technologies to cancer and viral genomics.

11. I currently serve or have served on technical advisory boards for a number of NGS companies, including Genturi Inc., Swift Biosciences, and FYR Diagnostics. I have been a member of a Key Opinion Leader panel for New England Biolabs. I am an Adjunct Assistant Professor in the Department of Biomedical Engineering at Tufts University, where I teach Molecular Biotechnology to graduate students.

12. In addition to membership of advisory groups for NGS reagent companies (NEB and Swift), I have extensive experience collaborating with, evaluating, and implementing novel reagent formulations and kits for sample preparation and sequencing. I have worked closely over the years with the R&D teams of several NGS kit and instrumentation companies including Kapa Biosystems (now part of Roche), NEB, Swift Biosciences, Watchmaker Genomics, IDT, Twist Biosciences, Illumina, Ion Torrent, and others. In many cases the purpose of the collaboration is to provide feedback and work with the product development teams who are hoping to bring new methods and kits to the market to support a particular aspect of NGS sample preparation.

13. Exhibit 1005 is a copy of my curriculum vitae.

IV. LEGAL STANDARDS

14. I am not a lawyer. I have been informed by counsel regarding various legal standards, which are described below. I have applied these legal standards in forming my opinions in this declaration.

A. Claim Construction

15. I understand that the words of the claims of a patent are generally given their plain and ordinary meaning from the perspective of a person of ordinary skill in the art (“POSA”) at the time of the claimed invention. I also understand that the claims of a patent are construed in light of the language of the claims, the patent specification, and the prosecution history of the patent.

16. I understand that, in the co-pending litigation, certain terms may be in dispute although, as of the date of the signing of this declaration, the parties have not completed claim construction. I was asked to assume for the purposes of this IPR proceeding the following.

17. First, “performing high-throughput sequencing of the amplified DNA” encompasses intervening steps between performing targeted multiplex PCR amplification and performing high-throughput sequencing.

18. Second, “the isolated cell-free DNA” does not require that cell-free DNA isolated from a plurality of biological samples be pooled.

19. I have applied these understandings, which I am told reflect how Patent Owner currently interprets the claims in the co-pending litigation. But my application of these understandings should not be understood to mean that I agree to them.

B. Obviousness

20. I understand that a claimed invention is “obvious” if the differences between the claimed subject matter and the prior art are such that the subject matter as a whole would have been obvious at the to a POSA at the priority date of the claimed invention.

21. I understand that a POSA provides a reference point from which the prior art and claimed invention should be viewed. This reference point prevents a POSA from using one’s hindsight in deciding whether a claim is obvious.

22. I understand that the obviousness analysis requires a comparison of the claim language to the prior art on a limitation-by-limitation basis.

23. I understand that in assessing whether a claimed invention would have been obvious at the priority date of the claimed invention to a POSA, the following factors are considered: (1) the scope and content of the prior art; (2) the differences between the art and the claims at issue; (3) the level of ordinary skill in the art; and (4) the existence of secondary indicia of non-obviousness or obviousness.

24. I understand that a claim can be obvious in light of a single reference, without the need to combine multiple prior art references, if the elements of the claim that are not found explicitly or inherently in the reference can be supplied by the knowledge or common sense of one of skill in the art.

25. I understand that an obviousness evaluation can be based on a combination of multiple prior art references. I understand that such a combination must meet certain requirements.

26. I understand the prior art must teach or suggest all the requirements of the invention as recited in the claims. I understand the prior art, coupled with the knowledge generally available in the art by the priority date of the claimed invention, must contain some suggestion or incentive that would have motivated a POSA to modify a reference or to combine references. I also understand the proposed modification of the prior art must have had a reasonable expectation of success, determined from the vantage point of a POSA by the priority date of the claimed invention.

27. I understand that the prior art references themselves may provide a suggestion, motivation, or reason to combine, but other times the nexus linking two or more prior art references is simply common sense. I further understand that an obviousness analysis recognizes that market demand, rather than scientific

literature, often drives innovation, and that a motivation to combine references may be supplied by the direction of the marketplace.

28. I understand that if a technique has been used to improve one device, and a POSA would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond the person's skill.

29. I understand that practical and common sense considerations should guide a proper obviousness analysis, because familiar items may have obvious uses beyond their primary purposes. I further understand that a POSA looking to overcome a problem will often be able to fit the teachings of multiple publications together like pieces of a puzzle, although the prior art need not be like two puzzle pieces that must fit perfectly together.

30. I understand that obviousness analysis therefore takes into account the inferences and creative steps that a POSA would employ under the circumstances.

31. I understand that a particular combination may be proven obvious by showing that it was obvious to try the combination. For example, when there is a design need or market pressure to solve a problem and there are a finite number of identified, predictable solutions, a POSA has good reason to pursue the known

options within the person's technical grasp because the result is likely the product not of innovation but of ordinary skill and common sense.

32. I understand that the combination of familiar elements according to known methods may be proven obvious when it does no more than yield predictable results. When a work is available in one field of endeavor, design incentives and other market forces can prompt variations of it, either in the same field or a different one. If a POSA can implement a predictable variation, then that obviousness likely bars its patentability.

33. I understand that a proper obviousness analysis focuses on what was known or obvious to a POSA, not just to the patentee. Accordingly, I understand that any need or problem known in the field of endeavor at the priority date of the claimed invention and addressed by the patent can provide a reason for combining the elements in the manner claimed.

34. I understand that a POSA would have combined two pieces of prior art or substituted one prior art element for another if the substitution can be made with predictable results, even if the swapped-in element is different from the swapped out element. In other words, the relevant question is whether prior art techniques are interoperable with respect to one another, such that a POSA would

view them as a design choice, or whether a POSA could apply prior art techniques into a new combined method or system.

35. In sum, my understanding is that prior art teachings are properly combined where a POSA, having the understanding and knowledge reflected in the prior art and motivated by the general problem facing the inventor, would have been led to make the combination of elements recited in the claims. Under this analysis, the prior art references themselves, or any need or problem known in the field of endeavor at the priority date of the claimed invention, can provide a reason for combining the elements of multiple prior art references in the claimed manner.

36. I understand that obviousness must be shown by a preponderance of the evidence.

37. I understand that certain objective factors, sometimes known as objective indicia must be taken into account in determining whether a claimed invention would have been obvious. I understand that the following is a nonexclusive list of secondary considerations to analyze for this inquiry: (i) a strong showing of commercial success of a product covered by the patent claims that can be attributed to the merits of the invention; (ii) a long felt but unmet need in the prior art that was satisfied by the invention of the patent; (iii) the willingness of industry members to license the patent at issue; (iv) evidence that those of skill

in the art were skeptical as to the merits of the invention, or that prior art references taught away from the invention; (v) evidence that those of skill in the art were surprised by the capabilities of the claimed invention; (vi) praise of the invention by others skilled in the art; and (vii) deliberate copying of the invention. I further understand that contemporaneous and independent invention by others is a secondary consideration supporting an obviousness determination. I understand that Natera has not raised such evidence to date, and I reserve the right to address any such evidence if raised by Natera.

38. I understand that any assertion of the above indications must be accompanied by a nexus between the merits of the purported invention and the evidence offered. If there is no such connection between the claimed technology and the secondary evidence, then that evidence does not actually establish the purported invention was nonobvious.

C. Written Description And Priority

39. I understand that the claimed invention(s) in a patent must be supported by written description in any application to which it is entitled to the benefit of priority.

40. I understand that the written description in the specification of the application must describe the claimed invention sufficiently to convey to a POSA that the patentee had possession of the claimed invention at the time of the

application. I also understand the specification must describe the claimed invention sufficiently to convey to a POSA that the applicant actually invented the claimed invention. I also understand that to show possession, the specification must convey the invention to a POSA in such a way that the POSA understands that the invention is possessed by the inventor, and not merely laying out a research plan for a POSA to follow. In other words, I understand that describing a desired outcome without describing how to achieve it is not sufficient.

41. I understand that to show possession of an invention, a patent owner must show that they are in possession of the invention by describing the invention, with all its claimed limitations. I understand that, even if an invention would have been obvious over certain disclosure, that disclosure does not provide written description support for what it might render obvious.

42. I understand that a claim that relies on new matter for written description support is only entitled to the date the new matter was added. I understand that a claim with a limitation only described in disclosure of a continuation-in-part application will only have priority to the effective filing date of the continuation-in-part application.

V. THE '596 PATENT

A. Subject Matter Of The '596 Patent

43. The '596 patent's Abstract states that it "provides methods, systems, and computer readable medium . . . for detecting single nucleotide variants" (Ex.1001, Abstract.) The patent's "Field of the Invention" section states that the "present invention generally relates to . . . methods and systems for detecting a single nucleotide variant." (*Id.*, 1:28-31.)

44. Broadly speaking, the '596 patent summarizes methods along the lines of those described in its Abstract and then provides specific examples of the same. (Ex.1001, [Examples 1–15].) For example, the '596 patent's Example 13 relates to "PCR assays for each SNV mutation for VAF detection in both biopsied and corresponding ctDNA from plasma." (*Id.*, 168:59–61.) FIG. 53B of the '596 patent (reproduced below) shows the results of "variant allele frequency" (VAF) "detection in both biopsied and corresponding ctDNA from plasma." (*Id.*) According to FIG. 53B (right column labeled "VAF plasma"), mutations were detected in plasma at VAF ranging from 0.15[%] to 23%. (*Id.*, FIG. 53B; *see also id.*, 168:62 ("a zero value represents no VAF detected").)

Sample	Gene	Chrom	ChromStart	Natera			
				VAF R1	VAF R2	VAF R3	VAF Plasma
L12	BRIP1	chr17	59924572	22.6	7.74	10.07	3.71
L12	CARS	chr11	3062181	22.39	10.47	18.21	5.02
L12	CIC	chr19	42797381	0	0	8.17	1.71
L12	CYFIP1	chr15	22940733	0	0	0	0
L12	FAT1	chr4	187519147	9.81	4.26	0.77	0.57
L12	KDM6A	chrX	44921898	6.33	3.54	0	0.28
L12	MLLT4	chr6	168347475	9.04	4.26	0.73	0.95
L12	NFE2L2	chr2	178098801	73.12	39.92	53.94	23.25
L12	RASA1	chr5	86642517	7.37	0	0.35	0
L12	TP53	chr17	7578406	22.93	9.17	16.97	4.89
L12	TP53	chr17	7578190	21.48	8.67	22.09	5.77
L13	EGFR	chr7	55241708	27.06	23.14	60.79	1.16
L13	EGFR	chr7	55242511	23.93	18.01	56.31	1.09
L13	HERC4	chr10	69793756	0.16	1.92	5.72	0
L13	JAK2	chr9	5022084	0.27	2.79	6.09	0
L13	KMT2C	chr7	151947008	0	0	6.69	0
L13	MSH2	chr2	47693816	5.04	3.33	12.67	0
L13	MTOR	chr1	11292495	2.4	1.38	6.01	0
L13	PLCG2	chr16	81942036	2.8	1.7	6.89	0
L13	TP53	chr17	7579509	6.11	3.76	15.4	0.4
L15	ALK	chr2	29940530	7.2	1.39		0
L15	GABRG1	chr4	46060315	11.84	2.04		0
L15	KDM6A	chrX	44922755	6.54	0.91		0.17
L15	MLL2	chr12	49443815	12.41	0		
L15	ROS1	chr6	117687379	10.82	2.94		0.15
L15	SLC39A4	chr8	145638322	18.59	0		0
L15	TP53	chr17	7578254	12.6	2.5		0
L15	ZFHX4	chr8	77776735	10.26	2.3		0
L15	ZMYM4	chr1	35827319	12.84	3.46		0
L17	BRCA2	chr13	32914959	15.03	27		0
L17	KRAS	chr12	25398284	14.22	23.79		0
L17	NF1	chr17	29653134	18.6	23.1		0
L17	NF1	chr17	29528088	0	0	17.79	0.37
L17	PAX8	chr2	113984793	6.6	0		0
L17	TP53	chr17	7577610	0	0	18.97	0
L17	TP53	chr17	7577079	15.36	33.7		0
L17	TRIM67	chr1	231299607	18.38	30.95		0.57
L17	RIP11	chr14	92471631	6.44	0		0

 = mmPCR-NGS

FIG. 53B

(Ex.1001, FIG. 53B.)

45. As another example, the '596 patent's Example 1 describes plasma sample preparation methods wherein "[u]niversal ligation adapters were appended to the end of each molecule of . . . purified plasma DNA and libraries were amplified for 9 cycles using adaptor specific primers." (*Id.*, 152:33–36.) The resulting libraries "were purified with AGENCOURT AMPURE beads and eluted in 50 ul DNA suspension buffer." (*Id.*, 152:36–38.) Example 9 describes a "Breast Cancer Feasibility SNV Panel from Plasma." (*Id.*, 164:51.) In this example, "600 ng" of "amplified and purified Natera plasma library" was used as input for multiplex PCR. (*Id.*, 164:57–59.) Example 14 relates to "using low primer concentrations such that primer amount is the limiting reactant in multiplex PCR in a workflow that is followed by next generation sequencing." (*Id.*, 169: 22–25.) According to Example 14, this use of low primer concentrations improves "uniformity of density of reads, and therefore limits of detection, across a pool of amplification reactions." (*Id.* 169:25–27.) For SNV detection, "experiments were carried out using the four 84-plex pools on breast cancer samples according to the protocol of Example 9 except that primer concentrations were 2 nM and PCR amplification was carried out for 15, 20, or 25 cycles." (*Id.*, 169:31–35.) Example 14 further explains that the "number of cycles" for primer limited multiplex PCR were calculated assuming a starting "DNA input level" of "100k copies of each

target (10^5 ; this is easily achieved with using [*sic*] amplified library).” (*Id.*, 169:54–59.)

46. The '596 patent also contains a number of disclosures indicating the state of the art before it was filed. For example, the '596 patent describes using Illumina (third-party) HiSeq and MiSeq devices and Ion Torrent (third-party) PGM® instruments to perform next generation sequencing, confirming that next generation sequencing was known before the '596 patent was written. (Ex.1001, 168:39–42; 169:38–41; *see also id.*, 96:38–40 (describing sequencing using an ILLUMINA IIGAX or HiSeq instrument).) As another example reflecting the state of the prior art, the '596 patent states in its description of multiplex PCR primer design that “the PCR primers may be designed using the Primer3 program (the worldwide web at primer3.sourceforge.net; libprimer3 release 2.2.3. . .).” (*Id.*, 106:35–39.) The third-party “Primer3” primer design software was also used for primer design in asserted prior art reference Kinde 2013 (Ex.1007). The '596 patent further describes isolating cell-free DNA from plasma using commercially available kits from Qiagen (third-party). (*See* Ex.1001, 152:30–33; 160:54–56.) Example 9 of the '596 patent also describes performing the multiplex PCR to detect SNVs in plasma libraries using a commercially available multiplex PCR

master mix (“mp-PCR master mix (2xMM final conc.)”) from Qiagen. (*Id.*, 163:62–165:5; *see also* 159:11–25.)

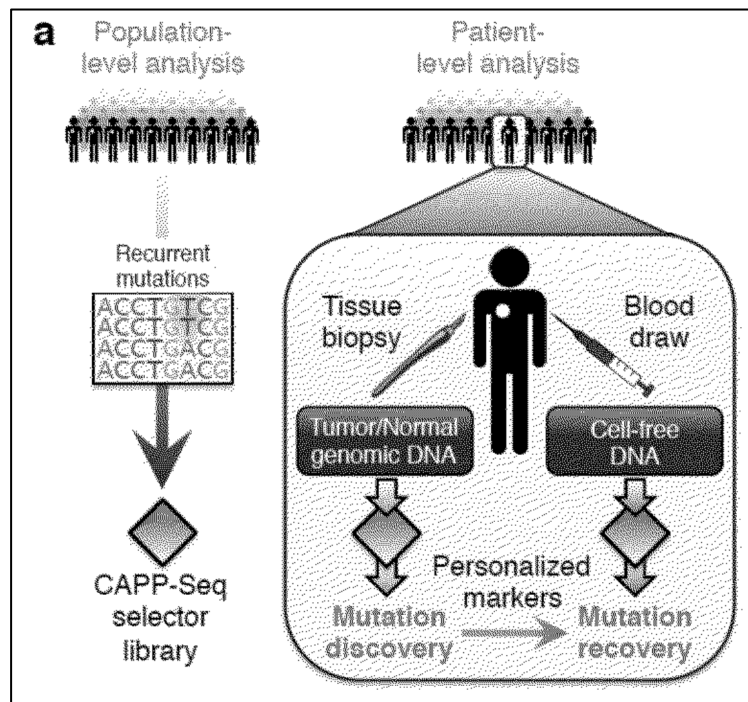
47. The ’596 patent includes 18 claims, 12 of which I understand are challenged in this IPR proceeding. According to the ’596 patent’s claim 1, a tumor biopsy sample of a subject is sequenced and a plurality of tumor-specific mutations are identified “wherein the tumor-specific mutations comprise one or more single nucleotide variant (SNV) mutations.” (Ex.1001, 171:56–58.) The results of the sequencing are evaluated to determine “a plurality of target loci specific to the subject.” (*Id.*, 171:60–61.) Then, cell-free DNA isolated from a plurality of biological samples obtained from the subject at multiple time points is assayed. The assaying comprises “performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA” and performing “high-throughput sequencing” to obtain sequence reads, “wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.” (*Id.*, 172:12–14.) In this declaration, I may refer to that last “wherein” limitation as the 0.015% limitation. Challenged dependent claims 2, 5, and 10–18 further refine the same method. (*Id.*, 172:15–16; 172:26–27; 172:38–62.)

B. Prosecution History

48. I understand the prosecution history of the ’596 patent as follows.

49. The '596 patent was filed on February 28, 2019. Original claim 1 recited only two steps: “performing whole exome sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor-specific mutations” and “assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points for the presence or absence of the tumor-specific mutations, thereby monitoring the progression of the cancer.” (Ex.1003, 1597.)

50. As discussed below, tumor-informed assays were well-known, and the Examiner rejected claim 1 as anticipated by Diehn et al. (Ex.1030) based in part on Fig. 1a below. (Ex.1003, 682.)



51. The Examiner also rejected dependent claims 14–16—directed to targeted multiplex PCR amplification, 10–50 target loci, and high-throughput sequencing—as obvious based on Diehn in view of Mertes et al., Nguyen-Dumont et al. and Wang et al. (Exs.1032, 1033, 1034). (Ex.1003, 684–85.) For example, she cited Mertes for the benefits of PCR-based enrichment over Diehn’s hybridization capture. (*Id.*, 685.)

52. On October 16, 2022, Patent Owner conducted an interview with the Examiner, where it argued that “the invention lies in the determination of mutations in a tumor obtained from a subject and following up the mutations in cell-free DNA obtained from the subject” unlike Diehn that “tests for mutations in a pre-selected set of genes.” (*Id.*, 647.) The Examiner apparently did not find the argument persuasive.

53. Patent Owner then amended claim 1 to recite a new step of “evaluating results of the sequencing on the tumor biopsy sample to determine a plurality of target loci specific to the subject” (*Id.*, 622) For the assaying step, Patent Owner added that the step comprises “amplifying the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject” and “sequencing the amplified DNA comprising the plurality of target loci.” (*Id.*) It then argued that Diehn and the other references did not meet the new

recitations directed to target loci specific to the subject. (*Id.*, 618–19.) But, once again, the Examiner was not convinced and rejected the claims, this time citing another Diehn publication with additional details regarding tumor-informed assays (Ex.1031). (Ex.1003, 561–66.)

54. Based on dependent claims 14 and 16, Patent Owner further amended the assaying step of claim 1 to recite performing “targeted multiplex PCR amplification” of target loci “together in a same reaction volume,” and performing “high-throughput” sequencing. (*Id.*, 469.) In addition, Patent Owner added “wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.” (*Id.*) As previously noted, I may refer to this limitation as the “0.015% limitation” in this declaration. Patent Owner argued that none of the cited references disclosed performing targeted multiplex PCR on cfDNA (as opposed to cellular DNA). (*Id.*, 474–75.) Patent Owner also argued that none of the references taught high-throughput sequencing and the 0.015% limitation. (*Id.*)

55. The Examiner was apparently aware of a reference, Talasaz (Ex.1035), that taught utilizing multiplex PCR and high-throughput sequencing, but did not disclose the 0.015% limitation. Based on that limitation, she allowed the claims as shown below. (*Id.*, 334.)

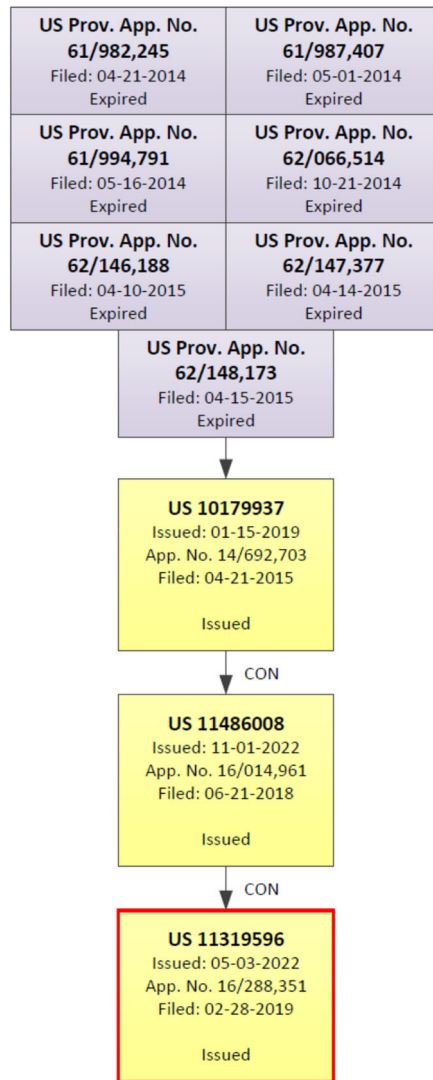
4. Applicant's claim amendments overcame all of the previously presented rejections. No new references were found teaching or suggesting claim 1 which now requires detection of SNV mutation that is present in less than or equal to 0.015% of the cell free DNA having the SNV locus. The closes prior art reference, Talasaz et al. (US 2014/0066317 A1; published March 2014), teaches detection limit of 0.1% for SNV mutations in cell-free DNA by sequencing (Example 4, Fig. 6B).

56. After allowance, Patent Owner submitted hundreds of references. In a follow-on Notice of Allowance, the Examiner noted that one reference (Kennedy et al.; Ex.1036) taught "detection of mutations with frequencies around 10^{-6} " but that the method was incompatible with target-specific multiplex amplification according to Kennedy. (Ex.1003, 62–63.) Kennedy teaches that duplex sequencing is not compatible with PCR-based enrichment of target sequences, which requires denaturing the complementary strands of DNA. (Ex.1036, 8.)

57. The '596 patent subsequently issued on May 3, 2022 with 18 claims.

C. Priority Date

58. I understand that patents may claim the benefit of, or priority to, prior application(s). The '596 patent includes a listing of earlier-filed patent applications which has been mapped visually below:



I understand that, in the co-pending litigation, Patent Owner has asserted that the priority date for the '596 patent is “no later than April 21, 2015.”

59. During prosecution of the '596 patent, when Patent Owner amended the claims to introduce the 0.015% limitation and “targeted multiplex PCR amplification,” in claim 1, it relied on supposed support in “claims 3, 14, and 16 and in the published application (US2019/0194758) at paragraphs 0052, 0060,

0412, and 0538.” (Ex.1003, 472.) Claims 3, 14, and 16 apparently refer to previously pending claims, which recited “wherein the tumor-specific mutations comprise one or more single nucleotide variant (SNV) mutations,” “wherein amplifying comprises targeted multiplex amplification of the plurality of target loci comprising the tumor-specific mutations from the cell-free DNA,” and “wherein sequencing comprises high-throughput sequencing,” respectively. A POSA would not understand any of these claims to support the 0.015% limitation.

60. Cited paragraphs [0052] and [0060] of US2019/0194758 both recite “In certain embodiments of this method for detecting one or more SNVs the limit of detection is 0.015%, 0.017%, or 0.02%.” (Ex.1027.) Cited paragraph [0412] discloses that “[the] improved methods provided herein can achieve limits of detection of 0.015, 0.017, 0.02, 0.05, 0.1, 0.2, 0.3, 0.4 or 0.5 percent SNV in a sample.” (*Id.*) These three paragraphs are the only disclosures of “0.015%” as a threshold in the ’596 patent specification that could potentially support the 0.015% limitation. I have been informed that these three mentions of 0.015% first appeared in U.S. Patent Application No. 14/692,703 filed on April 21, 2015 (Ex.1019) (“the ’703 application”), which is the top yellow box above.

61. The fourth paragraph cited by Patent Owner as purported support for the claim amendments (paragraph [0538] of US2019/0194758) provides (excerpted):

In some embodiments, a limit of detection is such that a mutation (such as an SNV or CNV) that is present in less than or equal to 10, 5, 2, 1, 0.5, 0.1, 0.05, 0.01, or 0.005% of the DNA or RNA molecules with that locus in a sample (such as a sample of cfDNA or cfRNA) is detected (or is capable of being detected).

(Ex.1027, [0538].) I have been informed that this disclosure first appeared in U.S. Provisional Application No. 62/066,514, filed on October 21, 2014 (Ex.1015), which is the second blue box from the top right. A POSA would not understand this passage to support the 0.015% limitation, because this paragraph does not mention (nor would a POSA understand this paragraph as describing) the detection of an SNV that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus. As discussed above, the only disclosures of 0.015% SNV in a sample were added in the '703 application filed on April 21, 2015.

62. Therefore, the critical date (or priority date) of the '596 patent is no earlier than April 21, 2015. Natera has not contended that the '596 patent is entitled to any priority date before April 21, 2015, and I reserve the right to address any arguments regarding priority which may be raised by Natera. But even if the '596 patent's priority date were as early as October 21, 2014 (the filing date of U.S. Provisional Application No. 62/066,514), or even April 21, 2014 (the earliest

U.S. provisional filing date)—in my opinion, the challenged claims still would have been obvious and unpatentable for the reasons I set forth below.

VI. PERSON OF ORDINARY SKILL IN THE ART

63. I understand that claims of a patent are construed from the perspective of a POSA at the priority date of the claimed invention. I understand that whether claims of a patent are obvious under 35 U.S.C. § 103 is also analyzed from the perspective of a POSA at the priority date of the claimed invention.

64. In my opinion, a POSA would have had at least (1) a Master's Degree and/or Ph.D. in biochemistry, molecular genetics, pharmacology, or an equivalent discipline with applications in genomics and (2) at least five years of academic or industrial laboratory experience.

65. In my opinion, a POSA would have a deep understanding of genomics, including nucleic acid detection, quantification, and genotyping and would understand the underlying scientific principles to such.

66. In my opinion, a POSA would stay apprised of recent literature on genomic assays, such as multiplex PCR, next-generation nucleic acid sequencing techniques, methods for detecting SNVs, and the use of cfDNA in genotyping. A POSA would also generally understand the processes behind the development of DNA mutation-based illnesses, such as cancers, and would stay apprised of the recent literature in those fields.

67. It is my opinion, as set forth herein, that at least claims 1, 2, 5, and 10–18 of the '596 patent are obvious under this definition of a POSA, or any other reasonable definition of a POSA. As I noted immediately above, the critical date (or priority date) of the '596 patent is no earlier than April 21, 2015. I understand that the Patent Owner has not asserted that the priority date of the '596 patent is any earlier than April 21, 2015. A POSA's qualifications would have been, for the purposes of this proceeding, roughly the same in 2014 as in 2015.

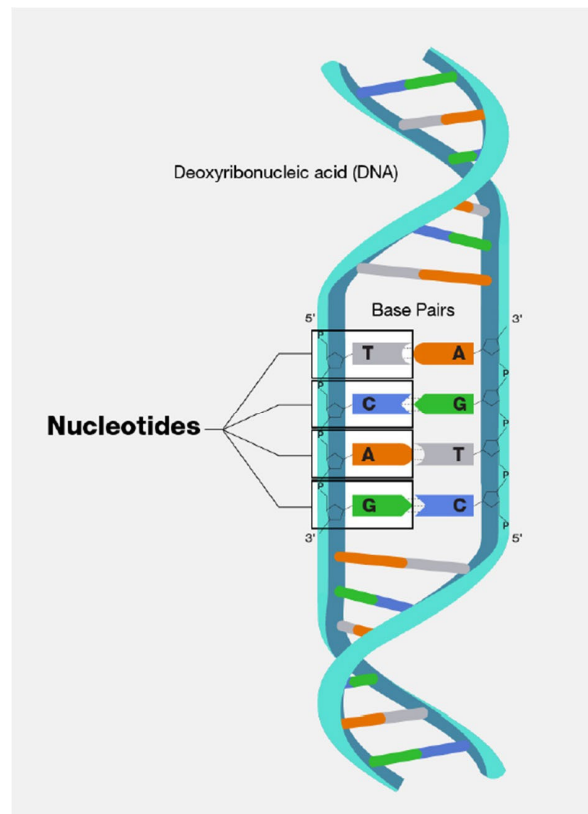
VII. TECHNOLOGY BACKGROUND

68. I summarize below the state of the art by 2014, which is before the 2015 critical date of the '596 patent asserted by Patent Owner as discussed above. In this summary, I sometimes cite references that were published after that date. These citations reflect the state of the art by 2014, and they are not meant to suggest or invoke developments in the art after the critical date of the '596 patent.

A. Cell-Free DNA

69. DNA is a double-stranded polymer comprised of nucleotides. Each nucleotide comprises (1) a deoxyribose sugar molecule, (2) one or more phosphate groups, and (3) a nitrogenous base. There are four types of bases that make up DNA: adenine ("A"), thymine ("T"), cytosine ("C"), and guanine ("G"). Each base has a complementary base—A is complementary to T, while C is complementary to G. (Ex.1052, 1; Ex.1053, 1.)

70. The two strands of DNA are complementary, connected via complementary base pairing. For example, a single strand of DNA with a sequence of GACT would bind to a complementary strand of DNA with a sequence of CTGA, as shown below:



(Ex.1052, 2; *see also* Ex.1053, 1.)

71. In humans, most DNA is generally found within the nucleus of cells, specifically, in large, complex structures called chromosomes. (Ex.1053, 1; Ex.1054, 1.) The nucleus contains all of the material required to maintain, replicate, and use DNA. (Ex.1054, 1.) DNA is also found outside of cells, and has been linked to apoptosis (programmed cell death), as well as signaling between

cells. (Ex.1055, 3.) This DNA found outside cells is DNA digested or fragmented by natural processes and then released into the tissue environment, eventually finding its way into the blood. (Ex.1056, 2; Ex.1057, 4; Ex.1058, 3.)

72. DNA located outside of the cells is referred to as “cell-free” DNA (cfDNA) or circulating DNA, and can be extracted from certain bodily fluids, such as blood plasma. (Ex.1006, 1.) cfDNA in blood plasma may originate from both normal cells and cancer cells and is “detectable in healthy volunteers, patients without cancer, patients with benign tumors, and cancer patients.” (Ex.1059, 2; *see also id.* (“Circulating tumor DNA (ctDNA) is the portion of circulating DNA specifically derived from cancer cells.”); Ex.1060, 2 (“Circulating DNA fragments carrying tumor-specific sequence alterations (circulating tumor DNA) are found in the cell-free fraction of blood.”).

B. Tumor-Informed Assays of Cell-Free DNA

73. Cancer is associated with mutations in DNA. These mutations can affect large or small portions of DNA. (Ex.1060, 5–6 (discussing single nucleotide mutations and structural variants, such as deletions).) Cancer mutations can be detected in ctDNA. (Ex.1006, 1.)

74. Cancers may be diagnosed and monitored by detecting and analyzing the presence of circulating tumor DNA (“ctDNA”), which contains cancer mutations. In 2014, ctDNA assays fell into two broad categories: (1)

mutation-agnostic “screening assays” (sometimes targeting commonly mutated regions of DNA called “hotspots”), and (2) tumor-informed assays. (Ex.1006, 4.) Tumor-informed assays involve first identifying mutations in a tumor biopsy from a subject, and then tracking those mutations by assaying cfDNA from the subject.

75. Tumor-informed assays were well known to a POSA by 2014. (See, e.g., *id.*; Ex.1060, 1 (“We used targeted or whole-genome sequencing to identify somatic genomic alterations and designed personalized assays to quantify circulating tumor DNA in serially collected plasma specimens.”); Ex.1031, Fig. 1A; Ex.1037, 4 (“massively parallel sequencing can be used to develop personalized biomarkers based on somatic rearrangements” which “can be used to detect tumor DNA in the presence of large quantities of normal DNA and in patient plasma”); Ex.1008, 6–7 (“[the] specific mutations [assayed in ctDNA] are defined by evaluation of the primary tumor” which “may be less of an obstacle in the future because more cancer patients will have their tumors genetically analyzed to guide therapeutic decisions”); Ex.1072, Figure 1, 5 (describing personalized ctDNA assays using “identification of mutations in the subject’s tumor as a preliminary step”).) Tumor-informed assays were routinely performed on plasma samples collected at different time points in order to monitor cancer progression over time. (See, e.g., Ex.1060, 1; Ex.1006, 1, 5; Ex.1072, 1.)

C. Whole Exome Sequencing

76. Whole-exome sequencing (WES) is a method to sequence only the regions of a genome that code for proteins, called the exome. (Ex.1038, 1). WES involves the capture of fragmented genomic DNA using probes targeting the exome, followed by sequencing. (*Id.*) By 2014, WES had been extensively used for clinical studies, in part because it avoids the expense of whole genome sequencing while still covering ~85% of known disease-causing variants. (*Id.*, 5.) These applications of WES included sequencing tumor biopsies for tumor-informed assays of cfDNA. (*See, e.g.*, Ex.1008, 2.)

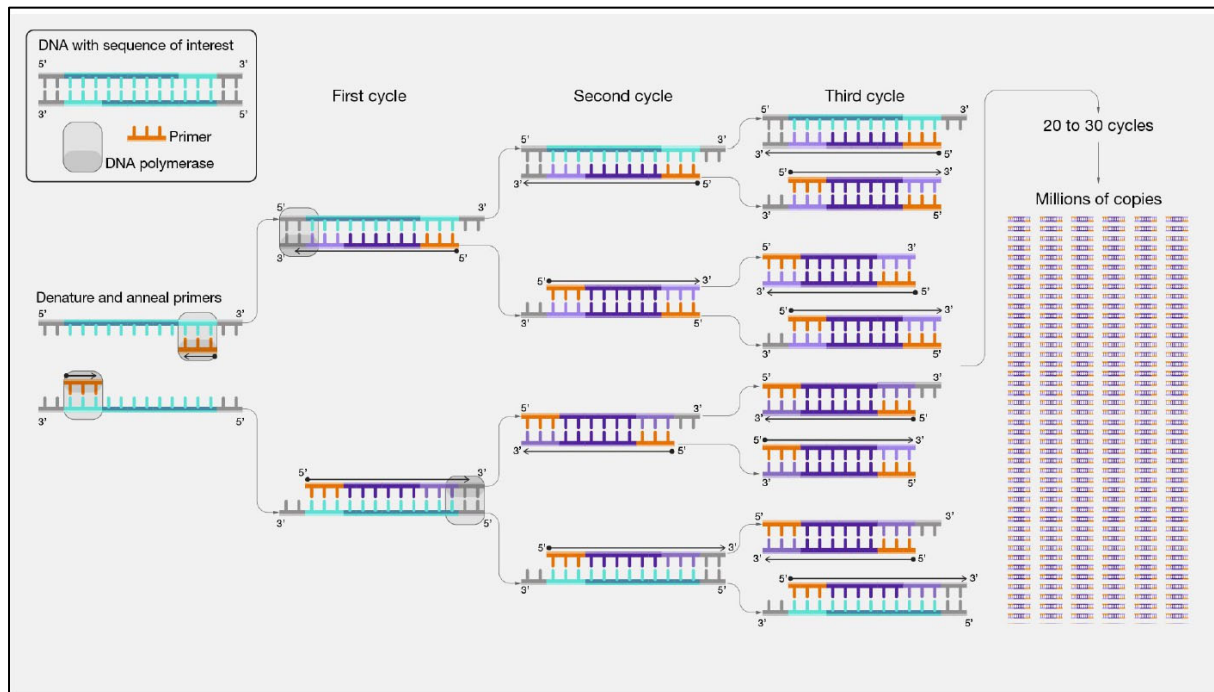
D. Multiplex PCR Amplification of Cell-Free DNA

77. A minimum amount of DNA is often required to perform different biotechnology applications or processes, such as DNA sequencing. A sample with a limited amount of DNA must, accordingly, sometimes be amplified or copied prior to performing DNA sequencing with certain sequencing platforms. Amplification is the process of creating multiple copies of DNA. PCR is one method of amplification, wherein DNA fragments are amplified or copied in cycles using an enzyme called DNA polymerase.

78. A typical PCR cycle requires three steps: 1) denaturing the double-stranded DNA to form single-stranded DNA, 2) annealing primers to the resulting single-stranded DNA molecules, and 3) primer extension using the single-stranded

DNA molecules as templates for the complementary strands formed. (Ex.1066, 2.)

As shown below, this process provides many copies of the original DNA template.



(Ex.1067, 1.)

79. Multiplex PCR involves the use of multiple primer pairs to amplify many different target DNA loci in multiple sequences in a single reaction or container, rather than using a single primer pair to amplify a single target sequence. (See Ex.1064, Abstract (“The present invention provides a method for multiplex polymerase chain reaction (mpxPCR), the simultaneous amplification of different target nucleic acid sequences in a single PCR reaction.”); *id.*, [0029].) Multiplex PCR is faster, less expensive, and less cumbersome compared to performing PCR for each target DNA sequence in a separate reaction or container. (Ex.1065,

Abstract.) The '596 patent describes several methods for multiplex PCR amplification of plasma libraries derived from isolated cfDNA.

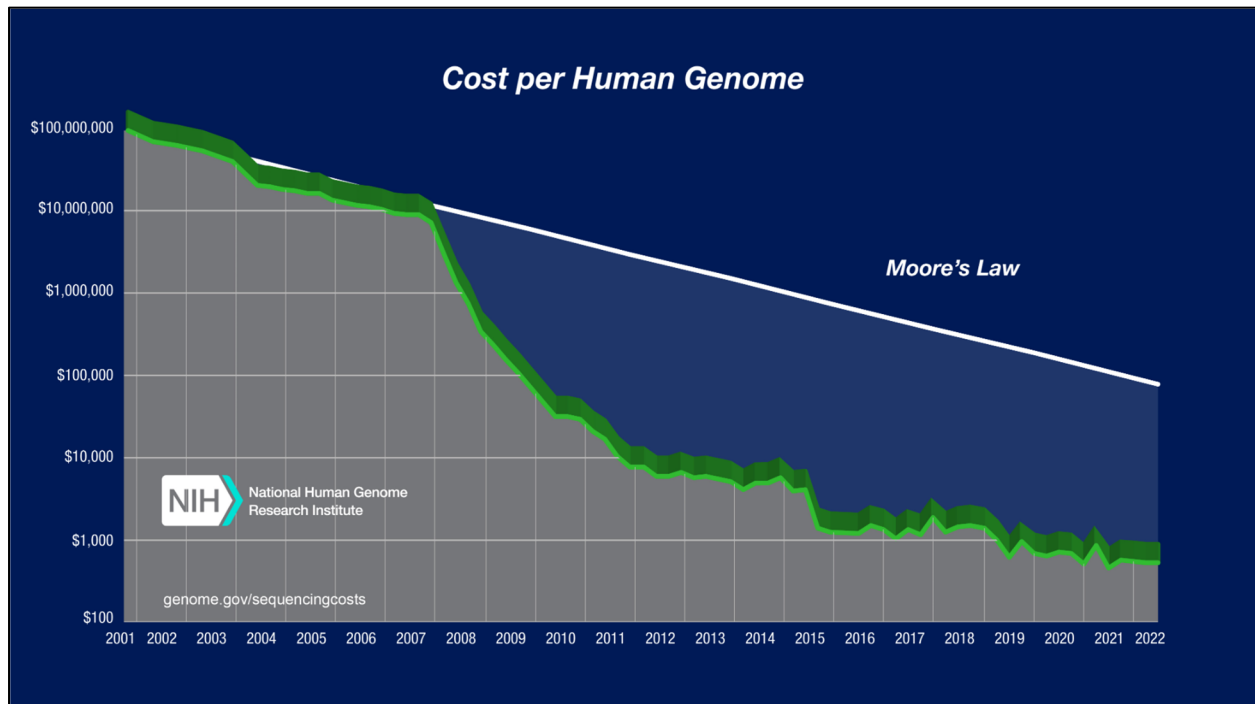
80. Multiplex PCR methods for samples containing isolated cfDNA or DNA derived therefrom were well known by 2014. (*See, e.g.*, Ex.1039, 3–4 (describing multiplex PCR reactions to amplify 12 target loci from isolated cfDNA comprising circulating tumor DNA, followed by NGS of the amplified DNA); Ex.1040, [0053]-[0057] (describing multiplex PCR from cell-free tumor DNA followed by sequencing to determine p53 mutation status); Ex.1041, 32:26–33:19 (“isolated extracellular nucleic acids” can be analyzed by techniques including multiplex PCR... Respective technologies are well-known to the skilled person and thus, do not need further description here).) For example, U.S. Patent Application Publication No. 2012/0270212 A1 (“Rabinowitz”; Ex.1009), filed and published in 2011 and 2012, respectively, discloses multiplex PCR assays (e.g., of more than fifty loci) that “may be combined in a single reaction” with an isolated cfDNA sample or a further processed derivative of isolated cfDNA. (Ex.1009, [0267]; *see also id.*, [0284] (“the target DNA” for the assays disclosed “may originate from . . . cancer patient plasma.”). I will discuss Rabinowitz further in this Declaration.

E. High-Throughput Sequencing

81. DNA sequencing is a process wherein the sequence of bases in DNA (i.e., the order of bases in a DNA strand) is determined. In the early 2000s, massively parallel sequencing technologies emerged that allowed for high throughput sequencing of many DNA fragments. (Ex.1104, 2; Ex.1105, Abstract.) At the time, these technologies were referred to as next-generation sequencing (NGS). NGS sequences many fragments of DNA at the same time. Parallel sequencing allows the processing of millions of DNA molecules in parallel, producing millions of “sequencing reads.” (Ex.1068, 2.) Each “read” is a short sequence of bases identified, or “called” by analyzing the raw sequencing data. (Ex.1043, 1.) Because base calls are sometimes incorrect due to sequencing errors, NGS platforms typically also generate quality scores for each sequenced base. (*Id.*) Sequencing platforms that carry out NGS use a variety of sequencing and detection biochemistry but generally have a common, i.e., conceptually similar, workflow to produce these sequence reads. (Ex.1068, 2.)

82. The introduction and refinement of the NGS methods mentioned above led to an exponential decrease in DNA sequencing costs, with the cost to sequence one human genome falling from around sixteen million dollars in 2005 to around ten thousand dollars in 2014, and still dropping. (Ex.1068; Ex.1070; Ex.1071, 6 (“[S]equencer speeds have improved dramatically in just the past

several months, and this trend is likely to continue. Thus, it does not seem that cost and time will remain significant impediments for much longer.”.)



(Ex.1069, 2 (*see* at 2014).)

83. By 2014, it was also well-known in the art to apply NGS methods for analysis of amplified cfDNA. (*See, e.g.*, Ex.1039, 3–4 (describing multiplex PCR reactions to amplify 12 target loci from isolated cfDNA comprising circulating tumor DNA followed by NGS of the amplified DNA); Ex.1009, [0229] (“once a mixture has been preferentially enriched at the set of target loci, it may be sequenced using any one of the previous, current, or next generation of sequencing instruments”).) As these and other references illustrate, NGS technology was

being adopted for the analysis of cfDNA, including ctDNA, by the earliest critical date of the '596 patent.

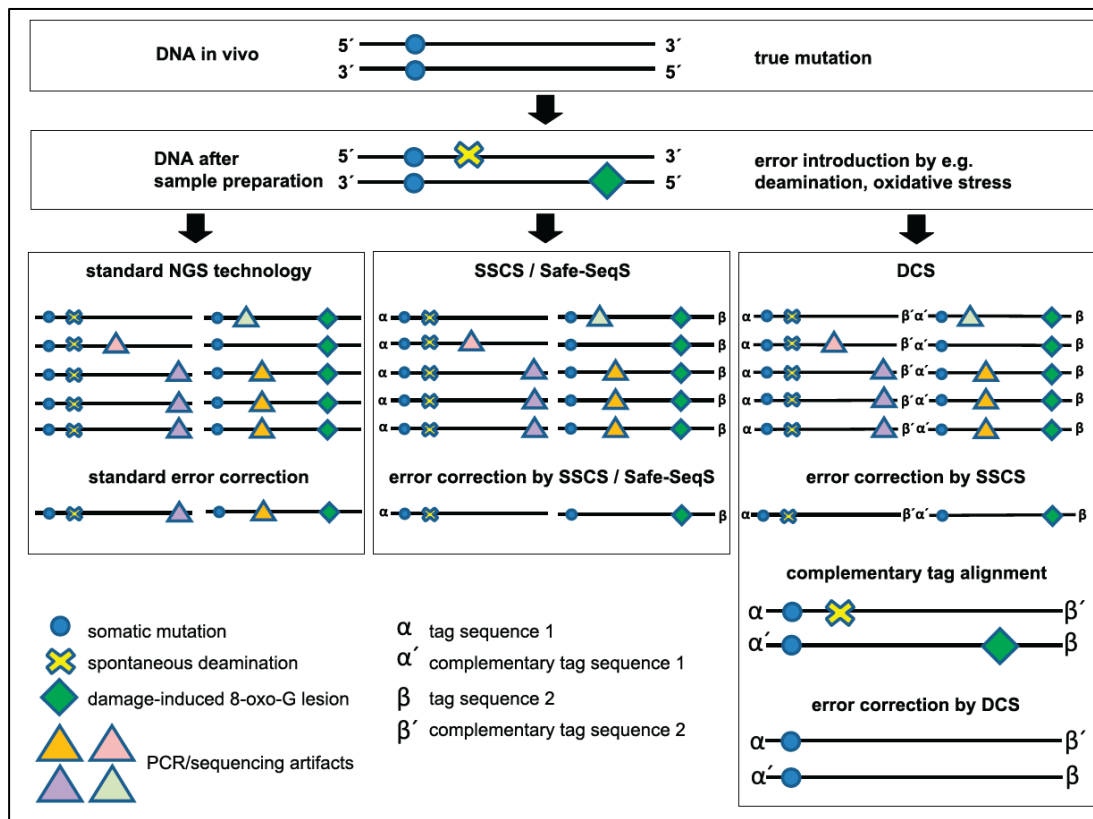
F. Detecting an SNV from Sequence Reads

84. Point mutations that occur in cancer cells in an individual and that differ from the individual's germ-line (original) DNA are often referred to as "single nucleotide variants" (SNVs). For example, if the germline of DNA of an individual includes a AGTCGT nucleotide sequence, then the tumor DNA of the individual diagnosed with cancer may instead include a AGTTGT sequence (i.e., T instead of C at the fourth nucleotide position).

85. As discussed above, NGS generates short sequences of base calls for sequenced DNA fragments, termed "sequencing reads." (Ex.1043, 1.) Detecting point mutations from these sequence reads requires matching the sequence read with the genetic locus from which it originated. Typically, this is done by aligning the reads to a reference sequence. (*Id.*) Once the location of the reads is identified, variants are identified or "called" from the sequencing reads. (*Id.*, 2.) This process is used to detect either novel SNVs or known SNVs. (*Id.*, 7.) When detecting known SNVs from sequence reads (as is the case in tumor-informed assays), less stringent parameters can be used for SNV detection. (*See* Ex.1042, 12 ("For example, SAMtools (MAQ) sets the prior probability for a heterozygous SNV at 0.001 for novel variants, and 0.2 for known SNVs").)

86. Some sequencing platforms provided integrated pipelines for detecting mutations such as SNVs from sequencing reads using a single platform. For example, Illumina NGS platforms included CASAVA 1.6 software, which could be used to detect SNVs. (Ex.1007, 16.) Similarly, the IonTorrent PGM sequencer (Life Technologies) provided a suite of tools to align sequencing reads to a reference and detect mutations from the reads after alignment (the “IonTorrent Suite V3.4.2,” including the “IonTorrent Variant Caller (ITVC) v3.4”). (Ex.1039, 3.) Numerous other SNV callers were also available to detect SNVs from sequence reads. (Ex.1043, 1.)

87. One challenge for SNV detection is that sequencing error rates can overwhelm the true SNV signal for rare SNVs. (*Id.*) By 2014, several approaches had emerged to “discriminate between sequencing error-derived noise and real genetic variation” using unique identifier sequences to label DNA templates. (Ex.1044, 1.) Fig. 1 of Kirsch (reproduced below) summarizes two widely cited approaches, termed “Safe-SeqS” and “duplex sequencing”:



(Ex.1044, Fig. 1.)

88. Both Safe-SeqS and duplex sequencing involve molecular barcoding, which allows identification of families of nucleic acid sequencing reads which share the same tag sequence. (*Id.*, 1.) True mutations can thus be distinguished from errors: the true mutation will be common among reads in the same family. Methods such as Safe-SeqS incorporated such tags into PCR primers, and successfully achieved detection of SNVs present at frequencies as low as 1 mutant template among 1,000,000 wild-type templates (0.0001%). (*See* Ex.1007, 4.) Safe-SeqS was also compatible with multiplex PCR. (Ex.1007, 5.)

Duplex sequencing provides an even lower limit of detection by distinguishing base changes present in only one strand of complementary DNA. (Ex.1036.)

Duplex sequencing is not compatible with PCR-based enrichment, however, which requires denaturing the complementary strands of DNA. (Ex.1036, 8.)

89. Barcoding-based error correction methods were widely cited by 2014. (*See, e.g.*, Ex.1045, 2 (noting that Safe-SeqS is a “highly sensitive and specific assay” which is “immune to the presence of a vast excess of normal alleles”); Ex.1047, 9 (explaining that sequencing error rates limit the accurate detection of rare mutations, which can be addressed using “single-molecule barcoding methods such as duplex sequencing or Safe-Seq, which reduce the sequencing error rate from 10^{-2} to 10^{-10} ”).)

90. A POSA also would have understood that barcoding-based error correction could be applied to cfDNA analysis. *See, e.g.*, Ex.1048, 7 (for cfDNA analysis, explaining that “[t]argeted assays have the promise of reduced cost and can, in principle, be combined with molecular bar-coding strategies that may reduce sequencing and PCR error rates”) (citing Ex.1049). Additionally, well before 2014, barcoding-based error correction had already been applied to cfDNA. (*See, e.g.*, Ex.1008, 2, 16 (disclosing that Safe-SeqS amplification and sequencing

was able to detect one mutant template in the DNA purified from up to 5 ml of plasma).)

VIII. PRIOR ART GROUNDS

91. As discussed in the previous section, it is my opinion the challenged claims are entitled to a priority date of no earlier than April 21, 2015. Based on that date, I believe the challenged claims are unpatentable based on at least four Grounds as set forth below:

Ground	Claims	Prior Art
1	1, 2, 5, 10, 12–14, and 16–18	Diaz (Ex.1006) & Kinde 2013 (Ex.1007)
2	1, 2, 5, 10–14, and 16–18	Diaz, Kinde 2013, & Rabinowitz (Ex.1009)
3	15	Diaz, Kinde 2013, & Fleischhacker (Ex.1010)
4	1, 2, 10–13, and 15–18	Rabinowitz & Bettegowda (Ex.1008)

92. Diaz was publicly available online “ahead of print” as of January 21, 2014 according to the left-hand column on the first page. (Ex.1006.) In addition, I note that Diaz indicates on its first-page header that it was published on February 20, 2014. I understand the February 2014 date to be the date of print publication. Consistent with that understanding, Ex.1092 is a date-stamped version of Diaz having a date stamp of February 28, 2014 from what I believe is Georgia Southern University. Exhibit 1006 appears to be an authentic copy of Diaz.

93. Kinde 2013 (including its supplementary materials) was publicly available online as of January 9, 2013 as shown on the bottom right footer. (Ex.1007, 1.) In my experience, articles published in scientific journals, including any supplemental materials, are publicly available online no later than their online publication date. In addition, Ex.1085 is a date-stamped version of Kinde 2013 from the National Library of Medicine. The date stamp is March 12, 2013. Exhibit 1007 appears to be an authentic copy of Kinde 2013.

94. Rabinowitz is a U.S. patent publication on October 25, 2012. (Ex.1009.)

95. Fleischhacker was publicly available online as of October 7, 2006 as shown under the heading of the article. (Ex.1010.) In addition, I note that Fleischhacker also indicates on its first page that it was published in 2007. (*Id.*) I further note that the '596 patent (Ex.1001) references Fleischhacker at Col. 133, lines 15–20 and 26–32 with a date of 2007. Ex.1010 appears to be an authentic copy of Fleischhacker.

96. Bettgowda (including its supplementary materials) was publicly available online as of February 19, 2014 as shown on the bottom right footer. (Ex.1008.) In my experience, as I have noted above, articles published in scientific journals, including any supplemental materials, are publicly available online no

later than their online publication date. Exhibit 1008 appears to be an authentic copy of Bettegowda.

97. Based on an April 21, 2015 priority date, I understand that each reference would qualify as prior art against the '596 patent under AIA § 102(a)(1). Diaz, Kinde 2013, Rabinowitz, Fleischhacker, and Bettegowda were published before the '596 patent's April 21, 2015 priority date as discussed above. (Exs.1006-1012.)

98. Even if Patent Owner asserts that the challenged claims are entitled to an earlier filing date, such as October 21, 2014 or even April 21, 2014, it is my opinion that the challenged claims are unpatentable based on at least the above Grounds. I understand each reference cited above would qualify as prior art under AIA § 102(a)(1) even if the Patent Office determines that the challenged claims are entitled to the filing date of the first provisional application on April 21, 2014.

A. GROUND 1: DIAZ & KINDE 2013

99. In my opinion, the combination of Diaz and Kinde renders obvious claims 1, 2, 5, 10, 12–14, and 16–18 of the '596 patent. Summaries of these references, the combination, and the bases for my opinions are set forth below.

1. Summary Of Diaz

100. Diaz is a review article that discloses various methods for performing “liquid biopsies” involving genotyping circulating tumor DNA at multiple time points. (Ex.1006, 1.) It is in the same field of endeavor as the '596 patent because it relates to analysis of cfDNA, and to circulating tumor DNA in particular. (*Id.*)

101. Diaz discloses various approaches to performing ctDNA analysis, including tumor-informed assays comprising “identifying a specific mutation or mutations in the tumor tissue” and then targeting and quantifying the level of ctDNA at a particular time point. (*Id.*, 4.) For example, Diaz discloses a method wherein, for a cohort of cancer patients, “[a] mutation profile was determined from each patient’s resected tumor, and this personal and unique molecular signature was used” to develop targeted assays to detect and quantify ctDNA over time following surgery. (*Id.*). According to Diaz, such tumor-informed assays have particular advantages for liquid biopsies to monitor ctDNA over time. Specifically, identification of “personalized markers based on the unique

mutational profiles of resected tumors” is advantageous because it allows for “exquisitely high specificity” to evaluate postoperative levels of ctDNA. (*Id.*, 4.)

102. Diaz also teaches that “PCR-based digital approaches have been updated with techniques that use next-generation sequencing (NGS) to identify rare variants in complex mixtures of DNA,” and that these techniques allowed “multiple genes of interest” to be investigated “in one sample.” (*Id.*, 2.) A POSA would have understood that Diaz’s references to “next-generation sequencing” or “NGS” meant high-throughput sequencing.

103. In the context of identifying rare variants, Diaz discusses the problem of distinguishing mutations from errors. (Ex.1006, 3.) Polymerase errors, according to Diaz, impose a technical limit on sensitivity for detecting a true mutation. (*Id.*) To address this challenge, Diaz points to Kinde 2011 (Ex.1049). Kinde 2011 discloses a method called “Safe-Sequencing System” or “Safe-SeqS” that, according to Diaz, is a solution “aimed at increasing overall sensitivity” for detecting mutations in ctDNA in the context of next-generation sequencing. (Ex.1006, 3 (citing Ex.1049).)

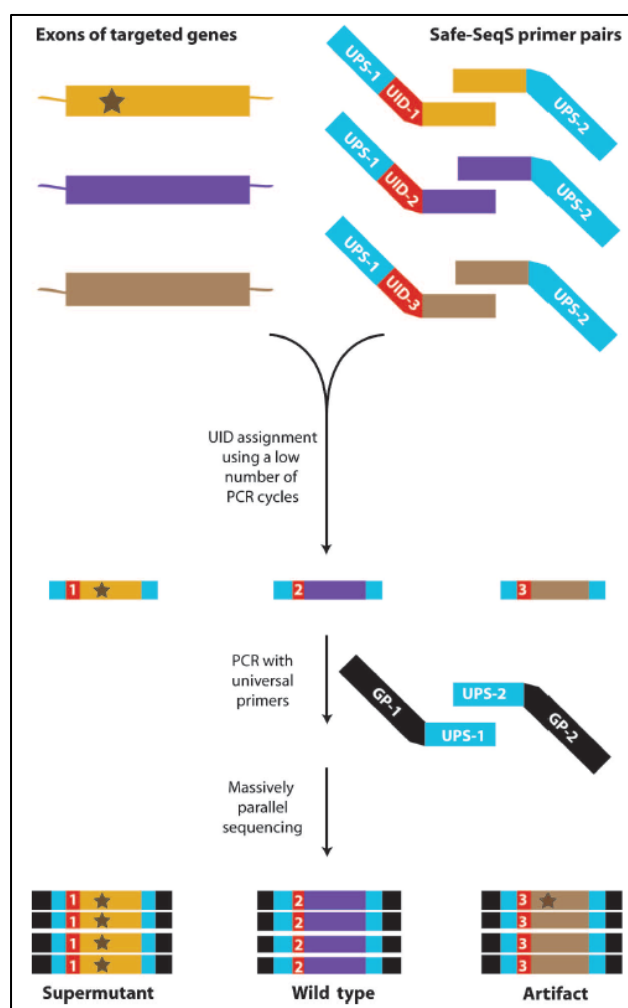
2. Summary Of Kinde 2013

104. Kinde 2013 discloses application of an error-corrected sequencing method to detect rare “somatic mutations” in liquid Pap smears. (Ex.1007, 1, 4.)

It is in the same field of endeavor as the '596 patent because it relates to detecting SNVs. (*Id.*)

105. Kinde 2013's method is an improved version of "Safe-SeqS" originally described in 2011 and referenced by Diaz as discussed above. (Ex.1006, 3; Ex.1049.)

106. As shown in Kinde 2013's Fig. 2, the improved Safe-SeqS method comprises amplifying template nucleic acids using primers comprising binding sites for universal primers ("UPS," blue), a unique identifier ("UID," red) and target-specific sequences (colors matching the target in Fig. 2.). (Ex.1007, 5.) Kinde discloses that the templates and primers are then "combined into a single PCR compartment and a UID is assigned to each targeted template, along with UPS binding sites, after a low number of PCR cycles ("UID assignment")." (*Id.*) Next, the "Safe-SeqS primers are removed, and subsequent PCR is performed with primers containing UPS sites, as well as sequences for attachment to the sequencing instrument ("GP," black) to prepare the templates for massively parallel sequencing." (*Id.*). The "multiplexed assays" using multiplex PCR are "performed in a similar fashion." (*Id.*, 8, 18.)



(Ex.1007, Fig. 2.)

107. Kinde 2013 discloses that the UID assignment, which can be performed as a multiplex PCR, allows true SNVs to be distinguished from errors. Kinde 2013 explains that the true SNV present in template DNA before sample preparation will be present in the majority “of the sequenced daughter molecules sharing the same UID[.]” (*Id.*, 5.) According to Kinde 2013, “[in] contrast, artifactual mutations caused by sample preparation or sequencing are unlikely to be

observed in most other daughter molecules sharing the same UID (‘Artifact’).”

(*Id.*) Based on this principle, Kinde 2013 distinguished true SNVs from artifactual mutations “by requiring that >50% of reads sharing the same UID contained the identical mutation.” (*Id.*, 19.)

108. The original Safe-SeqS method of Kinde 2011 “decreased presumptive sequencing errors by at least 70-fold,” thereby improving detection of true variants. (Ex.1049, 2.) Kinde 2013 disclosed that its improved Safe-SeqS approach is capable of detecting 1 mutant template among as many as 1,000,000 wild-type templates, including a specific example of detecting a mutation present at 0.01% of the DNA having the locus. (*Id.*, 4, 6.) Kinde’s improved Safe-SeqS approach in 2013 included several modifications from original Safe-SeqS of 2011. First, while original Safe-SeqS required a gel purification step following PCR amplification (Ex.1049, 7), the improved Safe-SeqS simply uses a bead clean-up step, greatly simplifying the workflow (Ex.1007, 18 (“PCR products were purified with AMPure XP beads and used directly for sequencing on either Illumina MiSeq or GA IIX instruments”).) Second, Kinde 2013 discloses an improved method for removing unincorporated primers after the UID assignment PCR cycles that resulted in “more robust amplification products.” (Ex.1007, 18.) Third, Kinde 2013 included “four “N” bases in the reverse target-specific primer to facilitate

sequencing analysis of paired-end libraries. (Ex.1007, 17.) Kinde 2013 teaches that the modifications in its improved Safe-SeqS “facilitated the amplification of multiple gene regions in a single well of a 96-well PCR plate.” (*Id.*)

3. Combination Of Diaz and Kinde 2013

109. A POSA would have found it obvious to use Kinde 2013’s Safe-SeqS “unique identifier” barcodes and error-correction algorithm with Diaz’s teachings regarding a tumor-informed PCR and next-generation sequencing assay for circulating tumor DNA to detect an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV.

110. Both Diaz and Kinde 2013 are in the same field as the ’596 patent. (*See* Ex.1001, 1:28–31 (stating that the invention of the ’596 patent “generally relates to methods and systems for detecting ploidy of a chromosome segment, and methods and systems for detecting a single nucleotide variant”).) Both references relate to detection of tumor-specific single nucleotide variants. (*See, e.g.*, Ex.1006, 2–6 (describing methods to detect mutations including “somatic point mutations” (SNVs) in circulating tumor DNA), Ex.1007, 309–10 (table S3) (showing detection of single nucleotide variants).)

111. Both Diaz and Kinde 2013 sought to address the same problem of detecting rare cancer mutations in a wild-type background. As discussed above, in this context, Diaz discusses the problem of distinguishing mutations from errors in

order to determine that a mutation is present. (*See* Ex.1006, 3.) According to Diaz, polymerase errors impose a technical limit on sensitivity for detecting a true mutation. (*Id.*) Diaz also specifically discloses Safe-SeqS (citing Kinde 2011) as a solution “aimed at increasing overall sensitivity” for detecting mutations in ctDNA in the context of next-generation sequencing. (Ex.1006, 3 (citing Ex.1049).)

112. Although the Pap smear liquid biopsy in Kinde 2013 is not a ctDNA assay, Kinde 2013 also sought to detect somatic cancer mutations in samples wherein “the amount of DNA derived from neoplastic cells . . . would be relatively small compared to the DNA derived from normal cells.” (Ex.1007, 3–4.) According to Kinde 2013, “[t]his necessitated the use of an analytic technique that could reliably identify a rare population of mutant alleles among a great excess of wild-type alleles. A modification of one of the Safe-SeqS (Safe-Sequencing System) procedures described in (37), in which DNA templates are amplified with modified gene-specific primers, was designed for this purpose (Fig. 2).” (*Id.*, 4.)

113. A POSA would also have been motivated to develop assays for detection of tumor-specific SNVs present in less than or equal to 0.015% of the cfDNA having the SNV locus. Diaz discloses that “[m]ultiple methods have shown that the fraction of circulating DNA contributed from the tumor varies greatly, between 0.01% and more than 90%,” and the ctDNA fraction can be even

lower than 0.01%. (Ex.1006, 2; *see also id.*, 3.) Therefore, a POSA would have understood that tumor-specific SNVs in ctDNA are often present at levels well below 0.015%. Diaz further explains that sensitivity is currently limited by the error rate of DNA polymerase, “which is generally considered to be 0.01%.” (*Id.*, 3.) As noted above, Diaz specifically suggests barcoding strategies to increase sensitivity in the context of NGS by citing an earlier version of the Safe-SeqS method. (*Id.*) Kinde 2013, in turn, discloses that its Safe-SeqS approach is capable of enhanced mutation detection for mutant templates present at levels even below 0.01% (down to 1 mutant template in 1,000,000 wild-type templates, or 0.0001%). (Ex.1007, 4.) Kinde 2013 further discloses using its improved Safe-SeqS for multiplex amplification. (*See, e.g., id.*, 5.)

114. The problem of distinguishing SNVs from artifactual errors and the Safe-SeqS error correction solution were also widely discussed in the art by the earliest possible priority date, including application of Safe-SeqS to cfDNA. (*See, e.g., Ex.1048*, 7 (for cfDNA analysis, explaining that “[t]argeted assays have the promise of reduced cost and can, in principle, be combined with molecular barcoding strategies that may reduce sequencing and PCR error rates”) (citing Ex.1049).)

115. A POSA would also have reasonably expected this combination to succeed. Diaz discloses that technically, “sensitivity is limited by the error rate of DNA polymerase, which is generally considered to be 0.01%.” (Ex.1006, 3.) Kinde 2013 teaches that Safe-SeqS allows detection of SNVs as distinct from errors, because “[m]utations not occurring in the original templates, such as those occurring during the amplification steps or through errors in base calling, should not give rise to supermutants.” (Ex.1007, 4.) A POSA would reasonably expect to detect an SNV present in less than or equal to 0.015% of the DNA containing the locus: the 0.01% error rate and sensitivity limit in Diaz (Ex.1006, 3) is already below 0.015%, and the Safe-SeqS method of Kinde 2013 allows true SNVs to be distinguished from errors, overcoming the technical limit imposed by errors in amplification and base calling.

116. A POSA would also have reasonably expected to succeed in applying Safe-SeqS barcodes and error-correction methods to cell-free DNA. Before the earliest possible priority date for the '596 patent, Safe-SeqS had already been applied in a tumor-informed assay to analyze tumor-specific mutations in serial plasma samples. (*See, e.g.*, Ex.1050 (“Tie”)). According to Tie, tumor tissue was first “analyzed for hotspot mutations in *TP53*, *APC*, *KRAS*, *BRAF*, *PIK3CA* and *FBXW7*” and “[the] same mutation was queried and quantified in

plasma using a MPS platform (Safe-SeqS).” (*Id.*) Using Safe-SeqS, “matching ctDNA [was] found for each [patient]” for which a mutation was identified in the tumor. (*Id.*)

117. Additionally, before the earliest possible priority date for the ’596 patent, a POSA would understand that Safe-SeqS had already been applied to cfDNA, which further supports a reasonable expectation of success. (*See, e.g.,* Ex.1008, 2, 16 (disclosing that Safe-SeqS amplification and sequencing was able to detect one mutant template in the DNA purified from up to 5 ml of plasma).)

118. A POSA would also have found it obvious to perform the PCR as a multiplex PCR amplifying multiple target loci in one reaction. First, Kinde 2013 teaches that Safe-SeqS barcodes can be used in multiplex PCR, as discussed in paragraphs 106–108 and 113 of my Declaration. Additionally, Diaz discloses that “PCR-based [] approaches” for assaying ctDNA have been updated to use “next-generation sequencing (NGS) to identify rare mutant variants in complex mixtures of DNA” wherein “multiple genes of interest can be investigated in one sample.” (*Id.*) A POSA would have been motivated to perform multiplex PCR followed by next-generation sequencing to investigate “multiple genes” or loci of interest “in one sample” as suggested by Diaz because multiplex PCR of cfDNA followed by

next-generation sequencing was well-known by 2014, as described in paragraph 80 of my Declaration.

119. Furthermore, a POSA would reasonably have expected to succeed with inclusion of the Safe-SeqS “unique identifier” (UID) barcodes in primers for multiplex amplification. First, Kinde demonstrated successful multiplex PCR amplification in a single reaction volume using primers containing Safe-SeqS UIDs. (Ex.1007, 5–8, 312(table S5).) Although Kinde disclosed a 0.1% limit of detection in the context of its multiplex Safe-SeqS assay, Kinde explained that different parameters were used for SNV detection in that experiment because it was a screening assay (covering 10,257 bp) in which the potential SNVs were not known *a priori*. (Ex.1007, 19; *see also* paragraph 145 of my Declaration below.) Therefore, a POSA reading Kinde would understand that the 0.1% limit of detection was chosen only for mutation-agnostic screening assays, and would not apply to multiplex amplification reactions wherein the SNVs *are* known *a priori*, which is the case for tumor-informed assays as described by Diaz.

120. A POSA would also reasonably have expected to succeed with inclusion of the Safe-SeqS “unique identifier” (UID) barcodes in primers for multiplex amplification from isolated cell-free DNA. As discussed in paragraph 80 of my Declaration, by 2014, methods for multiplex amplification from cell-free

DNA were already well-known to a POSA. Use of molecular barcodes in primers for multiplex amplification of cfDNA had also been described previously. (*See* Ex.1009, [0201] (describing multiplex PCR of “10,000 primers in one reaction” to efficiently enrich “plasma derived DNA,” wherein the “enrichment and/or amplification may involve tagging each individual molecule with different tags, molecular barcodes, tags for amplification, and/or tags for sequencing”), [0207] (“In some embodiments, the primers may contain a molecular barcode”), [0019]–[0020] (describing multiplex PCR and disclosing that “[in] some embodiments, at least some of the primers additionally contain a molecular barcode”), [0267] (disclosing that multiplex PCR reactions may be performed “in a single reaction with the entirety of a cfDNA sample”).) A POSA would understand that “molecular barcodes” are synonymous with “unique identifiers” as described in Kinde 2013. Therefore, a POSA would reasonably expect to succeed in using Kinde 2013’s unique identifiers in primers for multiplex amplification from cfDNA.

4. Claims 1, 2, 5, 10, 12–14, and 16–18 Are Obvious Over Diaz and Kinde 2013

a. Claim 1

Preamble: *A method for preparing biological samples useful for monitoring the progression of cancer in a subject, the method comprising:*

121. If limiting, the combined teachings of Diaz and Kinde 2013 disclose methods for preparing biological samples useful for monitoring the progression of cancer in a subject.

122. Diaz Table 1 discloses applications of liquid biopsies including monitoring tumor dynamics, monitoring minimal residual disease, and evaluation of early treatment response:

Table 1. Applications of Liquid Biopsy	
	Application
	Early detection
	Assessment of molecular heterogeneity of overall disease
	Monitoring of tumor dynamics
	Identification of genetic determinants for targeted therapy
	Evaluation of early treatment response
	Monitoring of minimal residual disease
	Assessment of evolution of resistance in real time

(Ex.1006, 2.)

123. Diaz further discloses that “[t]here are rapid increases in ctDNA levels with disease progression and corresponding declines in levels after successful treatment.” (*Id.*, 4.) Furthermore, Diaz discloses that “[a]nalysis of

ctDNA in plasma samples obtained before and after treatment can ultimately provide a global picture of the molecular genetics of a patient's tumor." (*Id.*, 5.)

Limitation 1[A]: (a) performing sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor-specific mutations, wherein the tumor-specific mutations comprise one or more single nucleotide variant (SNV) mutations;

124. The combined teachings satisfy Limitation 1[A]. Diaz discloses that one approach for performing ctDNA analysis requires "identifying a specific mutation or mutations in the tumor tissue to target and quantify the level of ctDNA at a particular time point." (Ex.1006, 4.) Diaz discloses a specific example "in a cohort of patients with colorectal cancer." (*Id.*) "A mutation profile was determined from each patient's resected tumor, and this personal and unique molecular signature was then used" to track the particular tumor-specific mutations for each patient in a personalized assay. (*Id.*) Diaz teaches that this method was used to "detect minimal residual disease after surgical resection." (*Id.*) Furthermore, Diaz discloses that identification of "personalized markers based on the unique mutational profiles of resected tumors" is advantageous because it allows for "exquisitely high specificity" to evaluate postoperative levels of ctDNA. (*Id.*)

125. A POSA would understand that "identifying a specific mutation or mutations in the tumor tissue" to determine a "mutation profile" from each

patient's resected tumor in Diaz involves sequencing a tumor biopsy. Mutations such as somatic point mutations are "[c]hanges in the DNA sequence" that are "the most frequent class of variants associated with the development of solid tumors." (*Id.*, 5.) Diaz further discloses that "next-generation sequencing (NGS)" can be used to identify rare mutant variants in complex mixtures of DNA and that sequencing of tumor tissue is "routine." (*Id.*, 2.) Diaz further discloses that Sanger sequencing, pyrosequencing, and next-generation sequencing are all approaches to detect mutations in tumor tissue. (*Id.*, Fig. 1.) Furthermore, Diaz teaches that "[m]olecular tools, such as NGS, can be implemented to find genetic differences between the tissue [biopsies] collected before and after therapy." (*Id.*, 4.)

126. To the extent Natera asserts that Diaz does not specifically teach that determining the mutation profile of the tumor biopsy involves sequencing the tumor biopsy, Kinde 2013 teaches sequencing a tumor biopsy to identify mutations that are then tracked in liquid biopsies. (Ex.1007, 3 ("somatic mutations in [] tumors were identified through whole exome sequencing In each of the 46 cases, we identified at least one somatic mutation (table S3) that was confirmed by an independent assay"), 5 (describing identifying the tumor-specific mutations in Pap specimens from the corresponding patients).)

127. Diaz also discloses that the tumor-specific mutations comprise one or more single nucleotide variant (SNV) mutations. For example, Diaz teaches that “[d]iscriminating ctDNA from normal cfDNA is aided by the fact that tumor DNA is defined by the presence of mutations” and that these somatic mutations are “commonly single-base-pair substitutions,” also known as single-nucleotide variants (SNVs). (Ex.1006, 1.)

Limitation 1[B]: (b) evaluating results of the sequencing on the tumor biopsy sample to determine a plurality of target loci specific to the subject, wherein each target locus spans a tumor-specific mutation of the identified plurality of tumor-specific mutations; and

128. Limitation 1[B] is also obvious in view of Diaz and Kinde 2013. As discussed above, Diaz discloses determining a plurality of target loci comprising tumor-specific mutations specific to the subject. (See, e.g., Ex.1006, 3.) Diaz discloses that the mutations are specific to the subject, “because mutations found in cfDNA are in essence integral agents of an individual’s cancer and are defined by their presence in tumor DNA and absence in matched normal DNA.” (*Id.*, 6; see also *id.*, 4 (explaining that the mutation profile from “each patient’s resected tumor” provides a “personal and unique molecular signature”).) And Diaz teaches that the mutations identified in the tumor tissue are used to “target and quantify the level of ctDNA.” (*Id.*, 4.) A POSA would understand that

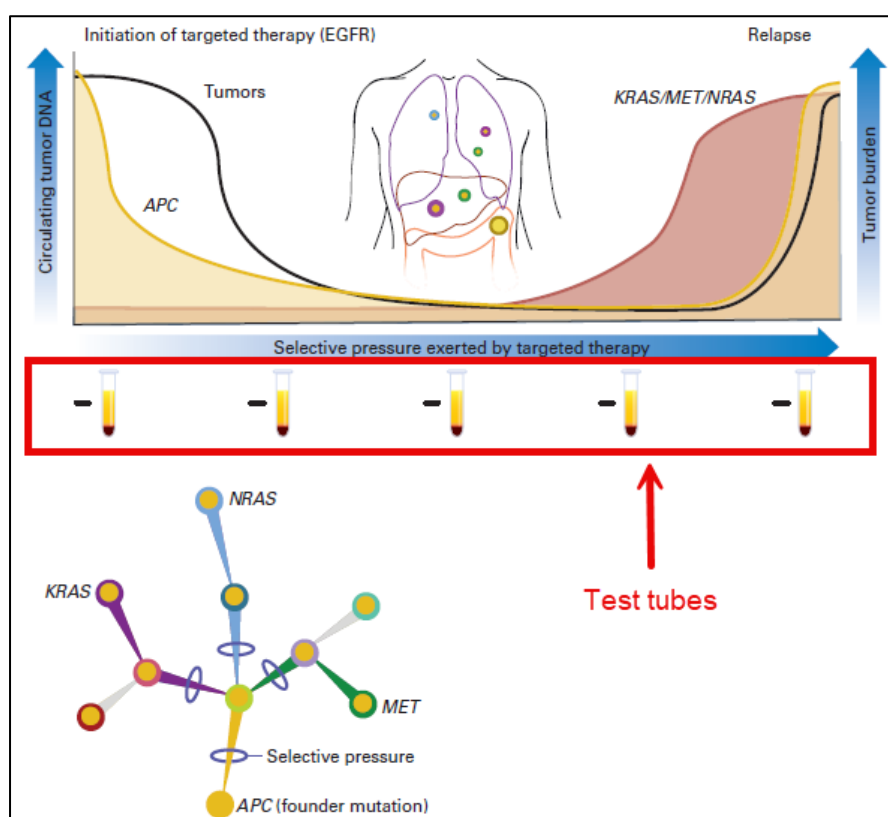
targeting in this context involves identifying and targeting loci comprising the tumor-specific mutations taught by Diaz.

129. To the extent Natera argues that Diaz does not specifically teach that determining the mutation profile and tracking said mutations in cfDNA involves evaluating results of the sequencing on the tumor biopsy sample to determine a plurality of target loci specific to the subject, Kinde 2013 also discloses this limitation. (See Ex.1007, 16 (describing evaluating the results of the sequencing by aligning reads to a reference genome, performing variant calling with the ELANDv2 algorithm in the CASAVA 1.6 software (Illumina), and removing known polymorphisms), 3 (explaining that 12,795 somatic mutations were identified in the 22 sequenced cancers).) Kinde 2013 also discloses designing primers to detect mutations identified in the sequenced cancers. (Ex.1007, 17.) A POSA would understand that the primers to detect mutations identified in the sequenced cancers are designed to amplify target loci, wherein each target locus spans a tumor-specific mutation.

Limitation 1[C][i]: (c) assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points, wherein the assaying comprises:

130. Limitation 1[C][i] is also obvious over Diaz and Kinde 2013. Diaz teaches assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points, wherein the biological sample is

a plasma sample. Referring to Fig. 3, for example, Diaz discloses “[c]ontinuous monitoring of plasma DNA” to detect mutations in tumor DNA. (Ex.1006, 5.) Diaz further explains that in Fig. 3 “[t]est tubes represent samples of plasma from which circulating free DNA is extracted and used to monitor the presence of cancer-specific aberrations.” (*Id.*)



(Ex.1006, Fig. 3 (cropped and annotated).)

131. Diaz discloses various applications for assaying cell-free DNA obtained from the subject at different time points, including to “effectively [] monitor the emergence of multiple resistance clones during the course of treatment (Fig 3)” (*id.*, 4) and to monitor tumor dynamics and minimal residual disease (*id.*,

Table 1). Furthermore, Diaz explains that one of the distinct advantages of ctDNA for monitoring cancer is that “blood can be drawn at any time during the course of therapy and allow for dynamic monitoring of molecular changes in the tumor rather than relying on a static time point.” (*Id.*, 3.)

Limitation 1[C][ii]: *performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject; and*

132. Based on the combined teachings of Diaz and Kinde 2013, it would have been obvious to perform targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject.

133. Diaz summarizes methodologies for detecting ctDNA. (Ex.1006, 2.) In this context, Diaz discloses that “PCR-based [] approaches” have been updated to use “next-generation sequencing (NGS) to identify rare mutant variants in complex mixtures of DNA” wherein “multiple genes of interest can be investigated in one sample.” (*Id.*) A POSA would have been motivated to perform multiplex PCR followed by next-generation sequencing to investigate “multiple genes” or loci of interest “in one sample” as taught by Diaz because multiplex PCR of cfDNA followed by next-generation sequencing was well-known by 2014, as

described in paragraph 80 of my Declaration. Multiplex PCR also has the advantage of requiring only a single reaction to analyze multiple genes or loci of interest in one sample.

134. Kinde 2013 discloses Safe-SeqS primers and an improved Safe-SeqS method for multiplex amplification in a single reaction volume. (Ex.1007, 5–8.) The multiplexed assays of Kinde were performed with “50 primer pairs” divided into “six independent amplifications” using Safe-SeqS primers described in table S5. (*Id.*, 4, 8.) An excerpt of table S5 showing the target loci in each of the six multiplex reactions is reproduced below.

Set #	Gene (codons)
1	<i>CTNNB1</i> 5-64 <i>EGFR</i> 697-728 <i>PIK3CA</i> 23-88 <i>PTEN</i> 1-26 <i>PTEN</i> 85-123 <i>TP53</i> 126-186 <i>TP53</i> 308-331
2	<i>BRAF</i> 439-475 <i>EGFR</i> 738-761 <i>KRAS</i> 1-21 <i>PIK3CA</i> 62-117 <i>PIK3CA</i> 980-1049 <i>PTEN</i> 100-164 <i>TP53</i> 186-224 <i>TP53</i> 332-366
3	<i>AKT1</i> 17-58 <i>EGFR</i> 762-823

	<i>NRAS</i> 1-14 <i>PIK3CA</i> 272-347 <i>PPP2R1A</i> 176-217 <i>PTEN</i> 56-70 <i>PTEN</i> 165-211 <i>TP53</i> 33-102 <i>TP53</i> 225-260
4	<i>EGFR</i> 830-875 <i>KRAS</i> 38-61 <i>NRAS</i> 38-62 <i>PTEN</i> 71-84 <i>PTEN</i> 213-267 <i>TP53</i> 63-125 <i>TP53</i> 262-306
5	<i>APC</i> 1254-1328 <i>APC</i> 1403-1476 <i>APC</i> 1537-1617 <i>FBXW7</i> 474-548 <i>FBXW7</i> 558-618 <i>TP53</i> 1-24
6	<i>TP53</i> 368-393 <i>APC</i> 1326-1405 <i>APC</i> 1475-1545 <i>FBXW7</i> 243-287 <i>FBXW7</i> 329-374 <i>FBXW7</i> 413-472 <i>FBXW7</i> 549-593 <i>KRAS</i> 134-150 <i>TP53</i> 26-32

(Ex.1007, 312 (table S5) (excerpted).)

135. As shown in table S5, Kinde 2013 disclosed multiplex reactions of up to nine different target loci in a single reaction using Safe-SeqS primers. (*Id.*) Kinde also teaches that the primers were designed using Primer3 software. (*Id.*,

17.) The '596 patent itself discloses embodiments in which the multiplex PCR primers are designed “using the Primer3 program (the worldwide web at primer3.sourceforge.net; libprimer3 release 2.2.3 . . .” (Ex.1001, 106:35–39.)

Therefore, a POSA would have a reasonable expectation of success for performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers that contain Safe-SeqS barcode sequences, wherein the primers are specific to the plurality of target loci for the individual subject.

Limitation 1[C][iii]: performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads,

136. Based on the combined teachings of Diaz and Kinde 2013, it would have been obvious to perform high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads. Diaz teaches that “PCR-based [] approaches” have been updated to use “next-generation sequencing (NGS) to identify rare mutant variants in complex mixtures of DNA” wherein “multiple genes of interest can be investigated in one sample.” (Ex.1006, 2.) A POSA would understand that NGS involves high-throughput sequencing. Diaz also teaches using barcoding strategies such as Safe-SeqS in the context of NGS in order to “increase overall sensitivity.” (*Id.*, 3.)

137. Kinde 2013 similarly discloses that Safe-SeqS involves massively parallel sequencing (*see* Ex.1007, 5 (Fig. 2)), which a POSA would understand is synonymous with NGS or high-throughput sequencing. Specifically, Kinde 2013 discloses that the Safe-SeqS PCR amplification products “were purified with AMPure XP beads and used directly for sequencing on either Illumina MiSeq or GA IIX instruments, with equivalent results.” (*Id.*, 18.) The Illumina MiSeq and GA IIX instruments were also listed as examples of sequencers used in the ’596 patent itself. (*See, e.g.*, Ex.1001 at 152:63–65 (describing sequencing amplicons using an “ILLUMINA GAIIX sequencer”), 169:37–45 (Example 14, describing performing “multi-read sequencing, such as sequencing on an Illumina HiSeq or MiSeq system”).)

138. A POSA would also have a reasonable expectation of success because by 2014, high throughput sequencing of amplified DNA from multiplex amplification of isolated cell-free DNA was within the knowledge of a POSA. (*See* paragraph 80 of my Declaration).

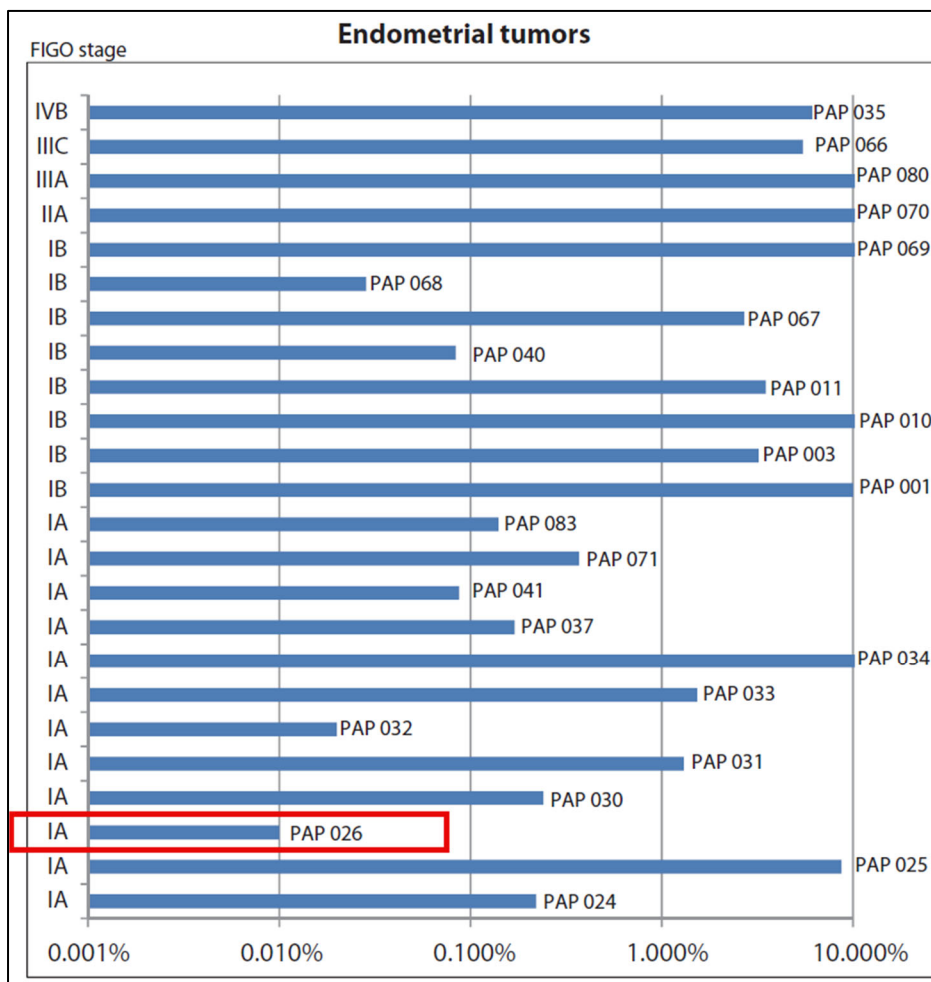
Limitation 1[C][iv]: *wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.*

139. Based on the combined teachings of Diaz and Kinde 2013, it would have been obvious to perform the claimed method wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV

locus is detected from the sequence reads. Kinde 2013's Safe-SeqS approach detects an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus. For example, Kinde 2013 teaches that the Safe-SeqS approach described "is capable of detecting 1 mutant template among 5000 to 1,000,000 wild-type templates, depending on the amplicon and the position within the amplicon that is queried." (Ex.1007, 4.) Mutations that preexist in template DNA before sample preparation (e.g., true SNVs) will be present in "all of the sequenced daughter molecules sharing the same UID" ("Supermutant" in Fig. 2). (*Id.*, 5.) In contrast, "artifactual mutations caused by sample preparation or sequencing are unlikely to be observed in most other daughter molecules sharing the same UID" ("Artifact" in Fig. 2). (*Id.*) Kinde 2013 therefore identified true SNVs, or "supermutants," only when ">50% of reads sharing the same UID contained the identical mutation," thereby distinguishing the SNVs from errors. (*Id.*, 19.)

140. Kinde 2013 specifically also discloses detection of mutations wherein "[t]he median fraction of mutant alleles was 3% and ranged from 0.01 to 80% (Fig. 3 and table S3)." (Ex.1007, 4; *see also id.*, 6 (Fig. 3), 309–10 (table S3).) Kinde 2013's Fig. 3, for example, shows "[p]ercent mutant alleles in liquid Pap smear specimens" as detected by Safe-SeqS. Fig. 3 illustrates the variant

allele frequency that was actually present in the samples (up to or more than 10% in some samples) rather than the minimal allele frequency detectable by Safe-SeqS. As shown in Fig. 3, Kinde 2013 detected a mutation present at 0.01% in a liquid Pap smear specimen PAP 026 using Safe-SeqS. A POSA would understand that Safe-SeqS was capable of detecting mutations present at even lower frequencies (as low as 1 in 1,000,000 depending on the amplicon and the position in the amplicon that is queried). (*Id.*, 4.)



(Ex.1007, 6 (Fig. 3) (cropped and annotated to highlight PAP 026).)

141. As shown in Kinde 2013's supplemental Table S3, the mutation detected at 0.01% in the PAP 026 sample was an SNV (a single base substitution from G to A, described in genomic coordinates as g.chr10:89710752G>A). (Ex.1007, 309 (table S3).) Although Fig. 3 shows results for a liquid Pap smear (not for cfDNA), a POSA would reasonably expect to achieve detection of an SNV present in less than 0.015% of the cell-free DNA having the SNV locus in the context of a cfDNA assay, as explained in paragraphs 116–117 of my Declaration.

142. Kinde 2013 also teaches that its improved Safe-SeqS assay is compatible with multiplex PCR, and demonstrates multiplex PCR followed by high-throughput sequencing. (*See* Ex.1007, 5; *see also id.* (Fig. 2).) As an example, Kinde 2013 demonstrates application of its improved Safe-SeqS assay with multiplex PCR as a screening test to target regions from commonly mutated cancer genes. (*Id.*, 5.)

143. A POSA would reasonably expect to detect an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus in view of the teachings of Diaz and Kinde 2013 because Kinde 2013 teaches that improved Safe-SeqS is capable of detecting one mutant template among as many as 1,000,000 wild-type templates (*id.*, 4), and Kinde 2013 demonstrates detection

of an SNV present at less than 0.015% for PAP 026 (*id.*, 6 (Fig. 3), 309–10 (table S3).)

144. A POSA would also have a reasonable expectation of success to perform Kinde 2013’s improved Safe-SeqS on cfDNA to detect one mutant template in a reaction containing cfDNA template copies to achieve the 0.015% limitation. For example, Kinde 2013 discloses that “[in] control experiments, 46 of the 50 amplicons were shown to provide information on a minimum of 2500 templates, as the number of sequenced templates can be determined directly from Safe-SeqS data.” (*Id.*, 5.) Because Kinde 2013 disclosed that improved Safe-Seq could work with a minimum of 2,500 templates in a reaction, a POSA would reasonably expect to succeed in performing improved Safe-SeqS on reasonable input amounts of cfDNA of at least around 7,000 templates per reaction. (*See, e.g.*, Ex.1051, 4 (disclosing that a commercially available Qiagen cfDNA prep kit yields about 8,000-80,000 genome equivalents per 5 ml plasma).)

145. This reasonable expectation of success is not contradicted by Kinde 2013’s disclosure of “comfortably detect[ing] mutations existing in >0.1% of template molecules” for Kinde 2013’s multiplex PCR-based screening method. (Ex.1007, 5.) For starters, Kinde 2013 discloses that this >0.1% threshold for detection is in the context of “2500 templates” and for an “agnostic” screening

assay encompassing 10,257 bp. (*Id.*; *see also id.*, 19.) In this context, because “mutations are not known a priori in a screening environment,” Kinde 2013 applied a “more agnostic metric to detect mutations.” (*Id.*, 19.) This mutation-agnostic metric required “supermutants” to be identified for “at least two template molecules” to be designated as mutations, and to meet other threshold requirements. (*Id.*) A POSA reading Kinde 2013 would understand that no such “agnostic” method is required when the mutations to be detected are known *a priori*. (*Id.*) Therefore, a POSA would expect Kinde 2013’s general teachings regarding the mutation-detection capabilities of improved Safe-SeqS to apply in a tumor-informed method, wherein the tumor- and patient-specific mutations are known before detecting the SNV in the cfDNA as taught by Diaz.

b. Claim 2

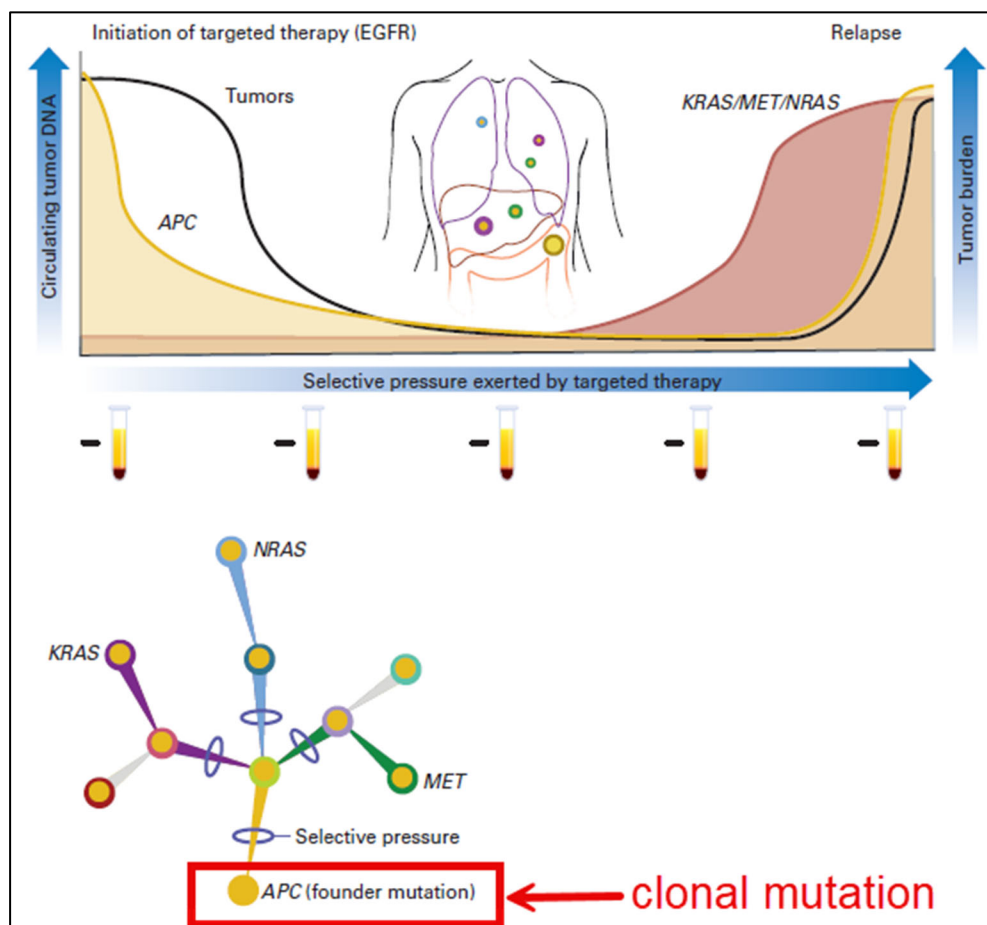
The method of claim 1, wherein the cell-free DNA comprises circulating tumor DNA.

146. Claim 2 is obvious over Diaz and Kinde 2013. Diaz discloses that cell-free DNA comprises “[c]irculating tumor DNA,” also referred to as “ctDNA.” (Ex.1006, 3, 4.) And, again, Diaz explains that “[c]irculating tumor DNA fragments contain genetic defects identical to those of the tumors themselves” including “point mutations,” which a POSA would understand are SNVs. (*Id.*).

c. Claim 5

The method of claim 1, wherein the tumor-specific mutations comprise one or more clonal SNV mutations.

147. Claim 5 is also obvious over Diaz and Kinde 2013. Diaz teaches using DNA from a primary tumor to identify a “founder mutation,” which a POSA would understand is a clonal mutation.



(Ex.1006, 5 (Fig. 3) (cropped and annotated)).

148. The '596 patent describes clonal SNV mutations as mutations that had “VAF allele calls in all biopsied samples assayed and in plasma” while

“subclonal populations had VAF allele calls in at least one biopsy sample, but not all biopsy samples.” (Ex.1001, 168:49–52.) A POSA would understand that the founder *APC* mutation taught by Diaz is a clonal mutation. As the founder mutation at the base of the evolutionary tree shown in Diaz’s Fig. 3, the founder mutation would be present in the other tumor regions. Diaz discloses that “[a]t the time of presentation, DNA from the primary tumor is used to identify the baseline mutation profile,” which includes the clonal or “founder” mutation. (Ex.1006, 5.) As shown in Fig. 3, the clonal or founder mutation (*APC*) is also detected in the plasma before initiation of targeted therapy. (*Id.*, 5 (Fig. 3).) Diaz also teaches assaying the clonal mutation in the plasma at multiple time points. (*Id.*)

d. Claim 10

The method of claim 1, wherein the method further comprises designing targeted PCR assays for the tumor-specific mutations identified in the cancer sample.

149. Claim 10 is obvious over Diaz and Kinde 2013. As discussed above, Kinde 2013 discloses identifying tumor-specific mutations in the cancer sample. (See Ex.1007, 16 (describing evaluating the results of the sequencing by aligning reads to a reference genome, performing variant calling with the ELANDv2 algorithm in the CASAVA 1.6 software (Illumina), and removing known polymorphisms); *id.*, 3 (explaining that 12,795 somatic mutations were identified in the 22 sequenced cancers).) Kinde 2013 also discloses designing

primers for targeted PCR assays to detect the tumor-specific mutations identified in the sequenced cancers. (Ex.1007, 17.) Kinde 2013 teaches that the primers were designed using Primer3 software (*id.*, 17), one of the same primer design methods disclosed in the '596 patent (Ex.1001, 106:35–39). Kinde 2013 also discloses designing Safe-SeqS primers and an improved Safe-SeqS method for multiplex amplification in a single reaction volume. (Ex.1007, 5–8.) Therefore, a POSA would have found it obvious to design targeted PCR assays for the tumor-specific mutations identified in the cancer sample in view of Diaz and Kinde 2013.

e. Claim 12

The method of claim 1, wherein the method further comprises detecting recurrence and/or metastases of the cancer from the tumor-specific mutations detected in the cell-free DNA.

150. Based on the combined teachings of Diaz and Kinde 2013, it would have been obvious to perform the claimed method wherein the method further comprises detecting recurrence and/or metastases of the cancer from the tumor-specific mutations detected in the cell-free DNA. Diaz discloses detecting recurrence and/or metastases of the cancer from the tumor-specific mutations detected in the cell-free DNA. (*See, e.g.*, Ex.1006, 4, 2 (Table 1), 5 (Fig. 3).) Diaz teaches in an example that “[a]ll patients with detectable postoperative levels of ctDNA experienced recurrence, whereas all patients with undetectable postoperative levels of ctDNA remained disease free.” (*Id.*, 4.) Diaz further

teaches that studies “evaluating postoperative levels of ctDNA could offer personalized markers based on the unique mutational profiles of resected tumors and thus having exquisitely high specificity” which will “rely on the ability to detect low levels of ctDNA released from *micrometastatic deposits* not detectable by imaging or other diagnostic modalities.” (*Id.*, 4 (emphasis added); *see also id.*, 5 (Fig. 3) (teaching a method comprising monitoring for metastatic mutations in plasma DNA).) Furthermore, a POSA would understand that applications of the cfDNA assay such as minimal residual disease monitoring described by Diaz include detecting recurrence.

f. Claims 13, 14, and 16

Claim 13: *The method of claim 1, wherein the cancer is colorectal cancer.*

Claim 14: *The method of claim 1, wherein the cancer is lung cancer.*

Claim 16: *The method of claim 1, wherein the cancer is breast cancer.*

151. These claims are obvious over Diaz and Kinde 2013. Diaz discloses that detection of tumor-specific mutations in cell-free DNA has been demonstrated for multiple types of cancer, including colorectal cancer (Ex.1006, 4; *see also id.*, 5), lung cancer (*id.*, 5), and breast cancer (*id.*, 4, 5). Diaz teaches that “[s]tudies in melanoma, ovarian, breast, and colon cancers have solidified the potential utility of [monitoring mutations in cfDNA over time] to more precisely define tumor

dynamics during therapy for patients with advanced disease.” (*Id.*, 4.) Similarly, Diaz teaches that “genetic markers of resistance can be noninvasively tracked throughout the course of treatment in breast, ovarian, and lung cancers.” (*Id.*, 5.)

152. As another example, Diaz discloses that “[a]ll patients with detectable postoperative levels of ctDNA experienced recurrence, whereas all patients with undetectable postoperative levels of ctDNA remained disease free. Similar studies have been performed blindly, examining KRAS mutations in patients with resected colorectal cancer, and shown a strong correlation between postoperative detection of mutant DNA in circulation and recurrence.” (*Id.*, 4.)

153. As another example, Diaz discloses that “[in] a comprehensive study by Murtaza et al, it was shown that genetic markers of resistance can be noninvasively tracked throughout the course of treatment in breast, ovarian, and lung cancers.” (*Id.*, 5.)

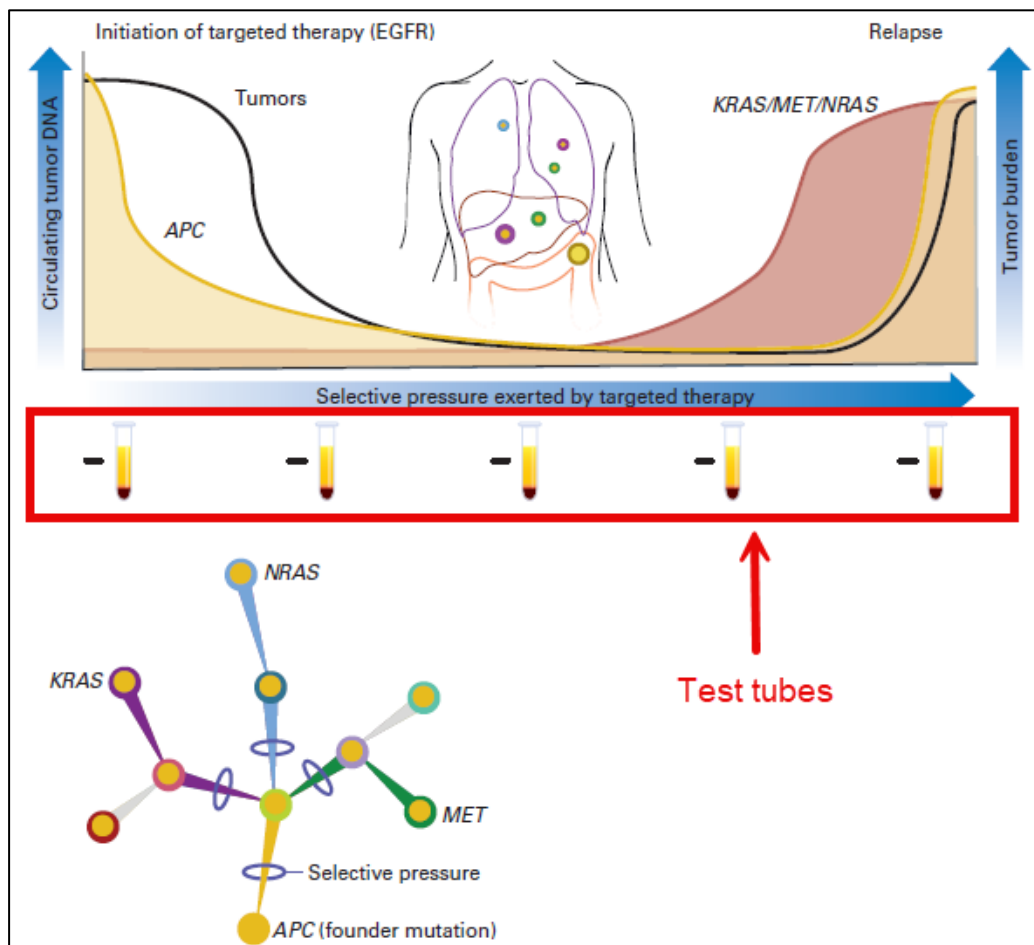
g. Claim 17

The method of claim 1, wherein the biological sample is a blood, serum, plasma, or urine sample.

154. Claim 17 is also obvious in view of Diaz and Kinde 2013. Diaz discloses assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points, wherein the biological sample is a plasma sample. Referring to Fig. 3, for example, Diaz discloses “[c]ontinuous

monitoring of plasma DNA” to detect mutations in tumor DNA. (Ex.1006, 5.)

Diaz further explains that in Fig. 3 “[t]est tubes represent samples of plasma from which circulating free DNA is extracted and used to monitor the presence of cancer-specific aberrations.” (*Id.*)



(Ex.1006, Fig. 3 (cropped and annotated).)

h. Claim 18

The method of claim 1, wherein step (a) comprises performing whole exome sequencing on a tumor biopsy sample of the subject to identify the plurality of tumor-specific mutations.

155. Claim 18 is also obvious in view of Diaz and Kinde 2013. Kinde 2013 teaches whole exome sequencing of a tumor biopsy to identify mutations that are then tracked in liquid biopsies. (Ex.1007, 3 (“somatic mutations in [] tumors were identified through whole exome sequencing (table S2) . . . In each of the 46 cases, we identified at least one somatic mutation (table S3) that was confirmed by an independent assay”), *id.* (disclosing that the somatic mutations identified by WES in table S2 include 12,795 somatic mutations identified in the 22 sequenced ovarian cancers), 5 (describing identifying the tumor-specific mutations in Pap specimens from the corresponding patients).)

156. A POSA would have been motivated to use whole exome sequencing as taught by Kinde 2013 to identify a plurality of tumor-specific mutations in order to “target and quantify the level of ctDNA at a particular time point” as taught by Diaz. (Ex.1006, 4.) Diaz teaches the advantages of identifying a “personal and unique molecular signature” based on tumor-specific mutations, which allows for “exquisitely high specificity” to evaluate postoperative levels of ctDNA. (*Id.*) A POSA would have been motivated to use whole exome sequencing to identify the tumor specific mutations because Kinde 2013 teaches

that whole-exome sequencing allows analysis of tumor-specific mutations at “a genome-wide level.” (Ex.1007, 2.)

157. A POSA would also reasonably expect to succeed because Kinde 2013 successfully identified somatic mutations in 22 endometrial cancer tumors “through whole exome sequencing (table S2).” (*Id.*, 3; *see also id.*, 21–308 (table S2).) As shown in table S2, Kinde 2013 identified a plurality of tumor-specific mutations for each of the endometrial cancer tumors, including one or more single nucleotide variant (SNV) mutations for each tumor. (*Id.*, 21–308 (table S2).)

158. By the critical date, performing whole exome sequencing to identify a plurality of tumor-specific mutations comprising one or more SNVs was within a POSA’s skill. According to Kinde 2013, “[p]reparation of Illumina genomic DNA libraries and selection for exomic DNA were performed according to the manufacturer’s recommendations.” (*Id.*, 8.) Kinde 2013 further explains that “[e]xomic capture was performed with the SureSelect Human Exome Kit V 4.0 (Agilent).” (*Id.*) As illustrated by the availability of whole exome sequencing kits and manufacturer recommendations, whole exome sequencing was within the skill and knowledge of a POSA.

159. Additionally, a POSA would have been motivated to use whole exome sequencing to detect the tumor-specific mutations and would reasonably

expect to succeed because tumor-specific mutations often occur within coding regions for genes, which are located in exons. For example, Diaz teaches identifying tumor-specific mutations in genes such as *KRAS*, *MET*, *NRAS*, *APC*, *EGFR*, *PIK3CA*, and *BRAF*. (Ex.1006, 5, Fig. 3.) Diaz teaches that “[c]hanges in the DNA sequence (eg, somatic point mutations or deletions) are the most frequent class of variants associated with the development of solid tumors and span common oncogenic events, such as *KRAS*, *PIK3CA*, *EGFR*, and *BRAF* mutations.” (*Id.*) A POSA would understand that such oncogenic mutations are often within exons. For example, Kinde 2013 detected tumor-specific mutations in *KRAS* (*see, e.g.*, Ex.1007, 236), *PIK3CA* (*see, e.g., id.*, 274–75) and *EGFR* (*see, e.g., id.*, 228) using whole-exome sequencing. (*Id.*, 21–308 (table S2).) These mutations included SNVs, such as a 35G>C mutation in *KRAS* (PAP50) (*id.*, 236), 3062A>G (*id.*, 275) and 263G>A (*id.*, 275) mutations in *PIK3CA* (PAP54), and a 1978G>A mutation in *EGFR* (PAP48) (*id.*, 228), to name just a few examples among the 12,795 somatic mutations identified in the 22 ovarian cancers by whole-exome sequencing. (*Id.*, 21–308 (table S2), 3.) Therefore, a POSA would reasonably expect to identify such mutations using whole exome sequencing.

B. GROUND 2: DIAZ, KINDE 2013, & RABINOWITZ

160. In my opinion, the combination of Diaz, Kinde, and Rabinowitz renders obvious claims 10 and 11 of the '596 patent. Summaries of Rabinowitz, the combination, and the bases for my opinions are set forth below.

1. Summary Of Rabinowitz

161. Rabinowitz is a U.S. Patent Application publication originally assigned to Gene Security Network Inc., which later changed its name to Natera, Inc. (Ex.1009, 1.) Rabinowitz shares several named inventors with the '596 patent, and is in the same field as the '596 patent because it relates to detecting mutations in cell-free DNA, including single base changes and ploidy mutations. (Ex.1009, [0283], [0249].) Rabinowitz was published on October 25, 2012, more than a year before the earliest provisional application to which the '596 patent purports to claim priority.

162. In Rabinowitz, the inventors of the '596 patent admit that “the prior art includes disclosures of successful multiplexed PCR reactions involving pools of up to about 50 or 100 primers” (Ex.1009, [0217].) Rabinowitz states that it provides methods that include “highly efficient highly multiplexed targeted PCR to amplify DNA followed by high throughput sequencing to determine the allele frequencies at each target locus.” (*Id.*, [0064].) According to Rabinowitz, the provided methods include “the ability to multiplex more than about 50 or 100 PCR

primers in one reaction in a way that most of the resulting sequence reads map to targeted loci.” (*Id.*)

163. Rabinowitz also teaches generally that the multiplex assays can be applied to isolated cell-free DNA from maternal plasma or cancer patient plasma. For example, Rabinowitz teaches that “[a]ssays may be combined in a single reaction with the entirety of a cfDNA sample isolated from maternal plasma, a fraction thereof, or a further processed derivative of the cfDNA sample.” (*Id.*, [0267].) Rabinowitz further explains that the same assays can be applied to determine the genetic state of a cancer from measurements on host blood. (*Id.*, [0209] (“Any claim in the instant disclosure that reads on determining the ploidy state or genetic state of a fetus from the measurements made on maternal blood can equally well read on determining the ploidy state or genetic state of a cancer from the measurements on host blood.”).) Rabinowitz further discloses that “[in] some embodiments, the target DNA may originate from . . . cancer patient plasma.” (*Id.*, [0284].)

164. Rabinowitz also demonstrates that the multiplex PCR methods described in the ’596 patent were already within the prior art. For example, *compare* Ex.1001 at 97:32–98:17 (incorporating Rabinowitz by reference for description of mini-PCR and its combination with multiplexed PCR) *with* Ex.1009,

[0302]–[0322] (describing methods of multiplexed mini-PCR); *compare* Ex.1001, 105:50–110:21 (describing primer design methods that are characterized as “essential” for high levels of multiplexing) *with* Ex.1009, [0291]–[0298] (same). The ’596 patent discloses and incorporates by reference these prior art methods for multiplex PCR, as well as other prior art primer design methods such as Primer3. (Ex.1001, 106:35–39.)

2. Combination Of Diaz, Kinde, And Rabinowitz

165. A POSA would have been motivated to combine Diaz, Kinde, and Rabinowitz to design targeted PCR assays and to perform targeted multiplex amplification of 10 to 50 of the target loci spanning tumor-specific mutations.

166. A POSA would have been motivated to combine Diaz and Rabinowitz because both references relate to methods for analyzing plasma DNA using PCR to amplify multiple targets followed by next-generation sequencing. Diaz teaches that with PCR and NGS-based methods, “multiple genes of interest can be investigated in one sample.” (Ex.1006, 2.) Rabinowitz discloses multiplex PCR methods for analyzing multiple target loci in the same reaction, which can be used together with NGS. (*See, e.g.*, Ex.1009, [0287] (“mini-PCR methods described herein may be used along with next-generation sequencing”), [0229] (“once a mixture has been preferentially enriched at the set of target loci, it may be sequenced using any one of the previous, current, or next generation of sequencing

instruments that sequences a clonal sample (a sample generated from a single molecule; examples include ILLUMINA GAIIx, ILLUMINA HISEQ, LIFE TECHNOLOGIES SOLiD, 5500XL))), [0064] (“[in] an embodiment, a method disclosed herein uses highly efficient highly multiplexed targeted PCR to amplify DNA followed by high throughput sequencing to determine the allele frequencies at each target locus”).)

167. A POSA also would have been motivated to combine Diaz, Kinde 2013, and Rabinowitz because Rabinowitz describes its “highly multiplexed targeted PCR” as “highly efficient.” (*Id.*, [0064].) A POSA would have been motivated to perform multiplex amplification of 10 to 50 of the target loci in order to more accurately detect circulating tumor DNA by analyzing more tumor-specific mutations without requiring additional reactions. According to Rabinowitz, “[g]reater multiplexing allows more alleles to be targeted, giving more accurate results.” (*Id.*, [0239].)

168. A POSA would have been motivated to combine Diaz, Kinde 2013, and Rabinowitz because all three references discuss advantages of PCR-based molecular barcoding methods. Diaz specifically suggests barcoding strategies to increase sensitivity in the context of NGS, citing an earlier Kinde 2011 version of the Safe-SeqS method. (Ex.1006, 3.) Kinde 2013, in turn, discloses that its

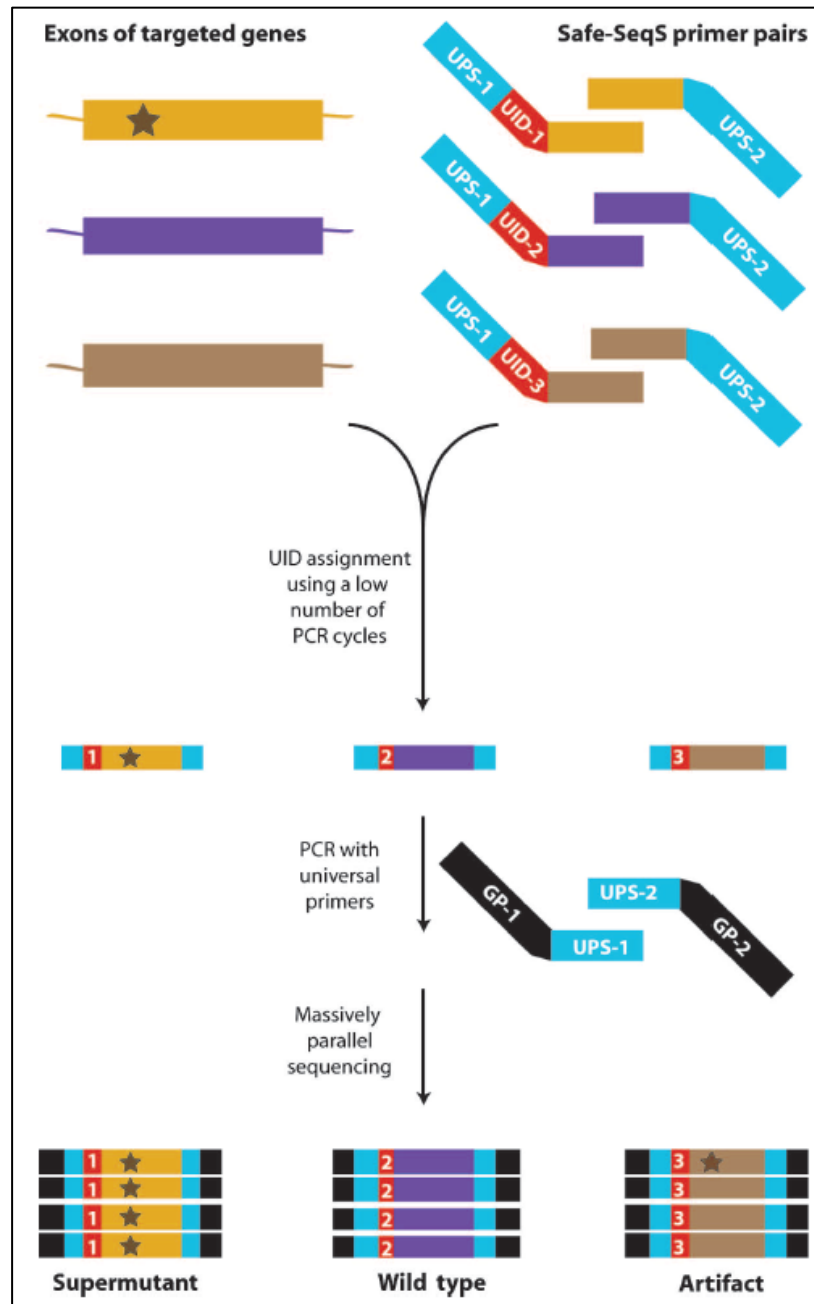
improved Safe-SeqS approach is capable of enhanced mutation detection for mutant templates present at levels even below 0.01%. (*See, e.g.*, Ex.1007, 4.) Rabinowitz discloses that “tails with no homology to the target genome may also be added to the 3-prime or 5-prime end of any of the primers” and that “[t]hese tails facilitate subsequent manipulations, procedures, or measurements . . . In some embodiments, the primers may contain a molecular barcode.” (Ex.1009, [0277].)

169. A POSA would also reasonably expect the combination to succeed because Rabinowitz discloses that “[the] targeted PCR may be massively multiplexed” and “[the] selective [] amplification may involve tagging each individual molecule with different tags, molecular barcodes, tags for amplification, and/or tags for sequencing.” (*Id.*, [0201].) A POSA would understand that Kinde 2013’s Safe-SeqS barcode sequences are a type of molecular barcode. Because Rabinowitz discloses that the amplification can involve adding molecular barcodes, a POSA would expect that Kinde 2013’s Safe-SeqS barcode sequences could be included in primers designed as described in Rabinowitz. (*Id.*, [0277], [0020] (“In some embodiments, at least some of the primers additionally contain a random region that differs for each individual primer molecule. In some embodiments, at least some of the primers additionally contain a molecular barcode.”); *see also id.*, [0120].)

170. Additionally, a POSA would reasonably expect the combination to succeed because Rabinowitz discloses that primers comprising molecular barcodes can be used to identify “tagged molecules” that originate from the same “original” DNA molecule. (*Id.*, [0488].) Specifically, Rabinowitz discloses a molecular barcoding approach that “entails targeting one or more specific loci and generating a tagged copy of the original molecules such manner [*sic*] that most or all of the tagged molecules from each targeted locus will have a unique tag and can be distinguished from one another upon sequencing of this barcode using clonal or single molecule sequencing.” (*Id.*)

171. A POSA would understand that Rabinowitz’s molecular barcoding approach is substantially similar to Kinde 2013’s improved Safe-SeqS approach, as shown in Fig. 2 (reproduced below). (Ex.1007, 5 (Fig. 2).) In Kinde 2013’s method, UID-tagged primers also generate “tagged [copies] of the original molecules” such that “most or all of the tagged molecules” will have a “unique tag” and can be “distinguished from one another upon sequencing.” Like Kinde 2013, Rabinowitz discloses that “[e]ach unique sequenced barcode represents a unique molecule in the original sample.” (Ex.1009, [0488].) According to Rabinowitz, “sequencing data is used to ascertain the locus from which the molecule originates.” (*Id.*) A POSA would therefore consider Rabinowitz as

further support for the combination of Diaz and Kinde 2013, because Rabinowitz discusses the advantages of molecular barcoding in the context of cfDNA assays.



(Ex.1007, Fig. 2.)

172. A POSA would also expect to succeed because Rabinowitz teaches that “the prior art includes disclosures of successful multiplexed PCR reactions involving pools of up to about 50 or 100 primers.” (Ex.1009, [0217].) Rabinowitz discloses that multiplexing difficulties arise when multiplexing more than about one hundred assays. (*See, e.g., id.*, [0257] (“the interactions between different PCR assays makes it difficult to multiplex them beyond about one hundred assays”), [0291] (“the general belief in the art is that multiplexing PCR for sequencing is limited to about 100 assays in the same well”), [0267] (“traditional multiplex PCRs evaluate up to fifty loci simultaneously”).) According to Rabinowitz, the approaches disclosed also “enable simultaneous evaluation of more than 50 loci simultaneously.” (*Id.*, [0267] (“Experiments have shown that up to, including and more than 10,000 distinct loci can be evaluated simultaneously, in a single reaction, with sufficiently good efficiency and specificity to make non-invasive prenatal aneuploidy diagnoses and/or copy number calls with high accuracy.”).)

173. A POSA would also have a reasonable expectation of success for performing high-throughput sequencing of the amplified DNA because Rabinowitz discloses that its multiplex PCR methods result in a high percentage of sequencing reads that map to targeted loci (e.g., greater than 99% DNA molecules that map to

targeted loci). (*Id.*, [0064]; *see also id.*, [0515] (for multiplex amplification from a DNA library prepared from plasma DNA using 800 different primer pairs, “1.9 million reads” were obtained from an Illumina HIGAX sequencer, “of which 92% mapped to the genome; of those reads that mapped to the genome, more than 99% mapped to one of the regions targeted by the targeted primers.”).)

3. Claims 1, 2, 5, 10–14, And 16–18 Are Obvious Over Diaz, Kinde 2013, And Rabinowitz

a. Claim 1

Preamble: *A method for preparing biological samples useful for monitoring the progression of cancer in a subject, the method comprising:*

174. If limiting, the combined teachings of Diaz, Kinde 2013, and Rabinowitz disclose methods for preparing biological samples useful for monitoring the progression of cancer in a subject. As discussed in paragraphs 121–123 of my Declaration, Diaz discloses methods for preparing biological samples useful for monitoring the progression of cancer in a subject.

Limitation 1[A]: *(a) performing sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor-specific mutations, wherein the tumor-specific mutations comprise one or more single nucleotide variant (SNV) mutations;*

175. As discussed in paragraphs 124–127 of my Declaration, this limitation is obvious in view of Diaz and Kinde 2013.

Limitation 1[B]: (b) evaluating results of the sequencing on the tumor biopsy sample to determine a plurality of target loci specific to the subject, wherein each target locus spans a tumor-specific mutation of the identified plurality of tumor-specific mutations; and

176. As discussed in paragraphs 128–129 of my Declaration, this limitation is obvious in view of Diaz and Kinde 2013.

Limitation 1[C][i]: (c) assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points, wherein the assaying comprises:

177. As discussed in paragraphs 130–131 of my Declaration, Diaz teaches this limitation.

Limitation 1[C][ii]: performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject; and

178. Based on the combined teachings of Diaz, Kinde 2013, and Rabinowitz, it would have been obvious to perform targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject.

179. As discussed in paragraphs 132–135 of my Declaration, this limitation is obvious in view of Diaz and Kinde 2013. Diaz discloses that for ctDNA assays, “PCR-based [] approaches” have been updated to use “next-generation sequencing (NGS) to identify rare mutant variants in complex mixtures

of DNA” wherein “multiple genes of interest can be investigated in one sample.” (Ex.1007, 2.) A POSA reading Diaz would have been motivated to perform multiplex PCR followed by NGS to detect tumor-specific mutations as taught by Diaz for the same reasons discussed in paragraph 132 of my Declaration. Kinde 2013 also discloses Safe-SeqS primers and an improved Safe-SeqS method for multiplex amplification in a single reaction volume.

180. To the extent Natera asserts that Diaz and Kinde 2013 alone do not teach performing multiplex PCR with primers having Safe-SeqS barcodes, this would have been obvious in view of Rabinowitz. A POSA reading Diaz would naturally turn to Rabinowitz to design targeted multiplex PCR assays to assay cfDNA for target loci spanning tumor-specific mutations such as SNVs. Rabinowitz discloses designing primers for multiplexed PCR of target loci followed by NGS, with a particular focus on cfDNA assays. (*See* paragraphs 162–164 of my Declaration.)

181. Rabinowitz generally states that the same assays can be applied to determine the genetic state of a cancer from measurements on host blood. (Ex.1009, [0209] (“Any claim in the instant disclosure that reads on determining the ploidy state or genetic state of a fetus from the measurements made on maternal blood can equally well read on determining the ploidy state or genetic

state of a cancer from the measurements on host blood.”.) Rabinowitz further discloses that “[in] some embodiments, the target DNA may originate from . . . cancer patient plasma.” (*Id.*, [0284].)

182. Rabinowitz discloses that its methods are useful for a wide variety of applications “where there is a target set of DNA that is mixed with an amount of contaminating DNA,” such as wherein the “target and contaminating DNA may be from the same individual, but where the target and contaminating DNA are different by one or more mutations, for example in the case of cancer.” (Ex.1009, [0283].) Rabinowitz further explains that the target DNA may be from “cancer patient plasma” which comprises a mix between “healthy and cancer DNA.” (*Id.*, [0284].)

183. Rabinowitz also demonstrates that the multiplex PCR methods described in the ’596 patent were already within the prior art. For example, *compare* Ex.1001 at 97:32–98:17 (incorporating Rabinowitz by reference for description of mini-PCR and its combination with multiplexed PCR) *with* Ex.1009, [0302]–[0322] (describing methods of multiplexed mini-PCR); *compare* Ex.1001, 105:50–110:21 (describing primer design methods that are characterized as “essential” for high levels of multiplexing) *with* Ex.1009, [0291]–[0298] (same). The ’596 patent discloses and incorporates by reference these prior art methods for

multiplex PCR, as well as other prior art primer design methods such as Primer3.
(Ex.1001, 106:35–39.)

Limitation 1[C][iii]: *performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads,*

184. Based on the combined teachings of Diaz, Kinde 2013, and Rabinowitz, it would have been obvious to perform high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads. As discussed in paragraphs 136–138 of my Declaration, this limitation is obvious in view of Diaz and Kinde 2013.

185. Rabinowitz also teaches performing high-throughput sequencing of amplified DNA from targeted multiplex PCR of isolated cfDNA. (Ex.1009, [0064] (stating that the provided methods include “highly efficient highly multiplexed targeted PCR to amplify DNA followed by high throughput sequencing to determine the allele frequencies at each target locus”); *id.* (stating that the provided methods include “the ability to multiplex more than about 50 or 100 PCR primers in one reaction in a way that most of the resulting sequence reads map to targeted loci”).

186. A POSA would also have a reasonable expectation of success because by 2014, high throughput sequencing of amplified DNA from multiplex

amplification of isolated cell-free DNA was within the knowledge of a POSA.

(See paragraph 80 of my Declaration).

Limitation 1[C][iv]: wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.

187. Based on the combined teachings of Diaz, Kinde 2013, and Rabinowitz, it would have been obvious to perform the claimed method wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads. As discussed in paragraphs 139–140 of my Declaration, this limitation is obvious in view of Diaz and Kinde 2013. A POSA would also expect to succeed because Rabinowitz discloses that molecular barcodes and/or random sequences can be incorporated into any of Rabinowitz’s primer designs for multiplex PCR from isolated cfDNA, as discussed in paragraphs 169–171 of my Declaration.

b. Claim 2

The method of claim 1, wherein the cell-free DNA comprises circulating tumor DNA.

188. As discussed in paragraph 146 of my Declaration, Diaz discloses this limitation.

c. Claim 5

The method of claim 1, wherein the tumor-specific mutations comprise one or more clonal SNV mutations.

189. As discussed in paragraphs 147–148 of my Declaration, Diaz discloses this limitation.

d. Claim 10

The method of claim 1, wherein the method further comprises designing targeted PCR assays for the tumor-specific mutations identified in the cancer sample.

190. Claim 10 is obvious over Diaz, Kinde 2013, and Rabinowitz. As discussed in paragraph 149 of my Declaration, Kinde 2013 discloses designing targeted PCR assays for the tumor-specific mutations identified in a cancer sample. Rabinowitz discloses methods for designing primers for multiplex amplification of target loci in isolated cfDNA, and teaches that any of the primers can comprise molecular barcodes, as discussed in paragraphs 162–164 of my Declaration. Therefore, a POSA would reasonably expect to succeed in using Safe-SeqS molecular barcodes in targeted PCR assays designed for the tumor-specific mutations identified in the cancer sample in view of the combined teachings of Diaz, Kinde 2013, and Rabinowitz.

191. A POSA would also reasonably expect to be able to remove primer dimers with the design constraints of “mini-PCR” for short DNA fragments

because Rabinowitz discloses that its method of primer design “may be used in combination with the mini-PCR method described elsewhere in this document” and that “the primer design method may be used as part of a massive multiplexed PCR method.” (Ex.1009, [0295].)

e. Claim 11

The method of claim 1, wherein amplifying comprises targeted multiplex PCR amplification of 10 to 50 of the target loci.

192. Claim 11 is also obvious over Diaz, Kinde 2013, and Rabinowitz. Claim 11 of the '596 patent only requires multiplex amplification of 10 to 50 target loci. This was within the skill of a POSA according to Rabinowitz. As discussed in paragraph 172 of my Declaration, Rabinowitz teaches that “the prior art includes disclosures of successful multiplexed PCR reactions involving pools of up to about 50 or 100 primers.” (*See, e.g.*, Ex.1009, [0217].) And as discussed in paragraphs 162–164 of my Declaration, Rabinowitz discloses primer design methods for much higher levels of multiplexing, including for cfDNA. And by 2014, a POSA would also have been aware that multiplex PCR reactions of more than 10 target loci in a reaction followed by NGS had successfully been applied to detect circulating tumor DNA. (Ex.1039, 1–3.)

193. Rabinowitz discloses techniques that “allow[] highly multiplexed targeted PCR to perform in a highly efficient manner” which “involve[] designing

primers that are unlikely to hybridize with one another.” (Ex.1009, [0064].)

Rabinowitz further discloses that this can be accomplished by “creating a thermodynamic model of potentially adverse reactions.” (*Id.*) According to Rabinowitz, this primer design approach allows multiplexing of “more than about 50 or 100 PCR primers in one reaction in a way that most of the resulting sequence reads map to targeted loci.” (*Id.*)

194. A POSA would have reasonably expected to succeed with targeted multiplex PCR amplification of 10 to 50 of the target loci based on the combined teachings of Diaz, Kinde, and Rabinowitz. As shown in table S5, Kinde 2013 disclosed multiplex reactions of up to nine different target loci in a single reaction using Safe-SeqS primers. (Ex.1007, 312 (table S5).) Even if Safe-SeqS barcodes were incorporated into 10 or more pairs of primers, a POSA would reasonably expect to succeed because the modification would only require one additional primer pair. A POSA would also expect to succeed because Rabinowitz discloses that molecular barcodes and/or random sequences can be incorporated into Rabinowitz’s primer designs, as discussed in paragraphs 168–171 of my Declaration.

f. Claim 12

The method of claim 1, wherein the method further comprises detecting recurrence and/or metastases of the cancer from the tumor-specific mutations detected in the cell-free DNA.

195. As discussed in paragraph 150 of my Declaration, Diaz teaches this limitation.

g. Claims 13, 14, and 16

Claim 13: ***The method of claim 1, wherein the cancer is colorectal cancer.***

Claim 14: ***The method of claim 1, wherein the cancer is lung cancer.***

Claim 16: ***The method of claim 1, wherein the cancer is breast cancer.***

196. As discussed in paragraphs 151–153 of my Declaration, Diaz teaches the limitations of claims 13–16.

h. Claim 17

The method of claim 1, wherein the biological sample is a blood, serum, plasma, or urine sample.

197. As discussed in paragraph 154 of my Declaration, Diaz discloses this limitation.

i. Claim 18

The method of claim 1, wherein step (a) comprises performing whole exome sequencing on a tumor biopsy sample of the subject to identify the plurality of tumor-specific mutations.

198. As discussed in paragraphs 155–159 of my Declaration, Kinde 2013 discloses this limitation.

C. GROUND 3: DIAZ, KINDE, & FLEISCHHACKER

199. In my opinion, the combination of Diaz, Kinde 2013, and Fleischhacker renders obvious claim 15 of the '596 patent. Summaries of Fleischhacker, the combination, and the bases for my opinions are set forth below.

1. Summary Of Fleischhacker

200. Fleischhacker is a review article that describes detection of tumor-associated mutations in circulating nucleic acids (CNAs) of tumor origin in a wide variety of cancers. Fleischhacker is in the same field as the '596 patent because it relates to detecting mutations including SNVs. Fleischhacker teaches that “[it] has been known for decades that it is possible to detect small amounts of extracellular nucleic acids in plasma and serum of healthy and diseased human beings.”

(Ex.1010, 1.) According to Fleischhacker, these CNAs are “extracellular nucleic acids found in cell-free plasma/serum of humans.” (*Id.*) Fleischhacker also confirms that by 2007 (when the review article was published), there was “unequivocal proof that part of these circulating nucleic acids (CNAs) is of tumor

origin” and “many experiments showed that tumor-associated alterations can be detected at the DNA and RNA level.” (*Id.*)

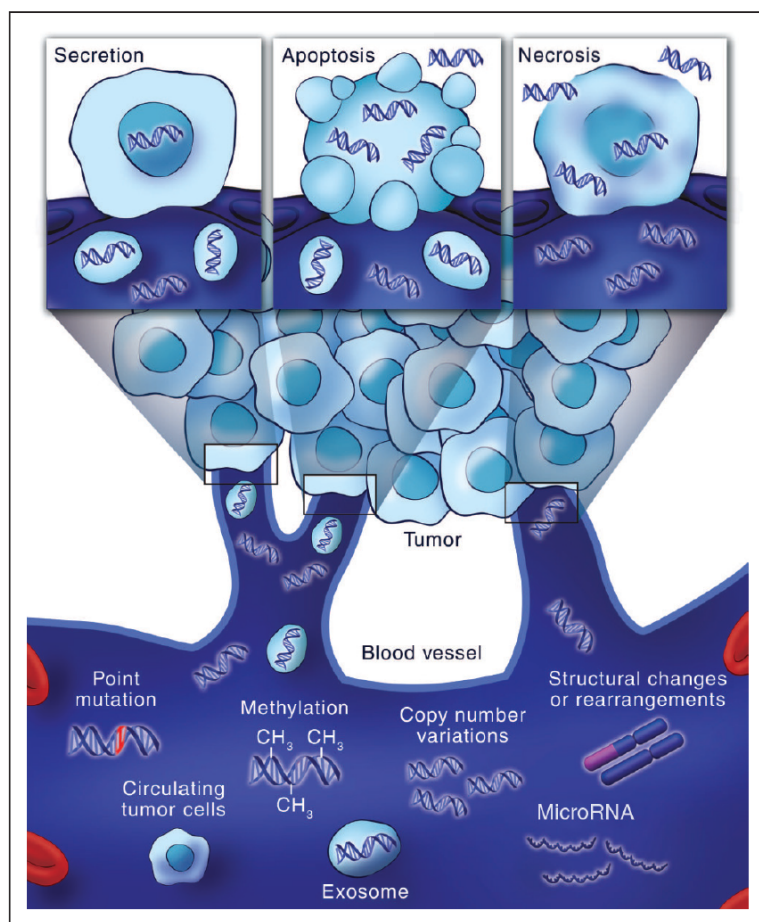
201. At the circulating DNA level, Fleischhacker discloses that “the detection of point mutations, microsatellite alterations, [and] chromosomal alterations, *i.e.* inversion and deletion, were demonstrated.” (*Id.*) Fleischhacker further teaches that both point mutations and microsatellite alterations had been detected in plasma or urine of bladder cancer patients. (*See, e.g.*, Ex.1010, 9–13 (Table 2) (disclosing point mutations detectable in different bodily fluids of cancer patients, including for bladder cancer), 15–21 (Table 3) (disclosing detection of microsatellite alterations in cell-free DNA from plasma/serum and urine of cancer patients, including for bladder cancer); 14 (“While in the first papers patients with small cell lung cancer (SCLC) and head and neck tumor were analyzed, other groups made similar observations in patients with a tumor of the . . . bladder.”).)

2. Combination Of Diaz, Kinde 2013, And Fleischhacker

202. A POSA would have found it obvious to combine Diaz, Kinde 2013, and Fleischhacker. For starters, Diaz, Kinde 2013, and Fleischhacker are in the same field as the ’596 patent. All three references relate to detection of tumor-specific single nucleotide variants. (*See* Ex.1001, 1:28–31 (stating that the invention of the ’596 patent “generally relates to methods and systems for

detecting ploidy of a chromosome segment, and methods and systems for detecting a single nucleotide variant”).)

203. A POSA reading Diaz would reasonably look to articles such as Fleischhacker for examples of types of cancers and their associated tumor mutations that have been detected in cell-free DNA. Both references disclose detection of the same types of mutations in cell-free DNA. For example, Diaz teaches that mutations released from tumors into the bloodstream include point mutations (SNVs), copy number variations, and structural changes or rearrangements. (*See, e.g.*, Ex.1006, 2 (Fig. 2, reproduced below).) Fleischhacker similarly discloses that “extracellular DNA changes such as point mutations, microsatellite alterations, single nucleotide polymorphisms (SNPs), hypermethylated sequences, [and] rearranged genomic sequences” have been detected. (Ex.1010, 8.)



(Ex.1006, 2 (Fig. 2).)

3. Claim 15 Is Obvious Over Diaz, Kinde 2013, & Fleischhacker

Claim 15: *The method of claim 1, wherein the cancer is bladder cancer.*

204. In view of the combined teachings of Diaz, Kinde 2013, and Fleischhacker, it would have been obvious to perform the method of claim 1 wherein the cancer is bladder cancer.

205. As discussed in paragraphs 121–145 of my Declaration, the method of claim 1 is obvious over Diaz in view of Kinde 2013. Fleischhacker teaches

detection of tumor-specific SNVs in cell-free DNA present in plasma or in urine for bladder cancer, including by methods involving PCR and sequencing. (*See, e.g.,* Ex.1010, 12 (Table 2 titled “Point mutations detectable in different body fluids of cancer patients”).) For example, Fleischhacker teaches that in a study of bladder cancer patients, a K-ras point mutation was detected in a subset of patients. (*Id.*, (in Table 2 titled “Point mutations detectable in different body fluids of cancer patients,” disclosing detection of K-ras mutations in both tumor and “Plasma/serum or other body fluids”) (citing Dominguez et al. 2002, which is submitted herewith as Ex.1061); *see also* Ex.1061, Fig. 1D, 4 (teaching that for bladder cancer “[in] 2 cases, the same K-*ras* mutations were identified in both tumor tissue and plasma (Fig. 1D)”), 3 (explaining that the mutational study of the K-*ras* gene in bladder cancer plasma involved PCR using 50 ng of DNA as template, followed by sequencing).)

206. The '596 patent itself admits that detection of mutations in cell-free DNA for bladder cancer patients was known in the prior art, citing Fleischhacker as an example. (*See, e.g.,* Ex.1001 at 133:15–20 (“K-ras is an oncogene that is activated in many cancers. Exemplary K-ras mutations are mutations in codons 12, 13, and 61. K-ras cfDNA mutations have been identified in pancreatic, lung, colorectal, bladder, and gastric cancers (Fleischhacker & Schmidt “Circulating

nucleic acids (CNAs) and can[c]er—a survey,” Biochim Biophys Acta 1775:181-232, 2007, which is hereby incorporated by reference in its entirety”), 133:26–32 (“p53 cfDNA mutations have been identified in breast, lung, ovarian, bladder, gastric, pancreatic, colorectal, bowel, and hepatocellular cancers (Fleischhacker & Schmidt “Circulating nucleic acids (CNAs) and can[c]er—a survey,” Biochim Biophys Acta 1775:181-232, 2007, which is hereby incorporated by reference in its entirety)”).)

207. A POSA would reasonably have expected to be able to perform the method wherein the cancer is bladder cancer based on the combined teachings of Diaz, Kinde 2013, and Fleischhacker. Doing so would be a simple matter of applying a tumor-informed ctDNA assay based on the combined teachings of Diaz and Kinde 2013 to a bladder cancer patient as taught by Fleischhacker.

D. GROUND 4: RABINOWITZ & BETTEGOWDA

208. In my opinion, the combination of Rabinowitz and Bettegowda renders obvious claims 1, 2, 10–13, and 15–18 of the ’596 patent. Rabinowitz is summarized in paragraphs 161–164 of my Declaration. A summary of Bettegowda, the combination, and the bases for my opinions are set forth below.

1. Summary Of Bettegowda

209. Bettegowda is a research article published in February 2014, describing detection of circulating tumor DNA in early- and late-stage human

malignancies. (Ex.1008, 1.) Bettegowda is in the same field as the '596 patent because it relates to methods for detecting “single-base substitutions” or SNVs. (*Id.*) Bettegowda discloses tumor-informed assays for ctDNA. (*See, e.g., id.*, 4 (explaining that results were “obtained by first identifying a mutation in a tumor and then determining whether that same mutation was detectable in the plasma”). In order to “directly compare different tumor types and to determine the spectrum of cancers in which ctDNA measurements could prove clinically useful,” Bettegowda “evaluated a large number of tumor types” and found that ctDNA could be detected “in most of the studied patients with solid tumors outside the brain.” (*Id.*, 2.)

210. Bettegowda compared multiple different methods to “evaluate ctDNA levels from each tumor” so that they could “report the number of mutant templates per milliliter of plasma in each case (Fig. 1).” (*Id.*) Specifically, Bettegowda compared the results obtainable using BEAMing with results from Safe-SeqS, and found that the sensitivity of the two different methods were comparable. (*Id.*, 16 (citing Ex.1049); *see also id.*, 4.) Bettegowda teaches that the results from both methods were able to detect “one mutant template in the DNA from 5 ml plasma, as determined by spiking known amounts of mutant KRAS DNA in plasma DNA from normal individuals.” (*Id.*, 16.)

2. Combination Of Rabinowitz And Bettegowda

211. A POSA would have found it obvious to combine the teachings of Rabinowitz and Bettegowda to arrive at the presently claimed method. Both references relate to detection of single nucleotide variants and other genetic mutations, which is the same field as the '596 patent. (*See* Ex.1001, 1:28–31 (stating that the invention of the '596 patent “generally relates to methods and systems for detecting ploidy of a chromosome segment, and methods and systems for detecting a single nucleotide variant”).)

212. Rabinowitz suggests applying its multiplex PCR and next-generation sequencing assays to determine the genetic state of a cancer from measurements on host blood. (Ex.1009, [0209] (“Any claim in the instant disclosure that reads on determining the ploidy state or genetic state of a fetus from the measurements made on maternal blood can equally well read on determining the ploidy state or genetic state of a cancer from the measurements on host blood.”).) Rabinowitz further discloses that “[in] some embodiments, the target DNA may originate from. . . cancer patient plasma.” (*Id.*, [0284].) Rabinowitz also suggests using “tumor DNA [that] may be present in many different body fluids [] of tumor patients” for “detection of tumor recurrence.” (*See id.*, [0289].) In my opinion, a POSA seeking to configure Rabinowitz’s cfDNA assays for detecting

cancer recurrence would reasonably turn to references in the field of ctDNA analysis and detection, including references like Bettgowda.

213. A POSA reading Bettgowda would understand that Safe-SeqS provides advantages for detecting mutations such as SNVs in cfDNA because it achieves detection of rare SNVs while being compatible with multiplex PCR, as discussed in more detail below. Bettgowda teaches that Safe-SeqS achieved sensitivities comparable to BEAMing, which detects mutations in ctDNA at levels as low as 0.005% (Ex.1072, 1). (Ex.1008, 16.) While achieving the similarly high sensitivity as the older BEAMing technology, Safe-SeqS allows profiling of more target loci simultaneously. (See, e.g., Ex.1062, 4 (explaining that Safe-SeqS provides a variant of “digital” technology by tagging individual template molecules with unique identifiers which enables researchers to analyze “many more loci—up to 30 or more—than BEAMing can reasonably handle”).)

a. Modification 1: tumor-informed assays

214. A POSA would have been motivated to modify the Rabinowitz method by sequencing a tumor biopsy to identify a plurality of tumor-specific mutations comprising one or more SNV mutations and then assaying those mutations in cfDNA, as taught by Bettgowda.

215. A POSA would have been motivated to make this modification because of the well-known advantages of sequencing a tumor biopsy to identify

mutations that serve as highly specific biomarkers in cfDNA. (*See* Ex.1072, 5, 1.)

Additionally, Bettegowda teaches that some cancer patients have their tumors sequenced to guide therapeutic decisions, and the “genetic alterations used to guide therapies can also be used for ctDNA analysis.” (Ex.1008, 6–7.)

216. A POSA would also have a reasonable expectation of success for performing sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor-specific mutations, wherein the tumor-specific mutations comprise one or more single nucleotide variant (SNV) mutations which could then be assayed in cfDNA because Bettegowda actually achieved this. (*See, e.g.*, Ex.1008, 3.) Additionally, Bettegowda teaches that sequencing a tumor sample to identify mutations is already performed in some cases to guide therapeutic decisions. (*Id.*, 6–7.) Tumor-informed assays and their advantages were also well-known to a POSA, as explained in paragraphs 73–75 of my Declaration. Therefore, this modification would involve simply combining prior art elements according to known methods to yield predictable results.

b. Modification 2: error-correction to detect an SNV present in less than or equal to 0.015% of the cfDNA having the locus

217. A POSA reading Rabinowitz in view of Bettgowda would also be motivated to use Safe-SeqS molecular barcodes and error correction in the multiplex PCR-based cfDNA assay of Rabinowitz. With this combination, a POSA would reasonably expect to detect an SNV present in less than or equal to 0.015% of the cfDNA having the SNV locus. A POSA would be motivated to make this combination based on the teachings of Bettgowda.

218. Bettgowda teaches that the Safe-SeqS method was able to achieve detection of SNVs present with sensitivity comparable to BEAMing. (Ex.1008, 16.) BEAMing was able to detect mutations in ctDNA at levels as low as 0.005% (Ex.1072, 1). A POSA would understand, however, that BEAMing does not readily allow multiplex analysis of different target loci in the same reaction. Additionally, BEAMing requires generation of fluorescently labeled probes for each mutation and wild-type allele analyzed (Ex.1072, 2), which is costly. Therefore, a POSA would be motivated to use the newer Safe-SeqS method that allows multiplexing and does not require fluorescent probes.

219. A POSA would also have a reasonable expectation of success in using Safe-SeqS molecular barcodes in primers for multiplex amplification from isolated cfDNA because Rabinowitz discloses that a “molecular barcode” can be

included in “any of the primers” disclosed in Rabinowitz. (Ex.1009, [0277].)

Rabinowitz further discloses that “[the] targeted PCR may be massively multiplexed” and “[the] selective [] amplification may involve tagging each individual molecule with different tags, molecular barcodes, tags for amplification, and/or tags for sequencing.” (*Id.* at [0201].) A POSA would understand that the Safe-SeqS barcode sequences used in Bettegowda are molecular barcodes.

Because Rabinowitz discloses that the amplification can involve adding molecular barcodes, a POSA would expect that Bettegowda’s Safe-SeqS barcode sequences could be included in primers designed as described in Rabinowitz. (*Id.*, [0277], [0020] (“In some embodiments, at least some of the primers additionally contain a random region that differs for each individual primer molecule. In some embodiments, at least some of the primers additionally contain a molecular barcode.”); *see also id.*, [0120].)

220. Additionally, a POSA would reasonably expect the combination to succeed because Rabinowitz discloses that primers comprising molecular barcodes can be used to identify “tagged molecules” that originate from the same “original” DNA molecule. (*Id.*, [0488].) Specifically, Rabinowitz discloses a molecular barcoding approach that “entails targeting one or more specific loci and generating a tagged copy of the original molecules such manner [*sic*] that most or all of the

tagged molecules from each targeted locus will have a unique tag and can be distinguished from one another upon sequencing of this barcode using clonal or single molecule sequencing.” (*Id.*) Thus, Rabinowitz suggests that molecular barcodes can be used to identify sequences that originate from the same original DNA template. A similar principle underlies the Safe-SeqS error correction method of Bettegowda. (*See* Ex.1008, 16 (citing Kinde 2011 (Ex.1049) for details of Safe-SeqS method).)

3. Claims 1, 2, 10–13, and 15–18 Are Obvious Over Rabinowitz and Bettegowda

a. Claim 1

Preamble: *A method for preparing biological samples useful for monitoring the progression of cancer in a subject, the method comprising:*

221. If limiting, the combined teachings of Rabinowitz and Bettegowda disclose methods for preparing biological samples useful for monitoring the progression of cancer in a subject.

222. Rabinowitz generally suggests applying its multiplex PCR and next-generation sequencing assays to determine the genetic state of a cancer from measurements on host blood. (Ex.1009, [0209] (explaining that “[any] claim in the instant disclosure” for determining the “genetic state” of a fetus from the measurements made on maternal blood “can equally well read on determining the ploidy state or genetic state of a cancer from the measurements on host blood.”).)

Rabinowitz further discloses that “[in] some embodiments, the target DNA may originate from . . . cancer patient plasma.” (*Id.*, [0284].) Rabinowitz suggests using “tumor DNA [that] may be present in many different body fluids [] of tumor patients” for “detection of tumor recurrence.” (*See id.*, [0289].) And as discussed above, by 2014 a POSA would also have been aware that multiplex PCR reactions followed by NGS had successfully been applied to detect circulating tumor DNA. (Ex.1039, 1–3.)

223. Bettegowda discloses methods for preparing samples comprising ctDNA useful for “monitoring patients with advanced cancers” which Bettegowda teaches may at least have “psychological benefits” for patients who are “anxious to know, as soon as possible, whether disease has progressed.” (*See* Ex.1008, 7.) Bettegowda further teaches that monitoring ctDNA is advantageous compared to imaging because it is “noninvasive” and does not expose patients to radiation. (*Id.*) Additionally, Bettegowda teaches that “[l]iquid biopsies can also be used to monitor patients being treated with targeted agents, providing an early warning of recurrence and information about the genetic basis of resistance.” (*Id.*, 4.)

Limitation 1[A]: (a) performing sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor-specific mutations, wherein the tumor-specific mutations comprise one or more single nucleotide variant (SNV) mutations;

224. Limitation 1[A] is obvious over Rabinowitz and Bettegowda.

225. Rabinowitz does not specifically teach performing sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor specific mutations that are then assayed in cfDNA. But sequencing a tumor biopsy for a tumor-informed assay of cfDNA would have been obvious to a POSA reading Rabinowitz in view of Bettegowda.

226. A POSA reading Rabinowitz in view of Bettegowda would have been motivated to perform sequencing on a tumor biopsy of the subject to identify a plurality of tumor-specific mutations, wherein the tumor-specific mutations comprise one or more single nucleotide variant (SNV) mutations which are then assayed in cfDNA (*see* Limitations 1[C][i]-1[C][iv] below). Bettegowda discloses performing sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor-specific mutations which are then assayed in cfDNA. (*See* Ex.1008, 2 (“Targeted sequencing, exomic sequencing, or whole-genome sequencing was used to identify mutations in the tumors, as described in Supplementary Materials and Methods”); *id.*, 6 (explaining that in this study “[the] specific mutations [assayed in ctDNA] are defined by evaluation of the primary

tumor” which “may be less of an obstacle in the future because more cancer patients will have their tumors genetically analyzed to guide therapeutic decisions.”.) Bettgowda teaches that by sequencing the tumor sample to identify mutations, the “genetic alterations used to guide therapies can also be used for ctDNA analysis.” (*Id.*, 6–7.) Additionally, Bettgowda teaches identifying a plurality of tumor-specific mutations comprising one or more SNVs. (*See, e.g., id.*, 3 (“tumor-specific rearrangements as well as tumor-specific point mutations were identified in 19 patients (table S2)”); *see also id.*, 24 (table S2) (showing point mutations and rearrangement mutations identified for the same specimens).)

Limitation 1[B]: (b) evaluating results of the sequencing on the tumor biopsy sample to determine a plurality of target loci specific to the subject, wherein each target locus spans a tumor-specific mutation of the identified plurality of tumor-specific mutations; and

227. Limitation 1[B] is also obvious over Rabinowitz and Bettgowda. Bettgowda discloses evaluating results of the sequencing on the tumor biopsy sample to determine a plurality of target loci specific to the subject, wherein each target locus spans a tumor-specific mutation of the identified plurality of tumor-specific mutations. (*See, e.g., Ex.1008*, 15 (“Once putative rearrangements were identified on the basis of sequencing data, PCR primers were designed to amplify PCR products of 100 bp that spanned the rearrangement”).) Bettgowda also discloses that “single base substitutions and small insertions or deletions (indels)”

identified in the tumor sequencing were assessed in ctDNA by BEAMing, by a PCR/ligation method, or by SafeSeqS. (*Id.*) A POSA would understand that all of these methods involve targeting loci that each span a mutation identified in the tumor.

Limitation 1[C][i]: (c) assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points, wherein the assaying comprises:

228. Limitation 1[C][i] is also obvious over Rabinowitz and Bettgowda. Rabinowitz suggests using “tumor DNA [that] may be present in many different body fluids [] of tumor patients” for “detection of tumor recurrence.” (*See* Ex.1009, [0289].) To detect tumor recurrence using cell-free DNA, a POSA would be motivated to monitor the ctDNA over time.

229. Bettgowda discloses assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points. (*See* Ex.1008, 14 (explaining that for some patients, “two plasma draws were obtained: one prior to initiating EGFR blockade and one sample when the tumors had recurred after a clinical response”); *see also id.*, 15–16 (“SafeSeqS was used to assess all of the 206 metastatic colorectal cancer patients assayed for mutations in the liquid biopsy study (table S3) as well as to assess the 24 patients assayed for resistance mutations after EGFR blockade”).) Bettgowda also discloses the advantages of assaying cfDNA at multiple timepoints because it is “noninvasive”

compared to other types of repeated assays. (*See id.*, 7 (explaining that unlike assaying cfDNA to monitor cancer progression, “[r]epeated imaging also subjects patients to radiation”).)

230. In view of the teachings of Rabinowitz and Bettegowda, a POSA would have been motivated to assay cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points in order to monitor cancer progression over time. A POSA would be motivated to do so because Bettegowda teaches the advantages of ctDNA monitoring over other repeated methods for detecting recurrence, such as imaging, as discussed above. A POSA would also have a reasonable expectation of success in doing so, because it would simply involve performing the same assay for multiple samples obtained at different time points. Additionally, tumor-informed assays and serial monitoring of ctDNA in plasma were well-known to a POSA by 2014, as discussed in paragraphs 73–75 of my Declaration.

Limitation 1[C][ii]: *performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject; and*

231. Based on the combined teachings of Rabinowitz and Bettegowda, it would have been obvious to perform targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the

isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject.

232. Rabinowitz discloses methods for multiplex PCR amplification to amplify a plurality of target loci together in the same reaction volume from the isolated cell-free DNA using target-specific primers. For example, Rabinowitz teaches that “[w]hereas traditional multiplex PCRs evaluate up to fifty loci simultaneously” the disclosed multiplex PCR assays can involve many more than fifty target loci, and “[a]ssays may be combined in a single reaction with the entirety of a cfDNA sample isolated from maternal plasma, a fraction thereof, or a further processed derivative of the cfDNA sample.” (Ex.1009, [0267].)

233. Rabinowitz generally states that the same assays can be applied to determine the genetic state of a cancer from measurements on host blood. (*Id.*, [0209] (“Any claim in the instant disclosure that reads on determining the ploidy state or genetic state of a fetus from the measurements made on maternal blood can equally well read on determining the ploidy state or genetic state of a cancer from the measurements on host blood.”).) Rabinowitz further discloses that “[in] some embodiments, the target DNA may originate from . . . cancer patient plasma.” (*Id.*, [0284].) A POSA would understand that plasma DNA is cell-free DNA. And, as discussed above, by 2014 a POSA would also have been aware that multiplex PCR

reactions followed by NGS had successfully been applied to detect circulating tumor DNA. (Ex.1039, 1–3.)

234. A POSA would have been motivated to apply multiplex PCR as taught by Rabinowitz in a tumor-informed assay in order to analyze target loci spanning a plurality of tumor-specific mutations as taught by Bettegowda without requiring separate reactions. As discussed above, Bettegowda discloses assaying the cell-free DNA at multiple target loci spanning multiple tumor-specific mutations. For example, Bettegowda teaches assaying cell-free DNA by performing PCR reactions on both target loci spanning tumor-specific rearrangements and point mutations (SNVs). (*See* Ex.1008, 3 (“We were also interested in comparing the quantity of two different types of genetically altered DNA fragments in the circulation of the same patients ... tumor-specific rearrangements as well as tumor-specific point mutations were identified in 19 patients (table S2)”).)

235. A POSA would also reasonably expect to succeed because Rabinowitz discloses primer design methods for multiplex amplification from cfDNA, including that there are “a number of ways to choose primers for a library where the amount of non-mapping primer-dimer or other primer mischief products are minimized.” (Ex.1009, [0292].) Even without the primer design methods of

Rabinowitz, a POSA would reasonably expect to succeed because Rabinowitz discloses that primer side products, such as primer dimers, and other artifacts typically only present challenges for “reactions of more than 5 to 10 targets” (*Id.*, [0258]) and that “the prior art includes disclosures of successful multiplexed PCR reactions involving pools of up to about 50 or 100 primers.” (*Id.*, [0217].)

236. Additionally, a POSA would reasonably expect to succeed because Bettgowda teaches use of a “multiplexed, sequencing-based assay to query known mutated hotspots of several genes in the EGFR pathway.” (Ex.1008, 4.) Bettgowda further teaches that Safe-SeqS was used in that EGFR hotspot assay. (*See id.*, 15–16 (disclosing that Safe-SeqS was used to assess the 24 patients assayed for resistance mutations after EGFR blockade).) Therefore, a POSA would understand that Safe-SeqS can be used in the context of a multiplexed assay, as well as for individual assays.

Limitation 1[C][iii]: *performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads,*

237. Based on the combined teachings of Rabinowitz and Bettgowda, it would have been obvious to perform high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads.

238. Rabinowitz states that the provided methods include “highly efficient highly multiplexed targeted PCR to amplify DNA followed by high

throughput sequencing to determine the allele frequencies at each target locus.”

(Ex.1009, [0064].) According to Rabinowitz, the provided methods include “the ability to multiplex more than about 50 or 100 PCR primers in one reaction in a way that most of the resulting sequence reads map to targeted loci.” (*Id.*)

Bettegowda also discloses detecting tumor-specific mutations in cell-free DNA using Safe-SeqS (Ex.1008, 16 (citing Ex.1049)), which a POSA would understand involves performing next-generation (high-throughput) sequencing of the amplified DNA.

Limitation 1[C][iv]: wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.

239. Based on the combined teachings of Rabinowitz and Bettegowda, it would have been obvious to perform the claimed method wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads. A POSA would have been motivated to detect an SNV that is present in less than or equal to 0.015% of the cfDNA having the SNV locus because a POSA was aware that “circulating mutant DNA represents only a tiny fraction of the total circulating DNA, sometimes less than 0.01%.” (Ex.1072, 1.)

240. Bettegowda teaches that Safe-SeqS can be applied to cfDNA to detect SNVs and can achieve results comparable to BEAMing in that context.

(*See, e.g.*, Ex.1008, 2, 16 (disclosing that both BEAMing and Safe-SeqS amplification and sequencing was able to detect one mutant template in the DNA purified from up to 5 ml of plasma); *see also id.*, 18.) Although Bettegowda uses alternative units (mutant templates per cfDNA purified from up to 5 ml of plasma) to describe the mutant allele fraction, a POSA would have understood that the isolated cfDNA purified from 5 ml of plasma contained sufficient template copies such that a single mutant template would be present in less than or equal to 0.015% of the cfDNA having the SNV locus. (*See, e.g.*, Ex.1051, 4 (for the cfDNA purification kit used by Bettegowda, explaining that circulating cell-free DNA yields per 5 mL sample varied from about 8,000 to more than 80,000 genome equivalents).) A POSA would understand that detecting one mutant template among 8,000–80,000 genome equivalents ($1/8,000$ – $1/80,000$) in a single reaction would be equivalent to detecting an SNV that is present in 0.0125%–0.00125% of the cfDNA having the SNV locus, which is less than 0.015%. To illustrate this concept: if a reaction contains at least 7,000 copies of a particular cfDNA template locus (e.g., 7,000 genome equivalents) and only one of those copies is a mutant copy, then a POSA would understand that the single mutant copy of that template would be present in less than 0.015% of the cfDNA containing the locus. This is because $1/7,000$ is about 0.014%, which is less than 0.015%.

241. A POSA in 2014 would reasonably expect to be able to perform the multiplex PCR reaction disclosed by Rabinowitz with Bettegowda's Safe-SeqS barcodes and error correction in a single reaction containing at least 7,000 templates of a cfDNA locus. First, a POSA would expect to be able to prepare a reaction containing sufficient cfDNA template copies because the cfDNA purification method used by Bettegowda yields about 8,000 to more than 80,000 genome equivalents per 5 mL sample. (*See, e.g.,* Ex.1051, 4.) Obtaining such quantities of cfDNA was also routine to a POSA. For example, as early as 2008, cfDNA assays were performed that involved PCR amplification with 8,000 or more copies of a cfDNA template in the reaction. (*See* Ex.1072, 1, 6 (describing BEAMing assays was performed using DNA purified from 2 ml of plasma containing median of 4,000 cfDNA fragments of a given locus per milliliter of plasma (*i.e.*, a median of 8,000 fragments per reaction)).

242. Bettegowda also discloses that “comparison between DNA from [circulating tumor cells (CTCs)] and ctDNA cannot easily be performed with point mutations” because the limit of detection is too high to detect a few CTCs among the millions of wild-type cells present per milliliter of blood. (Ex.1008, 7.) According to Bettegowda, the sensitive methods used in their study (which included Safe-SeqS) did not allow detection of point mutations at “levels less than

1 in 100,000 cells,” (0.001%, fifteen-fold below the 0.015% level required by the present claims). (Ex.1008, 7 (citing Ex.1049).) Because Bettgowda describes 0.001% as the level below which point mutations could not be detected, a POSA would understand that the limit of detection for SNVs was on the order of 0.001%, and well below 0.015%. Therefore, a POSA would reasonably expect to detect an SNV mutation present in less than or equal to 0.015% of the cfDNA having the SNV locus by using the Safe-SeqS barcodes and error correction method in a multiplex PCR reaction with at least 7,000 cfDNA templates in the reaction.

b. Claim 2

The method of claim 1, wherein the cell-free DNA comprises circulating tumor DNA.

243. Claim 2 is obvious over Rabinowitz and Bettgowda. First, Rabinowitz discloses that “[a]ssays may be combined in a single reaction with the entirety of a cfDNA sample isolated from maternal plasma, a fraction thereof, or a further processed derivative of the cfDNA sample.” (Ex.1009, [0267].) Rabinowitz states generally that the same assays can be applied to determine the genetic state of a cancer from measurements on host blood. (*Id.*, [0209] (“Any claim in the instant disclosure that reads on determining the ploidy state or genetic state of a fetus from the measurements made on maternal blood can equally well read on determining the ploidy state or genetic state of a cancer from the

measurements on host blood.”).) Rabinowitz also teaches that “[in] some embodiments, the target DNA may originate from... cancer patient plasma.” (*Id.*, [0284].) By the filing date of the first provisional application for the ’596 patent in 2014, a POSA would understand that target DNA originating from “cancer patient plasma” and used to determine the “genetic state of a cancer” comprises circulating tumor DNA.

244. Additionally, Bettegowda teaches that the cell-free DNA comprises circulating tumor DNA. (*See, e.g.*, Ex.1008, 3 (“ctDNA was detectable in most patients with metastatic cancers”), *id.*, 2 (“ctDNA was detected in most of the studied patients with solid tumors outside the brain”).)

a. Claim 10

The method of claim 1, wherein the method further comprises designing targeted PCR assays for the tumor-specific mutations identified in the cancer sample.

245. Rabinowitz discloses designing primers for multiplexed PCR of target loci followed by NGS, with a particular focus on cfDNA assays. (*See* paragraphs 162–164 of my Declaration.)

246. Furthermore, a POSA would reasonably expect to be able to remove primer dimers with the design constraints of “mini-PCR” for short DNA fragments because Rabinowitz discloses that its method of primer design “may be used in combination with the mini-PCR method described elsewhere in this

document” and that “the primer design method may be used as part of a massive multiplexed PCR method.” (Ex.1009, [0295].)

247. A POSA would also reasonably expect to succeed in designing targeted PCR assays including Safe-SeqS molecular barcodes in the primers because Rabinowitz describes methods by which “the design and generation of molecular barcoding primers may be reduced to practice.” (*Id.*, [0496].)

b. Claim 11

The method of claim 1, wherein amplifying comprises targeted multiplex PCR amplification of 10 to 50 of the target loci.

248. Claim 11 is also obvious over Rabinowitz and Bettegowda. As discussed in paragraph 172 of my Declaration, Rabinowitz teaches that “the prior art includes disclosures of successful multiplexed PCR reactions involving pools of up to about 50 or 100 primers.” (*See, e.g.*, Ex.1009, [0217].) And as discussed in paragraphs 162–164 of my Declaration, Rabinowitz discloses primer design methods for much higher levels of multiplexing, including for cfDNA.

249. A POSA would expect to be able to identify 10 to 50 target loci spanning tumor-specific mutations in a solid tumor biopsy, because tumors were known to have more than 10 to 50 mutations. (*See, e.g.*, Ex.1072, 1 (“[in] solid tumors such as those of the colon or breast, it has been shown that, on average, approximately 80 genes harbor subtle mutations that are present in virtually every

tumor cell but are not present in normal cells.”.) Therefore, a POSA would also be motivated to perform targeted multiplex amplification of 10 to 50 of the target loci because this would allow tracking of more tumor-specific mutations in cfDNA without requiring additional reactions. According to Rabinowitz, “[g]reater multiplexing allows more alleles to be targeted, giving more accurate results.” (*Id.*, [0239].)

250. A POSA would also expect to succeed because Rabinowitz discloses that molecular barcodes and/or random sequences can be incorporated into Rabinowitz’s primer designs, as discussed in paragraphs 168–171 of my Declaration.

c. Claim 12

The method of claim 1, wherein the method further comprises detecting recurrence and/or metastases of the cancer from the tumor-specific mutations detected in the cell-free DNA.

251. Based on the combined teachings of Rabinowitz and Bettgowda, it would have been obvious to perform the claimed method wherein the method further comprises detecting recurrence and/or metastases of the cancer from the tumor-specific mutations detected in the cell-free DNA.

252. Rabinowitz discloses that “tumor DNA may be present in many different body fluids or tissues of tumor patients” and that “[it] may be used for detection of tumor recurrence.” (Ex.1009, [0289].) Rabinowitz also teaches that

“[in] some embodiments, the target DNA may originate from... cancer patient plasma.” (*Id.*, [0284].) By 2014, a POSA reading Rabinowitz would have understood that the “tumor DNA” present in body fluids such as cancer patient plasma was circulating tumor DNA.

253. Bettegowda discloses detecting tumor recurrence by detecting ctDNA mutations. For example, Bettegowda discloses that “once a targeted therapy is initiated in a patient with multiple metastases, clinicians frequently search for early evidence of recurrence or mechanisms underlying resistance, a scenario in which liquid biopsies are particularly valuable. For example, they can provide temporal measurements of the total tumor burden” (Ex.1008, 2; *see also id.* (“We also used liquid biopsies to identify mutations that were responsible for recurrence in patients who initially responded to EGFR blockade.”), *id.*, 4 (“Liquid biopsies can also be used to monitor patients being treated with targeted agents, providing an early warning of recurrence and information about the genetic basis of resistance.”).)

d. Claims 13, 15, and 16

Claim 13: *The method of claim 1, wherein the cancer is colorectal cancer.*

Claim 15: *The method of claim 1, wherein the cancer is bladder cancer.*

Claim 16: *The method of claim 1, wherein the cancer is breast cancer.*

254. These claims are also obvious over Rabinowitz and Bettegowda.

255. Bettegowda discloses detection of ctDNA in colorectal, bladder, and breast cancers. (Ex.1008, 6 (Fig. 3) (showing detection of ctDNA for colorectal and breast cancers), 5 (Fig. 2) (showing detection of ctDNA for bladder, colorectal, and breast cancers), 1 (“We found that ctDNA was detectable in >75% of patients with advanced ... colorectal, bladder, [and] breast [cancers]”), 2 (“most patients with stage III ovarian and liver cancers and metastatic cancers of the pancreas, bladder, colon, [and] breast, ... harbored detectable levels of ctDNA”).) Therefore, it would have been obvious to apply the method of claim 1 to colorectal, bladder, and breast cancers.

e. Claim 17

The method of claim 1, wherein the biological sample is a blood, serum, plasma, or urine sample.

256. Claim 17 is also obvious in view of Rabinowitz and Bettegowda.

257. First, Rabinowitz discloses that “tumor DNA may be present in many different body fluids or tissues of tumor patients” and that “[it] may be used for detection of tumor recurrence.” (Ex.1009, [0289].) Rabinowitz also teaches that “[in] some embodiments, the target DNA may originate from... cancer patient plasma.” (*Id.*, [0284].)

258. Bettgowda discloses that the cfDNA was isolated from plasma samples. (*See, e.g.*, Ex.1008, 2 (“We purified plasma and tumor DNA using regimented protocols for all samples and used digital technologies to evaluate ctDNA levels from each tumor so that we could report the number of mutant templates per milliliter of plasma in each case (Fig. 1)”); *see also id.*, 16 (describing quantifying mutations in 20 plasma samples using three different methods for detecting point mutations: “BEAMing (58), PCR-Ligation (53), or Safe-SeqS (40)”)). According to Bettgowda, “[t]hese methods yielded comparable results when applied to the same plasma samples (fig. S1) and all were able to detect one mutant template in the DNA purified from up to 5 ml of plasma.” (*Id.*, 2.) Bettgowda further discloses results “obtained by first identifying a mutation in a tumor and then determining whether that same mutation was detectable in the plasma.” (*Id.*, 4.)

f. Claim 18

The method of claim 1, wherein step (a) comprises performing whole exome sequencing on a tumor biopsy sample of the subject to identify the plurality of tumor-specific mutations.

259. Claim 18 is also obvious in view of Rabinowitz and Bettgowda.

260. Bettgowda teaches identifying a plurality of tumor-specific mutations in a tumor biopsy using “[t]argeted sequencing, exomic sequencing, or whole-genome sequencing ... to identify mutations in the tumors, as described in

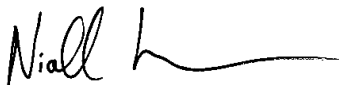
Supplementary Materials and Methods.” (Ex.1008, 2.) Bettegowda further teaches that the “exomic sequencing was performed after capture of the same libraries via SureSelect (Agilent), as previously described (56, 57).” (*Id.*, 15.)

261. A POSA would be motivated to identify the tumor-specific mutations using exomic sequencing as taught by Bettegowda. Doing so would simply involve combining prior art elements according to known methods to yield predictable results. A POSA would also be motivated to use exomic sequencing and would have a reasonable expectation of success in doing so because Bettegowda teaches that kits for performing whole exome sequencing were commercially available. (*Id.*, 15.)

* * *

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code.

Dated: Jan 14, 2025



Niall Lennon, Ph.D.