

**IN THE UNITED STATES DISTRICT COURT
FOR THE MIDDLE DISTRICT OF NORTH CAROLINA**

NATERA, INC.,

Plaintiff,

v.

NEOGENOMICS LABORATORIES,
INC.,

Defendant.

C.A. No. 1:23-CV-629

**DECLARATION OF DR. BRIAN VAN NESS IN SUPPORT OF NEOGENOMICS
LABORATORIES, INC.'S RESPONSE IN OPPOSITION TO NATERA, INC.'S
MOTION FOR A PRELIMINARY INJUNCTION**

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I. SUMMARY OF OPINIONS

1. My name is Dr. Brian Van Ness. I have been retained as an independent expert witness on behalf of Defendant NeoGenomics Laboratories, Inc. in the above-captioned action to provide testimony regarding whether NeoGenomics' RaDaR® product infringes certain claims of U.S. Patent Nos. 11,530,454 (the "454 patent") and 11,519,035 (the "035 patent") (collectively, the "asserted patents"), and whether the asserted patents are invalid.

II. BACKGROUND AND QUALIFICATIONS

2. I have over forty years of experience in the fields of Biochemistry, Molecular Immunology, and Genetics. I am currently a Professor in the Department of Genetics, Cell Biology & Development at the University of Minnesota, where I served as head for 9 years (2000-2009), followed by my appointment as Director of the Institute of Human Genetics (2009-2011). For over 10 years (2019-2022) I served as the Chair of the University of Minnesota Genomics Center Advisory Committee, which functions to oversee and evaluate new technologies being considered by the Genomics Center, and oversees the financial structure of the Center. My research focuses on molecular genetics and, in particular, how different genes influence disease progression and therapeutic response. I also serve as a consultant to a number of genomics companies, and have founded several genomics companies of my own. I am the author of over 170 peer-reviewed publications. My full credentials are set forth in my CV, which is Exhibit 1.

III. UNDERSTANDING OF THE LAW

3. I have been informed by counsel of the various legal standards that apply and have applied those standards in arriving at my conclusions expressed in this Report.

A. Legal Standard for Non-Infringement

4. I understand that there are a number of legal factors or requirements that may be considered in determining whether the claims of a patent are infringed.

5. I understand that patent infringement consists of making, using, offering to sell, or selling a patented invention within the United States, or importing a patented invention into the United States, without authorization. I further understand that determining whether there is infringement of a patent involves two steps. First, each asserted claim must be construed to determine its proper scope and meaning to a person of ordinary skill in the art. Second, each limitation of the asserted claim is compared to the accused product or process to determine whether the limitation is found in the accused product or process. For there to be infringement, the accused product or process must practice each and every limitation of an asserted claim.

6. It is my understanding that, in order to infringe a dependent claim, the accused product or process must practice each and every limitation of all claims from which the dependent claim depends. Therefore, a dependent claim cannot be infringed if the accused product or process does not infringe the independent claim from which the dependent claim depends.

7. To infringe a patent, I understand that only one claim needs to be infringed.

8. I understand that the party asserting infringement (Natera for the purposes of this report), has the burden of proving by a preponderance of the evidence that all limitations of at least one of the asserted claims are satisfied. I am informed that a “preponderance of the evidence” means that the evidence is sufficient to persuade a factfinder that the fact sought to be proved is more likely than not to be true.

9. I have been informed and understand that patent claims can be infringed either literally or under the doctrine of equivalents. While literal infringement requires each claim

element to be literally present in the accused device or method, infringement under the doctrine of equivalents can be found when every claim element that is not literally present is nonetheless equivalently present. I am informed and understand that, under the doctrine of equivalents, the accused system or method may infringe that claim if the differences between the requirements of the accused system or method and the requirements of the claimed system or method are insubstantial. I am further informed and understand that one test for the applicability of the doctrine of equivalents is that the accused system or method performs substantially the same function in substantially the same way to achieve substantially the same result as the claimed system or method.

B. Legal Standard for Obviousness Under 35 U.S.C. § 103

10. 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 person of ordinary skill in the pertinent art.

11. I understand that a person of ordinary skill in the art provides a reference point from which the prior art and claimed invention should be viewed. This reference point prevents a person of ordinary skill from using one’s insight or hindsight in deciding whether a claim is obvious.

12. I understand that the obviousness analysis requires a comparison of the claim language, including as construed by the Court, to the prior art on a limitation-by-limitation basis.

13. 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 nonobviousness or obviousness.

14. 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.

15. 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.

16. 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.

17. 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.

18. I understand that if a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill.

19. I also 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 person of ordinary skill in the art 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.

20. I understand that obviousness analysis therefore takes into account the inferences and creative steps that a person of ordinary skill in the art would employ under the circumstances.

21. 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 person of ordinary skill in the art has good reason to pursue the known options within his or her technical grasp because the result is likely the product not of innovation but of ordinary skill and common sense.

22. 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 person of ordinary skill in the art can implement a predictable variation, then that obviousness likely bars its patentability.

23. It is further my understanding that a proper obviousness analysis focuses on what was known or obvious to a person of ordinary skill in the art, not just 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.

24. I understand that a person of ordinary skill in the art could 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 that a person of ordinary skill in the art would view them as a design choice, or whether a person of ordinary skill in the art could apply prior art techniques into a new combined method or system.

25. 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.

26. I have written this report with the understanding that obviousness must be shown by clear and convincing evidence.

27. I understand that certain objective factors, sometimes known as “secondary considerations” 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.

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

C. Legal Standard for Patent Eligibility Under 35 U.S.C. § 101

29. I understand that a patent claim may be invalid if its subject matter is not patentable. I understand that laws of nature, natural phenomena, and abstract ideas are not patentable. I also understand that steps of “determining” a variable using mathematical methods or “performing” a mathematical operation may also be considered unpatentable mathematical calculations. I understand that claims that are directed to one of these categories of unpatentable subjects (e.g. genes in nature), may still be patentable if the claims contain an inventive concept or some element or combination of elements that is sufficient to ensure that the patent, in practice, amounts to significantly more than a patent on the ineligible concept itself. Those additional features must be more than well-understood, routine, and conventional activity.

30. I understand that the Supreme Court has established a two-part test to determine if a patent claims patentable subject matter:

(i) determine whether the claims at issue are directed to one of those patent-ineligible concepts, and

(ii) determine whether additional claim elements transform the nature of the claim into a patent-eligible application.

I understand that the elements of each claim are to be considered both individually and as an ordered combination to determine whether the additional claim elements transform the nature of the claim into patent-eligible subject matter. Regarding whether additional elements transform the nature of the claim into a patent-eligible application, I understand that the claims, for example,

must recite more than well-understood, routine, conventional activities, or a non-conventional and non-generic arrangement of known, conventional components.

D. Legal Standard for Written Description Under 35 U.S.C. § 112

31. I understand that a patent must contain a written description of a claimed invention.

32. I understand that the written description in the specification 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 understand the specification must describe the invention sufficiently to convey to a POSA that the applicant actually invented what is now claimed. 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; describing a desired outcome without describing how to achieve it is not sufficient.

33. I understand that invalidity for lack of adequate written description must be shown by clear and convincing evidence.

E. Legal Standard For Inventorship

34. I understand that a patent is invalid if it fails to meet the requirement that all of the actual inventors, and only the actual inventors, be named as inventors in the patent.

35. I understand that to be an inventor, one must make a significant contribution to the conception of at least one of the claims of the patent, and that whether the contribution is significant in quality is measured against the scope of the full invention. I understand that if someone only explains to the actual inventors well-known concepts or the current state of the art, he or she is not an inventor. Merely helping with experimentation, by carrying out the inventor's instructions, also

does not make someone an inventor. What is required is some significant contribution to the idea claimed.

36. I understand that persons may be inventors even if they do not make the same type or amount of contribution, and even if they do not contribute to the subject matter of each claim of the patent. Persons may be joint or co-inventors even though they do not physically work together, but they must have some open line of communication during or at approximately the time of their inventive effort.

IV. LEVEL OF SKILL OF ONE OF ORDINARY SKILL IN THE ART

37. I understand that claims are construed from the perspective of a person of ordinary skill in the art (which I understand is referred to by “POSA”) at the time of the purported invention.

38. I understand that Natera contends that the priority date for the ’454 patent is April 21, 2015. For the purposes of this declaration only, I assume this is the appropriate priority date for the ’454 patent.

39. I understand that Natera contends that the priority date for the ’035 patent is May 18, 2011. For the purposes of this declaration only, I assume this is the appropriate priority date for the ’035 patent.

40. I understand that in determining whether patent claims are obvious under § 103, constitute patent ineligible subject matter under § 101, or lack written description under § 112, the claims are analyzed from the perspective of a POSA at the time of the invention.

41. According to Dr. Metzker, a POSA “at or prior to 2010 and through to the present, would have at least a bachelor’s degree (with several additional years of laboratory experience) in a life or physical sciences discipline with applications in genomics. Through further education or graduate or postdoctoral experience, such a person would have obtained a thorough understanding

of nucleic acid detection, quantification, and genotyping, and would have understood the basic underlying scientific principles of nucleic acid assay, detection, and genotyping techniques. One of ordinary skill in the art would also have had general knowledge of, and be able to read literature on, nucleic acid assay methods including, for example, amplification by PCR, nucleic acid sequencing including by multiplex or high-throughput sequencing, genotyping, and the detection and quantification of nucleic acids, including cell-free DNA, using techniques such as polymorphism-based genotyping. In determining the level of ordinary skill in the art, more education could compensate for less experience, and vice versa.” D.I. 13 ¶ 32.

42. For the purposes of this declaration, I adopt Dr. Metzker’s definition of the skilled artisan. I reserve the right to subsequently provide my own definition to the extent warranted by further developments in this case.

V. TECHNOLOGY BACKGROUND

A. Cell-Free DNA

43. Cell-free DNA (“cfDNA”), as its name suggests, describes broadly DNA that is derived from cell-free bodily fluids, such as blood plasma.

44. Applications for tumor derived cell-free DNA were known in the art. For example, cell-free DNA had long been proposed as a method for diagnosing cancer. For instance, a patent application to Stroun and co-workers that was filed in 1996 specifically described monitoring evolution of cancers using blood plasma:

The purpose of this invention is hence to provide a method for diagnosing cancers which, on the one hand, is more accurate and more reliable and which, on the other hand, is easier to carry out without the need of resorting to invasive tests on the patients. The method for diagnosing and/or monitoring the evolution of cancers, object of the invention and aimed at achieving the above purposes, includes the analysis of the deoxyribonucleic acid (DNA) contained in the blood plasma.

Ex. 2 (U.S. Patent No. 5,952,170), titled “Methods for Diagnosing Cancers,” which was published on September 14, 1999 at 1:19-28 (describing a method to measure cell-free tumor DNA and the motivation for doing so).

45. As another example, a patent application filed in 2009 was entitled “Circulating Mutant DNA to Assess Tumor Dynamics” and explained that tumor DNA could be found in the blood of cancer patients and that this can be used to “track disease:”

Cancers arise through the sequential alteration of genes that control cell growth. In solid tumors such as those of the colon or breast, it has been shown that, on average, approximately 80 genes harbor subtle mutations that are present in virtually every tumor cell but are not present in normal cells¹. These somatic mutations thereby have the potential to serve as highly specific biomarkers. They are, in theory, much more specific indicators of neoplasia than any other biomarker yet described. One challenge for modern cancer research is therefore to exploit somatic mutations as tools to improve the detection of disease and, ultimately, to positively affect individual outcomes. Tumor cells can often be found in the circulation of individuals with advanced cancers^{2,3}. It has been shown that tumor-derived mutant DNA can also be detected in the cell-free fraction of the blood of people with cancer⁴⁻⁶. Most of this mutant DNA is not derived from circulating tumor cells⁴⁻⁶ and, in light of the specificity of mutations, raises the possibility that the circulating mutant DNA fragments themselves can be used to track disease. However, the reliable detection of such mutant DNA fragments is challenging⁷. In particular, the circulating mutant DNA represents only a tiny fraction of the total circulating DNA, sometimes less than 0.01%⁸.

In the current study, we developed modifications of a technique called BEAMing (Beads, Emulsion, Amplification and Magnetism)^{8,9} to quantify ctDNA in serially collected plasma samples from subjects with colorectal cancers. We were interested in determining whether such measurements provided information about the dynamics of tumor burden in these subjects during the course of their disease.

There is a continuing need in the art for ways to better determine which patients will experience relapses of their cancer and which will not.

Ex. 3 (U.S. Patent App. Pub. No. 2010/0041048), titled “Circulating Mutant DNA to Assess Tumor Dynamics,” which was filed on July 30, 2009 ¶¶ 3-5.

46. As yet another example, a patent application filed in 2000 noted the presence of tumor specific mutations in the plasma or serum fraction of blood and how this could be used for early detection of cancer:

This invention relates to detection of specific extracellular nucleic acid in plasma or serum fractions of human or animal blood associated with neoplastic or proliferative disease. Specifically, the invention relates to detection of nucleic acid derived from mutant oncogenes or other tumor-associated DNA, and to methods of detecting and monitoring extracellular mutant oncogenes or tumor-associated DNA found in the plasma or serum fraction of blood, using DNA extraction followed by nucleic acid amplification with or without enrichment for mutant DNA. In particular, the invention relates to detection, identification, or monitoring of the existence, progression or clinical status of benign, premalignant, or malignant neoplasms in humans or animals that contain a mutation associated with the neoplasm through detection of the mutated nucleic acid of the neoplasm in plasma or serum fractions. The invention permits the detection of extracellular, tumor-associated nucleic acid in the serum or plasma of humans or other animals recognized as having a neoplastic or proliferative disease or in individuals without any prior history or diagnosis of neoplastic or proliferative disease. The invention provides the ability to detect extracellular nucleic acid derived from genetic sequences known to be associated with neoplasia, such as oncogenes, as well as genetic sequences previously unrecognized as being associated with neoplastic or proliferative disease. The invention thereby provides methods for early identification of colorectal, pancreatic, lung, breast, bladder, ovarian, lymphoma and all other malignancies carrying tumor-related mutations of DNA and methods for monitoring cancer and other neoplastic disorders in humans and other animals.

Ex. 4 (U.S. Patent No. 6,156,504), titled “Detection of extracellular tumor-associated nucleic acid in blood plasma or serum using nucleic acid amplification assays,” which was filed on December 5, 2000 at Abstract (describing detection of cell-free tumor DNA to detect and monitor disease progression).

B. Multiplex PCR

47. In my opinion, the concept of multiplex PCR was known in the art in the relevant time period. For example, Varley et al. teaches “nested patch PCR” and explains how this can be used to amplify 90 exons simultaneously for subsequent sequencing:

We have developed Nested Patch PCR, a novel method for multiplex PCR that is

capable of amplifying 90 exons from genomic DNA simultaneously. The target selection protocol is an addition only reaction and can be performed in a single tube per sample, making it amenable to automation. We demonstrated that Nested Patch PCR can be performed on multiple samples in parallel, which can then be labeled with sample-specific DNA barcodes and sequenced as a pool. The choice of targets and target boundaries is flexible, and a wide range of sizes can be amplified simultaneously (here, 74 bp to 438 bp). We performed Nested Patch PCR on genomic DNA and obtained sequence for 90 of the 94 exons we targeted, indicating this method is robust. This method is also highly specific: 90% of reads matched targeted exons. The number of reads per exon was highly correlated across samples indicating high reproducibility. We identified both alleles of heterozygous SNPs at near-even frequencies across samples, demonstrating that this method has the allele sensitivity necessary for variant discovery in personal genome sequencing. Furthermore, we demonstrate the applied utility of this method by discovering a colon tumor-specific mutation in an individual.

D.I. 52-5, Ex. 5 (Varley et al., “*Nested Patch PCR enables highly multiplexed mutation discovery in candidate genes*,” *Genome Research* 18:1844-1850 (2008) (“Varley”)) at 1847. Even further, Varley teaches that the method “should prove useful for the amplification of an intermediate number (100-1000) of candidate regions in a large number of samples” as it is “well suited to these applications because it incorporates sample-specific DNA barcodes, allows for the precise definition of the boundaries of targeted sequences, is reproducible, is highly specific, and uniformly amplifies different alleles at a given locus.” D.I. 52-5 at 1848.

48. As another example, consider the Wang reference, a publication that was available years before the patents-in-suit. Wang describes how several researchers had achieved large scale multiplex PCR methods to facilitate sequencing:

Second-generation methods were initially marked by the protocol developed in our laboratory (Lin et al. 1996), which features using 5’ universal sequences (tails) on specific PCR primers that were attached to the ends of the amplicons during the early stage of PCR amplification. All specific primers could then be replaced by only two primers identical to the 5’ universal tails. This improvement allowed amplification of 26 DNA sequences in a single tube (Lin et al. 1996). It was shown that 41 sequences could be amplified very specifically and the resultant fragments could be resolved by gel electrophoresis (X. Cui and H. Li, unpubl.).

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). However, attaching the universal tails to the amplified sequences requires additional experimental steps in comparison with amplification by regular PCR, limiting the detection sensitivity. Some of these procedures also require pooling of individually amplified PCR products from multiple tubes, followed by column purification. Requirements of specialized probes and detection platforms and long oligonucleotides also limit the flexibility and cost-effectiveness of these systems.

D.I. 52-6, Wang, et al. *“A genotyping system capable of simultaneously analyzing > 1000 single nucleotide polymorphisms in a haploid genome,”* Genome research 15.2 (2005): 276-283. (“Wang Paper”) at 277.

49. Likewise, U.S. Patent Application Publication 2004/0126760 (“Broude”), published on July 1, 2004 from a PCT application filed May 17, 2001, teaches use of nested PCR approaches to simultaneously amplify at least 100 target loci. *See* Ex. 21 at 0025 (“The present invention is directed to a method for multiplex polymerase chain reaction (PCR), the simultaneous amplification of different target nucleic acid sequences in a single PCR reaction.”), 0057 (“The present invention is preferably used with 5 targets...and even more preferably with at least 100 targets.”).

50. U.S. Patent No. 4,683,195 (“Mullis”) is a patent filed in 1986 and issued in 1987 that describes the use of nested primers in the very first patent for PCR. *See* Ex. 40 at 30:20-31:17. Mullis demonstrates how old and well-known these concepts were to a POSA.

51. As another example, U.S. Patent No. 7,790,393 (“Lyamichev”), which issued on September 7, 2010 from an application filed July 16, 2008, which itself was a continuation of an application filed December 17, 2002, discloses a method of “highly multiplex PCR” that could amplify at least 100 targets in a single volume. *See* Ex. 22 at 26:63-27:3 (disclosing that in some embodiments “the number of PCR reactions is from about than [*sic*] 100 to 1,000”), 25:7-14 (explaining that the “highly multiplex PCR includ[es] hundreds to thousands of products in a single reaction); *see also generally id.* at 25:19-32:19.

52. As another example, U.S. Patent Application Publication 2010/0120038 (“Fluidigm”), which published on May 13, 2010 from an application filed August 26, 2009, teaches amplifying at least 100 targets, including SNP loci, in a single volume. *See* Ex. 13 at 0119 (“In the illustrative embodiments, the encoding reaction can be a preamplification reaction....To increase target nucleic acid concentration prior to encoding, an optional pre- preamplification reaction can be carried out before....The pre-preamplification can be carried out in multiplex. For example, target-specific primers for 9216 different target nucleic acids can be employed in one mixture.”), 0175 (“The methods of the invention are applicable to any technique aimed at detecting the presence or amount of one or more target nucleic acids in a nucleic acid sample. Thus, for example, these methods are applicable to identifying the presence of particular polymorphisms (such as SNPs), alleles....The methods may be employed in genotyping, which can be carried out in a number of contexts....”).

53. As yet another example, WO 2007/147079 (“Shoemaker”), filed June 14, 2007 and published on December 21, 2007, discloses amplifying more than 100 SNP loci in a single reaction volume using more than 100 PCR primer pairs. *See* Ex. 23 at 117 (“Preferably, the amplified/tagged nucleic acids include at least...100 polymorphic genomic DNA regions....”),

119 (“The primer element is configured to amplify the genomic DNA of interest (e.g. STR)....Primer elements can be chosen which are multiplexible with other primer pairs from other tags in the same amplification reaction (e.g. fairly uniform melting temperature, absence of cross-priming on the human genome, and absence of primer-primer interaction based on sequence analysis).”).

54. As another example, U.S. Patent Application Publication 2010/0285478 (“Chen”), which published on November 11, 2010 from a March 26, 2010 application, also teaches a multiplex amplification reaction with at least 100 targets and at least 100 primer pairs. *See* Ex. 41 at 212 (“In some embodiments, the first reaction is a multiplex reaction and said second reaction is a single-plex reaction. In some preferred embodiments, the multiplex reaction involves the use of at least two complete sets of primers..., each set of which is suitable or operative for amplifying a specific polynucleotide of interest.”). Chen discloses that “[t]he number of different amplification primer pairs utilized in the multiplex amplification is not critical and can range from as few as two, to as many as tens, hundreds, thousands, or even more. Thus...the multiplex amplifications permit the simultaneous amplification of from as few as two to as many as...thousands, or even more polynucleotide sequences of interest.” *Id.* at 214.

55. As yet another example, U.S. Patent No. 9,206,475 (“Gerdes”), which issued on December 8, 2015 from a June 5, 2015 application and traces its priority through continuation and divisional applications back to May 19, 2003, teaches a multiplex amplification method that works with any number greater than 2 primer sets. In fact, Gerdes teaches that its method is preferable for a greater number of primer sets. *See* Ex. 42 at 8:57-67 (“This invention reveals a robust and simple method for multiplex amplification of large numbers of gene targets. The invention teaches that preferably 2-40 or more primer sets can be combined to permit multiplex

amplification....However, it should be understood that any number of primer sets greater than two may be used according to the present invention. This has been designated as a ‘booster round’ or booster amp....”); *see also id.* at 7:34-43, 16:4-21. Gerdes teaches that this multiplex amplification occurs in a single reaction volume. *See id.* at 7:33-44, 8:24-51, Fig. 1.

56. A POSA would have been aware of and understood the aforementioned methods relating to amplifying at least 100 SNP loci in a single volume.

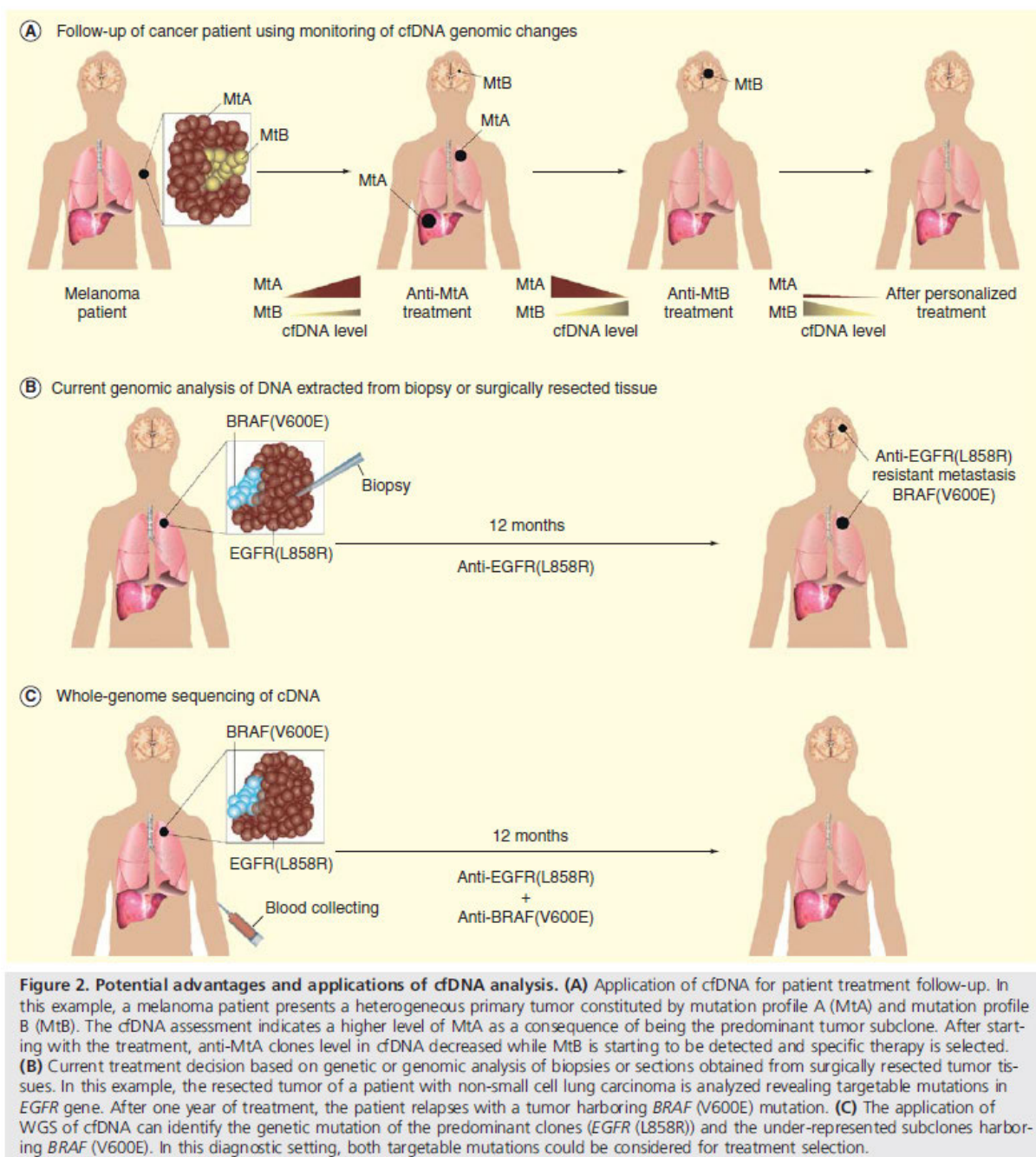
C. Minimal Residual Disease Monitoring Using Cell Free DNA

57. In my opinion, the concept of minimal residual disease monitoring by identifying tumor DNA sequences and monitoring the presence of tumor DNA sequence in cell free DNA over time to was known in the art in the relevant time period. There are numerous prior art references showing this to be the case, some of which I identify below.

58. For example, Chan et al. describe shotgun sequencing of plasma DNA to identify mutations, followed by subsequent targeting of these sequences for “serial monitoring purposes:”

One can also envision the possibility of using shotgun sequencing of a plasma sample upon presentation of a cancer patient, mining the shotgun sequencing data of plasma DNA for copy number aberrations and SNVs, and then specifically targeting such sequences for serial monitoring purposes. In this regard, solution-phase hybridization-based capture approaches have already been successfully used to enrich plasma DNA for noninvasive prenatal diagnosis (34, 35). Hence, we see that the targeted and shotgun approaches can be used in combination for cancer detection and monitoring.

Ex. 43 (K.C. Allen Chan, et al., *Cancer Genome Scanning in Plasma: Detection of Tumor-Associated Copy Number Aberrations, Single-Nucleotide Variants, and Tumoral Heterogeneity by Massively Parallel Sequencing*, 59 CLIN. CHEM. 211 (2013) (“Chan”)) at 223. Likewise, Marzese et al. describe and depict the use of MRD monitoring in Figure 2:



Ex. 53 at 833 (Figure 2).

59. As yet another example, ForsheW teaches the presence of tumor DNA in the plasma of cancer patients and using a technique known as TAm-Seq to monitor the cancer:

Plasma of cancer patients contains cell-free tumor DNA that carries information on

tumor mutations and tumor burden. Individual mutations have been probed using allele-specific assays, but sequencing of entire genes to detect cancer mutations in circulating DNA has not been demonstrated. We developed a method for tagged-amplicon deep sequencing (TAm-Seq) and screened 5995 genomic bases for low-frequency mutations. Using this method, we identified cancer mutations present in circulating DNA at allele frequencies as low as 2%, with sensitivity and specificity of >97%. We identified mutations throughout the tumor suppressor gene TP53 in circulating DNA from 46 plasma samples of advanced ovarian cancer patients. We demonstrated use of TAm-Seq to noninvasively identify the origin of metastatic relapse in a patient with multiple primary tumors. In another case, we identified in plasma an EGFR mutation not found in an initial ovarian biopsy. We further used TAm-Seq to monitor tumor dynamics, and tracked 10 concomitant mutations in plasma of a metastatic breast cancer patient over 16 months. This low-cost, high-throughput method could facilitate analysis of circulating DNA as a noninvasive “liquid biopsy” for personalized cancer genomics.

D.I. 13-7, Ex. 7, Forshew et al., “*Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA*,” *Sci Transl Med* 4, 136ra68 (2012) (including Supplementary Materials available at www.sciencetranslationalmedicine.org/cgi/content/full/4/136/136ra68/DC1) (“Forshew (2012)”) at Abstract (“Forshew (2012)”). Forshew goes on to explain how the cell free tumor DNA can be used for screening, prognosis, monitoring, and detection:

Evolutionary changes within the cancer can alter the mutational spectrum of the disease and its responsiveness to therapies, which may necessitate repeat biopsies (14–17). Biopsies are invasive and costly and only provide a snapshot of mutations present at a given time and location. For some applications, mutation detection in plasma DNA as a “liquid biopsy” could potentially replace invasive biopsies as a means to assess tumor genetic characteristics (2–7). Sensitive methods for detecting cancer mutations in plasma may find use in early detection screening (1), prognosis, monitoring tumor dynamics over time, or detection of minimal residual disease (3, 18, 19).

Id.

60. As yet another example, another publication by Chan expressly notes that the presence of cell-free tumor DNA has “remarkable potential” for cancer monitoring:

The analysis of circulating cell-free tumor DNA has remarkable potential for the detection and monitoring of cancers. Genetic alterations are present in virtually all

types of cancers, allowing broad applicability of this approach regardless of the primary organ and histologic type of the cancer.

Chan, “*Scanning for Cancer Genomic Changes in Plasma: Toward an Era of Personalized Blood-Based Tumor Markers*,” *Clinical Chemistry* 59:11 (2013), 1553-1555 (“Chan”) at 1553.

In summary, the large number of genetic alterations in cancer may potentially allow utilization of genomic changes in circulating cell-free DNA as personalized tumor markers for the screening, monitoring, and prognostication of cancers. With the rapid reduction in the cost of massively parallel sequencing, this approach may soon be adapted for routine clinical care.

Id. at 1554.

61. As yet another example, Dawson et al. teach that in advance of the filing of the ’454 patent the feasibility of using circulating tumor DNA to monitor cancer had already been established:

Circulating DNA fragments carrying tumor-specific sequence alterations (circulating tumor DNA) are found in the cell-free fraction of blood, representing a variable and generally small fraction of the total circulating DNA.^{11,12} Advances in sequencing technologies have enabled the rapid identification of somatic genomic alterations in individual tumors, and these can be used to design personalized assays for the monitoring of circulating tumor DNA. Studies have shown the feasibility of using circulating tumor DNA to monitor tumor dynamics in a limited number of patients with various solid cancers, but few cases of breast cancer have been analyzed.¹³⁻²⁰ Here, we provide a direct comparison between circulating tumor DNA and other circulating bio-markers (CA 15-3 and circulating tumor cells) and medical imaging, the current standard of care, for the noninvasive monitoring of meta-static breast cancer.

Dawson et al., “*Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer*,” *NEJM* 368:13 nejm.org (March 28, 2013), 1199-1209 at 1200.

62. As yet another example, Leary et al. teach using rearrangements to monitor and detect residual disease:

Tumor-specific (somatic) chromosomal rearrangements have the potential to serve as highly sensitive biomarkers for tumor detection. Such alterations are not present in normal cells and should be exquisitely specific. Rearrangement-associated biomarkers therefore offer a reliable measure that would be useful for monitoring

tumor response to specific therapies, detecting residual disease after surgery, and long-term clinical management. Recurrent somatic structural alterations, such as those involving the BCR-ABL oncogene (the target of the Philadelphia chromosome translocation), immunoglobulin genes, T cell receptor genes, and the retinoic acid receptor gene, have been shown to be useful as diagnostic markers in certain hematopoietic malignancies (16–20). However, recurrent structural alterations do not generally occur in most solid tumors. We reasoned that any structural alteration identified in an individual’s tumor could be used for analogous purposes, whether it was found in tumors of the same type in other individuals and whether it was a “driver”—causing a selective growth advantage—or a “passenger.” We describe herein our efforts to implement this concept in representative examples of common solid tumors.

Ex. 44 (Leary et al., “*Development of Personalized Tumor Biomarkers Using Massively Parallel Sequencing*,” Sci Transl Med, 2010 (“Leary”)) at 2.

VI. ASSERTED CLAIMS

63. I have been informed by counsel that Natera asserts claims 1, 8 and 11 of the ’454 patent and claims 1, 12, and 13 of the ’035 patent (collectively, the “Asserted Claims”).

VII. CLAIM CONSTRUCTION

64. The ’035 patent states that “Amplification refers to a method that increases the number of copies of a molecules, such as a molecule of DNA.” D.I. 1-2 at 42:4-5. The ’035 patent further states that “Selective Amplification may refer to a method that increases the number of copies of a particular molecule of DNA, or molecules of DNA that correspond to a particular region of DNA. It may also refer to a method that increases the number of copies of a particular targeted molecule of DNA, or targeted region of DNA more than it increases non-targeted molecules or regions of DNA. Selective amplification may be a method of preferential enrichment.” D.I. 1-2, Ex. 2 at 42:6-13.

65. In view of the foregoing, I construe the term “amplifying” in claims 1 and 13 of the ’035 patent to mean “increasing the number copies of a molecule, such as a molecule of DNA.” Moreover, a person of ordinary skill in the art would understand that these “copies” that result

from amplifying, such as in a multi-step PCR process, are not just the copies that result from the initial copying of the original sample DNA strand, but also the copies of those copies and any subsequent copies. I understand that Natera's expert, Dr. Metzker agrees with this:

Q. Now, the only thing that people understand to be a copy in the context of PCR amplification is the product that results when the primer hybridizes to one of the original sample molecules and a complementary strand is generated. But that's – that's the only thing people call a copy; correct?

A. I'm not sure I agree with that.

Q. Okay. Well, what else would be a copy?

A. Well, first, I'm not sure what people would agree with. I mean, I'm not -- I can tell you what I think, but I don't know what people would agree with.

Q. What do you think?

A. Well, I think you can make a copy from an original strand. You can make a copy from a copy. I don't think -- at the end of the day, they think of it as a product.

Q. When you say "you can make a copy from a copy," what did you mean by that?

A. Well, we talked about this in step 1B where the amplification can occur from a tagged product using a target-specific primer. So in that case, the tagged product would represent a copy because it's now tagged, and that can serve as a template in the next primer extension reaction.

Q. And will that lead to a new copy.

A. Well, I don't know about a new copy, but it's another copy.

Q. It's another copy, okay. Why do you distinguish between "a new copy" and "another copy"?

A. Well, I guess I don't think of copies in time – old copy, new copy. They're just copies.

Ex. 6 at 110:5-111:12.

VIII. OVERVIEW OF THE ASSERTED PATENT CLAIMS

A. The '454 Patent

66. Claim 1 of the '454 patent reads as follows:

1. A method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:

performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;

performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume; and

sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads, wherein the sequencing has a depth of read of at least 50,000 per target locus.

D.I. 1-1, Ex.1 at Claim 1.

67. Independent claim 1 of the '454 patent is thus directed to a method for detecting single nucleotide variant (SNV) mutations in a plasma sample of a subject having or suspected of having cancer.

68. In a first step, whole exome or whole genome sequencing on a tumor sample of the subject is used to identify tumor-specific mutations.

69. In a second step, targeted multiplex amplification is used to amplify 10 to 500 target loci from DNA to form amplicons. The target loci are amplified together in the same reaction volume. The amplicons have a length of 50-150 bases. The target loci each encompass a different

tumor-specific SNV mutation. The DNA is cfDNA isolated from plasma of the subject, or is derived from the cfDNA from plasma.

70. In a third step, the amplicons are sequenced to obtain sequence reads. The sequencing has a depth of at least 50,000 per target locus.

71. In a fourth step, tumor-specific SNV mutations are detected by detecting tumor-specific SNV mutations present in the cfDNA from the sequence reads.

B. The '035 Patent

72. Claim 1 of the '035 patent reads as follows:

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.

D.I. 1-2 at claim 1.

73. Independent claim 1 of the '035 patent is directed to a method for amplifying and sequencing DNA.

74. In a first step, isolated cell free DNA is tagged with one or more universal tail adaptors to generate tagged products. The isolated cfDNA used is from a blood sample of a patient other than a pregnant woman.

75. In a second step, tagged products are amplified to generate final amplification products. An initial amplifying step includes targeted amplification of more than one single nucleotide polymorphism (SNP) loci. A secondary amplifying step introduces a barcode and sequencing tag(s). The final amplification products include 25-2,000 SNP loci associated with cancer (as set forth in claim's description of the third step).

76. In a third step, the final amplification products including 25-2,000 SNP loci associated with cancer are sequenced.

IX. NON-INFRINGEMENT

77. Based on my knowledge and expertise and a careful review of documents and evidence in this litigation, it is my opinion that NeoGenomics's RaDaR Assay does not infringe any of the Asserted Claims.

A. The '454 Patent

1. NeoGenomics's RaDaR Assay Does Not Directly Sequence the Targeted Amplicons

78. NeoGenomics' RaDaR Assay does not infringe the claims of the '454 patent because it does not sequence amplicons generated by targeted amplification, but rather sequences amplicons generated by a universal amplification process. All of the Asserted Claims of the '454 patent recite "performing targeted multiplex amplification to amplify 10 to 500 target loci...to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume" and "sequencing the amplicons to obtain sequence reads." D.I. 1-1 at claims 1, 8, 11. The Asserted Claims thus require obtaining amplicons by performing targeted multiplex amplification in the same reaction volume and *directly* sequencing such amplicons without any intervening step. The RaDaR process, however, includes an intervening step—a universal, non-targeted amplification prior to sequencing.

79. The fact that the Asserted Claims require that the amplicons obtained by performing targeted multiplex amplification in a single reaction volume be directly sequenced is demonstrated by Natera's own admissions during the prosecution of U.S. Patent No. 11,486,008 (the "'008 patent"). The '008 patent is directly related to the '454 patent. The two patents are based on the same parent applications, have the same inventors, and share the same specification and title and have similar claims. D.I. 1-1; Ex. 55. Indeed, claim 16 of the '008 patent recites the use of "whole exome sequencing" to identify tumor-specific mutations and, as recited in claim 1, these mutations are then detected in cell-free DNA following "multiplex targeted amplification." Just like the '454 patent, the sole support for "whole exome sequencing" in the '008 patent resides in Example 13.

80. During the prosecution of the '008 patent, the Examiner issued a Non-Final Rejection, rejecting all of the then-pending claims as obvious over Forshew and other prior art references. Ex. 5 ('008 12/19/2019 Non-Final Rejection) at 4-8. Similar to the Asserted Claims of the '454 patent, all of the then-pending claims of the '008 patent recited "performing multiplex targeted amplification to amplify at least 100 target loci...to obtain amplicons, wherein the target loci are amplified together in the same reaction volume" and "performing high-throughput sequencing to sequence the obtained amplicons to obtain sequence reads." Ex. 8 ('008 Pending Claims) at 2. Like the '454 patent, the Examiner rejected the claims as obvious over Forshew (2012) and in response Natera asserted that its alleged distinction involved "performing high-throughput sequencing to sequence the amplicons obtained in the multiplex targeted amplification reaction to obtain sequence reads"—not the product of any subsequent amplification. Ex. 9 ('008 1/08/2021 Applicant Arguments) at 7 (emphasis in original). Natera further asserted that "Forshew requires a subsequent and separate targeted amplification step...therefore Forshew's amplicons are not obtained from the alleged multiplex targeted

amplification (i.e., ‘*limited-cycle pre-amplification step*’).” *Id.* (emphasis in original). Natera also asserted in an Examiner interview including inventor Bernard Zimmermann (also an inventor of the ’454 patent) that “the highly accurate mutation detection obtained are *due to lack of intermediate steps between the multiplex amplification and sequencing step.*” Ex. 45 (’008 5/22/2021 Interview Summary (emphasis supplied). The assertions that Natera made in order to overcome an obviousness rejection against highly similar and related claims show that the Asserted Claims, to be patentable over the prior art, require that any amplicons generated by performing a targeted multiplex amplification must be *directly* sequenced without any intervening step.

81. NeoGenomics’s RaDaR Assay does not infringe any of the Asserted Claims of the ’454 patent because it does not directly sequence the amplicons obtained by performing targeted multiplex amplification in a single reaction volume, as required by the claims of the ’454 patent. Instead, the RaDaR process includes an intervening step between targeted multiplex amplification and sequencing—a universal amplification. This universal amplification is a separate, distinct step from the preceding targeted multiplex amplification in the RaDaR process, which Dr. Metzker confirmed:

Q. All right. Based on that testimony, you can confirm that you’re using slide 21 in Exhibit 4 to contrast target-specific PCR with universal PCR; correct?

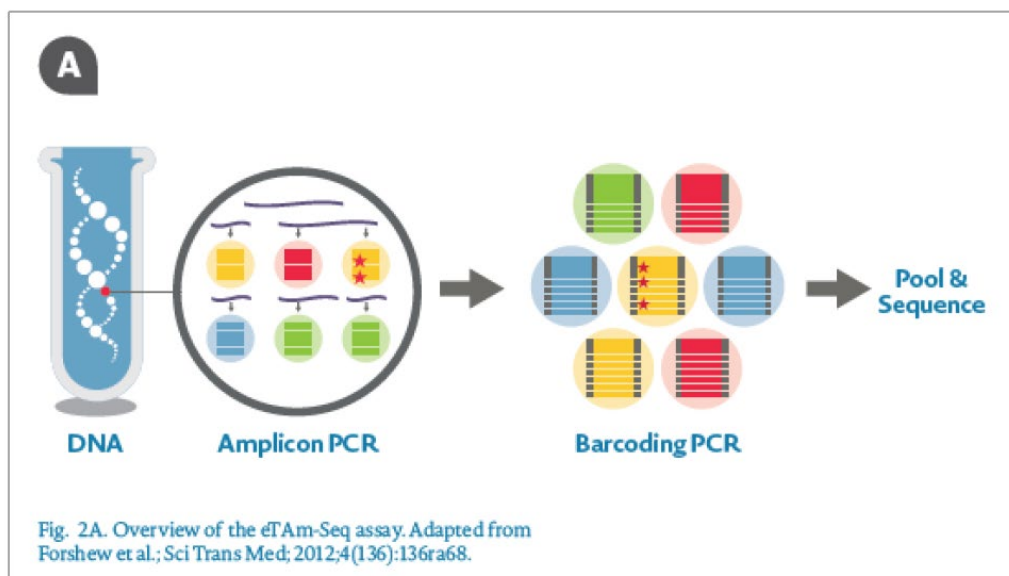
A. That’s correct.

Q. And the barcoding PCR that’s done in RaDaR, you understand that to be a universal PCR?

A. Well, I think I’ve testified *there are two barcoding steps: One involving target-specific amplification, and then there’s the second step involving universal amplification.*

Ex. 6 (Deposition of Dr. Metzker) at 73:7-18 (objection omitted).

82. The figure below cited by Dr. Metzker in his declaration further illustrates this point. In the RaDaR process, prior to sequencing, PCR reactions are performed on DNA samples. First, amplicons are obtained by performing “Amplicon PCR,” a targeted multiplex amplification. Then, “Barcoding PCR,” a universal, non-targeted amplification step, is performed on the amplicons from the prior targeted multiplex amplification step. Finally, the DNA samples are pooled and sequencing is performed. These steps of the RaDaR process are depicted in the figure below:



D.I. 13-6.

83. Natera’s expert, Dr. Metzker, testified regarding this figure as follows:

Q. The circled figure that’s labeled as “amplicon PCR,” that you refer to as a “targeted multiplex PCR”; correct?

A. They’re using target-specific primers, and they’re amplifying multiple loci. So you would refer to this as “targeted multiplex PCR.”

Q. And the barcoding PCR, you would only refer to that as “multiplex PCR”; correct?

A. That is the way I’ve characterized it.

Q. And why would you characterize it differently?

A. Because the second step is using common primers, not target-specific primers.

Ex. 6 at 77:10-22.

84. Dr. Metzker's testimony shown above demonstrates that the "Amplicon PCR" step of the RaDaR process is a targeted multiplex amplification, which is distinct from the "Barcoding PCR" step, a non-targeted amplification. In RaDaR, this "Barcoding PCR" happens after the "Amplicon PCR" but before the sequencing, such that the amplicons that result from a targeted amplification are not directly sequenced, as the claims of the '454 patent require.

85. As another example confirming this, I note that the Lipsyc-Sharf article that Natera relies upon for how RaDaR operates states that "[a]fter multiplex PCR, *reaction-specific index barcode sequences were added to the amplified sequences* and then libraries were pooled, combined with a PhiX control, and sequenced using the NovaSeq 6000 system (Illumina Inc)." D.I. 13-9 at Appx 1 (emphasis added). This additional intermediate universal amplification step, wherein barcode sequences are added to the amplicons obtained from targeted multiplex amplification prior to sequencing, shows that RaDaR does not sequence the amplicons generated by the targeted multiplex amplification and therefore does not infringe the claims of the '454 patent.

86. Natera's expert, Dr. Metzker, admitted that the RaDaR process involves an intervening step (non-targeted, universal barcoding PCR) between targeted multiplex amplification and sequencing:

Q. Let me start again. Following the multiplex PCR in RaDaR, you contend that there's an additional PCR process to introduce sample barcodes and sequencing adaptors; correct?

A. That's correct.

Q. And that barcoding process that happens after the targeted multiplex PCR and before the sequencing; correct?

A. Well, in Forshew (2012), I believe that is correct.

Ex. 6 at 54:14-25 (objection omitted).

Q. So you think it's possible that in reference 26, there's a suggestion of a reaction barcode that's introduced in connection with the targeted multiplex PCR? Assuming that there's a reaction barcode that's introduced in the targeted multiplex PCR, whatever that might be, you do understand that there's a sample barcoding PCR that takes place after the targeted multiplex PCR and before the sequencing; correct?

A. I believe that's correct. The sample barcode is added in the second round.

Q. And a sequencing adaptor is also added in the second round after the targeted multiplex PCR; correct?

A. Correct. You'd have to do that if you were going to do Illumina sequencing.

Ex. 6 at 56:10-57:4 (objections omitted).

Q. Now, after the barcoding PCR you contend that's in RaDaR, the barcoded products are subject to sequencing?

A. Yes. I state that in my report at paragraph 69.

Ex. 6 at 58:5-9. This all again confirms non-infringement because it shows that RaDaR does not sequence directly the amplicons that emerge from targeted amplification, but instead sequences other products that emerge from a non-targeted universal amplification.

87. I understand that Natera has waived any doctrine of equivalents theory with respect to any of the Asserted Claims in connection with the preliminary injunction proceedings in this case because they did not present such a theory in their opening papers. Moreover, with regard to the "sequencing the amplicons" limitation, I further understand that Natera is unable to assert a doctrine of equivalents theory because of its prosecution disclaimer in connection with the prosecution of the '008 patent. *See* Ex. 9. Nevertheless, in my opinion, the RaDaR process does not infringe this limitation under a doctrine of equivalents theory. Specifically, the universal

barcoding PCR will serve to increase the overall quantities of the target mutations that were obtained from the targeted multiplex amplification, and it will do so in an indiscriminate way such that information regarding the relative amounts of the mutations present in the original sample will be lost. As another example, the additional rounds of PCR can introduce further polymerase error that can impact the ability to detect the small amount of mutations that are present in the circulating tumor DNA. Natera confirmed how different the two processes are during prosecution, where inventor Bernhard Zimmerman explained that “the highly accurate mutation detection obtained are *due to lack of intermediate steps between the multiplex amplification and sequencing step.*” Ex. 45 (’008 5/22/2021 Interview Summary) (emphasis supplied).

88. Therefore, the RaDaR Assay does not directly infringe the Asserted Claims of the ’454 patent.

B. The ’035 Patent

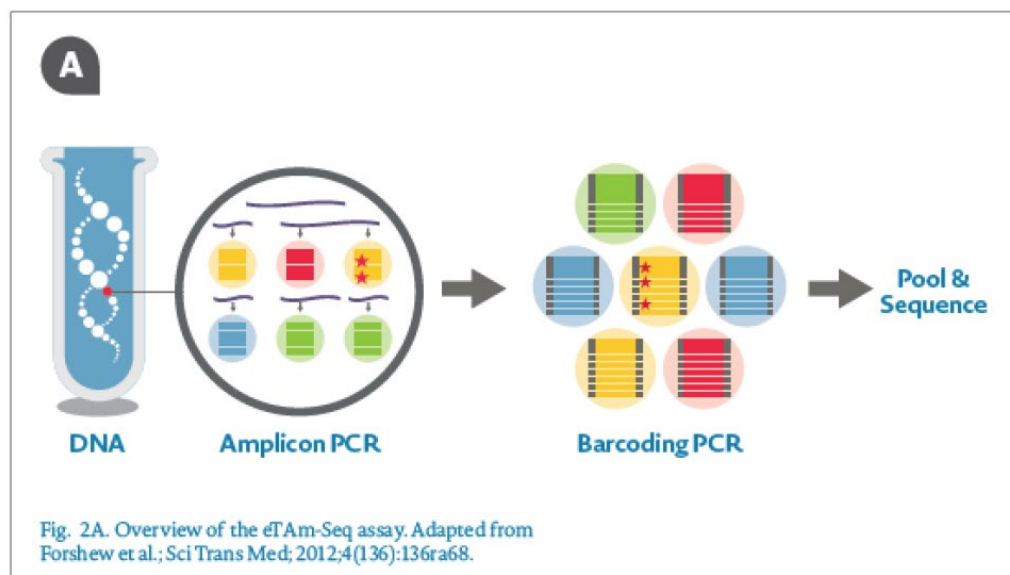
1. NeoGenomics’s RaDaR Assay Does Not Use Targeted Amplification to Amplify Tagged Products

89. NeoGenomics’s RaDaR Assay does not infringe the Asserted Claims of the ’035 patent because it does not amplify tagged products via targeted amplification. This is required by the Asserted Claims, which recite “tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products” and then “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.” D.I. 1-2 at claims 1, 12, 13 (emphasis added).

90. NeoGenomics’s RaDaR Assay does not infringe any of the Asserted Claims of the ’035 patent because to the extent it does “generate tagged products,” such “tagged products” are

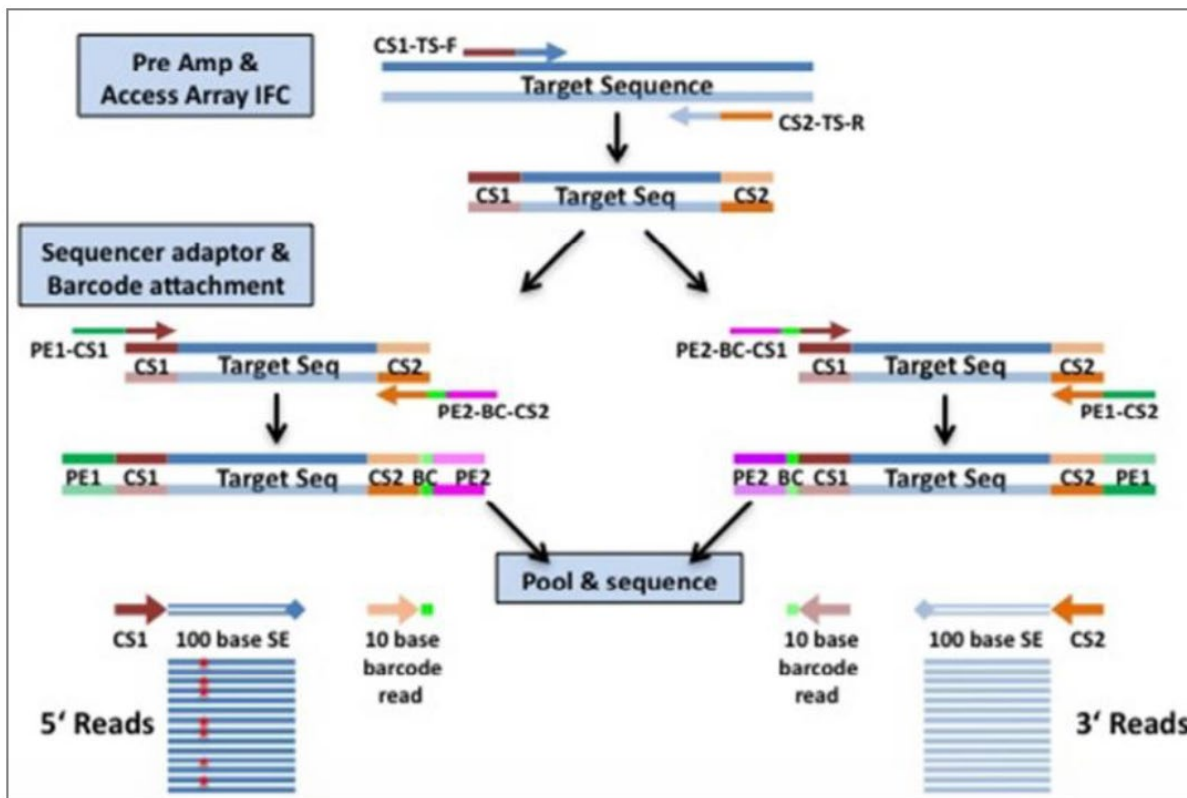
generated via targeted multiplex amplification, which are then, at most, amplified via a universal, *non-targeted* amplification. These “tagged products” are not amplified via *targeted* amplification as required by the Asserted Claims.

91. The Figure below provides an overview of certain steps of the RaDaR process. As shown below, first a DNA sample is subjected to “Amplicon PCR,” which is a targeted multiplex amplification. To the extent that “tagged products” are generated in the RaDaR process, this is achieved during this targeted multiplex amplification step. Next, “Barcoding PCR” is performed on the “tagged products.” The “Barcoding PCR” step is a non-targeted, universal amplification wherein reaction-specific barcode sequences are added to the “tagged products.” This is the only amplification step that the “tagged products” are subjected to—there is no targeted amplification of the “tagged products.”



D.I. 13-6.

92. This is also shown in Forsheew (2012), which depicts a targeted preamplification process followed by universal barcoding PCR:



D.I. 13-7 at Fig. S1.

93. To reiterate, to the extent that the RaDaR process generates any “tagged products” that are subject to further amplification, this is done in the first targeted multiplex amplification step. Any subsequent amplifications are *not* targeted. Because there is no targeted amplification of the tagged products, there can be no infringement of the '035 patent.

94. I understand that at deposition Dr. Metzker nonetheless advanced an infringement theory based on the contention that the preamplification step can satisfy multiple claim elements. The theory is based on distinguishing among the different cycles of the preamplification. Dr. Metzker’s opinion to this effect is not in his declaration. D.I. 13; *see* Ex. 6 at 93:5-95:15; 96:12-23; 98:13-99:8. Nonetheless, I explain his theory immediately below and explain why it is incorrect for completeness.

95. Dr. Metzker testified that his infringement opinion relies on the preamplification step of Forshew, *i.e.* the targeted multiplex amplification step, to satisfy claim 1a: “tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products” (“tagging limitation”). Ex. 6 at 89:19-24 (“[t]he preamplification step is creating the tagged products in Claim 1A of the ’035 Patent”). Dr. Metzker further testified at deposition that he also relies on the same preamplification step to satisfy a second claim limitation, claim 1b: “amplifying the tagged products one or more times to generate final amplification products, wherein one of the amplification steps comprises targeted amplification...” (“amplifying limitation”). D.I. 1-2 at claims 1, 12, 13; Ex. 6 at 47:24-48:1; 88:18-22; 89:19-90:5. In order to do so, Dr. Metzker attempts to distinguish between the first PCR cycle of the preamplification step (as satisfying the “tagging” limitation) and the 14 subsequent PCR cycles of the preamplification step (as satisfying the “tagging” and/or “amplifying” limitation):

Q. For Claim Element 1A of the process of generating the tagged products, you are relying upon the first cycle of the PCR preamplification; correct?

A. Yes, that would be the first time the target-specific primers will hybridize to the cell-free DNA.

Q. And then for step 1B, you’re relying upon the subsequent 14 cycles; correct?

A. Well, I’m only relying on those cycles that amplify the tagged products.

Ex. 6 at 92:3-13; *see also id.* at 99:20-100:13.

96. More specifically, Dr. Metzker’s opinion is that the “tagging” limitation is satisfied when a target-specific primer with a 5’ universal tail adaptor hybridizes to a cfDNA molecule and extends it to generate a complementary cfDNA with a 5’ tail in the RaDaR process:

Q. Now, the process that you contend satisfies that claim element in RaDaR is the process wherein a primer that’s specific to a target and that has a 5 prime universal tail adaptor hybridizes to a cell-free DNA molecule and extends it to generate a new DNA molecule that is complementary to the cell-free DNA

molecule and has the 5 prime tail; is that right?

A. Well, it generates a tagged product. It does -- it does do that. But it's all based -- all of my information is based on publicly available documents; and if there's more documents out there, I would reserve the right to review those as well.

Q. And that process that we've just discussed, that happens if the first cycle of the preamplification PCR; correct?

A. Well, I think as I've testified, it does happen in the first cycle; but it can also happen in subsequent cycles.

Q. Are there any other processes, other than the one we've just discussed, that you believe satisfies that tagging claim element in Element 1A of the claim?

A. Well, based on the public documents I have right now, that appears to be the only information that I have; but that information seems sufficient to render my opinion that it meets the claim limitation.

Ex. 6 at 103:17-104:24 (objection omitted).

97. With respect to the claim element in step 1b that requires targeted amplification of the tagged products, Dr. Metzker contends that this is also fulfilled by the preamplification step in RaDaR, albeit only by PCR cycles *after* the first cycle that involve hybridization of a primer to some copy of the cell-free DNA molecule:

Q. In Claim Element 1B, which you rely upon, are the courses of the preamplification process wherein a high primer hybridizes to a copy of a cell-free DNA molecule that was generated in a previous cycle of the PCR process; correct?

A. I guess it's easier just to stay to the claim language in what you're referring to as the "copied CDNA -- cfDNA molecule with a tag on it." It's just the tagged product; right? And I am relying on those products that have been generated in step 1A are now subject to one or more amplifications where one is a target amplification or wherein one is subject to introducing barcodes or one or more sequencing tags.

Q. Now, that process, that can only happen in the 14 cycles of the preamplification step that happened after the first PCR step; correct?

A. That is correct. So step 1B can start as early as cycle 2, but it doesn't necessarily -- is limited to cycle 2.

Ex. 6 at 105:16-107:5. This theory is erroneous for several reasons, as I discuss below.

98. First, as Dr. Metzker admitted, *all* of the 15 PCR cycles of the preamplification step generate tagged products:

Q. In the subsequent 14 cycles of the preamplification, there will be DNA fragments that include the universal tail sequence; correct?

A. They will be the product of the previous amplification cycle.

Q. And those DNA fragments that include the universal tail sequence, they can be subject to amplification by one of the primers in the pool; correct?

A. One of the target-specific primers in the pool that's specific to that target.

Q. *And that will yield an additional tagged product; correct?*

A. *That is correct.*

Q. And you rely upon that process for step 1B of the claim; correct?

A. Which process are you referring to?

Q. The process I just described, wherein a DNA fragment which includes a universal tail sequence is subject to amplification by one of the primers in the pool that's specific to the target.

A. I rely on the product that contain- -- that is now tagged with a universal tail adaptor is now subject to the wherein clause of one of the amplification steps comprising target amplification. So there are target-specific primers. They target the tagged product, and *they create a new tagged product.*

Q. And that happens only in the last 14 cycles of the PCR preamplification step in Forsheew (2012); correct?

A. That's correct. *Every time a primer hybridizes, it is tagging the product with a new tag.*

Ex. 6 at 97:4-98:12 (emphasis added).

Q. Okay. Now, those processes wherein the primers hybridize to tagged products that are generated as early as the first step of the preamplification process, that will generate new tagged products; correct?

A. *A target-specific primer with a universal adaptor -- that hybridizes to a tagged product will create a new tagged product.*

Ex. 6 at 107:6-17 (objection omitted). As such, the 15 PCR steps in the preamplification all must pertain to what Dr. Metzker refers to as step 1(a), which recites generating the “tagged products.” There is no principled basis for one to contend that some of the “tagged products” that are generated in the preamplification satisfy the step of the claim relating to generating “tagged products” while the other “tagged products” do not satisfy this claim element and only pertain to another claim element regarding targeted amplification of tagged products.

Second, and related to the first reason, claim 13 of the '454 patent recites that the tagging is accomplished by “amplifying.” As I explain above in connection with claim construction, this term refers to making copies of a molecule. Dr. Metzker appears to agree with this:

Q. Dr. Metzker, have you ever heard of the term “amplification” used in the context of PCR amplification?

A. In general, yes.

Q. What do you understand amplification to refer to in that setting?

A. Well, in the setting of PCR, it's where a template molecule is copied and that is repeated multiple times to make multiple copies.

Ex. 6 at 108:10-18. Therefore, one would understand that generating tagged products by amplification involves not just the initial hybridization of a primer to an original DNA molecule to generate an initial copy, but also all subsequent copies. This must be the case because, as Dr. Metzker confirmed at deposition, a person of skill in the art would understand that the products of all cycles of the PCR process are all just “copies:”

Q. Now, the only thing that people understand to be a copy in the context of PCR amplification is the product that results when the primer hybridizes to one of the original sample molecules and a complementary strand is generated. But that's – that's the only thing people call a copy; correct?

A. I'm not sure I agree with that.

Q. Okay. Well, what else would be a copy?

A. Well, first, I'm not sure what people would agree with. I mean, I'm not -- I can tell you what I think, but I don't know what people would agree with.

Q. What do you think?

A. Well, I think you can make a copy from an original stand. You can make a copy from a copy. I don't think -- at the end of the day, they think of it as a product.

Q. When you say "you can make a copy from a copy," what did you mean by that?

A. Well, we talked about this in step 1B where the amplification can occur from a tagged product using a target-specific primer. So in that case, the tagged product would represent a copy because it's now tagged, and that can serve as a template in the next primer extension reaction.

Q. And will that lead to a new copy.

A. Well, I don't know about a new copy, but it's another copy.

Q. It's another copy, okay. Why do you distinguish between "a new copy" and "another copy"?

A. Well, I guess I don't think of copies in time -- old copy, new copy. They're just copies.

Ex. 6 at 110:5-111:12.

99. Importantly, other than an amplification process, which Dr. Metzker agrees makes "copies" in all cycles, Dr. Metzker identified only one other possible process for generating "tagged products," which was through ligation and is hence not covered by claim 13:

Q. Is there more than one way to tag cell-free DNA isolated from a sample?

A. Yes.

Q. And what are they?

A. Well, one way which we've been talking about is taking the isolated cell-free DNA, hybridizing primer that has a 5 prime tag attached to it and extending that product to create a double-stranded DNA molecule. That is one way of tagging the cell-free DNA.

Q. Are there any others?

A. Yes. You can also use a process called “ligation,” where you can attach -- and you can do that enzymatically or chemically to attach the universal tailed adaptors to the cell-free DNA.

Q. Are there any others that you can think of?

A. Not off the top of my head. Ligation would be -- or PCR -- the two most common ways of doing that.

Ex. 6 at 114:8-115:1. Therefore, the process of “amplifying” that is recited in claim 13 and that Dr. Metzker contends satisfies the claims generates “tagged products” at all cycles, not just the first cycle. And, in view of the deposition testimony above, there is no other process Dr. Metzker can point to as satisfying the claims.

100. Third, and most important, Dr. Metzker’s opinion that the first PCR cycle in the preamplification step satisfies the “tagging” limitation (claim 1a) and the subsequent 14 PCR cycles of the preamplification step and the universal barcoding PCR step satisfy the “amplifying” limitation (claim 1b) contradicts and cannot be squared with the language of other claims, such as claims 12 and 13. Claim 1 recites “tagging isolated cell free DNA with *one or more universal tail adaptors* to generate tagged products.” D.I. 1-2 at claim 1 (emphasis added). Claim 12, which depends directly on claim 1, further recites “wherein the one or more universal tail adaptors comprise *a first universal tail adaptor* and *a second universal tail adaptor*.” D.I. 1-2 at claim 12 (emphases added). Thus, the “tagging” limitation of Claim 12 requires tagging cfDNA with a first universal tail adaptor and *a second universal tail adaptor* and cannot be satisfied by a PCR step that only incorporates a first universal tail adaptor (*i.e.*, when a target-specific primer with a 5’ universal tail adaptor hybridizes to a cfDNA molecule and extends it to generate a complementary cfDNA with a 5’ tail). Likewise, claim 13 recites “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.”

101. These claims establish unambiguously that Dr. Metzker's theory that the first part of a PCR process wherein a primer hybridizes to cell-free DNA is "tagging" but that subsequent steps where a primer hybridizes to a copy of the cell-free DNA are merely "amplification" is wrong. Specifically, I understand Dr. Metzker supported his theory based on the argument that the claims require tagging "isolated" cell-free DNA such that one would only view the initial hybridization of a primer to the original DNA molecule as being "tagging:"

Q. Do you rely upon those, quote, "new tagged products" that we just referred to for step 1A of the claim?

A. Oh, that doesn't meet step 1A because step 1A requires that tagged -- tagging isolated cell-free DNA. So -- so a tagged product that acts as a template to create a new tagged product does not meet the limitation of Claim 1A.

Ex. 6 at 107:19-108:1. The problem with this theory, however, is that claim 13 requires that the "tagging" 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." By calling for the use of a "second primer," this process necessarily involves using what Dr. Metzker refers to as a "tagged product" to create a "new tagged product." It is impossible for this to take place any other way. Specifically, the tagging process of Claim 13 cannot be satisfied by the first step of PCR because tagged products with both first and second universal tail adaptors are only generated in cycles after a first PCR cycle. During the first cycle a first tail adaptor is introduced. It is only after the first PCR cycle, at which point a second primer with a second tail adapter hybridizes to a copy of the original molecule, that products with the second tail adaptor are generated. As such, Dr. Metzker's infringement theory based on the notion that only the first round of a PCR wherein a primer hybridizes to an original cell free DNA molecule generates the tagged products that are then amplified is irreconcilable with the claim language and is thus wrong.

102. Beyond the foregoing, I note that there are other peculiarities in Dr. Metzker's changed infringement theory establishing that it is erroneous.

103. Dr. Metzker, for instance, testified as follows with regard to barcoding PCR in RaDaR as allegedly satisfying the "universal tail adaptors" element:

Q. Okay. Let's go look at Claim 12. If you look at the bottom of page -- paragraph 108, you state: "According to Defendant, Gale (2018) uses the eTAm-Seq technology that employs barcoding PCR using universal tailed adaptors." Do you see that?

A. I do see that as the last sentence.

Q. Based on that, you would agree that you are relying upon the barcoding PCR in RaDaR for the universal tail adaptors element claim; correct?

A. Well, I think, as I testified, it's one of the adaptors that I'm relying on. The CS1 and CS2 adaptors are also incorporated, and they also -- person of ordinary skill in the art would have understood that would meet the limitation of the universal tail adaptor. I testified to that.

Q. All right. *So you rely upon what you contend is a universal tail adaptor in connection with the barcoding PCR's one feature that satisfies the universal tail adaptor element; is that correct?*

A. Well, *I think it meets the limitation of one or more universal tail adaptors.*

Ex. 6 at 79:5-80:3 (objection omitted, emphasis added); *see also id.* at 18:5-14.

104. However, to the extent the universal, barcoding PCR step satisfies the "tagging" limitation, the "amplifying" limitation, which requires targeted amplification of the tagged products, cannot be satisfied. This is because the only targeted amplification in the RaDaR process *precedes* the universal, barcoding PCR step as Dr. Metzker admitted:

Q. In the RaDaR process, what targeted amplifications are there, if any, following the barcoding PCR step?

A. *I don't think there are any targeted after the barcoding*, but the tagged products in 1C comes from the first target multiplex PCR step.

Ex. 6 at 80:23-81:3 (emphasis added). In this regard, Dr. Metzker's reliance on the barcoding PCR

in RaDaR as satisfying the “universal tail adapter” element is nonsensical.

105. As another example, Dr. Metzker also strangely opined that the preamplification step produces “final amplification products.” D.I. 13 ¶ 100 (“As shown in the ‘preamplification’ stage in the figure *infra*, Forshew (2012) illustrates in a first round of PCR amplifying the tagged products one or more times to generate final amplification products”). Dr. Metzker admitted, however, that the preamplification step does not yield products with a barcode or sequencing tag:

Q. And that first round of PCR is the process that is referred to in the diagram as “preamp.” Is that right?

A. It is preamp, and it’s also inclusive of the access array IFC.

Q. Now, at the end of that preamp and access array IFC, the amplified products do not yet have barcodes or sequencing tags; is that correct?

A. That is correct.

Ex. 6 at 14:21-15:4.

106. The Asserted Claims require that the “final amplification product” have “a barcode and one or more sequencing tags.” Ex. D.I. 1-2 at claims 1, 12, and 13. Therefore, the preamplification step does not “generate final amplification products” as Dr. Metzker opines. *Id.* Dr. Metzker’s opinion that the preamplification generates “final amplification products” thus makes no sense.

107. As explained above, I understand that Natera has waived any DOE theory with respect to any of the Asserted Claims in connection with the preliminary injunction proceedings in this case. *See supra*. Nevertheless, in my opinion, the RaDaR Assay does not infringe the Asserted Claims under the DOE because targeted PCR and universal PCR are not insubstantially different. This is confirmed by the specification of the ’035 patent. The specification states that “[i]n some embodiments, the non-specific amplification comprises universal polymerase chain

reaction (PCR).” D.I. 1-2 at 6:18-20; *see also id.* at 33:19-21. “Targeting refers to a method used to selectively amplify or otherwise preferentially enrich those molecules of DNA that correspond to a set of loci, in a mixture of DNA.” *Id.* at 42:31-33. Universal priming sequences are “DNA sequence[s] that may be appended to a population of target DNA molecules....Once added to the population of target molecules, primers specific to the universal priming sequences can be used to amplify the target population using a single pair of amplification primers. Universal priming sequences are typically not related to the target sequences.” *Id.* at 42:14-21; *see also id.* at 42:22-30. In other words, a single pair of primers with universal priming sequences used in a universal PCR do not generate targeted amplicons. Thus, the RaDaR Assay does not infringe the Asserted Claims under the DOE.

108. NeoGenomics’s RaDaR Assay does not subject any tagged products to targeted amplification as required by the Asserted Claims. The second amplification is non-targeted by universal primers that do not distinguish between targets. Therefore, the RaDaR Assay does not infringe any of the Asserted Claims.

X. INVALIDITY UNDER 35 U.S.C. § 103—OBVIOUSNESS

109. As I explain above, it is my opinion that RaDaR does not infringe the Asserted Claims of the ’454 and ’035 patents. Nevertheless, to the extent one agrees with Natera’s infringement theories, then the Asserted Claims of both of these patents are invalid in view of the prior art. I detail my opinions to this effect below.

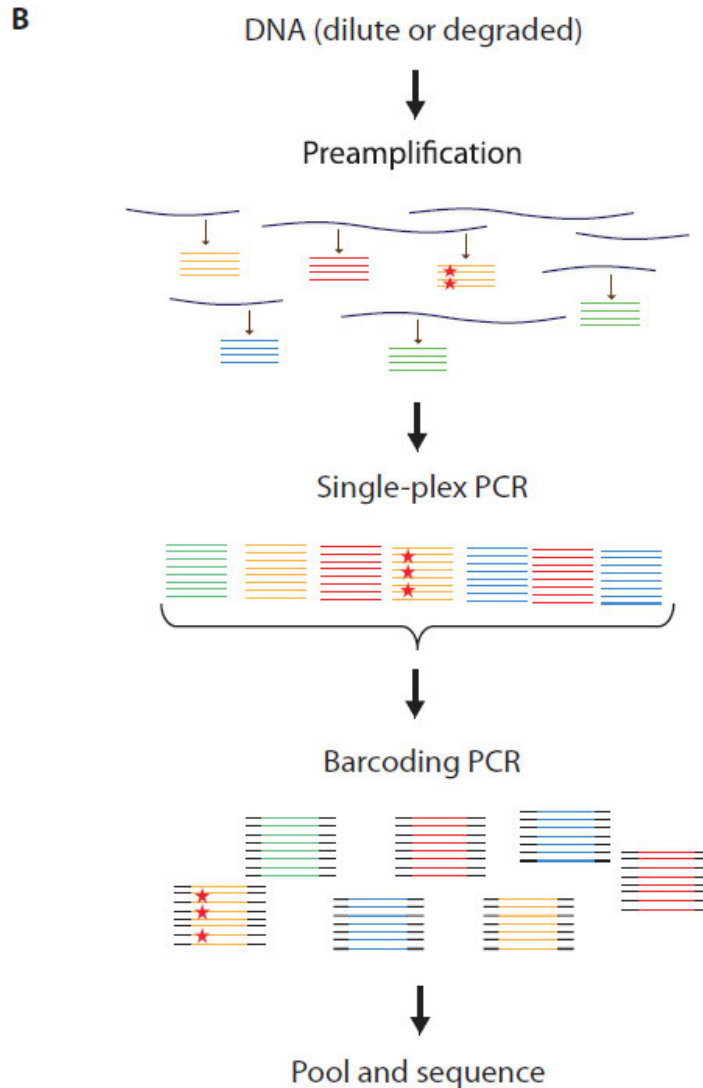
A. The '454 Patent is Invalid as Obvious

1. The Asserted Claims Of The '454 Patent Are Rendered Obvious By Forshew (2012) In View Of The Skill and Knowledge Of a POSA

a. Overview of Forshew (2012)

110. Forshew et al. published on May 30, 2012, with supplementary material that was available online at the same date. *See* D.I 13-7. It teaches tagged-amplicon deep sequencing (“TAm-Seq”), a particular method for detecting low frequency mutations and its use as a tool for identifying and monitoring cancer mutations in patients. D.I. 13-7 at 1, Abstract.

111. Forshew (2012) illustrates the basic overall workflow of TAm-Seq in Figure 1(b), reproduced below:



D.I. 13-7 at 2, Fig. 1(b). The legend for Figure 1(B) summarizes the figure above as follows:

Multiple regions were amplified in parallel. An initial preamplification step was performed for 15 cycles using a pool of the target-specific primer pairs to preserve representation of all alleles in the template material. The schematic diagram shows DNA molecules that carry mutations (red stars) being amplified alongside wild-type molecules. Regions of interest in the preamplified material were then selectively amplified in individual (single-plex) PCR, thus excluding nonspecific products. Finally, sequencing adaptors and sample-specific barcodes were attached to the harvested amplicons in a further PCR.

D.I. 13-7 at 2 (Fig. 1(B) legend).

112. By way of further summary, the TAm-Seq method includes first identifying mutations or single-nucleotide variants (“SNVs”) that are associated with cancer or tumors. D.I. 13-7 at 2, 9. At a high level, TAm-Seq accomplishes this by performing whole genome sequencing of both healthy and tumor tissue and then comparing the sequencing data to identify tumor-specific mutations, in particular, SNVs. D.I. 13-7.

113. Once the SNVs of interest are identified based on whole-genome sequencing of the tumor sample, primer pairs are designed to amplify the tumor-specific mutations in the cell-free DNA of a cancer patient. D.I. 13-7.

114. TAm-Seq is set forth in Forshaw (2012) as including a first PCR reaction (a so-called “Pre Amp”) using the target-specific primers. Importantly, this first PCR is conducted using mixtures of sequence-specific primer pairs, allowing generation of products from different loci. Specifically, Forshaw (2012) discloses that in the “Pre Amp” step “all primer sets were used together to capture the starting molecules present in the template.” D.I. 13-7 at 3.

115. After an additional amplification step to introduce barcodes and sequencing-adapters, the TAm-Seq method set forth in Forshaw (2012) proceeds by sequencing the amplified cell-free DNA followed by a subsequent bioinformatics analysis of the resulting sequence data to ascertain whether the cell-free DNA includes the tumor-specific mutations.

116. I understand that Natera contends that NeoGenomics’s RaDaR product infringes by its use of eTAm-Seq, which I understand is an updated version of the TAm-Seq method that is set forth in Forshaw (2012). I understand that the differences between eTAm-Seq and TAm-Seq are not pertinent relative to the elements of the ’454 patent.

117. For instance, I understand that Natera’s expert, Dr. Metzker, testified that the only differences included that eTAm-Seq “does not require the microfluidic portion of the TAm-Seq

technology” and that “there are also some statistical methods for reducing errors.” Ex. 6 at 31:4-32:5, 32:13-17 (“Q. And the differences you’re able to identify sitting here today are the two that we’ve discussed, the lack of a microfluidic device and the statistical methods; is that right? A. Right.”). I further understand that Natera and its expert rely extensively upon Forshew (2012) for its understanding of how the RaDaR product works.

118. In these circumstances, it seems clear that to the extent RaDaR is deemed to actually infringe, then the ’454 patent must be invalid in view of Forshew (2012). This is because Forshew (2012) appeared three years before the earliest priority date of the ’454 patent in April of 2015. And, according to Dr. Metzker’s infringement theory, every key element of the claims of the ’454 patent are identically disclosed in Forshew (2012). As such, it is my opinion that the asserted claims of the ’454 patent are unpatentable as obvious over Forshew (2012) to the extent RaDaR infringes the ’454 patent.

b. Claim 1 Preamble: A method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:

119. To the extent that the preamble of the claim is limiting, Forshew (2012) discloses a method of preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more SNV mutations in the plasma sample.

120. Forshew (2012) teaches preparation of a plasma sample of a subject having cancer in applying TAm-Seq to monitor cancer patients. Forshew (2012) states the “[p]lasma of cancer patients contains cell-free tumor DNA that carries information on tumor mutations and tumor burden” and “demonstrated use of TAm-Seq to noninvasively identify the origin of metastatic relapse in a patient with multiple primary tumors.” D.I. 13-7 at Abstract.

121. Specifically, Forshew (2012) teaches the use of its TAm-Seq method to identify mutations in cfDNA obtained from plasma of cancer patients. D.I. 13-7 at 3 (“[W]e applied TAm-Seq to directly identify mutations in plasma of cancer patients.”); *id.* at S1 (“Dilution of plasma DNA samples to determine allele quantification accuracy”). Forshew (2012) teaches whole-genome sequencing to identify tumor mutations in conjunction with TAm-Seq:

Whole-genome sequencing of tumor material was used to identify tumor mutations in a patient with metastatic breast cancer undergoing two phases of chemotherapy. Ten mutations were selected, and short amplicons (<120 bp) were designed to cover the mutation loci (table S7). Serial plasma samples were collected over the course of 497 days, both before and after treatment. We performed TAm-Seq in duplicate, *using DNA from 0.08 ml of plasma per amplification*, and tracked dynamics of all mutations in parallel (Fig. 4D).

D.I. 13-7 at 9 (emphasis added).

122. The mutations identified included single base changes to the genome, that is, SNVs, that reflect substitutions of a single nucleotide for another in a genome. *See* D.I. 13-7 at 9, 17-19, Tables S6-S7.

c. Claim 1[a]: performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;

123. Forshew (2012) repeatedly teaches performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations.

124. Forshew (2012), for instance, teaches using “[w]hole-genome sequencing of tumor material [] to identify tumor mutations in a patient with metastatic breast cancer.” D.I. 13-7 at 9. Forshew (2012) teaches that the whole genome sequencing was conducted using “an Illumina HiSeq 2000 sequencer to 30x genomic coverage” and “the standard Illumina paired-end sample preparation kit according to the manufacturer’s instructions.” D.I. 13-7 at Table S7. I understand

that one of Natera's preferred experts, Dr. John Quackenbush, has opined that by "2010, whole-genome sequencing to 30x coverage was routine on the commercially available HiSeq 2000." Ex. 7 ¶ 64. Thus, there is no question that Forshew (2012) teaches whole genome sequencing, by routine commercially available methods, of a tumor sample to identify tumor-specific mutations.

125. Forshew (2012) further teaches using the whole genome sequence data to identify tumor-specific SNV mutations. Indeed, as I note immediately above, Forshew (2012) unambiguously refers to using "[w]hole-genome sequencing of tumor material [] to identify tumor mutations in a patient with metastatic breast cancer." D.I. 13-7 at 9. As Forshew (2012) explains, these mutations are then subject to amplification.

126. These mutations are SNVs. For instance, Table S7 details the affected gene, genomic location of the amplicon, genomic location of mutation, reference allele, and mutant allele.

Table S7: Mutations and amplicons studied in one breast cancer patient. To identify multiple mutations, genomic libraries from tumour and matched normal tissues were prepared using the standard Illumina paired-end sample preparation kit according to the manufacturer's instructions. DNA fragments of 300 bp were sequenced using paired-end 100-bp reads on an Illumina HiSeq 2000 sequencer to 30× genomic coverage. Sequencing reads were aligned to the hg19 reference genome using ELAND (Illumina) and subsequent data analysis was performed using the CASAVA 1.8 analysis pipeline (Illumina) to identify somatic mutations. We selected 10 mutations and designed an amplicon of <120 bp to cover each mutation. We validated these mutations by TAM-Seq in the tumour material and then followed them in serial plasma samples collected from the same patient.

Gene	Genomic location of amplicon (genome build hg19)	Genomic location of mutation (genome build hg19)	Reference allele	Mutant allele
<i>CD1A</i>	chr1:158226710-158226810	chr1:158226762	C	T
<i>BUB1</i>	chr2:111430288-111430374	chr2:111430343	G	A
<i>IQCA1</i>	chr2:237402413-237402499	chr2:237402439	G	A
<i>PIK3CA</i>	chr3:178936048-178936130	chr3:178936091	G	A
<i>ARAP3</i>	chr5:141041694-141041773	chr5:141041739	G	A
<i>MET</i>	chr7:116398593-116398675	chr7:116398635	C	T
<i>GIMAP5</i>	chr7:150439702-150439805	chr7:150439730	C	T
<i>ZFYVE21</i>	chr14:104195437-104195539	chr14:104195486	G	A
<i>KIAA0406</i>	chr20:36641162-36641252	chr20:36641201	C	T
<i>ADAMTS1</i>	chr21:28214239-28214357	chr21:28214274	C	G

D.I. 13-7 at Table S7. As the columns “Reference allele” and “Mutant allele” show, the variants that were identified are SNVs consisting of a change of a single nucleotide.

127. Forsheew (2012) also teaches the identification of SNVs by sequencing of exons on a tumor sample to identify tumor mutations. D.I. 13-7 at 3 (Validation and sensitivity for mutation identification in ovarian tumor samples), S1 (Sanger Sequencing). In addition to using TAM-Seq, the “tumor specimens of ovarian cancers (table S3),...were also sequenced for *TP53* by Sanger sequencing (36) (Supplementary Methods).” D.I. 13-7 at 3. Forsheew (2012) reports 43 single-base substitutions were called (table S3)” and that “[t]hese matched 100% of mutations identified

by Sanger sequencing and included three additional mutations at low AFs [allele frequencies] that were below detection thresholds of Sanger sequencing (fig. S2).” D.I. 13-7 at 3. These single-base substitutions are SNVs identified by routine sequencing. For instance, Table S3 details the affected gene, mutation and base change, and whether it was identified by Sanger sequencing.

Table S3: Mutations identified in FFPE samples. Diagnosis: type (FT, fallopian tube; O, ovarian; PP, primary peritoneal), histiotype (END, endometrioid, grade III; HGSO, high-grade serous ovarian carcinoma; S/CC, mixed serous and clear cell; S/END, mixed serous papillary and endometrioid, high grade), and stage. FFPE source: sample type (B, biopsy; IDS, interval-debulking surgery; PS, primary surgery), sample tissue (FT, fallopian tube; O, ovary; OM, omentum; P, peritoneum; SC, sigmoid colon; U, uterus). Cellularity: estimated from H&E-stained slide of the FFPE block. Identified by Sanger sequencing: I, identified; FAIL, samples where one duplicate had a technical fault; Indel, indel identified (indels were not analyzed by the calling algorithm); M, missed; U, unsequenced region or sample. S-135 and S-165 were from the same patient, but collected at different timepoints. S-140 and S-106 were from the same block, cut in different batches. S-111, S-143, and S-122 had two mutations called each. S-133, S-169, and S-147 had known indels, and did not have any point mutations called. S-119 had no mutations called. S-176, S-177, and S-178 failed harvesting in one of the repeats and calling algorithm was not applied.

Patient ID	Age at diagnosis	Diagnosis: type, histiotype, and stage.	Sample ID	FFPE source	Cellularity	Gene	Mutation and base change (genome build hg19)	cDNA change	Protein change	TAm-Seq Allele frequency (repeat 1)	TAm-Seq Allele frequency (repeat 2)	Identified by Sanger sequencing
1	60	O HGSO III	S-131	PS O	60%	TP53	17:7577570 C>T	c.711G>A	p.M237I	0.72	0.70	I
2	63	O HGSO III	S-142	IDS O	40%	TP53	17:7578203 C>A	c.646G>T	p.V216L	0.42	0.43	I
3	54	O HGSO III	S-150	IDS O	70%	TP53	17:7577538 C>T	c.743G>A	p.R248Q	0.87	0.91	I
4	46	O HGSO I	S-167	PS O	70%	TP53	17:7578290 C>T	c.560-1G>A	p.?	0.73	0.73	I
5	38	O HGSO III	S-174	PS O	90%	TP53	17:7578268 A>G	c.581T>C	p.L194P	0.69	0.72	I
6	63	O HGSO III	S-102	IDS FT	60%	TP53	17:7578271 T>C	c.578A>G	p.H193R	0.39	0.34	I
7	68	O HGSO III	S-112	IDS O	60%	TP53	17:7578263 G>A	c.586C>T	p.R196*	0.30	0.28	I
8	60	O HGSO III	S-132	PS O	90%	TP53	17:7577120 C>T	c.818G>A	p.R273H	0.74	0.75	I
9	62	PP HGSO IV	S-151	PS SC	70%	TP53	17:7578479 G>A	c.451C>T	p.P151S	0.39	0.38	I
10	58	PP HGSO IV	S-168	IDS O	90%	TP53	17:7578508 C>T	c.422G>A	p.C141Y	0.82	0.86	I
11	55	O HGSO III	S-104	IDS O	15%	TP53	17:7578370 C>T	c.559+1G>A	p.?	0.12	0.12	M
12	62	O HGSO IV	S-115	IDS O	70%	TP53	17:7577579 G>T	c.702C>A	p.Y234*	0.57	0.59	I
13	45	O HGSO IV	S-123	IDS O	80%	TP53	17:7577574 T>C	c.707A>G	p.Y236C	0.53	0.55	I
14	58	O HGSO III	S-153	IDS O	95%	TP53	17:7578212 G>A	c.637C>T	p.R213*	0.56	0.56	I
15	61	O HGSO IV	S-116	IDS O	98%	TP53	17:7577121 G>A	c.817C>T	p.R273C	0.76	0.75	I
16	65	O HGSO III	S-127	IDS O	90%	TP53	17:7577094 G>A	c.844C>T	p.R282W	0.76	0.74	I
17	64	O HGSO III	S-145	PS FT	60%	TP53	17:7578176 C>T	c.672+1G>A	p.?	0.52	0.55	I
18	63	FT HGSO III	S-161	PS O	95%	TP53	17:7578534 C>G	c.396G>C	p.K132N	0.86	0.88	I
19	68	PP HGSO III	S-170	IDS U	50%	TP53	17:7579312 C>A	c.375G>T	p.T125T	0.22	0.20	I

20	67	PP	HGSOC	III	S-108	IDS	OM	20%	TP53	17:7578431	G>A	c.499C>T	p.Q167*	0.74	0.93	I
21	64	O	HGSOC	I	S-117	PS	O	95%	TP53	17:7574012	C>A	c.1015G>T	p.E339*	0.70	0.70	I
22	65	O	HGSOC	III	S-128	IDS	O	95%	TP53	17:7579521	C>A	c.166G>T	p.E56*	0.68	0.67	I
23	54	O	HGSOC	III	S-138	IDS	OM	95%	TP53	17:7577569	A>C	c.712T>G	p.C238G	0.79	0.79	I
24	63	FT	HGSOC	III	S-146	PS	O	60%	TP53	17:7577559	G>T	c.722C>A	p.S241Y	0.62	0.62	I
25	61	PP	HGSOC	III	S-162	IDS	OM	40%	TP53	17:7578404	A>T	c.526T>A	p.C176S	0.11	0.19	I
26	72	PP	HGSOC	III	S-171	IDS	OM	50%	TP53	17:7578265	A>T	c.584T>A	p.I195N	0.71	0.70	I
27	68	O	HGSOC	III	S-179	IDS	O	90%	TP53	17:7578262	C>G	c.587G>C	p.R196P	0.83	0.86	I
28	46	O	HGSOC	III	S-109	IDS	O	50%	TP53	17:7577548	C>T	c.733G>A	p.G245S	0.40	0.39	I
29	82	O	HGSOC	III	S-118	IDS	OM	80%	TP53	17:7577568	C>G	c.713G>C	p.C238S	0.74	0.75	I
30	69	FT	HGSOC	III	S-130	PS	O	80%	TP53	17:7579389	G>A	c.298C>T	p.Q100*	0.75	0.79	I
31	64	O	HGSOC	III	S-172	IDS	OM	70%	TP53	17:7578406	C>T	c.524G>A	p.R175H	0.48	0.42	I
32	71	O	HGSOC	III	S-101	IDS	P	90%	TP53	17:7578370	C>T	c.559+1G>A	p.?	0.95	0.94	I
33	73	O	HGSOC	III	S-144	IDS	OM	70%	TP53	17:7577557	A>C	c.724T>G	p.C242G	0.44	0.47	I
34	54	O	HGSOC	IV	S-165	IDS	O	95%	TP53	17:7578527	A>G	c.403T>C	p.C135R	0.47	0.46	I
					S-135	B	OM	80%	TP53	17:7578527	A>G	c.403T>C	p.C135R	0.57	0.60	I
35	55	PP	SI/END	III	S-106	PS	O	90%	TP53	17:7574021	C>A	c.1006G>T	p.E336*	0.96	0.96	I
					S-140			90%	TP53	17:7574021	C>A	c.1006G>T	p.E336*	0.96	0.96	I
36	52	O	HGSOC	III	S-111	PS	O	95%	TP53	17:7578502	A>T	c.428T>A	p.V143E	0.45	0.44	I
								95%	TP53	17:7579642	C>T	Intron	Intron	0.22	0.28	M
37	45	O	END	II	S-143	PS	O	95%	TP53	17:7577538	C>T	c.743G>A	p.R248Q	0.75	0.77	I
								95%	PTEN	10:89720778	A>G	c.929A>G	p.D310G	0.59	0.52	I
38	55	O	END	IV	S-122	IDS	O	80%	PTEN	10:89692791	A>G	c.275A>G	p.D92G	0.19	0.18	M
								80%	PTEN	10:89685287	A>G	c.182A>G	p.H61R	0.17	0.17	I
39	54	O	HGSOC	III	S-119	PS	OM	80%								U
40	71	O	HGSOC	III	S-133	IDS	OM	80%								Indel
41	66	O	HGSOC	III	S-169	IDS	OM	50%								Indel
42	68	FT	S/CC	III	S-147	IDS	O	90%								Indel
43	49	FT	HGSOC	III	S-176	PS	O	95%							FAIL	
44	73	PP	HGSOC	IV	S-177	PS	OM	50%							FAIL	
45	44	O	HGSOC	III	S-178	IDS	OM	30%							FAIL	

D.I. 13-7 at S13-S14. A POSA would understand from this that ForsheW (2012) identified 40 single-base substitutions, indicated by “I” in the last column, in the ovarian tumor sample, using Sanger Sequencing. A POSA would also understand this as showing the identification of tumor-specific SNVs by sequencing on a tumor sample of the subject. It is also my opinion that a POSA would understand that tumor-specific single-base substitutions (or SNVs) could be identified in the same manner using whole exome or whole genome sequencing because the information used to do so, sequence that includes the SNV, is also provided in whole exome and whole genome sequences.

- d. **Claim 1[b]: performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume**

128. It is my opinion that ForsheW (2012) discloses to a POSA the method of using targeted multiplex amplification to amplify 10 to 500 target loci encompassing different tumor-

specific SNV mutations from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume.

129. Forsheew (2012), for instance, teaches the amplification of 10 mutations identified in a tumor sample based on the whole-genome sequencing data:

Whole-genome sequencing of tumor material was used to identify tumor mutations in a patient with metastatic breast cancer undergoing two phases of chemotherapy. ***Ten mutations were selected, and short amplicons (<120 bp) were designed to cover the mutation loci (table S7).*** Serial plasma samples were collected over the course of 497 days, both before and after treatment. We performed TAm-Seq in duplicate, using DNA from 0.08 ml of plasma per amplification, and tracked dynamics of all mutations in parallel (Fig. 4D). The patient was treated with single-agent epirubicin (gray-shaded region). After 4 months off treatment, a CT scan showed progressive disease and the patient commenced further treatment with paclitaxel chemotherapy. The 10 mutations followed a common pattern of sharp decline in AF upon onset of therapy and an increase in AF upon disease progression after termination of therapy (Fig. 4D).

D.I. 13-7 at 9.

130. The ten mutations and corresponding amplicons are described in Table S7, which details the affected gene, genomic location of the amplicon, genomic location of mutation,

reference allele, and mutant allele.

Table S7: Mutations and amplicons studied in one breast cancer patient. To identify multiple mutations, genomic libraries from tumour and matched normal tissues were prepared using the standard Illumina paired-end sample preparation kit according to the manufacturer's instructions. DNA fragments of 300 bp were sequenced using paired-end 100-bp reads on an Illumina HiSeq 2000 sequencer to 30× genomic coverage. Sequencing reads were aligned to the hg19 reference genome using ELAND (Illumina) and subsequent data analysis was performed using the CASAVA 1.8 analysis pipeline (Illumina) to identify somatic mutations. We selected 10 mutations and designed an amplicon of <120 bp to cover each mutation. We validated these mutations by TAM-Seq in the tumour material and then followed them in serial plasma samples collected from the same patient.

Gene	Genomic location of amplicon (genome build hg19)	Genomic location of mutation (genome build hg19)	Reference allele	Mutant allele
<i>CD1A</i>	chr1:158226710-158226810	chr1:158226762	C	T
<i>BUB1</i>	chr2:111430288-111430374	chr2:111430343	G	A
<i>IQCA1</i>	chr2:237402413-237402499	chr2:237402439	G	A
<i>PIK3CA</i>	chr3:178936048-178936130	chr3:178936091	G	A
<i>ARAP3</i>	chr5:141041694-141041773	chr5:141041739	G	A
<i>MET</i>	chr7:116398593-116398675	chr7:116398635	C	T
<i>GIMAP5</i>	chr7:150439702-150439805	chr7:150439730	C	T
<i>ZFYVE21</i>	chr14:104195437-104195539	chr14:104195486	G	A
<i>KIAA0406</i>	chr20:36641162-36641252	chr20:36641201	C	T
<i>ADAMTS1</i>	chr21:28214239-28214357	chr21:28214274	C	G

D.I. 13-7 at Table S7. As I note above, these mutations are SNVs that arise from different genes and hence different genomic locations. D.I. 13-7 at Table S7.

131. Further, the amplification process that was used was a targeted multiplex amplification. D.I. 13-7 at S2 (“Pre-amplification reactions were carried out in 10-μl reaction volumes containing 50 nM of each forward and reverse target-specific primer....Reactions were subjected to 15 cycles of amplification.”). Specifically, Forshew (2012) discloses that pre-amplification reactions were carried out in volumes with “each forward and reverse target-

specific primer” and “[r]eactions were subjected to 15 cycles of amplification.” D.I. 13-7 at S2 (“Preamplification for TAm-Seq”). The multiplex PCR reaction is also depicted and described in Figure 1B, with Forshew (2012) stating “[m]ultiple regions were amplified in parallel. An initial preamplification step was performed for 15 cycles using a pool of the target-specific primer pairs.” D.I. 13-7 at 2 (Fig. 1B Legend).

132. Natera’s expert Dr. Metzker has confirmed that Forshew (2012)’s preamplification step is amplification of targets together in the same volume:

Q: So you understand that the preamplification step that’s described in Forshew (2012) is a reaction where all the targets are amplified together in the same tube?

A: That’s my understanding.

Ex. 6 at 39:19-23.

133. As to cell-free DNA, Forshew (2012) states that “[s]erial plasma samples were collected over the course of 497 days, both before and after treatment. We performed TAm-Seq in duplicate, using DNA from 0.08 ml of plasma per amplification, and tracked dynamics of all mutations in parallel.” D.I. 13-7 at 9. Forshew (2012) likewise discloses that “[p]lasma of cancer patients contains cell-free tumor DNA that carries information on tumor mutations and tumor burden” and focuses throughout on monitoring that cell-free DNA using the TAm-Seq process. D.I. 13-7 at Abstract. A POSA would also recognize that the testing using DNA isolated from plasma teaches using cell-free DNA from plasma. Natera details in the ’035 patent, for instance, “cell-free DNA from plasma.” D.I. 1-2 at 210:1-5, 214:5-13, 217:55-58.

134. As to the length of amplicons, Forshew (2012) discloses its amplicons within the recited range of 50-150 bases because each was “<120 bp to cover each mutation” (Table S7) and listed primers defining amplicons indicate an amplicon length of greater than 50 bases (Table S1),

as does the listed genomic location of each amplicon with a start location and stop location (S7). Each of these indicate amplicons with lengths within the recited range of 50-150 bases.

135. Forshew (2012) also discloses TAm-Seq being conducted using higher numbers of amplicons, teaching that the degree of multiplex is not limited to ten, but can be higher. Specifically, Forshew (2012) teaches using TAm-Seq with 48 sequence-specific primer pairs to amplify 5995 bases of genomic sequence covering coding region (exons and exon junctions) of TP53 and PTEN, and selected regions in EGFR, BRAF, KRAS, and PIK3CA (table S1) by overlapping short amplicons (Fig. 1A). D.I. 13-7 at 3 (“We designed a set of 48 primer pairs to amplify 5995 bases of genomic sequence covering coding regions (exons and exon junctions) of TP53 and PTEN, and selected regions in EGFR, BRAF, KRAS, and PIK3CA (table S1) by overlapping short amplicons (Fig. 1A)”); Ex. 6 at 39:19-40:10. Dr. Metzker also testified that Forshew (2012) discloses using more than 20 pairs of target-specific primers in a multiplex reaction:

Q. Now, let's go to Table S1.

A. Yes, I'm there.

Q. This is listing the target-specific primers that were used in that preamplification reaction?

A. That's correct.

Q. And there's more than 20 targets that are listed there?

A. Well, I haven't counted them. Looks like 20.

Q. There's more than 20; right? Because the table continues on the next page; right?

A. You are correct.

Ex. 6 at 39:24-40:10.

136. Forshew (2012)'s Table S6 also shows 23 point mutations, e.g., SNVs. These mutations were identified in plasma samples from ovarian cancer patients. D.I. 13-7 at Table S6 (titled "Additional data for Table 2"), 6 (Table 2 Legend) (Table 2 reports "[m]utations identified by TAm-Seq in a set of 62 plasma samples from ovarian cancer patients."). As discussed above, TAm-Seq includes multiplex amplification with "each forward and reverse target-specific primer" combined together in the same volume. D.I. 13-7 at 2.

137. The Table S6 heading lists "Gene," "Location (build is hg19)," "base change" for different samples. D.I. 13-7 at Table S6. Unique SNVs in Table S6 can be recognized by reading down the column looking at the Location and base change information; there are 23 different point mutations reported in Table S6. D.I. 13-7 at Table S6.

- e. **Claim 1[c]: sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads, wherein the sequencing has a depth of read of at least 50,000 per target locus.**

138. It is my opinion that Forshew (2012) teaches a POSA sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads, wherein the sequencing has a depth of read of at least 50,000 per target locus.

139. As to sequencing, as set forth above, Forshew (2012) discloses using TAm-Seq for sequencing to obtain sequence reads:

Here, we demonstrate noninvasive identification of mutant alleles in plasma, at AFs as low as 2%, by targeted deep sequencing of circulating DNA. Our TAm-Seq method uses a combination of short amplicons, two-step amplification, sample barcodes, and high-throughput PCR. Because the amplicons are short, this method effectively amplifies even small amounts of fragmented DNA such as are present in circulating DNA.

D.I. 13-7 at 9; D.I. 13-7 at S2-S3, S19, Table S7.

Table 1. Mutations identified by TAm-Seq in plasma samples from seven ovarian cancer patients. TAm-Seq was used to sequence DNA extracted from plasma of subjects with HGSOE (stage III/IV at diagnosis). Plasma was collected when patients presented with relapse disease, before initiation of chemotherapy. For patient 46, DNA from a formalin-fixed, paraffin-embedded (FFPE) sample was not included in the TAm-Seq set and the mutation was validated in FFPE by Sanger sequencing. CA125 was measured at time of plasma collection. Mean depth of coverage at the mutation locus in the TAm-Seq data was averaged over the repeats (RMS deviation = 850). AF, allele frequency; N, no; Y, yes.

Patient ID	Age at diagnosis	Time elapsed since surgery (months); number of previous lines of chemotherapy	CA125 (U/ml)	Plasma per amplification reaction (μl)	Gene	Mutation and base change (genome build hg19)	Protein change	Detected in FFPE	Mean depth (sequencing reads)	Mean AF using TAm-Seq	Mean AF using digital PCR
8	60	13; 1	2122	50	TP53	17:7577120 C>T	p.R273H	Y	5000	0.09	0.10
12	62	27; 3	365	50	TP53	17:7577579 G>T	p.Y234*	Y	5000	0.10	0.08
14	58	50; 3	260	120	TP53	17:7578212 G>A	p.R213*	Y	5800	0.15	0.12
25	61	9; 1	944	110	TP53	17:7578404 A>T	p.C176S	Y	4800	0.04	0.08
27 [†]	68	15; 1	1051	90	TP53	17:7578262 C>G	p.R196P	Y	7700	0.06	0.14
					EGFR	7:55259437 G>A	p.R832H	N	5700	0.06	0.05
31	64	12; 1	313	30	TP53	17:7578406 C>T	p.R175H	Y	4500	0.44	0.56
46	56	30; 2	1509	30	TP53	17:7578406 C>T	p.R175H	Y	4200	0.23	0.30

*Indicates stop codon.

[†]Both a TP53 and an EGFR mutation were identified in this sample (Fig. 4A).

D.I. 13-7 at Table 1.

140. As to sequencing “depth,” as stated in the ’454 patent, and as well-known to a POSA, the depth of read is a measure of how many times a particular sequence is read. D.I. 1-1 at 100:9-15 (“Depth of read can be expressed in variety of different ways,...for example in a highly parallel DNA sequencer such as an Illumina HISEQ, which, e.g., produces a sequence of 1 million clones, the sequencing of one locus 3,000 times results in a depth of read of 3,000 reads at that locus.”).

141. In Table 1, reproduced above, Forshew (2012) disclosed actual examples that informed a POSA of sequencing depths of up to 15,400x in the validation and sensitivity for mutation identification in ovarian tumor samples. D.I. 13-7 at 3, 5, Table 1. In this testing, Forshew (2012) disclosed that they “amplified DNA from each sample in duplicate, tagging each replicate with a different barcode” and then these were sequenced, such that duplicate sequencing data was obtained for each. D.I. 13-7 at 3. Forshew (2012) then reports results, including the mean sequencing depth for each of the reported identified mutations, including one with a mean depth of 7,700 in Table 1. As the mean of sequencing data from two replicates, the reported mean

depth (sequencing runs) informs a POSA that the total sequencing depth for the two replicates was twice 7,700 or 15,400.

142. Forshew (2012) also taught a POSA the desirability of higher read depth in disclosing examples with sequencing depths of up to 15,400, as explained above, and *teaching using “[h]igher read depth...[to] allow for enhanced mutation detection* without change to protocols.” D.I. 13-7 at 10 (emphasis added). Based on this, one would have understood Forshew (2012) to teach sequencing depths in excess of 15,400, including 50,000 and beyond.

143. To the extent Natera contends that Forshew (2012) does not disclose a read depth of at least 50,000, this would have been obvious to a POSA. In this regard, I note that the ’454 patent does not identify any particular sequencing depth as being inventive or special. Just the opposite, as far as I can tell, the ’454 patent only refers to a depth of 50,000 once, and then only in a long list of possible sequencing depths that spans four orders of magnitude. Specifically, the patent states that “[i]n certain embodiments, a depth of read of greater than 100, 250, 500, 1,000, 2000, 2500, 5000, 10,000, 20,000, 25,000, 50,000, or 100,000 on the low end of the range and 2000, 2500, 5,000, 7,500, 10,000, 25,000, 50,000, 100,000, 250,000 or 500,000 reads on the high end, is attained in the sequencing run for each single nucleotide variant position in the set of single nucleotide variant positions.” D.I. 1-1 at 68:33-39. This broad disclosure of potential sequencing depths is consistent with the understanding of a skilled artisan that sequencing depth is a parameter that would be routinely adjusted to achieve whatever sensitivity was required.

144. A POSA would have understood that increasing sequencing depth increases sensitivity in detecting SNPs and it would be routine to increase sequence read depth to obtain greater sensitivity as required. For example, published U.S. Patent Application US 2010/0261189 A1 (Ex. 46) (“Bentley”), which published October 14, 2010, expressly teaches that the greater the

number of reads, the lower the minimum frequency of SNPs in a population that is detectable. Ex. 46 ¶ 85. Bentley also teaches this to a POSA in showing the relationship between “Number of Reads” and the “Minimum frequency of SNP in population detectable with 95% confidence...[and]...99% confidence.”

TABLE 2

SNP Classes	Number of Reads	Minimum frequency of SNP in population detectable with 95% confidence	Minimum frequency of SNP in population detectable with 99% confidence
1	200000	0.002%	0.003%
2	100000	0.005%	0.007%
5	40000	0.014%	0.018%
10	20000	0.028%	0.037%
50	4000	0.14%	0.18%
100	2000	0.28%	0.37%
200	1000	0.55%	0.74%
500	400	1.39%	1.85%
1000	200	2.76%	3.64%

Ex. 46 ¶ 88 (Table 2). Bentley’s disclosure of potential sequencing depths and sensitivity is also consistent with the understanding of a skilled artisan that sequencing depth is a parameter that would be routinely adjusted to achieve whatever sensitivity was required.

145. Natera has taken that very position on many occasions. For example, in a case related to organ transplant, Natera argued that “[i]t was well-known by 2009 that the number of molecules analyzed is one component of sensitivity, and that higher sensitivity could be achieved by sequencing more molecules” and “that by sequencing more molecules, sensitivity will necessarily increase.” Ex. 10 (Case No. 19-567 (CFC)(CJB), D.I. 101, Natera’s Opening Brief In Support Of Summary Judgement (D. Del.)) at 26-27.

146. Another of Natera's preferred experts, Dr. Quackenbush, testified that sequencing to even greater depth when required by an experiment also would have been routine, as it would require only running additional sequencing assays: "it is very easy to increase the coverage or sequence depth, if you later decide you need more data. Provided you still have your original sample, you can just sequence more, and combine the sequencing output from different flow cells." Ex. 7 ¶ 65.

147. In a case involving Natera and CareDx, Dr. Quackenbush submitted a declaration that Natera relied upon stating that "POSAs prior to November 6, 2009 were well aware that sequencing more molecules, or increasing sequencing depth, improves sensitivity" and that "the detection limit is determined by sequencing error rate and sequencing read depth (i.e., how many molecules are sequenced), reflecting the fact that there was a well-known correlation between the number of molecules sequenced and sensitivity." Ex. 20 ¶ 119 (citing Voelkerding, et al., "**Next-generation Sequencing**: From Basic Research to Diagnostics", Clinical Chemistry, vol. 55, No. 4, Apr. 1, 2009, 641-658. Thomas, Sensitive Mutation Detection in Heterogeneous Cancer Specimens by Massively Parallel Picoliter Reactor Sequencing, Nature Medicine, Vol. 12, No. 7, pp. 852-855 (July 2006)).

148. Dr. Quackenbush further testified in the same case, during the hearing on summary judgment regarding § 101, that to get higher sensitivity, you simply sequence more molecules, and that this was well-known:

Q. Does the written description state anything about how a person of skill in the art can increase the sensitivity of high-throughput sequencing?

A. So *they do provide a description of how to increase sensitivity*, which is simply to obtain more sequence, right, which is well-known with any kind of measurement. *If you want to make a better measurement, you measure more.*

Q. Can you point to an example of that in the written description?

A. Sure. It's right here. It's called out. It just says, *higher sensitivity can be achieved simply by sequencing more molecules*. That means use more channels. And when they refer to channels, the Illumina sequencer had something that looked like a microscope slide added onto individual channels. Simply showed you load one, you load two, you load three, you load four.

Q. And is there anything nonconventional about achieving higher sensitivity by sequencing more molecules?

A. No. I mean, this is standard practice in many fields. To make a better measurement, you make more measurements. *To get higher sensitivity for detecting molecules, you simply sequence more molecules*.

Ex. 11 (Case No. 19-567 (CFC)(CJB), Motion for Summary Judgement Hearing) at 98:10-99:6 (emphasis added).

149. In a proceeding to cancel another party's patent claims, Dr. Metzker has also testified that it would have been obvious to increase sequence coverage, i.e., reading depth, to improve the accuracy of next-generation sequencing platforms. Ex. 12 (Case No. IPR2018-01317, Declaration of Michael L. Metzker, Ph.D.) ¶¶ 211-215. Dr. Metzker relies on Harismendy for teaching that:

At high sequence coverage all NGS platforms have excellent variant calling accuracy (>95%) as assessed by the detection of known SNP variants.

Ex. 12 ¶ 213 (citing Harismendy).

150. In sum, increasing the depth of read for increased sensitivity would have been obvious and been driven by both the need for higher sensitivity for testing and the lower costs with improving technology.

151. It is also my opinion that Forshew (2012) discloses detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads.

152. Forshew (2012) discloses the bioinformatics methodology for measuring a pre-defined mutation, which corresponds to tumor-specific SNV mutations, stating:

Mutation detection. When applying TAm-Seq to measure a pre-defined mutation (as opposed to screening nearly 18,000 possible substitutions), the frequency of the mutant allele can be read out directly from the data at the desired locus. We used confidence margins of ~0.95 using a Normal distribution model for each examined substitution, and requiring a minimum of 10 representative reads, in at least one amplified library per sample. When a mutation was positively detected, its allele frequency was estimated by averaging the allele frequencies in all replicates after subtracting substitution-specific background frequencies.

D.I. 13-7 at S4 (“Mutation detection”).

153. Forshew (2012) thus discloses performing data analysis using “confidence margins of ~0.95 using a Normal distribution model for each examined substitution, and requiring a minimum of 10 representative reads, in at least one amplified library per sample” to detect mutations. D.I. 13-7 at S4 (“Mutation detection”).

154. As Forshew (2012) reports tumor-specific SNV mutations present in the cell-free DNA, for example, in Table S7, Forshew (2012) discloses detecting one or more of the tumor-specific SNV mutations present in the DNA from plasma samples. D.I. 13-7 at Table S7 (“subsequent data analysis was performed using the CASAVA 1.8 analysis pipeline (Illumina) to identify somatic mutations.”).

f. Claim 8: The method of claim 1, wherein the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation.

155. It is my opinion that Forshew (2012) discloses to a POSA the method of claim 1, wherein the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation.

156. As discussed above with respect to claim 1, which requires targeted multiplex amplification of 10 to 500 target loci, Forshew (2012) discloses TAm-Seq being conducted using higher numbers of amplicons and teaches that the degree of multiplex is not limited to ten, or even twenty, but can be higher.

157. Specifically, as discussed above, Forshew (2012) teaches using TAm-Seq with 48 sequence-specific primer pairs to amplify 5995 bases of genomic sequence covering coding region (exons and exon junctions) of *TP53* and *PTEN*, and selected regions in *EGFR*, *BRAF*, *KRAS*, and *PIK3CA* (table S1) by overlapping short amplicons (Fig. 1A). D.I. 13-7 at 3 (“We designed a set of 48 primer pairs to amplify 5995 bases of genomic sequence covering coding regions (exons and exon junctions) of *TP53* and *PTEN*, and selected regions in *EGFR*, *BRAF*, *KRAS*, and *PIK3CA* (table S1) by overlapping short amplicons (Fig. 1A)”).

158. As another example, as discussed above, Forshew (2012) discloses using more than 20 pairs of target-specific primers in a multiplex reaction, as Dr. Metzker has testified. Ex. 6 at 39:24-40:10 (referring to Forshew (2012) at Table S1).

159. As yet another example, also as discussed above, Forshew (2012)’s Table S6 also shows 23 point mutations that were identified in plasma samples from ovarian cancer patients using TAm-Seq. D.I. 13-7 at 6 (Table 2) (“Mutations identified by TAm-Seq in a set of 62 plasma samples from ovarian cancer patients.”), Table S6 (“Additional data for Table 2”). The Table S6 heading lists “Gene,” “Location (build is hg19),” and “base change” for different samples. D.I. 13-7 at Table S6. Unique SNVs in Table S6 can be recognized by reading down the column looking at the Location and base change information; there are 23 different point mutations reported in Table S6. D.I. 13-7 at Table S6.

160. In sum, Forshew (2012) discloses and teaches TAm-Seq being conducted using higher numbers of amplicons, including within the range of 20 to 50 multiplex.

161. The ability to use greater multiplex TAm-Seq would also have been obvious to a POSA prior to the priority date for the claim because even significantly greater multiplex amplification of DNA was already well established. For example, in the relevant art of preparing

DNA libraries, and in particular libraries to be used later for sequencing, the relevant art includes Published Patent Application US 2010/0120038 A1 (Ex. 13 (US20100120038A1 (“Fluidigm”))), which published May 13, 2010. I have been informed that Natera and Dr. Metzker have relied on Fluidigm teaching a multiplex PCR amplification reaction using 9,216 primer pairs, to amplify 9,216 target sequences, in a Patent Trial and Appeal Board proceeding seeking to have another party’s patent claims canceled. Ex. 12 ¶ 98.

162. In particular, I understand Dr. Metzker relied on Fluidigm as teaching preamplification using thousands of target-specific primer pairs in the same reaction volume, i.e., a highly multiplex reaction:

To increase target nucleic acid concentration prior to encoding, an optional pre-preamplification reaction be carried out before the encoding preamplification reaction. The pre-amplification reaction can be carried out in multiplex [sic: multiplex]. For example, target-specific primers for 9216 different target nucleic acids can be employed in one mixture.

Ex. 12 ¶ 98 (quoting Ex. 13 ¶ 119).

163. In the present case, Dr. Metzker was asked on cross-examination about the earlier testimony on Fluidigm about the use of highly multiplex PCR:

Q. Okay. So in the PCR – in the Fluidigm reference that you opined upon, that is teaching a single-tube multiplex PCR of over 9,000 targets for sequencing; correct?

A. It does have a preamplification step that does target at multiplex PCR.

Q. Of over 9,000 targets; correct?

A. That’s what they report.

Ex. 6 at 87:24-88:6.

g. Claim 11: The method of claim 1, wherein the method further comprises performing barcoding PCR prior to the sequencing.

164. It is my opinion that Forshew (2012) discloses to a POSA the method of claim 1 further comprising performing barcoding PCR prior to the sequencing.

165. Forshew (2012) discloses attaching barcodes by PCR prior to sequencing in that both “[p]latform-specific adaptors and barcodes are attached by PCR following the single-plex amplification step” (D.I. 13-7 at Table S2) and the sequencing adaptors are necessary for sequencing (D.I. 13-7 at 3). Further, Forshew (2012) discloses barcoding PCR before sequencing because “[s]equencing adaptors were separately attached at either end and the products [then] mixed together, such that single-end sequencing generated separate sets of forward and reverse reads.” Finally, this is depicted in Forshew (2012) Figure S1:

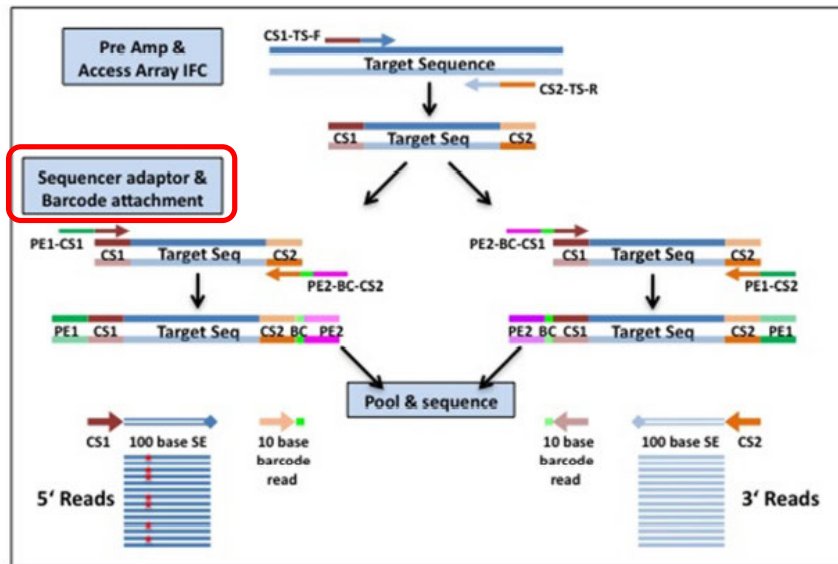


Figure S1: PCR strategy and primer design. Target-specific primers were synthesised with common adaptor sequences at their 5' ends (CS1 and CS2). These were used during the preamplification and single-plex stages to amplify the selected regions. Sequencing platform-specific adaptors and unique sample barcodes were attached during an additional round of PCR. The barcoding primers consist of Illumina sequencer adaptors (PE1 and PE2) at their 3' end, a unique 10 base barcode, and the common CS1 and CS2. Permutations of the universal tags and the sequencing adaptors enabled construction of bidirectional barcoded sequencing libraries. Using custom sequencing primers directed to the two tag sequences, sequence reads representing both strands of the amplicon pool were obtained from a single-end run. Both combinations were used in order to barcode and read amplicons in both directions. All samples were then pooled, cleaned, and then sequenced.

D.I. 13-7 at Fig. S1 (emphasis added) (“The barcoding primers consist of Illumina sequencer adaptors (PE1 and PE2) at their 3' end, a unique 10 base barcode, and the common CS1 and CS2”).

h. The Prosecution History Does Not Alter My Obviousness Opinions

166. I understand that the Patent Office considered Forshew (2012) during examination of the '454 patent, and I understand that Natera may contend this shows that the claims are not invalid in view of Forshew (2012).

167. Having considered the record, the fact that the '454 patent issued with Asserted Claim 1 from the Patent Office over rejections grounded on Forshew (2012) does not support the

validity of claim 1, but rather error in allowance of the claim. As I explain below, the Examiner made two errors.

168. First, the Examiner accepted erroneous arguments by Natera regarding whether Forshew (2012) discloses multiplex amplification. In response to a rejection in view of Forshew (2012), Natera argued as follows:

Furthermore, Forshew's TAm-Seq process was rationally and deliberately designed to rely on single-plex, "*individual amplification to purify and select for intended targets*" (pg. 3). In fact, Forshew had considered but dismissed multiplex targeted amplification – "*Multiplex amplification using a large set of primers could result in nonspecific amplification products and biased coverage*" (Fig. 1B, pg. 3). Forshew deliberately resorted to parallel single-plex amplification on Access Array, which physically separate individual primer pairs and individual reaction volumes to avoid issues such as nonspecific amplification and primer dimer formation. There was no reason or motivation for one of ordinary skill in the art to alter Forshew's TAmSeq process by substituting a step rationally and deliberately designed by Forshew for purpose of addressing the issue of nonspecific amplification, for a step already considered but dismissed by Forshew.

Ex. 47 at 9 (emphasis in original). Thus, Natera argued to the Examiner that Forshew (2012) did not utilize multiplex PCR. In view of these and the other arguments that Natera made, the Examiner allowed the claims. Ex. 48 at 3.

169. Yet, Natera's arguments, which the Examiner accepted, regarding whether Forshew (2012) teaches multiplex amplification were erroneous. As I explain above, the preamplification process in Forshew (2012) is in fact a multiplex amplification. Dr. Metzker agrees:

Q. And you do agree that the Forshew (2012) paper that does disclose the use of a multiplex amplification; correct?

A. Forshew (2012) does describe multiplex PCR.

* * *

Q. You do agree, though, that the Forshew (2012) publication we've been looking at, that it does use multiplex targeted application; correct?

A. In the preamplification step, it does use multiplex PCR.

Ex. 6 at 65:15-18, 66:19-24.

170. Second, the Examiner appears to have misapprehended or overlooked that increasing sequencing depth would have been obvious to a POSA before 2015.

171. During prosecution of the '454 patent, the Examiner issued a Non-Final Rejection, rejecting some of the then-pending claims as obvious over Forshew and other prior art references. Ex. 49 at 4-8. Then-pending claim 1 stood rejected over the combination of Forshew (2012) and Benesova. *Id.* at 4-6. Then-pending claim 2, adding the limitation “wherein the high-throughput sequencing has a depth of at least 50,000 per target locus,” was not the subject of rejection over the prior art. Ex. 50, 1; Ex. 49 at 4-8. As I explain herein, the use of such sequencing depth is not inventive, and it seems likely the Examiner simply overlooked this claim element.

172. Nevertheless, Natera responded to the obviousness rejection of claim 1 by amending claim 1 to incorporate a depth of sequencing limitation like that in then-pending claim 2. Ex. 47 at 8. I understand additional amendments to claim 1 addressed a Claim Objection and “alleged indefiniteness” (*Id.* at 2) and that these are immaterial to the question of obviousness (*Id.* at 6-7).

173. Natera argued for allowance of independent claim 1, over the combination with Forshew (2012), on the basis of the limitation to a sequencing depth of at least 50,000 per target locus:

Claim 2 stands free of the subject rejections. For the sole purpose of expediting prosecution and without acquiescing to the rejection, Applicant has amended independent claim 1 to incorporate the limitation of previous claim 2, thereby rendering the rejections moot. None of the cited references teaches or suggests sequencing each of the target loci with a depth of read of at least 50,000 per target locus. For at least these reasons, Applicant respectfully request withdrawal of the subject rejections against claims 1 and 3-14.

(*Id.* at 8.

174. The Office then withdrew the obviousness rejection of claim 1 over Forsheo (2012) and Benesova set forth in the Non-Final Office Action mailed “7 July 2022...in view of the arguments and amendment received 07 October 2022.” Ex. 48 at 2.

175. As discussed above, however, sequencing to “a depth of read of at least 50,000 per target locus” would have been obvious to a POSA before 2015, based on the teaching in Forsheo (2012), and as Natera and its experts have repeatedly argued. *See* § X.A.2.e. Indeed, as I explain above, Forsheo (2012) expressly teaches increasing sequencing depth so as to increase sensitivity. As I explain further below, this is also taught in the Bashashati prior art reference, which discloses sequencing depth up to 969,361.

176. It is my opinion, accordingly, that the Examiner misapprehended or overlooked that Forsheo (2012) would have taught or suggested sequencing with “a depth of read of at least 50,000 per target locus.”

2. The Asserted Claims Of The '454 Patent Are Rendered Obvious By Bashashati In View Of The Skill and Knowledge Of A POSA

177. It is my opinion that the asserted claims of the '454 patent are unpatentable as obvious over Bashashati.

a. Overview of Bashashati

178. Bashashati et al. published in 2013 with supplemental material that was also available in 2013. Ex. 51. It teaches sequencing exomes of tumor tissue and comparison of exome sequence with sequence of healthy tissue to identify tumor-specific mutations. Primers were then designed to amplify these mutations in circulating tumor DNA in patient plasma to monitor the tumor.

179. Specifically, Bashashati teaches whole exome sequencing on a tumor sample, stating, for instance that “[t]issue was obtained from a total of 31 tumour sites...matched normal

DNA for each patient (Table 1)” and “the exomes of 25 samples (19 tumour, six normal; Table 1) were sequenced.” Ex. 51 at 22; Ex. 52.

180. Bashashati further teaches identifying tumor-specific mutations, including SNVs, and using targeted amplification and sequencing to detect the presence of the tumor-specific mutations, stating that “tumour samples were used as index discovery samples to identify sets of mutations for interrogation by deep amplicon sequencing...to: (a) confirm predicted mutations; (b) determine the presence/absence of mutations in specific samples; and (c) infer clonal diversity estimates both within and between samples from the same patient.” Ex. 51 at 22; Ex. 52.

181. Bashashati then teaches targeted amplification and sequencing of target loci with SNVs from cell-free tumor DNA from plasma, stating that “for a subset of cases (four patients) plasma (pre-operative and pre-anaesthetic) was used to identify the presence of clonally dominant and subclonal mutations through deep sequencing of ctDNA.” Ex. 51 at 22; Ex. 52. For these four patients, selected clonal and subclonal variants were identified in these exomes and then assayed in plasma. Ex. 51 at 22; Ex. 52. (“We assayed 72 mutations in case 1, 49 in case 2, 63 in case 4 and 131 in case 5. Across the four cases, 215/312 (69%) mutations contained sequencing coverage and 38/215 (18%) were detectable with mutation allele frequency above statistical background noise (binomial exact test, false discovery rate < 0.05) (see supplementary material, Table S2)).

b. Claim 1 Preamble: A method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:

182. To the extent that the preamble of the claim is limiting, Bashashati discloses a method of preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more SNV mutations in the plasma sample:

women with ...cancer were included in this study...for a subset of cases (four patients), plasma...was used to identify the presence of clonally dominant and subclonal mutations through deep sequencing of ctDNA.

...

A total of 1349 somatic mutations in all samples were confirmed with deep amplicon resequencing (median 5079-fold coverage; see supplementary material, Table S2).

Ex. 51 at 22; Ex. 52. (citing Table S2); *id.* at 34 (“Table S2. Omnibus table of validated somatic SNVs and indels”)).

c. Claim 1[a]: performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;

183. It is my opinion that Bashashati teaches performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations.

184. Bashashati, for instance, teaches whole exome sequencing. Ex. 51 at 22; Ex. 52. (“the exomes of 25 samples (19 tumour, six normal; Table 1) were sequenced to a median exonic coverage of 70.7-fold across different samples (see supplementary material, Table S1 and Figure S1).”). Specifically, Bashashati teaches sequencing conducted on tumor samples of subjects, explaining that “multiple tissue samples were obtained from primary ovarian tumor and metastatic sites where adequate tumor volume permitted.” Ex. 51 at S6; Ex. 52. Bashashati further discloses that “[e]ach sampling is cut into three pieces, yielding two end-pieces for cryovials and a middle portion placed in 10% buffered formalin.” Ex. 51 at S5; Ex. 52. Bashashati also discloses that “[g]enomic DNA was extracted from frozen samples,” that is, from the cryovials, and that “[e]xome capture was achieved through solution hybrid selection with the Human All Exon kit SureSelect Target Enrichment System (Agilent) version 1 for Illumina Genome Analyzer paired-

end sequencing (Gnirke et al., 2009) and libraries were prepared as described in (Morin et al., 2011).” Ex. 51 at S6; Ex. 52. Given that Bashashati is using the “Human *All* Exon kit,” one would understand that Bashashati is teaching whole exome sequencing.

185. Bashashati further teaches identifying a plurality of tumor specific SNVs from the whole exome sequencing data. Specifically, Bashashati teaches that in “[e]xome alignment and mutation calling,” “[s]equence reads were aligned to the human reference sequence (NCBI build 36, hg18) and a compacted reference composed of the targeted exons.” Ex. 51 at S6; Ex. 52 (“Exome sequence data generation” and “Exome sequence alignment and mutation calling”). Then, “[u]sing the resulting tumour and normal pair of BAM files, somatic SNVs were called by JointSNVMix algorithm (Roth et al., 2012), sorted by marginal posterior probability and further run through the mutationSeq algorithm (Ding et al., 2012) to find high confidence candidate SNVs Ex. 51 at S6; Ex. 52.

186. Bashashati further teaches that these high confidence candidate SNVs were then validated using deep sequencing. Ex. 51 at S6; Ex. 52 (“Validation of mutations...Candidate SNVs and indels were subjected to deep sequencing using Illumina and Ion Torrent platforms (details below).”).

- d. **Claim 1[b]: performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume**

187. It is my opinion that Bashashati discloses to a POSA the method of using targeted multiplex amplification to amplify 10 to 500 target loci encompassing different tumor-specific SNV mutations from cell-free DNA isolated from a plasma sample of the subject or DNA derived

therefrom to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume.

188. Bashashati, for instance, teaches the amplification of 61 tumor-specific SNVs in Case 1a in an omnibus table of validated somatic SNVs and indels that was submitted with the supplementary material. *See* Ex. 52 (“Omnibus table of validated somatic SNVs and indels”). Columns T and U of this spreadsheet include 61 entries for Case1a showing counts of mutant SNV alleles and non-mutant, wild-type sequences. The table reports validated somatic SNVs, with column A stating the case identity, columns B and C stating the SNP location (chromosome and chromosome position), column E stating reference allele nucleotide (wild-type), column F stating variant allele nucleotide (mutant), column T (RefCountPlasma) listing the number of resequencing reads from the ctDNA matching the reference allele, and column U (AltCountPlasma) listing the number of resequencing reads from the ctDNA matching the variant allele. Ex. 52; Ex. 54.

189. Bashashati also teaches amplification of target loci from cell-free DNA isolated from a plasma sample. Bashashati first states that “[c]irculating tumour DNA was extracted from 1-2 ml of plasma.” Ex. 51 at S7. Bashashati then teaches amplifying circulating tumor DNA, by the method described in the Supplementary Appendix in the section titled: “Deep amplicon sequencing using the Illumina MiSeq platform for cell-free DNA in the plasma,” which refers back to the section titled “Deep amplicon sequencing using the Illumina GAII platform.” Ex. 51 at S6-7, S7 (“The primers used and the PCR cycling conditions were the same as in the previous section.”).

190. These sections teach the use of targeted PCR to amplify target loci encompassing different SNVs, disclosing that “[p]rimer pairs were designed to place the variant position within 75bps of either end of the amplicon and to be between 50-300bp in length” and that they “were

independently validated by in silico [sic, *silico*] PCR followed by BLAT against the human genome to ensure that the correct target was generated and that the resulting amplicon was unique within the genome.” Ex. 51 at 6.

191. As to length, a POSA would have understood that amplicons can have a length of 50-150 bases from the disclosure of amplicons having a length of 50-300 bases. Ex. 51 at 6 (“Primer pairs were designed to place the variant position within 75bps of either end of the amplicon and to be between 50-300bp in length.”). In teaching primer pairs with the variant position within 75 base pairs of either end of the amplicon, a POSA would understand that the primer pairs could be designed to place the variant position with 75 bps of not only one but both ends of the amplicon.

192. Further, a POSA would understand from the teaching to place the variant position within 75 base pairs of the ends of the amplicon, that it is likely preferable to have the variant position within 75 base pairs of both ends. It would follow, also, that a POSA would understand an amplicon with the variant position within 75 bps of both ends would be no longer than 149 base pairs in length, as there would be no more than 74 base pairs from the variant position to each end.

193. In any event, to the extent Bashashati is deemed to not disclose using an amplicon length of 50-150 base pairs, this would have been obvious, particularly in the context of cell-free DNA. The ’454 patent nowhere suggests that an amplicon length of 50-150 base pairs is special, unique, or inventive. Just the opposite, the ’454 patent broadly refers to a range of different amplicon lengths different from the claimed 50-150 base pair range and never calls out the 50-150 range as special:

In some embodiments, the amplicons are similar in size. In some embodiments, the range of the length of the target amplicons is less than 100, 75, 50, 25, 15, 10, or 5 nucleotides. In some embodiments (such as the amplification of target loci in

fragmented DNA or RNA), the length of the target amplicons is between 50 and 100 nucleotides, such as between 60 and 80 nucleotides, or 60 and 75 nucleotides, inclusive. In some embodiments (such as the amplification of multiple target loci throughout an exon or gene), the length of the target amplicons is between 100 and 500 nucleotides, such as between 150 and 450 nucleotides, 200 and 400 nucleotides, 200 and 300 nucleotides, or 300 and 400 nucleotides, inclusive.

* * *

In some embodiments, the length of the target amplicons is between 50 and 100 nucleotides, such as between 60 and 80 nucleotides, or 60 to 75 nucleotides, inclusive. In some embodiments, the range of the length of the target amplicons is less than 50, 25, 15, 10, or 5 nucleotides. In some embodiments, the range of the length of the target amplicons is between 5 to 50 nucleotides, such as 5 to 25 nucleotides, 5 to 15 nucleotides, or 5 to 10 nucleotides, inclusive.

D.I. 1-1 at 105:21-33, 111:32-40; *see also id.* at 97:36-40 (“In certain embodiments that relate most preferably to cfDNA from samples of individuals suspected of having cancer, the cfDNA is amplified using primers that yield a maximum amplicon length of 85, 80, 75 or 70 bp, and in certain preferred embodiments 75 bp....”).

194. Beyond this, it was known in the 2015 time frame that the sizes of circulating tumor DNA followed a certain size distribution. For instance, the '454 patent states that “cfDNA (such as fetal cfDNA in maternal serum or necroptically- or apoptotically-released cancer cfDNA) is highly fragmented. For fetal cfDNA, the fragment sizes are distributed in approximately a Gaussian fashion with a mean of 160 bp, a standard deviation of 15 bp, a minimum size of about 100 bp, and a maximum size of about 220 bp.” D.I. 1-1 at 97:20-24. These size distributions are applicable to tumor circulating DNA as well. Forsheew (2012), for instance, teaches that circulating tumor DNA has an average length of 140 to 170 base pairs. D.I. 13-7 at 1. As such, it would be natural to utilize an amplicon length of 50-150 base pairs, as this would match the average size of the circulating tumor DNA without being too long so as to miss mutations. Consistent with this, as I note above, Forsheew (2012) utilized an amplicon length of **120** base pairs when amplifying

cell-free DNA. *See id.* at 9. Far from being inventive or novel, an amplicon length of 50-150 base pairs would have been obvious and had, in fact, already been disclosed in the prior art.

195. Although the targeted amplification of SNVs from ctDNA was conducted using single primer pairs per reaction, Bashashati also teaches a POSA that SNVs can be amplified in multiplex reactions in disclosing that candidate SNVs were validated on an Ion Torrent PGM platform.

Validations of candidate single nucleotide variants (SNVs) identified from the initial sequencing of the primary ovarian cancer samples were achieved using a deep amplicon sequencing approach. Candidate SNVs were fed into the LIFE Technology AmpliSeq custom designer pipeline, after conversion to hg19 coordinates, and three multiplex primers pools were generated.

Ex. 51 at S7-S8. A POSA would have understood from this disclosure of multiplex amplification of candidate SNVs that identified target loci could be amplified in multiplex reactions in the same reaction volume. A POSA also would have been motivated to do so for the benefit of requiring fewer separate reactions.

196. Further, Bashashati cites Forsheo (2012) in support of the statement that “sequencing of cell-free circulating tumour DNA (ctDNA) extracted from plasma has been demonstrated to be an effective non-invasive tool for monitoring tumour burden [32, 33],” where endnote 32 is Forsheo (2012). Ex. 51 at 22, 33. A POSA would have thus also been informed by the reference to Forsheo (2012), and its teachings, to use multiplex amplification of target loci.

197. The ability to use greater multiplex targeted amplification would also have been obvious to a POSA prior to the priority date for the claim because even significantly greater multiplex amplification of DNA was already well established. As discussed above, in regard to Forsheo (2012), I have been informed that Natera and Dr. Metzker have relied on Fluidigm teaching a multiplex PCR amplification reaction using 9,216 primer pairs, to amplify 9,216 target

sequences, in a Patent Trial and Appeal Board proceeding seeking to have another party's patent claims canceled. Ex. 12 ¶ 98.

198. In particular, I understand Dr. Metzker relied on Fluidigm as teaching preamplification using thousands of target-specific primer pairs in the same reaction volume, *i.e.*, a highly multiplex reaction:

To increase target nucleic acid concentration prior to encoding, an optional pre-preamplification reaction be carried out before the encoding preamplification reaction. The pre-amplification reaction can be carried out in multiplex [sic: multiplex]. For example, target-specific primers for 9216 different target nucleic acids can be employed in one mixture.

Ex. 12 ¶ 98 (quoting Ex. 13 ¶ 119).

199. In the present case, Dr. Metzker was asked on cross-examination about the earlier testimony on Fluidigm about the use of highly multiplex PCR:

Q. Okay. So in the PCR – in the Fluidigm reference that you opined upon, that is teaching a single-tube multiplex PCR of over 9,000 targets for sequencing; correct?

A. It does have a preamplification step that does target at multiplex PCR.

Q. Of over 9,000 targets; correct?

A. That's what they report.

Ex. 6 at 87:24-88:6.

- e. **Claim 1[c]: sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads, wherein the sequencing has a depth of read of at least 50,000 per target locus.**

200. It is my opinion that Bashashati teaches a POSA sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the

cell-free DNA from the sequence reads, wherein the sequencing has a depth of read of at least 50,000 per target locus.

201. Bashashati, for instance, teaches sequencing amplicons on an Illumina GAII. Ex. 51 at S6-7, 7 (“Somatic mutation predictions were subjected to rigorous validation by targeted deep amplicon sequencing in the tumour and normal DNA templates.”).

202. Bashashati then further teaches sequencing amplicons generated from cell-free DNA in the plasma on an Illumina MiSeq platform, using the same primers and PCR cycling conditions as used to generate amplicons for sequencing on the Illumina GAII in section titled “Deep amplicon sequencing using the Illumini MiSeq platform for cell-free DNA in the plasma.” Ex. 51 at S7 (“Circulating tumour DNA was extracted from 1-2 ml of plasma....The primers used and the PCR cycling conditions were the same as in the previous section.”).

203. Bashashati also teaches detecting tumor-specific SNV mutations:

Mutational events in the ancestral clone are detectable in cell-free circulating tumour DNA from plasma

Plasma samples collected pre-operatively and pre-anaesthesia were available for four cases from which ctDNA was extracted and subjected to targeted deep-sequencing of validated somatic mutations (see supplementary material, Appendix). We assayed 72 mutations in case 1, 49 in case 2, 63 in case 4 and 131 in case 5. Across the four cases, 215/312 (69%) mutations contained sequencing coverage and 38/215 (18%) were detectable with mutation allele frequency above statistical background noise (binomial exact test, false discovery rate < 0.05) (see supplementary material, Table S2).

Ex. 51 at 25, Ex. 52.

204. The specific sequencing data showing the number of reads from the mutations appear in supplementary material Table S2, column U. Ex. 52 (“Table S2. Omnibus table of validated somatic SNVs and indels.”). There, Bashashati discloses non-zero “AltCountPlasma,”

which is the count of sequencing reads from the ctDNA matching the variant allele. Ex. 52 (“Table S2. Omnibus table of validated somatic SNVs and indels.”); Ex. 54.

205. For example, for Case 1a, Table S2 includes positive values for resequencing reads from the ctDNA matching variant alleles with single-nucleotide variations, that is, tumor-specific SNVs, including, for Column A, lines 48, 54, 82, 91, 98, 111, 121, 124, 129, 130, 144, 151-153, 156, 170, and 173. Ex. 52; Ex. 54. A POSA would understand that these positive resequencing read values for tumor-specific SNVs indicate that Bashashati detected tumor-specific SNV mutations present in the cell-free DNA from the sequence reads.

206. As to sequencing “depth,” as stated in the ’454 patent, and as well-known to a POSA, the depth of read is a measure of how many times a particular sequence is read. D.I. 1-1 at 100:9-15 (“Depth of read can be expressed in variety of different ways,...for example in a highly parallel DNA sequencer such as an Illumina HISEQ, which, e.g., produces a sequence of 1 million clones, the sequencing of one locus 3,000 times results in a depth of read of 3,000 reads at that locus.”).

207. Bashashati teaches that the sequencing has a depth of read of at least 50,000 in disclosing actual examples with the sequence depth for different SNVs, often in excess of 50,000, with a maximum depth of 969,361. Ex. 52 (column T (RefCountPlasma) listing the number of resequencing reads from the ctDNA matching the reference allele, and column U (AltCountPlasma) listing the number of resequencing reads from the ctDNA matching the variant allele); Ex. 54.

208. Further to the disclosure of many examples with read depths greater than 50,000, a POSA would understand the benefit to sequence with higher depths of read for greater sensitivity in detecting rare SNPs. As discussed above, Forshew (2012), which is cited by Bashashati for

demonstrating that “sequencing of cell-free circulating tumour DNA” is “an effective non-invasive tool for monitoring tumour burden” (Ex. 51 at 22 n.32) teaches using “[h]igher read depth...[to] allow *for enhanced mutation detection* without change to protocols” (D.I. 13-7 at 10 (emphasis added)). A POSA would have understood that increasing sequencing depth increases sensitivity in detecting SNVs and it would be routine to increase sequence read depth to obtain greater sensitivity as required. For example, U.S. Patent Application US 2010/0261189 A1 (Ex. 46) (“Bentley”), which published October 14, 2010, expressly teaches that the greater the number of reads, the lower the minimum frequency of SNPs in a population that is detectable. Ex. 46 ¶ 85. Bentley also teaches this to a POSA in showing the relationship between “Number of Reads” and the “Minimum frequency of SNP in population detectable with 95% confidence...[and]...99% confidence.”

TABLE 2

SNP Classes	Number of Reads	Minimum frequency of SNP in population detectable with 95% confidence	Minimum frequency of SNP in population detectable with 99% confidence
1	200000	0.002%	0.003%
2	100000	0.005%	0.007%
5	40000	0.014%	0.018%
10	20000	0.028%	0.037%
50	4000	0.14%	0.18%
100	2000	0.28%	0.37%
200	1000	0.55%	0.74%
500	400	1.39%	1.85%
1000	200	2.76%	3.64%

Ex. 46 ¶ 88 (Table 2). Bentley's disclosure of potential sequencing depths and sensitivity is also consistent with the understanding of a skilled artisan that sequencing depth is a parameter that would be routinely adjusted to achieve whatever sensitivity was required.

209. To the extent Natera contends that Bashashati does not disclose a read depth of at least 50,000, this would have been obvious to a POSA. In this regard, I note that the '454 patent does not identify any particular sequencing depth as being inventive or special. Just the opposite, as far as I can tell, the '454 patent only refers to a depth of 50,000 once, and then only in a long list of possible sequencing depths that spans four orders of magnitude. Specifically, the patent states that "[i]n certain embodiments, a depth of read of greater than 100, 250, 500, 1,000, 2000, 2500, 5000, 10,000, 20,000, 25,000, 50,000, or 100,000 on the low end of the range and 2000, 2500, 5,000, 7,500, 10,000, 25,000, 50,000, 100,000, 250,000 or 500,000 reads on the high end, is attained in the sequencing run for each single nucleotide variant position in the set of single nucleotide variant positions." D.I. 1-1 at 68:33-39. This broad disclosure of potential sequencing depths is consistent with the understanding of a skilled artisan that sequencing depth is a parameter that would be routinely adjusted to achieve whatever sensitivity was required.

210. Natera has taken that very position on many occasions. For example, in a case related to organ transplant, Natera argued that "[i]t was well-known by 2009 that the number of molecules analyzed is one component of sensitivity, and that higher sensitivity could be achieved by sequencing more molecules" and "that by sequencing more molecules, sensitivity will necessarily increase." Ex. 10 (Case No. 19-567 (CFC)(CJB), D.I. 101, Natera's Opening Brief In Support Of Summary Judgement (D. Del.)) at 26-27.

211. Another of Natera's preferred experts, Dr. Quackenbush, testified that sequencing to even greater depth when required by an experiment also would have been routine, as it would

require only running additional sequencing assays: “it is very easy to increase the coverage or sequence depth, if you later decide you need more data. Provided you still have your original sample, you can just sequence more, and combine the sequencing output from different flow cells.”

Ex. 7 ¶ 65.

212. In a case involving Natera and CareDx, Dr. Quackenbush submitted a declaration that Natera relied upon stating that “POSAs prior to November 6, 2009 were well aware that sequencing more molecules, or increasing sequencing depth, improves sensitivity” and that “the detection limit is determined by sequencing error rate and sequencing read depth (i.e., how many molecules are sequenced), reflecting the fact that there was a well-known correlation between the number of molecules sequenced and sensitivity.” Ex. 20 ¶ 119 (citing Voelkerding, et al., “*Next-generation Sequencing: From Basic Research to Diagnostics*”, Clinical Chemistry, vol. 55, No. 4, Apr. 1, 2009, 641-658. Thomas, Sensitive Mutation Detection in Heterogeneous Cancer Specimens by Massively Parallel Picoliter Reactor Sequencing, Nature Medicine, Vol. 12, No. 7, pp. 852–855 (July 2006)).

213. Dr. Quackenbush further testified in the same case, during the hearing on summary judgment regarding § 101, that to get higher sensitivity, you simply sequence more molecules, and that this was well-known:

Q. Does the written description state anything about how a person of skill in the art can increase the sensitivity of high-throughput sequencing?

A. So *they do provide a description of how to increase sensitivity*, which is simply to obtain more sequence, right, which is well-known with any kind of measurement. *If you want to make a better measurement, you measure more.*

Q. Can you point to an example of that in the written description?

A. Sure. It’s right here. It’s called out. It just says, *higher sensitivity can be achieved simply by sequencing more molecules*. That means use more channels. And when they refer to channels, the Illumina sequencer had

something that looked like a microscope slide added onto individual channels. Simply showed you load one, you load two, you load three, you load four.

Q. And is there anything nonconventional about achieving higher sensitivity by sequencing more molecules?

A. No. I mean, this is standard practice in many fields. To make a better measurement, you make more measurements. ***To get higher sensitivity for detecting molecules, you simply sequence more molecules.***

Ex. 11 (Case No. 19-567 (CFC)(CJB), Motion for Summary Judgement Hearing) at 98:10-99:6 (emphasis added).

214. In a proceeding to cancel another party's patent claims, Dr. Metzker has also testified that it would have been obvious to increase sequence coverage, i.e., reading depth, to improve the accuracy of next-generation sequencing platforms. Ex. 12 ¶¶ 211-215. Dr. Metzker relies on Harismendy for teaching that:

At high sequence coverage all NGS platforms have excellent variant calling accuracy (>95%) as assessed by the detection of known SNP variants.

Ex. 12 ¶ 213 (citing Harismendy).

215. In sum, increasing the depth of read for increased sensitivity would have been obvious and been driven by both the need for higher sensitivity for testing and the lower costs with improving technology.

f. Claim 8: The method of claim 1, wherein the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation.

216. It is my opinion that Bashashati discloses to a POSA the method claim 1, wherein the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation.

217. As discussed above with respect to claim 1, which requires targeted multiplex amplification of 10 to 500 target loci, Bashashati teaches the amplification of 61 tumor-specific

SNVs in Case 1a. *See* Ex. 52, cols. T, U (61 entries indicating counts of mutant SNV alleles and non-mutant, wild-type sequences). Bashashati also teaches multiplex amplification in its sequence validation using multiplex primer pools (Ex. 51 at 7-8). Further, as discussed above with respect to claim 1, 20- to 50-multiplex targeted amplification of loci encompassing tumor-specific SNVs would have been obvious because it was taught by Forsheo (2012) (cited by Bashashati), and because highly multiplex targeted amplification was known, a fact that both Natera and Dr. Metzker have relied on in other proceedings, and that Dr. Metzker confirms is reported in the prior art in this proceeding. Ex. 51 at 22, 33; Ex. 12 ¶ 98; Ex. 12 ¶ 98 (quoting Ex. 13 ¶ 119); Ex. 6 at 87:24-88:6.

g. Claim 11: The method of claim 1, wherein the method further comprises performing barcoding PCR prior to the sequencing.

218. It is my opinion that Bashashati discloses to a POSA the method of claim 1 further comprising performing barcoding PCR prior to the sequencing.

219. Bashashati teaches, for instance, that products were “PCR-amplified using Phusion DNA polymerase...using PE primer 1.0 (Illumina) and a custom multiplexing PCR Primer...with unique fault tolerant hexamer barcodes for each template.” Ex. 51 at 6-7.

220. Bashashati also teaches that after barcoding PCR, the PCR products were purified and prepared for sequencing, and then sequenced. Ex. 51 at 7 (“PCR products of the desired size range were purified...DNA quality was assessed...and quantified,...and libraries were pooled and diluted...600ul of 11pM pooled library was sequenced on an upgraded Illumina MiSeq”).

B. The '035 Patent is Invalid as Obvious

1. Overview of the Prior Art

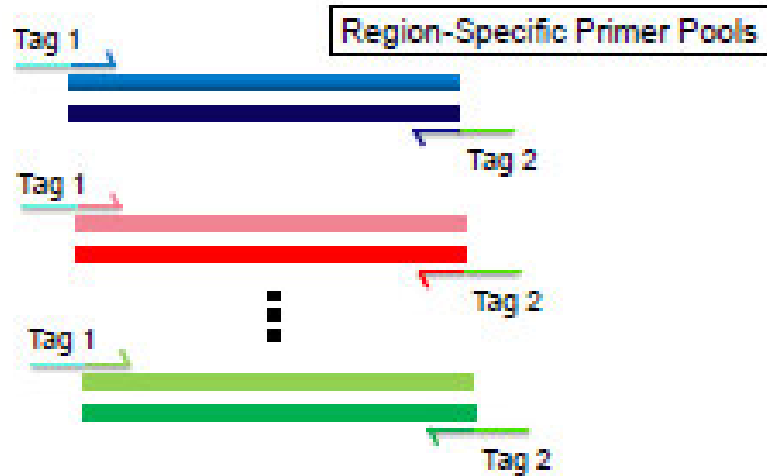
a. Kaper

221. *Parallel Preparation of Targeted Resequencing Libraries From 480 Genomic Regions Using Multiplex PCR on the Access Array™ System*, by Fiona Kaper, et al., is a poster teaching a prior art technique for tagging by PCR and sequencing using high throughput sequencing. This poster was presented at the American Association for Cancer Research (AACR) 101st Annual Meeting in 2010, and so is prior art. Ex. 14 (Abstract associated with AACR poster).

222. Kaper teaches the use of an “Access Array™” system to scale up PCR capabilities. This array system combines 48 sample inputs with 48 primer sets, permitting all 2304 possible combinations of samples and primers. This system is used in combination with primers designed for tagging and preparation for sequencing. Ex. 15. I understand that this Access Array platform was the platform used in Forsheo (2012), which Natera relies upon for its infringement case as evidence of how RaDaR operates. Therefore, as with the '454 patent, to the extent Natera contends that RaDaR infringes, the '035 patent is invalid. Below, I provide a brief summary of the design and operation of the Kaper reference.

223. To prepare targets for sequencing, Kaper uses a two-step tagging process that is depicted in Figure 2, reproduced below:

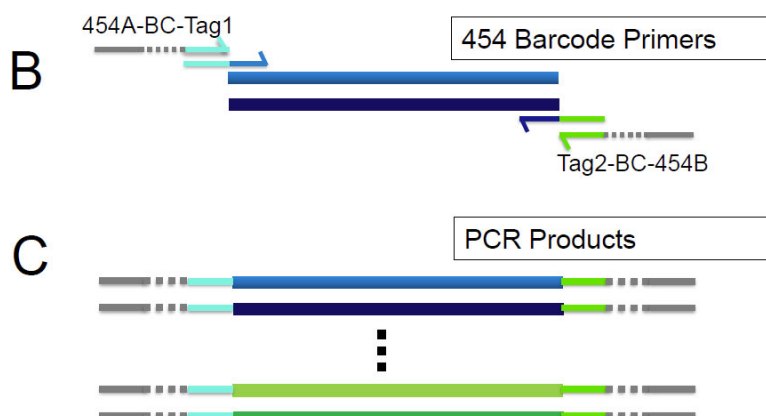
A



Id. at Fig. 2A. In this process, primers that are specific to loci of interest are used. These region-specific primers contain universal sequence tags, which are sequences of DNA that do not encode genes and are not complementary to the target DNA. This first amplification results in copies that are tagged with these universal sequence tags. Ex. 15.

224. The second amplification step uses primers that are specific to the universal sequence tags. These primers also include a barcode, which permits identification of the sample, and a sequencing tag, which permits sequencing of the sample in a high-throughput sequencing system. The barcode and sequencing tags are shown below as “BC” and “454A”/“454B,” respectively:

96-well PCR plate



Ex. 15 Figs. 2B-C. Kaper specifically uses the Roche 454 system for sequencing. Ex. 15. This is why the sequencing tags are denoted as “454A” and “454B.”

225. After PCR, the products can be sequenced. Kaper teaches “high quality sequence data” is obtained from the PCR products made by this technique. *Id.* at Fig. 3.

226. Kaper further teaches the amplification of 480 cancer gene exons. The amplification is multiplex, where 48 primer pools of 10 pairs each are used for the reactions. These PCR products are then sequenced. *Id.* at Fig. 2.

b. Prior Art Teaching ARM-PCR

227. Amplicon Rescue Multiplex Polymerase Chain Reaction (“ARM-PCR”) is a prior art technique to conduct multiplex amplification and analysis of several loci. I rely on two pieces of prior art in particular for my analysis.

228. The first is U.S. Patent Pub. No 2009/025183 A1 (“Han”). Han teaches ARM-PCR, which is demonstrated by Figure 1, reproduced below. In this method, two steps of PCR are used. The first of these steps is a target-specific amplification. In this step, nested primers are used that are specific to target loci. These primers may also contain a common primer sequence,

which is a universal sequence that does not hybridize to any part of the target loci. Once 10-15 cycles of this amplification are completed, the reaction is terminated. Ex. 16 ¶ 11.

229. After termination, the amplicons are “rescued,” or collected from the reaction mixture. Specifically, the amplicons, now tagged with a common primer sequence, are subjected to a second round of PCR (denoted as “PCR2” below). This round uses the common sequence as the primer, and permits amplification of all tagged amplicons.

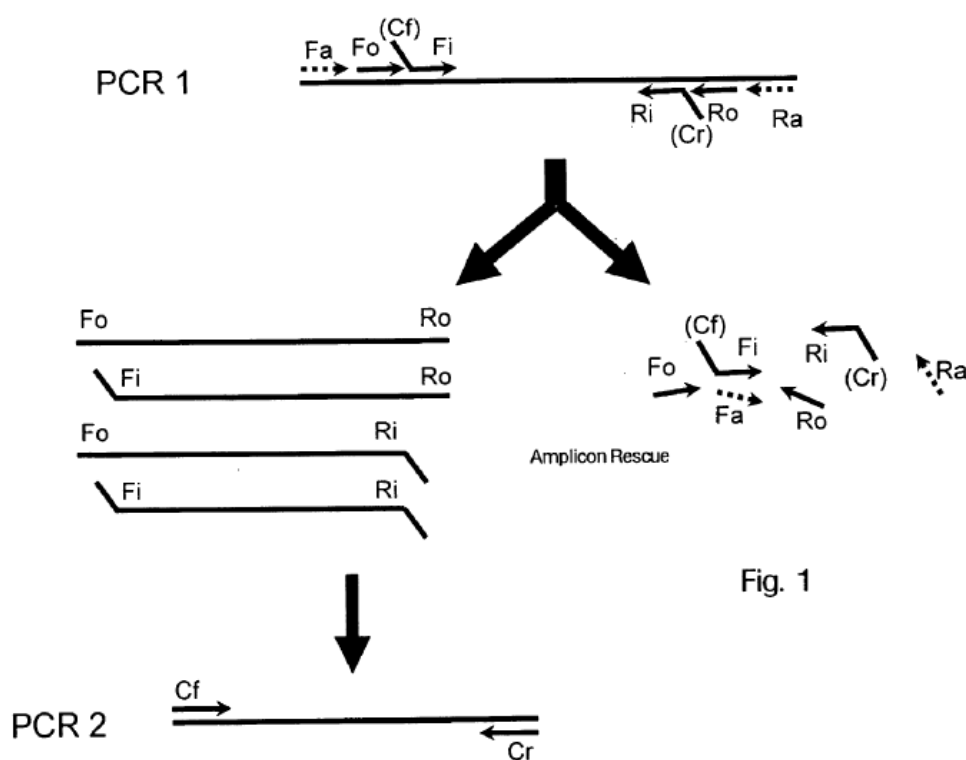


Fig. 1

Id. at Figure 1.

230. The second piece of prior art I rely on is Wang et al., *High Throughput Sequencing Reveals A Complex Pattern of Dynamic Interrelationships Among Human T Cell Subsets*, PNAS

(January 26, 2010) (“Wang”). This is a paper that teaches the use of ARM-PCR in the context of high throughput sequencing.

231. Wang studied the relationship between different lineages of human T cells by designing primers that were specific to sequences that coded for proteins expressed on the surface of those cells. Those primers were used in an ARM-PCR reaction.

232. After amplification, those primers were subject to high throughput sequencing on a Roche 454 system, which permitted analysis of the sequences to determine the lineage and relationships between different lineages of the studied cells.

233. Importantly, Wang established that ARM-PCR could be used to prepare a sample for high throughput sequencing.

2. The Asserted Claims Of The '035 Patent Are Rendered Obvious By Kaper In View Of The Skill and Knowledge Of A POSA

a. Claim 1[preamble]: A Method For Amplifying and Sequencing DNA, comprising:

234. It is my understanding that a claim preamble is only a limitation in specific circumstances, and I have been informed by counsel that this preamble does not recite a limitation. To the extent that Natera contends or the Court finds that the preamble does recite a limitation, however, it is my opinion that Kaper discloses it.

235. First, as I describe above, Kaper teaches a method for amplifying DNA. Specifically, Kaper discloses a two-step amplification method wherein the first step adds a universal tail sequence, and the second step adds a barcode and sequencing primer. Ex. 15.

236. Second, Kaper describes a method for sequencing DNA. Kaper teaches that PCR products are “sequencer-ready,” and that the added sequencing primer is used to sequence on a Roche 454 sequencer. Ex. 15.

- b. **Claim 1[a]: 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;**

237. It is my opinion that Kaper renders this claim limitation obvious.

238. First, Kaper teaches “tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products.” As depicted in Figure 2A of Kaper, the first step of amplification uses locus-specific primers “tagged at the 5’ end with universal sequence tags.” Ex. 15. This is tagging with one or more universal tail adaptors.



Ex. 15 at Figure 2A. As I explain above in detail in connection with non-infringement, a skilled artisan would view this as a single process that generates tagged products. Yet, to the extent Dr. Metzker’s infringement theory that he stated for the first time during his deposition is accepted, then the “tagging” aspect of the claims is taught in Kaper.

239. Second, it is my opinion that the application of Kaper’s technique to isolated cell-free DNA, and specifically isolated cell-free DNA that is not from a pregnant woman, would have been obvious in view of the skill of the POSA.

240. As an initial matter, Kaper teaches application of its method to cancer gene exons. As Kaper explains, to “maximally utilize the capacity of an 48.48 Access Array IFC, we carried out multiplexed amplification of a set of commonly mutated cancer gene exons across 48 genomic DNA samples.” *Id.* at Fig. 4. Given that Kaper teaches applications of its technique to cancer, it would have been natural and obvious to apply the method to cell-free DNA.

241. Indeed, long before the 2010 conference where Kaper disclosed this method, the use of cell-free DNA in cancer monitoring and detection was well-known. For example, in a PCT application that was published in 2005, Cantor et al. described the detection of “circulating tumor-specific nucleic acids in a blood, urine, or stool sample....” Ex. 17 at Abstract, ¶ 35.

242. In 2008, Ehrich et al. described a “method for the detection of specific polymorphic alleles in a mixed DNA population.” Ex. 18 at Abstract. Ehrich taught the use of cell-free DNA in the cancer context particularly, as it taught that a DNA sample may be “from an individual suspected of suffering from a disease,” and that in a related embodiment “the disease is cancer.” *Id.* at 4:24-29.

243. Lo et al. in 2009 also published a patent application, WO 2009/013492 A1, wherein a “sequencing technique on *plasma cell-free DNA* may be used to detect the chromosomal aberrations in the plasma DNA *for the detection of a specific cancer.*” Ex. 19 ¶ 248.

244. Thus, by Natera’s claimed priority date of May 18, 2011, a skilled artisan would have been well-aware of cell-free DNA and its use particularly in the cancer detection context, not only in the fetal cell-free DNA context. This is further confirmed by Natera’s own admissions and admissions by its experts in prior cases. For example, in support of *inter partes* review of Patent No. 9,493,831, Dr. Metzker opined that “creating a cell-free DNA library from maternal for aneuploidy detection [*sic*] was well known in the art,” and that the cell-free DNA was discovered in the fetal context as early as 1997. Ex. 12 ¶¶ 49-57.

245. As yet another example, Natera has called techniques to study cfDNA “routinely used before November 2009,” including techniques to detect “cell-free nucleic acids from analogous natural phenomena such as...tumor cfDNA in a cancer patient’s blood.” Ex. 10 at 3.

246. Dr. Quackenbush, an expert Natera often relies upon, has also noted that “scientists have long used cell-free nucleic acids to monitor disease.” Ex. 20 at ¶¶ 57-58. Dr. Quackenbush describes the presence of “cell-free nucleic acids with a different genotype than the patient’s normal genotype” as a “natural result” of biological processes that occur in a cancer patient’s body. *Id.* Indeed, Dr. Quackenbush described patent claims directed to detecting the presence of cell-free DNA as “directed to a natural law,” and thus unpatentable. *Id.* at ¶ 59-66. When testifying in that same case in support of summary judgement of invalidity, Dr. Quackenbush stated that as of November 2009, quantifying cell-free using SNPs is “completely conventional.” Ex. 11 at 108:8-20.

247. In an even more recent case, Dr. Quackenbush opined that by 2011, it was understood in the art that nucleic acid analysis could be applied to circulating tumor DNA, a type of cell free DNA specific to cancer cells circulating in a patient. Ex. 7 ¶¶ 84-87.

248. Because a POSA would have had familiarity with cell-free DNA, and specifically in the context of cancer detection and monitoring, it would have been obvious to apply Kaper’s tagging and sequencing and techniques to isolated cell-free DNA from a subject who is not a pregnant woman. Thus, to the extent that Natera contends that Kaper does not teach such analysis, the application to cell free DNA would have been an obvious variation yielding predictable results.

- c. **Claim 1[b]: 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**

249. It is my opinion that Kaper, in view of the skill and knowledge of a POSA, renders this claim limitation obvious.

250. As an initial matter, I have had the opportunity to review Dr. Metzker's deposition testimony clarifying his report as to how this claim should be interpreted. Ex. 6 at 109:8-23. According to Dr. Metzker, the first element of the claim, 1[a], is limited only to the first cycle of amplification, wherein a copy of the template cell-free DNA is generated with the tags from the primer. *Id.* at 110:23-111:6. This step, 1[b], can begin as early as a second cycle of amplification. That is, once cell-free DNA is copied once and tagged, a subsequent amplification of the tagged product with the same primer can satisfy this claim limitation. *Id.* at 109:8-23. Without opining on whether or not I agree with this interpretation, I have adopted it for the purposes of my invalidity analysis. It is my understanding that claim construction has not yet occurred in this case, and my opinions may change if the Court determines that the claim terms mean something other than what Dr. Metzker opined in his deposition.

251. First, Kaper discloses "amplifying the tagged products one or more times to generate final amplification products." As I discuss above, the references teach a two-step amplification process that generates a final amplification product of barcoded and tagged nucleic acids ready for sequencing. Ex. 15.

252. Second, Kaper obviates the limitation "wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume" in view of the skill and knowledge of a POSA.

253. SNPs are single-nucleotide variations in DNA, e.g. a variation in a gene where only one base in the gene is different. In the cancer context, SNPs are useful as they can differentiate cancer DNA, which has a mutation, from patient DNA, which is referred to as "wild-type" or lacks any cancerous mutation.

254. SNPs were well-known in the art long before the alleged priority date of the '035 patent. For example, the Ehrich reference from 2008 discloses the detection of fetal DNA using SNPs in circulating in cell-free DNA. Ex. 18 at 15:25-33.

255. As early as 2005, SNPs were understood as useful tools in the detection of cancer. The Cantor reference teaches that an approach to analyzing SNPs in fetal DNA could be “applied to other diagnostic applications of plasma DNA, such as *the detection of tumor-derived point mutations in cancer patients.*” Ex. 17 ¶ 90. These “tumor-derived point mutations” would have been understood to be SNPs, and thus a POSA would have been aware that the use of targeted amplification and detection of SNPs would be useful in the cancer context.

256. As yet another example, Natera’s own expert has admitted that such would have been routine and conventional. In testifying in support of summary judgement on behalf of Natera in the *CareDx* case, Dr. Quackenbush stated:

Q. What is your opinion regarding whether a person of skill in the art as of November 2009 would understand *quantifying donor cell-free DNA samples using SNPs to be conventional?*

A. So this type of analysis is *completely conventional* and it’s described in the written description as being conventional. So they tell us that this quantification involves a series of steps using methods that are known in the art and they simply saying [*sic*] steps for quantifying nucleic acids are known in the art. You know, having a number of molecules that come from the donor is a pretty simple approach.

Ex. 11 at 108:8-20 (emphasis added). This is further demonstrated in Dr. Quackenbush’s declaration in that case, wherein he states that laboratory techniques involving SNP association studies were “well understood, routine, and conventional by November 2009.” Ex. 20 ¶ 71.

257. Given such knowledge, and the two-step amplification process taught in Kaper, a POSA would have found it obvious to use Kaper’s amplification on a plurality of SNP loci associated with cancer. Kaper teaches the amplification of 480 cancer gene exons, wherein 48

primer pools of 10 pairs each were used to carry out multiplex PCR. Ex. 15. To take that exact technique and apply it to a plurality of SNP loci associated with cancer would be nothing more than an obvious arrangement of prior art elements.

258. Further, a skilled artisan would have understood that the first step, *e.g.* using region-specific primers, would have undergone several amplification cycles in a thermal cycler. Under Dr. Metzker's reading of the claims, summarized above, at least a second cycle of amplification with those primers would satisfy this limitation.

259. Third, Kaper teaches "wherein one of the amplifying steps introduces a barcode and one or more sequencing tags." The second, universal amplification step, depicted in Figure 2B of Kaper below, is such a step because it adds a barcode (BC) and sequencing tag (454A/B).



Id. at Figure 2B.

- d. **Claim 1[c]: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**

260. It is my opinion that Kaper renders this claim obvious in view of the skill and knowledge of a POSA.

261. First, Kaper teaches "conducting massively parallel sequencing on the final amplification products." Specifically, the final, barcoded products are sequenced on the Roche 454 system, a next-generation sequencing platform. Next generation sequencing is one method of massively parallel sequencing.

262. Second, Kaper teaches “wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.” I describe above why the application of Kaper’s technique to SNP loci associated with cancer would have been obvious to a POSA. Kaper also discloses at least 10-plex amplification in a single reaction volume. Increasing the degree of multiplex, however, would be an obvious variation of any multiplex technique.

263. The ’035 patent acknowledges that higher degrees of multiplex were commonplace in the art at the alleged time of invention. The patent states that “the general belief in the art is that multiplexing PCR for sequencing is limited to about 100 assays in the same well.” D.I. 1-2 at 48:25-27. Thus, a skilled artisan would have at the alleged time of invention would have found it obvious to perform multiplex amplification with at least around 100 assays.

264. This is consistent with what practitioners in the art were doing around 2010. Above, in my discussion of the background, I detail several additional references establishing how well-known it was to perform large scale multiplex PCR. *See supra* Part V.B. For example, Varley and Mitra published on nested patch PCR, a method wherein they were able to amplify 90 exons simultaneously. D.I. 52-5 at 1847.

265. However, much greater degrees of multiplex were available in the art. As yet another example of a high degree of multiplex, Fluidigm, in Patent Pub. No. 2010/0120038 A1, disclosed that the “the product of the total number of samples assayed in a single assay×T is at least a value selected from the group consisting of 2304, 3600, 4608, and 9216.” Ex. 13 ¶ 88. Thus, it would have been known that a single reaction volume can contain up to 9216 reactions and still be used for sequencing. As I explain above, Dr. Metzker previously argued that this very reference rendered obvious a large scale multiplex PCR method.

266. As yet another example, and even earlier than Fluidigm, U.S. Patent Application Publication 2004/0126760 (“Broude”), published on July 1, 2004, from a PCT application filed May 17, 2001, teaches use of nested PCR approaches to simultaneously amplify at least 100 target loci. Ex. 21 (Broude) ¶ 25 (“The present invention is directed to a method for multiplex polymerase chain reaction (PCR), the simultaneous amplification of different target nucleic acid sequences in a single PCR reaction.”), ¶ 14 (“According to the present invention, nucleic acid templates to be amplified in a multiplex PCR reaction are first prepared as tennis racquet structures to allow the PCR suppression effect.”), ¶ 57 (“The present invention is preferably used with at least 5 targets...and even more preferably with at least 100 targets.”), ¶ 124 (discussing the criteria for primer selection for multiplex PCR).

267. As another example, U.S. Patent No. 7,790,393 (“Lyamichev”), which issued on September 7, 2010 from an application filed July 16, 2008, which itself was a continuation of an application filed December 17, 2002, discloses a method of “highly multiplex PCR” that could amplify at least 100 targets in a single volume. *See* Ex. 22 at 26:63-27:3 (disclosing that in some embodiments “the number of PCR reactions is from about than [*sic*] 100 to 1,000”), 25:7-14 (explaining that the “highly multiplex PCR includ[es] hundreds to thousands of products in a single reaction”); *see also generally id.* at 25:19-32:19.

268. As yet another example, WO 2007/147079 (“Shoemaker”), filed June 14, 2007 and published on December 21, 2007, discloses amplifying more than 100 SNP loci in a single reaction volume using more than 100 PCR primer pairs. *See* Ex. 23 (Shoemaker) at 117 (“Preferably, the amplified/tagged nucleic acids include at least...100 polymorphic genomic DNA regions....”).

269. Thus, a POSA would have been aware of and understood that methods of amplifying 25-2,000 SNP loci for the purpose of sequencing were well-known in the art, and to

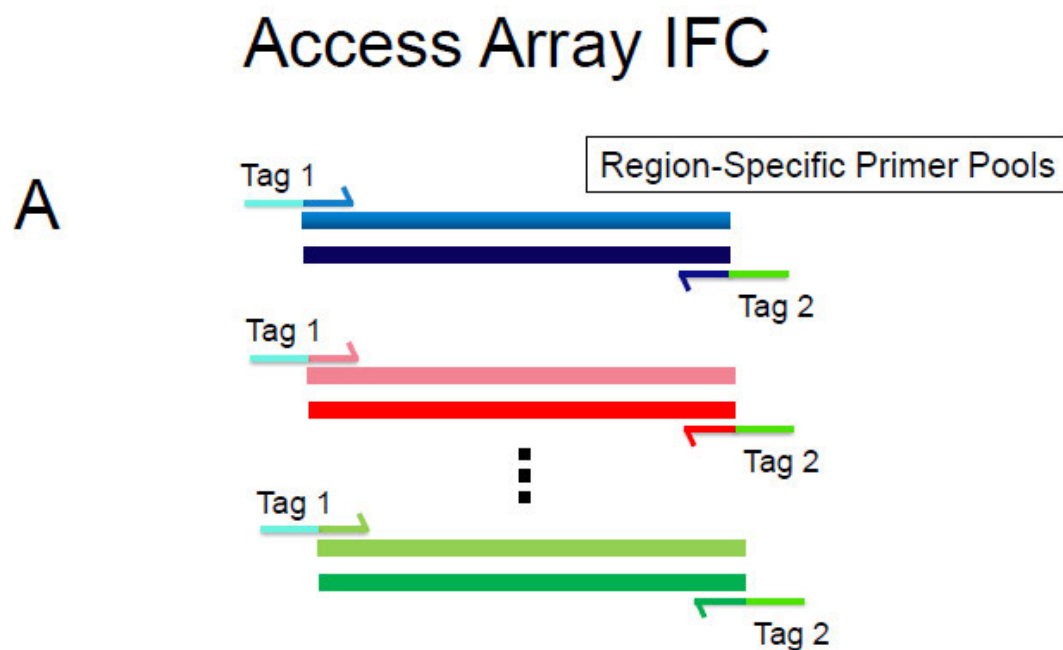
increase the degree of multiplex of the Kaper method would have been an obvious variation. It would have been unsurprising to a skilled artisan that a higher degree of multiplex could be accomplished with Kaper's method.

- e. **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**

270. It is my opinion that Kaper renders claim 12 of the '454 patent obvious.

271. First, for the reasons I describe above, Kaper renders obvious claim 1.

272. Second, Kaper teaches "wherein one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor." As shown in Figure 2A of Kaper below, the region specific primers comprise a "Tag 1" and a "Tag 2," which would be a first and second universal tail adaptor, respectively.



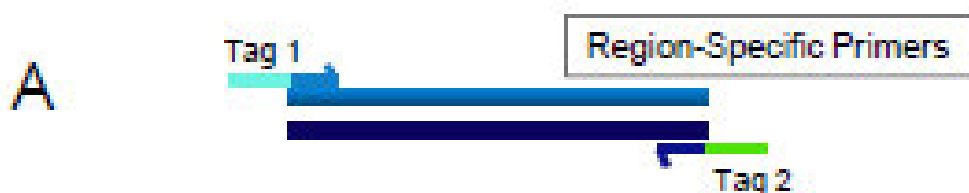
Ex. 15 at Figure 2A.

- f. **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

273. It is my opinion that Kaper renders claim 13 of the '035 patent obvious.

274. As I describe in my analysis of the invalidity of claim 1[a] and as is demonstrated in Figure 2 of Kaper, tagging with a universal sequence is accomplished by a first round of amplification wherein a region-specific primer with a universal sequence tag is used to generate copies that are tagged.



Id. at Figure 2A.

3. The Asserted Claims Of The '035 Patent Are Rendered Obvious By Prior Art Teaching ARM-PCR In View Of The Skill And Knowledge Of A POSA

a. Claim 1[preamble]: A Method For Amplifying and Sequencing DNA, comprising:

275. As I describe above, it is my understanding that a preamble is only a claim limitation in specific circumstances. To the extent that this may be considered a claim limitation, however, it is my opinion that the combination of Han and Wang disclose it.

276. First, Han teaches a method for amplifying DNA. Han's Amplicon Rescue Multiplex PCR, or ARM-PCR, is a "method for amplifying nucleic acids to enable detection of those nucleic acids," comprising a two-step amplification process. Ex. 16 ¶ 7.

277. Second, Wang teaches that ARM-PCR can be used to prepare DNA for sequencing, as Wang uses ARM-PCR to achieve a high level of multiplexing before rapidly sequencing on Roche 454 next generation sequencing platforms. Ex. 24 (Wang) at 1520.

b. Claim 1[a]: 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;

278. It is my opinion that that this claim is rendered obvious by the ARM-PCR art in view of the skill and knowledge as a POSA.

279. First, the ARM-PCR art teaches “tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products.” Han teaches that “in the first step of the method, high-concentration, target-specific, nested primers are used to perform a target-specific first amplification procedure,” and that

selected primers are “tagged” with additional nucleotides to provide an additional sequence that is not specific for the target nucleic acid(s) so that amplification of the target nucleic acid with such a primer will also incorporate into the resulting amplicon a binding site for a common primer that, unlike a target-specific primer, may be used to further amplify unrelated target nucleic acid amplicons

Ex. 16 ¶ 11. In this disclosure, a common primer binding site is a “universal tail adaptor.” Because Wang also used ARM-PCR, it also necessarily teaches this step because this is part and parcel of ARM-PCR. *See, e.g.*, Ex. 24 at 1522 (“For each target, a set of nested sequence specific primer was designed (Forward-out, Fo; Forward-in, Fi; Reverse-out, Ro; and Reverse-in, Ri). A pair of common sequence tags was linked to all internal primers (Fi and Ri).”).

280. Second, Han teaches the use of cell-free DNA, wherein the cell-free DNA is “isolated from a blood sample collected from a subject who is not a pregnant women.” Specifically, Han teaches that the method may be used for “clinical diagnosis,” including the “detection of genetic disorder, and/or the detection of disease conditions.” Given the state of the

art at the time of invention, a POSA would have understood such a disclosure to encompass or at least render obvious cell-free DNA. Indeed, as I explain above by the time of Han, the use of cell-free DNA for monitoring disease, including cancer, had been well-known and was already in use by researchers. *See supra* ¶¶ 241-248 (detailing the knowledge in the art regarding cell-free DNA, particularly in the context of cancer monitoring and disease monitoring).

- c. **Claim 1[b]: 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**

281. It is my opinion that Han, in view of the skill of a POSA, discloses this limitation.

282. As I describe above, I adopt my understanding of Dr. Metzker's explanation of this claim element for my analysis at this stage. Thus, first, Han teaches amplifying the products one or more times wherein one of the steps comprises targeted amplification of SNP loci in a single reaction volume.

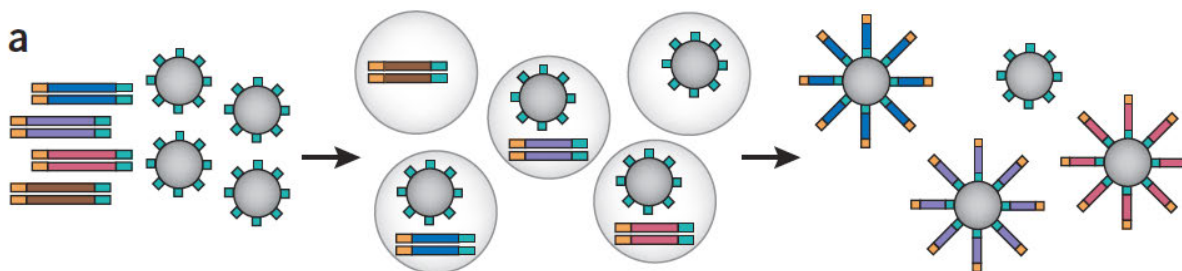
283. The first step of Han's ARM-PCR method is a target-specific amplification, wherein the primers are designed to anneal to a specific target. As described in my analysis of the invalidity of claim 1[a], the first step of amplification is a target-specific amplification, wherein the primers are designed to be specific to a locus of interest and include a tag. This amplification is "performed for approximately 10-15 cycles." Ex. 16 ¶ 11. After the first cycle, wherein tagged nucleic acids are generated by the amplification, the second and subsequent cycles using the target-specific primers to amplify the now-tagged products satisfies this claim element. This is at least consistent with Dr. Metzker's understanding of the limitation, wherein this step can occur as early as the second cycle of amplification with a target specific primer. *See* Ex. 6 at 109:8-23. What's more, because the target-specific PCR in ARM-PCR is a nested PCR, the PCR amplification using

the internal primers can be regarded as a second targeted amplification that introduces tagged products.

284. Wang also teaches this claim limitation because it uses the same ARM-PCR method set forth in Han. *See, e.g.*, Ex. 24 at 1522 (“For each target, a set of nested sequence specific primer was designed (Forward-out, Fo; Forward-in, Fi; Reverse-out, Ro; and Reverse-in, Ri). A pair of common sequence tags was linked to all internal primers (Fi and Ri).”).

285. Further, to the extent that Natera contends that Han does not teach the amplification of SNP loci, it would have been obvious in view of the skill and knowledge of a POSA. As I describe above in ¶¶ 254-256, SNP loci were well-known targets of amplification in a clinical setting. A POSA with Han in hand, using ARM-PCR for “clinical diagnosis” and the “detection of disease conditions,” would have found using the method to amplify SNP loci as an obvious variation and would have been motivated to apply this routine variation because SNP loci are frequently responsible for “disease conditions.” Ex. 16 ¶ 7.

286. Further, because Wang uses the 454 sequencing platform, it includes an emulsion PCR step that is a universal amplification that introduces a sequencing tag. Specifically, prior to sequencing the tagged products in Wang are subject to a ligation process to introduce sequencing adapters. These molecules are then subject to emulsion PCR wherein the sequencing tag will be introduced into the amplicons, which remain fixed to a bead. This is shown, for instance, in the figure below which shows the emulsion PCR process used in 454 sequencing:



Ex. 39 (Shendure) at Fig. 2a. In this figure, the sequencing tags that are introduced are denoted by the blue and orange boxes at the ends of the DNA fragments.

287. To the extent Natera contends that the ARM-PCR method does not involve an amplification that introduces a barcode, Natera has admitted throughout numerous litigations that the use of PCR to add not just a barcode but also sequencing tag has long been known in the art.

288. For example, Dr. Metzker, in the 2018 *inter partes* review of U.S. Patent No. 9,493,891, opined that the “use of indexed DNA library for multiplexed next generation sequencing was wide spread in the art.” Ex. 12 at 38. Indeed, as of 2010, commercially available systems were commonplace to permit barcoding and tagging with sequencing primers. Dr. Metzker provided numerous examples of the use of barcodes, or indexed DNA fragments: Bentley used such a system to analyze an individual human genome.

289. An “indexed DNA library” is the same as the addition of barcodes to DNA fragments to construct a library. *See, e.g. Id.* ¶ 59 (“In addition to the term barcode, these molecular codes have been referred to as tags, multiplex identifiers, and indexes”).

290. Dr. Metzker goes on to provide nine paragraphs of examples of the use of barcoding in the art:

- He cites to McKenna, who used barcoded primers for sequencing. These barcoded primers had a structure of Sequencing Primer-Barcode-Targeted primer, and serve the function to uniquely tag a given sample. *Id.* ¶¶ 60-61, 65.

- As another example, Dr. Metzker describes the Illumina GA sequencer, which he described as “the most commonly used next-generation sequencer” as of 2010. The first step of the workflow of the Illumina GA sequencer was DNA library preparation, which would have included barcoding. *Id.* ¶ 62.
- As yet another example, Dr. Metzker describes Bentley, which ligated adaptors to sheared DNA fragments for amplification and sequencing. *Id.* ¶ 63.
- As yet another example, Dr. Metzker cited to Quail, which used the Illumina GA system with barcoded primers to analyze DNA fragments and generate an indexed library. *Id.* ¶ 64.
- As yet another example, Dr. Metzker describes Craig, which provided a generalized framework for multiplexed resequencing of targeted genome regions. In the described protocol, “a unique indexed-adaptor sequences [*sic*] was ligated” to DNA fragments. *Id.* ¶ 67.

291. In addition to the Illumina GA sequencer system from 2010, Dr. Metzker described even earlier systems wherein barcoding was widespread and used in the art:

As discussed above, in the relevant timeframe, next-generation sequencing was in widespread use. In fact, 454 Life Sciences Corporation’s sequencer, known as Genome Sequencer 20, was commercially in use by 2005. Likewise, Solexa’s next generation sequencer was released in late 2006 and achieved widespread commercial use by 2007. ***Barcoding or sample tagging was equally prevalent.***

Id. ¶ 115.

292. Thus, a skilled artisan would have been well-aware of barcoding and found its use in the context of tagging and sequencing nucleic acid fragments to be an obvious modification to such techniques to permit identification of nucleic acid molecules.

293. As yet another example, as early as 2009, it was known that a universal PCR could be used with primers that included sequencing oligos and barcodes, such as what was taught by Varley and Mitra. D.I. 52-5 at 1849 (“The universal PCR used primers tailed with ***454 Life Sciences A or B oligo at the 5’ end, followed by a sample-specific DNA sequence*** and ending at the 3’ end with the same universal primer sequence ligated to the amplicons in the Nested Patch PCR procedure.”).

294. Thus, a POSA would have found it unsurprising and obvious that ARM-PCR, which can be used to generate tagged nucleic acids with a universal sequence, would be followed with a step using that universal sequence to add a barcode and sequencing tag.

d. Claim 1[c]: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

295. It is my opinion that this is rendered obvious by art teaching ARM-PCR in combination with the skill and knowledge of a POSA.

296. First, Wang teaches “sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products.” Wang teaches the use of Roche 454 sequencing technology to permit “rapid sequencing of millions of DNA fragments at low cost and without cloning bias.” Ex. 24 at 1520. Wang further teaches that by “combining this method with the ARM-PCR, which allows millions of highly similar sequences to be amplified in a semiquantified matter from a complex mixture, our approach has provided us with the necessary arsenal to characterize the majority of antigen receptor sequences at the level of sequence resolution.” *Id.*

297. Thus, a skilled artisan would have been aware that ARM-PCR could be used to prepare a sample for sequencing on a platform capable of massively parallel sequencing, and would have found it obvious to use the techniques taught in Han for such a purpose.

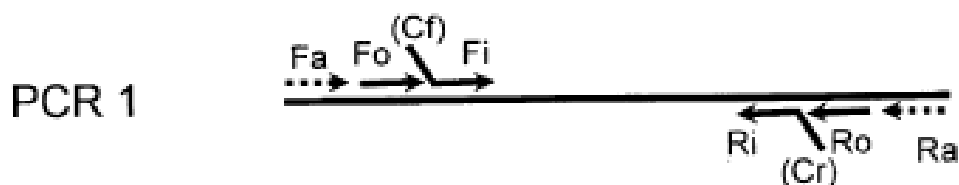
298. Further, Han teaches that the plurality of SNP loci comprises 25-2,000 loci associated with cancer. As I describe above, the use of amplification techniques to study SNP loci in the cancer context would have been obvious to a POSA, as they were well-known and practiced widely in the art. Table 2 of Han demonstrates that this technique is capable of the degree of

multiplex recited in the claim. This Table displays 72 forward and reverse primers, which when run in a single volume reaction would be within the multiplex range recited in this claim.

4. **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**

299. It is my opinion that the ARM-PCR art renders obvious this claim limitation.

300. As demonstrated in Figure 1 of Han, reproduced below, in the first step of amplification forward and reverse common primer sequence can be added (labeled Cf and Cr). These are a “first universal tail adaptor and a second universal tail adaptor.”



Excerpted from Ex. 16 at Figure 1.

5. **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**

301. It is my opinion that the ARM-PCR art renders this claim limitation obvious.

302. Specifically, Han discloses that in the first PCR step (as depicted in the above excerpt from Figure 1), selected primers may be tagged “with additional nucleotides to provide an additional sequence that is not specific for the target nucleic acid(s) so that amplification of the target nucleic acid with such a primer will also incorporate into the resulting amplicon a binding site for a common primer that, unlike a target-specific primer, may be used to further amplify unrelated target nucleic acid amplicons.” Ex. 16 ¶ 11.

303. The disclosed “common sequence” is a universal tail adaptor, and the Fi/Ri primers with the Cf/Cr sequences appended, respectively, are a first and second primer comprising, respectively, the first and second universal tail adaptor.

XI. INVALIDITY UNDER 35 U.S.C. § 101—INELIGIBLE SUBJECT MATTER

A. The Asserted Claims of the ’454 Patent Are Ineligible

1. The Asserted Claims of the ’454 Patent Are Directed to a Method of Detecting DNA to Identify Tumor-Specific Mutations

304. I am informed by counsel that Natera asserts claims 1, 8, and 11 of the ’454 patent against NeoGenomics. The Asserted Claims of the ’454 patent reads as follows:

1. A method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:

performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;

performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume; and

sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads, wherein the sequencing has a depth of read of at least 50,000 per target locus.

8. The method of claim 1, wherein the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation.

11. The method of claim 1, wherein the method further comprises performing barcoding PCR prior to the sequencing.

D.I. 1-1 at claims 1, 8, and 11.

305. It is my opinion that the Asserted Claims of the '454 patent are “methods-of-detection” claims. The '454 patent claims methods of detecting the presence in a blood (plasma) sample of DNA mutations associated with a cancerous tumor. The methods disclosed in claim 1 of the '454 patent include the following steps:

- a. performing DNA sequencing of a tumor sample “to identify a plurality of tumor-specific SNV mutations”;
- b. amplifying DNA strands with “tumor-specific SNV mutation(s)” to generate copies of DNA (“amplicons”) containing those mutations; and
- c. “sequencing the amplicons” and “detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads.”

306. The Asserted Claims of the '454 patent detect or measure the amount of certain genetic data (DNA mutations associated with a cancerous tumor)—in other words, the Asserted Claims detect a natural phenomenon. Natera did not invent or discover DNA mutations associated with cancerous tumors, the relationship between DNA mutations and cancerous tumors, or the correlation DNA mutations have with cancer.

307. Moreover, in my opinion, the step of “detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads” is a purely abstract, mental process implemented with a mathematical algorithm. Indeed, a significant portion of the disclosures of the '454 patent specification involves purely mathematical algorithms. *See generally* D.I. 1-1 at 27:1-39, 45:44-95:40, 105:48-110:5, 115:7-128:5, 152:55-155:35, 157:49-158:36, 163:10-167:2. Moreover, I note that claim 1 of the '454 patent (and indeed, all the claims) do not

purport to claim any particular algorithm, method, or formula disclosed within the specification of the '454 patent, or any novel procedures, steps, protocols, or parameters.

308. I understand that purely mathematical algorithms are non-patentable subject matter, regardless of their purported novelty. I understand that the algorithmic steps outlined in the patent specifications are ineligible for patenting.

309. As such, the steps of claim 1 of the '454 patent are nothing more than (1) identifying tumor-specific SNV mutations by performing DNA sequencing of a tumor sample, (2) amplifying cfDNA strands with tumor-specific SNV mutation(s) from a subject having cancer or suspected of having cancer using conventional techniques, including PCR amplification; (3) sequencing the amplified products using conventional techniques; (4) applying an abstract, mathematical process to detect a natural phenomenon: the presence in a blood (plasma) sample of DNA mutations associated with a cancerous tumor. In other words, the claims are directed to a method of detecting SNV tumor mutations, which is accomplished by applying a mathematical algorithm and routine and conventional steps (as explained below).

310. I also understand that Natera previously argued that (1) cfDNA occurs naturally as a result of cell death in a transplant recipient's body; and (2) correlating the detected quantity of donor-specific cfDNA to transplant health, are both natural phenomenon that are not patent eligible. Ex. 10 at 14 (arguing that "[t]he Claims are directed to detecting a natural phenomenon—donor-specific cfDNA from a transplanted organ that circulates in the blood of a transplant recipient. The '652 patent claims are directed to the additional unpatentable concept of correlating the detected quantity of donor-specific cfDNA to transplant health—another natural phenomenon"). I have reviewed the claims of the '652 patent, and it is my opinion that the "detecting" steps recited by the Asserted Claims of the '454 patent are broader, more generic, and

recite less technical detail than the “determining” or “quantifying” steps of the ’652 patent. Therefore, to the extent the ’652 patent is characterized as being directed to detecting natural phenomenon, the Asserted Claims of the ’454 patent are even more so.

311. Therefore, the Asserted Claims of the ’454 patent are directed to detecting a natural phenomenon.

2. The ’454 Patent, Natera’s Expert Witnesses, and Natera’s Own Admissions State That the Techniques Recited by the Asserted Claims Are Standard, Routine, and Conventional

312. In my opinion, the claims of the ’454 patent identify the recited steps only at a high level of generalization. The claims do not identify any particular approach for performing the steps. The written description states how different ways of carrying out the techniques recited in those steps, and the combinations of them, are conventional, common, or already in use.

313. Neither the patents’ written description nor the claims identify anything as being nonconventional or innovative about the limitations recited in the claims.

a. The ’454 Patent and Natera’s Own Admissions State that Obtaining a Blood Plasma Sample Was a Well-Understood, Routine, and Conventional Technique

314. The preamble of claim 1 of the ’454 patent states a “method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample.” D.I. 1-1 at claim 1. To the extent that the preamble of the claim is limiting, obtaining a blood plasma sample was well known to a POSA.

315. Nowhere does the ’454 patent state that the methods for obtaining a plasma sample described in the patent specification involve anything new, innovative, or novel. To the contrary,

the specification of the '454 patent makes clear that methods of obtaining plasma samples were known in the art:

In one experiment, plasma samples were prepared and amplified using a hemi-nested 19,488-plex protocol. The samples were prepared in the following way: up to 20 mL of blood were centrifuged to isolate the buffy coat and the plasma.

Id. at 152:5-9.

Blood samples were collected into EDTA tubes. Circulating tumor DNA was isolated from 1 mL plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Valencia, Calif.).

Id. at 160:33-36.

Blood samples were collected into EDTA tubes. The whole blood sample was centrifuged and separated into three layers: the upper layer, 55% of the blood sample, was plasma and contains cell-free DNA (cfDNA); the buffy coat middle layer contained leucocytes having DNA, <1% of total; and the bottom layer, 45% of the collected blood sample, contained erythrocytes, no DNA was present in this fraction as erythrocytes are enucleated. Circulating tumor DNA was isolated from at least 1 mL plasma using the QIAamp Circulating Nucleic Acid kit, Qia-Amp (Qiagen, Valencia, Calif.), according to the manufacture's [sic] protocol.

Id. at 163:61-164:4.

316. Additionally, in *Illumina, Inc. v. Natera, Inc.*, Natera argued that methods of obtaining blood samples were known in the art by 2010. Ex. 34 at 5. If such techniques were conventional in 2010, they were surely conventional by 2015.

317. In sum, the specification of the '454 patent and Natera's own admissions show that obtaining blood plasma samples as claimed in the '454 patent was well-known, routine, and conventional in 2015.

b. The '454 Patent, Natera's Expert Witnesses, and Natera's Own Admissions State That Whole Genome Sequencing Was a Well-Understood, Routine, and Conventional Method

318. The first step of claim 1 of the '454 patent involves whole genome sequencing to identify SNVs. D.I. 1-1 at claim 1. Whole genome sequencing and whole exome sequencing were well known to a POSA.

319. Nowhere does the '454 patent state that the methods for whole genome sequencing described in the patent specification involve anything new, innovative, or novel. To the contrary, the specification of the '454 patent states that “[s]tandard methods (such as sequencing or an array) can be used to identify the alleles on a single chromosome to determine a haplotype of the individual.” *Id.* at 116:17-19. The specification further identifies commercial techniques for whole exome sequencing that were available well before 2015. *Id.* at 168:12-14 (“Each biopsied sample was assayed by whole exome sequencing (Illumina HiSeq2000; Illumina, San Diego, Calif.)”). Indeed, the technique of whole genome sequencing was so commonplace by 2015 that the '454 patent mentions it without describing or explaining what it is. *Id.* at 41:59-61.

320. Likewise, the '454 patent cites to a wealth of prior art publications describing routine, standard, gene sequencing techniques known in the art, including whole genome sequencing (all emphases below are added):

'454 patent at 9:

- Bentley, David R. et al., “Accurate ***Whole Human Genome Sequencing*** Using Reversible Terminator Chemistry”, *Nature*, 456, 6, 2008, 53-59.

'454 patent at 10:

- Chu, Tianjiao et al., “Statistical Model for ***Whole Genome Sequencing*** and its Application to Minimally Invasive Diagnosis of Fetal Genetic Disease”, *Bioinformatics*, 25(10), 2009, 1244-1250.

'454 patent at 18:

- Li, R. et al., “SNP detection for massively parallel *whole-genome resequencing*”, *Genome Research*, vol. 19, 2009, 1124-1132.
- Leary, Rebecca J. et al., “Detection of Chromosomal Alterations in the Circulation of Cancer Patients with *Whole-Genome Sequencing*”, *Science Translational Medicine*, 4, 162, 2012, 12.

’454 patent at 19:

- Lu, S. et al., “Probing Meiotic Recombination and Aneuploidy of Single Sperm Cells by *Whole-Genome Sequencing*”, *Science*, vol. 338, Dec. 21, 2012, 1627-1630.

’454 patent at 23:

- Pinard, et al., “Assessment of Whole Genome Amplification-induced Bias Through High-throughput, Massively Parallel *Whole Genome Sequencing*”, *BMC Genomics*, vol. 7:216, Aug. 23, 2006, 1-21.

’454 patent at 25:

- Shi, H. et al., “Melanoma *whole-exome sequencing* identifies V 600E B-RAF amplification-mediated acquired B-RAF inhibitor resistance”, *Nature Communications*, vol. 3, No. 724, Mar. 6, 2012, 8 pages.

321. Further, Natera’s expert, Dr. Michael Metzker, has repeatedly acknowledged that gene sequencing, including whole genome sequencing, was well known in the art by 2015:

A 2008 publication by Bentley, et al. entitled “*Accurate whole human genome sequencing* using reversible terminator chemistry” reflected “the knowledge of a person of ordinary skill.”

Ex. 12 ¶ 93 (emphasis added).

Not only was the concept of creating an indexed DNA sequencing library routine and commonplace, the concept of using cell-free DNA from maternal blood to detect chromosomal aneuploidy, such as trisomy 21, the underlying cause of Down syndrome was equally well-known in the art.

Ex. 12 ¶ 56.

As discussed above, in the relevant timeframe, next-generation sequencing was in widespread use....Further, a kit for making a sequencing library for use with Solexa’s sequencer was commercially available in 2008, underscoring the routine nature of sequencing library preparation. Thus, one of ordinary skill would have expected success in being able to sequence cell-free DNA found in maternal blood.

Ex. 12 ¶ 115.

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....As such, one of ordinary skill would have had a reasonable expectation of success in using cell-free DNA from maternal blood in a multi-step PCR method (such as Fluidigm's). One of ordinary skill would have expected reasonable success in identifying sequences from particular chromosomes, because the human genome had already been sequenced by the relevant timeframe. Cell-free genomic DNA from maternal blood had also previously been successfully amplified and sequenced to detect aneuploidy, including fetal trisomy 21, trisomy 18 and trisomy 13. This typically involved amplifying DNA from a suspected chromosome and a reference chromosome and detecting whether levels of sequences of the suspect chromosome were elevated. Given the success in using cell-free DNA to perform amplification and sequencing, one of ordinary skill would have had a reasonable expectation of success to employ cell-free DNA from maternal blood in the Fluidigm method with the goal of testing for chromosomal aneuploidy.

Ex. 12 ¶ 116 (citations omitted).

Since their introduction, NGS platforms have allowed for high-throughput sequencing of nucleic acids on a genome-wide scale and highly multiplexed gene-specific sequencing.

Ex. 12 ¶ 41.

The first NGS platform to achieve widespread commercial use was 454 Life Sciences Corporation's sequencer, also known as Genome Sequencer 20 ("GS20"). The GS20 sequencer first became commercially available in 2005 and produced average read-lengths of ~100 bases.

Ex. 12 ¶ 42 (citations omitted).

The Genome Analyzer II ("GAII") was developed by Solexa and released in late 2006. After the acquisition of Solexa by Illumina in 2007, this massively parallel sequencing platform achieved widespread commercial use.

Ex. 12 ¶ 45.

As of January 2010, Illumina's GA sequencer was the most commonly used next-generation sequencer....The GA instrument was capable of sequencing a sample from an individual or multiple samples from many different individuals.

Ex. 12 ¶ 62.

As described above in this declaration, indexed sequences were routinely added by PCR as well. For example, Illumina described adding index sequences to its

adaptor-ligated DNA fragments using a PCR enrichment step. Following additional library steps, each HapMap sample was pooled into a single tube and subjected to a PCR enrichment step using Illumina compatible primers.

Ex. 12 ¶ 67.

322. Furthermore, Natera's expert witness, Dr. John Quackenbush, further posits that the sequencing limitations were routine and conventional as of 2015. Dr. Quackenbush states that a specific sequencing method (pyrosequencing), was well-known, commercially available, and routinely used by researchers well before 2015:

[A] method called pyrosequencing, which performs sequencing-by-synthesis, was developed and first published in 1993. It was the foundation for different companies. PYROSEQUENCING AB, out of Uppsala Sweden, launched its first commercial automated pyrosequencing instrument in 1999....Based on the machines made available by Pyrosequencing, by the year 2000, pyrosequencing had established itself as a standard and conventional means for multiplex or high-throughput sequencing, including for genotyping SNPs.

Id. ¶ 103 (citing Ex. 25 (S. Marsh, *Pyrosequencing Protocols*, Methods in Molecular Biology No. 373 (2007)) ("Marsh 2007"), Ex. 26 (A. Ahmadian, et al., *Single- Nucleotide Polymorphism Analysis by Pyrosequencing*, Analytical Biochemistry 280, pp. 103–110 (2000)) ("Ahmadian 2000"); Ex. 27 (T. Nordstrom, et al., *Direct Analysis of Single-Nucleotide Polymorphism on Double-Stranded DNA by Pyrosequencing*, Biotechnol. Appl. Biochem., 31:107–112 (2000)) ("Nordstrom 2000"); Ex. 28 (M. Ronaghi, *Pyrosequencing Sheds Light on DNA Sequencing*, Genome Research, 11:3–11 (2001)) ("Ronaghi 2001")).

Another high throughput sequencing-by-synthesis method developed by a group out of Cambridge University in 1998 became the foundation for a company called Solexa (later acquired by Illumina, Inc.)....As the group that developed this technology reported in Margulies 2005, the instrument branded as the Genome Analyzer could "sequence 25 million bases, at 99% or better accuracy, in one four-hour run," B11305- 1309 (Margulies 2005) at B1305 (Abstract), and had an average read length of 108 bases.

Id. ¶ 104 (citing Ex. 33 (M. Margulies, *Genome Sequencing in Microfabricated High-Density Picolitre Reactors*, *Nature*, (2005)) (“Marguiles 2005”)).

As another example, a high throughput sequencing method co-developed by the named inventor Dr. Quake became the foundation for his company, Helicos, which was founded in 2003, and on which I was a member of the Scientific Advisory Board.

Id. ¶ 106.

323. Dr. Quackenbush opined that in 2005, there was the expectation that high-throughput sequencing would generate large quantities of data to understand cancer—an expectation that was quickly realized:

For example, when I began working in the biological sciences at the Salk Institute in 1992, the first molecular biology techniques that I learned were PCR and Sanger DNA sequencing. As part of my move to Stanford University in 1994, I was tasked with developing a new large-scale DNA sequencing method based on PCR mapping of transposon insertions. My recruitment to the Dana-Farber Cancer Institute in 2005 was largely based on the expectation that high throughput DNA sequencing would soon generate unprecedented quantities of data that could be used to understand cancer. And that expectation very quickly was realized throughout the genomics field.

Id. ¶ 94.

324. Dr. Quackenbush also noted that in 2005, there was a rapid explosion in the use of sequencing by synthesis instruments:

Due to the rapid explosion in use of sequencing-by-synthesis instruments including the 454 sequencer in 2005, the Illumina Genome Analyzer and Applied Biosystems SOLiD sequencer in 2006, and others shortly thereafter, my group and I quickly became involved in multiplex / high-throughput (or Next Generation Sequencing (“NGS”)) DNA sequencing.

Id. ¶ 95.

325. Thus, according to Dr. Quackenbush, there were a number of commercially available sequencers. *See, e.g., Id.* at ¶¶ 103-113 (describing, various commercially available sequencing platforms, including, pyrosequencing, technology available by Helicos Biosciences

Corporation, technology available by 454 Lifesciences, Clonal Single Molecule Array (Solexa, Inc.) or sequencing-by-synthesis (SBS) utilizing reversible terminator chemistry; AnyDot chips (Gonovox, Germany); sequencers such as Illumina Genome Analyzer, etc.). Further, Dr. Quackenbush has opined that by “2010, whole-genome sequencing to 30x coverage was routine on the commercially available Illumina HiSeq 2000.” Ex. 7 ¶ 64. He likewise opined that by “approximately 2008, techniques for NGS were well-known and commercially available,” that “using NGS, the entire human genome can now be sequenced on a single instrument in a single day,” and that the “development of NGS made more widely available ‘[t]he ability to sequence entire human genomes.’” *Id.* ¶¶ 60-61, 63 (quoting Lam et al. *Performance comparison of whole-genome sequencing platforms*, *Nature Biotechnology* 30:78 (2012)).

326. Natera, through its expert’s declaration, has taken the position that whole genome sequencing to identify SNVs was a well-understood, routine, and conventional method by 2009. Ex. 20 ¶ 93 (describing that determining SNPs (SNVs) by “whole genome sequencing or exome sequencing” in 2009 was routine and conventional by reference to patents pre-dating 2009). If such techniques were conventional in 2009, they were surely conventional by 2015.

327. I understand that Natera has taken the position that “references cited in [Natera patent] prosecution history establish the patentees knew of ways to sequence-by-synthesis beyond pyrosequencing.” *See* Ex. 29, (Joint CC Brief, Case No. 1:20-cv-00038, D.I. 76) at 12 (citing Ex. 30, (Jarvie, *Next generation sequencing technologies*, *Drug Discovery Today* Vol. 2, 255-60 (2005)) (“Jarvie”); Ex. 31 (Harismendy and Frazer, *Method for improving sequence coverage uniformity of targeted genomic intervals amplified by LR-PCR using Illumina GA sequencing-by-synthesis technology*, *Bio Techniques* 46:229-31 (2009)) (“Harismendy and Frazer”); and Ex. 32 (Mardis, *The impact of next-generation sequencing technology on genetics*, *Cell press* 24:133-41

(2008)) (“Mardis”). Further illustrating that the claimed method was routine, Natera argued that the “patentees knew about different types of sequencing-by-synthesis...when they drafted the claims, evidenced by cites to Jarvie and other references during prosecution.” See Ex. 29 at 16 (citation omitted). In light of this, it is clear that Natera contends that the claimed sequencing was a routine, and well-understood technique in 2015.

328. In sum, the ’454 patent, Natera’s expert witnesses, and Natera’s own admissions show that sequencing as claimed in the ’454 patent was well-known, routine, and conventional in 2015.

c. The ’454 Patent, Natera’s Expert Witnesses, and Natera’s Own Admissions State That Amplification, Including Using Targeted and Universal PCR, Was a Well-Understood, Routine, and Conventional Technique

329. The second step of claim 1 of the ’454 patent involves multiplex amplification to amplify 10-500 loci of the DNA to result in amplified DNA for each such location. D.I. 1-1 at claim 1. Multiplex amplification, including PCR, was well known to a POSA.

330. The ’454 patent does not state that its methods involve anything new, innovative, or novel with respect to amplification. To the contrary, the ’454 patent cites to a wealth of prior art publications describing routine, standard, amplification techniques known in the art, including PCR (all emphases below are added):

’454 patent at 7:

- Abd-Elsalam, Kamel A., “Bioinformatic Tools And Guideline for **PCR** Primer Design”, African Journal of Biotechnology, vol. 2, 2003, pp. 91-95.

’454 patent at 8:

- Bartlett, John M. et al., “**PCR** Protocols”, PCR Protocols, vol. 226, 2003, 519 pages.
- Beck, J. et al., “Digital Droplet **PCR** for Rapid Quantification of Donor DNA in the Circulation of Transplant Recipients as a Potential Universal Biomarker of Graft Injury”, Clinical Chemistry, vol. 59, No. 12, 2013, 1732-1741.

'454 patent at 9:

- Binladen, J. et al., "The Use of Coded **PCR** Primers Enables High-Throughput Sequencing of Multiple Homolog Amplification Products by 454 Parallel Sequencing", PLOS One, Issue 2, Feb. 2007, 9 pages.
- Blomquist, T M. et al., "Targeted RNA-Sequencing with Competitive **Multiplex-PCR** Amplicon Libraries", Plos One, vol. 8, Issue 11, Nov. 2013, 14 pages.
- Broude, N. E. et al., "**High Multiplexity PCR** Based on PCR Suppression", **DNA Amplification** Current Technologies and Applications, 2004, 61-76.
- Broude, N. E. et al., "Multiplex Allele-specific Target **Amplification** based on PCR Suppression", PNAS, vol. 98, No. 1, Jan. 2, 2001, 206-211.
- Brownie, Jannine et al., "The Elimination of Primer-Dimer Accumulation in **PCR**", Nucleic Acids Research, 25(16), 1997, 3235-3241.

'454 patent at 10:

- Caliendo, Angela, "**Multiplex PCR** and Emerging Technologies for the Detection of Respiratory Pathogens", Clinical Infectious Diseases, 52(4), 2011, S326-S330.
- Cashon, J. A. et al., "A method for counting **PCR** template molecules with application to next-generation sequencing", Nucleic Acids Research, vol. 39, No. 12, Apr. 13, 2011, 1-8.
- Chen, Bing-Yuan et al., "**PCR** Cloning Protocols", **PCR** Cloning Protocols, vol. 192, 2002, 434 pages.

'454 patent at 11:

- D'Aquila, Richard et al., "Maximizing sensitivity and specificity of **PCR** by pre-amplification heating", Nucleic Acids Research, 19(13), 1991, p. 3749.
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331. Further, Natera’s expert, Dr. Michael Metzker, has repeatedly acknowledged that amplification, including PCR, was well known in the art by 2015:

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....As such, one of ordinary skill would have had a reasonable expectation of success in using cell-free DNA from maternal blood in a multi-step PCR method (such as Fluidigm’s).

Ex. 12 ¶ 116.

As described above in this declaration, indexed sequences were routinely added by PCR as well. For example, Illumina described adding index sequences to its adaptor-ligated DNA fragments using a PCR enrichment step. Following additional library steps, each HapMap sample was pooled into a single tube and subjected to a PCR enrichment step using Illumina compatible primers.

Id. 13 ¶ 67.

332. Likewise, Dr. Metzker admitted at deposition that a “person of ordinary skill in the art would understand how PCR works,” “one of ordinary skill would have understood how the PCR cycle works,” and that “[l]igation would be -- or PCR -- the two most common ways of [tagging cfDNA].” Ex. 6 at 93:1-2, 99:4-5, 114:24-115:1.

333. Further, Dr. Quackenbush testified that amplification was routine and conventional as early as 1985, well before 2015:

Q. And how far back were persons of skill in the art using PCR to do selected amplification?

A. So Kary Mullis, I described in 1985 Kary Mullis was doing it in 1985. It was a rapidly adopted technology because it was so powerful.

Ex. 11 at 88:14-18.

334. Dr. Quackenbush also opined that the claimed amplification technique had been “universally adopted” as a standard tool by 2000:

PCR-based tools for genotyping SNPs, including for genotyping up to hundreds of thousands of SNPs, were commercially available as far back as 1995. ***By 2000, PCR had been universally adopted as a standard tool*** in applications of molecular biology, and it was already successful in the simultaneous detection of thousands of SNPs...***PCR was a well-established, conventional and indispensable tool*** for genetic testing that was routinely used to target and amplify specific, pre- selected genes for further study.

Ex. 20 ¶¶ 82-83 (emphasis added).

335. Dr. Quackenbush has also stated that he had significant experience with PCR techniques in the 1990s.

Starting in 1992, I ran a large program at the Salk Institute using PCR to assay polymorphic genetic markers to produce a map of human chromosome 11, a project that required tens of thousands of multiplexed PCR reactions. Between 1994 and 1996, while at Stanford University, I was responsible for the design and conduct of hundreds of thousands of PCR reactions for mapping the entire human genome and as part of developing a strategy for sequencing regions of human chromosomes 4 and 21. After joining The Institute for Genomic Research in 1997, I was responsible for projects involving hundreds of thousands of PCR reactions for genome sequencing and microarray analysis. And after joining the faculty at the Dana-Farber Cancer Institute and Harvard School of Public Health, I led large-scale projects analyzing gene expression in cancer and establishing high-throughput genome sequencing that required PCR and other amplification reactions from diverse biological samples, including cell lines, human tissue samples, circulating tumor cells, and circulating cell-free DNA. In all of these projects, I used standard and routine PCR applications for genotyping.

Id. ¶ 87. Further, Dr. Quackenbush has opined that “well before 2013 several techniques had been developed to generate or isolate copies of DNA molecules of interest to facilitate sequencing.” Ex. 7 ¶ 66. Likewise, Dr. Quackenbush cites to a document he describes as stating that PCR is among the most widely used approaches to target enrichment. *Id.* at ¶ 68.

336. Natera has twice taken the position that selective multiplex amplification was a well-understood, routine, and conventional method by 2009. Ex. 20 ¶ 8; D.I. 52-9 at 50 (“There is a good reason for this: the patentee admitted selective amplification was routine and conventional—so much so that it was unnecessary to provide any guidance on how to apply it to 1,000 SNPs.”) (citations omitted). Additionally, in *Illumina, Inc. v. Natera, Inc.*, Natera argued that methods of DNA amplification were known in the art by 2010. Ex. 34 at 5. If such techniques were conventional in 2009 and 2010, they were surely conventional by 2015.

337. In sum, the ’454 patent, Natera’s expert witnesses, and Natera’s own admissions show that the technique of amplifying cfDNA as claimed in the ’454 patent was well-known, routine, and conventional in 2015.

d. The ’454 Patent, Natera’s Expert Witnesses, and Natera’s Own Admissions State That Sequencing Amplicons to Detect Tumor-

Specific SNV Mutations Was a Well-Understood, Routine, and Conventional Technique

338. The third step of claim 1 involves sequencing amplicons to detect tumor-specific SNV mutations using a “sequencing depth of at least 50,000 per target loci.” D.I. 1-1 at claim 1. As described above, sequencing techniques were well known in the art by 2015. *See* Section X.A. The addition of using a “sequencing depth of at least 50,000 per target loci” fails to add an inventive concept. Indeed, the ’454 patent never describes sequencing depth as inventive. D.I. 1-1 at 68:33-40 (giving laundry list of sequencing depths, just one of which includes 50,000). Likewise, Natera has argued that sequencing depth was a well-known, well-understood concept at that POSAs could readily increase sequencing depth sensitivity (i.e., the number of copies sequenced). Ex. 20 ¶ 119 (citing prior art and stating that “POSA’s prior to November 6, 2009, were well aware that sequencing more molecules, of increasing sequencing depth, improves sensitivity.”).

e. Claims 8 and 11 Add Only Conventional Steps

339. It is my opinion that claims 8 and 11 fail to add an inventive concept and add only conventional steps. Claim 8 simply specifies the number of target loci, which have been described in prior patent applications. D.I. 1-1 at claim 8; D.I. 52-8 at 78:17-21 (“Whereas traditional multiplex PCRs evaluate up to fifty loci simultaneously, the approach described herein may be used to enable simultaneous evaluation of more than...100 loci simultaneously....”). Claim 11 adds barcoding, which is conventional and described in prior patent publications cited by the ’454 patent. D.I. 1-1 at claim 11; Ex. D.I. 52-8 at 31:26 (“A primer may also contain a molecular barcode.”), 91:30-31 (“Primers may contain additional functional sequences, e.g. barcodes....”).

340. For the reasons discussed above, it is my opinion that the Asserted Claims of the ’454 patent are method of detection claims, that these claims only detect the presence of cfDNA in

a biological sample, which is a natural phenomenon, and that (based on the '454 patent, Natera's expert witnesses, and Natera's own admissions) the detecting is accomplished with no meaningful non-routine steps. There is nothing about the claimed steps, individually or in combination, that is unconventional.

B. The Asserted Claims of the '035 Patent Are Ineligible

1. The Asserted Claims of the '035 Patent Are Directed to Methods of Detecting the Presence of SNPs Associated With Cancer

341. I am informed by counsel that Natera asserts claims 1, 12, and 13 of the '035 patent against NeoGenomics. The Asserted Claims of the '035 patent reads as follows:

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.

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.

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.

342. It is my opinion that the Asserted Claims of the '035 patent are “methods-of-detection” claims. Claim 1 of the '035 patent recites methods for detecting the presence of SNPs (DNA code variants) “associated with cancer.” The methods disclosed in asserted claim 1 of the '035 patent include the following steps:

- a. tagging isolated cfDNA;
- b. amplifying the tagged cfDNA, including targeted amplification of a plurality of single nucleotide polymorphism (SNP); and
- c. sequencing the plurality of SNP loci on the cfDNA.

343. The Asserted Claims of the '035 patent detect or measure the amount of certain genetic data (SNPs associated with cancer)—in other words, the Asserted Claims detect a natural phenomenon. Natera did not invent or discover cfDNA associated with cancer, the relationship between cfDNA and cancer, or the correlation cfDNA has with cancer. Further, the disclosures of the '035 patent are generic and fail to provide guidance as to which SNPs are associated with any particular type of cancer or cancer in general, or even what SNPs are associated with healthy cells. The user is left to figure this information out for themselves.

344. The prosecution and allowance of the '035 patent confirm that the Asserted Claims are directed to generic detection of DNA code associated with cancer. In response to a rejection for obviousness, the Applicant amended the claims to recite sequencing SNP loci comprising 25-2000 loci “associated with cancer.” Ex. D.I. 53-1, Ex. D.I. 53-2 at 2. Further, the Applicant claimed that the prior art Chuu reference “fails to teach or suggest targeted amplification of 25-2,000 target loci associated with cancer in a single reaction volume.” Ex. D.I. 53-2 at 5. Following this amendment and argument, the claims of the '035 patent were allowed by the Examiner, who explained that the amendment to add the “associated with cancer” limitation, to the exclusion of

testing fetal cfDNA, led to allowance. Ex. D.I. 52-3 at 3. In my opinion, this confirms that the '035 patent is directed to a natural phenomenon.

345. Additionally, I understand that Natera and its inventors have repeatedly asserted that detecting tumor-derived cfDNA is an “equivalent condition” to detecting fetus-derived cfDNA. D.I. 53-3 ¶ 10 (Natera inventor arguing in affidavit that “it should be noted that cfDNA from a fetus in the plasma or serum is considered by a person of ordinary skill in the art as an ‘equivalent condition’ to the presence of ctDNA in the relevant art.”), Ex. D.I. 53-4 at 5 (Natera arguing during patent prosecution that “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 cancer DNA context.”); *see also* Ex. D.I. 53-5 at 1, 3, 6. Even if detecting SNPs associated with cancer was patent eligible, Natera has repeatedly argued that this is the same concept as detecting variants from a fetus, which Natera has also argued cannot be an inventive concept.

346. I also understand that Natera previously argued that (1) cfDNA occurs naturally as a result of cell death in a transplant recipient’s body; and (2) correlating the detected quantity of donor-specific cfDNA to transplant health, are both natural phenomenon that are not patent eligible. Ex. 10 at 14 (arguing that “[t]he Claims are directed to detecting a natural phenomenon—donor-specific cfDNA from a transplanted organ that circulates in the blood of a transplant recipient. The '652 patent claims are directed to the additional unpatentable concept of correlating the detected quantity of donor-specific cfDNA to transplant health—another natural phenomenon”). I have reviewed the claims of the '652 patent, and it is my opinion that the “detecting” steps recited by the Asserted Claims of the '035 patent are broader, more generic, and recite less technical detail than the “determining” or “quantifying” steps of the '652 patent.

Therefore, to the extent the '652 patent is characterized as being directed to detecting natural phenomenon, the Asserted Claims of the '035 patent are even more so.

347. Therefore, the Asserted Claims of the '035 patent are directed to detecting a natural phenomenon, and are hence, patent ineligible under §101.

2. The '035 Patent, Natera's Expert Witnesses, and Natera's Own Admissions State That the Techniques Recited by the Asserted Claim Are Standard, Routine, and Conventional

348. In my opinion, the claims of the '035 patent identify the recited steps only at a high level of generalization. The claims do not identify any particular approach for performing the steps. The written description states how different ways of carrying out the techniques recited in those steps, and the combinations of them, are conventional, common, or already in use.

349. Neither the '035 patent's written description nor the claims identify anything as being nonconventional or innovative about the limitations recited in the claims.

350. The '035 patent incorporates by reference a 2008 publication by Varley et al., which demonstrates that the claims were known in the art well in advance of the claimed inventions. D.I. 52-4 at 93:41-58 (citing D.I. 52-5), 250:9-11; D.I. 52-5 at Abstract, 1845.

a. The '035 Patent and Natera's Expert Witness State that Tagging DNA Was a Well-Understood, Routine, and Conventional Technique by 2011

351. Claim 1 of the '035 patent recites "tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products." D.I. 1-2 at claim 1. Tagging DNA was well known to a POSA.

352. Nowhere does the '035 patent state that the methods for tagging described in the patent specification involve anything new, innovative, or novel. To the contrary, the specification

of the '035 patent makes clear that DNA tagging and part of amplification and sequencing was known in the art:

As is known in the art, the term “sequence tag” refers to a relatively short (e.g., 15-100) nucleic acid sequence that can be used to identify a certain larger sequence, e.g., be mapped to a chromosome or genomic region or gene.

D.I. 1-2 at 187:16-20.

353. Likewise, the '035 patent cites to prior art publications describing routine, standard, tagging techniques known in the art, including barcoding (all emphases below are added):

'035 patent at 9:

- Craig, D. W. et al., “Identification of genetic variants using *barcoded multiplexed sequencing*”, Nature Methods, vol. 5, Oct. 2008, 887-893.

'035 patent at 13:

- McCloskey, M. L. et al., “Encoding PCR Products with *Batchstamps and Barcodes*”, Biochem Genet., vol. 45, Oct. 23, 2007, 761-767.

'035 patent at 14:

- Miner, B. E. et al., “Molecular *barcodes* detect redundancy and contamination in hairpin-bisulfite PCR”, Nucleic Acids Research, vol. 32, No. 17, Sep. 30, 2004, 1-4.

'035 patent at 19:

- Parameswaran, P., et al., “A pyrosequencing-tailored nucleotide *barcode* design unveils opportunities for large-scale sample multiplexing”, Nucleic Acids Research, Oct. 11, 2007, 9 pages.

354. Further, Natera’s expert, Dr. Michael Metzker, has repeatedly acknowledged that tagging was well known in the art by 2011:

Solexa’s next generation sequencer was released in late 2006 and achieved widespread commercial use by 2007. Barcoding or sample tagging was equally prevalent.

Ex. 12 ¶ 115; *see also* Ex. 6 at 114:24-115:1 (“Ligation would be -- or PCR -- the two most common ways of [tagging cfDNA.]”); Ex. 37 (“It has been well known for many years that barcodes can be added to target nucleic acid sequences either using PCR primers or using ligated adaptors”).

Next generation sequencers could sequence millions of DNA molecules in a single run and thereby made feasible sequencing DNA from different individuals at the same time. To distinguish samples from different individuals, DNA from a particular individual was “tagged” or “indexed” such that each individual sample had a separate tag, index or barcode. Thus, a “tagged” or “indexed” library of that individual’s DNA fragments could be created and the “tag” or “index” could be used to associate a particular DNA with an individual. The advantage of “indexing” was that DNA from different individuals could be pooled and sequenced together in a single reaction.

Ex. 12 ¶ 55.

[The 2010 Fluidigm reference] states: “[i]f desired, *tagged target nucleotide sequences generated as described herein* may be analyzed by *DNA sequencing*.”

Id. ¶ 94. Further, Dr. Metzker has opined that the “use of indexed DNA library for multiplexed next generation sequencing was widespread in the art,” and that the use of an “indexed DNA library” is the same as the use of tags. *Id.* at 38, ¶ 59 (“In addition to the term barcode, these molecular codes have been referred to as tags, multiplex identifiers, and indexes”). In a case before the Patent Trial and Appeal Board, Dr. Metzker provided an expert declaration on behalf of Natera in which he provided nine paragraphs of examples of the use of barcoding in the art:

- He cites to McKenna, who used barcoded primers for sequencing. These barcoded primers had a structure of Sequencing Primer-Barcode-Targeted primer, and serve the function to uniquely tag a given sample. *Id.* ¶¶ 60-61, 65.
- As another example, Dr. Metzker describes the Illumina GA sequencer, which he described as “the most commonly used next-generation sequencer” as of 2010. The first step of the workflow of the Illumina GA sequencer was DNA library preparation, which would have included barcoding. *Id.* ¶ 62.
- As yet another example, Dr. Metzker describes Bentley, which ligated adaptors to sheared DNA fragments for amplification and sequencing. *Id.* ¶ 63.
- As yet another example, Dr. Metzker cited to Quail, which used the Illumina GA system with barcoded primers to analyze DNA fragments and generate an indexed library. *Id.* ¶ 64.
- As yet another example, Dr. Metzker describes Craig, which provided a generalized framework for multiplexed resequencing of targeted genome regions. In the described protocol, “a unique indexed-adaptor sequences [*sic*] was ligated” to DNA fragments. *Id.* ¶ 67.

355. In addition to the Illumina GA sequencer system from 2010, Dr. Metzker described even earlier systems wherein barcoding was widespread and used in the art:

As discussed above, in the relevant timeframe, next-generation sequencing was in widespread use. In fact, 454 Life Sciences Corporation's sequencer, known as Genome Sequencer 20, was commercially in use by 2005. Likewise, Solexa's next generation sequencer was released in late 2006 and achieved widespread commercial use by 2007. ***Barcoding or sample tagging was equally prevalent.***

Id. ¶ 115.

356. As yet another example, as early as 2009, it was known that a universal PCR could be used with primers that included sequencing oligos and barcodes, such as what was taught by Varley and Mitra. D.I. 52-5 at 1849 (“The universal PCR used primers tailed with ***454 Life Sciences A or B oligo at the 5’ end, followed by a sample-specific DNA sequence*** and ending at the 3’ end with the same universal primer sequence ligated to the amplicons in the Nested Patch PCR procedure.”).

357. In sum, the '035 patent and Natera's expert witness show that the technique of tagging cfDNA as claimed in the '035 patent was well-known, routine, and conventional in 2011.

b. The '035 Patent, Natera's Expert Witnesses, and Natera's Own Admissions State That DNA Amplification Was a Well-Understood, Routine, and Conventional Technique by 2011

358. Claim 1 of the '035 patent recites methods for “amplifying the tagged products one or more times to generate final amplification products.” D.I. 1-2 at claim 1. DNA amplification, including PCR, was well known to a POSA.

359. The written description of the '035 patent includes the following exemplary disclosures, describing various amplification techniques, including PCR, that it characterizes as routine, and available at the time of the filing to perform the claimed methods:

Performing a highly multiplexed PCR amplification using methods known in the art results in the generation of primer dimer products that are in excess of the

desired amplification products and not suitable for sequencing.

Id. at 86:10-13 (emphasis added).

In some embodiments, the preparation of the DNA may involve ***amplification***, separation, purification by chromatography, liquid separation, isolation, preferential enrichment, preferential amplification, targeted amplification, ***or any of a number of other techniques either known in the art*** or described herein.

Id. at 131:32-37 (emphasis added).

If desired, any of the PCR conditions disclosed herein or 35 ***any standard PCR conditions can be used*** to test a primer library to determine, e.g., the percent of primer dimers, percent of target amplicons, and percent of target loci that are amplified. If desired, standard methods can be used to optimize the reaction conditions to improve the performance of a primer library.

Id. at 125:35-41 (emphasis added).

[S]imultaneous amplification of many target nucleic acids in a sample of interest can be carried out by combining many oligonucleotide primers with the sample and then subjecting the sample to polymerase chain reaction (PCR) conditions in a process known in the art as multiplex PCR.

Id. at 2:66-3:4.

Some embodiments of the present disclosure involve the use of “Linked Inverted Probes” (LIPs), which have been previously described in the literature, to amplify the target loci before or after amplification using primers that are not LIPs in the multiplex PCR methods of the invention.

Id. at 111:11-15.

In some embodiments, the PCR products are sequenced as described 60 in Example 15 or using standard sequencing methods.

Id. at 129:59-61.

There are also sequencing methods, for example the ILLUMINA SO LEXA GENOME SEQUENCER or the ABI SOLID GENOME SEQUENCER, wherein the genetic sequence of fragments of DNA are sequenced; upon extension of the strand of DNA complementary to the strand being sequenced, the identity of the extended nucleotide is typically detected via a fluorescent or radio tag appended to the complementary nucleotide.

Id. at 184:26-34.

The following protocol was used for 800-plex amplification of DNA isolated from maternal plasma from a euploid pregnancy and also genomic DNA from a triploidy 21 cell line using standard PCR (meaning no nesting was used).

Id. at 210:26-34.

An aliquot of the STA products was then amplified by standard PCR for 10 cycles with 1 uM of tag-specific forward and barcoded reverse primers to generate barcoded sequencing libraries.

Id. at 214:33-36.

An aliquot of the STA products was then amplified by standard PCR for 15 cycles with 1 uM of tag-specific forward and barcoded reverse primers to generate barcoded 45 sequencing libraries.

Id. at 214:41-43.

An aliquot of the STAR 2 products was then amplified by standard PCR for 12 cycles with 1 uM of tag-specific forward and barcoded reverse primers to generate barcoded sequencing libraries.

Id. at 218:12-16.

360. Likewise, the '035 patent cites to a wealth of prior art publications describing routine, standard, amplification techniques known in the art, including PCR (all emphases below are added):

'035 patent at 7:

- Binladen, J. et al., "The Use of Coded **PCR** Primers Enables High-Throughput Sequencing of Multiple Homolog **Amplification** Products by 454 Parallel Sequencing", PLOS One, Issue 2, Feb. 2007, 9 pages.
- Siebert, P. D. et al., "An improved **PCR** method for walking in uncloned genomic DNA", Nucleic Acids Research, vol. 23, No. 6, 1995, 1087-1088.
- Tewhey, R. et al., "Microdroplet-based **PCR enrichment** for largescale targeted sequencing", Nature Biotechnology, vol. 27, No. 11, Nov. 2009, 1025-1031.

'035 patent at 9:

- Cheung, V. G. et al., "**Whole genome amplification** using a degenerate oligonucleotide primer allows hundreds of genotypes to be performed on less than

one nanogram of genomic DNA”, Proceedings of the National Academy of Sciences, USA, vol. 93, Dec. 1996, 14676-14679.

'035 patent at 10:

- D'Aquila, Richard et al., “Maximizing sensitivity and specificity of **PCR** by pre-**amplification** heating”, Nucleic Acids Research, 19(13), 1991, p. 3749.
- Dietmaier, W. et al., “Multiple Mutation Analyses in Single Tumor Cells with Improved **Whole Genome Amplification**”, American Journal of Pathology, vol. 154, No. 1, Jan. 1999, 83-95.
- Fredriksson, et al., “**Multiplex amplification** of all coding sequences within 10 cancer genes by Gene-Collector”, Nucleic Acids Research, 2007, vol. 35, No. 7 e47, 1-6.

'035 patent at 11:

- Handyside, et al., “Isothermal **whole genome amplification** from single and small numbers of cells: a new era for preimplantation genetic diagnosis of inherited disease”, Molecular Human Reproduction vol. 10, No. 10 pp. 767-772, 2004.
- Harismendy, O. et al., “Method for Improving Sequence Coverage Uniformity of Targeted Genomic Intervals Amplified by LR-**PCR** Using Illumina GA Sequencing-By-Synthesis Technology”, Bio Techniques, 46(3), 2009, 229-231.
- Hawkins, T. et al., “**Whole genome amplification**-applications and advances”, Current Opinion in Biotechnology, 13, 2002, 65-67.
- Hayden, et al., “**Multiplex-Ready PCR**: A new method for multiplexed SSR AND SNP genotyping”, BMC Genomics 2008, 9(80), 1-12.
- Hellani, A. et al., “Clinical Application of Multiple Displacement **Amplification** in Preimplantation Genetic Diagnosis”, Reproductive BioMedicine Online, 10 (3), 2005, 376-380.
- Hellani, Ali et al., “Multiple displacement **amplification** on single cell and possible PGD applications”, Molecular Human Reproduction, 10(11), 2004 847-852.

'035 patent at 13:

- Liao, J. et al., “An Alternative Linker-Mediated Polymerase Chain Reaction Method Using a Dideoxynucleotide to Reduce **Amplification** Background”, Analytical Biochemistry 253, 137-139 (1997).
- Lo, et al., “Prenatal Sex Determination by **DNA Amplification** from Maternal Peripheral Blood”, The Lancet, 2, 8676, 1989, 1363-1365.
- Lo, Y-M.D et al., “Prenatal Determination of Fetal Rhesus D Status by **DNA Amplification** of Peripheral Blood of Rhesus-Negative Mothers”, Annals New York Academy of Sciences, 731, 1994, 229-236.
- Mamon, H. et al., “Letters to the Editor: Preferential **Amplification of Apoptotic DNA** from Plasma: Potential for Enhancing Detection of Minor DNA Alterations in Circulating DNA”, Clinical Chemistry, vol. 54, No. 9, 2008, 1582-1584.

'035 patent at 14:

- Paez, Guillermo J. et al., “Genome coverage and sequence fidelity of cp29 polymerase-based multiple strand displacement *whole genome amplification*”, Nucleic Acids Research, 32(9), 2004, 1-11.

’035 patent at 15:

- Pertl, B. et al., “Detection of Male and Female Fetal DNA in Maternal Plasma by Multiplex Fluorescent *Polymerase Chain Reaction Amplification* of Short Tandem Repeats”, Hum. Genet., 106, 2000, 45-49.
- Pirker, C. et al., “*Whole Genome Amplification* for CGH Analysis: Linker-Adapter PCR as the Method of Choice for Difficult and Limited Samples”, Cytometry Part A, vol. 61A, 2004, 26-34.
- Porreca, Gregory J et al., “*Multiplex Amplification* of Large Sets of Human Exons”, Nature Methods, 4, (advance online publication), 2007, 6.

’035 patent at 16:

- Rychlik, et al., “Optimization of the annealing temperature for *DNA amplification* in vitro”, Nucleic Acids Research, 18(21), 1990, 6409-6412.
- Spertini, D. et al., “Screening of Transgenic Plants by *Amplification* of Unknown Genomic DNA Flanking T-DNA”, Bio Techniques, vol. 27, Aug. 1999, 308-314.
- Spits, C et al., “Optimization and Evaluation of *Single-Cell Whole Genome Multiple Displacement Amplification*”, Human Mutation, 27(5), 496-503, 2006.

’035 patent at 17:

- Wang, W. P. et al., “Multiplex single nucleotide polymorphism genotyping by adapter ligation-mediated allele-specific *amplification*”, Analytical Biochemistry, vol. 355, May 5, 2006, 240-248.

’035 patent at 18:

- Wells, Dagan, “Detailed Chromosomal and Molecular Genetic Analysis of Single Cells by *Whole Genome Amplification* and Comparative Genomic Hybridisation”, Nucleic Acids Research, 27, 4, 1999, 1214-1218.
- You, Frank M. et al., “BatchPrimer3: A high throughput web application for *PCR* and Sequencing Primer Design”, BMC Bioinformatics, Biomed Central, London, GB, vol. 9, No. 1, (May 29, 2008), p. 253.
- Wong, K. K. et al., “Allelic imbalance analysis by high-density single nucleotide polymorphic allele (SNP) array with *whole genome amplified DNA*”, Nucleic Acids Research, vol. 32, No. 9, May 17, 2004, 8 pages.
- Zhang, L. et al., “*Whole genome amplification* from a single cell: Implications for genetic analysis”, Proc. Nat’l. Acad. Sci. USA, vol. 89, Jul. 1992, 5847-5851.

’035 patent at 19:

- Lasken, R. S., et al., “*Whole genome amplification*: abundant supplies of DNA from precious samples or clinical specimens”, Trends in Biotechnology, Dec. 2003, 531-535.

- Pask, R., et al., “Investigating the utility of combining **whole genome amplification** and highly multiplexed single nucleotide polymorphism BeadArray™ genotyping”, BMC Biotechnology, Jul. 27, 2004, 8 pages.
- Paunio, T., et al., “Preimplantation diagnosis by **whole-genome amplification, PCR amplification**, and solid-phase minisequencing of blastomere DNA”, Clinical Chemistry, 1996, 1382-1390.
- Ruano, G., et al., “Haplotype of multiple polymorphisms resolved by enzymatic **amplification** of single DNA molecules”, Proc. Nati. Acad. Sci. USA, Aug. 1990, 6296-6300.

’035 patent at 20:

- Fortina, P. et al., “DOP-PCR **Amplification of Whole Genomic DNA** and Microchip-Based Capillary Electrophoresis”, Methods in Molecular Biology: Capillary Electrophoresis of Nucleic Acids, vol. II Practical Applications of Capillary Electrophoresis, 2001, 211-219.
- Hosono, S. et al., “Unbiased **Whole-Genome Amplification** Directly From Clinical Samples”, Genome Research, vol. 13, 2003, 954-964.
- Nishigaki, K. et al., “Random **PCR**-Based Genome Sequencing: A Non-Divide-and-Conquer Strategy”, DNA Research, vol. 7, 2000, 19-26.
- Zheng, S. et al., “**Whole Genome Amplification** Increases the Efficiency and Validity of Buccal Cell Genotyping in Pediatric Populations”, Cancer Epidemiology, Biomarkers & Prevention, vol. 10, Jun. 2001, 697-700.

’035 patent at 21:

- Bergen, A. W. et al., “Effects of DNA mass on multiple displacement **whole genome amplification** and genotyping performance”, BMC Biotechnology, vol. 5, No. 24, Sep. 16, 2005, 11 pgs.

’035 patent at 22:

- Lovmar, L. et al., “Quantitative evaluation by minisequencing and microarrays reveals accurate multiplexed SNP genotyping of **whole genome amplified DNA**”, Nucleic Acids Research, vol. 31, No. 21, 2003, 9 pgs.
- McDonald, J. P. et al., “Novel thermostable Y-family polymerases: applications for the **PCR amplification** of damaged or ancient DNAs”, Nucleic Acids Research, vol. 34, No. 4, 2006, 1102-1111.
- Broude, N. E., et al., “**High Multiplexity PCR** Based on PCR Suppression”, **DNA Amplification Current Technologies and Applications**, 2004, 61-76.
- Broude, N. E., et al., “High-Level **Multiplex DNA Amplification**”, Antisense & Nucleic Acid Drug Development, vol. 11, 2001, 327-332.
- Broude, N. E., et al., “Multiplex Allele-specific Target **Amplification** based on PCR Suppression”, PNAS, vol. 98, No. 1, Jan. 2, 2001, 206-211.
- Frohman, M. A., et al., “On Beyond Classic RACE (Rapid **Amplification** of cDNA Ends)”, Genome Research, vol. 4, 1994, S40-S58.

'035 patent at 23:

- Kuhn, H., et al., "Rolling-circle **amplification** under topological constraints", Nucleic Acids Research, 2002, 574-580.
- Schoske, R., et al., "Multiplex PCR Design Strategy used for the Simultaneous **Amplification** of 10 Y Chromosome Short Tandem Repeat (STR) Loci", Analytical and Bioanalytical Chemistry, vol. 375, 2003, 333-343.
- Dhara, O., et al., "One-sided Polymerase Chain Reaction: The **Amplification** of cDNA", Proceedings of the National Academy of Sciences, vol. 86, 1989, 5673-5677.
- Treff, N. R., et al., "Single Cell **Whole Genome Amplification** Technique Significantly Impacts the Accuracy and Precision of Microarray Based 23 Chromosome Aneuploidy Screening", Poster Presentations Preimplantation Genetic Diagnosis, Sep. 01 2007, S231.
- Troutt, et al., "Ligation-anchored **PCR**: A Simple Amplification Technique with Single-sided Specificity", Proceedings of the National Academy of Sciences, vol. 89, Oct. 1992, 9823-9825.

'035 patent at 24:

- Dorit, D. L., "cDNA **Amplification** Using One-sided (Anchored) **PCR**", Current Protocols in Molecular Biology, vol. 17, 1992, pp. 15.6.1-15.6.10.
- Dorit, Robert L. et al., "One-sided Anchored **Polymerase Chain Reaction for Amplification** and Sequencing of Complementary DNA", Methods in Enzymology, vol. 218, 1993, pp. 36-47.
- Loh, Elwyn, "Anchored **PCR: Amplification** with Single-sided Specificity", Methods, vol. 2, 1991, pp. 11-19.
- Meuzelaar, Linda S. et al., "Megaplex b: A Strategy for **Multiplex Amplification**", Nature Methods, vol. 4, 2007, pp. 835-837.

'035 patent at 25:

- Vallone, Peter M. et al., "Demonstration of Rapid **Multiplex PCR Amplification** Involving 16 Genetic Loci", Forensic Science International: Genetics, vol. 3, 2008, pp. 42-45.
- Watt, Heather L., "Sex Diagnosis of Preimplantation Porcine Embryos through **PCR Amplification** of The SRY Gene", Sex Diagnosis of Preimplantation Porcine Embryos Through PCR Amplification of the Sry Gene (1998) 1998, 151 pages.

'035 patent at 26:

- Olerup, O. et al., "HLA-DR typing by **PCR amplification** with sequence-specific primers (PCR-SSP) in 2 hours: an alternative to serological Dr typing in clinical practice including donor-recipient matching in cadaveric transplantation", Tissue Antigens, vol. 39, No. 5, May 1992, 225-235.
- Dahl, et al., "**Multigene Amplification** and Massively Parallel Sequencing for Cancer Mutation Discovery", Proceedings of the National Academy of Sciences, vol. 104, No. 22, May 29, 2007, 9387-9392.

- Paruzynski, A. et al., “Genome-wide high-throughput integrome analyses by nrLAM-**PCR** and next-generation sequencing”, Nature Protocols, vol. 5, No. 8, Jul. 8, 2010, 1379-1395.

’035 patent at 28:

- Pinard, et al., “Assessment of **Whole Genome Amplification** induced Bias Through High-throughput, Massively Parallel Whole Genome Sequencing”, BMC Genomics, vol. 7:216, Aug. 23, 2006, 1-21.

’035 patent at 29:

- Glaab, W. E. et al., “A novel assay for allelic discrimination that combines the fluorogenic 5’ nuclease polymerase chain reaction (TaqMan) and mismatch **amplification** mutation assay”, Mutation Research, vol. 430, 1999, 12 pgs.

361. Likewise, Varley states:

This is the first method that couples multiplex PCR with sample-specific DNA barcodes and next-generation sequencing to enable highly multiplex mutation discovery in candidate genes for multiple samples in parallel.

Id. at Abstract.

Sample-specific DNA barcodes are then incorporated into the primers used for the final universal PCR by tailing the 5’ end with sample-specific DNA sequences and 454 sequencing primers.

Id. at 1845.

362. Further, Natera’s expert, Dr. Michael Metzker, has repeatedly acknowledged that amplification, including PCR, was well known in the art by 2011:

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....As such, one of ordinary skill would have had a reasonable expectation of success in using cell-free DNA from maternal blood in a multi-step PCR method (such as Fluidigm’s).

Ex. 12 ¶ 116 (emphasis added).

To perform NGS, sequencing libraries were often created from samples using PCR amplification. PCR-based library preparation was well-known and widely-used by the time the first application leading to the ’831 Patent was filed.

Id. ¶ 54.

As described above in this declaration, indexed sequences were routinely added by PCR as well. For example, Illumina described adding index sequences to its adaptor-ligated DNA fragments using a PCR enrichment step. Following additional library steps, each HapMap sample was pooled into a single tube and subjected to a PCR enrichment step using Illumina compatible primers.

Id. ¶ 67. Likewise, at deposition, Dr. Metzker admitted that the 2010 Fluidigm reference discloses multiplex PCR of over 9,000 targets, that a “person of ordinary skill in the art would understand how PCR works,” “one of ordinary skill would have understood how the PCR cycle works,” and that “[l]igation would be -- or PCR -- the two most common ways of [tagging cfDNA].” Ex. 6 at 87:24-88:6, 93:1-2, 94:6-7, 99:4-5, 114:24-115:1. Dr. Metzker acknowledged at trial that PCR “make copies of your target,” in other words, PCR is an amplification technique. Ex. 36 at 902:19-21.

363. Additionally, Natera’s expert, Dr. Quackenbush, testified that amplification was routine and conventional as early as 1985, well before 2011:

Q. And how far back were persons of skill in the art using PCR to do selected amplification?

A. So Kary Mullis, I described in 1985 Kary Mullis was doing it in 1985. It was a rapidly adopted technology because it was so powerful.

Ex. 11 at 88:14-18.

364. Dr. Quackenbush, also opined that the claimed amplification technique had been “universally adopted” as a standard tool by 2000:

PCR-based tools for genotyping SNPs, including for genotyping up to hundreds of thousands of SNPs, were commercially available as far back as 1995. ***By 2000, PCR had been universally adopted as a standard tool*** in applications of molecular biology, and it was already successful in the simultaneous detection of thousands of SNPs...***PCR was a well-established, conventional and indispensable tool*** for genetic testing that was routinely used to target and amplify specific, pre- selected genes for further study.

Ex. 20 ¶¶ 82-83 (emphasis added).

365. Dr. Quackenbush has also stated that he had significant experience with PCR techniques in the 1990s:

Starting in 1992, I ran a large program at the Salk Institute using PCR to assay polymorphic genetic markers to produce a map of human chromosome 11, a project that required tens of thousands of multiplexed PCR reactions. Between 1994 and 1996, while at Stanford University, I was responsible for the design and conduct of hundreds of thousands of PCR reactions for mapping the entire human genome and as part of developing a strategy for sequencing regions of human chromosomes 4 and 21. After joining The Institute for Genomic Research in 1997, I was responsible for projects involving hundreds of thousands of PCR reactions for genome sequencing and microarray analysis. And after joining the faculty at the Dana-Farber Cancer Institute and Harvard School of Public Health, I led large-scale projects analyzing gene expression in cancer and establishing high-throughput genome sequencing that required PCR and other amplification reactions from diverse biological samples, including cell lines, human tissue samples, circulating tumor cells, and circulating cell-free DNA. In all of these projects, I used standard and routine PCR applications for genotyping.

Id. ¶ 87. Further, Dr. Quackenbush has opined that “well before 2013 several techniques had been developed to generate or isolate copies of DNA molecules of interest to facilitate sequencing.” Ex. 7 ¶ 66. Likewise, Dr. Quackenbush cites to a document he describes as stating that PCR is among the most widely used approaches to target enrichment. *Id.* at ¶ 68.

366. Nor is multiplexing more than 25 loci in a single volume an inventive concept. The ’035 patent acknowledges that it was known in the art that up to 100 loci could be multiplexed in a single volume. D.I. 1-2 at 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....”), 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.”). Likewise, the ’035 patent states that it was built on prior art, which included “fundamental methods” that “underlie the methods disclosed.” *Id.* at 93:41-58 (citing D.I. 52-5, D.I. 52-6, and D.I. 53-6). Varley provides an example of these

“fundamental methods,” and teaches multiplexing of 90 loci in a single volume. D.I. 52-5 at 1845 (“we designed oligonucleotides for **94 exons** from six genes that cause cancer when mutated in the germline (TP53, APC, MLH1, RB1, BRCA1, VHL)”). The ’035 patent mischaracterizes Varley as only teaching multiplexing of 9 loci in a single volume. D.I. 1-2 at 53-58 (“A method comprising multiplexing of an average of 9 assays for sequencing is described in” Varley.). As the ’035 patent acknowledges that 90- and 100-plexes were in the prior art, the use of 25-plexes cannot be an inventive concept.

367. Natera has twice taken the position that selective multiplex amplification was a well-understood, routine, and conventional method by 2009. Ex. 20 ¶ 8; Ex. D.I. 52-9 at 50 (“There is a good reason for this: the patentee admitted selective amplification was routine and conventional—so much so that it was unnecessary to provide any guidance on how to apply it to 1,000 SNPs.”) (citations omitted). Additionally, in *Illumina, Inc. v. Natera, Inc.*, Natera argued that methods of DNA amplification were known in the art by 2010. Ex. 34 at 5. If such techniques were conventional in 2009 and 2010, they were surely conventional by 2011.

368. In sum, the ’035 patent, Natera’s expert witnesses, and Natera’s own admissions show that the technique of amplifying cfDNA as claimed in the ’454 patent was well-known, routine, and conventional in 2011.

c. The ’035 Patent, Natera’s Expert Witnesses, and Natera’s Own Admissions State That DNA Sequencing Was a Well-Understood, Routine, and Conventional Method in 2011

369. Claim 1 of the ’035 patent recites “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.” D.I. 1-2 at claim 1. Gene sequencing was well known to a POSA.

370. Nowhere does the '035 patent state that the methods for sequencing described in the patent specification involve anything new, innovative, or novel. To the contrary, the '035 patent cites to a wealth of prior art publications describing routine, standard, sequencing techniques known in the art (all emphases below are added):

'035 patent at 7:

- Binladen, J. et al., "The Use of Coded PCR Primers Enables **High-Throughput Sequencing** of Multiple Homolog Amplification Products by 454 Parallel Sequencing", PLOS One, Issue 2, Feb. 2007, 9 pages.
- Gnirke, A. et al., "Solution hybrid selection with ultra-long oligonucleotides for **massively parallel targeted sequencing**", Nature Biotechnology, vol. 27, No. 2, Feb. 2009, 182-189.
- Tewhey, R. et al., "Microdroplet-based PCR enrichment for **largescale targeted sequencing**", Nature Biotechnology, vol. 27, No. 11, Nov. 2009, 1025-1031.

'035 patent at 8:

- Bentley, David R et al., "Accurate **Whole Human Genome Sequencing** Using Reversible Terminator Chemistry", Nature, 456, 6, 2008, 53-59.
- Bermudez, M. et al., "**Single-cell sequencing and mini-sequencing** for preimplantation genetic diagnosis", Prenatal Diagnosis, 23, 2003, 669-677.
- Alkan, Can et al., "Personalized Copy Number and Segmental Duplication Maps Using **Next-Generation Sequencing**", Nature Genetics, 41, 10, 2009, 1061-1068.

'035 patent at 9:

- Chiu, Rossa W.K. et al., "Maternal Plasma DNA Analysis with **Massively Parallel Sequencing** by Litigation for Noninvasive Prenatal Diagnosis of Trisomy 21", Clinical Chemistry, 56, 3, 2010, 459-463.
- Chiu, Rossa W.K. et al., "Noninvasive Prenatal Diagnosis of Fetal Chromosomal Aneuploidy by **Massively Parallel Genomic Sequencing** of DNA in Maternal Plasma (with Supporting Information)", PNAS, vol. 105, No. 51, 2008, 20458-20463.
- Choi, M. et al., "Genetic diagnosis by whole exome capture and **massively parallel DNA sequencing**", PNAS, vol. 106, No. 45, Nov. 10, 2009, 19096-19101.
- Chu, Tianjiao et al., "Statistical Model for **Whole Genome Sequencing** and its Application to Minimally Invasive Diagnosis of Fetal Genetic Disease", Bioinformatics, 25(10), 2009, 1244-1250.
- Craig, D. W. et al., "Identification of genetic variants using **barcoded multiplexed sequencing**", Nature Methods, vol. 5, Oct. 2008, 887-893.

'035 patent at 10:

- Fan, Christina H et al., “Noninvasive Diagnosis of Fetal Aneuploidy by **Shotgun Sequencing** DNA from Maternal Blood”, PNAS, 105, 42, 2008, 16266-16271.
- Fan, H. Christina et al., “Sensitivity of Noninvasive Prenatal Detection of Fetal Aneuploidy from Maternal Plasma Using **Shotgun Sequencing** Is Limited Only by Counting Statistics”, PLoS One, vol. 5, Issue 5 (e10439), May 3, 2010, 1-6.
- Dohm, J. et al., “Substantial Biases in Ultra-Short Read Data Sets From **High-Throughput DNA Sequencing**”, Nucleic Acids Research, 36 (16), e105, 2008, 10 pgs.

’035 patent at 11:

- Harismendy, O. et al., “Method for Improving Sequence Coverage Uniformity of Targeted Genomic Intervals Amplified by LR-PCR Using Illumina GA **Sequencing-By-Synthesis** Technology”, Bio Techniques, 46(3), 2009, 229-231.
- Hollas, B. et al., “A stochastic approach to count RNA molecules using DNA **sequencing** methods”, Lecture Notes in Computer Science, vol. 2812, 2003, 55-62.

’035 patent at 12:

- Jarvie, T., “**Next generation sequencing technologies**”, Drug Discovery Today: Technologies, vol. 2, No. 3, 2005, 255-260.
- Leary, R. J. et al., “Development of Personalized Tumor Biomarkers Using **Massively Parallel Sequencing**”, Science Translational Medicine, vol. 2, No. 20, Feb. 24, 2010, 1-8.
- Li, R. et al., “SNP detection for **massively parallel whole-genome resequencing**”, Genome Research, vol. 19, 2009, 1124-1132.

’035 patent at 13:

- Lo, Y.M. Dennis et al., “Maternal Plasma DNA **Sequencing** Reveals the Genome-Wide Genetic and Mutational Profile of the Fetus”, Science Translational Medicine, 2 (61), 2010, 13.
- Mardis, E. R., “The impact of **next-generation sequencing** technology on genetics”, Trends in Genetics, vol. 24, No. 3, Feb. 11, 2008, 133-141.
- Margulies, M. et al., “Genome **sequencing** in microfabricated highdensity picolitre reactors”, Nature, vol. 437, Sep. 15, 2005, 376-380.
- Margulies, M. et al., “Genome **sequencing** in microfabricated highdensity picolitre reactors plus Supplemental Methods”, Nature, vol. 437, Sep. 15, 2005, 40 pgs.

’035 patent at 17:

- Ten Bosch, J., “Keeping Up With the **Next Generation Massively Parallel Sequencing** in Clinical Diagnostics”, Journal of Molecular Diagnostics, vol. 10, No. 6, 2008, 484-492.

’035 patent at 18:

- You, Frank M. et al., “BatchPrimer3: A high throughput web application for PCR and **Sequencing** Primer Design”, BMC Bioinformatics, Biomed Central, London, GB, vol. 9, No. 1, May 29, 2008 (May 29, 2008), p. 253.

’035 patent at 19:

- “Illumina, “Preparing Samples for **Sequencing** Genomic DNA”, Part # 11251892 Rev. A, 2007, 18 pages.
- “Illumina, “Technology: Solexa **Sequencing** Technology”, https://web.archive.org/web/20070521_081517_/http://www.Illumina.com/pages.ilmn?ID203, May 21, 2007, 1 page.
- Robertson, G., et al., “Genome-wide profiles of STAT! DNA association using chromatin immunoprecipitation and **massively parallel sequencing**”, Nature Methods, Aug. 2007, 651-657.

’035 patent at 20:

- Eltoukhy, H. et al., “Modeling and Base-Calling for DNA **Sequencing-By-Synthesis**”, IEEE, 2006, II-1032-II-1035.
- Meyerson, M. et al., “Advances in understanding cancer genomes through **second-generation sequencing**”, Nature Reviews: Genetics, vol. 11, Oct. 2010, 685-696.
- Nishigaki, K. et al., “Random PCR-Based Genome **Sequencing**: A Non-Divide-and-Conquer Strategy”, DNA Research, vol. 7, 2000, 19-26.
- Shendure, J. et al., “Next-generation DNA **sequencing**”, Nature Biotechnology, vol. 26, No. 10, Oct. 2008, 1135-1145.

’035 patent at 22:

- Campbell, P. J., et al., “Subclonal phylogenetic structures in cancer revealed by **ultra-deep sequencing**”, PNAS, Sep. 2, 2008, 13081-13086.

’035 patent at 23:

- Meyer, M, et al., “Illumina **Sequencing** Library Preparation for Highly Multiplexed Target Capture and **Sequencing**”, Cold Spring Harbor Protocols, vol. 2010, Issue 6, Jun. 2010, 1-10.

’035 patent at 24:

- Dorit, Robert L. et al., “One-sided Anchored Polymerase Chain Reaction for Amplification and **Sequencing** of Complementary DNA”, Methods in Enzymology, vol. 218, 1993, pp. 36-47.
- Hu, Hao et al., “Mutation Screening in 86 Known X-linked Mental Retardation Genes by Droplet-based Multiplex Per and **Massive Parallel Sequencing**”, Hao Hu. et al., "Mutation Screening in 86 Known X-linked Mental Retardation Genes by Droplet-based Multiplex Per and **Massive Parallel Sequencing**", Hugo J, Dec. 2009, vol. 3, pp. 41-49., Dec. 1, 2009, 41-49.
- Marguiles, M. et al., “Genome **Sequencing** in Open Microfabricated High Density Picoliter Reactors”, Nature, vol. 437, No. 7057, Sep. 15, 2005, 376-380.

'035 patent at 25:

- Witherspoon, David J. et al., "Mobile Element Scanning (Me-scan) by Targeted **High-throughput Sequencing**", BMC Genomics, vol. 410, 2010, 15 pages.
- Bau, Stephan et al., "Targeted **next-generation sequencing** by specific capture of multiple genomic loci using low-volume microfluidic DNA arrays", Anal Bioanal Chem, vol. 393, 2009, 171-175.

'035 patent at 26:

- Paruzynski, A. et al., "Genome-wide high-throughput integrome analyses by nrLAM-PCR and **next-generation sequencing**", Nature Protocols, vol. 5, No. 8, Jul. 8, 2010, 1379-1395.
- Ansorge, Wilhelm J., "**Next-generation DNA Sequencing** Techniques", New Biotechnology, vol. 25, No. 4, Feb. 2, 2009, 195-203.
- Barbazuk, et al., "SNP Discovery via 454 Transcriptome **Sequencing**", The Plant Journal, vol. 51, Jul. 27, 2007, 910-918.
- Beck, et al., "**Next Generation Sequencing** of Serum Circulating Nucleic Acids from Patients with Invasive Ductal Breast Cancer Reveals Differences to Healthy and Nonmalignant Controls", Molecular Cancer Research, vol. 8, No. 3, Mar. 1, 2010, 335-342.
- Bender, et al., "A Multiplex SNP Typing Approach for the DNA **Pyrosequencing** Technology", International Congress Series, vol. 1288, Apr. 20, 2006, 73-75.
- Bentley, et al., "High-resolution, High-throughput HLA Genotyping by **Next-generation Sequencing**", Tissue Antigens, vol. 74, No. 5, Nov. 1, 2009, 393-403.
- Bordoni, et al., "Evaluation of Human Gene Variant Detection in Amplicon Pools by the GS-FLX Parallel **Pyrosequencer**", BMC Genomics, vol. 9, Oct. 8, 2008, 1-8.
- Brockman, et al., "Quality Scores and SNP Detection in **Sequencing-by-synthesis** Systems", Genome Research, vol. 18, No. 5, May 1, 2008, 763-770.
- Church, et al., "**Multiplex DNA Sequencing**", Science, vol. 240, No. 4849, Apr. 8, 1988, 185-188.
- Dahl, et al., "Multigene Amplification and **Massively Parallel Sequencing** for Cancer Mutation Discovery", Proceedings of the National Academy of Sciences, vol. 104, No. 22, May 29, 2007, 9387-9392.
- Doostzadeh, et al., "High Throughput Automated Allele Frequency Estimation by **Pyrosequencing**", PLoS One, vol. 3, No. 7, Jul. 16, 2008, 1-4.
- Hoberman, Rose et al., "A Probabilistic Approach for SNP Discovery in High-throughput Human **Resequencing** Data", Genome Research, vol. 19, Jul. 15, 2009, 1542-1552.

'035 patent at 27:

- Holt, et al., "Detecting SNPS and Estimating Allele Frequencies in Clonal Bacterial Populations by **Sequencing** Pooled DNA", Bioinformatics, vol. 25, No. 16, Aug. 15, 2009, 2074-2075.

- Illumina, “Genomic **Sequencing**”, Data Sheet: Sequencing, 2010, 38939-38944.
- Ingman, et al., “SNP Frequency Estimation Using **Massively Parallel Sequencing** of Pooled DNA”, European Journal of Human Genetics, vol. 17, No. 3, Oct. 15, 2008, 383-386.
- Karger, et al., “DNA **Sequencing** by Capillary Electrophoresis”, Electrophoresis, vol. 30, Supplement 1, Jun. 1, 2009, 1-11.
- Koboldt, et al., “VarScan: Variant Detection in **Massively Parallel Sequencing** of Individual and Pooled Samples”, Bioinformatics, vol. 25, No. 17, Jun. 19, 2009, 2283-2285.
- Lavebrat, et al., “Single Nucleotide Polymorphism (SNP) Allele Frequency Estimation in DNA Pools Using **Pyrosequencing**”, Nature Protocols, vol. 1, No. 6, Jan. 11, 2007, 2573-2582.
- Lavebratt, Catharina et al., “**Pyrosequencing**-based SNP Allele Frequency Estimation in DNA Pools”, Human Mutation, vol. 23, Issue 1, Dec. 19, 2003, 92-97.
- Li, et al., “Mapping Short DNA **Sequencing** Reads and Calling Variants Using Mapping Quality Scores”, Genome Research, vol. 18, No. 11, Aug. 19, 2008, 1851-1858.
- Li, et al., “Multiplex Padlock Targeted **Sequencing** Reveals Human Hypermutable CpG Variations”, Genome Research, vol. 19, No. 9, Jun. 12, 2009, 1606-1615.
- Lo, et al., “**Next-generation Sequencing** of Plasma/Serum DNA: an Emerging Research and Molecular Diagnostic Tool”, Clinical Chemistry, vol. 55, No. 4, Apr. 1, 2009, 607-608.
- Neve, B. et al., “Rapid SNP Allele Frequency Determination in Genomic DNA Pools by **Pyrosequencing**”, Bio Techniques, vol. 32, No. 5, May 1, 2002, 1138-1142.

'035 patent at 28:

- Ng, et al., “**Multiplex Sequencing** of Paired-end Ditags (MS-PET): A Strategy for the Ultra-high-throughput Analysis of Transcriptomes and Genomes”, Nucleic Acids Research, vol. 34, No. 12, Jul. 13, 2006, 1-10.
- Pinard, et al., “Assessment of Whole Genome Amplification-induced Bias Through **High-throughput, Massively Parallel Whole Genome Sequencing**”, BMC Genomics, vol. 7:216, Aug. 23, 2006, 1-21.
- Pourmand, et al., “**Multiplex Pyrosequencing**”, Nucleic Acid Research, vol. 30, No. 7, Apr. 1, 2002, 1-5.
- Smith, et al., “Rapid Whole-genome Mutational Profiling using **Next-generation Sequencing** Technologies”, Genome Research, vol. 18, Sep. 4, 2008, 1638-1642.
- Stiller, et al., “Direct Multiplex Sequencing (DMPS)-A Novel Method for Targeted **High-throughput Sequencing** of Ancient and Highly Degraded DNA”, Genome Research, vol. 19, No. 10, Jul. 27, 2009, 1843-1848.
- Voelkerding, et al., “**Next-generation Sequencing**: From Basic Research to Diagnostics”, Clinical Chemistry, vol. 55, No. 4, Apr. 1, 2009, 641-658.

- Wasson, Jon et al., “Assessing Allele Frequencies of Single Nucleotide Polymorphisms in DNA Pools by **Pyrosequencing** Technology”, BioTechniques, vol. 32, No. 5, May 1, 2002, 1144-1152.
- Wiedmann, Ralph T. et al., “SNP Discovery in Swine by Reduced Representation and **High Throughput Pyrosequencing**”, BMC Genetics, vol. 9, Article No. 81, Dec. 4, 2008, 1-7.

'035 patent at 29:

- Xie, et al., “CNV-SEQ, A New Method to Detect Copy Number Variation Using **High-throughput Sequencing**”, BMC Bioinformatics, vol. 10:80, Mar. 6, 2009, 1-9.
- Zhou, et al., “**Pyrosequencing**, A High-throughput Method for Detecting Single Nucleotide Polymorphisms in the Dihydrofolate Reductase and Dihydropteroate Synthetase Genes of Plasmodium Falciparum”, Journal of Clinical Microbiology, vol. 44, No. 11, Nov. 1, 2006, 3900-3910.

371. Further, Varley states:

This is the first method that couples multiplex PCR with sample-specific DNA barcodes and next-generation sequencing to enable highly multiplex mutation discovery in candidate genes for multiple samples in parallel.

D.I. 52-5 at Abstract.

Sample-specific DNA barcodes are then incorporated into the primers used for the final universal PCR by tailing the 5' end with sample-specific DNA sequences and 454 sequencing primers.

Id. at 1845.

372. Likewise, Natera's expert, Dr. Michael Metzker, has repeatedly acknowledged that sequencing was well known in the art by 2011:

As discussed above, in the relevant timeframe, next-generation sequencing was in widespread use....Further, a kit for making a sequencing library for use with Solexa's sequencer was commercially available in 2008, underscoring the routine nature of sequencing library preparation. Thus, one of ordinary skill would have expected success in being able to sequence cell-free DNA found in maternal blood.

Ex. 12 ¶ 115.

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....As such, one of ordinary skill would have had a reasonable

expectation of success in using cell-free DNA from maternal blood in a multi-step PCR method (such as Fluidigm's). One of ordinary skill would have expected reasonable success in identifying sequences from particular chromosomes, because the human genome had already been sequenced by the relevant timeframe. Cell-free genomic DNA from maternal blood had also previously been successfully amplified and sequenced to detect aneuploidy, including fetal trisomy 21, trisomy 18 and trisomy 13. This typically involved amplifying DNA from a suspected chromosome and a reference chromosome and detecting whether levels of sequences of the suspect chromosome were elevated. Given the success in using cell-free DNA to perform amplification and sequencing, one of ordinary skill would have had a reasonable expectation of success to employ cell-free DNA from maternal blood in the Fluidigm method with the goal of testing for chromosomal aneuploidy.

Ex. 12 ¶ 116 (citations omitted).

Since their introduction, NGS platforms have allowed for high-throughput sequencing of nucleic acids on a genome-wide scale and highly multiplexed gene-specific sequencing.

Ex. 12 ¶ 41.

The first NGS platform to achieve widespread commercial use was 454 Life Sciences Corporation's sequencer, also known as Genome Sequencer 20 ("GS20"). The GS20 sequencer first became commercially available in 2005 and produced average read-lengths of ~100 bases.

Ex. 12 ¶ 42.

The Genome Analyzer II ("GAII") was developed by Solexa and released in late 2006. After the acquisition of Solexa by Illumina in 2007, this massively parallel sequencing platform achieved widespread commercial use.

Ex. 12 ¶ 45.

Not only was the concept of creating an indexed DNA sequencing library routine and commonplace, the concept of using cell-free DNA from maternal blood to detect chromosomal aneuploidy, such as trisomy 21, the underlying cause of Down syndrome was equally well-known in the art.

Ex. 12 ¶ 56.

As of January 2010, Illumina's GA sequencer was the most commonly used next-generation sequencer....The GA instrument was capable of sequencing a sample from an individual or multiple samples from many different individuals.

Ex. 12 ¶ 62.

As described above in this declaration, indexed sequences were routinely added by PCR as well. For example, Illumina described adding index sequences to its adaptor-ligated DNA fragments using a PCR enrichment step. Following additional library steps, each HapMap sample was pooled into a single tube and subjected to a PCR enrichment step using Illumina compatible primers.

Ex. 12 ¶ 67.

373. At trial, Dr. Metzker admitted that he “first started working with PCR and DNA sequencing technologies in 1987,” that he published on NGS as early as 1994, that NGS technologies became commercially available in 2005, that between 2005 and 2010 there were “a lot of breakthroughs in new technologies” in NGS, and that by 2011 there were “numerous [MGS] instruments on the market.” Ex. 36 at 974:14-16, 888:21-889:1, 894:12-14, 900:22-901:2, 957:2-6. Likewise, Dr. Metzker wrote a 2010 review article that discussed, *inter alia*, that people had “begun sequencing complete human genomes.” *Id.* at 900:22-901:22; Ex. 37 at 6.

374. Furthermore, Dr. Quackenbush posits that high-throughput sequencing was routine and conventional as of 2010. In his declaration, Dr. Quackenbush states that the sequencing method, claimed in the '035 patent was well-known, commercially available, and routinely used by researchers well before 2011:

[A] method called pyrosequencing, which performs ***sequencing-by-synthesis***, was developed and first published in 1993. It was the foundation for different companies. PYROSEQUENCING AB, out of Uppsala Sweden, launched its first commercial automated pyrosequencing instrument in 1999....Based on the machines made available by Pyrosequencing, by the year 2000, pyrosequencing had established itself as a ***standard and conventional*** means for multiplex or ***high-throughput sequencing***, including for genotyping SNPs.

Ex. 20 ¶103 (citing Marsh 2007, Ahmadian 2000; Nordstrom 2000; Ronaghi 2001).

Another ***high throughput sequencing-by-synthesis*** method developed by a group out of Cambridge University in 1998 became the foundation for a company called Solexa (later acquired by Illumina, Inc.)....As the group that developed this

technology reported in Margulies 2005, the instrument branded as the Genome Analyzer could “sequence 25 million bases, at 99% or better accuracy, in one four-hour run,” B11305- 1309 (Margulies 2005) at B1305 (Abstract), and had an average read length of 108 bases

Id. ¶ 104 (citing Marguiles 2005).

As another example, a high throughput sequencing method co- developed by the named inventor Dr. Quake became the foundation for his company, Helicos, which was founded in 2003, and on which I was a member of the Scientific Advisory Board.

Id. ¶ 106.

375. I understand that Dr. Quackenbush opined that in 2005, there was the expectation that high-throughput sequencing would generate large quantities of data to understand cancer—an expectation that was quickly realized:

For example, when I began working in the biological sciences at the Salk Institute in 1992, the first molecular biology techniques that I learned were PCR and Sanger DNA sequencing. As part of my move to Stanford University in 1994, I was tasked with developing a new large-scale DNA sequencing method based on PCR mapping of transposon insertions. My recruitment to the Dana-Farber Cancer Institute in 2005 was largely based on the expectation that high throughput DNA sequencing would soon generate unprecedented quantities of data that could be used to understand cancer. And that expectation very quickly was realized throughout the genomics field.

Id. ¶ 87.

376. Dr. Quackenbush also noted that in 2005 there was a rapid explosion in the use of sequencing by synthesis instruments:

Due to the rapid explosion in use of sequencing-by-synthesis instruments including the 454 sequencer in 2005, the Illumina Genome Analyzer and Applied Biosystems SOLiD sequencer in 2006, and others shortly thereafter, my group and I quickly became involved in multiplex / high-throughput (or Next Generation Sequencing (“NGS”)) DNA sequencing.

Id. ¶ 95.

377. According to Dr. Quackenbush, there were a number of commercially available sequencers. *See, e.g., Id.* at ¶¶ 103-113 (describing, various commercially available sequencing platforms, including, pyrosequencing, technology available by Helicos Biosciences Corporation, technology available by 454 Lifesciences, Clonal Single Molecule Array (Solexa, Inc.) or sequencing-by-synthesis (SBS) utilizing reversible terminator chemistry; AnyDot chips (Gonovox, Germany); sequencers such as Illumina Genome Analyzer, etc.). Further, Dr. Quackenbush has opined that by “2010, whole-genome sequencing to 30x coverage was routine on the commercially available HiSeq 2000.” Ex. 7 ¶ 64. He likewise opined that by “approximately 2008, techniques for NGS were well-known and commercially available,” that “using NGS, the entire human genome can now be sequenced on a single instrument in a single day,” and that the “development of NGS made more widely available ‘[t]he ability to sequence entire human genomes.’” *Id.* ¶¶ 60-61, 63 (quoting Lam et al. *Performance comparison of whole-genome sequencing platforms*, *Nature Biotechnology* 30:78 (2012)).

378. Natera, through its expert’s declaration, has taken the position that sequencing to identify SNVs was a well-understood, routine, and conventional method by 2009. Ex. 20 ¶ 93 (describing that determining SNPs (SNVs) by “whole genome sequencing or exome sequencing” in 2009 was routine and conventional by reference to patents pre-dating 2009). Additionally, in *Illumina, Inc. v. Natera, Inc.*, Natera argued that methods of sequencing were known in the art by 2010. Ex. 34 at 5 (“the ’831 patent does not recite any new or unknown type of sequencing library”). If such techniques were conventional in 2009 and 2010, they were surely conventional by 2011.

379. I understand that Natera has taken the position that references cited in the prosecution histories of the ’454 patent establish that a POSA would have been known of ways to

perform sequencing before 2005. *See* Ex. 29 at 12 (citing Exs. 31-33). Further illustrating that the claimed method was routine, Natera argued that the “patentees knew about different types of sequencing-by-synthesis...when they drafted the claims, evidenced by cites to Jarvie and other references during prosecution.” *See* Ex. 29 at 16 (citation omitted).

380. In sum, the '035 patent, Natera's expert witnesses, and Natera's own admissions show the method of high-throughput sequencing as claimed in the '035 patent was well-known, routine, and conventional in 2011.

d. Claims 12 and 13 Add Only Conventional Steps

381. It is my opinion that claims 12 and 13 fail to add an inventive concept and add only conventional steps. Claim 12 depends from claim 1 and simply specifies that the “one or more universal tail adaptors” comprise “a first universal tail adaptor and a second universal tail adaptor.” D.I. 1-2 at claim 12. Indeed, “universal tail adaptors” were so well known in the art that the '035 patent specification did not even include a single reference to them. Ex. 6 at 34:15-35:1. Claim 13 depends from claim 12 and adds that the first and second universal tail adaptors are comprised of first and second primers. Primers were likewise ubiquitous in the art. *See, e.g.*, D.I. 1-2 at 7-26 (citing to a wealth of prior art publications describing the use of primers); D.I. 52-5 at 1845 (Sample-specific DNA barcodes are then incorporated into the *primers* used for the final universal PCR by tailing the 5' end with sample-specific DNA sequences and 454 sequencing *primers*.) (emphasis added); Ex. 12 ¶ 67 (discussing the use of “Illumina compatible primers”). Neither claim 12 nor claim 13 add an inventive concept.

382. For the reasons discussed above, it is my opinion that the Asserted Claims of the '035 patent are method of detection claims, that these only detect cfDNA in a biological sample,

which is a natural phenomenon, and that (based on the '454 patent, Natera's expert witnesses, and Natera's own admissions) the detecting is accomplished with no meaningful non-routine steps. There is nothing about the claimed steps, individually or in combination, that is unconventional.

XII. INVALIDITY OF THE '454 PATENT FOR IMPROPER INVENTORSHIP

383. As I explain above, the claims of the '454 patent are not inventive, but are rather invalid as obvious in view of multiple prior art references. To the extent the claims of the '454 patent are deemed to reflect a bona fide invention, however, it is my opinion that they are invalid for failure to name the proper inventors. Specifically, as I explain below, the claimed approach based on whole exome sequencing was invented not by Natera alone, but rather in collaboration with researchers at University College London ("UCL"), including, for instance, Dr. Miriam Jamal-Hanjani.

A. Disclosure Relating to Whole Exome and Whole Genome Sequencing In The '454 Patent

384. The independent claims of the '454 patent recite the step of:

performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;

D.I. 1-1 at claims 1, 14. Thus, the claims recite the use of either "whole exome" or "whole genome" sequencing.

385. There is no description of "whole genome sequencing" in the '454 patent, a point that I discuss further below in connection with written description. Although the description for Figure 51B refers to "whole genome sequencing," this is an error because Figure 51 is actually only "whole exome" sequencing. As to "whole exome" sequencing, it is my opinion that the '454 patent has limited written description. Specifically, it is my opinion that the sole support for "whole exome" sequencing appears in the '454 patent's Example 13.

386. Specifically, Example 13 provides as follows:

Two to three biopsies from various regions from the entire cancerous lung were taken from each patient (FIG. 51A). Each biopsied sample was assayed by whole exome sequencing (Illumina HiSeq200; Illumina, San Diego, Calif.), followed by Amp-15 liSeq® sequencing (Ion Torrent, South San Francisco, Calif.) on a PGM® for identification of underlying clonal heterogeneity. Following sequencing and SNV analyses, the variant allele frequency (VAF) was determined for each biopsy sample (FIG. 51B).

Id. at 168:10-19. I have reviewed the entirety of the '454 patent in the context of identifying written description support in the disclosure for whole exome sequencing and identified nothing other than the foregoing in Example 13.

B. The '454 Patent Example 13 and the Jamal-Hanjani Thesis

387. I have reviewed and compared disclosures of the '454 patent (D.I. 1-1) and the Jamal-Hanjani Thesis (Ex. 38).

388. It is my opinion that the limited written description for “whole exome” sequencing in the '454 patent is the same as a disclosure of research based on the use of “whole exome” sequencing that is appears in the Jamal-Hanjani Thesis.

389. Based on comparison of the disclosures, I focus my discussion below on “Section 5.4 Multiplex PCR and targeted HiSeq sequencing” of the Jamal-Hanjani Thesis. Ex. 38 at 155-160.

390. Section 5.4 includes Table 22 (Clinical characteristics of the patient cohort), Table 23 (List of selected mutations for the Natera approach), and Figure 49 (Concordance between VAFs). Ex. 38, 155 (Table 22), 156 (Figure 49), 157-158 (Table 23).

Patient ID	Age	Gender	Histology	Stage	Vascular invasion	Pleural invasion	Smoking status (pack years)
L012	69	F	LUSC	IB	Y	Y	Smoker (40)
L013	68	F	LUSC	IB	Y	Y	Smoker (50)
L015	68	M	LUSC	IA	N	N	Smoker (100)
L017	61	F	LUAD	IIB	Y	N	Smoker (48)

Table 22 Clinical characteristics of the patient cohort

Abbreviations: LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma.

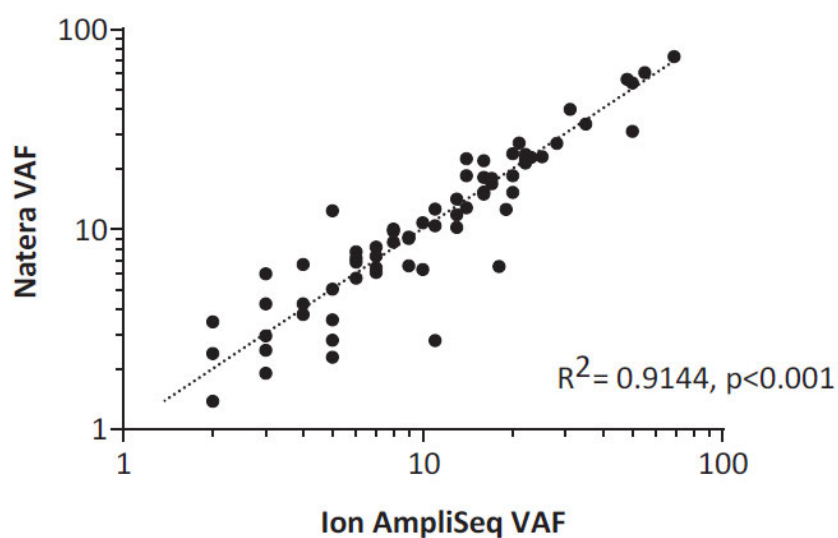


Figure 49 Concordance between VAFs

Concordance between individual mutation variant allele frequencies (VAFs) and mean VAF (vertical line) identified by prior sequencing (WES and Ion AmpliSeq) and the Natera approach by direct comparison (A), and by linear regression modeling (B).

Patient	Gene (amino acid change)	WES/Ion (VAF %)			AmpliSeq			Natera (VAF %)			cfDNA (VAF %)	Truncal/ Branch
		R1	R2	R3	R1	R2	R3	R1	R2	R3		
L012 LUSC Stage IB Smoker (40)	BRIP1 (R173C)	14.0	6.0	8.0				22.6	7.7	10.1	3.71	Truncal
	CARS (T51A)	22.0	11.0	16.0				22.4	10.5	18.2	5.02	Truncal
	CIC (E61X)	0.1	nd	7.0				nd	nd	8.2	1.71	Branch
	FAT1 (D924Y)	8.0	4.0	1.2				9.8	4.3	0.8	0.57	Branch
	KDM6A (R4079K)	10	5.0	0.8				6.3	3.5	nd	0.28	Branch
	MLLT4 (E478Q)	9.0	3.0	0.3				9.0	4.3	0.7	0.95	Branch
	NFE2L2 (E1142D)	69.0	31.0	50				73.1	39.9	53.9	23.25	Truncal
	RASA1 (E183X)	6.5	nd	0.7				7.4	nd	0.4	nd	Branch
	TP53 (R136H)	23.0	9.0	17.0				22.9	9.2	17.0	4.89	Truncal
	TP53 (Y181C)	22.0	8.0	16.0				21.5	8.7	22.1	5.77	Truncal
L013 LUSC Stage IB Smoker (50)	EGFR (G719A)	21.4	24.8	55.2				27.1	23.1	60.8	1.16	Truncal
	EGFR (D761Y)	20.1	17.3	48.0				23.9	18.0	56.3	1.09	Truncal
	HERC4 (N217K)	0.1	3.0	6.0				0.2	1.9	5.7	nd	Branch
	JAK2 (K33X)	0.2	11.1	6.8				0.3	2.8	6.1	nd	Branch
	MLL3 (K589R)	0.2	0.2	4.1				nd	nd	6.7	nd	Branch
	MSH2 (Q444H)	4.8	4.2	11.0				5.0	3.3	12.7	nd	Truncal
	MTOR (Q838E)	2.0	0.6	3.0				2.4	1.4	6.0	nd	Truncal
	PLCG2 (E525X)	5.1	1.7	10.5				2.8	1.7	6.9	nd	Truncal
	TP53 (P60S)	7.0	4.3	15.6				6.1	3.8	15.4	0.4	Truncal
L015 LUSC Stage IA Smoker (100)	ALK (P234R)	6.0	2.0	NS				7.2	1.4	NS	nd	Truncal
	GABRG1 (I279F)	13.0	0.8	NS				11.8	2.0	NS	nd	Truncal
	KDM6A (S539C)	18.0	6.0	NS				6.5	0.9	NS	0.17	Truncal
	MLL2 (E1186X)	5.0	0.3	NS				12.4	nd	NS	nd	Branch
	ROS1 (Y891C)	10.0	3.0	NS				10.8	2.9	NS	0.15	Truncal
	SLC39A4 (A546T)	14.0	nd	NS				18.6	nd	NS	nd	Branch
	TP53 (G199X)	19.0	3.0	NS				12.6	2.5	NS	nd	Truncal

Ex. 38 at 155 (Table 22), 156 (Figure 49), 157-158 (Table 23).

391. Jamal-Hanjani Thesis Table 22 sets forth data mirroring that in '454 patent Figure 51A. Ex. 38 at 155 (Table 22); D.I. 1-1 at Fig.51A.

392. Jamal-Hanjani Thesis Table 23 sets forth data regarding WES/Ion Ampliseq (VAF %) and Natera (VAF%) mirroring that in '454 patent Figures 51B and 53A. Ex. 38 at 157-158 (Table 23); D.I. 1-1 at Figs 51B, 53A-B.

393. Jamal-Hanjani Thesis Figure 49 appears the same as '454 patent Figure 54B.

394. I have also considered other relevant portions of the Jamal-Hanjani Thesis, including lists of genes location referred to in Example 13, which are identical. Ex. 38 at 68-70; D.I. 1-1 at Figs.51B, 53A-B.

395. Based on my comparison of the work reported in the Jamal-Hanjani Thesis and '454 patent Example 13, it is my opinion that research reported in the Jamal-Hanjani Thesis is disclosed in the '454 patent in Example 13.

C. The UCL Contribution To The Claims

396. As I explain above, the sole support in the specification for “whole exome” sequencing as recited in the claims appears in Example 13, and this example in the patent matches the disclosure in Chapter 5 of the Jamal-Hanjani thesis. For several reasons, it is evident that researchers at UCL, including at least Dr. Jamal-Hanjani, contributed to the conception of the approach in Example 13, including the use of whole exome sequencing and analysis of cell-free DNA to detect tumor-specific mutations.

1. The Jamal-Hanjani Thesis Itself

397. I start with the Jamal-Hanjani thesis itself. The fact the content of Example 13 matches Chapter 5 of the Jamal-Hanjani Thesis is evidence that she made a significant contribution to the claimed inventions of the '454 patent.

398. Beyond this, the thesis states that Dr. Hanjani wrote the protocol for the TRACERx study (without input from Natera), which started in April 2014:

I wrote and developed the protocol for this study under the guidance of my supervisor during the production of this thesis, with the input of the TRACERx consortium and the support of the UCL Cancer Trials Centre (Alan Hackshaw, Yentig Ngai, and Natasha Iles). This study started recruitment in April 2014, and is currently open in London, Manchester, Leicester, Birmingham, Aberdeen, and Cardiff.

Ex. 38 at 26. The protocol included determining whether cfDNA includes mutations found in tumor sequencing. *Id.* at 25 (“Surgically resected primary NSCLC tumours and associated lymph nodes, surplus to diagnostic requirements, will be subjected to multi-region sampling and subsequent WES and/or WGS.”). The protocol also involved tracking those mutations over time in cell free DNA to monitor residual disease. *Id.* at 26 (“To determine if cfDNA and CTCs can be used to track actionable mutations to guide therapeutic intervention, monitor residual disease and predict tumour recurrence.”).

399. Given that Dr. Hanjani wrote the protocol and research plan involving whole exome sequencing and monitoring of cell-free DNA in mutations (without input from Natera), it seems clear that she would have made a significant contribution to the alleged inventions of the '454 patent, which include steps pertaining to these very concepts.

400. Consistent with the foregoing, the Materials and Methods section of the Jamal-Hanjani thesis describes how Dr. Hanjani provided variants and samples for to collaborators, including Natera, for processing and that it was her that lab analyzed the results:

2.12 cfDNA analyses

2.12.1 Selection of mutations

Non-silent mutations identified by WES and subsequently validated by Ion AmpliSeq sequencing were considered for detection in cfDNA. The majority of the mutations investigated were SNVs, but in some cases indels were tested and in one case an EML4-ALK translocation was tested (collaboration with Illumina). All approaches involved multiplex PCR and PCR primers were designed using the specified genomic coordinates for the selected mutations. In order to address the question as to whether the heterogeneous genetic landscape of a tumour could be explored using cfDNA, both truncal and branch mutations were selected. cfDNA analyses involved collaborations with companies outside of our laboratory (as described below), whereby cfDNA extracted at diagnosis from 2ml of plasma for each patient was sent to these companies along with specified mutations for detection. ***The results of these experiments were analysed in our laboratory.*** Significant associations between VAFs for selected mutations in these analyses were tested for using the Mann-Whitney U test.

Id. at 53. The foregoing further shows that Dr. Jamal-Hanjani made a significant contribution to the claims with regard to sequencing of tumor mutations and subsequent monitoring of such mutations in cell-free DNA. Indeed, it appears she was not just conceiving of the experiments, but also participating in the analysis of the results to confirm the utility of the approach.

2. Testimony From Dr. Zimmermann

401. I also understand that Natera made Dr. Bernhard Zimmerman, a named inventor on Natera's patents, available to testify as its corporate representative regarding Natera's collaboration with UCL. His testimony further confirms my opinions.

402. For instance, section 5.4 of the Jamal-Hanjani thesis discloses work to detect mutations in cfDNA. As the Jamal-Hanjani thesis explains, the mutations used were those that were identified using whole exome sequencing. *See* Ex. 38 at 158 (Table 23 caption, "List of mutations and variant allele frequencies (VAFs) identified using WES/AmpliSeq sequencing and the Natera approach."). [REDACTED]

[REDACTED]

[illegible]

In view of the foregoing evidence, it is my opinion that researchers at UCL would have made a significant contribution to the claims of the '454 patent, including by conceiving

of the use of whole-exome sequencing of tumor tissue followed by the analysis of cell-free DNA to detect tumor-specific mutations. Also, I am informed that conception, as demonstrated here, indicates inventorship.

XIII. INVALIDITY UNDER 35 U.S.C. § 112—LACK OF WRITTEN DESCRIPTION

404. It is my opinion that to the extent the claims of the '454 and '035 patents are not invalid as being obvious, they are invalid for lack of written description.

405. As to the '454 patent specifically, I note that independent claims of the '454 patent recite the step of:

performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;

D.I. 1-1 at claims 1, 14. Thus, thus the claims recite the use of either “whole exome” or “whole genome” sequencing.

406. There is, however, no description of “whole genome sequencing” in the '454 patent. Although the description for Figure 51B refers to “whole genome sequencing,” this is an error because Figure 51 is actually only “whole exome” sequencing. Dr. Zimmerman confirmed that this is the case:

[REDACTED]

[REDACTED]

[REDACTED]

407. One can further confirm this by comparing the data that appears in Fig. 51b of the '454 patent to the data that appears in Table 23 of the Jamal-Hanjani thesis. As one can see, the

data in the Jamal-Hanjani Thesis was generated by “WES,” which is “whole exome sequencing,” not “whole genome sequencing.” Ex. 38 at 157 (Table 23). This data matches exactly what is in Figure 51b of the patent, confirming that Figure 51b of the patent is showing whole exome sequencing data, not whole genome sequencing data.

408. Thus, the claims lack written description because there is no description in the ’454 patent of the “whole genome sequencing” claim element.

409. The claims of the ’035 patent and ’454 patents are also invalid for failure to describe techniques of PCR amplification without the use of techniques for selecting primers.

410. The claims of the ’035 and ’454 patents both recite steps related to performing PCR on multiple nucleic acid targets in the same reaction volume at the same time, a process referred to as “multiplex PCR.” *See* D.I. 1-2 at 2:65-3:4; D.I. 1-1 at 58:40-46 (“multiplex amplification reaction...such as PCR”).

411. For the ’454 patent, claim 1 recites

performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume

D.I. 1-1 at claim 1.

412. For the ’035 patent, claim 1 recites

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

D.I. 1-2 at claim 1.

413. It is my understanding that in a co-pending case involving organ transplant, Natera put forth the position that their patents, which are in the same family as the patents in this case,

cover techniques for multiplex PCR with an unlimited number of randomly selected targets (i.e., without the use of any techniques for loci/primer selection) that supposedly yields usable nucleic acid for high throughput sequencing. The claims for the patents in this case also include techniques for multiplex PCR. I understand that Natera may be bound to that position in this case. If so, the patents in this case, nor any of the family member patents to the patents in this case, disclose such a technique for multiplex PCR. Rather, the inventors were in possession solely of techniques that involved target loci selection and primer design for achieving multiplex PCR.

414. As an initial matter, as I explain above in connection with obviousness, the individual concepts recited in the claims, such as multiplex amplification, molecular barcodes, and sequencing amplicons were all known in the art. Natera has acknowledged that these were not new things, as I explain in Section X. Therefore, to the extent there is anything inventive in the claims of the asserted patents, it must reside in the combination of recited elements. Having reviewed the patents, however, I do not believe there is any embodiment that discloses the elements of the claimed inventions of the '454 or '035 Patents as arranged in the claims. This alone establishes that the inventors were not in possession of the claimed inventions.

415. For example, the '454 patent does not disclose an example that discusses sequencing a tumor sample and then obtaining any depth of read for sequenced cell-free DNA. As for the '035 patent, claim 1 requires the use of "universal tail adaptors," which is not a term used or defined anywhere in the specification, let alone in conjunction with the other steps of claim 1 in any kind of example. This establishes that the inventors were not in possession of the claimed inventions.

416. As discussed, to the extent the claims are not invalid as obvious, the specification also makes clear to the skilled artisan that the inventors were solely in possession of techniques that involved target loci selection and primer design.

417. First, as recited above, the claims of the '454 and '035 patents recite *inter alia* steps related to performing PCR on multiple nucleic acid targets in the same reaction volume at the same time, a process referred to as “multiplex PCR.” Specifically, claim 1 of the '454 patent, the sole asserted independent claim, recites “performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor specific SNV mutation from cell-free DNA.” D.I. 1-1 at 171:33-34. Likewise, claim 1 of the '035 patent, the sole asserted independent claim, recites amplifying tagged nucleic acid samples “wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume” and sequencing the plurality of SNP loci, wherein the plurality comprises “25-2,000 loci associated with cancer.” D.I. 1-2 at 249:43-62.

418. As an example of disclosures of only techniques that involved target loci selection and primer design, the very first sentences of the Detailed Description of the invention explained that the alleged invention is about targeting sequences for amplification to avoid undesirable side products like primer dimers. As the specification explains, the “present invention is based in part on the surprising discovery that often only a relatively small number of primers in a library of primers are responsible for a substantial amount of the amplified primer dimers that form during multiplex PCR reactions.” *Id.* at 46:37-41. Accordingly, the specification provides that “[m]ethods have been developed to select the most undesirable primers for removal from a library of candidate primers.” *Id.* at 46:41-43. “By reducing the amount of primer dimers to a negligible amount...these methods allow the resulting primer libraries to simultaneously amplify a large

number of target loci in a single multiplex PCR reaction.” *Id.* at 46:43-47. As such, the specification discloses that because “the primers hybridize to the target loci and amplify them rather than hybridizing to other primers and forming amplified primer dimers, the number of different target loci that can be amplified is increased.” *Id.* at 46:47-451.

419. Further confirmation that the inventors were solely in possession of techniques that involved target loci selection and primer design is established in the specification’s disclosure that it is essential to remove such primer-dimers by selecting primers to avoid primer dimers: “[a]t high multiplexing it is not possible to eliminate all spurious interactions, but *it is essential* to remove the primers or pairs of primers with the highest interaction scores in silico as they can dominate an entire reaction, greatly limiting amplification from intended targets.” *Id.* at 54:46-50; D.I. 1-1 at 108:18-22. Likewise, “when multiple pairs are added to the same PCR reaction, non-target amplification products may be generated, such as amplified primer dimers. The risk of generating such products increases as the number of primers increases. These non-target amplicons significantly limit the use of the amplified products for further analysis and/or assays. Thus, improved methods are needed to reduce the formation of non-target amplicons during multiplex PCR.” D.I. 1-2 at 3:6-14; *see also id.* at 47:53-55, 96:27-30; *see also* D.I. 1-1 at 106:2-6. Additionally, the patents explain that without target loci selection and primer design techniques, the PCR reaction yields essentially nothing but primer dimer: “Sequencing of a 1042-plex without design and selection of assays resulted in >99% of sequences being primer dimer products.” D.I. 1-2 at 96:28-30. If the inventors were in possession of techniques that did not require target loci selection and primer design for use with the claimed techniques, they would not state that such techniques are “essential” and point out that without the use of such techniques the yield is almost nothing but primer dimer.

420. In fact the specification relies on the avoidance of primer side products to distinguish the prior art. The specification identifies the problem of “[h]ighly multiplexed PCR [that] can often result in the production of...unproductive side reactions such as primer dimer formation.” *Id.* at 48:7-10. Positing the alleged invention as a solution to this problem, the specification provides that the invention is an “alternative to microarrays” for sequencing, since the invention provides for the “high level of multiplexing with minimal nontarget amplicons that has now been achieved.” *Id.* at 47:40-49.

421. Moreover, the specification frames the alleged invention’s removal of “problematic primers, that is, those primers that are particularly likely to f[o]rm dimers” as the element that “unexpectedly enabled extremely high PCR multiplexing[.]” *Id.* at 48:14-17; *see also id.* at 48:32-36 and D.I. 1-1 at 105:64-106:1 (“Empirical data indicate that a small number of ‘bad’ primers are responsible for a large amount of non-mapping primer dimer side reactions. Removing these ‘bad’ primers can increase the percent of sequence reads that map to targeted loci.”); D.I. 1-2 at 48:30-32 (disclosing that there “are a number of ways to choose primers for a library where the amount of non-mapping primer dimer...products are minimized.”). In this way, the patentee distinguished the invention from the prior art by directly connecting the removal of the primers that cause primer dimers as something “unexpected.” *Id.* at 48:10-13.

422. The increase in amplification accuracy and efficiency is the stated solution to the problem the patentee identified in conventional DNA amplification techniques in the Background. *Id.* at 2:64-3:13. For example, the specification discloses that, in “systems such as sequencing, where performance significantly degrades by primer dimers...greater than 10, greater than 50, and greater than 100 times higher multiplexing than other described multiplexing has been achieved”

due to the selection of primers that “hybridize to the target loci and amplify them rather than hybridizing to other primers and forming primer dimers[.]” *Id.* at 48:18-24.

423. The individual embodiments disclosed in the specification again emphasize avoiding primer side products:

In various embodiments, less than 60, 50, 40, 30, 20, 10, 5, 4, 3, 2, 1, 0.5, 0.25, 0.1, or 0.05% ***of the amplified products are primer dimers.***

D.I. 1-2 at 8:60-63 (emphasis added).

In some embodiments, the library includes primers that simultaneously amplify 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; 30,000; 40,000; 50,000; 75,000; or 100,000 ***different target loci such that less than 60, 40, 30, 20, 10, 5, 4, 3, 2, 1, 0.5, 0.25, 0.1, or 0.05% of the amplified products are primer dimers.***

Id. at 19:1-9 (emphasis added).

In some embodiments, ΔG values for each possible combination of two primers (***each possible primer dimer***) in a library are all equal to or greater than -20, -18, -16, -14, -12, -10, -9, -8, -7, -6, -5, -4, -3, -2, or -1 kcal/mol.

Id. at 19:54-62 (emphasis added).

In some embodiments, (i) less than 60% of the amplified products ***are primer dimers*** and at least 40% of the amplified products are target amplicons, (ii) less than 40% of the amplified products ***are primer dimers*** and at least 60% of the amplified products are target amplicons, (iii) less than 20% of the amplified products ***are primer dimers*** and at least 80% of the amplified products are target amplicons, (iv) less than 10% of the amplified products ***are primer dimers*** and at least 90% of the amplified products are target amplicons, or (v) less than 5% of the amplified products ***are primer dimers*** and at least 95% of the amplified products are target amplicons.

Id. at 22:3-14 (emphasis added).

424. The examples in the patents further confirm that the inventors were in possession solely of techniques that utilized target loci selection and primer design for multiplex PCR. All of the working examples use such techniques. I was unable to identify any working example in the patent that did not use the techniques for targeted loci selection and primer design that are disclosed

in the specification. If the inventors were in possession of techniques for carrying out large scale multiplex PCR that would generate usable nucleic acid for high throughput sequencing and that did not involve loci/primer selection techniques, they would have disclosed such techniques and would have provided a working example

425. As to the '035 patent specifically, the level of written description support is particularly thin in my opinion. The concept of a “universal tail adaptor” is never mentioned in the entirety of the specification. I could not identify any description of a “universal tail adaptor” being used in the context of an embodiment that corresponds to the claims.

426. In view of this, there is unsurprisingly no disclosure in the '454 and '035 patents of a method for carrying out the claimed multiplex PCR without the primer design approaches. Therefore, it is my opinion that '454 and '035 patents are invalid because the claims lack written description support for multiplex PCR amplification that does not utilize any techniques for selection of primers to avoid primer side products, such as primer dimers.

XIV. NATERA'S SIGNATERA™ ASSAY

427. I understand that Dr. Metzker is of the opinion that Signatera™ practices the '454 and '035 patents. D.I. 13 ¶ 124.

A. Signatera™ is Not Shown to Practice the '454 Patent

428. Dr. Metzker sets forth the basis for Signatera™ practicing claim 1 of the '454 patent relying on various exhibits he identifies as disclosing or teaching elements of the claim. *Id.* ¶¶ 124-131.

429. Having considered the cited evidence, I disagree that Dr. Metzker has set forth a basis for Signatera™ practicing claim 1 of the '454 patent. For example, Dr. Metzker fails to

establish the “amplicons having a length of 50-150 bases” in paragraphs 127 to 129, in which he addresses:

Claim 1[b]: performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume; and

Id. ¶¶ 127-129. Dr. Metzker relies on Signatera White Paper (D.I. 13-17), Coombes (2019) (D.I. 13-19), Christensen (2019) (D.I. 13-21), Kotani (2023) (D.I. 13-22), Reinert (D.I. 13-18), and Kirkizlar (2015) (D.I. 13-2). D.I. 13 ¶¶ 127-129. Paragraph 127 of Dr. Metzker’s declaration is silent as to amplicon length. D.I. 13 ¶ 127 (citing D.I. 13-17 at 2).

430. Paragraph 128 of Dr. Metzker’s declaration states:

Coombes (2019), Christensen (2019), and Kotani (2023), which all used Signatera™, illustrate the presence of this claim limitation in Signatera™. For example, Coombes (2019), Christensen (2019), and Kotani (2023) performed targeted multiplex amplification in the same reaction volume to amplify 16 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample and sequencing the amplicons to generate sequence reads having a length of 50 bases.¹⁶⁴

¹⁶⁴ See Coombes (2019) at Abstract, 4256-4258, 4261, Figure 1 and legend, Figure 2 and legend, Supplementary Data at 1-2; see also Christensen (2019) at 1548-1549, Figure 4 and legend, Data Supplement at 3; Kotani (2023) at Methods, citing to Reinert (2019); see Reinert (2019) at eMethods 6.

D.I. 17 ¶ 128. Having reviewed the cited exhibits carefully, I determine that Coombes (2019) discloses that “[s]equencing was performed on an Illumini HiSeq 2500 Rapid Run with 50 cycles of paired-end reads using the Illumina Paired End v2 kit with an average read depth of >100,000 per amplicon.” D.I. 13-19 at 11-12 (Coombes (2019) at Supplementary Data at 1-2).

431. It is my opinion that Coombes (2019)’s disclosure of 50 cycles of paired-end reads does not establish a basis for Signatera employing “amplicons having a length of 50-150 bases” because the number of cycles of paired-end reads is a measure of how many sequencing cycles are conducted to generate sequence reads, and not of the length of the amplicon that is being sequenced. An amplicon being subjected to sequencing could be shorter than fifty bases or longer than 150 bases. Further, Dr. Metzker provides no explanation or reasoning relating to amplicon length. D.I. 17 ¶ 128.

432. The other cited portions of Coombes (2018) are also silent as to the length of amplicons. D.I.13-19 at 2 , 3-5, 11-12 (Coombes (2019) at Abstract, 4256-4258, 4261, Figure 1 and legend, Figure 2 and legend, Supplementary Data at 1-2).

433. Christensen (2019), likewise, discloses that “[s]equencing was performed on an Illumina HiSeq 2500 Rapid Run with 50 cycles of paired-end reads using the Illumina Paired End v2 kit” (D.I. 13-21 at 16 (Data Supplement at 3)), with the other cited portions also being silent as to the length of amplicons (D.I. 13-21 at 3-4, 8 (Christensen (2019) at 1548-1549, Figure 4 and legend).

434. Kotani (2023) and Reinert (2019) are similarly limited, with Reinert (2019) eMethods 6 having the same disclosure that “[s]equencing was performed on an Illumina HiSeq 2500 Rapid Run with 50 cycles of paired-end reads using the Illumina Paired End v2 kit,” with the other cited portions also being silent as to the length of amplicons. D.I. 13-22 (Kotani (2023)); D.I. 13-18 (Reinert (2019)). Also, D.I. 13-18, as filed by Natera, does not include eMethods 6. D.I. 13-18; Ex. 59 (Reinert eMethods).

435. Paragraph 129 of Dr. Metzker’s declaration states:

Kirkizlar (2015) explains that Signatera employs multiplex PCR in a single reaction volume to amplify a bespoke set of targets identified from a patient's tumor sample.¹⁶⁵ The bespoke amplification products from the multiplex PCR “were designed to have a maximum amplicon length of 75 bp” (*i.e.*, 75 bases).¹⁶⁶

¹⁶⁵ See Kirkizlar (2015) at 409.

¹⁶⁶ *Id.*

D.I. 17 ¶ 129.

436. It is my opinion that Kirkizlar does not establish a basis for Signatera employing “amplicons having a length of 50-150 bases” because there is no showing that what is described is Signatera, and I disagree with Dr. Metzker’s opinion that Kirkizlar (2015) provides information about Signatera. *Id.* ¶ 129. First, the multiplex PCR in Kirkizlar differs dramatically from what is employed in Signatera where Natera and Dr. Metzker repeatedly describe Signatera as employing a 16-plex PCR reaction. *Id.* 17 ¶¶ 119, 121; D.I. 13-17 at 2. In contrast to Signatera, in Kirkizlar, the PCR assay is “massively multiplexed PCR” (mmPCR) in which “3168 SNPs were amplified using one primer pair for each SNP.” D.I. 13-2 at 4 (Kirkizlar at 409). In my opinion, such a dramatic difference in multiplex alone establishes that the mmPCR disclosed is not Signatera. Second, Kirkizlar was published in October 2015, while Natera did not launch Signatera until 2017. *Id.* at 2 (indicating publication in 2015: “© 2015 Published by Elsevier Inc. on behalf of Neoplasia Press, Inc.”); D.I. 1 ¶ 17 (“Building on these innovations, in 2017, Natera launched...Signatera®”). This is also contrary to the disclosed assay using mmPCR being Signatera. Third, having reviewed Kirkizlar carefully, there is no mention of Signatera in Kirkizlar. D.I. 13-2. This is also contrary to the disclosed assay using mmPCR being Signatera. In sum, it is my opinion that what Kirkizlar discloses is not Signatera.

437. It is further my opinion, that because Kirkizlar does not disclose Signatera, but rather a different assay using mmPCR, Kirkizlar provides no basis for Signatera employing “amplicons having a length of 50-150 bases.”

438. Accordingly, with no basis provided for Signatera employing “amplicons having a length of 50-150 base,” as required by claim 1, it is my opinion that it is not shown that Signatera™ practices the ’454 patent.

B. Signatera™ is Not Shown to Practice the ’035 Patent

439. Dr. Metzker sets forth the basis for Signatera™ practicing claim 1 of the ’035 patent relying on various exhibits he identifies as disclosing or teaching elements of the claim. D.I. 17 ¶¶ 132-137.

440. Having considered the cited evidence, I disagree that Dr. Metzker has set forth a basis for Signatera™ practicing claim 1 of the ’035 patent. For example, for Claim 1[b]: “amplifying the tagged products..., wherein one of the amplifying steps introduces a barcode and one or more sequencing tags,” Dr. Metzker again relies on Kirkizlar:

In Signatera, one or more sequencing tags are added during one of the amplification steps that also introduces the barcode in the Signatera™ assay. Kirkizlar (2015) explains that Signatera employs multiplex PCR in a single reaction volume to amplify a bespoke set of targets identified from a patient’s tumor sample.¹⁸⁶ The bespoke amplification products are further amplified by a barcoding PCR reaction, which adds “sequencing tags and index sequences,” resulting in final barcoded products.¹⁸⁷

¹⁸⁶ See Kirkizlar (2015) at 409.

¹⁸⁷ *Id.*

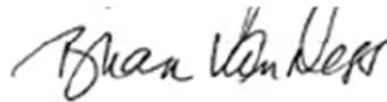
Id. ¶ 136 (citing 13-2 at 4 (Kirkizlar at 409)). As discussed above, however, there is no showing that what Kirkizlar describes is Signatera, and I disagree with Dr. Metzker’s opinion that Kirkizlar (2015) provides information about Signatera.

441. It is further my opinion, that because Kirkizlar does not disclose Signatera, Kirkizlar provides no basis for Signatera “amplifying the tagged products..., wherein one of the amplifying steps introduces a barcode and one or more sequencing tags.”

442. Accordingly, with no basis provided for Signatera “amplifying the tagged products..., wherein one of the amplifying steps introduces a barcode and one or more sequencing tags,” as required by claim 1, it is my opinion that it is not shown that SignateraTM practices the '035 patent.

I declare under penalty of perjury the laws of the United States of America that the foregoing is true and correct.

Date: October 18, 2023

A handwritten signature in black ink, appearing to read "Brian Van Ness". The signature is written in a cursive, flowing style.

Dr. Brian Van Ness