

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 NIALL LENNON, PH.D IN SUPPORT OF
PETITION FOR INTER PARTES REVIEW OF U.S. PATENT NO.
11,519,035**

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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 1-5 and 10-15 of U.S. Patent No. 11,519,035 (“the ’035 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, 2, 12, and 13 against NeoGenomics in *Natera, Inc. v. NeoGenomics Labs, Inc.*, No. 23-cv-00629 (M.D.N.C.) (the “co-pending litigation”). I also understand that the ’035 patent is currently the subject of an appeal at the U.S. Court of Appeals for the Federal Circuit, styled as *Natera, Inc. v. NeoGenomics Labs., Inc.*, Nos. 24-1324, 24-1409 (Fed. Cir.) (the “co-pending appeal”).

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 '035 patent are obvious and unpatentable. The following is a summary of my opinions:

- The '035 patent's priority date is, at the earliest, October 20, 2015 because earlier applications in the '035 patent's priority chain do not provide sufficient written description support for the challenged claims.
- Claims 1, 12, and 13 of the '035 patent are rendered obvious by Forshew *et al.*, "Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA," Science Translational Medicine, Vol. 4, Issue 136 (May 30, 2012) ("Forshew") (Ex.1005).
- Claims 1 and 10-15 are obvious in view of "Assays for the Detection of Genotype, Mutations, and/or Aneuploidy," International Publication No. WO 2011/142836 ("Pieprzyk") (Ex.1006) and "Multi-Primer Amplification Method for Barcoding of Target Nucleic Acids," International Publication No. WO 2010/115154 ("May") (Ex.1007).
- Claims 1-3, and 10-15 are obvious in view of "Methods and Compositions for Evaluating Genetic Markers," International Publication No. WO 2010/126614 ("Porreca") (Ex.1008) and "Methods and Compositions for the Extraction and Amplification of Nucleic Acid from a Sample," International Publication No. WO 2007/140417 ("Hoyal") (Ex.1009).

- Claims 1-5 and 10-15 are obvious in view of Porreca and “Miniature Inverted Repeat Transposable Elements and Methods of Use,” U.S. Patent No. 6,420,117 (“Wessler”) (Ex.1010).

III. QUALIFICATIONS

4. 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.

5. Prior to my current position, 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 18 years of experience studying and researching molecular biotechnology, including nucleic acid sequencing and its application to cancer, germline, and viral genomics.

6. 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.

7. 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.

8. 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.

9. Exhibit 1051 is a copy of my curriculum vitae.

IV. MATERIALS CONSIDERED

10. In preparing this declaration, I considered at least the following materials:

- The exhibits cited in this Declaration; and
- a publicly-available, redacted version of the Declaration of Dr. Brian Van Ness that was filed in the co-pending litigation (Ex.1103).

While there may be similarities in the wording or opinions recited in this Declaration and the Van Ness Declaration, the analysis and opinions expressed herein are my own.

V. LEGAL STANDARDS

11. 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.

B. Claim Construction

12. 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 (including prior art references that were cited during the prosecution of the patent).

13. In forming my opinions, I have given all other claim terms of the ’035 patent their plain and ordinary meaning as would have been understood by a POSA at the time of the claimed invention.

14. I understand that, in the co-pending litigation, two terms in independent claim 1 are in dispute: (1) “amplifying the tagged products...wherein one of the amplification steps comprises targeted amplification”; and (2) “SNP loci...associated with cancer.” (Ex.1035, 3, 7.) I was asked to assume for the purposes of this IPR proceeding: (1) that the “amplifying” term encompasses targeted amplification occurring in the same reaction as a claimed tagging step; and (2) that “SNP loci...associated with cancer” broadly refers to any single-point mutation in human DNA that is associated with cancer. I have applied these constructions, which I understand reflect how Patent Owner interprets the claims in the co-pending litigation, herein. But my application of those constructions should not be understood to mean that I agree to them.

C. Obviousness

15. 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 time the invention was made to a POSA.

16. 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.

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

18. I understand that in assessing whether a claimed invention would have been obvious at the time of the purported 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.

19. 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.

20. 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.

21. 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 at the time of the purported 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 at the time the purported invention was made.

22. 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.

23. 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.

24. 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.

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

26. 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.

27. 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.

28. 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 time of invention and addressed by the patent can provide a reason for combining the elements in the manner claimed.

29. 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.

30. 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 time of the purported invention, can provide a reason for combining the elements of multiple prior art references in the claimed manner.

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

32. 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.

33. 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.

D. Written Description And Priority

34. 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.

35. 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.

36. 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.

37. 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.

VI. THE '035 PATENT

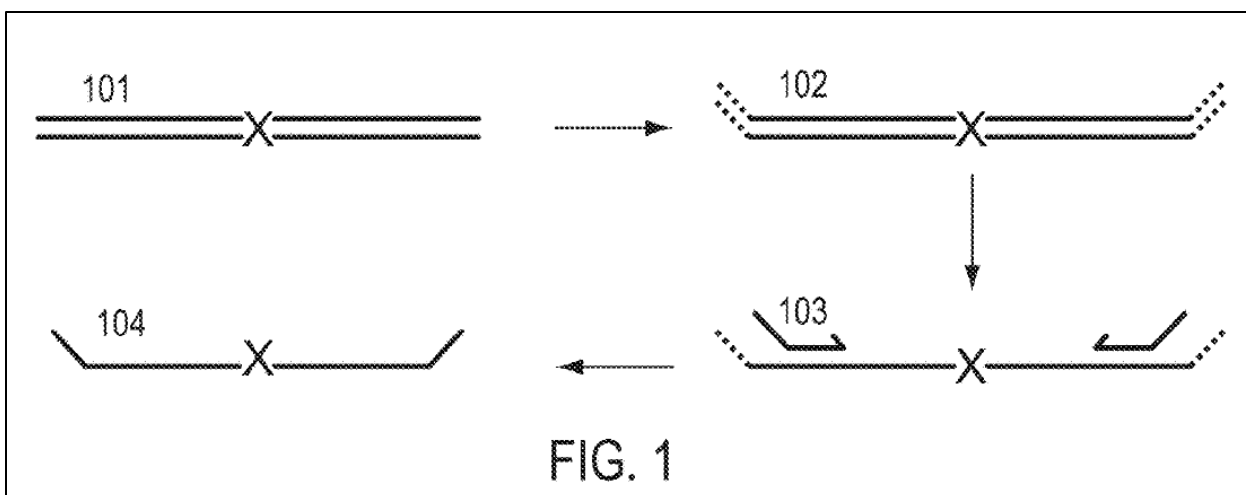
A. Subject Matter Of The '035 Patent

38. The '035 patent's Abstract states that it "provides methods for simultaneously amplifying multiple nucleic acid regions of interest in one reaction volume as well as methods for selecting a library of primers for use in such amplification methods." (Ex.1001, Abstract.) That abstract further refers to providing a "library of primers with desirable characteristics, such as minimal formation of amplified primer dimers or other non-target amplicons." (*Id.*) The patent's "Field of the Invention" section states that "the present invention generally relates to methods and compositions for simultaneously amplifying multiple nucleic acid regions of interest in one reaction volume." (*Id.*, 2:57-61.) In my opinion, the field of endeavor of the '035 patent is broader than the patent's "Field of the Invention" cited above and includes any targeted analysis of cell-free DNA

(also called “cfDNA”) or other targeted analysis of genomic regions to detect mutations. As discussed in more detail below, most inventions within the field of genomics and its various subfields would reasonably commend themselves to the problems faced by the inventor of the ’035 patent.

39. Broadly speaking, the ’035 patent summarizes methods along the lines of those described in its Abstract and then provides specific examples of the same. (Ex.1001 (Examples 1-29).) For example, the ’035 patent’s Example 27 relates to performing PCR to amplify fragments of cfDNA naturally found in plasma. (*Id.*, 239:8-17.) In that example, “[m]assively multiplex PCR and DNA sequencing methods...were used to determine allele counts at a plurality of polymorphic loci with...18-25 M reads/sample for plasma.” (*Id.*, 240:14-19.) According to the ’035 patent, the multiplex PCR involved “using [a] 3,168 SNP primer pair pool.” (*Id.*, 20-21.)

40. The ’035 patent depicts one such multiplex PCR method, which it refers to as a “multiplexed mini-PCR method,” in its Figure 1:



(*Id.*, Fig. 1, 42:54-55; *see also id.* 88:52-89:4 (“mini-PCR may equally well refer to normal PCR with no additional restrictions or limitations”), 95:58-65.) In Figure 1, element 101 depicts “double stranded DNA with a polymorphic locus of interest at X.” (*Id.*, 95:58-60-61.) In element 102, “ligation adaptors [are] added for universal amplification,” *i.e.*, to increase the initial copy numbers of every sequence in solution. (*Id.*, 95:61-63.) In element 103, what appear to be target-specific PCR primers hybridize to single stranded amplicons resulting from the universal amplification. (*Id.*, 95:63-65.) And in element 104, Figure 1 depicts “the final PCR product,” which may be tagged with various sequences for downstream applications. (*Id.*, 95:65, 96:3-9.)

41. The '035 patent also contains a number of disclosures about the state of the art before it was filed. For example, the '035 patent states: “the general belief in the art is that multiplexing PCR for sequencing is limited to about 100 assays in the same well.” (Ex.1001, 48:25-27,; 85:14-20, 87:32-33.) This is not

entirely accurate—in fact, multiplexing of more than 100 sequences in a single reaction volume was known by 2010—but it is consistent with a POSA’s understanding that multiplexing more than 25 sequences in a single reaction volume was known. And to the extent that Patent Owner asserts that the above quoted passage is not directed to circulating tumor DNA (“ctDNA”), I disagree. The passage does not exclude ctDNA and, as I discuss below, multiplexing more than 25 sequences of ctDNA in a single reaction volume was known. As another example in the ’035 patent about the state of the art, it describes using Illumina (third-party) HiSeq and MiSeq devices to perform next generation sequencing, confirming that next generation sequencing was known before the ’035 patent was written. (Ex.1001, 90:60-64.)

42. The ’035 patent includes 15 claims, 11 of which I understand are challenged in this IPR proceeding. According to the ’035 patent’s claim 1, isolated cfDNA is first tagged with one or more “universal tail adaptors.” (Ex.1001 (claim 1).) Next, the tagged DNA, including 25-2,000 loci “associated with cancer,” is amplified to generate “final amplification products,” where the amplifying comprises targeted amplification in a single reaction volume and introducing a barcode and one or more sequencing tags. (*Id.*) The final amplification products are then sequenced. (*Id.*) Challenged dependent claims 2-5 and 10-15 further refine the same method. (Ex.1001 (claims 2-5, 10-15).)

B. Prosecution History

43. The application (16/934,407) that matured into the '035 patent was filed on July 21, 2020. Original independent claim 1 was directed to a method for amplifying and sequencing DNA. It recited three steps: (1) “tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products, wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject”; (2) “amplifying the tagged products one or more times to generate final amplification products, wherein one of the amplifying steps introduces a barcode and one or more sequencing tags”; and (3) “sequencing a plurality of loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products, wherein the plurality of loci comprises 25-2,000 loci.” (Ex.1003, 1635.) The application also includes dependent claims 2-15. (*Id.*, 1635-37.)

44. The Examiner rejected all of the claims in a non-final Office Action on September 16, 2020 based on Fan (Ex.1022), entitled “Noninvasive Diagnosis of Fetal Aneuploidy by Sequencing,” either alone (claims 1-5, 10-15) or in combination with two references disclosing a nested PCR primer (claim 6) and a ligated index tag (claims 7-9), respectively. (Ex.1003, 957-58.)

45. Patent Owner submitted a response on December 2, 2020. To overcome the rejection based on Fan, Patent Owner added “wherein one of the

amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci” to its claims and then argued that Fan did not teach “targeted amplification of 25-2,000 SNP...or...massively parallel sequencing of the amplicons produced.” (Ex.1003, 853, 858-59.) Patent Owner further argued that “Fan fails to teach or suggest the targeted amplification of 25-2,000 SNP loci,” citing Fan’s disclosure that its diagnostic test is “polymorphism-independent.” (*Id.*, 859.)

46. The Examiner then rejected the claims again on January 6, 2021 based on Chuu (Ex.1023), entitled “Methods of Fetal Abnormality Detection,” either alone (claims 1-5, 10-15) or in combination with the same two references in the first Office Action disclosing a nested PCR primer (claim 6) and a ligated index tag (claims 7-9), respectively. (Ex.1003, 635-36.)

47. To overcome this rejection, Patent Owner amended the claims again in a response on March 3, 2021. (Ex.1003, 626.) It added that the cfDNA is isolated from a blood sample collected from a subject “who is not a pregnant women [sic],” that the targeted amplification is “in a single reaction volume,” and that the 25-2,000 SNP loci are “associated with cancer.” (*Id.*) In doing so, Patent Owner explained that “[s]upport for the amendments to claim 1 can be found in the published application at paragraphs 0041, 0082, 0388, 0397, 0450, 0956, and Examples 26-29.” (Ex.1003, 628.) Patent Owner then remarked that “Chuu is

directed to analysis of maternal plasma samples for detection of fetal aneuploidy, but fails to teach or suggest anything about amplifying and sequencing cell-free DNA isolated from a blood sample collected from a subject who is not a pregnant women [sic].” (Ex.1003, 629.) That is, Patent Owner argued that the remaining scope of the claim, i.e., amplifying and sequencing cfDNA associated with cancer and isolated from a blood sample of a non-pregnant woman, was inventive and non-obvious. Patent Owner also argued that “Chuu fails to teach or suggest targeted amplification of 25-2,000 target loci associated with cancer in a single reaction volume.” (Ex.1003, 629.)

48. The Examiner then allowed the '035 patent on April 9, 2021—less than one year from the filing of the application. (Ex.1003, 573.) The Examiner remarked that the prior art disclosed “multiplex amplification and sequencing of 25 to 2000 SNP targets in a sample” but that the amendment—directed to excluding “samples of cell free nucleic acid obtain from a pregnant female” and 25-2000 loci “associated with cancer”—was sufficient to overcome the art. (*Id.*, 578-79.) After allowance, Patent Owner then submitted hundreds and hundreds of references in multiple information disclosure statements, including a 2014 version of the Pieprzyk reference and the May and Hoyal references, discussed below. (*Id.*, 2, 16, 50, 56, 144, 151, 171, 230, 301, 379, 492, 547, 555.)

49. In a subsequent Notice of Allowance on July 9, 2021, the Examiner noted that one submitted 2011 reference—Su (Ex.1104.)—“may appear relevant but there are substantial differences between Su and the method as claimed.” (Ex.1003, 333-34.) The Examiner explained that “Su does not antedate the priority dates of the instant application”, that Su’s “SNaPshot format of multiplex is not applied to cell free nucleic acid” and that “the multiplex format of Su does not include simultaneous amplification and analysis of plural SNP loci, as claimed.” (*Id.*) Instead, according to the Examiner, “the panel of SNP loci of Su are analyzed in grouped panels of four or six mutations instead of all separate loci together.” (*Id.*) The ’035 patent issued on November 16, 2022.

C. Priority Date

50. I understand that patents may claim the benefit of, or priority to, prior application(s). The ’035 patent includes a listing of earlier-filed patent applications which has been mapped visually in the following accompanying exhibit, Ex. 1002. I understand that, in the co-pending litigation, Patent Owner has asserted that the priority date for the ’035 patent is “by May 18, 2011.”

51. I disagree with Patent Owner’s asserted May 18, 2011 priority date. As I discuss in further detail below, the ’035 patent’s priority date is no earlier than October 20, 2015, because that is when Patent Owner first provided support for certain elements of independent claim 1.

52. But even if the '035 patent's priority date is May 18, 2011 (the earlier U.S. non-provisional filing date)—or even as early as May 18, 2010 (the earliest U.S. provisional filing date)—in my opinion, the challenged claims still would be obvious and unpatentable over at least Pieprzyk, May, Porreca, Hoyal, and Wessler for the reasons I set forth below.

VII. PERSON OF ORDINARY SKILL IN THE ART

53. I understand that claims of a patent are construed from the perspective of a POSA at the time 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 time of the claimed invention.

54. 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.

55. 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.

56. In my opinion, a POSA would stay apprised of recent literature on genomic assays, such as multiplex PCR, next-generation nucleic acid sequencing techniques, 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.

57. It is my opinion, as set forth herein, that all of the claims of the '035 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 '035 patent is no earlier than October 20, 2015, whereas I understand that Patent Owner has asserted in the co-pending litigation that the critical date is by May 18, 2011. A POSA's qualifications would have been, for the purposes of this proceeding, roughly the same in 2011 as in 2015, or even as early as 2010.

VIII. TECHNOLOGY BACKGROUND

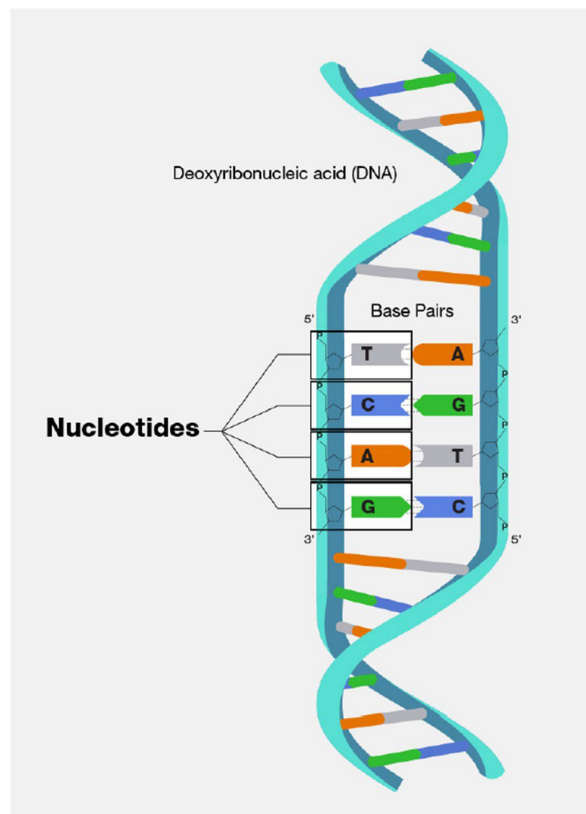
58. I summarize below the state of the art by 2010, which is before the 2011 critical date of the '035 patent as asserted by Patent Owner. In this summary, I sometimes cite references that were published after that date. These citations reflect the state of the art by 2010, and they are not meant to suggest or invoke developments in the art after the critical date of the '035 patent.

B. Cell-Free DNA

59. 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.)

60. 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.)

61. Each strand of DNA has a 5' end and a 3' end. DNA synthesis occurs in the 5' to 3' direction. (Ex.1053, 2.) The complementary nature of DNA

has great significance in biological techniques such as polymerase chain reaction (“PCR”) and DNA sequencing that rely on the binding of complementary strands together, a process called hybridization, and the synthesis of a second strand of DNA complementary to a first strand that acts as a template for the synthesis. (*See id.* (“The annealing of complementary nucleotide sequences, a process called hybridization, forms the basis of several DNA technologies that helped launch the biotechnology industry and modern genomics.”).)

62. 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.)

63. DNA located outside of the cells is referred to as cfDNA and can be extracted from certain bodily fluids, such as blood plasma. (Ex.1005, 2, 11.) cfDNA has been known since 1947 and its presence has been correlated to illnesses, including cancer, since the 1970s. cfDNA may also be referred to as free-floating DNA. 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.”); Ex.1061, ¶ 3 (“Tumor cells can often be found in the circulation of individuals with advanced cancers.”); Ex.1055, 2 (“The cellular origin of the extracellular DNA was not determined until the 1980s, when Stroun and Anker showed that circulating free DNA was derived from tumor cells. They showed that certain characteristics of tumor DNA were also present in circulating DNA.”); Ex.1062, 191 (“Cell-free DNA is supposed to be released into the blood circulation by apoptotic and necrotic cells of the primary tumor early during tumorigenesis. Moreover, it has been suggested that living cells may actively release their DNA into the blood.”).)

64. In the late 1980s, Lo discovered that the blood plasma of pregnant women contains circulating fetal DNA. (Ex.1055, 2.) This opened up a new field of investigation of noninvasive prenatal diagnosis, which enabled clinicians to determine fetal gender, assess chromosome ploidy, and monitor for pregnancy-related complications. This was a particularly important development because it presented an alternative to amniocentesis, which poses risks to fetal and maternal

health. Because of the immediate clinical significance, there were strong incentives to pursue research in this area and many academic and commercial investigators alike engaged in this research.

65. cfDNA also has clinical significance to the treatment of cancer. “[W]ork in the 1990s showed that oncogene mutations, loss of heterozygosity (LOH), and microsatellite shifts can be found in the plasma and serum matching those occurring in primary tumors.” (Ex.1055, 2.)

66. Cancers may be diagnosed and monitored by detecting and analyzing the presence of circulating tumor DNA (“ctDNA”), as well as any changes in the ctDNA. (See Ex.1063, 1:19-28; Ex.1061, Abstract (“We apply a highly sensitive approach to quantify circulating tumor DNA (ctDNA) in body samples of patients.”); Ex.1061, ¶ 3 (“It has been shown that tumor-derived mutant DNA can also be detected in the cell-free fraction of the blood of people with cancer.”); *see also* Ex.1064, Abstract (“[M]ethods of detecting and monitoring extracellular mutant oncogenes or tumor-associated DNA found in the plasma or serum fraction of blood”); Ex.1065, 3; Ex.1066, 2 (“Detection of microsatellite alterations in plasma DNA and serum DNA was first described in small cell lung cancer (SCLC) patients, and head and neck cancer patients, respectively. Subsequently, microsatellite alterations have been identified in the plasma or serum of patients suffering from clear cell renal carcinoma, colorectal cancer, non-

small-cell lung cancer (NSCLC), melanoma, ovarian cancer, breast cancer, lung cancer, acute myeloid leukemia and myelodysplasia. In many of these studies, the levels and persistence of the microsatellite alterations were found to have prognostic correlations with disease progression or recurrence.”) (internal citations omitted).) I note that three of these citations, namely Ex.1061, Ex.1063 and Ex.1064, are cited in the Van Ness Declaration submitted in co-pending litigation.

67. cfDNA may be analyzed to determine: 1) whether tumor DNA is present, thus showing a recurrence or new tumor development, 2) how much tumor DNA is present, which can indicate the cancer’s severity or reaction to treatment, and 3) the differences within the cfDNA collected, which further indicates tumor progression. (Ex.1062, 195; Ex.1067, 36:885-92 (abstract).)

C. Single Nucleotide Variants (SNVs) And Single Nucleotide Polymorphisms (SNPs)

68. DNA variants can occur naturally (hereditary differences between people) or through mutations to an individual’s DNA. Cancer is associated with mutations in DNA. These mutations can affect large or small portions of DNA. (Ex.1068, 1-3 (discussing deletions of spans of DNA associated with lung cancer); Ex.1060, 5-6 (discussing single nucleotide mutations and structural variants, such as deletions of nucleotide spans).)

69. Mutations that affect a single nucleotide are called single nucleotide variants (“SNVs”) or single nucleotide polymorphisms (“SNPs”). SNVs and SNPs

are both single nucleotide variations in DNA. (Ex.1069, Abstract; Ex.1060, 5-6.) For example, if the DNA of a healthy individual includes a TAGTGC nucleotide sequence, then the DNA of an individual diagnosed with cancer may instead include a TAGCGC sequence (i.e., C instead of T at the fourth nucleotide position).

70. Somatic SNVs are generally defined as single nucleotide mutations that differ from the individual's germ-line (original) DNA, and are often identified in cancerous DNA. (Ex.1069, Abstract (“Somatic (cancer-only) single nucleotide variants (SNVs) are the simplest class of mutation, yet their identification in DNA sequencing data is confounded by germline polymorphisms, tumour heterogeneity and sequencing and analysis errors.”).)

71. The '035 patent defines SNP as “a single nucleotide that may differ between the genomes of two members of the same species. The usage of the term should not imply any limit on the frequency with which each variant occurs.” (Ex.1001 at 34:46-50.; *see also* Ex.1116, 8, Lela Buckingham & Maribeth L. Flaws, *Molecular Diagnostics Fundamentals Methods and Clinical Applications* (2007).) Single nucleotide changes in a person's germline DNA are typically referred to as SNPs in the art, including in 2010 and before. (Ex.1070, Abstract, 5 (discussing “detecting genetic variation” and SNPs); Ex.1071, Abstract (discussing SNPs in human height variation); *see also id.* (“Single nucleotide

polymorphisms (SNPs)...account for only a small fraction of the genetic variation of complex traits in human populations....We estimated the proportion of variance for human height explained by 294,831 SNPs genotyped on 3,925 unrelated individuals....We show that 45% of variance can be explained by considering all SNPs simultaneously.”.)

D. Multiplex PCR

72. 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.1074, 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.*, ¶29.) 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.1075, Abstract.) Multiplex PCR also reduces the likelihood of missing rare molecules because it avoids the need to partition the sample into individual reactions, each of which will start with less genetic material. (Ex.1005, 4 (“Performing single-plex amplification with each of these primer pairs would require dispersing the initial sample into many separate reactions, considerably increasing the probability of sampling errors and allelic loss.”).)

73. Multiplex PCR has been well-known since long before 2010. The '035 patent cites references showing multiplex amplification as early as 2000, and describes that 100 loci could be multiplexed in a single reaction as of its purported priority date. (Ex.1001, 85:14-16 (“Methods described in the prior art used to multiplex more than 50 or 100 [loci] in one reaction volume followed by sequencing.”); *id.*, 48:25-29 (“Also note that the general belief in the art is that multiplexing PCR for sequencing is limited to about 100 assays in the same well. Fluidigm and Rain Dance offer platforms to perform 48 or 1000s of PCR assays in parallel reactions for one sample.”); *id.*, 210:35-211:43 (Experiment 4 using 800-plex amplification of DNA).)

74. As another example, U.S. Patent Application No. 13/300,235 (“the '235 application”), related to the '035 patent and filed and published in 2011 and 2012, respectively, discloses multiplexing greater than “500 loci simultaneously.” (Ex.1076, ¶267 (“Whereas traditional multiplex PCRs evaluate up to fifty loci simultaneously, the approach described herein may be used to enable simultaneous evaluation of more than...500 loci simultaneously”); *see also id.*, ¶303 (“Prior art demonstrates that ordinary people skilled in the art typically design 100+ bp amplicons...In an embodiment, the amplicons may be designed to be less than 50 bp.”); *id.*, ¶¶515-16 (Experiment 4 using 800-plex amplification of DNA).)

75. The prior art more generally establishes that multiplex PCR was well-known in the art. For example, Kaper *et al.*, “Parallel Preparation of Targeted Resequencing Libraries from 480 Genomic Regions Using Multiplex PCR on the Access Array™ System,” a poster presented at the American Association for Cancer Research (AACR) 101st Annual Meeting in 2010, teaches the use of an “Access Array™” system for scaling up PCR capabilities that comes with 48 sample inputs with 48 primer sets and, thus, performs multiplex PCR. (Ex.1077; Ex.1078.) Kaper further teaches the multiplex amplification of 480 cancer gene exons, where 48 primer pools of 10 primer pairs are used for the reactions, and where the PCR products are then sequenced. (Ex.1078, Fig. 2.) May *et al.*, “Parallel Production of Barcoded Amplicon Libraries for 454 Sequencing using Individual and Multiplex PCR in Nanoliter Volumes,” another poster presented at the American Association for Cancer Research (AACR) 101st Annual Meeting in 2010, uses a similar technique to achieve multiplex PCR. (Ex.1079.)

76. Multiplex PCR reactions involving multiplexing thousands of DNA locations across thousands of sequences were also well-known in the art. (Ex.1080, ¶214 (“multiplex amplifications permit the simultaneous amplification of...as many as tens, hundreds, thousands, or even more polynucleotide sequences of interest.”); Ex.1081, ¶119 (“The pre-preamplification can be carried out in

multiplex [sic]. For example, target-specific primers for 9216 different target nucleic acids can be employed in one mixture.”); *id.*, ¶175 (“these methods are applicable to identifying the presence of particular polymorphisms (such as SNPs), alleles, or haplotypes, or chromosomal abnormalities, such as amplifications, deletions, or aneuploidy”); Ex.1082, 4-5 (describing “nested patch PCR” capable of amplifying 90 gene fragments in the same reaction and useful for amplifying 100-1000 DNA fragments); Ex.1074, Abstract, ¶57 (“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” preferably with “at least 100 targets.”); Ex.1083, 1 (“A high-throughput genotyping system for scoring single nucleotide polymorphisms (SNPs) has been developed. With this system, >1000 SNPs can be analyzed in a single assay.”).)

77. By 2005, these kinds of multiplex PCR reactions were commercialized by several companies:

Very recently, the universal tail concept was applied by a few newly developed systems (Yeakley et al. 2002; Hardenbol et al. 2003; Kennedy et al. 2003; Fan et al. 2004; Matsuzaki et al. 2004). With these systems, universal sequences are attached to the amplicons with different approaches before PCR, allowing >1000 SNP-containing sequences to be amplified in a single reaction. This advance has significantly facilitated high-throughput genotyping. The technique initially described by Yeakley et al. (2002) has been commercialized by Illumina and used in ~65% of the HapMap project (<http://www.hapmap.org>). The technologies described by Hardenbol et

al. (2003) and Kennedy et al. (2003) have also been commercialized and used in largescale genetic analyses (Butcher et al. 2004; Zhou et al. 2004).

(Ex.1083, 2.)

78. Well before 2010, various DNA polymerases were commercially available for use in PCR reactions, including polymerases that were able to amplify DNA fragments while preventing mispriming and primer dimers formation.

(Ex.1084, 1-2 (introduction).) For example, the “Phusion” Hot Start DNA Polymerase, commercially available from Thermo Scientific was designed to “overcome or reduce problems that were limiting PCR development.” (Ex.1085, 2.) The Hot Start technology improved DNA polymerase processivity and fidelity, and reduced nonspecific binding in the form of primer dimers. (Ex.1086.) The first version of this technology was available in 2006 and the second version was available in 2009. (Ex.1085, 3.)

E. PCR Amplification

79. 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. The '035 patent discusses using various PCR methods to amplify DNA.

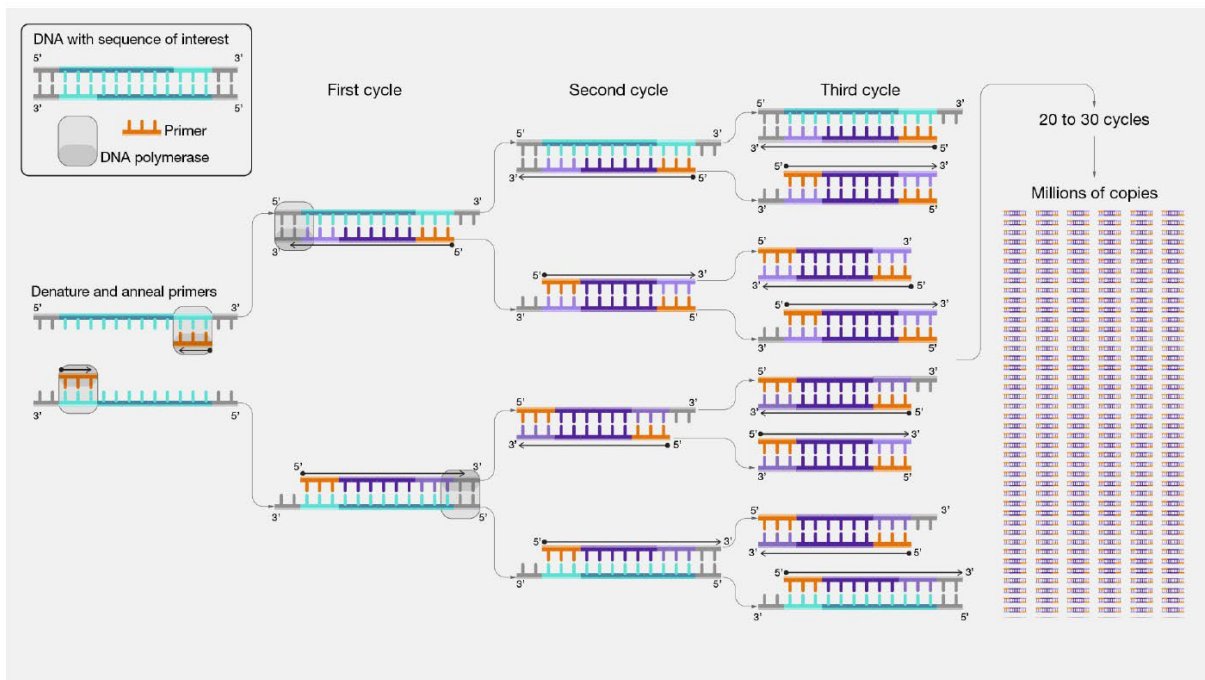
80. As described in further detail below, a typical PCR cycle requires three steps: 1) denaturing the double-stranded DNA to form single-stranded DNA, 2) annealing primers to the resulting single-stranded DNA molecules, and 3) primer extension using the single-stranded DNA molecules as templates for the complementary strands formed. (Ex.1087, 2.)

81. First, denaturation is the separation of the double-stranded DNA by using heat or through other chemical or physical means. (Ex.1087, 2.)

82. Second, annealing primers is the process of binding primers through base pairing to the complementary single-stranded DNA template strands that result from denaturation. Primers can be designed to bind to almost any sequence of DNA. Generally, PCR uses pairs of primers where each individual primer in a pair binds to a complementary sequence at the 3' end of each of the complementary single-stranded DNA molecules, and where each of the single-stranded DNA molecules of the double-stranded DNA can then act as a template for extension of the primer in the 5' to 3' direction to form a further complementary DNA strand. (Ex.1087, 2.)

83. Third, primer extension is the process wherein the annealed primers are extended in the 5' to 3' direction by an enzyme, typically a form of DNA

polymerase. In extending the primers, the DNA polymerase synthesizes new strands of DNA complementary to the single-stranded template strands, forming double-stranded DNA molecules. (Ex.1087, 2.) In extending the primers in the 5' to 3' direction, DNA polymerase synthesizes the new strands by the addition of nucleotides to the 3' end of the annealed primer. This process may be repeated in multiple PCR cycles, as shown below:



(Ex.1088, 1.)

84. PCR was well-established and well-known in the art prior to 2010.

(See, e.g., Ex.1089, 30:20-31:17; Ex.1087.)

F. Sequencing

85. DNA sequencing is a process wherein the sequence of bases in DNA (i.e., the order of bases in a DNA strand) is determined.

86. In 1977, Frederick Sanger developed a dideoxy chain termination method of DNA sequencing (now called Sanger sequencing). (Ex.1090, 1-3.) Sanger sequencing is based on parallel polymerase extension reactions, with each incorporating a different dideoxynucleotide analog (*i.e.*, of the A, C, G, and T bases) into extending primer strands, where incorporation of a dideoxynucleotide analog prevents addition of a further nucleotide to a DNA strand, terminating extension. (*Id.*) The resulting chains can be separated based on size using gel electrophoresis to determine the sequence. (*Id.*) The amount of nucleotide sequences that could be determined was limited, and the cost was relatively high. (*See id.*, 3-5.)

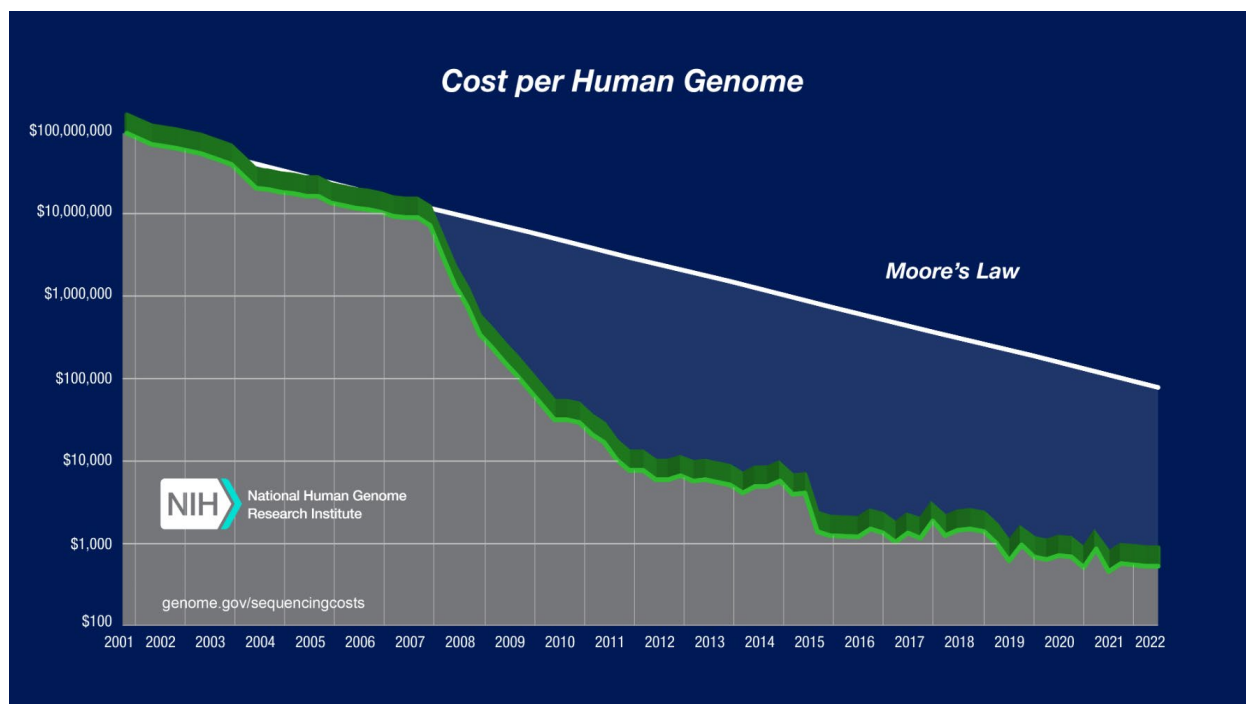
87. In the early 2000s, massively parallel sequencing technologies emerged that allowed for higher throughput of DNA and lower cost. (Ex.1091, 2; Ex.1092, Abstract.) At the time, these technologies were referred to as next-generation sequencing. By approximately 2008, NGS was commercially available, including through the Roche/454 FLX, Illumina/Solexa Genome Analyzer and Applied Biosystems SOLiD System. (Ex.1091, 2; Ex.1093, 2.)

88. 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.1093, 2.) These sequence reads, the individual DNA base sequences, must then be aligned (mapped) so that the

sequences cover the full region of interest, thus providing the sequence of the full region rather than disparate parts of the region. This is achieved through alignment programs, which match up the overlapping parts of the individual reads into a cohesive DNA sequence for that region. (*Id.*, 8.) 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. (*Id.*, 2.)

89. Sequencing was also well-known in the art to be useful for cancer diagnostics and prognostics. (Ex.1094.)

90. 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 tens of thousand dollars in 2010, and still dropping at a rate exceeding Moore's law. (Ex.1095; Ex.1096; Ex.1097, 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.1095, 2 (*see* at 2010).)

91. It was also well-known in the art to apply NGS methods for analysis of cfDNA. In 2008 Chiu *et al.* applied “...massively parallel genomic sequencing to quantify maternal plasma DNA sequences for the noninvasive prenatal detection of fetal trisomy 21” (Ex.1098, Abstract), noting that such a “locus-independent method” of sequence analysis permitted “...potentially every DNA fragment...[to] contribute to the measurement of the amount of that chromosome” (*id.*, 2.) NGS thereby ensured that “for any fixed volume of maternal plasma, the number of quantifiable sequences would be much greater than the number of DNA molecules that could serve as templates for detection by gene locus-specific assays.” (*Id.*) Also in 2008, Korshunova *et al.* disclosed use of massively parallel bisulphite sequencing for “the detection of DNA with cancer-

associated changes (mutations, methylation, rearrangements) in the serum of patients.” (Ex.1099, 1.) 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 ’035 patent.

92. I understand that Dr. Metzker has summarized the state of the art in a declaration supporting Natera against Illumina in IPR2018-01317. His conclusions relating to what was known in the art are similar to mine. For example, in Paragraph 116 of his declaration, Dr. Metzker stated that “it was further well-known by 2008 that one could isolate, amplify and sequence cell-free genomic DNA from maternal blood to detect aneuploidy, including fetal trisomy 21.” (Ex.1100, ¶ 116 (internal citations removed).) Although that sentence refers to cfDNA from maternal blood, Dr. Metzker cites to paragraph [0175] of Mir (Ex.1081), which refers to applications well-beyond fetal diagnostics. (*Id.*) Mir states that its methods are widely applicable to nucleic acid analysis in various contexts, and the very next paragraph of Mir explicitly contemplates using these methods “in the diagnosis and/or prognosis of genetic defects and diseases, such as cancer.” (Ex.1081, [0176].)

IX. THE CHALLENGED CLAIMS OF THE ’035 PATENT DO NOT HAVE WRITTEN DESCRIPTION SUPPORT BEFORE 2015

93. I understand that patent claims may be entitled to the filing date of prior applications provided that the claims have written description support in the

prior application(s). As discussed above, I have set forth my understanding of what constitutes written description support.

94. I have reviewed portions of the file history for the application that became the '035 patent and portions of the patent applications to which the '035 patent claims priority. The portions that I reviewed are set out in this declaration.

95. In my opinion, based on the amendments made to the independent claim of the '035 patent during prosecution, the claims are entitled to a priority date no earlier than October 20, 2015.

96. I understand that, while prosecuting the patent application that issued as the '035 patent, Patent Owner amended claim 1 on March 3, 2021 to overcome a final rejection of anticipated by Chuu. (Ex.1003, 626, 635-36.) The amendments are shown below as underlined text.

1. A method for amplifying and sequencing DNA, comprising:
tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products, wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;
amplifying the tagged products one or more times to generate final amplification products, wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume, wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and
sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products, wherein the plurality of SNP loci comprises 25-2,000 loci

associated with cancer.

(Ex.1003, 626.)

97. As shown above, Patent Owner added three limitations: “in a single reaction volume,” “not a pregnant women,” and “associated with cancer.” The “not a pregnant women” and “associated with cancer” amendments together form what I refer to as the “cancer limitation.” More specifically, the cancer limitation can be articulated as requiring cfDNA isolated from a blood sample collected from a subject who is not a pregnant woman and amplification of regions of the cfDNA containing 25-2,000 SNPs associated with cancer.

A. Example 27, Example 28, And Example 29 Provide The Only Potential Support For The Cancer Limitation

98. In amending claim 1, Patent Owner cited a number of paragraphs and examples that purportedly support the cancer limitation. I was asked to review these paragraphs to form an opinion on whether they adequately support the cancer limitation. I understand that, as support for the amendments, Patent Owner cited “paragraphs 0041, 0082, 0388, 0397, 0450, 0956, and Examples 26-29” of the published application (US202000347454A1). (Ex.1003, 628.)

99. Having considered the disclosure of the published applications in view of the meaning of the terms used in the cancer limitation as a POSA would understand them, and applying Patent Owner’s constructions, I conclude that the

cancer limitation is only possibly supported by Examples 27, 28, and 29 (if at all).

I will address each of the cited paragraphs and examples in turn.

100. Paragraph [0041] of the published application (US20200347454A1) provides:

In various embodiments of any of the aspects of the invention, the selection method selects candidate primers and divides them into different pools (e.g., 2, 3, 4, 5, 6, or more different pools). Each pool can be used to simultaneously amplify a large number of target loci (or a subset of target loci) *in a single reaction volume*. In some embodiments, a graph coloring algorithm is used to divide candidate primers into different pools. If desired, this method can be used to minimize the number of different pools needed to amplify most or all of the target loci.

(Ex.1101, ¶41 (emphasis added).)

101. I have been informed that this disclosure appeared first in Patent Owner's U.S. Provisional Application No. 61/994,791, filed on May 16, 2014. A POSA would understand this passage to be directed to the single reaction volume limitation of claim 1 of the '035 patent. A POSA would not understand this passage to support the cancer limitation.

102. Paragraph [0082] of the published application (US20200347454A1) provides:

In one aspect, the invention features methods of quantitatively measuring a plurality of genetic targets in a sample for analysis. In some embodiments, the method includes (i) mixing genetic material derived from the sample for analysis with a plurality of target specific

amplification reagents, and a plurality of standard sequences corresponding to the target specific amplification reagent targets; (ii) amplifying target regions of the genetic material and the standard sequences to produce target amplicons and standard sequence amplicons; and (iii) measuring the quantity of target amplicons and standard sequence amplicons produced. In some embodiments, the genetic material is present in a genetic library. In some embodiments, the genetic targets are polymorphic loci (such as SNPs). In some embodiments, the measuring of quantity is achieved by counting sequences. In some embodiments, the method further includes determining the estimated copy number of at least one chromosome in a sample from which the genetic library was derived, wherein the determination involves comparing the number of sequence reads of a target amplicon with the number of sequence reads of a standard amplicon. In some embodiments, the standard sequences and the genetic library include universal priming sites cable of being primed by the same primer. In some embodiments, the mixing step includes at least 10; 100, 500; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target specific amplification reagents and at least 10; 100, 500; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 standard sequences. In various embodiments, the method involves using any of the primer libraries of the invention. In various embodiments, the method involves simultaneously amplifying at least 25; 50; 75; 100; 300; 500; 750; 1,000; 2,000; 5,000; 7,500; 10,000; 15,000; 19,000; 20,000; 25,000; 27,000; 28,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target regions. In some embodiments, the relative amounts of each of the standard sequences are known. In some embodiments, the relative amounts of each of the sequences is has been calibrated with respect to a reference genome. ***In some embodiments, the sample for analysis includes a mixture of fetal and maternal genomes. In some embodiments, the sample for analysis is derived from the blood of a pregnant woman or derived from blood plasma. In some embodiments, the reference genome has***

at least one aneuploidy, such as an aneuploidy at chromosome 13, 18, 21, X, or Y. In some embodiments, the reference genome is diploid.

(Ex.1101, ¶82 (emphasis added).)

103. I have been informed that this disclosure first appeared in U.S. Patent Application No. 13/683,604, filed on November 21, 2012. A POSA would not understand this passage to support the cancer limitation. The amplification is not of cfDNA “isolated from a blood sample collected from a subject who is not a pregnant women.” (Ex.1001 (claim 1).) On the contrary, a POSA would understand “the sample for analysis is derived from the blood of a pregnant woman or derived from blood plasma” as referring to maternal blood or maternal plasma. Additionally, aneuploidy in the listed genomic regions are traditionally tested in prenatal testing. This is further supported by the reference to “a mixture of fetal and maternal genomes,” which a POSA would understand referred to genomes tested using samples of maternal blood or blood plasma.

104. Paragraph [0388] of the published application (US20200347454A1) provides:

In an embodiment, highly multiplex PCR followed by evaluation of amplified material by clonal sequencing may be used for various applications such as the detection of fetal aneuploidy. Whereas traditional multiplex PCRs evaluate up to fifty loci simultaneously, the approach described herein may be used to enable simultaneous evaluation of more than 50 loci simultaneously, more than 100 loci simultaneously, more than 500 loci simultaneously, more than 1,000

loci simultaneously, more than 5,000 loci simultaneously, more than 10,000 loci simultaneously, more than 50,000 loci simultaneously, and more than 100,000 loci simultaneously. 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. Assays may be combined in a single reaction with the entirety of a sample such as *a cfDNA sample isolated from maternal plasma*, a fraction thereof, or a further processed derivative of the cfDNA sample. The sample (e.g., cfDNA or derivative) may also be split into multiple parallel multiplex reactions. The optimum sample splitting and multiplex is determined by trading off various performance specifications. Due to the limited amount of material, splitting the sample into multiple fractions can introduce sampling noise, handling time, and increase the possibility of error. Conversely, higher multiplexing can result in greater amounts of spurious amplification and greater inequalities in amplification both of which can reduce test performance.

(Ex.1101, ¶388 (emphasis added).) I have been informed that this disclosure first appeared in U.S. Provisional Application No. 61/542,508, filed on October 3, 2011. A POSA would understand this passage to be directed to the single reaction volume limitation of claim 1 of the '035 patent, but not to the cancer limitation. The disclosure is directed to cfDNA isolated from maternal plasma.

105. Paragraph [0397] of the published application (US20200347454A1) provides:

In an embodiment, a multiplex pool of PCR assays are designed to amplify potentially heterozygous SNP or other polymorphic or non-polymorphic loci on one or more chromosomes and these assays are *used in a single reaction to amplify DNA*. The number of PCR assays

may be between 50 and 200 PCR assays, between 200 and 1,000 PCR assays, between 1,000 and 5,000 PCR assays, or between 5,000 and 20,000 PCR assays (50 to 200-plex, 200 to 1,000-plex, 1,000 to 5,000-plex, 5,000 to 20,000-plex, more than 20,000-plex respectively). In an embodiment, a multiplex pool of about 10,000 PCR assays (10,000-plex) are designed to amplify potentially heterozygous *SNP loci on chromosomes X, Y, 13, 18, and 21 and 1 or 2 and these assays are used in a single reaction to amplify cfDNA obtained from a material plasma sample, chorion villus samples, amniocentesis samples, single or a small number of cells, other bodily fluids or tissues, cancers, or other genetic matter*. The SNP frequencies of each locus may be determined by clonal or some other method of sequencing of the amplicons. Statistical analysis of the allele frequency distributions or ratios of all assays may be used to determine if the sample contains a trisomy of one or more of the chromosomes included in the test. In another embodiment the original cfDNA samples is split into two samples and parallel 5,000-plex assays are performed. In another embodiment the original cfDNA samples is split into n samples and parallel ($\sim 10,000/n$)-plex assays are performed where n is between 2 and 12, or between 12 and 24, or between 24 and 48, or between 48 and 96. Data is collected and analyzed in a similar manner to that already described. Note that this method is equally well applicable to detecting translocations, deletions, duplications, and other chromosomal abnormalities.

(Ex.1101, ¶397 (emphases added).) I have been informed that this disclosure first appeared in U.S. Patent Application No. 13/300,235, filed on November 18, 2011.

106. A POSA would not understand the foregoing passage to support the cancer limitation. The paragraph discloses a list of samples from which genetic material could be obtained and then analyzed in diagnostic methods, including “cfDNA from a plasma sample, chorion villus samples, amniocentesis samples,

single or a small number of cells, other body fluids or tissues, cancers, or other genetic material.” In my opinion, it does not disclose collecting cfDNA from each of those samples. For example, it would not make sense to read this passage to refer to collecting cfDNA from chorion villus samples, which are biopsies of placental tissue and do not contain cfDNA. Nor would it make sense to read this passage as collecting cfDNA from “a small number of cells.” And even if the passage did refer to collecting cfDNA from each listed source, it still would not support isolation of cfDNA “from a blood sample collected from a subject who is not a pregnant women [sic].” (Ex.1001 (claim 1).) Cancer is a group of diseases; it is not a biological material like the other listed samples. Finally, I note that SNP loci on the majority of the recited chromosomes are traditionally amplified to diagnose aneuploidy in prenatal testing, not to diagnose cancer.

107. Paragraph [0450] of the published application (US20200347454A1) provides:

Improved PCR amplification methods have also been developed that minimize or prevent interference due to the amplification of nearby or adjacent target loci ***in the same reaction volume (such as part of the sample multiplex PCR reaction that simultaneously amplifies all the target loci)*** (see, U.S. Ser. No. 61/982,245, filed Apr. 21, 2014; U.S. Ser. No. 61/987,407, filed May 1, 2014, and U.S. Ser. No. 62/066,514, filed Oct. 21, 2014, which are each hereby incorporated by reference in its entirety). These methods can be used to simultaneously amplify nearby or adjacent target loci, which is faster and cheaper than having to separate nearby target loci into different reaction volumes so that

they can be amplified separately to avoid interference. In particular embodiments, these methods are used to tile a region such that the amplicons include all the nucleotides in the region (such as an exon or all the exons of a gene such as cystic fibrosis).

(Ex.1101, ¶450 (emphasis added)).

108. I have been informed that this disclosure first appeared in U.S. Patent Application No. 14/538,982 filed on November 11, 2015. A POSA would understand this passage as justifying the use of multiplexed PCR. A POSA would not understand this passage to support the cancer limitation.

109. Paragraph [0956] of the published application (US20200347454A1) provides:

The following Example illustrates an exemplary method for designing and selecting primers that can be used in any of the multiplexed PCR methods of the invention. In some embodiments, the primers are divided into different pools (e.g., 2, 3, 4, 5, 6, or more different pools) such that each pool is used to amplify target loci in a different reaction volume. ***Each pool is used to simultaneously amplify a large number of target loci (or a subset of target loci) in a single reaction volume.*** Preferably, primers are designed and selected for a large portion of the most desirable target loci or for all of the target loci. A set of candidate target loci can be selected as described in Examples 16 or 20 based on the particular polymorphisms or mutations of interest. In some embodiments, one or more of the following type of target loci are included: SNPs, short indels, long indels, exons, and combinations thereof. In some embodiments for target loci that are short indels, the PCR primer or primer pair targets a sequence of adjacent base pairs; and the indel is completely covered by one sequencing read. In some embodiments for target loci that are large indels, two primer pairs are used to target a pair of breakpoints at the boundaries of the indel. In this

case, the two primer pairs are designed such that when the deletion is present there is a PCR product and the two primer pairs are selected together for inclusion in the same pool (the four primers are treated by the algorithm as a single assay rather than two assays). In some embodiments for target loci that are exons, a set of primers pairs are designed to tile the full exon.

(Ex.1101, ¶956 (emphasis added).) I have been informed that this disclosure first appeared in U.S. Provisional Application No. 61/994,791, filed on May 16, 2014.

A POSA would understand this paragraph to be directed to amplifying a large number of target loci in a single reaction volume. I also understand this paragraph to set up the primer selection example that follows. A POSA would not understand this passage to support the cancer limitation.

110. Example 26 of the published application (US20200347454A1) provides:

This example describes an exemplary method for detection of copy number variations in breast cancer samples using SNP-targeted massively multiplexed PCR. Evaluation of CNV in tumor tissues typically involves SNP microarray or aCGH. These methods have high whole-genome resolution, but require large amounts of input material, have high fixed costs, and do not work well on formaldehyde fixed-paraffin embedded (FFPE) samples. For this example, 28,000-plex SNP-targeted PCR with next generation sequencing (NGS) was used to target ip, 1q, 2p, 2q, 4p16, 5p15, 7q11, 15q, 17p, 2211, 2213 and chromosomes 13, 18, 21 and X for detection of CNVs in breast cancer samples. Accuracy was validated on 96 samples with aneuploidies or microdeletions. Single-molecule sensitivity was established by analyzing single cells. Of 17 *breast cancer samples (15 fresh frozen and 2 FFPE tumor tissues)*, 5 pairs of matched tumor and normal cell

lines) analyzed, 16 (including both FFPEs) were observed with full or partial CNVs in one to 15 targets (average: 7.8); evidence of tumor heterogeneity was observed. The three tissues with one CNV all had a 1q duplication, the most frequent cytogenetic abnormality in breast carcinoma. The most frequent regions with CNVs were 1q, 7p, and 221. Only one tumor tissue (with 9 CNVs) had a region with LOH; this LOH was also detected in adjacent putatively normal tissue that lacked the other 8 CNVs. By contrast, 5 or more regions with LOH and a high total CNV incidence (average: 12.8) was detected in cell lines. Thus, massively multiplexed PCR offers an economical high-throughput approach to investigate CNVs in a targeted manner, and is applicable to difficult-to-analyze samples, such as FFPE tissues.

(Ex.1101, ¶999 (emphasis added).)

111. A POSA would not understand this example to be support for the cancer limitation because the DNA is extracted from fresh frozen and formaldehyde fixed paraffin embedded breast cancer tumor tissue samples. Amplification of tumor DNA from a tumor tissue sample is distinct from amplification of “cell-free DNA . . . isolated from a blood sample collected from a subject who is not a pregnant women.” (Ex.1001 (claim 1).)

112. Example 27 of the published application (US20200347454A1) provides, in part:

We acquired matched buffy coat samples for eight patients and matched plasma samples for nine patients. FF tumour tissues and matched buffy coat and *plasma samples from five ovarian cancer patients* were from North Shore-LIJ. . . .

Blood samples were collected into EDTA tubes. Circulating cell free

DNA (containing ctDNA) was isolated from 1 mL plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Valencia, Calif.). Genomic DNA (gDNA) from FF tumor tissues, blood, and buccal samples was extracted using the DNeasy Blood and Tissue Kit (Qiagen)

An additional relevant part of Example 27 provides:

Multiplexed PCR allows simultaneous amplification of many targets in a single reaction. In this study, ***we targeted 3,168 SNPs, which were distributed across five chromosome arms as follows: 646 on 1p, 602 on 1q, 541 on 2p, 707 on 2q, and 672 on the 2211.2 focal region. These genomic regions were selected for convenience from SNP panels available in our laboratory. Target SNPs had at least 10% population minor allele frequency (1000 Genomes Project data; Apr. 30, 2012 release)*** to ensure that a sufficient fraction would be heterozygous in any given patient. For each SNP, multiple primers were designed to have a maximum amplicon length of 75 bp and a melting temperature between 54.0-60.5° C.

For PCR amplifications, 3,168 SNPs were amplified in a multiplex PCR reaction using one primer pair for each SNP, during 25 cycles, and sequencing barcodes were added in 12 additional cycles. Prior to sequencing, the barcoded products were pooled, purified with the QIAquick PCR Purification Kit (Qiagen), and quantified using the Qubit™ dsDNA BR Assay Kit (Life Technologies). Amplicons were sequenced using an Illumina HiSeq 2500 sequencer with 1.5-7 M reads/sample for tumor tissue DNA and 18-25 M reads/sample for plasma cfDNA.

An additional relevant part of Example 27 provides:

CNVs were identified by a maximum likelihood algorithm that searched for plasma CNVs in regions where the tumor sample from the same individual also had CNVs, using haplotype information deduced from the tumor sample. This algorithm modeled expected

allelic frequencies across a set of average allelic imbalances at 0.025% intervals for three sets of hypotheses: (1) all cells are normal (no allelic imbalance), (2) some/all cells have a homolog 1 deletion or homolog 2 amplification, or (3) some/all cells have a homolog 2 deletion or homolog 1 amplification. For at least some of the analysis, modeling was performed up to 15% average allelic imbalance, although for the vast majority of samples AAI was less than or equal to 5%. ***The likelihood of each hypothesis was determined at each SNP using a Bayesian classifier based on a beta binomial model of expected and observed allele frequencies at all heterozygous SNPs, and then the joint likelihood across multiple SNPs was calculated taking linkage of the SNP loci into consideration.*** The maximum likelihood hypothesis from the comparison of expected to observed allele frequencies was then selected.

This algorithm also calculates the confidence of each CNV call by comparing the likelihoods of different hypotheses. A confidence threshold of 99.9% was used in plasma samples to minimize false positive results.

(Ex.1101, ¶¶1002-03, 1008-10, 1016-17 (emphases added).)

113. A POSA might understand Example 27 to be potential support for the cancer limitation of claim 1 of the '035 patent. Example 27 describes isolation of cfDNA from plasma from five ovarian cancer patients. It also describes amplification and sequencing of 3,168 SNPs and an algorithmic model used to identify CNVs associated with cancer based on the presence of those SNPs.

114. Example 28 of the published application (US20200347454A1), provides, in part:

Plasma CNV Protocol for 3,168-Plex for Chromosomes 1p, 1q, 2p, 2q, and 22q11

Plasma DNA libraries were prepared and amplified using a mmPCR 3,168-plex protocol. The samples were prepared in the following way: Up to 20 mL of blood was centrifuged to isolate the buffy coat and the plasma. Plasma extraction of cfDNA and library preparation was performed. DNA was eluted in 50 uL TE buffer. The input for mmPCR was 6.7 uL of amplified and purified Natera plasma library at an input amount of approximately 1200 ng. The plasma DNA was amplified in a 20 uL reaction volume containing Qiagen mp-PCR master mix (2×MM final conc), 2 nM tagged primer conc. (total 12.7 uM) in 3,168-plex primer pools and PCR amplified: 95 C 10 min, 25×[96 C 30 sec, 65 C 20 min, 72 C 30 sec], 72 C 2 min, 4 C hold. The amplification product was diluted 1:2,000 in water and 1 ul added to the Barcoding-PCR in a 10 uL reaction volume. The barcodes were attached to the amplification products via PCR amplification for 12 cycles using tag specific primers. Products of multiple samples were pooled and then purified with QIAquick PCR Purification Kit (Qiagen) and eluted in 50 ul DNA suspension buffer. Samples were sequenced by NGS as described for the Single Cell CNV Protocol for 28,000-plex PCR.

Breast Cancer Feasibility SNV Panel from Plasma

cfDNA from breast cancer patient blood samples was prepared and amplified using 336 primer pairs that were distributed into four 84-plex pools. Natera plasma libraries were prepared as described for Plasma CNV Protocol for 3,168-plex for Chromosomes 1p, 1q, 2p, 2q, and 22q11. DNA was eluted in 50 uL TE buffer. The input for mPCR was 2.5 uL of amplified and purified Natera plasma library at an input amount of approximately 600 ng. ***SNPs were selected from the 1000 Genomes map for Humans, Group 19 and dbSNP to pick targets, but only SNPs from the 1000 Genomes were used to screen for minor allele frequencies. The plasma DNA was amplified in four parallel reactions of 84-plex primer pools,*** a 10 uL reaction volume containing

Qiagen mp-PCR master mix (2×MM final conc.), 4 mM EDTA, 7.5 nM primer concentration (total 1.26 uM) and PCR amplified: 95 C 15 min, 25×[94 C 30 sec, 65 C 15 min, 72 C 30 sec], 72 C 2 min, 4 C hold. The amplification product of the 4 subpools were each diluted 1:200 in water and 1 ul added to the Barcoding-PCR reaction in a 10 uL reaction volume containing Q5 HS HF master mix (1×final), and 1 uM each barcoding primer and each of the pools were amplified in the following reaction: 98 C 1 min, 25×[98 C 10 sec, 70 C 10 sec, 60 C 30 sec, 65 C 15 sec, 72 C 15 sec], 72 C 2 min, 4 C hold. Libraries were purified with QIAquick PCR Purification Kit (Qiagen) and eluted in 50 ul DNA suspension buffer. Samples were sequenced by paired end sequencing.

(Ex.1101, ¶¶1043-44 (emphases added).)

115. A POSA might understand Example 28 to be potential support for the cancer limitation of claim 1 of the '035 patent. The example describes isolation of circulating tumor DNA from plasma, which a POSA would understand as “isolate[ion of] cell-free DNA...from a blood sample collected from a subject who is not a pregnant women [sic].” (Ex.1001 (claim 1).)

116. As described above regarding Example 27 in paragraph 114 of this Declaration, Example 28 describes the same 3,168-panel used to detect CNVs associated with cancer.

117. Example 28 also describes using a separate panel of 336 primers distributed into four 84-plex pools. While Example 28 does not specify what types of mutations the 336 primer panel targeted, in combination with the disclosure of a similar panel in Example 29, a POSA would understand the panel to likely

comprise SNV primer pools. (Ex.1101, ¶1055 (“For each of four 84-plex SNV PCR primer pools we observed improved DOR efficiency with increasing cycles from 15 to 20 to 25.”)) Under Patent Owner’s construction of “SNP loci . . . associated with cancer,” a POSA might understand this 336 primer panel to support the cancer limitation.

118. Example 29 of the published application (US20200347454A1), provides, in part:

This example demonstrates that by 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, uniformity of density of reads, and therefore limits of detection, across a pool of amplification reactions is improved. *Some experiments were carried out for plasma CNV using the 3,168-plex panel according to Example 28 above* except that the total reaction volume was 10 uL instead of 20 uL. Furthermore, PCR was carried out for 15, 20, or 25 cycles. *Other experiments were carried out using the four 84-plex pools on breast cancer samples according to the protocol of Example 28* except that primer concentrations were 2 nM and PCR amplification was carried out for 15, 20, or 25 cycles.

(Ex.1101, ¶¶1045-46 (emphasis added).)

119. An additional relevant part of Example 29 provides:

For each of four 84-plex SNV PCR primer pools we observed improved DOR efficiency with increasing cycles from 15 to 20 to 25. Similar results were obtained for experiments using the 3,168-plex panel. *The limit of detection decreased (i.e. SNV sensitivity increased) with increasing depth of read.* Furthermore, the sensitivity was

consistently better when detecting transversion mutations than transition mutations. It is likely that additional increases in DOR efficiency can be obtained with additional cycles when using primer-limiting multiplex PCR before multi-read sequencing.

(Ex.1101, ¶1055 (emphasis added)).

120. A POSA might understand Example 29 to be potential support for the cancer limitation of claim 1 of the '035 patent. The experiment describes primer concentrations and PCR conditions. Reading this example in connection with the examples above, a POSA would understand that the use of the 3,168-plex panel for plasma might provide potential support for the cancer limitation.

121. As discussed above regarding the SNV panel described in Example 28, a POSA might also understand the four 84-plex SNV PCR primer panel in Example 29 to support the cancer limitation under Patent Owner's construction of "SNP loci . . . associated with cancer."

122. In my opinion, the 3,168-plex panels described in Example 27, Example 28, and Example 29 are the only disclosures that might provide potential written description support for the cancer limitation. The four 84-plex SNV panel described in Examples 28 and 29 would only provide written description support for the cancer limitation under Patent Owner's construction of "SNP loci . . . associated with cancer" as any single-point mutation in human DNA that is associated with cancer.

B. Examples 27, 28, And 29 Were New Matter Added In 2015 Without Which The Cancer Limitation Would Lack Written Description Support

123. I believe the first disclosure of Example 27, Example 28, or Example 29 of the '035 patent occurred when the Patent Owner added Example 27, Example 28, and Example 29 in U.S. Patent Application 14/918,544 ("544 application"), filed on October 20, 2015. (Ex.1102.)

124. I understand that versions of Example 27, Example 28, and Example 29 are included in some patent applications that Patent Owner filed in 2014 and 2015. For example, U.S. Provisional Application Nos. 62/066,514 (Ex.1105) and 62/148,173 (Ex.1106) and U.S. Patent No. 10,179,937 (Ex.1107). I have reviewed these examples. In my opinion, the previous versions of Example 27, Example 28, and Example 29 are different than the disclosures of Examples 27, Example 28 and 29 in the '544 application. For example, the previous versions of Example 27 do not disclose massively multiplexed PCR and DNA sequencing or the necessary data analysis steps for the circulating Tumor DNA experiments. (Ex.1107, (Example 8).) In addition, previous versions of Example 27 do not provide the comparative analysis of variation identified in tumor tissue and in plasma cfDNA in order to show the efficacy of the cfDNA approach. (*Id.*) The previous versions of Example 28 did not include the necessary data analysis steps for the circulating Tumor DNA experiments. (*Id.* (Example 9).) The previous

versions of Example 29 included data in the form of figures that are not included in the '544 application. (*Id.* (Example 14).)

125. As I mentioned above, 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 further understand that a claim with a limitation only described in disclosure added in a continuation-in-part (CIP) application will only have priority to the effective filing date of the continuation-in-part application. I understand that the '544 application is a continuation-in-part application and, thus, if the disclosure that supports the claims of the issued '035 patent first appeared in that CIP application, then the issued claim will only be entitled to the priority date of the CIP application.

126. Thus, it is my opinion that the claims of the '035 patent do not have written description support prior to the filing of the '544 CIP and are not entitled to priority before the filing date of the '544 CIP, October 20, 2015.

C. The Parent '925 Application

127. The prosecution history of a parent application, 14/877,925 ("'925 application") (Ex.1024 (excerpted)) which precedes Patent Owner's filing of the '544 CIP, also confirms my opinion regarding the '035 patent's priority date.

128. I understand that, during prosecution of the '925 application, Patent Owner amended that application's claim 1 as shown below with added text underlined and removed text crossed out.

1. (Currently Amended) A method for determining a ploidy status of a chromosome or chromosome segment of a cancer in a host, the method comprising:

measuring ~~the~~ free-floating DNA in a ~~mixed plasma sample of the host~~ that comprises both DNA from normal cells ~~from the host~~ and DNA from ~~the cancer~~ at a plurality of at least 1000 polymorphic loci on the chromosome or chromosome segment, by simultaneously amplifying the at least 1000 polymorphic loci to obtain amplicon and sequencing the amplicons;

determining allele counts at the plurality of polymorphic loci from the DNA measurements made on the sample;

fitting, on a computer, the determined allele counts to expected allele counts at the plurality of polymorphic loci for each of a plurality of ploidy hypotheses each specifying a different possible ploidy state of the chromosome or chromosome segment for the cancer, using a joint distribution model that takes into account ~~the~~ an expected linkage between the plurality of polymorphic loci on the chromosome or chromosome segment, to determine a relative probability of each the ploidy hypotheses; and

outputting the ploidy hypothesis with the greatest probability of being true as ~~an indication~~ determination of the ploidy status of the chromosome or chromosome segment of the cancer.

(Ex.1024, 32.)

129. The '925 application is relevant to this IPR proceeding because the measuring step of independent claim 1 of the '925 application is similar to what Patent Owner contends the '035 patent's cancer limitation covers (as shown below)

and because the Patent Office rejected the '925 application's measuring step limitation for lack of written description support. A side-by-side comparison of the '925 application's measuring step and the cancer limitation is shown below:

'925 Application (Claim 1)	'035 Patent Cancer Limitation
“measuring free-floating DNA in a plasma sample of the host that comprises both DNA from normal cells and DNA from cancer” and “by simultaneously amplifying the at least 1000 polymorphic loci to obtain amplicons and sequencing the amplicons	“isolated cell free DNA...collected from a subject who is not a pregnant women [and]...targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci...wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer”

130. **First**, I will address the similarity in claim scope (as asserted by Patent Owner) in the above table. In my opinion, a POSA would read “free-floating DNA” in abandoned claim 1 of the '925 application as covering the same subject matter as “cell-free DNA” in claim 1 of the '035 patent. A POSA would understand “free-floating DNA” to refer to extracellular DNA, in the same way that “cell-free DNA” refers to extracellular DNA. Although cfDNA may also refer to DNA in vesicles, exosomes, or apoptotic bodies that might not be considered truly “free-floating,” a POSA would understand these terms to be the same in this context, *i.e.*, in the context of analyzing DNA using the methods described in the application.

131. In my opinion, a POSA would read “plasma sample of the host” in abandoned claim 1 of the ’925 application as covering the same subject matter as “isolated from a blood sample” in claim 1 of the ’035 patent. A POSA would read these terms as covering the same subject matter because plasma is a component of blood. Although blood is comprised of plasma and other components, such as the buffy coat, a POSA would know to isolate plasma from blood in order to further isolate cfDNA.

132. In my opinion, a POSA would read “polymorphic loci” in abandoned claim 1 of the ’925 application as covering the same subject matter as “single nucleotide polymorphism” in claim 1 of the ’035 patent under Patent Owner’s construction of that term. Specifically, and under that construction, a POSA would read both “polymorphic loci” and “single nucleotide polymorphism” to cover mutations specific to tumor cells. Furthermore, applying Patent Owner’s understanding of “SNP loci . . . associated with cancer,” I believe that a POSA would understand “SNP loci . . . associated with cancer” to cover the same subject matter as “polymorphic loci” measured in “DNA from cancer.”

133. In my opinion, a POSA would read “by simultaneously amplifying” in abandoned claim 1 of the ’925 application as covering the same subject matter as “targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume” in claim 1 of the ’035 patent. A POSA would

understand amplification in a single reaction volume to be a method to perform simultaneous amplification.

134. **Second**, I have been informed that the Patent Office rejected the measuring step of the '925 application for lack of written description. (Ex.1024, 9-11.) Because the measuring step in claim 1 of the '925 application has the same scope as what Patent Owner contends its claims cover, the Patent Office's finding that the measuring step lacked written description support confirms my opinion that the cancer limitation lacks written description support before October 2015.

135. **Third**, in making the written description rejection, the Office considered paragraphs 0010, 0233, 0298, and 0569 cited by Patent Owner for support. (Ex.1024, 10, 48.) Because at least some of these paragraphs are also found in the '035 patent, their failure to provide support for the '925 application's measuring step also demonstrates that they do not provide support for the cancer limitation of the '035 patent. I address each of those paragraphs below.

136. Paragraph [0010] of the application (US14/877,925) provides:

In one aspect, the invention features methods of amplifying target loci in a nucleic acid sample. In some embodiments, the method involves (i) contacting the nucleic acid sample with a library of test primers that simultaneously hybridize to at least 1,000; 2,000; 5,000; 7,500; 10,000; 20,000; 25,000; 30,000; 40,000; 50,000; 75,000; or 100,000 different target loci to produce a reaction mixture; and (ii) subjecting the reaction mixture to primer extension reaction conditions to produce amplified products that include target amplicons. In some embodiments, the method also includes determining the presence or absence of at least

one target amplicon (such as at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5% of the target amplicons). In some embodiments, the method also includes determining the sequence of at least one target amplicon (such as at least 50, 60, 70, 80, 90, 95, 96, 97, 98, 99, or 99.5 % of the target amplicons).

(Ex.1024, 609-610.)

137. A POSA would not understand this paragraph to support “measuring free-floating DNA in a plasma sample of the host that comprises both DNA from normal cells and DNA from cancer.”

138. Paragraph [00233] of the application (US14/877,925) provides:

In some embodiments, the target DNA may originate from single cells, from samples of DNA consisting of less than one copy of the target genome, from low amounts of DNA, from DNA from mixed origin (*e.g.* pregnancy plasma; placental and maternal DNA: ***cancer patient plasma and tumors: mix between healthy and cancer DNA***, transplantation *ect*), from other body fluids, from cell cultures, from culture supplements, from forensic samples of DNA, from ancient samples of DNA (*e.g.* insects trapped in amber), from other samples of DNA, and combinations thereof.

(Ex.1024, 670 (emphasis added).)

139. A POSA would understand this paragraph to describe how healthy and cancer DNA can originate from patient plasma and tumors. A POSA would not understand this paragraph to disclose free-floating tumor DNA in patient plasma. Further, this paragraph does not disclose measuring DNA using the claimed method of amplifying 25-2,000 SNPs associated with cancer. Thus, I do

not believe this paragraph supports “measuring free-floating DNA in a plasma sample of the host that comprises both DNA from normal cells and DNA from cancer.”

140. I reviewed paragraph [00298] of the application (US14/877,925).

That paragraph is reproduced below:

Another way to increase the number of reads that interrogate the position of interest is to decrease the length of the probe, as long as it does not result in bias in the underlying enriched alleles. The length of the synthesized probe should be long enough such that two probes designed to hybridize to two different alleles found at one locus will hybridize with near equal affinity to the various alleles in the original sample. Currently, methods known in the art describe probes that are typically longer than 120 bases. In a current embodiment, if the allele is one or a few bases then the capture probes may be less than about 110 bases, less than about 100 bases, less than about 90 bases, less than about 80 bases, less than about 70 bases, less than about 60 bases, less than about 50 bases, less than about 40 bases, less than about 30 bases, and less than about 25 bases, and this is sufficient to ensure equal enrichment from all alleles. When the mixture of DNA that is to be enriched using the hybrid capture technology is a mixture comprising free floating DNA isolated from blood, for example maternal blood, the average length of DNA is quite short, typically less than 200 bases. The use of shorter probes results in a greater chance that the hybrid capture probes will capture desired DNA fragments. Larger variations may require longer probes. In an embodiment, the variations of interest are one (a SNP) to a few bases in length. In an embodiment, targeted regions in the genome can be preferentially enriched using hybrid capture probes wherein the hybrid capture probes are of a length below 90 bases, and can be less than 80 bases, less than 70 bases, less than 60 bases, less than 50 bases, less than 40 bases, less than 30 bases, or less than 25 bases. In an embodiment, to increase the chance that the desired

allele is sequenced, the length of the probe that is designed to hybridize to the regions flanking the polymorphic allele location can be decreased from above 90 bases, to about 80 bases, or to about 70 bases, or to about 60 bases, or to about 50 bases, or to about 40 bases, or to about 30 bases, or to about 25 bases.

(Ex.1024, 696-97.)

141. A POSA would understand this paragraph to describe methods for amplifying regions of DNA. A POSA would not understand this paragraph to support “measuring free-floating DNA in a plasma sample of the host that comprises both DNA from normal cells and DNA from cancer.”

142. Paragraph [00569] of the application (US14/877,925) provides:

Note that it has been demonstrated that DNA that originated from cancer that is living in a host can be found in the blood of the host. In the same way that genetic diagnoses can be made from the measurement of mixed DNA found in maternal blood, genetic diagnoses can equally well be made from the measurement of mixed DNA found in host blood. The genetic diagnoses may include aneuploidy states, or gene mutations. *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.*

(Ex.1024, 804-805 (emphasis added).)

143. In my opinion, this paragraph does not provide the necessary specificity to demonstrate that Patent Owner possessed claim 1 as modified by the cancer limitation. A POSA would understand this paragraph as a general claim

that methods developed for cfDNA fetal diagnostics could help in cancer diagnosis, but without the technical features necessary to achieve the method. Thus, I believe this paragraph without additional disclosures does not support “measuring free-floating DNA in a plasma sample of the host that comprises both DNA from normal cells and DNA from cancer.”

144. My conclusion that the cited paragraphs do not provide written description support is consistent with the determination of the Patent Office. After Patent Owner submitted the above amendment, I understand that the Examiner rejected the claim for lack of written description support, noting: “While the specification supports free floating fetal DNA in blood or plasma the cited [*sic*] sections for fetal DNA and review of the specification did not reveal support for, ‘measuring free-floating DNA in a plasma sample of the host that comprises both DNA from normal cells and DNA from cancer.’” (Ex.1024, 9-11.) I understand that Patent Owner abandoned the ’925 application (and thus claim 1) after this rejection.

D. No Disclosure Prior To 2015 Provides Written Description Support For The Cancer Limitation

145. I was asked to review certain paragraphs from patents and patent applications in the ’035 priority chain and assess whether they provide support for the ’035 cancer limitation.

146. As I mentioned above, I understand that written description requires a showing that the inventor was “in possession” of the invention by describing the invention, with all its claimed limitations.

147. I reviewed the below paragraph [0400] of the published application that became U.S. Patent No. 8,825,412.

Note that it has been demonstrated that DNA that originated from cancer that is living in a host can be found in the blood of the host. In the same way that genetic diagnoses can be made from the measurement of mixed DNA found in maternal blood, genetic diagnoses can equally well be made from the measurement of mixed DNA found in host blood. The genetic diagnoses may include aneuploidy states, or gene mutations. *Any claim in that patent 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.*

(Ex.1110, ¶400 (emphasis added).) This paragraph provides the same disclosure as paragraph [00569] of the '925 application, which Patent Owner unsuccessfully cited as disclosure supporting “measuring free-floating DNA in a plasma sample of the host that comprises both DNA from normal cells and DNA from cancer.”

(Ex.1024, 10, 40.) Thus, as explained above, I believe this paragraph does not provide support of the cancer limitation.

148. In addition, as I noted above, paragraph [0400] does not provide the necessary description to demonstrate Patent Owner possessed the cancer limitation.

During the prosecution of the '035 patent, Patent Owner amended claim 1 to exclude blood samples from pregnant women. It then remarked that “Chuu is directed to analysis of maternal plasma samples for detection of fetal aneuploidy, but fails to teach or suggest anything about amplifying and sequencing cell-free DNA isolated from a blood sample collected from a subject who is not a pregnant women [sic].” (Ex.1003, 629.) That is, Patent Owner argued that the remaining scope of the claim, i.e., amplifying and sequencing cfDNA isolated from a blood sample of a non-pregnant woman, was inventive and non-obvious. But that assertion is inconsistent with the statement in paragraph [0400], which provides no indication that anything is inventive about monitoring cfDNA in non-maternal applications.

149. I also reviewed the below paragraph [0401] of the published application that became U.S. Patent No. 8,825,412.

In some embodiments, the method may allow one to determine the ploidy status of a cancer, the method comprising obtaining a mixed sample that contains genetic material from the host, and genetic material from the cancer, ***measuring the DNA in the mixed sample, calculating the fraction of DNA that is of cancer origin in the mixed sample, and determining the ploidy status of the cancer*** using the measurements made on the mixed sample and the calculated fraction. In some embodiments, the method may further comprise administering a cancer therapeutic based on the determination of the ploidy state of the cancer. In some embodiments, the method may further comprise administering a cancer therapeutic based on the determination of the ploidy state of the cancer, wherein the cancer therapeutic is taken from

the group comprising a pharmaceutical, a biologic therapeutic, and antibody based therapy and combination thereof.

(Ex.1076, ¶401 (emphasis added).)

150. In my opinion, this paragraph alone or in connection to the preceding paragraph discussed above, does not provide support for the cancer limitation. A POSA would understand the paragraph to teach calculating the fraction of DNA that is cell-free derived from cancer in a sample, but without using the claimed method of amplifying 25-2,000 SNPs associated with cancer.

151. I also reviewed the below paragraph [0276] of the published application that became U.S. Patent No. 10,017,812.

In an embodiment, a multiplex pool of PCR assays are designed to amplify potentially heterozygous SNP or other polymorphic or non-polymorphic loci on one or more chromosomes and these assays are used in a single reaction to amplify DNA. The number of PCR assays may be between 50 and 200 PCR assays, between 200 and 1,000 PCR assays, between 1,000 and 5,000 PCR assays, or between 5,000 and 20,000 PCR assays (50 to 200-plex, 200 to 1,000-plex, 1,000 to 5,000-plex, 5,000 to 20,000-plex, more than 20,000-plex respectively). In an embodiment, a multiplex pool of about 10,000 PCR assays (10,000-plex) are *designed to amplify potentially heterozygous SNP loci on chromosomes X, Y, 13, 18, and 21 and 1 or 2* and these assays are used in a single reaction *to amplify cfDNA obtained from a material plasma sample, chorion villus samples, amniocentesis samples, single or a small number of cells, other bodily fluids or tissues, cancers, or other genetic matter*. The SNP frequencies of each locus may be determined by clonal or some other method of sequencing of the amplicons. Statistical analysis of the allele frequency distributions or ratios of all assays may be used to determine if the sample contains a trisomy of one

or more of the chromosomes included in the test. In another embodiment the original cfDNA samples is split into two samples and parallel 5,000-plex assays are performed. In another embodiment the original cfDNA samples is split into n samples and parallel (10,000/n)-plex assays are performed where n is between 2 and 12, or between 12 and 24, or between 24 and 48, or between 48 and 96. Data is collected and analyzed in a similar manner to that already described. Note that this method is equally well applicable to detecting translocations, deletions, duplications, and other chromosomal abnormalities.

(Ex.1076, ¶276 (emphasis added).)

152. This paragraph provides the same disclosure as paragraph [0397] of the published application for the '035 patent. (Ex.1111, ¶397.) As I described above in paragraph 105 of this declaration, a POSA would not understand the mention of cancer in this list to support isolation of cfDNA “from a blood sample collected from a subject who is not a pregnant women [sic].” (Ex.1001 (claim 1).) Once again, the passage does not refer to collecting cfDNA from each listed sample type. And even if otherwise, cancer is a group of diseases; it is not a biological material like the other listed samples. Additionally, SNP loci on the majority of the recited chromosomes are traditionally amplified to diagnose aneuploidy in prenatal testing.

153. I also reviewed below paragraph [0283] of the published application that became U.S. Patent No. 10,017,812.

The methods described herein may be used for a number of purposes where there is a target set of DNA that is mixed with an amount of

contaminating DNA. In some embodiments, the target DNA and the contaminating DNA may be from individuals who are genetically related. For example, genetic abnormalities in a fetus (target) may be detected from maternal plasma which contains fetal (target) DNA and also maternal (contaminating) DNA; the abnormalities include whole chromosome abnormalities (e.g. aneuploidy) partial chromosome abnormalities (e.g. deletions, duplications, inversions, translocations), polynucleotide polymorphisms (e.g. STRs), single nucleotide polymorphisms, and/or other genetic abnormalities or differences. In some embodiments, 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. *(see e.g. H. Mamon et al. Preferential Amplification of Apoptotic DNA from Plasma: Potential for Enhancing Detection of Minor DNA Alterations in Circulating DNA. Clinical Chemistry 54:9 (2008).* In some embodiments, the DNA may be found in cell culture (apoptotic) supernatant. In some embodiments, it is possible to induce apoptosis in biological samples (e.g. blood) for subsequent library preparation, amplification and/or sequencing. A number of enabling workflows and protocols to achieve this end are presented elsewhere in this disclosure.

(Ex.1076, ¶283 (emphasis added).)

154. This paragraph cites a scientific publication directed to amplification of apoptotic DNA from plasma. A POSA would not understand the paragraph to support the cancer limitation because the paragraph does not disclose amplification of SNPs associated with cancer.

155. I reviewed the below paragraph [0289] of the published application that became U.S. Patent No. 10,017,812.

In some embodiments, it may be used for tumor analysis: tumor biopsies are often a mixture of health and tumor cells. Targeted PCR

allows deep sequencing of SNPs and loci with close to no background sequences. It may be used for copy number and loss of heterozygosity analysis on tumor DNA. Said tumor DNA may be present in many different body fluids or tissues of tumor patients. It may be used for detection of tumor recurrence, and/or tumor screening. It may be used for quality control testing of seeds. It may be used for breeding, or fishing purposes. Note that any of these methods could equally well be used targeting non-polymorphic loci for the purpose of ploidy calling.

(Ex.1076, ¶289.) A POSA would understand this paragraph to support analysis of tumor biopsies by multiplex PCR. The brief mention of tumor DNA in other body fluids of patients does not provide support for amplification of DNA from cfDNA isolated from a blood sample. Thus, the paragraph does not provide support for the '035 cancer limitation.

156. In my opinion none of the paragraphs I discuss above from the patents and patent applications in the '035 patent family, prior to those added in the 2015 '544 application, provides adequate written description for the '035 patent's cancer limitation.

X. PRIOR ART GROUNDS

157. As discussed in the previous section, it is my opinion the challenged claims are entitled to a priority date of no earlier than October 20, 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, 12, 13	ForsheW (Ex.1005)
2	1, 10-15	Pieprzyk (Ex.1006) & May (Ex.1007)
3	1-3, 10-15	Porreca (Ex.1008) & Hoyal (Ex.1009)
4	1-5, 10-15	Porreca & Wessler (Ex.1010)

158. Based on a 2015 priority date, I understand that each reference would qualify as prior art against the '035 patent under AIA § 102(a)(1). ForsheW, Pieprzyk, May, Porreca, Hoyal, and Wessler were published or issued on May 30, 2012, November 17, 2011, October 7, 2010, November 4, 2010, December 6, 2007, and July 16, 2002, respectively—all of which predate the '035 patent's 2015 priority date by years. (Exs.1005-1010.)

159. If the Patent Office determines that the challenged claims are entitled to an earlier filing date, such as the May 2011 or even May 2010, it is my opinion that the challenged claims are unpatentable based on at least the above Grounds, with the exception of the ground based on ForsheW (Ground 1). I understand each reference would qualify as prior art under pre-AIA § 102. Wessler issued on July 16, 2002 and Hoyal published on December 6, 2007, and thus both are prior art under § 102(b). I understand that Porreca, Pieprzyk and May were PCT applications filed on April 30, 2010, May 16, 2011 and April 2, 2010, respectively, that designated the United States and were published in English. (Ex.1006-1008.) I understand that they thus qualify under § 102(e) based on their international or PCT filing dates. *See* M.P.E.P. 2136.03(II)(A).

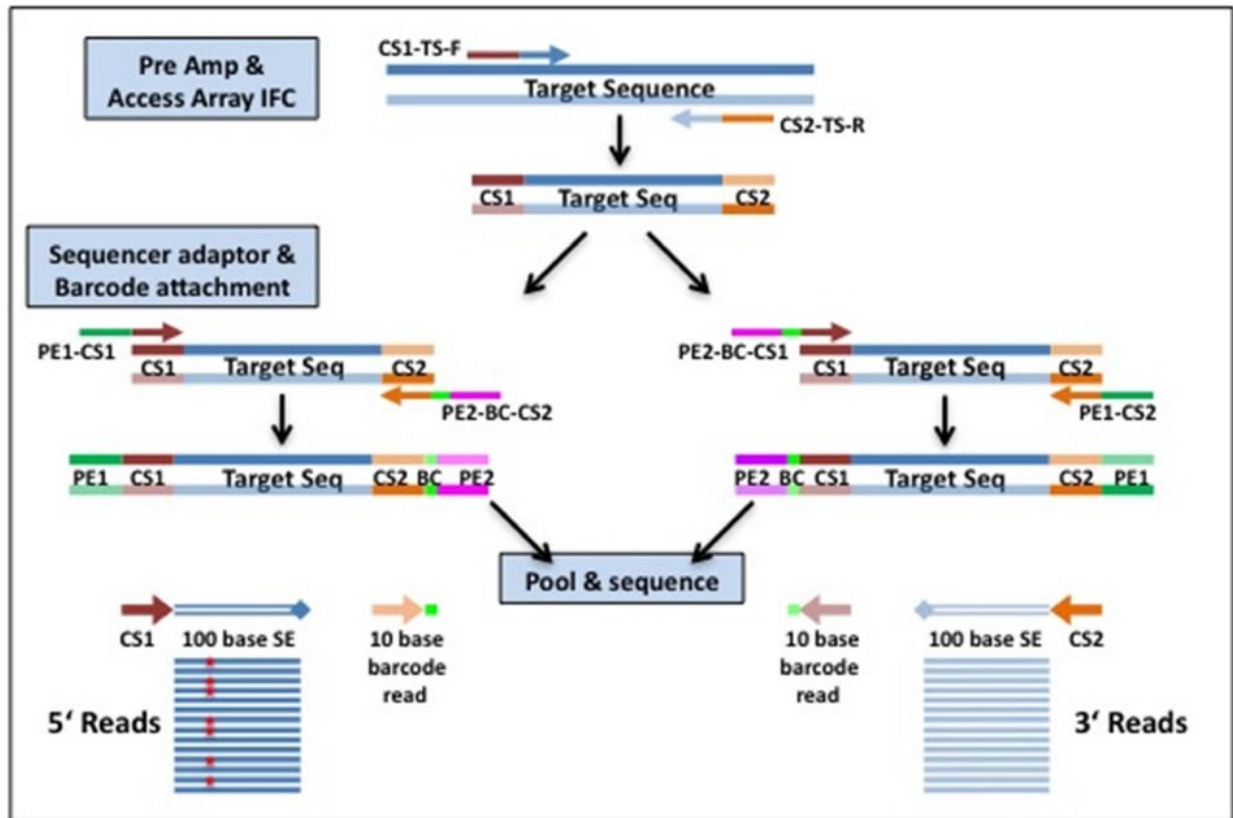
160. Moreover, if Patent Owner asserts an even earlier priority date, such as the May 18, 2010 U.S. provisional date on the face of the '035 patent, I understand that Pieprzyk, May, Porreca, Hoyal, and Wessler of Grounds 2-4 would still qualify as prior art. Wessler and Hoyal would qualify as prior art under at least pre-AIA § 102(b), and the other references also would qualify as prior art under § 102(e) based on *their* U.S. provisional filing dates, as detailed below.

B. GROUND 1: FORSHEW

161. In my opinion, Forshew anticipates or renders obvious claims 1, 12, and 13 of the '035 patent. A summary of Forshew and the bases for my opinions are set forth below.

2. Summary Of Forshew

162. Forshew discloses a method called “TAm-Seq” for identifying point mutations in a tumor sample and then tracking them by monitoring ctDNA. (Ex.1005, 2-10.) It is in the same field of endeavor as the '035 patent because it relates to simultaneously amplifying multiple nucleic acid regions of interest in a single reaction volume. In Forshew’s method (below), target-specific primers CS1-TS-F and CS2-TS-R amplify a plurality of target sequences in a pre-amplification step. (*Id.*, 3-4, 19.)



(Id., Fig. S1.)

163. Those target-specific primers include common adapter sequences (CS1 and CS2) at their ends. Then, in later amplifications, Forshew's method adds sequencing adapters and barcodes with barcoding primers PE1-CS1 and PE2-BC-CS1. Finally, Forshew's method sequences the resulting amplicons via next-generation Illumina sequencing.

164. Forshew (including its supplementary material) was publicly available online as of May 30, 2012. (Ex.1005.) In my experience, articles published in scientific journals, including any supplemental materials, are publicly available online as of the date that they publish. The right-hand margin of the

body of the article also states “[d]ownloaded from stm.sciencemag.org on June 6, 2012.” (*Id.*) Exhibit 1005 appears to be an authentic copy of Forshew downloaded from the publisher’s website (www.ScienceTranslationalMedicine.org).

3. Forshew Anticipates Or Renders Obvious Claims 1, 12, And 13

165. In district court, I understand that Patent Owner has used Forshew to show how Petitioner’s product allegedly practices claims 1, 12, and 13. (Ex.1038, *passim.*) But I also understand that, because the ’035 patent’s priority date is 2015, Forshew is actually prior art to the ’035 patent’s claims. Therefore, Patent Owner’s infringement contentions demonstrate how, under Patent Owner’s infringement theory, Forshew renders its own claims unpatentable. Although I do not believe Patent Owner’s infringement theory is correct, I have applied Patent Owner’s infringement theory in the following analysis.

b. Claim 1:

Preamble: *A method for amplifying and sequencing DNA, comprising:*

166. If limiting, Forshew discloses methods of amplifying and sequencing DNA.

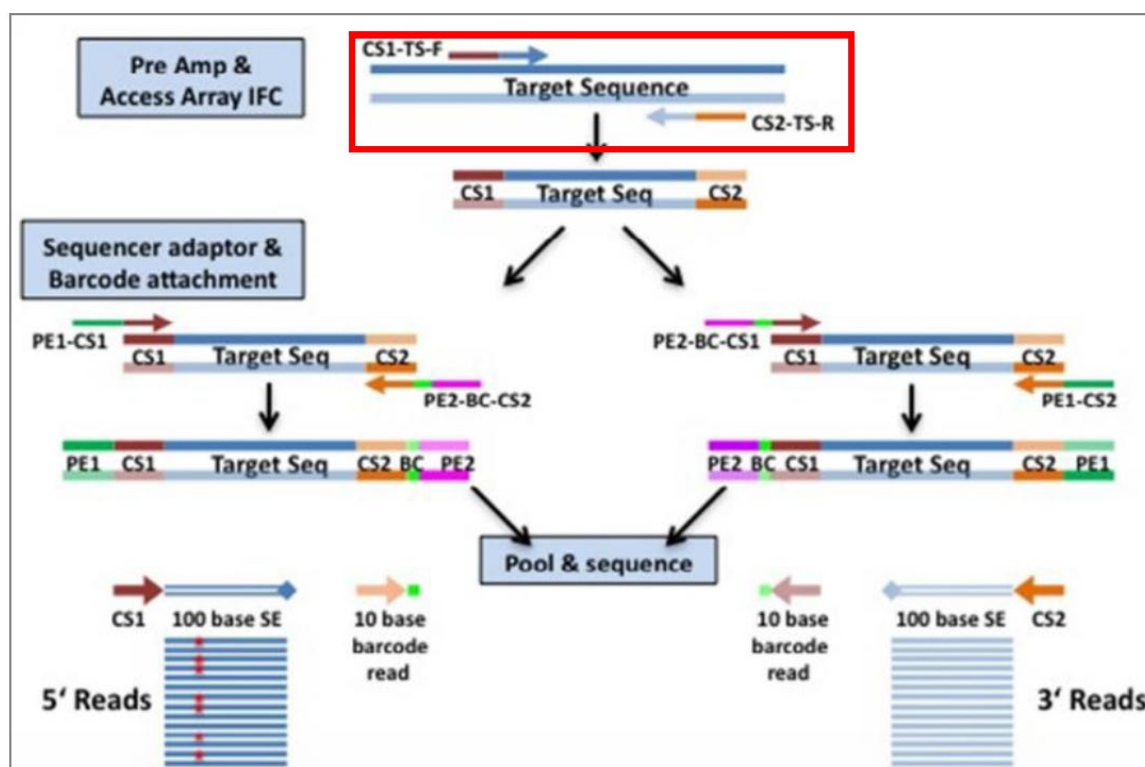
Limitation 1[A]: *tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,*

167. Forshew discloses applying TAM-Seq to isolated ctDNA. (*E.g.*, Ex.1005, 5 (“[w]e applied TAM-Seq to directly identify mutations in plasma of cancer patients”), 11 (“[c]irculating DNA was extracted from between 0.85 and 2.2

ml of plasma”); *see also id.*, 2, 10.) According to Patent Owner’s infringement contentions, Forshew also discloses the other elements of this limitation:

Forshew discloses that common tail sequences (i.e., CS1 and CS2) are added to each end of a target sequence in a pre-amplification step via PCR to generate a tagged product, as reproduced in the figure below. One of ordinary skill in the art would have understood that an adaptor with a common tail sequence is a universal tail adaptor.

(Ex.1038, 8.) The foregoing excerpt from Patent Owner’s infringement contentions refers to Forshew’s Figure S1 below, as annotated by Patent Owner:



(Ex.1038, 9 (reproducing Ex.1005, Fig.S1).)

Limitation 1[B]: *wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;*

168. Under Patent Owner’s infringement theory, and as explained above, Forshew teaches applying TAM-Seq to cfDNA isolated from cancer patients’

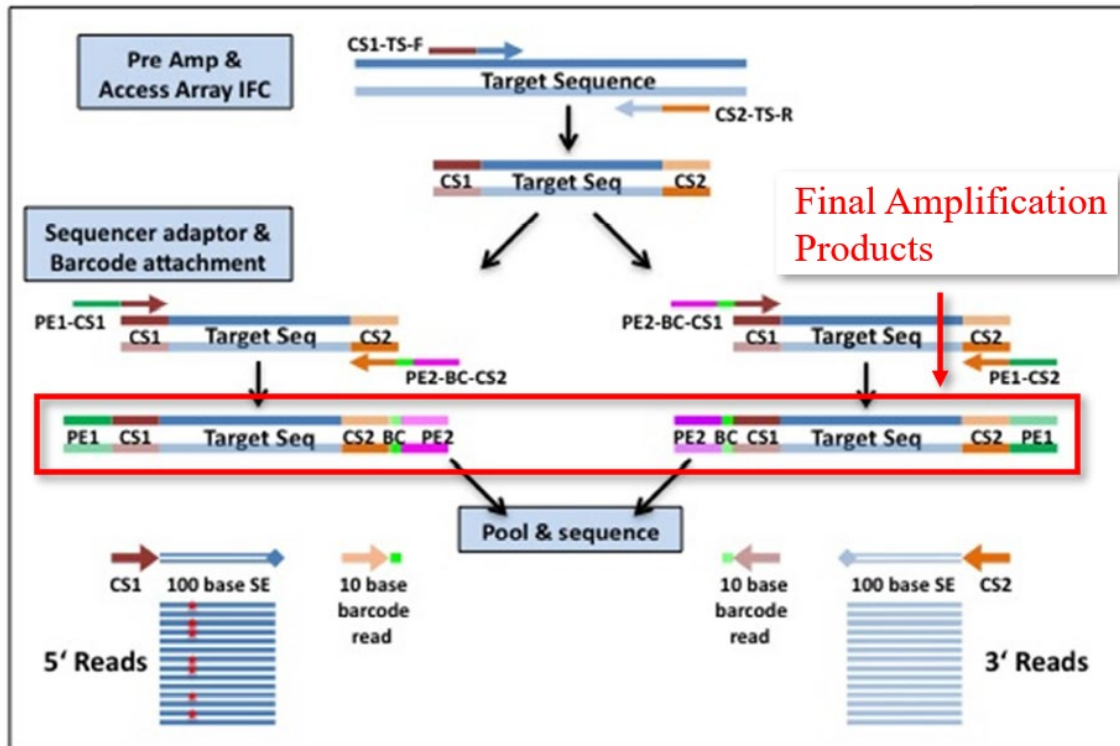
blood. (Ex.1005, 2-3, 5, 10-11.) It also discloses collecting samples from subjects in their late 60's and 70's with ovarian cancer, *i.e.*, non-pregnant women, and then applying TAm-Seq to those samples.

169. Additionally, in my opinion, it would have been obvious to use Forshew's method on subjects who are not pregnant women. A POSA would have been motivated to apply Forshew's methods to humans who are not pregnant women because many humans with cancer are not pregnant. They also would have reasonably expected success in doing so. Forshew discloses that its methods are suitable for monitoring for breast and ovarian cancers, and many non-pregnant people have breasts and ovaries. (Ex.1005, 5-11.) Thus, there is no doubt that TAm-Seq was suitable for assaying cfDNA from non-pregnant people.

Limitation 1[C]: amplifying the tagged products one or more times to generate final amplification products,

170. TAm-Seq's preamplification step amplifies tagged products because "[a]t the end of each of the remaining amplification cycles in the PCR process, one of ordinary skill would have understood that the output (amplified tagged products) is used as the input for the following amplification cycle, thus thereby performing targeted amplification of already tagged products." (Ex.1038, 21.) In other words, Patent Owner asserts claim 1's separate tagging and amplifying steps can be different cycles in one PCR reaction. (Ex.1038, 22 (citing Ex.1005, Fig.S1).)

171. Patent Owner also contends that ForsheW teaches “an additional round of PCR,” *i.e.*, a second amplification, to generate “final amplification products.” (*Id.* at 23.) Those alleged final amplification products are shown below in the following annotated version of ForsheW’s Figure S1:



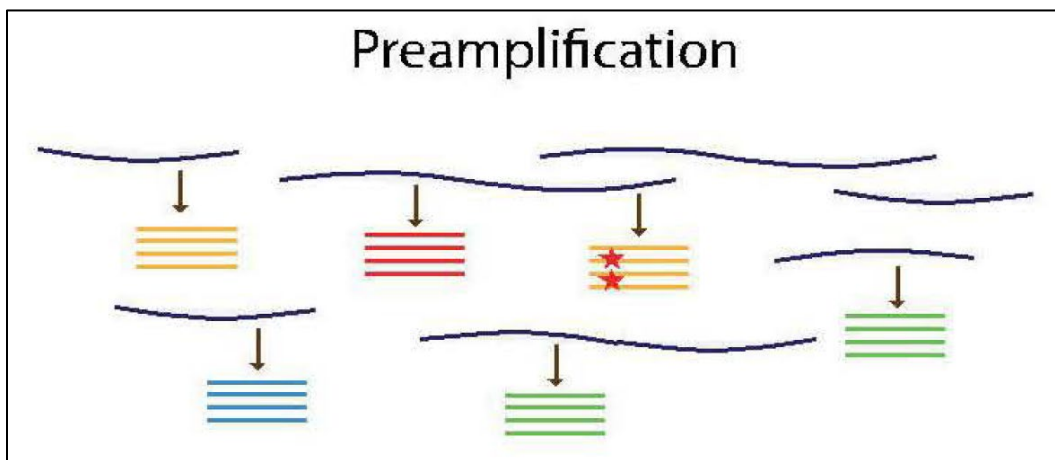
(Ex.1005, Fig. S1 (annotated).)

Limitation 1[D]: *wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume,*

172. According to Patent Owner, ForsheW’s preamplification is a “targeted amplification of already tagged products.” (Ex.1038, 21.) Patent Owner explains that, in ForsheW’s “preamplification...involv[ing]...targeted multiplex amplification,” the products of the first amplification cycle are “the input for the

subsequent PCR cycle wherein the tagged products are amplified.” (*Id.*; *see also* Ex.1005, 4, Figs. 1A-B, S1.)

173. Those tagged products include a plurality of SNP loci under Patent Owner’s interpretation of that term. TAm-Seq’s preamplification uses “48 primer pairs to amplify 5995 bases of genomic sequence covering coding regions...of *TP53* and *PTEN*, and selected regions in *EGFR*, *BRAF*, *KRAS*, and *PIK3CA*...by overlapping short amplicons.” (Ex.1005, 4-5.) Those regions were known to contain locations of common point mutations associated with cancer, which, again, I understand Patent Owner contends are SNP loci. (*Id.*, 4 (target regions “cover mutations that account for 38% of all point mutations in the COSMIC database”).) Forsheiw depicts preamplifying one point mutation (starred red) in its Figure 1b:



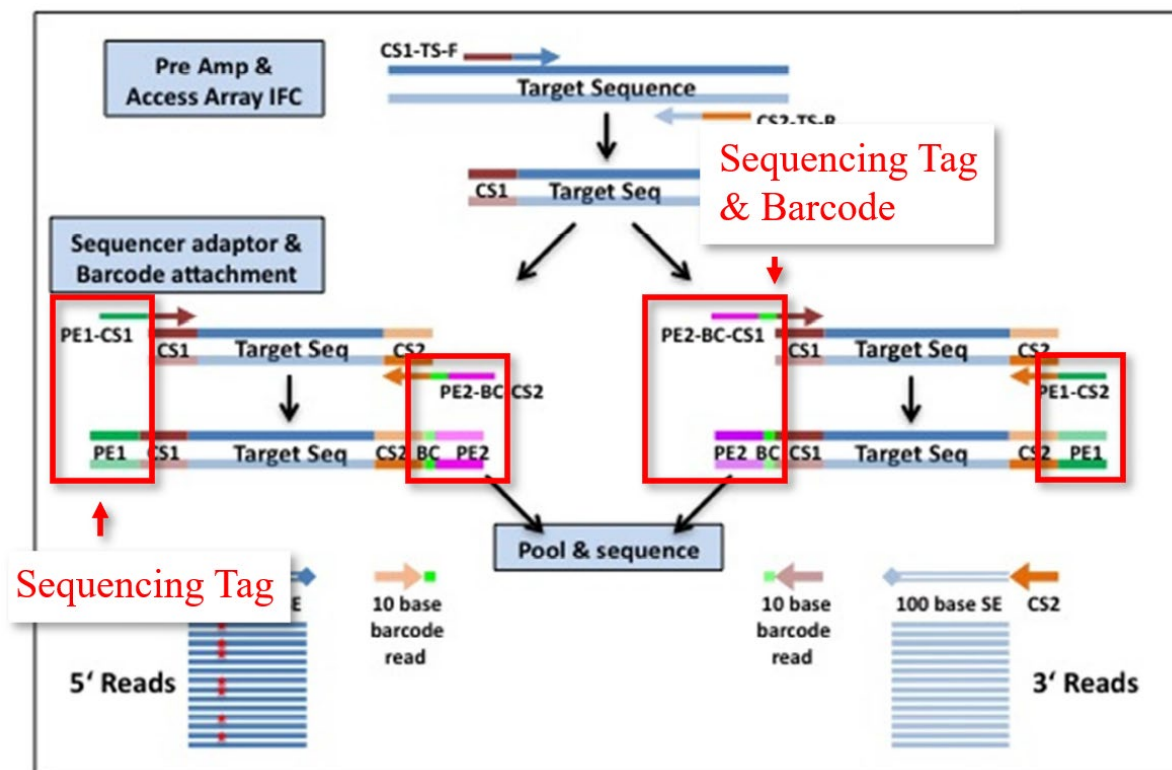
(Ex.1005, 3 (Fig. 1b (cropped))).)

174. TAm-Seq’s preamplification occurs in a single reaction volume:
“[w]e...applied a two-step amplification process: a limited-cycle preamplification

step *where all primer sets were used together.*” (*Id.*, 4, 16, Figs. 1A-B, S1 (emphasis added).)

Limitation 1[E]: *wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and*

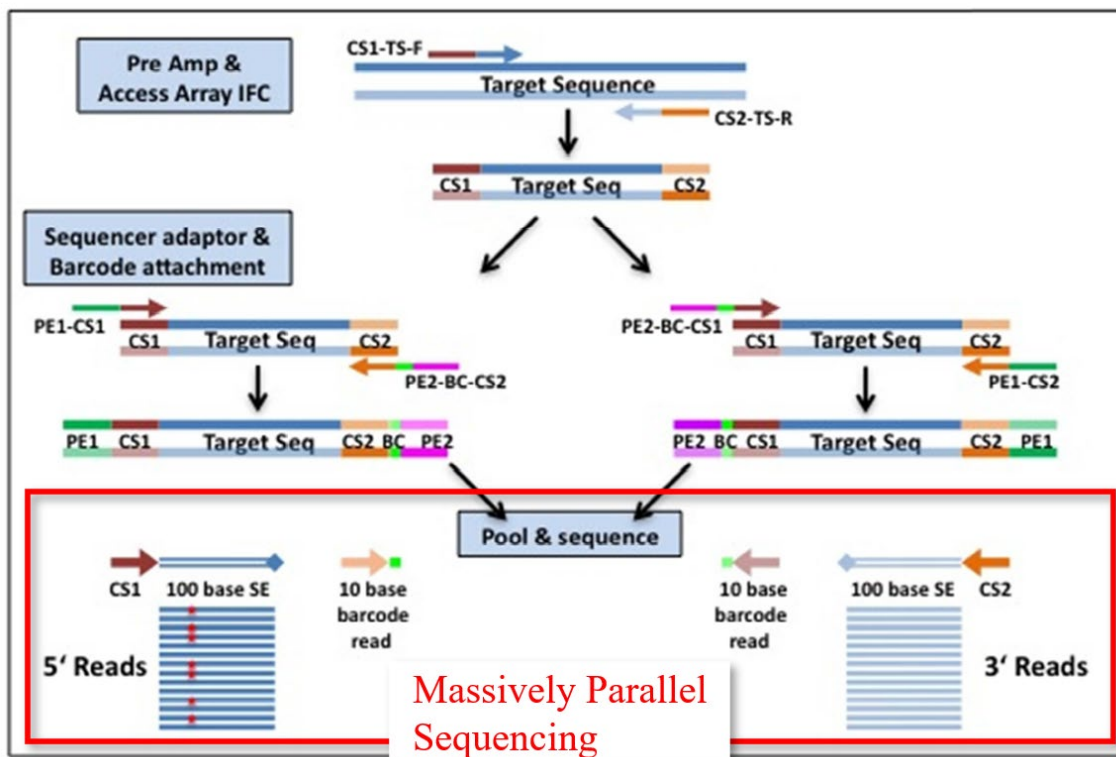
175. According to Patent Owner, “Forsheew discloses[,] in an additional round of PCR[,] introducing a barcode and one or more sequencing tags.” (Ex.1038, 21, 22 (quoting Ex.1005, 19 (“Sequencing platform-specific adaptors and unique sample barcodes were attached during an additional round of PCR.”)); *see also* Ex.1005, 4 (“barcode sequencing primer”); Fig. S1 (“sequencing adaptor and barcode attachment”) (cleaned up).) Patent Owner identifies Illumina sequencing adapters (PE1 and PE2) and a 10-base barcode as the claimed barcode and sequencing tags:



(Ex.1005, Fig.S1 (annotated).)

Limitation 1[F]: sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products,

176. Under Patent Owner's theory, Forshew depicts sequencing its final amplification products in its Figure S1's "Pool & sequence step" (boxed red):



(Ex.1005, Fig. S1 (annotated); *see also id.*, 3, 19, Fig. 1.) Forshew discloses TAm-Seq performs massively parallel sequencing of those products. (*Id.*, 4 (“100-base single-end sequencing (GAIIx sequencer, Illumina)...generating ~30 million reads per lane”); *see also id.*, 5 (“the mutation was also identified by TAm-Seq”).)

Limitation 1[G]: wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.

177. As explained above, TAm-Seq uses 48 primer pairs to amplify and then sequence 48 genomic regions containing point mutations associated with cancer, which I understand that Patent Owner contends are SNP loci. Forshew thus discloses 48 SNP loci associated with cancer under Patent Owner’s view of the claims. Indeed, I note that, in its infringement contentions in the co-pending

litigation, Patent Owner asserted that a method using a “primer panel of up to 48 primer pairs capturing at least one somatic variant” practices the limitation.

(Ex.1038, 24.)

c. Claim 12

The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.

178. According to Patent Owner, Forshew’s “common tail sequences (i.e., CS1 and CS2)” are “universal tail adaptors.” (Ex.1038, 8.) Under that view, Forshew’s CS1 is a “first” universal tail adapter and “CS2” is a second universal tail adapter.

d. Claim 13

The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.

179. Forshew’s CS1 and CS2 sequences are part of its “[t]arget-specific primers.” (Ex.1005, 19.) According to Patent Owner, Forshew teaches tagging target cfDNA via amplification with those primers. (Ex.1038, 8; *see also* Ex.1005, 3-5, 11, Fig. S1.)

C. GROUND 2: PIEPRZYK & MAY

180. In my opinion, the combination of Pieprzyk and May renders obvious all of claims 1 and 10-15 of the '035 patent. Summaries of these references, the combination, and the bases for my opinions are set forth below.

2. Summary Of Pieprzyk

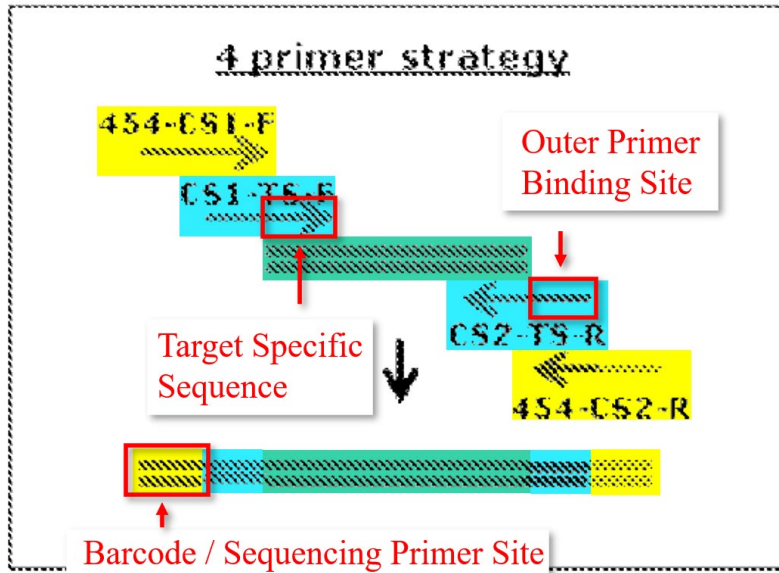
181. Pieprzyk is an international or PCT application originally assigned to Fluidigm Corporation. (Ex.1006.) Pieprzyk teaches methods for analyzing “cell-free DNA in the bodily fluid (*e.g.*, plasma) of subjects with cancer.” (Ex.1006, ¶111; *see also id.*, ¶¶3, 116, 138.) Its methods can be applied to “up to...10000 or more amplification reactions...carried out in each individual reaction chamber.” (*Id.*, ¶218.) They also can “identify[] the presence of particular polymorphisms (such as SNPs)” and facilitate the detection or quantification of nucleic acid samples like “tumor DNA in plasma from cancer patients.” (*Id.*, ¶¶116, 221.)

182. In one of Pieprzyk’s methods, cfDNA is tagged and amplified in a four-primer PCR reaction. (*Id.*, ¶10.) Two of the four primers are “inner forward” and “inner reverse” primers, which include “target specific portion[s]” and universal “nucleotide tags.” (*Id.*; *see also id.*, ¶¶57, 113-14.) The other two primers, “outer forward” and “outer reverse” primers, anneal to the first two primers’ nucleotide tags and can “include one or more additional nucleotide

sequences to be added to the target nucleic acid.” (*Id.*, ¶10.) Pieprzyk teaches sequencing the amplified DNA via “any available sequencing method,” including “high-throughput sequencing.” (*Id.*, ¶187.) A POSA would understand that Pieprzyk’s reference to “high-throughput sequencing” meant massively parallel sequencing.

3. Summary Of May

183. May is also a Fluidigm Corporation PCT application. (Ex.1007.) May teaches the same four-primer strategy as Pieprzyk. Like Pieprzyk, it teaches inner primers with universal “nucleotide tag[s]” and outer primers including additional sequences. (*Id.*, ¶11.) May adds that its outer “barcode primers,” can contain both a “barcode nucleotide sequence” and “additional primer binding sites...capable of being bound by DNA sequencing primers.” (*Id.*, ¶115.) May illustrates its approach the outer barcode primers (yellow), inner target-specific primers (blue), a target sequence (green), and an amplification product (multicolor) in its Figure 18:



(*Id.*, Fig. 18 (cropped and annotated).)

184. May discloses using its four-primer strategy for similar applications as Pieprzyk. May teaches using its method for “identifying the presence of particular polymorphisms (such as SNPs).” (*Id.*, ¶235.) And it teaches analyzing “[n]ucleic acids [] extracted or amplified from...blood [or] a blood fraction,” including via “multiplex detection...[in an] individual amplification mixture.” (*Id.*, ¶¶132, 210.) Like Pieprzyk, May also teaches sequencing the amplified DNA via “any available sequencing method,” including “high throughput sequencing.” (*E.g., id.*, ¶221.) Again, a POSA would have recognized that high throughput sequencing meant massively parallel sequencing.

4. Combination Of Pieprzyk And May

185. A POSA would have found it obvious to use May’s four primers, with their barcodes and sequencing tags in the outer primers, in place of Pieprzyk’s

four primers. For starters, both Pieprzyk and May are in the same field as the '035 patent. Like the '035 patent, both relate to the targeted analysis of cfDNA. Pieprzyk is directed to “the area of detecting genotype and/or aneuploidy.” (Ex.1006, ¶2.) And, similarly, May is directed to the “detection and/or sequencing of particular target nucleic acids” and its “methods can be performed in determining genotypes.” (Ex.1007, ¶¶2, 28.) Both references were, at one time, assigned to Fluidigm, a company working in this field by 2010, further confirming a POSA would have looked to their disclosures. Finally, both references provide solutions for one of the problems also facing the '035 patent's inventors: how to detect mutations in cfDNA. (*See, e.g.*, Ex.1001, 3:47-52; Ex.1006, ¶¶2-3; Ex.1007, ¶¶2, 132.)

186. A POSA would have been motivated to make this combination. In my opinion, doing so was a simple substitution of one known element (May's primers) for another (Pieprzyk's primers) between two references describing the same, underlying Fluidigm technology. A POSA would have viewed May as disclosing just one more way to configure Pieprzyk's outer primers. Pieprzyk discloses a four-primer strategy, and May teaches how to configure those four primers. Specifically, May discloses a particular arrangement of common adapters, barcodes, and sequencing tags organized across its four primers.

(Ex.1007, ¶¶11, 115.) Adopting May's 4-primer strategy in Pieprzyk's method would simplify experimental design for a POSA seeking to practice that method.

187. In my opinion, a POSA also would have been motivated to use May's four-primer configuration because, in the context of Pieprzyk's four-primer system, that configuration would simplify primer design. May's inner primers include target-specific portions and "nucleotide tags." (Ex.1007, ¶¶11, 115; Fig. 18.) May's outer primers contain portions specific (*i.e.*, that anneal to and extend from) the inner primers' nucleotide tags, and the outer primers further include barcodes and sequencing tags. (*Id.*) Pieprzyk discloses a similar configuration, but provides less detail about the outer primers. (Ex.1006, ¶10.) May's disclosure of placing barcodes and sequencing tags on outer primers, rather than on inner primers, results in short inner primers that would have been easier to design than longer inner primers with additional sequences.

188. A POSA also would have been motivated to adopt May's four primer configuration when using Pieprzyk's method because, in that context, May's configuration would be economical. That is, by locating the sequencing tags and barcodes—tags that are typically common across all targets of a given sample—on the outer primers and annealing outer primers to universal adapters on the target-specific inner primers, May's configuration eliminates the need to design unique, target specific inner primers including the barcodes. Furthermore, a POSA

would have recognized that, because barcodes are often used to differentiate between different patients, May's configuration would allow the same set of inner, target specific primers to be used across all patients.

189. To give an example, if a POSA were to analyze 100 target regions in 100 patients, with each patient sample receiving a unique barcode, a method in which the inner primers included barcodes would require ordering a unique pair of target-specific inner primers comprising a barcode for each patient sample. That is, such a method would require ordering 10,000 unique primer pairs to barcode all 100 target sequences across 100 samples ($100 \times 100 = 10,000$). In contrast, May's 4-primer strategy requires only one target-specific inner primer pair per target sequence, meaning one would only need to order 200 total primer pairs (100 inner primer pairs and 100 outer primer pairs with sample-specific barcodes).

190. This problem and solution were also widely discussed in the art by the earliest possible priority date. For instance, Cuppens in 2010 noted that "[a]ddition of a DNA-tag to a DNA fragment of a sample...[with] at least one amplicon-specific primer contain[ing] the tag as an adaptor...[is] very costly" and provides a solution wherein a "...first PCR reaction [uses] amplicon-specific primers with an adaptor...[and] a second PCR reaction [with] primers...directed against these adaptor sequences" and "...includ[ing] a unique tag sequence, and possibly additional adaptor sequences for further processing" thereby decreasing

the number of primers needed and reducing costs. (Ex. 1049, ¶¶96-97.) It is my belief that a POSA concerned with economy thus would have been motivated to adopt May's four primer configuration.

191. A POSA also would have reasonably expected this combination to succeed. Both Pieprzyk and May relate to the same Fluidigm technology. Both references teach using four primers to tag and amplify DNA. And May even discloses that its four primers work for multiplex reactions like those in Pieprzyk. (Ex.1007, ¶¶210, 276, Fig. 18; Ex.1006, ¶¶201, 218.) The modification would have been trivial. Finally, I should add that what was known or obvious as of the critical date in this ground (as well as Grounds 3 and 4) applies equally regardless of whether the Patent Office concludes that the critical date is 2015, 2011, or even 2010.

5. Claims 1, 10-15 Are Obvious Over Pieprzyk And May

a. Claim 1:

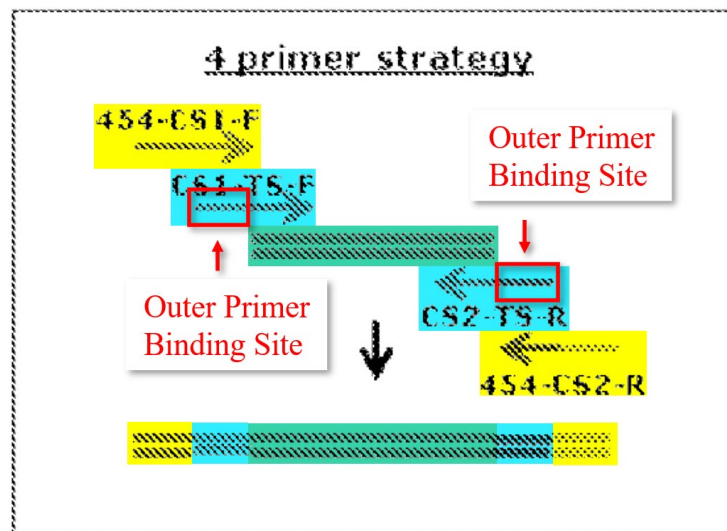
Preamble: *A method for amplifying and sequencing DNA, comprising:*

192. If limiting, Pieprzyk and May disclose methods for amplifying and/or sequencing DNA.

Limitation 1[A]: *tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,*

193. In the combined method, May's inner primers (blue) comprise universal tail adaptors because they contain nucleotide tags, which are not target

specific and to which May's outer primers anneal. (Ex.1007, ¶¶11, 115.) Put differently, the inner primers (blue) comprise universal tail adapters because their nucleotide tags serve as an annealing site for the outer primers, which permit later, universal amplification of all target sequences with a single set of outer. May depicts an example of its 4-primer strategy in its Figure 18:

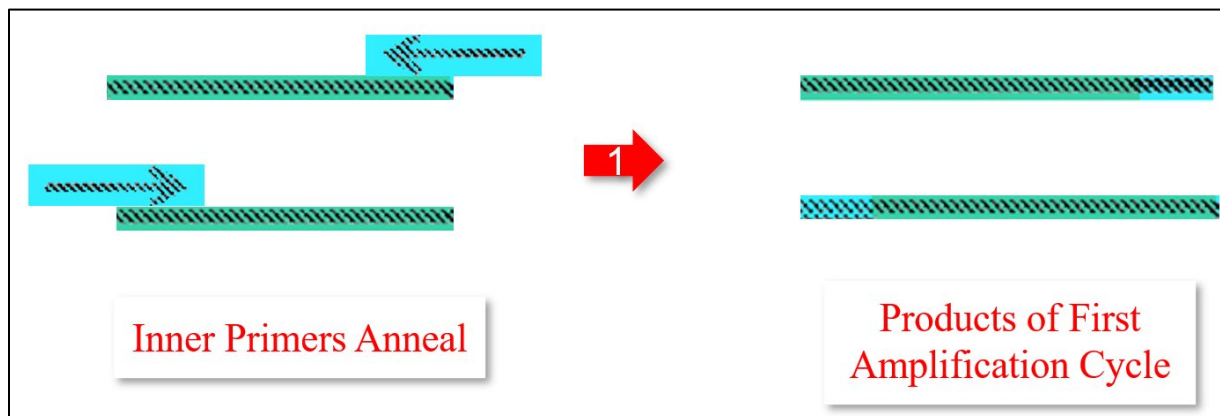


(*Id.*, Fig. 18 (cropped and annotated).)

194. Under Patent Owner's view of the claims, May's inner primers would tag their target DNA in the first amplification cycle of a PCR reaction and thereby yield a tagged product.¹ For instance, the following graphic, which is adapted from May's Figure 18, depicts forward and reverse inner primers

¹ If tagging is required across more than one cycle (*e.g.*, due to variability in the PCR reaction), my analysis set forth herein would simply shift by one cycle.

annealing to two complementary strands of target DNA and also the products of that first amplification cycle. Those products, shown in the right-hand side of the graphic, are DNA strands including one of the inner primer's nucleotide tags.



195. In the combined method, May's inner primers would tag isolated cfDNA. Pieprzyk discloses performing "the amplification steps" on "[n]ucleic acids of interest...isolated using methods well known in the art." (Ex.1006, ¶149.) And it further discloses applying its methods to "cell-free DNA in the bodily fluid (e.g., plasma) of subjects with cancer." (*Id.*, ¶111; *see also id.*, ¶¶3, 116, 138, 147, 149.)

Limitation 1[B]: *wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;*

196. As discussed above, Pieprzyk discloses tagging cfDNA isolated from plasma, which is part of blood. (Ex.1006, ¶¶3, 111, 116, 138, 147, 149.) It also discloses collecting those samples from a subject who is not a pregnant woman. When referring to pregnant women, it uses the adjective "maternal."

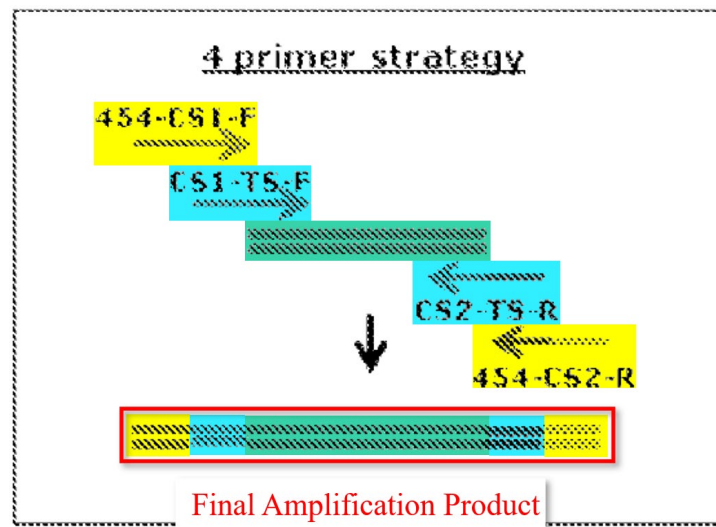
(E.g., Ex.1006, ¶138 (“[i]n various embodiments the nucleic acid are [sic] derived from *a maternal biological sample* (e.g....from a pregnant mammal (e.g., human))”).) But when discussing monitoring cancer, it does not. (*Id.* (“derived from *a biological sample*...having, suspected of having, or at risk for a cancer” (emphases added)); *see also, e.g., id.*, ¶¶111, 116.) In my opinion, Pieprzyk’s omission of the limiting adjective “maternal” in that second phrase teaches to a POSA that its method extends to non-pregnant women.

197. Alternatively, in my opinion, it would have been obvious to isolate the cfDNA from non-pregnant women. Pieprzyk discloses detection of ctDNA, and any POSA would understand that there are plenty (in fact, the majority) of cancer patients who are not pregnant. A POSA would have been motivated to apply the combined method to non-pregnant females because many humans with cancer are not pregnant. A POSA would have reasonably expected success because Pieprzyk’s methods are agnostic as to whether a subject is pregnant. (Ex.1006, ¶138; *see also id.*, ¶221.)

Limitation 1[C]: *amplifying the tagged products one or more times to generate final amplification products,*

198. As discussed above, the combined method tags cfDNA via amplification. (Ex.1007, ¶¶11, 115.) In a single PCR reaction, May’s inner primers generate tagged products by amplifying DNA and May’s outer primers then amplify those tagged products. (*Id.*, ¶¶11, 115.) The products of those later

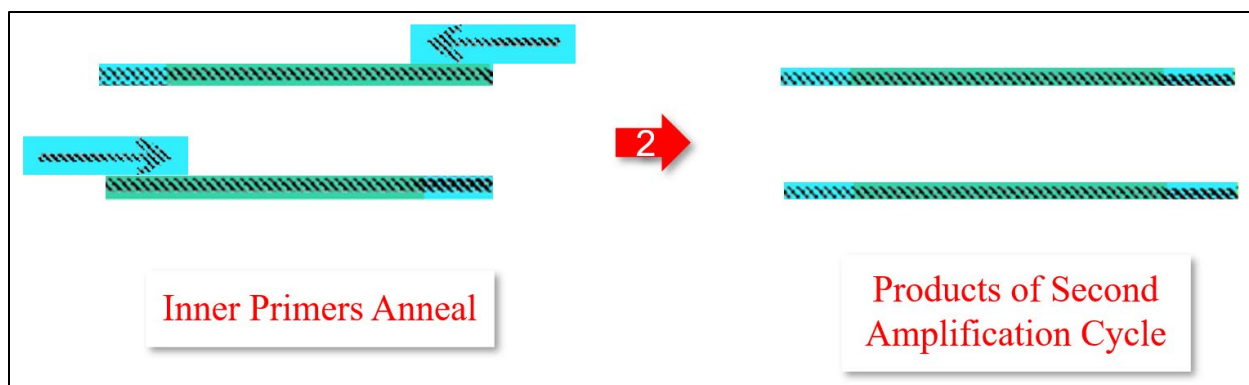
amplification cycles are the final amplification products. (See Ex.1006, ¶¶187, 210; Ex.1007, ¶¶121, 164.)



(Ex.1007, Fig. 18 (cropped and annotated).)

Limitation 1[D]: *wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume,*

199. In the combined method, May's target-specific primers perform target-specific amplification in the second amplification cycle of the PCR reaction with the inner target-specific primers. (Ex.1007, ¶11.) That second cycle is illustrated in the below figure adapted from May's Figure 18. The left-hand side of the below figure shows May's inner primers annealing to the products of the first amplification cycle and the right-hand side of the below figure shows the products of the second amplification cycle, which are DNA strands now bearing the inner primers' nucleotide tags on both ends:



200. In the combined method, May’s inner primers amplify the plurality of SNP loci disclosed in Pieprzyk in a single reaction chamber. Pieprzyk discloses targeting “genomic DNA, which can be analyzed for specific polymorphisms (such as SNPs).” (Ex.1006, ¶151; *see also id.*, ¶221 (similar).) It further discloses targeting “genomic DNAs that are altered (e.g., amplified, deleted, and/or mutated) in genetic diseases or other pathologies.” (*Id.*) And it discloses performing 50, 100, 500, and 1000 “multiplex detection” reactions “in each individual reaction chamber” of a device, *i.e.*, in a single reaction volume. (*Id.*, ¶218.) May also contains similar disclosure, confirming that its primers also are suitable for multiplexing. (Ex.1007, ¶210.)

201. If Pieprzyk had not disclosed targeted amplification of a plurality of SNP loci in a single reaction volume, that step would have been obvious. Disclosures in Pieprzyk would have motivated a POSA to conduct multiplex amplification in a single reaction volume on ctDNA. In the context of detecting fetal cfDNA, Pieprzyk teaches that “[p]lasma contains a limited concentration of

target nucleic acids (...SNPS...etc.).” (Ex.1006, ¶379.) There, “[m]ultiplexing will be necessary” because the alternative, “[d]ividing the sample[,] will reduce the number of target[s] per assay and increase sampling error.” (*Id.*) Specifically, dividing a cfDNA sample in this method would potentially lead to, among other issues, loss of sample due to dead volume, dilution of target sequences leading to failed amplification, and skewed amplification results. Pieprzyk also teaches that “[s]imilar challenges exist for mutation detection by mutation dosage in...cancer, and therefore this approach can be used in these contexts.” (*Id.*, ¶387; *see also* Ex.1025, 5.) Finally, Pieprzyk proposes analyzing between “30” and “1000 or more target and/or internal control sequences,” which falls within the claimed bounds of 25-2,000 SNP loci under Patent Owner’s construction. (Ex.1006, ¶110.)

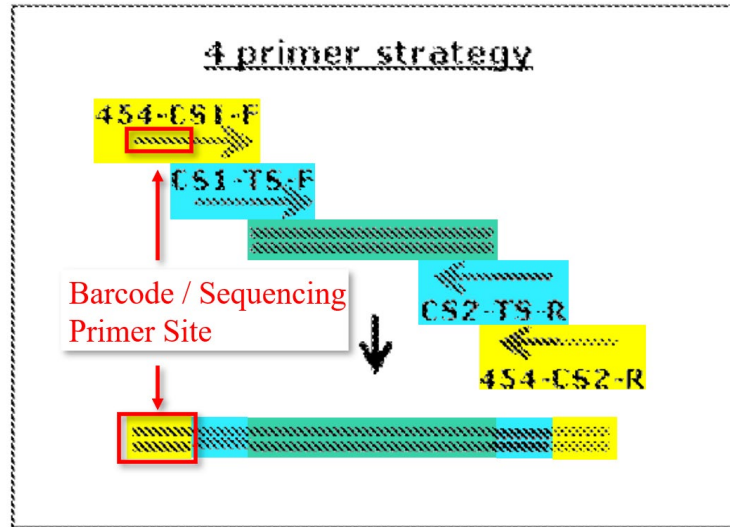
202. A POSA also would have reasonably expected success in performing multiplex amplification. By the early 2000s, multiplex amplification was routine. In 1995, Schuber *et al.* described designing primers for a 15-plex PCR reaction, where the primers included both target specific sequences and 20-nucleotide universal adapters. (Ex.1026, 1, 3.) In 2004, Inagaki *et al.* described designing primers a 39-plex multiplex PCR reactions. (Ex.1027, 2-3.) Also by 2004, software called “AutoDimer” could screen sets of up to 1,000 primers for simultaneous use in multiplex reactions. (Ex.1028, 1; *see also* Ex.1006, ¶¶484-86.) Even the ’035 patent itself confirms that “multiplexing PCR for sequencing”

of up to “100 assays in the same well” was known, showing a POSA would have had no difficulty multiplexing at least that many sequences. (Ex.1001, 48:25-27; 85:14-20, 87:32-33.)

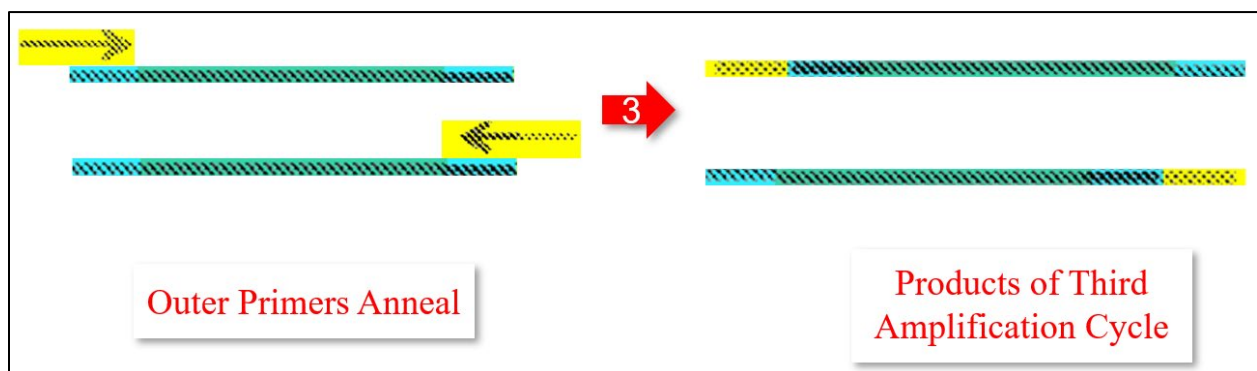
203. Furthermore, other publicly available art as of 2010 confirms multiplex amplification was well with the skill of a POSA by that date. A 2010 published United States patent application to Mir disclosed performing “an optional pre-preamplification reaction...[wherein] target-specific primers for 9216 different target nucleic acids can be employed in one mixture.” (Ex.1081, ¶119.) Mir taught that its methods could be used “in the diagnosis and/or prognosis of genetic defects and diseases, such as cancer.” (Ex.1081, ¶0176.) In fact, in another proceeding, Patent Owner’s expert testified that Mir’s methods were “well-understood and predictable” by “the relevant timeframe [2010].” (Ex.1040, ¶¶91, 98, 114.) I agree with Patent Owner’s expert on this point: by 2010, multiplex amplification was well-known and well-within a POSA’s skill.

Limitation 1[E]: *wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and*

204. In the combined method, May’s outer primers introduce barcodes and sequencing tags beginning in the third amplification cycle of the PCR reaction because they include barcode sequences and sequencing tags and, thus, so do their amplification products. (*Id.*, ¶¶11, 115.)



(Ex.1007, Fig.18 (cropped and annotated).) The below graphic, adapted from May's Figure 18, shows one amplification that occurs in the third amplification cycle. Specifically, the left-hand side of the graphic depicts May's outer primers annealing to the nucleotide tags added in the second amplification cycle. The right-hand side of the graphic depicts some products of the third amplification cycle, which bear outer primer sequencing sites and barcodes and inner primer nucleotide tags at one end and inner primer nucleotide tags at the other.



Limitation 1[F]: sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products,

205. Pieprzyk's methods "can include subjecting at least one target amplicon to DNA sequencing using any available DNA sequencing," including "high throughput sequencing." (Ex.1006, ¶187; *see also id.*, ¶207.) And, as I discuss above in paragraphs 199-203 of my Declaration, Pieprzyk also discloses or renders obvious that the target amplicons (*i.e.*, the final amplification products) can include a plurality of SNP loci on cfDNA. Once again, a POSA would have understood that high throughput sequencing meant massively parallel sequencing.

Limitation 1[G]: wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.

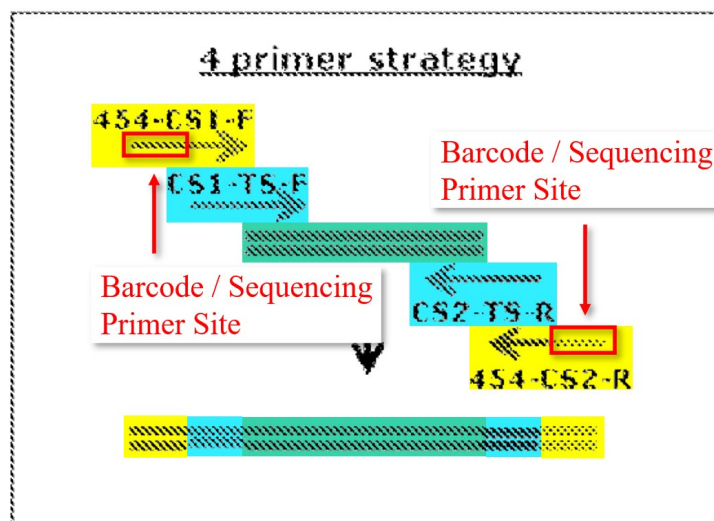
206. As explained above in paragraphs 199-203 of my Declaration, Pieprzyk discloses or renders obvious applying the combined method to between 30 and 1000 or more ctDNA sequences, which would contain SNP loci under Patent Owner's interpretation of that term. Specifically, Pieprzyk teaches using its methods in "multiplex detection...carried out in individual amplification mixture" where each mixture can comprise "up to 2, 3, 4, 5, 6, 7, 8, 9, 10, 50, 100, 500, 1000, 5000, 10000, or more amplification reactions." (Ex.1006, ¶218; *see also* Ex.1006, ¶¶116, 221.) Thus, Pieprzyk provides ample instruction to a POSA to use its methods in a multiplex reaction of "25-2,000 loci associated with cancer" as recited in claim 1 of the '035 patent.

b. Claims 10 & 11

Claim 10: *The method of claim 1, wherein the one or more universal tail adaptors comprise a second barcode.*

Claim 11: *The method of claim 1, wherein the one or more universal tail adaptors comprise a second sequencing tag.*

207. These claims are obvious over Pieprzyk and May. May's 4-primer strategy uses two outer primers that include barcodes and sequencing tags. (Ex.1007, ¶¶118 ("each of the outer primers contains a unique barcode"), 115 ("first additional primer binding site" and "second additional primer binding site").) The outer primers comprise universal tail adapters because their "additional primer binding sites" for "DNA sequencing primers" allow for universal amplification with sequencing primers. (Ex.1007, ¶¶115, 129.)



(Ex.1007, Fig.18 (cropped and annotated).)

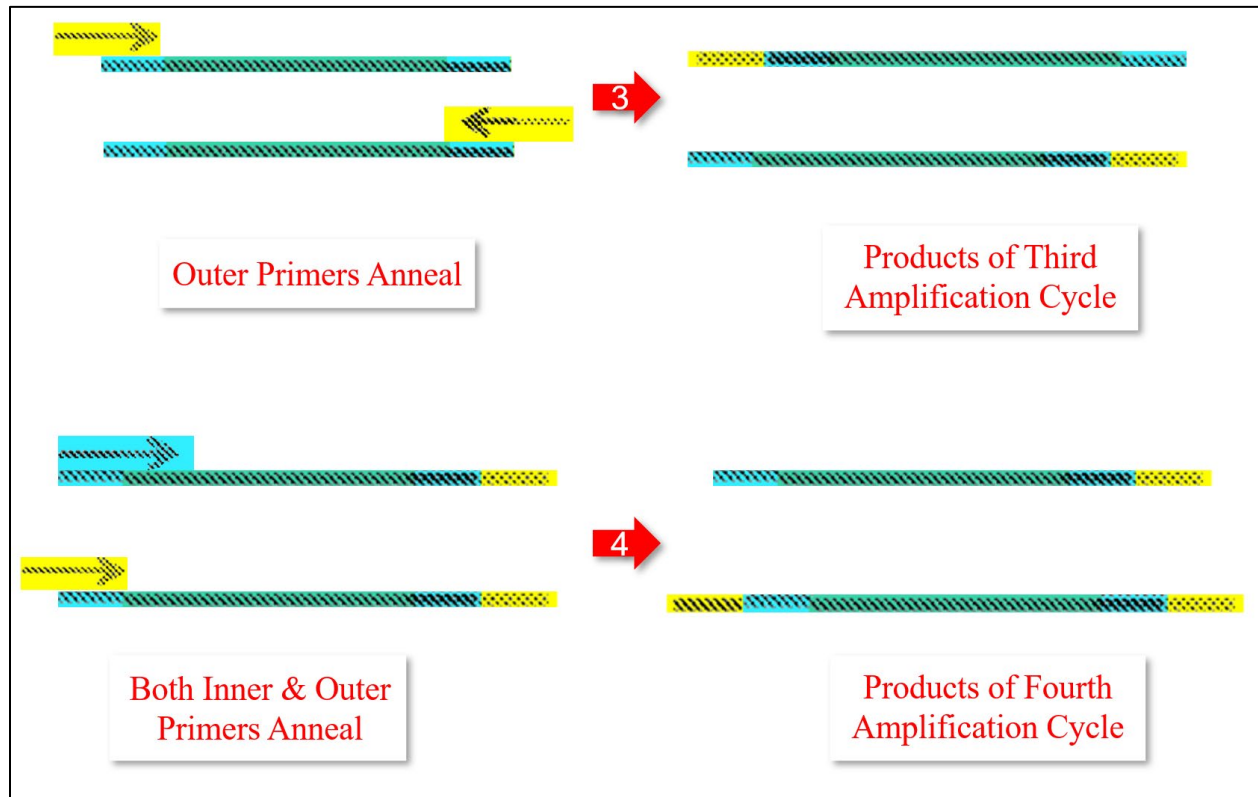
208. Indeed, a POSA, by 2010, would have understood that DNA sequencing primer binding sites could be used for universal amplification of target

sequences. For instance, as early as 2008, Craig *et al.* disclosed methods of amplify sequences by performing universal amplification with Illumina adapter-compatible primers (Ex.1113, 15.) Thus, like May's inner primers, May's outer primers also comprise universal tail adapters.

209. Moreover, under Patent Owner's view of the claims, May's outer primers generate tagged products beginning in the third amplification cycle of a PCR reaction, and those tagged products then would be amplified in a targeted amplification with inner primers in a fourth amplification cycle.

210. To illustrate, the top half of the below figure, adapted from May's Figure 18, again shows the third amplification cycle discussed above. The bottom half of that figure shows some of the reactions that would occur in the fourth amplification cycle. The lower left-hand corner of the below figure depicts (1) an inner primer annealing to a product of the third amplification cycle and (2) an outer primer annealing to a product of the third amplification cycle. The lower right-hand corner of the figure depicts some products of the fourth amplification cycle. In the amplification in the fourth amplification cycle involving the inner primer, the DNA strand tagged with one outer primer would undergo targeted

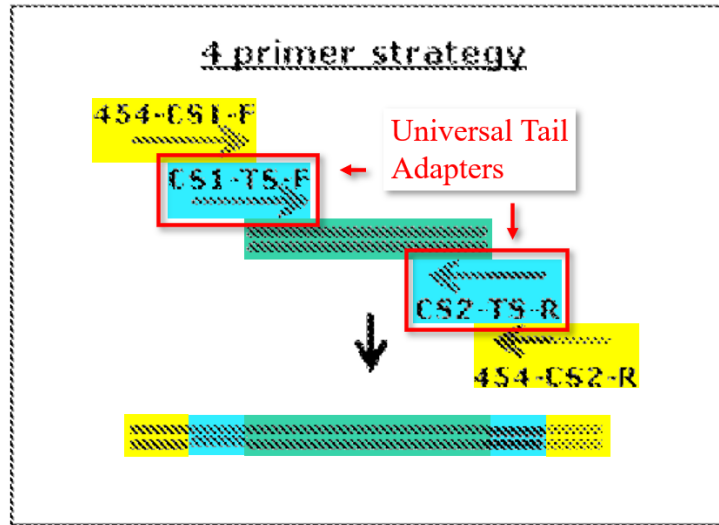
amplification because May's inner primers include target-specific sequences.



c. Claim 12

The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.

211. Claim 12 is obvious over Pieprzyk and May. As I explained above, May's two inner primers comprise universal tail adapters.

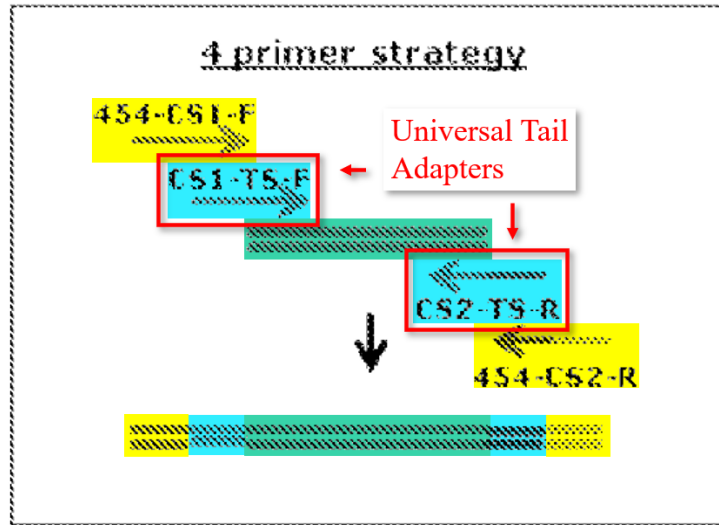


(Ex.1007, Fig.18 (cropped and annotated).)

d. Claim 13

The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.

212. Claim 13 is obvious over Pieprzyk and May. In the combined method, cfDNA is tagged (according to Patent Owner's view) by amplifying it with May's inner primers, both of which comprise universal tail adapters.



(Ex.1007, Fig.18 (cropped and annotated).)

e. **Claim 14**

The method of claim 12, wherein amplifying the tagged products comprises a single amplifying step.

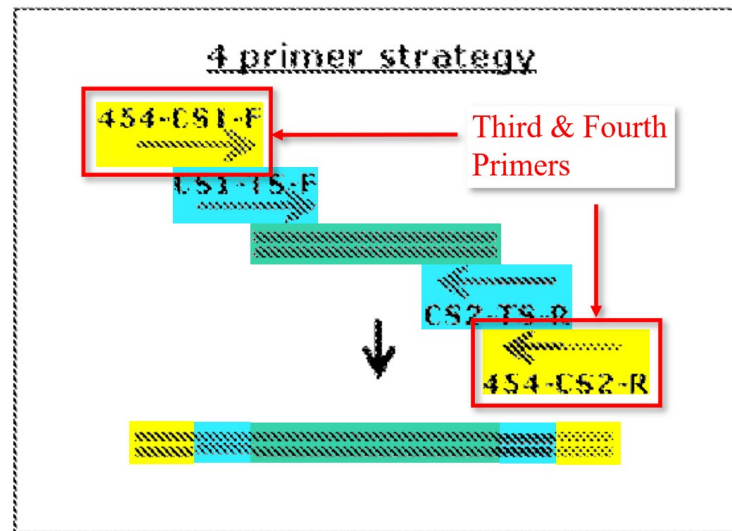
213. Claim 14 also is obvious over Pieprzyk and May. Both references teach using four primers in one amplification. (Ex.1007, ¶11 (“an amplification mixture”); Ex.1006, ¶10 (same).) Alternatively, if “a single amplifying step” is a single PCR cycle, both references teach amplifying the tagged products in one or more PCR cycles, which is what I have been asked to assume this claim requires.

f. **Claim 15**

The method of claim 14, wherein amplifying the tagged products comprises using a third primer and a fourth primer, wherein the third primer comprises a first sequencing tag and wherein the fourth primer comprises a second sequencing tag.

214. Claim 15 is obvious over Pieprzyk and May. May’s 4-primer strategy amplifies the tagged products with all four primers. (Ex.1007, ¶¶11, 115.)

And, again, May's outer primers can include "additional primer binding sites [that] are capable of being bound by DNA sequencing primers," *i.e.*, sequencing tags. (*Id.*, ¶115.) Those outer primers, which are May's third and fourth primers, therefore comprise first and second sequencing tags.



(Ex.1007, Fig.18 (cropped and annotated).)

6. Pieprzyk's Provisional Application

215. In my opinion, Pieprzyk's provisional Application No. 61/395,551 ("551 application") discloses what is claimed in at least Pieprzyk's claim 1. *Id.* (*Compare* Ex.1006 (claim 1) *with* Ex.1017, ¶49.) In my opinion, it also includes substantively the same disclosure from Pieprzyk cited in paragraphs 185-214 of my Declaration. (*Compare* Ex.1006, ¶¶2, 3, 10, 57, 110, 111, 113, 114, 116, 138, 147, 149, 151, 187, 201, 207, 218, 221, 379, 387 *with* Ex.1017, ¶¶1, 2, 25, 55, 78-79, 81-82, 84, 106, 115, 117, 119, 155, 169, 190, 240, 246, Example 6.)

7. May's Provisional Applications

216. In my opinion, May's provisional Application No. 61/305,907 ("907 Application") discloses what is claimed in at least May's claim 1. (*Compare* Ex.1007 (claim 1) *with* Ex.1016 (claims 1, 2).) In my opinion, May's provisional applications also include substantively the same disclosures from May cited in paragraphs 185-214 of my declaration. (*Compare* Ex.1007, ¶¶2, 11, 28, 115, 118, 121, 129, 132, 164, 210, 235, 276, and Fig. 18 *with* Ex.1016, ¶¶2, 71-73, 83, 86, 89, 97, 100, 132, 178, 203, 245, Fig. 18, and Claim 39.)

D. GROUND 3: PORRECA & HOYAL

217. In my opinion, Porreca and Hoyal render obvious all of claims 1-3 and 10-15 of the '035 patent. Summaries of these references, the combination, and the bases for my opinions are set forth below.

2. Summary Of Porreca

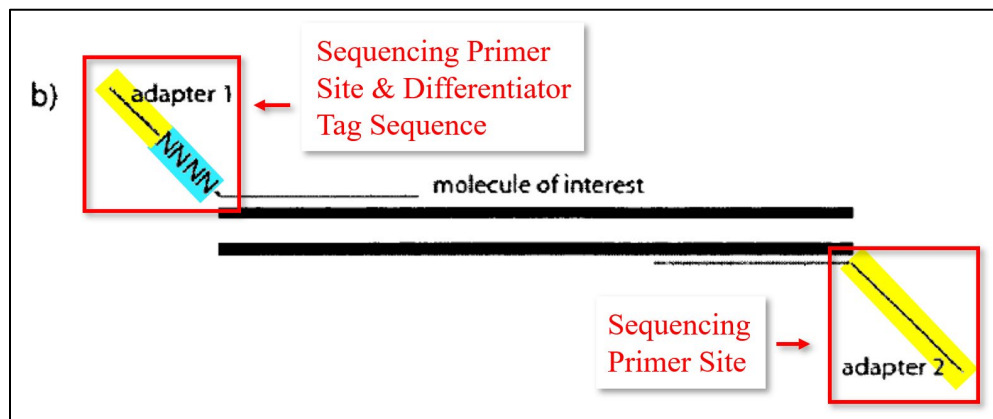
218. Porreca is a PCT application that discloses a method of analyzing "multiple genetic loci...from a patient sample, such as...blood." (Ex.1008, 4:3-5; Ex.1001, 1-2.) According to Porreca, "10-25, 25-50, 50-75, 75-100 or more loci may be evaluated for each subject." (Ex.1008, 29:15-16.) "The genetic loci may be analyzed by sequencing to obtain a genotype at one or more polymorphisms (e.g., SNPs)[,]" including "any locus associated with a disease or condition of interest." (*Id.*, 4:21-22, 45:11-12; *see also id.*, 10:22-23.) Porreca also describes

“monitoring tumor progression/regression,” and detecting “mutations” in some cfDNA. (*Id.*, 4:23-33, 7:5-10, 29:5.)

219. Porreca discloses several methods for multiplex tagging and detection of genetic loci including SNPs from samples. These preparative methods include amplification-based methods such as PCR, hybridization-based methods, and circularization selection-based methods utilizing molecular inversion probes (MIPs) (Ex.1008, 22:1-33.) Porreca provides robust disclosure for its PCR-based amplification method and importantly notes that a genotyping method of its invention “may include several steps, each of which independently may involve one or more different preparative techniques described herein...and the nucleic acid preparation may be analyzed using one or more different techniques (e.g., amplification, hybridization capture, circular probe capture, etc., or any combination thereof) that are selected independently of the techniques used for the initial preparation.” (*Id.*, 24:1-9.) Furthermore, Porreca explains, “[i]t should be appreciated that any of the circularization, amplification, and/or hybridization based methods described herein may be used in connection with one or more of the tiling/staggering, tagging, size detection, and/or sensitivity enhancing algorithms described herein.” (*Id.*, 8:28-31.) It is therefore my understanding, and it would have been the understanding of a POSA by 2010, that Porreca’s disclosures

regarding each of its preparative methods would apply equally to all of its preparative methods and were not simply limited to each method.

220. Porreca's amplification method, which from here forward I will simply refer to as "Porreca's method," includes two steps. First, "a polymerase chain reaction ...selectively amplifies each locus using one or more oligonucleotide primers." (Ex.1008, 8:5-8.) An early step in that amplification, annealing target-specific primers having sequencing primer sites (yellow) and differentiator tag sequences (blue) to a target sequence, is shown below in Porreca's Figure 4b:



(Ex.1008, Fig. 4b (annotated), 8:13-15, 8:28-31, 43:29-33.) Porreca's amplification can be a "multiplex amplification (amplification of multiple target nucleic acids in parallel)." (*Id.*, 8:15-17, 22:11-13.)

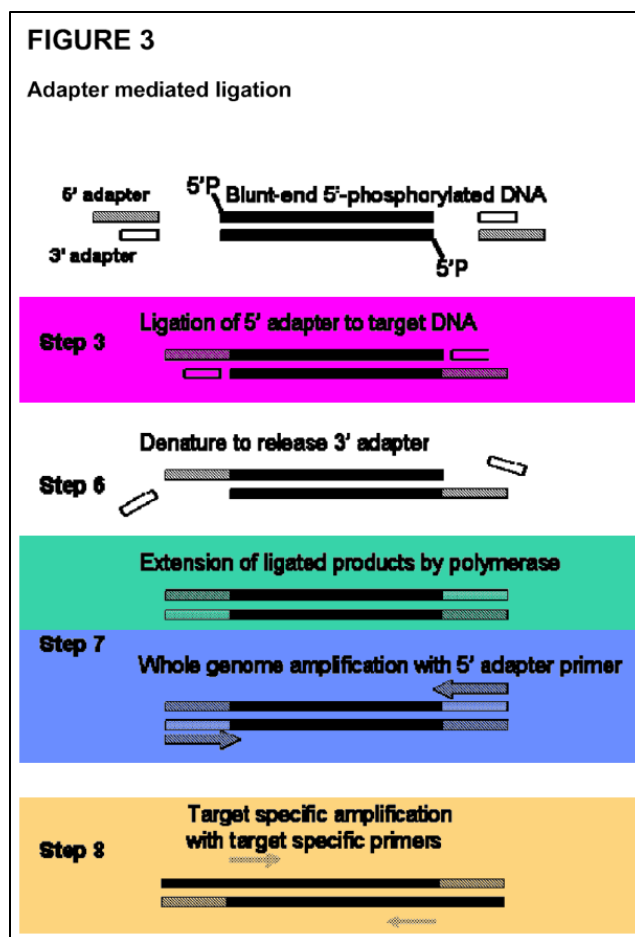
221. Second, Porreca's method sequences the amplified products. Porreca teaches "[n]ext-generation sequencing may be used to quantitatively measure the abundance of each isolated target nucleic acid obtained from a certain

preparative method[,]” including from its “amplification technique.” (Ex.1008, 23:4-13, 34:10-13.) Up to “100 or more loci may be evaluated for each subject out of any number of subject samples that may be processed in parallel.” (*Id.*, 29:15-18.) Once again, a POSA would have recognized that “next-generation sequencing” referred to massively parallel sequencing.

3. Summary Of Hoyal

222. Hoyal discloses methods for isolating and amplifying DNA from human blood and plasma. (Ex.1009, 1 (Title), 4.) Specifically, it teaches “detecting and monitoring extracellular mutant oncogenes or tumor-associated DNA found in the plasma or serum fraction of blood by using DNA extraction with enrichment for mutant DNA,” *i.e.*, detecting ctDNA. (*Id.*, 20.)

223. One such amplification method is “[a]dapter-mediated ligation.” (Ex.1009, 27.) Adapter-mediated ligation ligates “a non-genome specific adapter to the blunt ends” of DNA in a sample, extends the ligated adapter, performs “whole genome amplification” with primers specific to the ligated adapter, and performs “specific amplification of a nucleic acid region of interest for further analysis.” (*Id.*, 27-28.) Hoyal depicts ligation (lavender), primer extension (green), universal whole-genome amplification (navy), and target-specific amplification (gold) in Figure 3:



(*Id.*, Fig. 3 (annotated).)

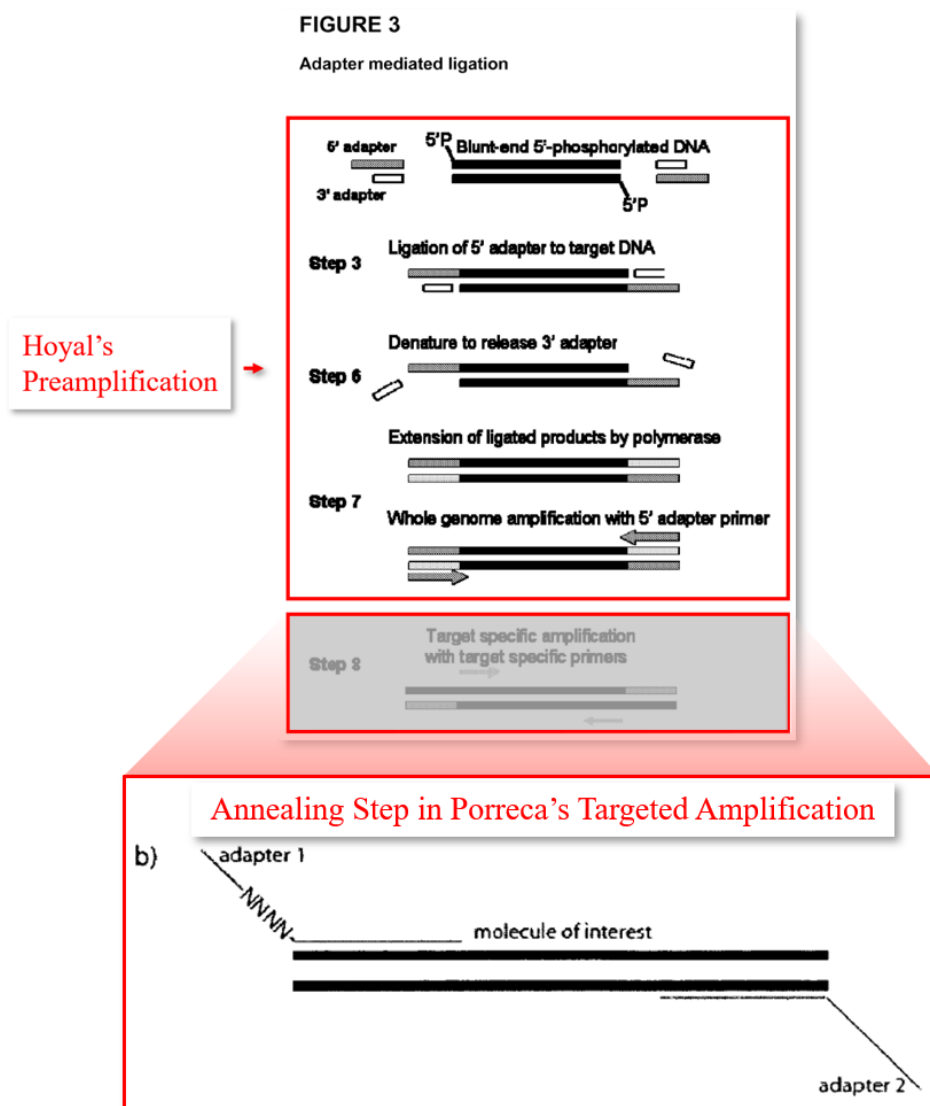
4. Combination Of Porreca And Hoyal

224. Porreca and Hoyal both are in the same field as the '035 patent. Like the '035 patent, both related to the targeted analysis of cfDNA. Porreca teaches “methods for analyzing multiple genetic loci...from a patient sample, such as a blood.” (Ex.1008, 4:3-5.) And, similarly, Hoyal teaches “monitoring...tumor-associated DNA found in the plasma or serum fraction of blood by using DNA extraction with enrichment for mutant DNA.” (Ex.1009, 10, 20.) Both references also provide solutions for one of the problems facing the '035 patent's inventors:

how to detect mutations in cfDNA. (*See, e.g.*, Ex.1001, 3:47-52; Ex.1008, 4:4-5; Ex.1009, 10, 20.) As set forth below, it would have been obvious to combine two aspects of Hoyal with Porreca.

5. Modification 1: Universal Preamplification

225. A POSA would have found it obvious to modify Porreca's method by adding Hoyal's isolation and adapter-mediated ligation preamplification steps to Porreca's method before Porreca's targeted amplification step. The following graphic showing Hoyal's Figure 3 (top) and Porreca's Figure 4b (bottom) illustrates how the combined method's amplifications would flow together by depicting Hoyal's steps 3, 6 and 7 preceding Porreca's targeted amplification step:



(Ex.1008, Fig. 4b; Ex.1009, Fig. 3 (combined and annotated).)

226. Teachings from both Porreca and Hoyal would have motivated a POSA to combine these methods. Porreca explains that some methods, like its targeted amplification, can produce a skewed pool of amplicons that “does not adequately represent the input abundance distribution.” (Ex.1008, 67:27-28.) This flaw “reduce[s] the sensitivity of the sequencing method” by increasing the risk

that “potentially the ‘mutant’ allele was the one not observed.” (*Id.*, 67:33-68:2.)

Porreca calls this flaw “acute” in the context of monitoring cancer. (*Id.*, 68:2-7.)

227. A POSA, motivated by Porreca’s teachings to seek out solutions to reduce sampling errors, would naturally turn to Hoyal, which teaches overlapping issues and offers reasonable solutions to reduce sampling errors. Like Porreca, Hoyal acknowledges that there was “a need for improved extraction methods capable of capturing small nucleic acid molecules”—cfDNA—because “the ability to successfully analyze short, fragmented DNA in the presence of more abundant, longer DNA ha[d] remained elusive.” (Ex.1009, 1-2, 20.) Hoyal solved that problem with isolation followed by universal preamplification. By isolating and then pre-amplifying the ctDNA, Hoyal’s method increased the initial number of copies of all target sequences and thereby improved the odds that a later, targeted amplification and sequencing could detect sequences with low prevalence. (*Id.*, 7.) Put another way, by increasing the initial number of copies of each sequence in solution, Hoyal’s preamplification facilitates subsequent targeted amplification by ensuring that target sequences will be present in high enough abundance to serve as templates for targeted PCR. A POSA thus would have been motivated to add a pre-amplification step to Porreca’s method to achieve that benefit.

228. A POSA would have had a reasonable expectation of success in adding Hoyal’s preamplification steps to Porreca’s protocol. Porreca already

discloses applying its method to nucleic acids from blood. (Ex.1008, 8:5-8, 18:31-32, 19:3-4, 22:1-4, 67:20-21.) It already discloses ligating adapters to cfDNA to provide primer sites for non-targeted amplification. (*Id.*, 8:5-17, 43:17, 43:23-29; *see also id.*, 8:28-31.) And it already discloses using primers for targeted amplification. (*Id.*, 8:5-7, 43:29-33, Fig. 4b; *see also id.*, 8:28-31.) A POSA would reasonably have expected success in sequentially performing these routine steps, as in the combined method.

6. Modification 2: SNP Loci Associated With Cancer In Isolated Cell-Free DNA

229. A POSA would have been motivated to apply the combined method to SNP loci associated with cancer in cfDNA (according to Patent Owner's construction) in view of disclosures from Porreca and Hoyal. As I understand Patent Owner has stated in another proceeding, techniques for studying "tumor [cell-free] DNA in a cancer patient's blood" were "routinely used before November 2009." (Ex.1039, 9.) Porreca discloses one such technique for studying cfDNA. It teaches that its method is suitable for the "detection of mutations in maternally-circulating fetal DNA," meaning fetal cfDNA. (Ex.1008, 7:5-8.) It further teaches its method is useful for detecting cancer. (*See, e.g.*, Ex.1008, 18:32-33 ("[i]n some embodiments, the biological sample is a...tumor sample, circulating tumor cells"), 29:5-6 ("monitoring tumor progression/regression [or] analyzing circulating tumor cells"), 25:3-7 ("detection of cancer mutations"), 4:23-

33, 7:5-10.) And it teaches that its method is “useful for any application where sensitivity is important.” (*Id.*, 25:3-7; *see also id.*, 5:1-3, 20:16-17, 25:3-7, 25:28-29, 26:18-25, 28:7-10, 30:9-10, 87:31-88:4.) Moreover, it has been known since the 1990s that cancers may be diagnosed and monitored by detecting and analyzing the presence of ctDNA, as well as any changes in the ctDNA. (*See* Ex.1034, 1:19-28; Ex.1033, Abstract.) Therefore, a POSA reading Porreca’s disclosures would have been motivated to apply the combined method to SNP loci associated with cancer in cfDNA.

230. To the extent Patent Owner argues that Porreca does not suggest applying its method to ctDNA, Hoyal does so. Hoyal states its method is useful for “detecting and monitoring extracellular mutant oncogenes or tumor-associated DNA found in the plasma or serum fraction of blood by using DNA extraction with enrichment for mutant DNA as provided herein.” (Ex.1009, 20.) A POSA thus would have been motivated to extend the combination to detecting mutations in ctDNA isolated from blood.

231. A POSA would have had a reasonable expectation of success in applying the combined method to cfDNA containing SNP loci associated with cancer, including ctDNA. For one thing, Hoyal already teaches that its preamplification steps, plus a targeted amplification step, can enrich ctDNA. (Ex.1009, 20.) For another, as Patent Owner told the USPTO when seeking other

claims, the “same lab techniques including extraction of cell-free DNA, amplification of targeted loci, and sequencing of the amplicons can be used in both [the] fetal DNA context and [the] cancer DNA context.” (Ex.1025, 5). And as I already have explained in this Declaration, designing primers suitable for multiplex PCR was well within a POSA’s skill by 2010. Finally, as for sequencing, ctDNA, or other cfDNA containing SNP loci associated with cancer, was no more difficult to sequence than fetal cfDNA.

7. Claims 1-3, 10-15 Are Obvious Over Porreca And Hoyal

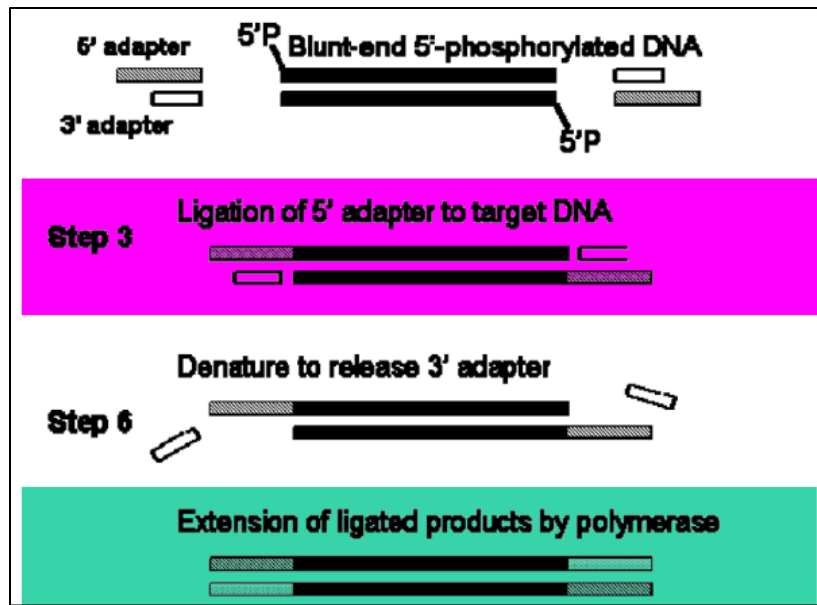
a. Claim 1

Preamble: *A method for amplifying and sequencing DNA, comprising:*

232. If limiting, Porreca and Hoyal teach methods of amplifying and/or sequencing DNA.

Limitation 1[A]: *tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,*

233. The combined method satisfies Limitation 1[A] in Hoyal’s preamplification step. In that step, DNA is tagged by ligating adapter complexes made up of a 5’ adapter and a 3’ adapter (lavender) and then extending the ligated products (green):



(Ex.1009, Fig. 3 (cropped and annotated), 16, 27-28.)

234. The adapter complexes comprise universal tail adapters because their constituent adapters contain sequences used for non-targeted “whole genome amplification.” (*Id.*, 16, 27-28.) “[W]hole genome amplification” refers to a universal, non-specific amplification of all DNA in solution. Alternatively, the 5’ adapters themselves comprise universal tail adapters. In either scenario, the green highlighted DNA is a tagged product. Finally, as I already have explained above in paragraphs 229-231 of my Declaration, a POSA would have found it obvious to apply the combined method to isolated cfDNA.

Limitation 1[B]: wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;

235. The combined method satisfies this limitation. Porreca teaches collecting the target “nucleic acid from a biological sample obtained from a

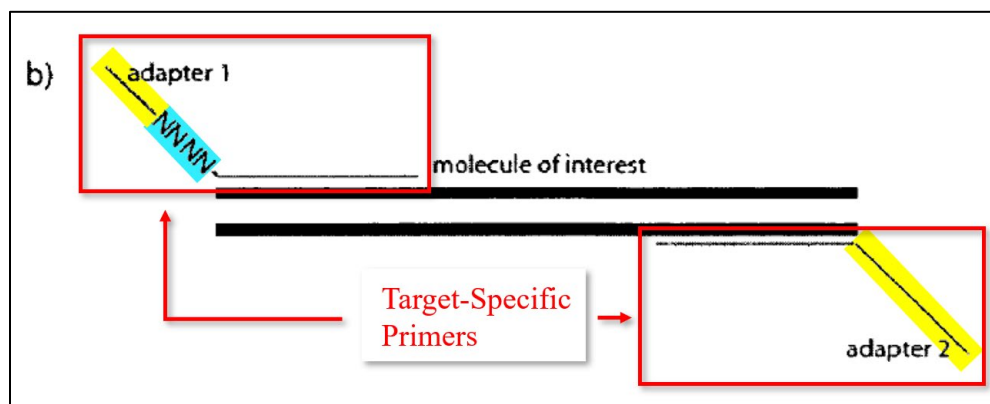
subject,” such as “blood.” (Ex.1008, 18:30-32.) And it further teaches that the “subject or patient may be male,” *i.e.*, not a pregnant woman. (*Id.*, 31:29-30.)

Limitation 1[C]: *amplifying the tagged products one or more times to generate final amplification products,*

236. The combined method has two amplification steps. First, the tagged products from Hoyal’s ligation steps undergo “[w]hole genome amplification”:



(Ex.1009, Fig. 3 (cropped and annotated), 27-28.) Second, the products of that first amplification undergo targeted amplification with Porreca’s primers:



(Ex.1008, Fig. 4b (annotated), 8:5-8, 8:15-17, 8:28-31, 43:29-33.) The products of both amplification steps—target sequences tagged with Porreca’s target-specific primers—are final amplification products.

Limitation 1[D]: wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume,

237. The combined method discloses or renders obvious every element of Limitation 1[D]. First, the combined method's second amplification step is a "targeted amplification" because it selectively amplifies the cfDNA with target-specific primers. (Ex.1008, 8:5-8, 43:29-33, Fig. 4b.)

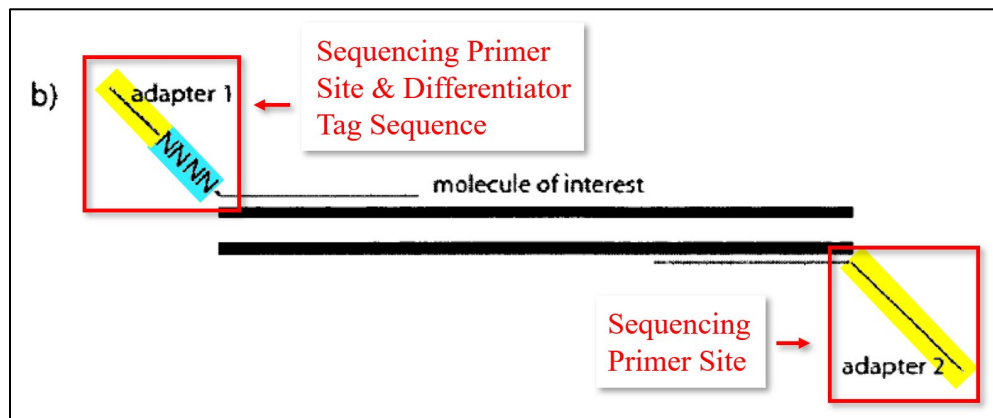
238. Second, that targeted amplification amplifies a plurality of SNP loci in a single reaction volume. Porreca discloses that its "PCR based methods may include...multiplex amplification (amplification of multiple target nucleic acids in parallel)." (Ex.1008, 22:11-13.) It discloses its invention is "particularly useful for analyzing multiple loci in a multiplex assay" and for the "detection of cancer mutations," which Patent Owner contends are SNPs. (*Id.*, 10:22-23, 25:3-7.) And it discloses that its "[m]ultiplex amplification strategies disclosed herein may be used analytically, as in detection of SNPs." (*Id.*, 37:24-26; *see also id.*, 13:9-10, 28:7-10, 29:15-18, 45:11-12.) A POSA would have known that "multiplex amplification" meant amplifying multiple sequences in a single reaction volume. (*See also, e.g., id.*, 8:15-17; 29:23-25.)

239. Alternatively, if Porreca had not disclosed this limitation, the foregoing disclosures render it obvious. A POSA would have been motivated by the foregoing disclosures to selectively amplify sequences containing SNP loci

associated with cancer in a single reaction volume because doing so is more efficient than amplifying them separately. (Ex.1008, 29:15-18.) A POSA would have reasonably expected the combined amplification to succeed because, as the '035 patent confirms, multiplexing up to “100 assays in the same well” was known. (Ex.1001, 48:25-27; 85:14-20, 87:32-33; *see also* Ex.1040, ¶98; Ex.1041, ¶202.)

Limitation 1[E]: wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and

240. The combined method's targeted amplification step introduces a sequencing adapter and differentiator tag, which correspond to the claimed sequencing tag and barcode, respectively. (Ex.1008, 8:5-8, 43:29-33.) Porreca's Figure 4b, below, depicts its target-specific primers having two sequencing primer sites (yellow) and one differentiator tag (blue):



(Ex.1008, Fig. 4b (annotated).) Through amplification, the barcode and sequencing tags are incorporated into final amplification products.

Limitation 1[F]: sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products,

241. Porreca teaches “sequencing to obtain a genotype at one or more polymorphisms (e.g., SNPs).” (Ex.1008, 45:9-15; *see also id.*, 21:8-32.) It also discloses using “[n]ext-generation sequencing” to analyze “each isolated target nucleic acid,” including those isolated via its “amplification technique[s]” (discussed above). (*Id.*, 23:4-13; *see also id.*, 21:8-32.) Thus, Porreca discloses sequencing the plurality of SNP loci on the cfDNA in its final amplification products via massively parallel sequencing.

242. Alternatively, had Porreca not disclosed the foregoing method, in my opinion, it would have been obvious to use massively parallel sequencing to sequence the final amplification products. A POSA would have been motivated to use massively parallel sequencing because, as Patent Owner’s own expert has admitted, before 2010, “next-generation sequencing was in widespread use,” including for cfDNA. (Ex.1040, ¶115.) Unlike prior Sanger sequencing, such massively parallel sequencing was accessible and easy-to-use. And as the ’035 patent itself indicates, Illumina’s HiSeq and MiSeq massively parallel sequencers were widely available before its drafting. (Ex.1001, 90:60-64.) Given the method’s widespread use and ready availability, a POSA would also have found it

trivial to perform massively parallel sequencing on the final amplification products by the critical date.

Limitation 1[G]: *wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.*

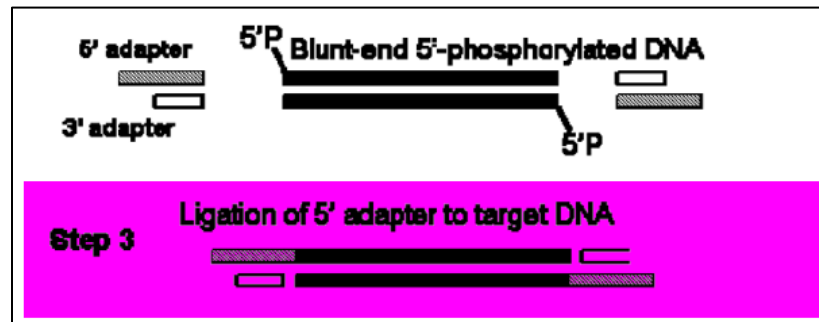
243. Porreca discloses genotyping (including sequencing) “10-25, 25-50, 50-75, 75-100 or more loci...for each subject.” (Ex.1008, 29:10-18; *see also id.*, 12:25-26, 25:28-31, 31:15-19.) It discloses that “[e]mbodiments of [its] invention may be used for any of a number of different settings,” including “identifying subjects having cancer.” (*Id.*, 30:9-18.) It even recites that its “multiplex diagnostic methods comprise capturing a plurality of genetic loci in parallel,” and gives specific examples of genes containing SNP loci associated with cancer, like ATRX/RAD54 and FANCC. (*Id.*, 31:15-21, 45:16-49:33.) Finally, as discussed above, it would have been obvious to apply the combined method to ctDNA, which contains SNP loci associated with cancer (under Patent Owner’s construction).

b. Claims 2 & 3

The method of claim 1, wherein tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA.

The method of claim 2, wherein the one or more universal tail adaptors each comprise a first strand and a second strand, wherein a first end of each of the universal tail adaptors comprises a double-stranded section comprising the 5’ portion of the first strand and the 3’ portion of the second strand, wherein the first end is ligated to the cell free DNA.

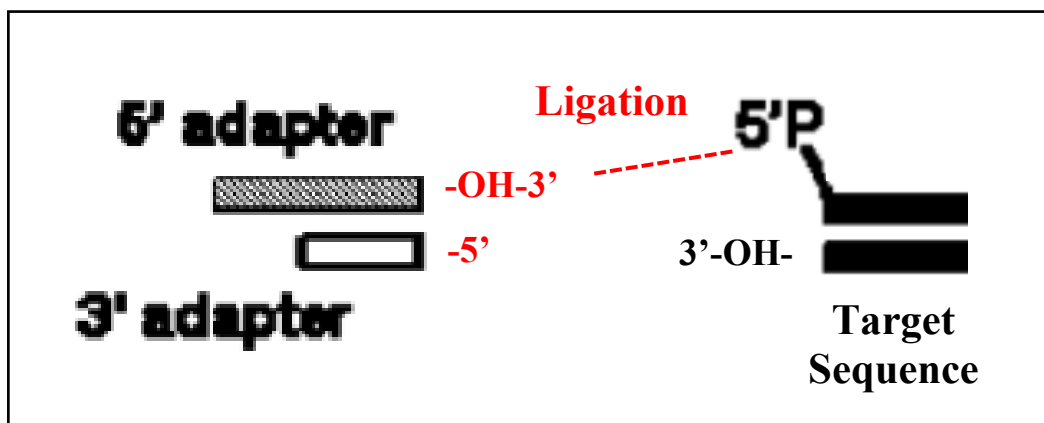
244. As explained above, the combined method tags cfDNA by ligating universal tail adapters to it. (Ex.1009, 16, 27-28, Fig. 3.) Those adapter complexes are double-stranded:



(Ex.1009, Fig. 3 (cropped and annotated), 27-28.) Each adapter complex has two ends, and each end includes a 5' portion of one strand and a 3' portion of a second strand (that is how double-stranded DNA is structured). The first, blunt ends of the adapter complexes are ligated to the cfDNA via a covalent link between the 5' adapters and the target DNA. (*Id.*, 16, 27-28, Fig. 3.)

245. Alternatively, the additional limitation of claim 3 would have been obvious. Hoyal explains that “3; and 5; dephosphorylated adapters [that] are complementary form a double-stranded blunt end adapter complex” and that its method “...capitalizes on the blunt end and 5' phosphorylated nature of the target nucleic acid as a means to attach a non-genome specific adapter to the blunt ends using the ligation process.” (Ex.1009, 15.) Ligation between two DNA strands is accomplished by DNA ligases that catalyze the formation of phosphodiester bonds between the 5' phosphate group of the first DNA strand and the 3' hydroxyl group

of the second DNA strand. Hoyal therefore discloses universal adaptors lacking a 5' phosphate group on the 3' adapter, which results in this single DNA strand not attaching to the target sequence during ligation.



(*Id.*, Fig. 3 (cropped and annotated).)

246. It would have been obvious to modify Hoyal's preamplification to use (and ligate) a double-stranded adapter with two strands of equal length and with a phosphate group on the 3' adapter in place of Hoyal's single-stranded 5' adapter. First, the governing biological principles of this ligation reaction are universal. That is, the ability of a DNA ligase to form a phosphodiester bond between a 5' phosphate group and a 3' hydroxyl group would similarly apply to the "3' adapter" of Hoyal if it also comprised a 5' phosphate group. A POSA would undoubtedly understand this fact, as well as the fact that these reactions are readily performed by the same lab techniques.

247. Furthermore, a POSA would also be familiar with blunt-end ligations wherein both strands of a double-stranded adapter are ligated to a target

DNA molecule would find the design and implementation of said reaction to be trivial. A POSA would have known that cfDNA typically is blunt ended because, during its formation, “Caspase-activated DNase (CAD)...generate[s] double-stranded DNA breaks.” (Ex.1009, 14.) Similarly Porreca discloses performing its methods on blunt-ended DNA: “nucleic acids comprising differentiator tag sequences may be incorporated by ligation...[t]his is a flexible method, because molecules having differentiator tag sequence[s] can be ligated *to any blunt-ended nucleic acids.*” (Ex.1008, 43:22-25 (emphasis added).) Thus, in practical terms, modifying Hoyal’s preamplification as proposed here would require nothing more than using double stranded adapters (*i.e.*, Hoyal’s 5’ adapter with an equally long complimentary sequence) where the elongated 3’ adapter” included a 5’ phosphate group in place of Hoyal’s original adapter complexes. Such adapters could have been ordered from any number of companies by 2010, and thus incorporating the modified adapters into Hoyal’s preamplification would have been trivial to a POSA by that date.

248. A POSA would be motivated to make this modification to Hoyal’s preamplification. Doing so would obviate Hoyal’s extension reaction and thereby simplify Hoyal’s method. (Ex.1009, Fig. 3 (step 6).) The extension step of Hoyal’s protocol requires entirely different reaction conditions and reagents (e.g., DNA polymerases and unique buffer and temperature conditions), the introduction

of which is time-consuming and costly. By contrast, ligating a double-stranded adapter at both strands can be accomplished in a single ligation reaction with no extra effort, thereby saving time and resources.

c. Claims 10 & 11

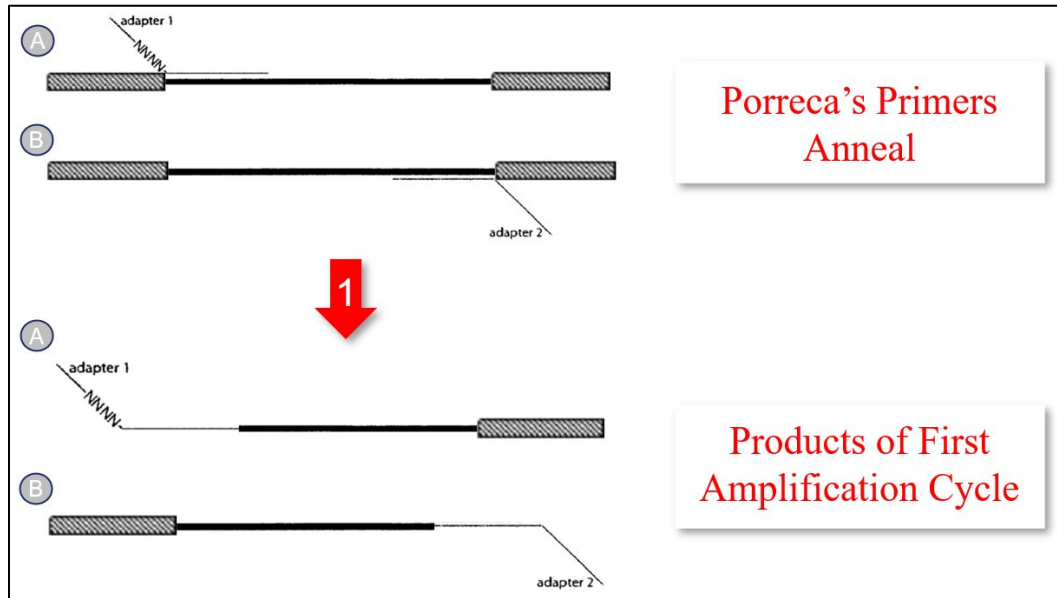
Claim 10: *The method of claim 1, wherein the one or more universal tail adapters comprise a second barcode.*

Claim 11: *The method of claim 1, wherein the one or more universal tail adapters comprise a second sequencing tag.*

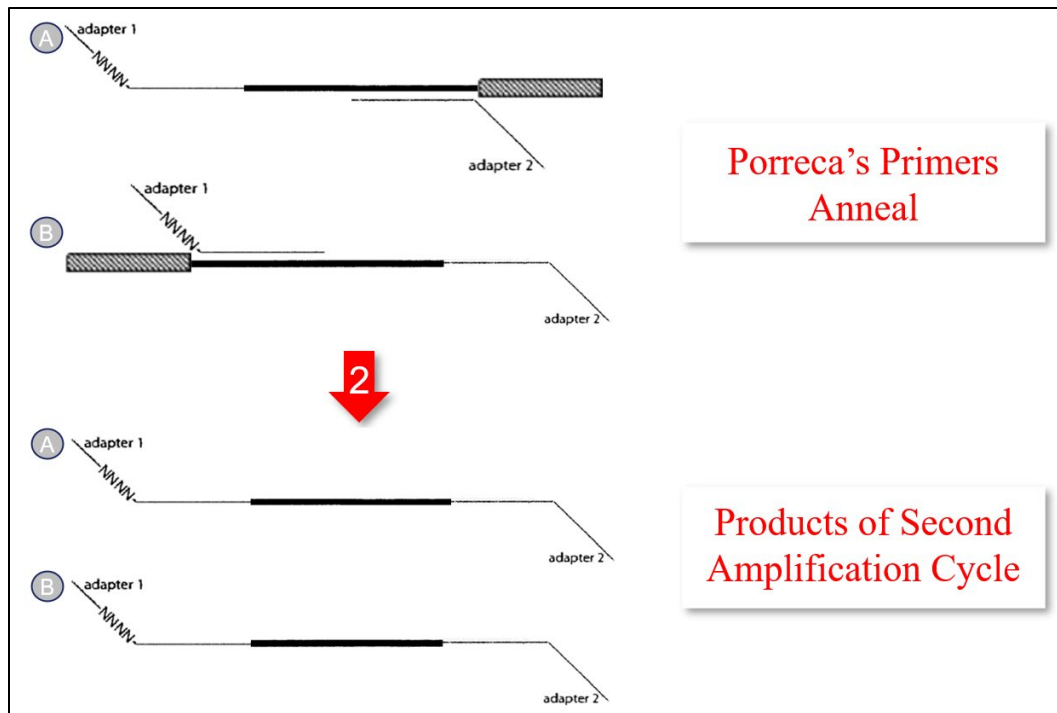
249. These claims are obvious over the combined method of Porreca and Hoyal. Porreca's target-specific primers comprise universal tail adapters because they include "common adapter sequence[s]." (Ex.1008, 43:29-33.) The common adapter portions of the primers comprise sequencing tags because they "contain the sequencing primer site." (*Id.*, 43:29-33.) The primers also can contain differentiator tags, which are barcodes. (*Id.*, 6:29-30, 22:9-11, 43:29-33, 63:16, 71:21.)

250. Under Patent Owner's construction, amplifying with Porreca's target-specific primers would generate tagged products in the first amplification cycle in which they anneal to the products of Hoyal's whole genome amplification step. (*Id.*, 8:5-8, 43:29-33.) For instance, the upper half of the following graphic, adapted from Hoyal's Figure 3 and Porreca's Figure 4b, illustrates Porreca's first and second target specific primers annealing to regions of DNA strands. The lower half of the below graphic depicts products of the first amplification cycle.

As shown in the graphic, the first amplification cycle tags the DNA strands with either Porreca's first or second adapter.



Then, as shown in the following graphic, the tagged products undergo targeted amplification. The amplification is targeted because Porreca's target-specific primers anneal to and then extend from specific sequences in the target DNA.



(*Id.*) I also note that, although the foregoing graphics depict only one of Porreca's target-specific primers as including an "NNNN" barcode, Porreca's text indicates that both of its primers can include barcodes. (*See, e.g., Ex.1008, 22:9-11* ("Primers may also include additional nucleic acids that are not complementary to target sequences but that facilitate downstream applications, including for example restriction sites and *identifier sequences (e.g., source sequences)*"); 71:21 ("sequence tags (also referred to as barcodes)").)

d. Claim 12

The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.

251. The combined method tags isolated cfDNA with two universal tail adapters in the ligation step. Alternatively, and under Patent Owner's view of the

claims, the combined method tags isolated cfDNA with two universal tail adapters—Porreca’s target-specific primers—in the first amplification cycle of Porreca’s targeted amplification.

e. Claim 13

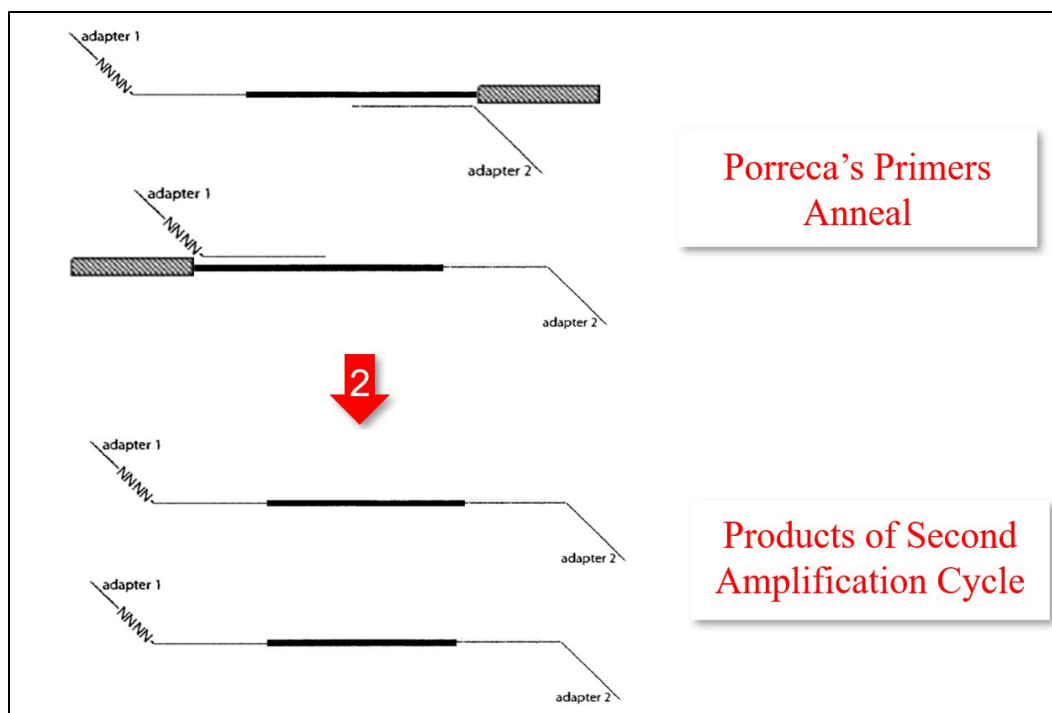
The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.

252. The combined method satisfies claim 13’s additional limitation. In the first amplification cycle of Porreca’s targeted amplification, the combined method tags the cfDNA according to Patent Owner’s view of the claims with Porreca’s two, target specific-primers, which comprise universal tail adapters.

f. Claim 14

The method of claim 12, wherein amplifying the tagged products comprises a single amplifying step.

253. The combined method amplifies tagged products in one or more single amplification steps, which is what I have been asked to assume this claim requires. Alternatively, if required, the targeted amplification also performs all of targeted multiplex amplification and introducing barcodes and sequencing tags to tagged DNA strands in the second amplification cycle of its reaction:



(Ex.1008, 43:29-33.)

g. Claim 15

The method of claim 14, wherein amplifying the tagged products comprises using a third primer and a fourth primer, wherein the third primer comprises a first sequencing tag and wherein the fourth primer comprises a second sequencing tag.

254. The combined method amplifies the tagged products using four primers: first and second, Hoyal's forward and reverse 5' adapter primers; and third and fourth, Porreca's forward and reverse target-specific primers. (Ex.1008, 8:5-8, 8:15-17, 8:28-31, 43:29-33; Ex.1009, 27-28, Fig. 3.) As I explained above, Porreca's forward and reverse target-specific primers contain sequencing tags. (*Id.*, 8:5-8, 43:29-33.)

8. Porreca's Provisional Applications

255. In my opinion, Porreca's U.S. provisional Application No. 61/182,089 ("089 Application") discloses what is claimed in at least Porreca's claim 1. (*Compare* Ex.1008 (claim 20) *with* Ex.1014, 4:26-30.) In my opinion, Porreca's provisional applications also include substantively the same disclosure from Porreca cited in paragraphs 224-253 of my Declaration. (*Compare* Ex.1008, 4:4-5, 4:21-33, 5:1-3, 6:29-30, 7:5-10, 8:5-17, 8:28-31, 10:22-23, 12:25-26, 13:9-10, 18:31-33, 19:3-4, 20:16-17, 21:8-32, 22:1-4, 22:9-13, 23:4-13, 25:3-7, 25:28-31, 26:18-25, 28:7-10, 29:5, 29:10-18, 29:23-25, 30:9-18, 31:15-21, 31:29-30, 34:10-13, 37:24-26, 43:17, 43:23-33, 45:9-49:33, 63:16, 67:20-21, 67:27-68:7, 71:21, 87:31-88:4, Fig. 4b *with* Ex.1011, 6:18-21, 9:11-13; Ex.1012, 2:27-3:16, 4:15-27, 5:8-16; 5:31-34; 7:32-33, 8:5-13; Ex.1013, 3:6-10, 4:4-7, 4:11-15, 7:6-9, 7:21-32, 8:2-3, 10:28-29, 11:1-11, 12:18-24, 13:25-19:10, 28:23-33:4, Fig. 1b; *and* Ex.1014, 2:5-6, 4:3-4, 4:17-21, 7:15-17, 7:18-19, 7:27-28, 9:14-24, 10:22-24.)

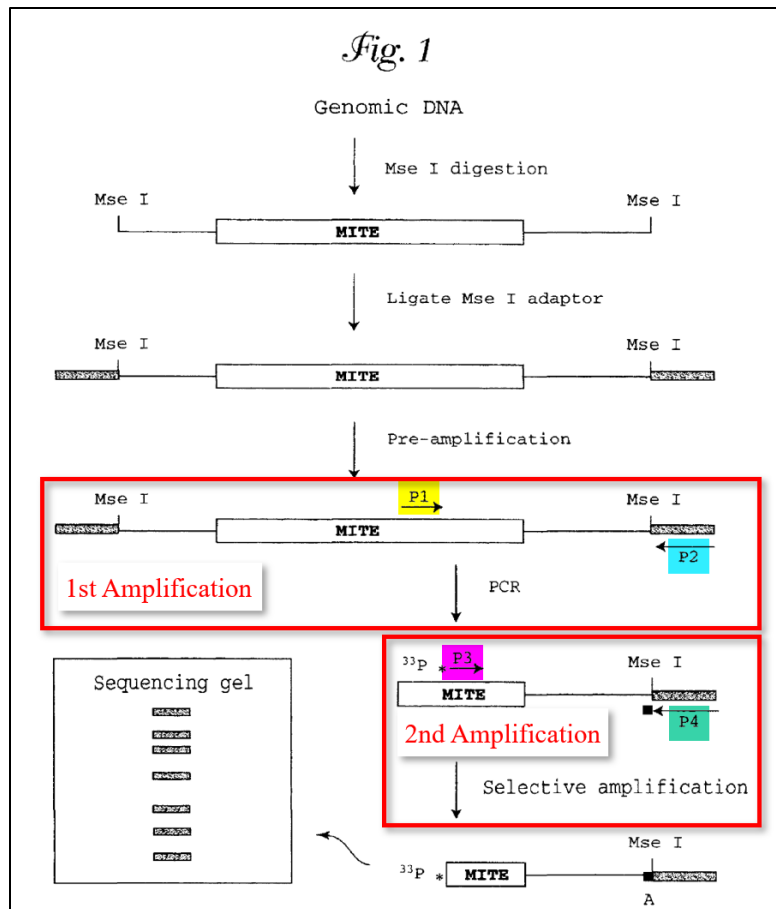
E. GROUND 4: PORRECA & WESSLER

256. In my opinion, the combination of Porreca and Wessler renders obvious all of claims 1-5 and 10-15. A summary of Wessler, the combination, and the bases for my opinions are set forth below.

2. Summary Of Wessler

257. Wessler discloses methods of "detecting at least one polymorphism between the nucleic acid fragments of two individuals." (Ex.1010, Abstract.)

First, Wessler teaches ligating double-stranded adapters onto target DNA. (*Id.*, 10:53-62, Fig. 1.) It then describes a targeted amplification involving selectively amplifying part of a target sequence (“MITE”) via a first primer that anneals to MITE and a second primer complementary to part of a ligated sequencing adapter. (*Id.*, 10:63-11:7, 11:45-60, 12:5-15; *see also id.*, 3:36-42.) Then, in a second, optional targeted amplification, Wessler uses third and fourth primers to amplify part of the earlier amplification product. (*Id.*, 10:63-11:7, 11:45-60, 12:16-37; *see also id.*, 3:36-42.) Like the first primer, the third primer anneals to part of MITE and, like the second primer, the fourth primer contains a nucleotide sequence identical to a portion of the adapter’s first strand. (*Id.*, 11:45-60, 12:16-37.) Wessler depicts these amplifications and its first, second, third, and fourth primers (yellow, blue, lavender, and green, respectively), in its Figure 1:



(*Id.*, Fig. 1 (annotated).)

3. Combination Of Porreca And Wessler

258. In my opinion, a POSA considering Porreca's disclosures also would have looked to Wessler. Wessler is in the same field as the '035 patent: both Wessler and the '035 patent relate to the targeted analysis of genomic regions. And while Wessler applies its methods to plant genomes, similar polymorphisms exist in humans, and Wessler's methods are not limited to plants. (Ex.1010, 2:1-4, 3:6-4:41, 29:22-30:4.)

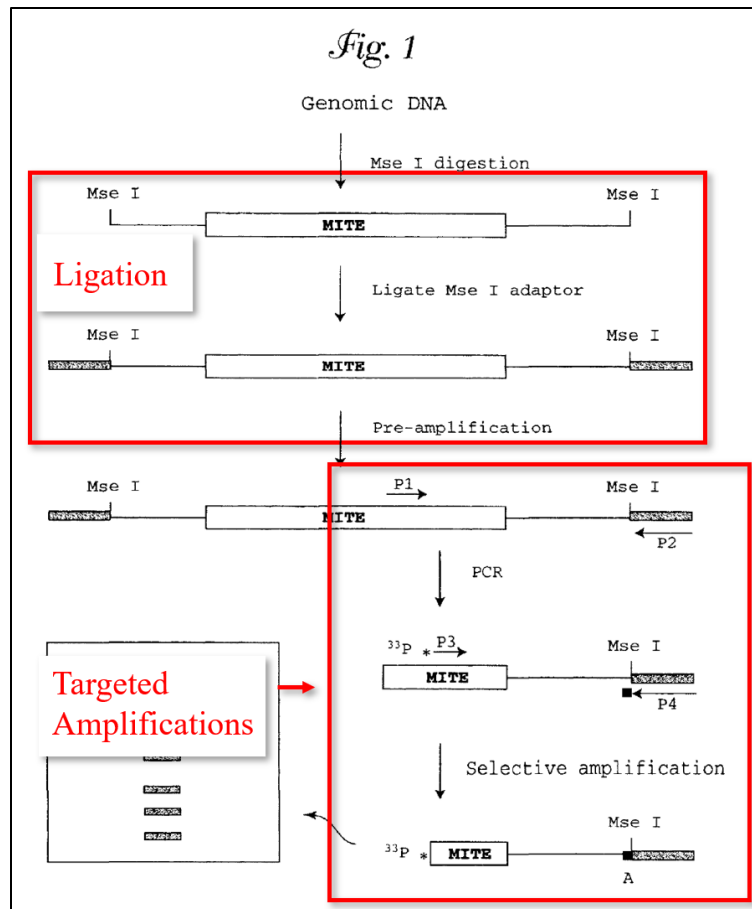
259. Wessler also is reasonably pertinent to the problems facing the '035 patent's inventors. Like the '035 patent, Wessler teaches solutions for detecting polymorphisms in DNA. (Ex.1010, 3:6-4:41; Ex.1001, 1:1-2.) Wessler also addresses a concern faced by the '035 patent's inventors—cost—by teaching a solution for ameliorating the “cost in generating and mapping excessive numbers of markers.” (Ex.1010, 3:2-3; *see* Ex.1001, 3:50-52 (citing need to reduce “time and cost” for fetal DNA genotyping).) Wessler's method—including ligation and nested, target-specific amplifications—also provides a solution for selectively amplifying certain sequences of DNA, which was another problem faced by the '035 patent's inventors. (*E.g.*, Ex.1010, Fig. 1; Ex.1001, (abstract) (invention directed to primers yielding “minimal formation of amplified primer dimers or *other non-target amplicons*”) (emphasis added).)

260. Furthermore, I believe a POSA seeking to configure a protocol for detecting SNP loci would reasonably turn to other references in the field of genomics, specifically references—like Wessler—focused on identification of polymorphisms. A POSA would naturally consider references within any of the subfields of genomics and, in particular, plant genomics because plant genomics was widely recognized as an advanced field in detection of polymorphisms by 2010. To illustrate with a recent example, I understand that in 2017, a company called Guardant Health licensed certain patents relating to sequencing technologies

from KeyGene, an agricultural company. (Ex.1114, 2.) Guardant Health advertises assays for detecting ctDNA in blood, and its decision to license patents from an agricultural company further confirms a POSA configuring a method of monitoring ctDNA from humans would have looked to references like Wessler. Thus, and as set forth below, it would have been obvious to combine Porreca and Wessler.

4. Modification 1: First And Second Targeted Amplifications

261. A POSA would have been motivated to modify the Porreca method by performing Wessler's ligation and one or two targeted amplifications in lieu of Porreca's targeted amplification. The following graphic from Wessler shows its ligation and targeted amplification steps:



(Ex.1010, Fig. 1 (annotated).)

262. Teachings from Porreca and Wessler would have motivated a POSA to combine these methods. **First**, using multiple amplifications would improve performance. Porreca's single targeted amplification can sometimes produce a skewed pool of amplicons that limits the method's accuracy. (Ex.1008, 67:27-68:7.) Wessler's ligation followed by preamplification provides a solution. (Ex.1010, Fig. 1.) By preamplifying isolated, cfDNA, the combined method would increase the initial numbers of copies of all target sequences and thereby improve

the odds that a later targeted amplification and sequencing could detect sequences with low initial prevalence.

263. ***Second***, a POSA also would have recognized that Wessler's optional second targeted amplification offered improved specificity over Porreca's method. Amplifying a first sequence and then amplifying a second sequence within the first sequence was a well-known method of reducing the abundance of non-specific or off-target amplification products compared to a single PCR reaction.

264. Indeed, it is my understanding that the first and second targeted amplification steps of Wessler are a specific example of a broader method known as nested PCR. By using two sequential PCR steps, nested PCR allows for more sensitive detection of target sequences by reducing the abundance of non-specific or off-target amplification products from a single PCR alone. A POSA in the field of genomics broadly and cfDNA detection, specifically, would have understood the principles and benefits of nested PCR at least by 2010, and they would have seen examples of it used in this context. For example, Igetei *et al.* disclosed in 2008 the use of nested PCR on cfDNA from the plasma of eighty-five adults with hepatocellular carcinoma, a form of liver cancer, using nested PCR to assay for the presence of mutations in codon 249 of the p53 gene. (Ex.1115, 2.)

265. **Third**, Wessler's first and second targeted amplifications would significantly reduce the number of possible off-target interactions between primers. Each of the first and second targeted amplifications uses half as many target-specific primers as in Porreca's amplifications. To illustrate, a reaction with 100 pairs of unique forward and reverse primers involves 10,000 possible interactions between forward and reverse primers, whereas a reaction with 100 unique forward primers and one reverse primer involves only 100 such interactions.

266. **Fourth**, Wessler's targeted amplifications are better equipped to ameliorate primer dropout potentially caused by polymorphisms or mutations in the primer binding sites and Porreca's targeted amplifications. That is, genetic variation in the cfDNA surrounding target sequences of interest, particularly at primer binding sites, may impede hybridization of primers to these sites for PCR. By annealing one primer to a ligated adapter, which has a known sequence, Wessler's method halves the risk of primer dropout compared to Porreca's method. This benefit would have appealed to a POSA in the context of assays for detecting mutations associated with cancer because many tumor cells accumulate passenger mutations in their genomes, particularly in the case of late-stage cancers.

267. A POSA also would have reasonably expected the combination to succeed. By the critical date, adapting the Porreca method according to Wessler was within a POSA's skill. Porreca teaches its methods can "be implemented as

described herein using alternative techniques and/or protocols that are available to one o[f] ordinary skill in the art.” (Ex.1008, 87:31-33, 88:1-2.) The modification would have required nothing more than applying known ligation and amplification methods to isolated cfDNA.

5. Modification 2: SNP Loci Associated With Cancer In Isolated Cell-Free DNA

268. As I explained above in paragraphs 229-231, Porreca’s disclosures would have motivated a POSA to target SNP loci associated with cancer in cfDNA (under to Patent Owner’s construction). A POSA would have had a reasonable expectation of success in extending Porreca’s method to isolated cfDNA in the cancer context because, again, applying such methods to such DNA was no more difficult than applying them to fetal cfDNA, which Porreca already discloses. As I understand that Patent Owner has acknowledged in another proceeding, methods for studying “tumor [cell-free] DNA in a cancer patient’s blood” were “routinely used before November 2009.” (Ex.1039, 9.)

6. Claims 1-5, 10-15 Are Obvious Over Porreca And Wessler

a. Claim 1

Preamble: A method for amplifying and sequencing DNA, comprising:

269. If limiting, Porreca and Wessler teach methods of amplifying and/or sequencing DNA.

Limitation 1[A]: tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,

270. The combined method performs Limitation 1[A]’s step on isolated, ctDNA during Wessler’s ligation step. (Ex.1010, 10:53-62, Fig. 1.) The ligated adapters comprise universal tail adapters because they contain sequences used for non-target specific amplification. (*Id.*, 12:5-36, Fig. 1.) Ligating the adapters generates amplicons tagged with the adapters. (*Id.*, Fig. 1.)

Limitation 1[B]: *wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;*

271. As explained above, it would have been obvious to apply the combined method to isolated cfDNA. Porreca teaches collecting “nucleic acid from a biological sample obtained from a subject,” such as “a blood sample.” (Ex.1008, 18:30-32.) And it further teaches that the “subject or patient may be male,” *i.e.*, not a pregnant woman. (*Id.*, 31:29-30.)

Limitation 1[C]: *amplifying the tagged products one or more times to generate final amplification products,*

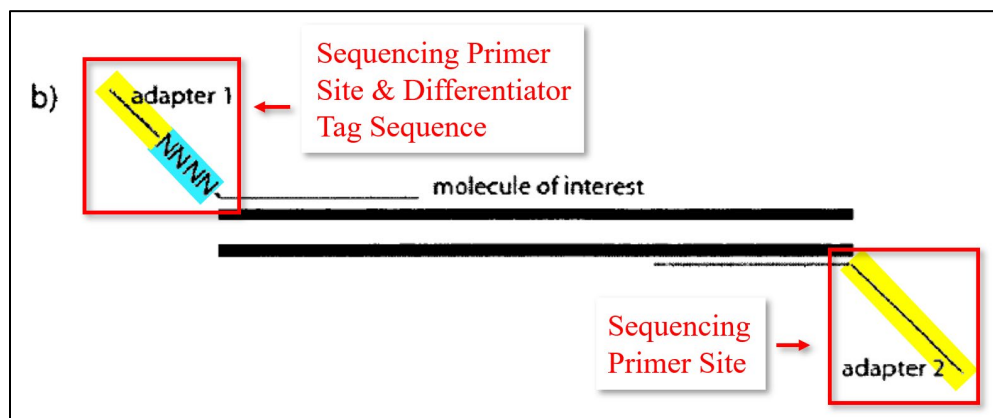
272. The combined method has two targeted amplification steps. First, the tagged products from Wessler’s ligation undergo targeted amplification using one of Porreca’s target-specific primers and one of Wessler’s adapter primers. Second (and optionally), the process repeats. The products of these amplification steps are final amplification products.

Limitation 1[D]: *wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume,*

273. In my opinion, the combined method discloses or renders obvious every element of Limitation 1[D]. First, the combined method's targeted amplifications are "targeted" because they selectively amplify the cfDNA with target-specific primers. (E.g., Ex.1008, 8:5-8, 43:29-33, Fig. 4b.) Second, those amplifications would amplify a plurality of SNP loci in a single reaction volume because, as explained above in paragraphs 237-239 of my Declaration, Porreca teaches those elements or renders them obvious.

Limitation 1[E]: wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and

274. The combined method's first and optional second amplification steps introduce Porreca's sequencing adapter and differentiator tags, which correspond to the claimed sequencing tag and barcode, respectively. (Ex.1008, 8:5-8, 43:29-33.) The left-hand portion of Porreca's Figure 4b depicts a sequencing adapter (yellow) and a barcode (blue):



(Ex.1008, Fig. 4b (annotated).) Through amplification, the barcode and

sequencing tags are incorporated into final amplification products.

Limitation 1[F]: *sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products,*

275. As I explain in paragraphs 241-242 of my Declaration, Porreca discloses or renders obvious this limitation.

Limitation 1[G]: *wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.*

276. As I explain in paragraph 243 of my Declaration, Porreca discloses or renders obvious this limitation.

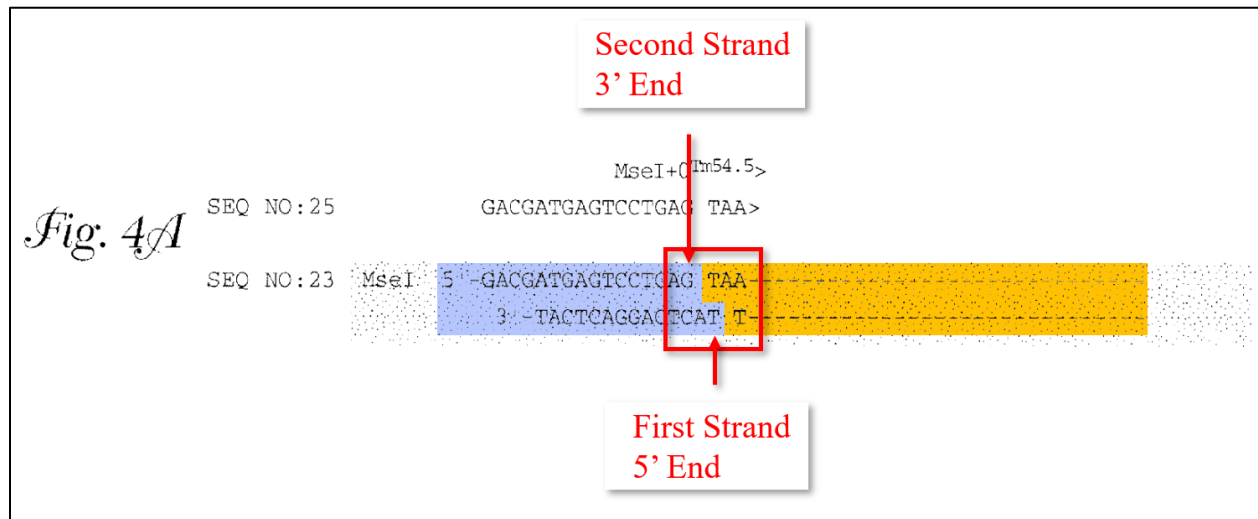
b. Claims 2 & 3

The method of claim 1, wherein tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA.

The method of claim 2, wherein the one or more universal tail adaptors each comprise a first strand and a second strand, wherein a first end of each of the universal tail adaptors comprises a double-stranded section comprising the 5' portion of the first strand and the 3' portion of the second strand, wherein the first end is ligated to the cell free DNA.

277. As explained above in paragraph 269 of my Declaration, the combined method tags cfDNA by ligating universal tail adaptors to it. Wessler teaches that its adaptors may comprise a double-stranded “SEQ ID No:23.” (Ex.1010, 6:28-34, 10:59-62, 13:9-14.) Those adaptors include a first strand and a second strand, wherein a first end of each adapter includes a 5' portion of the first strand and a 3' portion of the second strand that are ligated to the target, cfDNA.

(*Id.*, Fig. 4A.) Wessler depicts one of these adapters (navy) ligated to one end of target DNA (gold) with a first end in Figure 4A:



(*Id.*, Fig. 4A (annotated).)

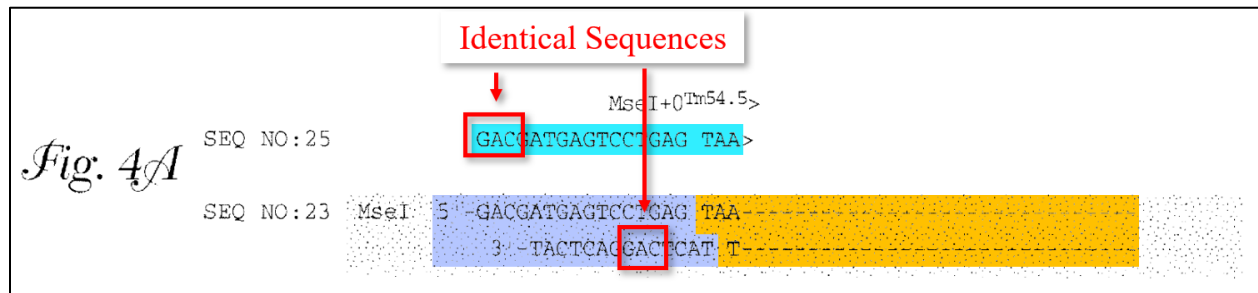
c. Claim 4

The method of claim 3, wherein amplifying the tagged products comprises a first amplifying step and a second amplifying step, wherein the first amplifying step comprises using a first target-specific primer that specifically anneals to a target sequence and a first adaptor primer having a nucleotide sequence identical to a first portion of the first strand to generate a first amplification product.

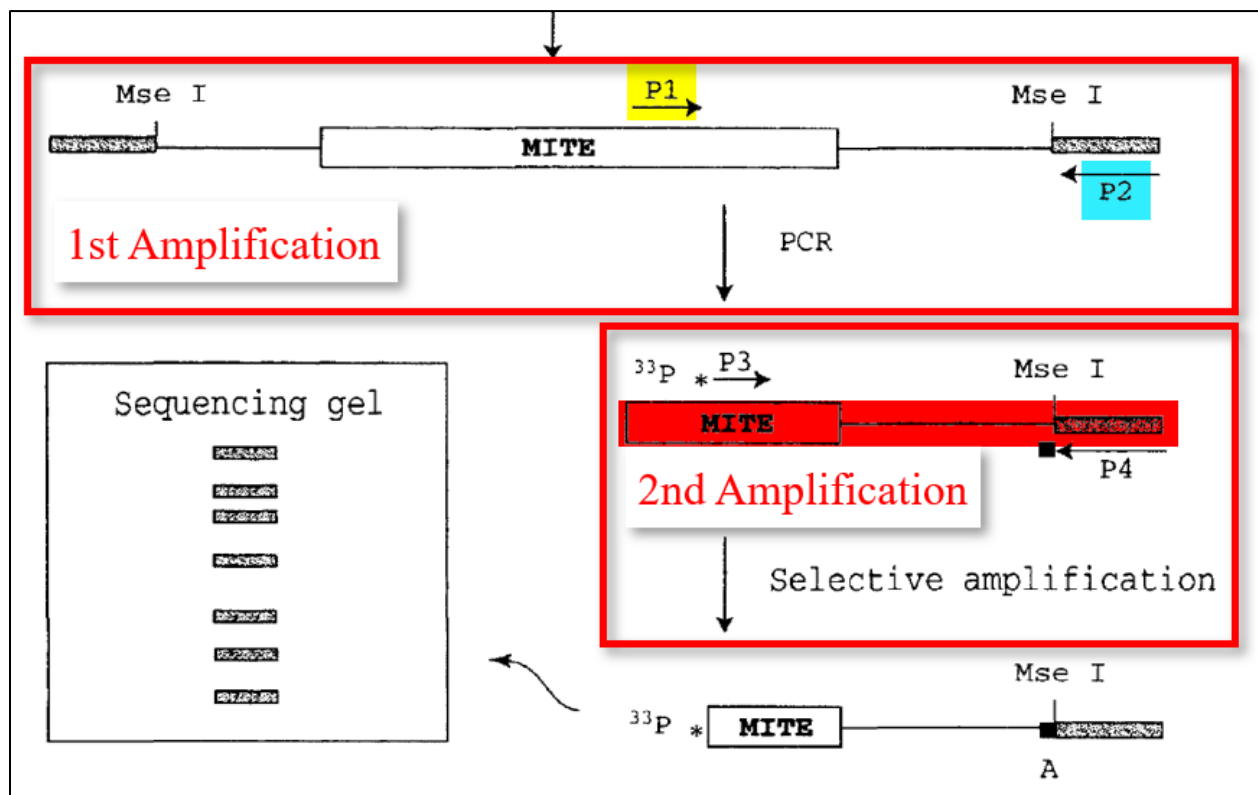
278. Claim 4 is obvious over the combination of Porreca and Wessler.

The combined method includes a first amplification step where Porreca's first target-specific primer specifically anneals to a target sequence. That amplification step also uses one of Wessler's adapter primers, such as "SEQ ID NO:25," to anneal to the portion of the target sequence tagged with an adapter. (Ex.1010,

13:9-19, Figs. 1, 4A.) SEQ ID NO:25 (blue) has a sequence identical to a first portion of the first strand of Wessler's adapter SEQ ID NO:23 (navy):



(*Id.*, Fig. 4A (annotated).) In combination with Porreca's target-specific primer, it then generates a first amplification product. Wessler's Figure 1, which is illustrative, shows using a first target-specific primer ("P1," (yellow)) and a first adapter primer ("P2," (blue)) to generate a first amplification product (red):



(Ex.1010, Fig. 1 (cropped and annotated).) The combined method also includes a second, optional amplifying step.

d. Claim 5

The method of claim 4, wherein the second amplifying step comprises using a second target-specific primer that specifically anneals to the first amplification product and a second adaptor primer having a nucleotide sequence identical to a second portion of the first strand to generate the final amplification product.

279. Claim 5 is obvious over the combination of Porreca and Wessler.

In the combined method's second, optional amplification step, it would use a second target-specific primer and a second 5' adapter primer. The second target-specific primer would specifically anneal to the first amplification product.

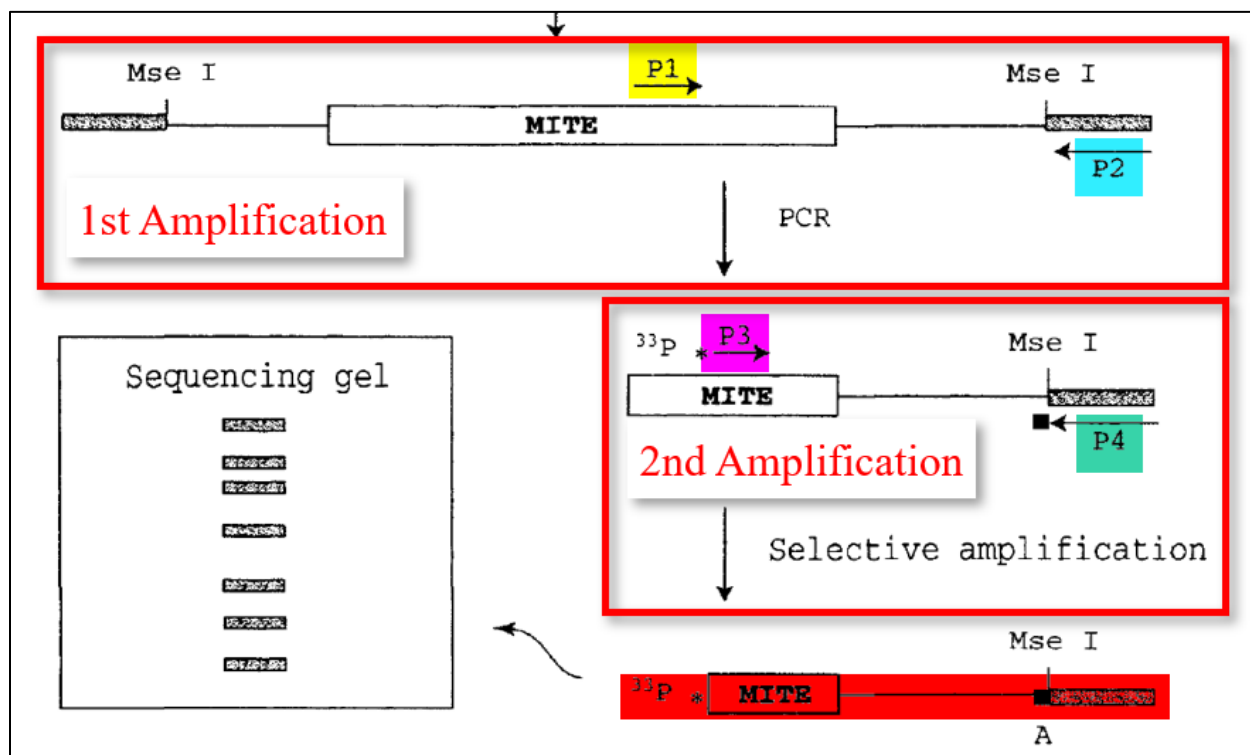
(Ex.1008, 43:29-32, Fig.4b.) And the 5' adapter primer would have, again, a sequence identical to a portion of a first strand of a 5' adapter introduced via ligation. (Ex.1010, 10:53-62, Fig. 1.) Wessler discloses, for example, a second adapter primer "SEQ ID NO: 3 1", which it calls a "fourth primer[]," composed of the following bases: "5' GACGATGAGTCCTGAGTAAN." (*Id.*, 13:9-22.)

Wessler's SEQ ID NO: 3 1 has a nucleotide sequence identical to a second portion of the first strand of SEQ ID NO:23 because both strands include the sequence "AC." (*See id.*, 13:9-22 (SEQ ID NO: 3 1), Fig. 4A (SEQ ID NO:23).)

280. This amplification would generate a final amplification product.

Wessler's Figure 1, which is illustrative, shows using third (lavender) and fourth

(green) primers to generate a final amplification product (red):



(*Id.*, Fig. 1 (cropped and annotated).)

e. Claims 10 & 11

Claim 10: *The method of claim 1, wherein the one or more universal tail adaptors comprise a second barcode.*

Claim 11: *The method of claim 1, wherein the one or more universal tail adaptors comprise a second sequencing tag.*

281. These claims are obvious over the combined method of Porreca and Wessler. As explained above, Porreca's target-specific primers comprise universal tail adaptors comprising barcodes and sequencing tags. Under Patent Owner's construction, the first amplification cycle in the combined method's second PCR reaction would generate products tagged with a second target-specific primer and

thus also a second barcode and sequencing tag. (Ex.1010, Fig. 1.) Then, under Patent Owner's construction, targeted amplification of the tagged products is performed in later cycles.

f. Claim 12

The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.

282. The combined method tags isolated cfDNA with two universal tail adapters in the ligation and, alternatively, the targeted amplification steps.

g. Claim 13

The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.

283. The combined method's amplification steps satisfy this limitation. The first and second target-specific primers comprise universal tail adapters and, under Patent Owner's view of the claims, tag the isolated cfDNA in the first amplification cycle of each targeted amplification.

h. Claim 14

The method of claim 12, wherein amplifying the tagged products comprises a single amplifying step.

284. The combined method amplifies tagged products in one or more single amplification steps, which is what I have been asked to assume this claim requires.

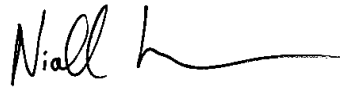
i. Claim 15

The method of claim 14, wherein amplifying the tagged products comprises using a third primer and a fourth primer, wherein the third primer comprises a first sequencing tag and wherein the fourth primer comprises a second sequencing tag.

285. The combined method amplifies the tagged products using four primers: Wessler's adapter primers and Porreca's target-specific primers. The target-specific primers contain sequencing tags and are third and fourth primers. (Ex.1008, 8:5-8, 43:29-33.)

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: 4/14/24

A handwritten signature in black ink, appearing to read "Niall" followed by a stylized surname.

Niall Lennon