

**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-CCE-JLW

**CONTAINS HIGHLY CONFIDENTIAL
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MATERIAL**

DEFENDANT'S AMENDED FINAL INVALIDITY CONTENTIONS

Pursuant to Local Rules for Patent Cases LR 103.6 and the Court's Order dated September 24, 2024 (Dkt. 330 at 5), Defendant NeoGenomics Laboratories, Inc. ("NeoGenomics" or "Defendant") provides the following amended final invalidity contentions for claims 1, 11, 12, and 18 of U.S. Patent No. 11,319,596 ("the '596 patent") and claims 1, 8, 10, 12, 14, 15, 22, 24, 26, and 28 of U.S. Patent No. 11,530,454 ("the '454 patent") (collectively, the "Asserted Patents" and the "Asserted Claims") that Plaintiff Natera, Inc. ("Natera") has asserted in its preliminary infringement contentions, served on November 25, 2024, and elected on December 11, 2024. Defendant reserves all rights with respect to these contentions.

The Court has yet to issue a claim construction order for the '596 patent, and Defendant's investigation regarding the invalidity of the Asserted Patents is ongoing. Although the Court has issued a claim construction order for the claims of the '454 patent (Dkt. 280), Defendant does not agree with Natera's apparent interpretation of that order as reflected in Natera's preliminary infringement contentions. Accordingly, these invalidity contentions do not represent an agreement of view as to either the proper construction of any term in any of the asserted claims or the proper application of the Court's claim construction order to the same. Similarly, nothing herein shall be construed as an admission regarding the application of the Asserted Claims to the

accused products or an admission as to claim scope.

Pursuant to the Federal Rules of Civil Procedure, the Local Rules, and Court Orders, Defendant also reserves the right to amend or supplement these invalidity contentions as additional research is conducted, prior art is discovered, discovery is obtained, supplements or modifications are made to the infringement theories advanced by Natera, claim construction positions are taken or subsequent orders are issued, expert discovery is obtained, and for any other reason permitted under the Federal Rules of Civil Procedure, the Local Rules, or Court Orders.

I. INTRODUCTION AND BACKGROUND

The following patents and claims were elected by Natera on December 11, 2024:

<u>Patent No.</u>	<u>Asserted Claims Against NeoGenomics</u>
US 11,319,596	1, 11, 12, and 18
US 11,530,454	1, 8, 10, 12, 14, 15, 22, 24, 26, and 28

A. The Asserted Claims of the '596 Patent

Natera asserts claims 1, 11, 12, and 18 of the '596 patent.

Asserted Claim 1 of the '596 patent recites:

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

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

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

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

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

performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads, wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.

Asserted Claim 11 of the '596 patent recites:

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

Asserted Claim 12 of the '596 patent recites:

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

Asserted Claim 18 of the '596 patent recites:

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

B. The Asserted Claims of the '454 Patent

Natera asserts claims 1, 8, 10, 12, 14, 15, 22, 24, 26, and 28 of the '454 patent.

Asserted Claim 1 of the '454 patent recites:

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.

Asserted Claim 8 of the '454 patent recites:

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.

Asserted Claim 10 of the '454 patent recites:

10. The method of claim 1, wherein the method further comprises designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample.

Asserted Claim 12 of the '454 patent recites:

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

Asserted Claim 14 of the '454 patent recites:

14. 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, 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 method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus.

Asserted Claim 15 of the '454 patent recites:

15. The method of claim 14, wherein the sequencing has a depth of read of at least 50,000 per target locus.

Asserted Claim 22 of the '454 patent recites:

22. The method of claim 14, wherein the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation.

Asserted Claim 24 of the '454 patent recites:

24. The method of claim 14, wherein the method further comprises designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample.

Asserted Claim 26 of the '454 patent recites:

26. The method of claim 14, wherein the method further comprises detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA.

Asserted Claim 28 of the '454 patent recites:

28. The method of claim 14, wherein the method is capable of detecting an SNV mutation that is present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus.

II. PRIORITY DATES

A. '596 Patent

The '596 patent is issued from U.S. Patent Application No. 16/288,351 (filed Feb. 28, 2019), which purports to be a continuation of application No. 16/014,961 (filed on June 21, 2018, now Pat. No. 11,486,008), which is a continuation of application 14/692,703 (filed on Apr. 21, 2015, now Pat. No. 10,179,937), which claims priority to U.S. Provisional Application No. 61/982,245 (filed on Apr. 21, 2014), U.S. Provisional Application No. 61,987,407 (filed on May 1, 2014), U.S. Provisional Application No. 62/066,514 (filed on Oct. 21, 2014), U.S. Provisional Application No. 62/146,188 (filed on Apr. 10, 2015), U.S. Provisional Application No. 62/147,377 (filed on Apr. 14, 2015), U.S. Provisional Application No. 62/148,173 (filed on Apr. 15, 2015).

Natera acknowledges “[t]he priority date of each of the asserted claims of the '596 patent is by April 21, 2015.” Natera’s Fourth Supplemental Response to NeoGenomics’ Interrogatory No. 1 at 14; *see also* Preliminary Infringement contentions at 8. For instance, with respect to the

claim limitation “an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA,” the disclosures of the priority applications provide no written description support until the application No. 14/692,703 (“the ’703 application”), filed on April 21, 2015. The only disclosures in the specification mentioning 0.015% were not added until the ’703 application, and Natera relied on these disclosures for support when they added the 0.015% limitation in their June 1, 2021 claim amendment. As another example, Example 14, the only specification example showing detection of any SNV with a limit of detection of 0.015%, first appeared in the priority chain in U.S. Provisional Application No. 62/148,173 (filed on Apr. 15, 2015). As yet another example, in a non-final Office Action mailed on June 18, 2020, the Examiner asserted that claims reciting clonal and subclonal SNV mutations and clonal tumor heterogeneity lacked support prior to filing of U.S. Provisional Application No. 62/146,188 (filed on Apr. 10, 2015). Natera did not dispute that at least claims reciting clonal and subclonal SNVs and determining clonal tumor heterogeneity lacked support prior to April 10, 2015.

B. ’454 Patent

The ’454 patent is issued from U.S. Patent Application No. 17/738,354 (filed on May 6, 2022), which purports to be a continuation of U.S. Patent Application No. 17/692,469 (filed on Mar. 11, 2022), which is a continuation of U.S. Patent Application No. 15/898,145 (filed on Feb. 15, 2018, now U.S. Patent No. 11,319,595), which is a continuation of U.S. Patent Application No. 14/692,703 (filed on April 21, 2015, now U.S. Patent No. 10,179,937), which claims priority to U.S. Provisional Application No. 61/982,245 (filed on Apr. 21, 2014), U.S. Provisional Application No. 61,987,407 (filed on May 1, 2014), U.S. Provisional Application No. 62/066,514 (filed on Oct. 21, 2014), U.S. Provisional Application No. 62/146,188 (filed on Apr.

10, 2015), U.S. Provisional Application No. 62/147,377 (filed on Apr. 14, 2015), U.S. Provisional Application No. 62/148,173 (filed on Apr. 15, 2015).

Natera acknowledges, for the '454 patent, "[t]he priority date of each of the asserted claims is by April 21, 2015." Natera's First Supplemental Response to NeoGenomics' Interrogatory No. 1 at 10; *see also* Preliminary Infringement Contentions at 8. For instance, Example 13, the only specification support for "whole exome sequencing" or "whole genome sequencing" or a tumor-informed assay as claimed, appeared in its entirety for the first time in the priority chain in U.S. Patent Application No. 14/692,703 filed on April 21, 2015. A version of Example 13 mentioning whole exome sequencing appeared for the first time in the priority chain in U.S. Provisional Application No. 62/146,188 (filed on Apr. 10, 2015). Earlier disclosures of "long read sequencing" and "phasing" are unavailing because a POSA at Natera's asserted priority date would have understood that "long read sequencing" and "phasing" could be, and often were, accomplished on smaller portions of genomic material than whole exomes or whole genomes. As another example, Example 14, the only specification example showing detection of any SNV with a limit of detection of 0.015% or sequencing any target locus with a read depth of 50,000, first appeared in the priority chain in U.S. Provisional Application No. 62/148,173 (filed on Apr. 15, 2015). As yet another example, with respect to the claim limitation "an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA," there is no written description support in the specification until the application No. 14/692,703, filed on April 21, 2015.

Regardless, the Asserted Claims of the '454 patent are only entitled to a priority date of May 6, 2022, when Natera filed the application leading up to the '454 patent with the claim limitation "*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 the cell-free DNA isolated from a plasma sample of the subject* or DNA derived therefrom.” The specification of the ’454 patent lacks adequate disclosure to support this claim limitation, much less in combination with the limitations an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA. *See* section (IV)(A)(2)(d) *infra*. Accordingly, the priority date of the ’454 patent is its filing date— May 6, 2022.

III. THE ASSERTED CLAIMS ARE INVALID BASED ON THE PRIOR ART

The Asserted Patents are invalid under 35 U.S.C. § 102 and/or 35 U.S.C. § 103 for being anticipated and/or obvious over the prior art and the knowledge of a POSA.

Under 35 U.S.C. § 102, “[a] claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.” *Verdegaal Bros., Inc. v. Union Oil Co. of California*, 814 F.2d 628, 631 (Fed. Cir. 1987). “When a claim covers several structures or compositions, either generically or as alternatives, the claim is deemed anticipated if any of the structures or compositions within the scope of the claim is known in the prior art.” *Brown v. 3M*, 265 F.3d 1349, 1351 (Fed. Cir. 2001).

The question of whether a claimed invention is obvious under § 103 considers the scope and content of the prior art as of the effective filing date of the claim, what the similarities are between the claimed invention and the prior art, and what the level of ordinary skill in the art was at the effective filing date. *KSR Int’l Co. v. Teleflex Inc.*, 550 U.S. 398, 406 (2007). Where the claimed subject matter is no more than a predictable result of using known prior art elements according to their established functions, the claim is obvious and hence, invalid. *See id.* at 416. In determining whether there are known elements in the prior art, a court can “look to

interrelated teachings of multiple patents; the effects of demands known to the design community or present in the marketplace; and the background knowledge possessed by a person having ordinary skill in the art.” *Id.* at 418. For example, obviousness can be demonstrated by showing “there existed at the time of invention a known problem for which there was an obvious solution encompassed by the patent’s claims.” *Id.* at 420. “[A]ny 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.” *Id.* Common sense also teaches that “familiar items may have obvious uses beyond their primary purposes, and in many cases a person of ordinary skill will be able to fit the teachings of multiple patents together like pieces of a puzzle.” *Id.*

A. The Prior Art

In the accompanying exhibits, Defendant identifies specific exemplary portions of prior art references that disclose the elements of the Asserted Claims. Although Defendant has identified at least one citation per element for each reference, each and every disclosure of the same element in the reference is not necessarily identified. In an effort to focus the issues, Defendant identifies specific portions of the cited references.

It should be recognized that a person of ordinary skill in the art (“POSA”) generally would read a prior art reference as a whole. It should also be recognized that, when evaluating a prior art reference, a POSA may consult multiple documents about that reference. One of ordinary skill in the art would read these prior art references in the context of other publications, literature, and general knowledge in the field available at Natera’s asserted priority date for the Asserted Claims. To understand and interpret any specific statement or disclosure in a prior art

reference, a POSA would, when helpful, rely upon other information, including other publications and general scientific or engineering knowledge.

Defendant therefore reserves the right to rely upon other unidentified portions of the prior art references and on other publications and expert testimony to provide context and to aid understanding and interpretation of the identified portions. Defendant also reserves the right to rely upon other portions of the prior art references, other publications, and the testimony of experts to establish that the alleged invention is anticipated or would have been obvious to a POSA, including on the basis of modifying or combining certain cited references. Defendant also reserves the right to rely upon any admissions relating to prior art in the Asserted Patents or their prosecution histories.

Where Defendant identifies a particular figure in a prior art reference, the identification should be understood to encompass the caption and description of the figure, as well as any text relating to the figure, in addition to the figure itself. Similarly, where an identified portion of text refers to a figure or other material, the identification should be understood to include the referenced figure or other material as well.

To the extent that they constitute prior art, Defendant reserves the right to rely upon foreign counterparts of the U.S. Patents identified in these contentions; U.S. counterparts of foreign patents and foreign patent applications identified in these contentions; U.S. and foreign patents and patent applications corresponding to articles and publications identified in these contentions; and any systems, products, or prior inventions that relate to any references identified in these contentions.

For the purposes of these contentions, the references are named with abbreviated nomenclature, but Defendant reserves the right to give references other nomenclature, labels or titles for expert discovery, trial, other proceedings, briefings, or uses in this litigation.

B. General Knowledge Of A POSA

1. Multiplex PCR

Performing multiplex PCR in a single reaction volume was well known in the art at the time of Natera's asserted priority date, including for samples derived from cell-free DNA. *See, e.g.,* Couraud et al., *Noninvasive Diagnosis of Actionable Mutations by Deep Sequencing of Circulating Free DNA in Lung Cancer from Never-Smokers: A Proof-of-Concept Study from BioCAST/ IFCT-1002*, Clin Cancer Res. 20(17) 4615–16 (Sept. 2014) (describing multiplex PCR reactions to amplify 12 target loci from isolated cfDNA comprising circulating tumor DNA, followed by Next-Generation Sequencing (NGS) of the amplified DNA); Kandioler, US2012/0171687 at [0053]-[0057] (describing multiplex PCR from cell-free tumor DNA followed by sequencing to determine p53 mutation status); Horlitz et al., WO2013/045432 at 32:26–33:19 (explaining that “isolated extracellular nucleic acids” can be analyzed by techniques including “multiplex polymerase chain reaction” and that such technologies “are well-known to the skilled person and thus, do not need further description”); Ronald Lebofsky, et al., *Circulating tumor DNA as a non-invasive substitute to metastasis biopsy for tumor genotyping and personalized medicine in a prospective trial across all tumor types*, 9 Molecular Oncology 783–90 (Dec. 2014) (“At least five nanograms of extracted DNA was used to generate amplicons in 46 oncogenes and tumor suppressor genes through optimized multiplex PCR” followed by next-generation sequencing); Gerald Schochetman, et al., *Polymerase Chain Reaction*, 158 J. of Infectious Diseases 6, 1154 (Dec. 1988) (describing PCR generally); Katherine Elea Varley and

Robi David Mitra, *Nested Patch PCR enables highly multiplexed mutation discovery in candidate genes*, 18 Genome Res. 1844, 1847–48 (2008) (teaching that “Nested Patch PCR 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”); U.S. Patent Application Publication 2004/0126760 at [0025] (filed May 17, 2001) (“[t]he 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] (“[t]he present invention is preferably used with at least 5 targets . . . and even more preferably with at least 100 targets”); U.S. Patent No. 7,790,393 at 25:7–14 (filed July 16, 2008) (explaining that the “highly multiplex PCR include[s] hundreds to thousands of products in a single reactions”), 26:63–27:3 (disclosing that in some embodiments “the number of PCR reactions is from about than [sic] 100 to 1,000”); U.S. Patent Application Publication 2010/0285478 at 212–214 (filed March 26, 2010) (teaching a multiplex reaction with at least 100 targets and at least 100 primer pairs and “[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”); U.S. Patent Application Publication 2012/0270212 at [0267] (filed No. 18, 2011) (describing multiplex PCR of DNA derived from cfDNA, and explaining that “[w]hereas traditional multiplex PCRs evaluate up to fifty loci simultaneously, the approach described herein may be used to enable simultaneous evaluation of more than . . .

500 loci simultaneously . . .”); *id.* at [0303] (“Prior art demonstrates that ordinary people skilled in the art typically design 100+ bp amplicons . . . In an embodiment, the amplicons may be designed to be less than 50 bp.”); *id.* at [0515]–[0516] (teaching an example using 800-plex amplification of a DNA library prepared from cell-free DNA); *id.* at [0232] (teaching the multiplex PCR can be performed on “the mixture of DNA molecules . . . derived from DNA isolated from biological material that has already liberated from cells such as blood plasma, which is known to contain cell free DNA”); Fiona Kaper et al., *Parallel Preparation of Targeted Resequencing Libraries from 480 Genomic Regions Using Multiplex PCR on the Access Array™ System*, American Association for Cancer Research (AACR) 101st Annual Meeting (2010) (teaching use of an “Access Array™” system for scaling up PCR capabilities by using 48 sample inputs with 48 primer sets, thus performing multiplex PCR); Hui-Yun Wang et al., *A genotyping system capable of simultaneously analyzing >1000 single nucleotide polymorphisms in a haploid genome*, 15 Genome Res. 276, 276–77 (2005) (demonstrating commercialization of systems wherein “universal sequences are attached to the amplicons with different approaches before PCR, allowing >1000 SNP-containing sequences to be amplified in a single reaction” and noting that such systems have “significantly facilitated high-throughput genotyping”).

Natera has admitted that multiplex PCR of targets up to about 100 assays in the same well was conventional before Natera’s asserted priority date for the ’596 and ’454 patents. For example, Natera’s U.S. Patent No. 11,519,035 (“the ’035 patent”) describes “the general belief in the art” was that amplifying multiple targets with “multiplexing PCR” was already feasible up “to about 100 assays in the same well.” ’035 patent at 48:25–29, 85:14–16 (similar), 87:29–57 (similar). *See also* ’035 patent at 2:65–3:4 (describing in the Background section how “simultaneous 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 128:57–130:22 (disclosing that some embodiments utilize third-party "multiplex PCR kits"). *See also* '454 patent at 44:40–42 (“The multiplex PCR and next generation sequencing that may be used increase throughput and reduces costs.”). *See also* United States Patent Application Publication No. 2012/0270212 at [0217] (“Note that the prior art includes disclosures of successful multiplexed PCR reactions involving pools of up to about 50 or 100 primers, and not more”), [0284] (describing application to cancer patient plasma samples), [0064] (disclosing “highly efficient highly multiplexed targeted PCR to amplify DNA followed by high throughput sequencing to determine the allele frequencies at each target locus” and stating that “[t]he ability to multiplex more than about 50 or 100 PCR primers in one reaction in a way that most of the resulting sequence reads map to targeted loci is novel and non-obvious”).

Methods to perform multiplex PCR of more than 100-targets followed by next-generation sequencing to detect mutations in samples derived from cell-free DNA without generating significant spurious products were also known in the prior art before Natera’s asserted priority date, including for cell-free DNA comprising circulating tumor DNA or DNA derived therefrom. *See, e.g.*, United States Patent Application Publication No. 2012/0270212 at [0291]–[0298] (describing primer design methods for highly multiplexed PCR while minimizing primer-dimers or other “mischief products”), [0271]–[0322] (describing nested multiplex PCR and mini-PCR methods that reduce the number of spurious amplification products), [0279] (explaining that partly nested multiplex amplification “greatly reduces the number of spurious amplicons”) [0209] (“[a]ny claim in the instant disclosure that reads on ... measurements made on maternal

blood can equally well read on determining the ploidy state or genetic state of a cancer from the measurements on host blood”).

Further, Natera agrees multiplex PCR was known in the art well before 2014. Natera, Inc.’s Response to Emergency Mot. to Stay P.I. Pending Appeal, 2, *NeoGenomics Lab ’ys, Inc. v. Natera, Inc.*, No. 24-1324, 24-1409 (Fed. Cir.) (Dkt. 25-1) (explaining “[p]rior art amplification methods like Multiplex PCR”); Dkt. 145, Michael Metzker Decl. ISO Natera’s Reply ISO Mot. for P.I., ¶255 (“By 2011, one of ordinary skill in the art would have been familiar with multiplex PCR amplification.”); Metzker 11/14/2023 Dep. Tr. at 27:9-17 (acknowledging a POSA would have been aware of methods to design primers for multiplex PCR amplification assays that would avoid primer dimers as of the Natera-asserted priority dates of the asserted patents). Accordingly, performing multiplex PCR in a single reaction volume was routine and conventional at Natera’s asserted priority date for the ’596 and ’454 patents.

Prior art methods included multiplex PCR followed by sequencing of the amplified cell-free DNA. *See, e.g.*, Couraud at 4615 (describing multiplex PCR of known cancer hotspot target regions from cell-free DNA followed by next-generation sequencing); Lebofsky at 786 (describing multiplex PCR amplification from cell-free DNA to generate amplicons in 46 oncogenes and tumor suppressor genes followed by next-generation sequencing).

Methods avoid or remove spurious amplification products such as primer dimers in multiplex PCR products were well-known in the art, including: (1) primer design methods that avoid primer dimers; (2) nested multiplex PCR; (3) multiplex PCR followed by single-plex PCR to exclude non-specific products; and (4) purification methods such as gel extraction or bead purifications performed under conditions that remove spurious amplicons from the amplified DNA.

First, primer design methods to avoid primer dimer formation in multiplex PCR were known in the art. *See, e.g.*, Shen et al. *MPprimer: a program for reliable multiplex PCR primer design*, BMC Bioinformatics 2010, 11:143 (“Shen”). Shen describes an algorithm for multiplex PCR primer design to “avoid primer dimerization” by “examin[ing] each pair of primers in a [primer set combination] using a dimer checking program” (*id.* at 2). This approach allowed successful multiplex PCR of 20 different targets in the same reaction. *See, e.g., id.* at 1, 5. Available primer design tools such as MPrimer use a “scoring matrix” based specificity and the likelihood of primer dimer formation to select primers for successful multiplex PCR. *See, e.g., id.* at 2-3. Natera’s own prior art publication US 2012/0270212 (“Rabinowitz”) describes a similar approach to primer design by removing primers likely to form primer dimers. *See, e.g.,* Rabinowitz at [0291]-[0293] (describing ranking primer interactions using a scoring function, and stating that this approach has been used to create “multiplex primer sets of up 10,000 primers”). The same basic approach to avoiding primer dimers is described in the ’596 and ’454 patents (*see, e.g.,* ’596 patent at 105:65-107:67¹), including the well-known Primer3 program for selecting initial primer candidates (*id.* at 111:32-34). *See also* Shen at 1-6 (describing use of Primer3 together with selection methods to avoid primer dimers). Rabinowitz discloses application of such primer design methods to cell-free DNA. *See, e.g.,* [0261]-[0262].

Second, nested multiplex PCR was known to avoid spurious amplification products in highly multiplexed PCR, including for cell-free DNA. *See, e.g.,* WO2013/138510 (“Patel”) at 65-73 (describing nested multiplex PCR to successfully amplify 40 different targets from cell-free DNA while avoiding spurious amplification products). Natera’s own prior art similarly

¹ For simplicity, these Invalidity Contentions sometimes cites only one version of the specification. The ’596 patent and ’454 patents share the same disclosure with slightly different line numbering. The corresponding disclosure of the other patent is incorporated by citation to one specification.

discloses nested multiplex PCR from cell-free DNA libraries to avoid spurious products. *See, e.g.,* Rabinowitz at [0275] (“Multiplex PCR may involve a single round of PCR in which all targets are amplified or it may involve one round of PCR followed by one or more rounds of nested PCR or some variant of nested PCR. . . . Nested PCR reduces the number of spurious amplification targets by amplifying, in subsequent reactions, only those amplification products from the previous one that have the correct internal sequence.”). *See also id.* at [0515]-[0516] (describing 800-plex amplification of target regions from a cell-free DNA library followed by sequencing), [0516] (describing 150-plex amplification of target regions from a cell-free DNA library followed by sequencing, [0522]-[0529] (describing a hemi-nested multiplex PCR reaction of 9,600 targets followed by sequencing); *see also id.* at [0530]-[0533].

Third, prior art methods such as TAm-Seq included an intervening single-plex PCR step between multiplex PCR and sequencing, “thus excluding nonspecific products.” *See, e.g.,* Forshew at 2.

Fourth, methods were available to remove non-specific products using intervening purification steps between multiplex PCR and sequencing. Such purification methods include gel extraction or bead purifications performed under conditions that remove spurious amplicons from the amplified DNA (*e.g.,* multiple rounds of bead purifications). *See, e.g.,* Nguyen-Dumont et al., *A High-Plex PCR Approach for Massively Parallel Sequencing*, *BioTechniques* 55:2, 69-74 (2013) at 72 (“the size selection step that follows PCR eliminates the great majority of off-target reaction by-products”). Nguyen-Dumont describes performing such size-selection using gel extraction (*id.* at 70) followed by sequencing (*id.* at 71), and further explains that “[t]he size selection step could optionally be automated using a system such as the Pippin Prep System (Sage Science).” *Id.* at 74. Automated gel extraction using the Pippin Prep System was a well-

known method to select nucleic acids based on size, removing spurious amplification products including primer dimers. *See, e.g.,* Sage Science, *Say Goodbye to Manual Gels: Automated DNA Size Selection Has Arrived* (2012); Thermo Fisher Scientific Inc. *Pippin™ DNA size selection systems: Improving NGS Data Everywhere* (2013); Quail et al., *Evaluation and optimisation of preparative semi-automated electrophoresis systems for Illumina library preparation*, *Electrophoresis*, 33(23):3521–3528 (Dec. 2012) (“Quail”). Performing multiple rounds of bead purification under certain conditions was another known method to remove some spurious products, but did not accomplish size selection as stringently as gel extraction. *See* Quail at 3523-3525.

2. Sequencing Technologies

The use of “next generation,” “high-throughput,” and “massively parallel” sequencing for cell-free DNA was also well known in the art prior to Natera’s asserted priority date for the ’454 and ’596 patents. For instance, as early as 2000, Lynx Therapeutics (USA) Company had launched one of the first next generation sequencing technologies, known as Massively Parallel Signature Sequencing (MPSS), which was later acquired by Illumina, Inc. *See* Marina Barba et al., *Historical Perspective, Development and Applications of Next-Generation Sequencing in Plant Virology*, 6 *Viruses* 106, 109 (Jan. 6, 2014). *See also* John R. ten Bosch & Wayne W. Grody, *Next-Generation Sequencing in Molecular Diagnostics*, *Molecular Diagnostics: Techniques and Applications for the Clinical Laboratory*, Chap. 6, 59–67 (1st ed. 2010) (describing “the uses and potential uses of [massively parallel sequencing] technology in the clinical molecular diagnostic laboratory”); Elaine R. Mardis, *Next-Generation DNA Sequencing Methods*, 9 *Annu. Rev. Genomics Hum. Genet.* 387, 388 (2008) (explaining that, in 2008, “[t]hree platforms for massively parallel DNA sequencing read production are in reasonably

widespread use at present: the Roche/454 FLX . . . the Illumina/Solexa Genome Analyzer . . . and the Applied Biosystems SOLiD™ System”); Jay Shendure & Hanlee Ji, *Next-generation DNA sequencing*, 26 *Nature Biotechnology* 10, 1136 (Oct. 2008) (teaching, in 2008, “[a]lternative strategies for DNA sequencing,” including “various implementations of cyclic-array sequencing that have recently been realized in a commercial product (e.g., 454 sequencing . . . Solexa technology . . . the SOLiD platform . . . and the HeliScope Single Molecule Sequencer technology”); Sarah-Jane Dawson et al., *Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer*, 368 *N. Engl. J. Med.* 13, 1200 (Mar. 28, 2013) (“Dawson”) (“Advances in sequencing technologies ha[s] enabled the rapid identification of somatic genomic alterations in individual tumors.”); Matthew Meyerson et al., *Advances in understanding cancer genomes through second-generation sequencing*, 11 *Nature Reviews* 685, Abstract (Oct. 2010) (explaining that the “application of second-generation DNA sequencing technologies (also known as next-generation sequencing)—through whole-genome, whole-exome and whole-transcriptome approaches—is allowing substantial advances in cancer genomics”).

The '035 patent describes “high-throughput next-generation sequencing techniques” or “massively parallel” sequencing technology as known technologies. *See, e.g.*, '035 patent at 109:43-49 (describing an embodiment that “may be sequenced using any one of the previous, current, or next generation of sequencing instruments that sequences a clonal sample . . . examples include ILLUMINA GAIIX, ILLUMINA HISEQ, LIFE TECHNOLOGIES SOLiD, 5500XL); 120:20-26 (“DNA sequencing techniques, particularly high-throughput next-generation sequencing techniques (often referred to as massively parallel sequencing techniques) such as those employed in MYSEQ (ILLUMINA), HISEQ (ILLUMINA), ION TORRENT (LIFE TECHNOLOGIES), GENOME ANALYZER ILX (ILLUMINA), GS FLEX+(ROCHE

454) etc., can be used . . .”). *See also* Natera’s own prior art publication U.S. 2012/0270212 at [0229] (describing an embodiment that “may be sequenced using any one of the previous, current, or next generation of sequencing instruments that sequences a clonal sample (a sample generated from a single molecule; examples include ILLUMINA GAIIx, ILLUMINA HISEQ, LIFE TECHNOLOGIES SOLiD, 5500XL”).

Further, Natera agrees that “high-throughput” sequencing was a “conventional technique” for cfDNA analysis since at least 2009. *See* Natera, Inc.’s Opening Brief in Support of its Mot. for Summary Judgment, *CareDx, Inc. et al v. Natera, Inc.*, No. 1:19-cv-00567 (D. Del. June 6, 2020) (Dkt. 101 at 2) (explaining that “the prior and contemporaneous scientific literature from 2009 and before confirms” that sequencing cfDNA “using ‘multiplex’ or ‘high-throughput’ sequencing” was a conventional technique); *see also* Natera, Inc.’s Concise Statement of Facts in Support of Mot. for Summary Judgment, *CareDx, Inc. et al v. Natera, Inc.*, No. 1:19-cv-00567 (D. Del. June 11, 2020) (Dkt. 102 at 7) (contending it was undisputed that “[b]y November 6, 2009 POSAs were using commercial laboratory instruments for high-throughput sequencing of samples comprising cell-free nucleic acids using sequencing-by-synthesis”).

Next-generation sequencing technologies improved dramatically between their initial introduction in 2005 and 2015, before Natera’s asserted priority date of the asserted patents. *See* Erwin L. van Dijk et al., *Ten years of next-generation sequencing technology*, 30 Trends Genet. 9, 418-420 (Sept. 2014) (“van Dijk”). In January 2014, Illumina released the HiSeq X Ten, a set of ten HiSeq X sequencing machines, “with the staggering capacity to generate up to 1.8 Tb of sequence per run.” van Dijk at 420; *see also* Press Release, Illumina, Illumina Introduces the HiSeq X™ Ten Sequencing System (Jan. 14, 2014) <https://investor.illumina.com/news/press-release-details/2014/Illumina-Introduces-the-HiSeq-X-Ten-Sequencing-System/default.aspx>

(captured Dec. 11, 2024). These improvements in sequencing technologies came with dramatically reduced costs for high-throughput sequencing. *See* van Dijk at Figure 1C (showing reduced sequencing costs through 2014); *see also* Kris A. Wetterstrand. DNA Sequencing Costs: Data from the NHGRI Genome Sequencing Program (GSP). www.genome.gov/sequencingcostsdata. (captured Dec. 12, 2024) (showing reduced costs for DNA sequencing through 2022).

3. Sequencing Depth

By Natera's asserted priority date, reduced sequencing costs were well known to facilitate sequencing at higher read depths. For example, "lower costs ... provide more widespread opportunities for laboratories to increase the sequencing depth that is used in their experiments." David Sims et al., *Sequencing depth and coverage: key considerations in genomic analyses*, 15 Nat. Rev. Genet. 121-132, 130 (Feb. 2014) ("Sims"). The advantages of sequencing at greater read depth were also well understood. *See, e.g., id.* ("Increased depth would be expected to improve the precision by which known phenomena can be defined and to reveal new phenomena that cannot be observed using current experimental designs."). In particular, increased read depth was known to improve detection of sequence variants as distinguished from sequencing errors. *See, e.g., id.* at 121 (explaining how "[i]ncreased depth of coverage therefore 'rescues' inadequacies in sequencing methods" and can "produce a strongly supported variant call with an associated error rate of 10^{-16} " even if reads themselves contain a higher error rate of 1%); Tim Forshew et al., *Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA*, 4 Sci. Transl., Med. 136, 10 (May 30, 2012) ("Forshew") ("Higher read depth or fidelity, additional replicates, or improved algorithms could allow for enhanced mutation detection without change to protocols.").

Sequencing with high read depths was also known to predictably improve detection of SNVs present at 0.01% or less of the DNA comprising the SNV locus. *See, e.g.,* Yoji Kukita et al., *Quantitative Identification of Mutant Alleles Derived from Lung Cancer in Plasma Cell-Free DNA via Anomaly Detection Using Deep Sequencing Data*, PLoS One. 8(11), 2 (Nov. 2013) (“Kukita”) (“With an increasing read depth and decreasing RER, the detection limit decreases” and a limit of detection of 0.01% “can be achieved with 100,000 reads when the read error rate is below 0.01%.”); Moritz Gerstung et al., *Reliable detection of subclonal single-nucleotide variants in tumour cell populations*. Nat. Comms. 3, 811 (May 1, 2012) (including its Supplementary Information, “Gerstung”), Supplementary Figure S5, panel b (showing that increasing read depth from 10,000 to 100,000 improves the significant fraction of variants detected at frequencies of 1e-04 (0.01% or 1 in 10,000)); U.S. Patent Application Publication No. 2010/0261189 at Table 2 (showing the relationship between the number of reads and the minimum frequency of SNP in population detectable with 99% confidence).

A POSA would also have been well aware of the desirability and feasibility of increasing read depth to sequence amplified DNA from multiplex amplification of cfDNA or DNA derived therefrom. *See, e.g.,* Kukita at 2 (suggesting a read depth of 100,000 to achieve detection of SNVs present at 0.01% in cfDNA); Forshew at Table 1 (showing mean sequencing depths of up to 7,700 for cfDNA amplified with a multiplex amplification step), 10 (“[h]igher read depth ... could allow for enhanced mutation detection without change to protocols”); Abhijit Ajit Patel, *Measurement of Nucleic Acid Variants Using Highly-Multiplexed Error-Suppressed Deep Sequencing*, Patent Application WO 2013/138510 AI (Sept. 19, 2013) at 72 (“Patel”) (for cfDNA amplified with a 40-plex multiplex amplification step, “40 different gene target regions were

fairly evenly represented among the filtered sequences. The median sequence count for the 40 gene-specific regions (all barcodes) was 166,867.”).

By 2014, it was also well-known in the art to apply NGS methods for analysis of amplified cfDNA. *See, e.g.*, Couraud at 4615–16 (describing multiplex PCR reactions to amplify 12 target loci from isolated cfDNA comprising circulating tumor DNA followed by NGS of the amplified DNA); US 2012/0270212 at [0229] (“once a mixture has been preferentially enriched at the set of target loci, it may be sequenced using any one of the previous, current, or next generation of sequencing instruments”). As these and other references illustrate, NGS technology was being adopted for the analysis of cfDNA, including circulating tumor (ctDNA), well before the earliest critical date of the ’596 and ’454 patents.

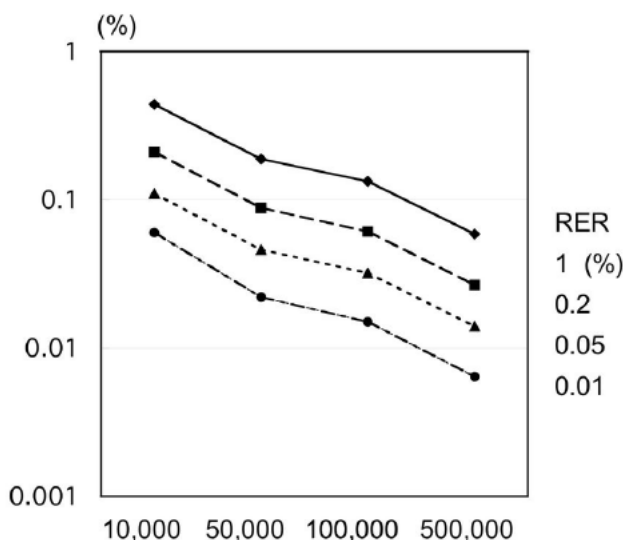
4. Detection of SNVs From Sequencing Reads

Before Natera’s asserted priority date for the ’454 and ’596 patents, several well-known approaches existed to detect rare SNVs in a large background of wild-type sequences, including for detection of an SNV present at less than or equal to 0.015% of the DNA comprising the SNV locus. As discussed below, it was also known that such techniques could be (and had been) applied to cell-free DNA assays.

A first family of approaches involved estimating background error rates for particular substitutions, and then sequencing at a sufficiently high read depth to detect the SNV based on the background error rate for the substitution. This was the approach taken, for example, by Forshew, who described calling point mutations in a sample “only if nonreference AFs were above the respective substitution-specific background distribution at a high confidence margin.” Forshew at 3; *see also id.* (explaining that when measuring a predefined mutation identified in a tumor “[the] minimal nonreference AFs that could be detected depend on the read depth and

background rates of nonreference reads, which vary per locus and substitution type”). Forshew explained that mutation detection could be enhanced by increasing read depth with this approach, without change to protocols. *See* Forshew at 10. The predictable relationship between read depth, background error frequency, and minimal detectable SNV allele frequency described by Forshew was well understood before Natera’s asserted priority date.

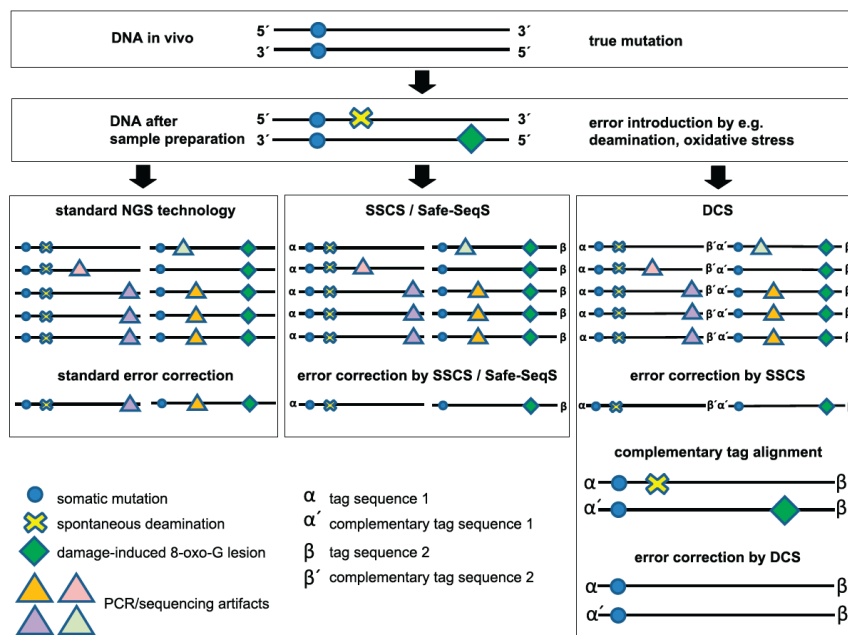
As one example, Kukita Figure 1a (reproduced below) shows the relationship between the mutation detection limit, read depth, and read error rate at a significance level of $p=2 \times 10^{-5}$ for each individual detection without multiplicity correction, assuming that read errors occur following a Poisson distribution. *See* Kukita at 2; *see also* Gerstung at Supplementary Figure S5B (showing that increasing read depth from 10,000 to 100,000 improves the significant fraction of variants detected at frequencies of 1×10^{-4} (0.01% or 1 in 10,000).



In sum, it was well understood before Natera’s asserted priority date that using higher read depths in combination with the well-understood approach of estimating local background error rates, an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus could be detected. *See, e.g.,* Kukita at Abstract, 3 (describing detection of SNVs from sequencing reads at levels as low as 1 in 10,000 and application of the method to

cfDNA); Gerstung at Abstract, 2 (describing method using estimated local error rate capable of accurately detecting SNVs with frequencies as low as 1/10,000 alleles).

A second group of well-known approaches involved use of molecular barcoding. While the local error-rate estimation approaches above achieved confident detection of SNVs at frequencies around 1 in 10,000, these barcoding-based approaches achieved detection of even rarer mutations by distinguishing between sequencing errors and true SNVs. *See generally* Stepan Kisch & Christoph A. Klein, *Sequence error storms and the landscape of mutations in cancer*, 109 PNAS 36, 14289–14290 (Sept. 4, 2012) (“Kirsch”) (describing barcoding-based error correction methods Safe-SeqS (PCR-based barcode attachment) and Duplex sequencing (ligation-based barcode attachment)). Fig. 1 of Kirsch (reproduced below) summarizes these two approaches.



The use of molecular barcoding allows identification of families of nucleic acid sequencing reads which share the same tag sequence, thereby allowing true mutations to be distinguished from errors: the true mutation will be common among reads in the same family.

Methods such as Safe-SeqS incorporated such tags into PCR primers, and achieved detection of SNVs present at frequencies as low as 1 mutant template among 1,000,000 wild-type templates (0.0001%). *See* Isaac Kinde et al., *Evaluation of DNA from the Papanicolaou Test to Detect Ovarian and Endometrial Cancers*, 5 Sci. Transl. Med. 167, (Jan. 9, 2013) (including its supplementary tables and Supplementary Materials, “Kinde”) at 4. An analogous method called “duplex sequencing” provided an even lower limit of detection by distinguishing base changes present in only one strand of complementary DNA, which are likely to represent errors. *See* Kirsch at 14289; *see also* Michael W. Schmitt, et al., *Detection of ultra-rare mutations by next-generation sequencing*, Proc. Natl. Acad. Sci. USA 109:36, 14508–14513 (Sept. 4, 2012) (“Schmitt”) (describing duplex sequencing).

Barcoding-based error correction methods were widely cited before Natera’s asserted priority date. *See, e.g.*, Eleftherios P. Diamandis, *Towards Identification of True Cancer Biomarkers*, 12 BMC Med. 156 (2014) at 2 (noting that Safe-SeqS is a “highly sensitive and specific assay” which is “immune to the presence of a vast excess of normal alleles”); Nicholas E. Navin, *Cancer Genomics: One Cell at a Time*, 15 Genome Biol. 452 (Aug. 30, 2014) at 9 (explaining that sequencing error rates limit the accurate detection of rare mutations, which can be addressed using “single-molecule barcoding methods such as duplex sequencing or Safe-Seq, which reduce the sequencing error rate from 10^{-2} to 10^{-10} ”) (citing Schmitt and Isaac Kinde et al., *Detection and quantification of rare mutations with massively parallel sequencing*, Proc. Natl. Acad. Sci. USA, . 7;108(23), 9530-5 (June 7, 2011) (“Kinde 2011”)). And Natera’s own specification admits that error-corrected sequencing methods such as duplex sequencing were available in the prior art and “greatly reduce[] errors by independently tagging and sequencing each of the two strands of a DNA duplex.” *See, e.g.*, ’596 patent at 98:66-99:41 (citing Schmitt).

A POSA also would have understood that barcoding-based error correction could be applied to cfDNA analysis. *See, e.g., Iwijn De Vlaminck et al., Circulating Cell-Free DNA Enables Noninvasive Diagnosis of Heart Transplant Rejection*, 6 Sci. Transl. Med. 241241ra77, at 8 (June 18, 2014) (for cfDNA analysis, explaining that “[t]argeted assays have the promise of reduced cost and can, in principle, be combined with molecular bar-coding strategies that may reduce sequencing and PCR error rates”) (citing Kinde 2011); Kinde 2011 at 9530 (describing cfDNA assays as an application of Safe-SeqS). Additionally, well before Natera’s asserted priority date, barcoding-based error correction had already been applied to cfDNA to detect SNVs present at levels below 0.015%. *See, e.g., C. Bettgowda et al., Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies*, 6 Sci. Transl. Med. 224:224ra24 (Feb. 19, 2014) at 4 (disclosing that Safe-SeqS amplification and sequencing were able to detect one mutant template in the DNA purified from up to 5 ml of plasma).²

5. Cell-Free DNA Technologies

Techniques for detecting and analyzing cell-free DNA were known in the art prior to Natera’s asserted priority date for the ’454 and ’596 patents, and it was known that such techniques could also be used with cell-free tumor DNA. For instance, in 2006, Yu-Kwan Tong, and Y.M. Dennis Lo published a review paper titled “Diagnostic developments involving cell-free (circulating) nucleic acids” which surveys the history of analyzing cell-free nucleic acids encompassing both circulating tumor and circulating fetal DNA. Yu-Kwan Tong & Y.M. Dennis Lo, *Diagnostic developments involving cell-free (circulating) nucleic acids*, Clin. Chim.

² Although Bettgowda uses alternative units to describe the mutant allele fraction, a POSA would have understood that detection of 1 mutant template from 5 ml of plasma corresponds to detection of an SNV present in less than or equal to 0.015% of the cell-free DNA comprising the locus. *See, e.g., Martin Horlitz et al., Yields of Viral and Circulating Cell-Free Nucleic Acids Using the QIAamp® Circulating Nucleic Acid Kit* (2011) 259-268 at 262 (for the cfDNA purification kit used by Bettgowda, explaining that circulating cell-free DNA yields per 5 mL sample varied from about 8,000 to more than 80,000 genome equivalents).

Acta. 363:187–196 (Jan. 2006). The paper discusses the “applications” of cancer testing and prenatal diagnosis and describes how “studies of circulating nucleic acids have opened up a new avenue for non-invasive prenatal diagnosis.” *Id.* at 188. *See also, e.g., id.* at Abstract (“Besides circulating DNA and RNA in plasma and serum, cell-free DNA in other body fluids, such as urine, has been detected in patients with different clinical conditions. Regardless of the sources of cell-free DNA for clinical use, the amount is frequently scarce.”); at 192 (“The discovery of circulating nucleic acids has opened up new possibilities for non-invasive detection and monitoring of various disease conditions. In cancer patients, detection and quantification of circulating nucleic acids have shown promise for cancer diagnosis and prognosis. Maternal plasma analysis can be used to detect or exclude certain diseases of the fetus, and quantification of fetal nucleic acids has the potential to be used to screen for certain pregnancy-associated complications. Other applications include monitoring following trauma and stroke, when circulating DNA level is correlated with the severity.”). *See also, e.g.,* Farideh Z. Bischoff et al., *Cell-free fetal DNA in maternal blood: kinetics, source and structure*, 11 Human Reproduction Update, 1:59-67 (2005), (describing the study of ctDNA beginning in the early 1970s and parallel studies demonstrating fetal DNA in plasma and serum from healthy pregnant women in the 1990s) (citing Yanni Y.N, Lui et al., *Circulating DNA in plasma and serum: biology preanalytical issues and diagnostic applications*, Clin. Chem. Lab. Med. 40 962-968 (Oct. 2002)); Vishnu Swarup & M.R. Rajeswari, *Circulating (cell-free nucleic acids) – A promising, non-invasive tool for early detection of several human diseases*, FEBS Lett. 5:795-799 (Mar. 6, 2007) (describing the use of quantitative PCR for quantifying circulating nucleic acids in cancer and prenatal contexts); Y.M. Dennis Lo & Rossa W.K. Chiu, *Next-Generation Sequencing of Plasma/Serum DNA: An Emerging Research and Molecular Diagnostic Tool*, 55 Clin. Chem.

4:607–608 (Apr. 1, 2009); Rossa W.K. Chiu et al., *Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma*, 105 PNAS 20458-20463 (Dec. 23, 2008); Yulia Korshunova et al., *Massively parallel bisulphite pyrosequencing reveals the molecular complexity of breast cancer-associated cytosine-methylation patterns obtained from tissue and serum DNA*, Genome Res. 18:19-29 (2008); John R. ten Bosch & Wayne W. Grody, *Next-Generation Sequencing in Molecular Diagnostics*, Molecular Diagnostics: Techniques and Applications for the Clinical Laboratory, Chap. 6, 59–67 (1st ed. 2010) (teaching methods of ligation- and PCR-mediated tagging of adapters to DNA fragments that can be used on fetal and tumor cfDNA).

6. Minimal Residual Disease Monitoring

Minimal residual disease (“MRD”) monitoring was known in the art prior to Natera’s asserted priority date for the ’454 and ’596 patents. MRD is the monitoring of residual cancer that remains after a patient has undergone treatment such as surgery, radiation, or chemotherapy. See, e.g., K.C. Allen Chan, *Scanning for Cancer Genomic Changes in Plasma: Toward an Era of Personalized Blood-Based Tumor Markers*, 59 Clin. Chem. 11, 1553-1555 (July 2013) (“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.”); 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 2013”) (explaining that MRD monitoring was developed to provide patient-specific care by evaluating a specific individual’s tumor, that tumor’s susceptibility to different treatments, and the individual’s

remission or likelihood of remission); Diego M. Marzese et al., *Diagnostic and prognostic value of circulating tumor-related DNA in cancer patients*, 13 Expert Rev. Mol. Diagn. 827-844 (2013) (reviewing “the diagnostic and prognostic values of cfDNA in cancer patients”).

MRD monitoring generally involves whole genome sequencing or whole exome sequencing of cancerous DNA to identify cancer-related mutations, as well as follow-up monitoring (involving multiplex PCR and sequencing) of these mutations in cfDNA. *See generally* Forshew; Ali Bashashati et al., *Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling*, 231 J. Pathol. 21-34 (2013); *see also* Dawson at 1200 (teaching use of whole genome sequencing for identification of somatic genomic alterations). Such assays are sometimes referred to as tumor-informed assays. Tumor-informed assays were well known to a POSA. *See, e.g.*, Diehn et al., US2016/0032396, Fig. 1A; Rebecca J. Leary et al., *Development of Personalized Tumor Biomarkers using Massively Parallel Sequencing*, 2 Sci. Transl. Med. 20 (Feb. 24, 2010) at 4 (“massively parallel sequencing can be used to develop personalized biomarkers based on somatic rearrangements” which “can be used to detect tumor DNA in the presence of large quantities of normal DNA and in patient plasma”); Bettegowda et al., *Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies*, 6 Sci. Transl Med. 224:224ra24 (Feb. 19, 2014) at 8 (“Bettegowda”) (“[the] specific mutations [assayed in ctDNA] are defined by evaluation of the primary tumor” which “may be less of an obstacle in the future because more cancer patients will have their tumors genetically analyzed to guide therapeutic decisions”); Frank Diehl et al., *Circulating mutant DNA to assess tumor dynamics*, 14 Nature Med. 985-990 at 986 (Sept. 2008) (“Diehl”), Figure 1, 989 (describing personalized ctDNA assays using “identification of mutations in the subject’s tumor as a preliminary step”). Tumor-informed assays were routinely

performed on plasma samples collected at different time points in order to monitor cancer progression over time. *See, e.g.*, Dawson at 1199; Diaz at 579, 583; Diehl at 985.

MRD monitoring was well known in the art and routinely conducted by at least 2013. *See, e.g.*, Chan 2013 at 223 (teaching an MRD approach called “shotgun sequencing”); Forsheew at Abstract (teaching an MRD approach called “TAm-Seq”); Rebecca J. Leary et al., *Development of Personalized Tumor Biomarkers using Massively Parallel Sequencing*, 2 Sci. Transl. Med. 20 (Feb. 24, 2010) (teaching an MRD approach called “chromosomal rearrangements”).

7. Whole Exome Sequencing

Whole-exome sequencing (WES) is a method to sequence only the regions of a genome that code for proteins, called the exome. *See* van Dijk at 418. WES involves the capture of fragmented genomic DNA using probes targeting the exome, followed by sequencing. *Id.* Whole genome and whole exome sequencing were generally understood to be suitable alternatives for obtaining alternatives for the purpose of “[o]btaining the genotype of the tumor in [a] subject” in order to design personalized assays for circulating tumor DNA. *See, e.g.*, US2016/0032396 at [0270] (describing obtaining the genotype of a tumor to design a personalized selector set for a cfDNA assay), [0271] (“Obtaining the genotype of the tumor in the subject may comprise conducting a sequencing reaction on a sample from the subject. Sequencing may comprise whole genome sequencing. Sequencing may comprise whole exome sequencing.”). By 2014, WES had been extensively used for clinical studies, in part because it avoids the expense of whole genome sequencing while still covering ~85% of known disease-causing variants. *van Dijk* at 422. These applications of WES included sequencing tumor

biopsies for tumor-informed assays of cfDNA. *See, e.g.*, Bettgowda at 2; US2016/0032396 at [0270]-[0271].

8. Assaying Cell-Free DNA At Different Time Points

Prior to Natera's asserted priority date for the '454 and '596 patents, it was well known in the art to assay cfDNA at different time points for purposes of monitoring the effect of a treatment or progression of a disease such as cancer. *See, e.g.*, Frank Diehl et al., *Circulating mutant DNA to assess tumor dynamics*, 14 Nature Med. 985-990 at 986 (Sept. 2008) ("We evaluated 18 subjects after a total of 22 surgeries during the course of this study (Supplementary Table 1) ... A sharp drop in the ctDNA level by the day of discharge (two to ten days after surgery) was observed in all subjects who underwent complete resections, with a 99.0% median decrease in ctDNA (range between 10th and 90th percentiles, 58.9–99.8%; Table 1"); *id.* at Figure 4 (showing the results of experiments assaying tumor-specific mutations in cell-free DNA over time); Patel at 2 (disclosing that "multiplex assays described herein can be used for clinical purposes" and "[in] an embodiment, nucleic acid variants within blood can be identified and measured before and after treatment"); *id.* at 4 ("Figs. 7A-7C show the changes in ctDNA levels with treatment or disease progression" with measurements made at "various times in relation to therapeutic interventions and disease status"); *id.* at 5 ("Figs. 8A-8C show the ctDNA levels in patients with fewer than 3 time-points"); Forsheew at 8 ("We studied serial plasma samples collected during follow-up and treatment of two patients with relapsed HGSOV, collected during 104 and 273 days of follow-up and treatment, respectively"); *id.* at 1 (disclosing that "[s]ensitive methods for detecting cancer mutations in plasma" may be useful for "monitoring tumor dynamics over time.")

C. Identification of References

NeoGenomics provides these final invalidity contentions with a reasonable number of prior art references per the Court's December 6, 2024 order. Dkt. 345. When Courts require the parties to narrow the asserted claims and identified prior art references, the accused infringer is routinely allotted a modestly higher number of references than the number of claims the patentee is allotted. *See, e.g., Fuma Int'l LLC v. R.J. Reynolds Vapor Co.*, No. 1:19-cv-00260 (M.D.N.C. Aug. 30, 2019), Dkt. 47 at 2 (limiting invalidity contentions to "no more than 6 per patent claim"); *Certusview Techs., LLC. v. S&N Locating Servs., LLC*, No. 2:13-cv-00346 (E.D. Va. Sept. 30, 2014, filed Oct. 1, 2014), Dkt. 159 (limiting contentions to 25 prior art references for 15 asserted claims); *Intellectual Ventures I LLC, et al. v. Capital One Financial Corp., et al.*, No. 8:14-cv-00111 (D. Md. April 15, 2014), Dkt. 93 at 5 (no limit required for the number of contentions in the invalidity contentions while limiting to "no more than 10 claims from each patent and no more than a total of 32 claims" in the infringement contentions); *see also* General Order 13-20 (E.D. Tex. Oct. 29, 2013) ¶¶ 3 (limiting contentions to "no more than a total of 16 claims" and "no more than a total of 20 references"). Here, Natera asserts 14 claims from the '596 and '454 patents, and NeoGenomics' final invalidity contentions present a total of 10 references and 17 combinations based thereon. The number of references and combinations identified in these final invalidity contentions are well within the limits of reasonable under the foregoing authorities, especially considering the Court has not issued a claim construction order on the '596 patent.

D. Prior Art to the '596 Patent

1. Patent or Patent Application Prior Art

<u>Patent No. & Country of Origin</u>	<u>Filing or Priority Date</u>	<u>Date of Issue or Publication</u>	<u>Cited As</u>	<u>Prior Art Status</u> ³	<u>Chart</u>
WO 2013/138510	March 13, 2012	September 19, 2013	Patel	102(a)	Exhibit 1-C

2. Non-Patent Literature Prior Art

<u>Title</u>	<u>Date of Publication</u>	<u>Cited As</u>	<u>Prior Art Status</u>	<u>Chart</u>
Ali Bashashati, <i>Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling</i> , 231 J. Patho. 21–34 (2013)	June 7, 2013	Bashashati	102(a)	Exhibits A-1, 1-B, and 1-C
Tim Forshew et al., <i>Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA</i> , 4 Sci. Transl., Med. 136 (May 30, 2012)	May 30, 2012	Forsheew	102(a)	Exhibits 1-A and 1-B
Luis A. Diaz Jr. & Albert Bardelli, <i>Liquid biopsies: genotyping circulating tumor DNA</i> , 32 J. Clin. Oncol. 6:579-86	Feb. 20, 2014	Diaz	102(a)	Exhibit 1-A
Isaac Kinde et al., <i>Evaluation of DNA from the Papanicolaou Test to Detect Ovarian and Endometrial Cancers</i> , 5 Sci. Transl. Med. (167):167ra4	Jan. 9, 2013	Kinde	102(a)	Exhibit 1-A
Moritz Gerstung et al., <i>Reliable detection of subclonal single-</i>	May 2012	Gerstung	102(a)	Exhibit 1-B

³ For purposes of these contentions, NeoGenomics discloses the prior art status of the asserted references under the AIA based on the priority of the claims as explained *supra* in Section II. Additionally, Natera does not contend that the '596 Patent claims priority to any application earlier than April 21, 2015.

<i>nucleotide variants in tumour cell populations</i> . Nat. Comms. 3, 811 (May 1, 2012)				
Y. Kukita, <i>Quantitative Identification of Mutant Alleles Derived from Lung Cancer in Plasma Cell-Free DNA via Anomaly Detection Using Deep Sequencing Data</i> . 8(11) PLOS ONE e81468 (Nov. 21, 2013)	Nov. 2013	Kukita	102(a)	Exhibit 1-B
A.L. Young, <i>Quantifying ultra-rare pre-leukemic clones via targeted error-corrected sequencing</i> . 29 Leukemia 1608–1611	Feb. 2015	Young	102(a)	Exhibit 1-A

E. Prior Art to the '454 Patent

1. Patent or Patent Application Prior Art

<u>Patent No. & Country of Origin</u>	<u>Filing or Priority Date</u>	<u>Date of Issue or Publication</u>	<u>Cited As</u>	<u>Prior Art Status</u> ⁴	<u>Chart</u>
WO 2013/138510	March 13, 2012	September 19, 2013	Patel	102(a)	Exhibits 2-A, 2-B, and 2-D

2. Non-Patent Literature Prior Art

<u>Title</u>	<u>Date of Publication</u>	<u>Cited As</u>	<u>Prior Art Status</u>	<u>Chart</u>
Ali Bashashati, <i>Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling</i> , 231 J. Patho. 21–34 (2013)	June 7, 2013	Bashashati	102(a)	Exhibit 2-A

⁴ For purposes of these contentions, NeoGenomics discloses the prior art status of the asserted references under the AIA based on the priority of the claims as explained *supra* in Section II. Additionally, Natera does not contend that the '454 patent claims priority to any application earlier than April 21, 2015.

Tim Forshew et al., <i>Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA</i> , 4 Sci. Transl., Med. 136 (May 30, 2012)	May 30, 2012	Forshew	102(a)	Exhibits 2-B and 2-D
Luis A. Diaz Jr. & Albert Bardelli, <i>Liquid biopsies: genotyping circulating tumor DNA</i> , 32 J. Clin. Oncol. 6:579-86	Feb. 20, 2014	Diaz	102(a)	Exhibit 2-D
A.L. Young, <i>Quantifying ultra-rare pre-leukemic clones via targeted error-corrected sequencing</i> . 29 Leukemia 1608–1611	Feb. 2015	Young	102(a)	Exhibit 2-D
Isaac Kinde et al., <i>Evaluation of DNA from the Papanicolaou Test to Detect Ovarian and Endometrial Cancers</i> , 5 Sci. Transl. Med. (167):167ra4	Jan. 9, 2013	Kinde	102(a)	Exhibit 2-D
Christopher Abbosh et al., <i>Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution</i> , 545 Nature 446 (2017)	May 25, 2017	Abbosh	102(a)	Exhibit 2-C
Y. Kukita, <i>Quantitative Identification of Mutant Alleles Derived from Lung Cancer in Plasma Cell-Free DNA via Anomaly Detection Using Deep Sequencing Data</i> . 8(11) PLOS ONE e81468 (Nov. 21, 2013)	Nov. 2013	Kukita	102(a)	Exhibit 2-B
Moritz Gerstung et al., <i>Reliable detection of subclonal single-nucleotide variants in tumour cell populations</i> , 3 Nat. Comms. 811	May 1, 2012	Gerstung	102(a)	Exhibit 2-B

3. Other Prior Art

<u>Systems</u>	<u>Dates Known, Used, Sold, or Offered for Sale</u>	<u>Identity of Persons with Knowledge</u>
RaDaR	2020	Tim Forshew, Giovanni Marsico

To the extent Natera accuses RaDaR of infringement of claims 1, 8, 10, 12, 14, 15, 22, 24, 26, and 28 the '454 patent, which NeoGenomics disagrees, RaDaR—predating the priority of

the '454 patent (*See* section (II)(B))—renders the claims invalid. *See* Natera's May 2, 2024 preliminary infringement contentions at Ex. 2 and June 14, 2024 final infringement contentions at Ex. 2.

F. Prior Art Combinations

1. '596 Patent

As referenced and discussed in the claim charts attached as Exhibits 1-A to 1-C, multiple prior art references teach or suggest the concepts claimed in the '596 patent. To the extent Natera argues that any concepts claimed in the '596 patent were not contained in any prior art reference, it would, at a minimum, have been obvious to adapt each reference to include the concept or combine it with other references that disclose the concept. In addition, each of the constituent techniques required to carry out the steps of the claimed methods were well-known to those of skill in the art. The reasons for modifying or combining references and for a reasonable expectation of success include at least those identified in Exhibits 1-A through 1-C.

The Asserted Claims of the '596 patent are obvious in light of at least the following combination(s) of prior art:

- Diaz and Forshew alone, optionally in view of Kinde or Young, optionally further in view of Bashashati (Ex. 1-A)
- Forshew alone, optionally in view of Gerstung or Kukita, optionally further in view of Bashashati (Ex. 1-B)
- Bashashati and Patel (Ex. 1-C)

2. '454 Patent

As referenced and discussed in the claim charts attached as Exhibits 2-A to 2-D, multiple prior art references teach or suggest the concepts claimed in the '454 patent. To the extent

Natera argues that any concepts claimed in the '454 patent were not contained in any prior art reference, it would, at a minimum, have been obvious to adapt each reference to include the concept or combine it with other references that disclose the concept. In addition, each of the constituent techniques required to carry out the steps of the claimed methods were well-known to those of skill in the art. The reasons for modifying or combining references and for a reasonable expectation of success include at least those identified in Exhibits 2-A to 2-D, which are incorporated by reference into this section.

The Asserted Claims of the '454 patent are obvious light of at least the following combination(s) of prior art:

- Patel and Bashashati (Ex. 2-A)
- Forsheew alone, optionally in view of Gerstung, Kukita or Patel (Ex. 2-B)
- Abbosh (Ex. 2-C)
- RaDaR system art
- Diaz and Forsheew alone, optionally in view of Kinde, Young, or Patel (Ex. 2-D)

To the extent Dr. Niall Lennon's opening expert report addresses any prior art references or combinations thereof for the '454 patent elected by NeoGenomics for trial, Natera is on notice of those arguments. *See* Niall Lennon August 2, 2024 Expert Report at Section (XV).

NeoGenomics reserves all rights to rely on those opinions.

G. Objective Indicia of Non-obviousness

Evidence of objective indicia must be considered in conducting an obviousness analysis. *In re Cyclobenzaprine Hydrochloride Extended-Release Capsule Pat. Litig.*, 676 F.3d 1063, 1080 (Fed. Cir. 2012). However, with any such showing, evidence of objective indicia of non-obviousness must be commensurate in scope with the claims. There must be a nexus between the

asserted indicia, such as unexpected results, and the claimed invention. *Wyers v. Master Lock Co.*, 616 F.3d 1231, 1246 (Fed. Cir. 2010); *Allergan, Inc. v. Apotex Inc.*, 754 F.3d 952, 963 (Fed. Cir. 2014). “For objective evidence of secondary considerations to be accorded substantial weight, its proponents must establish a nexus between the evidence and the merits of ***the claimed invention***.” *In re Huai-Hung Kao*, 639 F.3d 1057, 1068 (Fed. Cir. 2011) (quoting *Wyers*, 616 F.3d at 1246 (emphasis added)). Where objective indicia “result[] from something other than what is both claimed and *novel* in the claim, there is no nexus to the merits of the claimed invention.” *Id.* “We have repeatedly held that evidence of commercial success must have a nexus to the claims to be given weight in an obviousness analysis. In other words, there must be a ‘legally and factually sufficient connection’ between the evidence and the patent claims. . . . Simply speaking, there may be many reasons a product is commercially successful; it is only where the success is due to the claimed invention that commercial success can show nonobviousness.” *UCB, Inc. v. Actavis Lab’ys UT, Inc.*, 65 F.4th 679, 695 (Fed. Cir. 2023).

Plaintiff has not provided evidence that Signatera practices the Asserted Claims on a limitation-by-limitation basis for either of the Asserted Patents. With respect to the ’454 patent, Plaintiff has not provided evidence, for example, that Signatera achieves sequencing with a depth of read of at least 50,000 per target locus (claim 1); or that Signatera is capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus (claim 14, claim 28); or that Signatera achieves targeted multiplex amplification of 20 to 50 target loci each encompassing a different tumor-specific SNV mutation (claim 8, claim 22). With respect to the ’596 patent, Plaintiff has not provided evidence, for example, that Signatera detects an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus (claim 1); or that Signatera performs targeted multiplex PCR

amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA (claim 1); or that Signatera performs high-throughput sequencing of the amplified DNA (claim 1).

Plaintiff alleges that Signatera has received industry praise, awards, and is commercially successful. *See* Natera's Response to Interrogatory No. 13. But Plaintiff has not shown that the alleged commercial success, industry praise, or awards relating to Signatera are due to the "novel features" of any Asserted Claim of any of the Asserted Patents. For example, FDA Breakthrough Device Designation eligibility is based in part on the disease or condition that the product intends to treat rather than the product itself, and a product does not require breakthrough technology to be eligible. Nor has Plaintiff shown that Signatera is coextensive with any, let alone all, of the Asserted Claims. The specifications of the Asserted Patents describe many alternative methods and techniques for amplifying, sequencing, and detecting cfDNA, none of which are actually required by the Asserted Claims. As another example, Signatera's alleged commercial success is derived from false advertising practice against competitor products such as the Guardant RevealTM assay. Complaint (Dkt. 1) from *Guardant Health Inc. v. Natera, Inc.* (N.D. Cal. Case No. 21-4062) at 8-18. Natera falsely and misleadingly stated and implied that Reveal suffers from significant gaps in study design and performance, and that Signatera's performance is superior to Reveal. *See* Final Verdict Form in *Guardant Health Inc. v. Natera, Inc.* (N.D. Cal. Case No. 21-4062) (Dkt. 847) at 1 finding "Natera engaged in false advertising in violation of the Lanham Act." As a result, from the first quarter of 2021 to August of 2023, there was a market outperformance of Natera's Signatera assay and corresponding underperformance of the Guardant RevealTM assay. Trial transcript of *Guardant Health Inc. v. Natera, Inc.* (N.D. Cal. Case No. 21-4062) dated November 14, 2024

(Volume 6) (Dkt. 818) at 1316. As yet another example, the claims of the '454 and '596 patents are directed to a tumor-informed assay, while Natera tacitly acknowledges its Signatera product competes in the same market with tumor-naïve products such as the Guardant Reveal™ assay—ruling out any contribution from the tumor-informed feature as claimed to the alleged commercial success of Signatera. *See, e.g.,* trial transcript of *Guardant Health Inc. v. Natera, Inc.* (N.D. Cal. Case No. 21-4062) dated November 15, 2024 (Volume 7) (Dkt. 819) at 1503 (“it’s fair to compare information and data between tumor-informed and tumor-naïve MRD tests”).

Natera has not demonstrated a sufficient nexus between the alleged secondary considerations and the Asserted Claims. For example, in other contexts, Natera has stated that its use of an amplified library derived from cfDNA rather than isolated cfDNA as input for multiplex PCR may account for its “improvement in performance.” *See* Yücel Altuğ et al., *Analytical Validation of a Single-nucleotide Polymorphism-based Donor-derived Cell-free DNA Assay for Detecting Rejection in Kidney Transplant Patients*, 103:12 Transplantation 2657, 2663-2664 (Dec. 2019) (“Several factors may account for this improvement in performance. The library preparation step reduces variability caused by the long DNA fragments.”). Natera has also admitted that Signatera uses a universally amplified DNA library as input for multiplex PCR, and that the Natera plasma library preparation process alters characteristics such as DNA fragment size distribution that could affect assay results. *See A personalized, tumor-informed approach to detect molecular residual disease with high sensitivity and specificity*, 2019_08_27_NAT-801944 at 5 (2019), www.natera.com/wp-content/uploads/2020/11/Oncology-Clinical-A-personalized-tumor-informed-approach-to-detect-molecular-residual-disease-SGN_SR_WP.pdf. But the Asserted Claims do not recite that the

targeted multiplex amplification is from such an amplified library derived from isolated cfDNA—not from isolated cfDNA itself.

NeoGenomics reserves all rights to respond to any objective indicia of non-obviousness that Plaintiff may later assert. Should Plaintiff identify any additional objective indicia of non-obviousness, NeoGenomics reserves all rights to respond to and rely upon further references not cited herein to rebut those objective indicia of non-obviousness.

H. The Asserted Claims are Invalid for Obviousness-Type Double Patenting

The doctrine of obviousness-type double patenting was created by the courts to “prevent claims in separate applications or patents that do not recite the ‘same’ invention, but nonetheless claim inventions so alike that granting both exclusive rights would effectively extend the life of patent protection.” *In re Hubbell*, 709 F.3d 1140, 1145 (Fed. Cir. 2013) (quoting *Perricone v. Medicis Pharm. Corp.*, 432 F.3d 1368, 1373 (Fed. Cir. 2005)). Obviousness-type double patenting “prevent[s] the extension of the term of a patent . . . by prohibiting the issuance of the claims in a second patent not patentably distinct from the claims of the first patent.” *Boehringer Ingelheim Int’l GmbH v. Barr Lab’ys, Inc.*, 592 F.3d 1340, 1346 (Fed. Cir. 2010) (cleaned up). An obviousness-type double-patenting analysis, which compares claims in an earlier patent to claims in a later patent or application, requires that the court “construes the claims in the earlier patent and the claims in the later patent and determines the differences,” and then “determines whether those differences render the claims patentably distinct.” *Pfizer, Inc. v. Teva Pharms. USA, Inc.*, 518 F.3d 1353, 1363 (Fed. Cir. 2008). In considering whether the claims in the later patent are obvious, a court considers whether the claims are obvious in view of the prior art as a whole. *In re Longi*, 759 F.2d 887, 893 (Fed. Cir. 1985) (“Under that facet of the doctrine of double patenting, we must direct our inquiry to whether the claimed invention in the application

for the second patent would have been obvious from the subject matter of the claims in the first patent, *in light of the prior art.*” (emphasis added)). Patents may be shown to be invalid under statutory or non-statutory principles of obviousness-type double patenting. *Abbvie Inc. v. Mathilda & Terence Kennedy Inst. of Rheumatology Tr.*, 764 F.3d 1366, 1374 (Fed. Cir. 2014). The Federal Circuit made “explicit what was implicit in *Gilead*: the doctrine of obviousness-type double patenting continues to apply where two patents that claim the same invention have different expiration dates.” *Id.* The Court noted the doctrine also prevents an inventor from securing a second, later expiring patent for the same invention. *Id.* at 1373. “Obviousness-type double patenting is a judicially created doctrine that ‘prohibit[s] a party from obtaining an extension of the right to exclude through claims in a later patent that are not patentably distinct from claims in a commonly owned earlier patent.’” *Pfizer, Inc.*, 518 F.3d at 1363 (internal citation omitted). The same claims analyzed below are invalid under § 101 for statutory double patenting.

Natera touts having “a substantial intellectual property portfolio with over 150 issued patents and over 220 pending patent applications as of December 31, 2022.” (Dkt. 7, Malani Decl. ¶ 74). On information and belief, the Asserted Claims are invalid for obviousness-type double patenting over certain of the vast number of claims of earlier-expiring patents commonly owned with the Asserted Claims and/or with overlapping inventors.

NeoGenomics’s discovery into Natera’s expansive patent portfolio is ongoing and NeoGenomics reserves the right to supplement its contentions based the results of such discovery. To the extent Plaintiffs contend any reference is disqualified under pre-AIA § 103(c), NeoGenomics may also rely on that reference as an OTDP patent if it is an earlier-expiring patent (or has an earlier-expiring U.S. Patent in the same family).

IV. THE ASSERTED CLAIMS ARE INVALID UNDER 35 U.S.C. § 112

Defendant contends that the Asserted Claims are invalid under 35 U.S.C. § 112 for lack of written description and/or lack of enablement. Defendant reserves the right to supplement these disclosures based on further investigation and discovery.

A. Lack of Written Description and Enablement

The Asserted Patents fail to convey that the inventor(s) were in possession of the claimed subject matter because the following terms are not described in the respective specifications. Accordingly, the claims below and their dependent claims are invalid for lacking written description support.

A patentee is required to provide a disclosure sufficient to convey with reasonable clarity to persons skilled in the art, as of the effective filing date, that the patentee in fact invented what is claimed. *Centocor Ortho Biotech, Inc. v. Abbott Lab'ys*, 636 F.3d 1341, 1348 (Fed. Cir. 2014). That is, the patent specification, including its figures and claims, must allow a skilled artisan to easily recognize that the patentee was in possession of the claimed invention as of his/her asserted priority date. *Ariad Pharms., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1351 (Fed. Cir. 2010) (en banc). Compliance with the written description requirement involves both an assessment of the scope of the claims and the adequacy of the specification to support claims of such scope. *See id.* The test requires an objective inquiry into the four corners of the specification from the perspective of a person of ordinary skill in the art. *Id.* A description that merely renders the invention obvious does not satisfy the requirement. *Id.* at 1352.

If the claims of a patent cover generic-scope inventions, the specification is assessed for sufficient disclosure of species within the claimed genus. *See id.* at 1350. When a claim is phrased in functional language, a limited number of disclosed species may adequately support it if there is a known correlation between structure and function. *See id.* at 1350. However, where

there is no known structure/function correlation, an appropriate number of species representative of the claimed genus across its scope must be disclosed. *Id.* Furthermore, numerous species that are closely related, i.e., derived from a common parent or the like, are not considered independently representative. *AbbVie Deutschland GmbH v. Janssen Biotech, Inc.*, 759 F.3d 1285, 1300–01 (Fed. Cir. 2014). Their disclosure, thus, does not count as a disclosure of multiple representative species in the assessment of whether there is adequate support for the claim. *Id.*

Additionally, evidence showing that a claimed genus does not disclose a representative number of species may include evidence of species that fall within the claimed genus that are not disclosed by the patent, but, instead, postdate the priority date of the patent. *Amgen Inc. v. Sanofi*, 872 F.3d 1367, 1374 (Fed. Cir. 2017).

The specification must “demonstrate that the patentee possessed the full scope of the invention recited in” the claims. *LizardTech, Inc. v. Earth Res. Mapping, Inc.*, 424 F.3d 1336, 1345 (Fed. Cir. 2005). The claims may be no broader than the supporting disclosure, particularly where the patentee has indicated that a certain feature is an essential element of the invention. *Gentry Gallery, Inc. v. Berkline Corp.*, 134 F.3d 1473, 1479 (Fed. Cir. 1998) (“It is a truism that a claim need not be limited to a preferred embodiment. However, in a given case, the scope of the right to exclude may be limited by a narrow disclosure.”).

The Asserted Patents also fail to enable a POSA to practice the full scope of the Asserted Claims. Accordingly, the claims below are invalid for lack of enablement.

To satisfy the enablement requirement, a patent specification must teach a person of ordinary skill in the art how to make and use the claimed invention without undue experimentation. *Wyeth and Cordis Corp. v. Abbott Lab ’ys*, 720 F.3d 1380, 1384 (Fed. Cir.

2013). The person of ordinary skill can draw upon what is already known in the art as of the effective filing date of the patent. Thus, the extent to which a patent enables its claims to be practiced without undue experimentation is based on the teachings of the specification and the knowledge of one of ordinary skill in the art. A patent specification is not enabling if it amounts to no more than an invitation to a person of ordinary skill to engage in research that may or may not result in the claimed invention. *See Enzo Biochem, Inc. v. Calgene, Inc.*, 188 F.3d 1362, 1374-75 (Fed. Cir. 1999). The degree to which a patent specification is enabling must be at least commensurate with the scope of the claims. *MagSil Corp. v. Hitachi Glob. Storage Techs., Inc.*, 687 F.3d 1377, 1381 (Fed. Cir. 2012). If claims are not enabled across their scope, they are invalid for failure to comply with the enablement requirement of 35 U.S.C. § 112. *Id.* And, as the Supreme Court recently explained in *Amgen Inc. v. Sanofi*, 598 U.S. 594, 143 S. Ct. 1243, 1254 (2023), “[t]he more one claims, the more one must enable.” Though a specification need not always “describe with particularity how to make and use every single embodiment within a claimed class,” it must nevertheless “enable the full scope of the invention as de-fined by its claims,” for example by “disclosing [a] general quality” of the class that may “reliably enable a person skilled in the art to make and use all of what is claimed.” *Id.* at 1254–55.

The enablement requirement has both a “[how] to make” aspect and a “how to use” aspect. *Id.*; *see Rasmusson v. SmithKline Beecham Corp.*, 413 F.3d 1318, 1322–23 (Fed. Cir. 2005). A person of ordinary skill in the art must be able to make the claimed invention across its scope without undue experimentation. *Nat’l Recovery Techs., Inc. v. Magnetic Separation Sys., Inc.*, 166 F.3d 1190, 1195–96 (Fed. Cir. 1999). Under the “how to use” prong of enablement, the specification must disclose a practical utility for the invention and the specification must also

enable the skilled artisan to use the invention so as to achieve that utility. *Rasmusson*, 413 F.3d at 1322-23.

In determining whether the disclosure of a patent would require undue experimentation, several factors are considered, including: (1) the amount of experimentation necessary; (2) the amount of guidance presented in the patent; (3) whether the patent discloses specific working examples of the claimed invention; (4) the nature of the invention; (5) the state of the prior art; (6) the relative skill of those in the art; (7) the nature and predictability of the field; and (8) the breadth of the claims. *See In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988).

In judging whether or not a patent specification is enabling, post-filing date references may be considered. Post-filing date references that show a claimed invention remained unknown or was never achieved after the filing date is evidence that the patent lacks an enabling disclosure. *Chiron Corp. v. Genentech, Inc.*, 363 F.3d 1247, 1254–55 (Fed. Cir. 2004).

1. '596 Patent

The Asserted Claims of the '596 patent, claims 1, 11, 12, and 18, lack adequate written description and enablement.

a. Failure to Claim an Essential Element of the Invention

The '596 patent specification makes clear to the skilled artisan that the inventors were solely in possession of techniques that involved target loci selection and primer design for highly multiplexed PCR.

Claim 1 of the '596 patent recites 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.” In particular, claim 1 recites:

performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from

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

The '596 specification, however, shows that the inventors were only in possession of techniques involving target loci selection and primer design for highly multiplexed PCR in a single reaction volume. This is demonstrated by the specification's disclosure that "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." '596 patent at 108:35–37. Similarly, the specification discloses that "[o]ne way to identify the 'bad' primers is to look at the sequencing data of DNA that was amplified by targeted amplification; those primer dimers that are seen with greatest frequency can be removed to give a primer library that is significantly less likely to result in side product DNA." *Id.* at 106:17–23. If the inventors were in possession of a method that did not require target loci selection and primer design for highly multiplex amplification, they would not have explained that such techniques were "essential."

Further, the specification explains that removal of "problematic primers" is the element that "has unexpectedly enabled extremely high PCR multiplexing levels." *Id.* at 106:7–10. According to the specification, "[e]mpirical 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." *Id.* at 106:13–17.⁵ In this way, the patentee characterized the particular primer design method as essential to practice the full scope of the claim.

⁵ The primer design methods disclosed in the '596 patent for highly multiplexed PCR from DNA derived from cell-free DNA were known in the prior art at the time of filing. *See* U.S. Patent Application No. 2012/027021 ("Rabinowitz") at [0291]–[0298], as were the other variants of multiplex PCR disclosed in the '596 patent. *See, e.g., id.* at [0299]–[0329].

Accordingly, and because the specification does not disclose a method for carrying out the claimed multiplex PCR without primer design approaches, the '596 patent lacks written description support for multiplex PCR amplification that does not utilize techniques for selection of primers to avoid primer side products. Therefore, a POSA would understand that the Asserted Claims fail to recite essential steps involving target loci selection and removal of primer pairs likely to form primer-dimers for targeted, highly multiplexed PCR amplification in a single reaction volume.

For at least these reasons, the Asserted Claims of the '596 patent lack an essential element of the invention and therefore lack adequate written description support. *See LizardTech, Inc.*, 424 F.3d 1336; *Tronzo v. Biomet, Inc.*, 156 F.3d 1154 (Fed. Cir. 1998).

b. Detection of an SNV Present at Any Level Less Than or Equal to 0.015%

Asserted Claim 1 of the '596 patent requires “detect[ing] from the sequence reads” “an SNV mutation that is present in less than equal to 0.015% of the cell-free DNA having the SNV locus.” The '596 patent does not provide adequate written description and enablement support for the full scope of claim 1 and its dependents. Nor does the claim specify any limit or requirements on the sensitivity of the method. Therefore, the patent claims a method that achieves a broad range of sensitivities, without disclosing or enabling a method that is capable of achieving the full scope of SNV detection required. “A ‘mere wish or plan’ for obtaining the claimed invention does not satisfy the written description requirement.” *Novozymes v. DuPont Nutrition Biosciences APS*, 723 F.3d 1336, 1344 (Fed. Cir. 2013); *see also, e.g., Medytox, Inc. v. Galderma S.A.*, 71 F.4th 990, 998 (Fed. Cir. 2023) (affirming decision that undue

experimentation would be required to practice a claimed method reciting a responder rate of at least 50% where the specification did not demonstrate any responder rates greater than 62%).

The claim and related dependent claims therefore require support demonstrating that the inventors could perform sequencing and detection at all levels of sensitivity and for an SNV that is present at less than or equal to 0.015% of the cell-free DNA having the SNV locus. But the specification does not demonstrate that the inventors were in possession of a method wherein an SNV is detected across the full claimed range. Nor does the specification enable the claims for detection of an SNV across the full range of less than or equal to 0.015%. For example, the specification would have to demonstrate that the inventors could use the claimed method to detect tumor-specific SNV mutations at very low absolute and relative abundance, for example in a single molecule of cfDNA containing tumor-specific SNV mutations among many molecules of cfDNA not containing such mutations. A POSA reviewing the specification of the '596 patent at the time of Natera's claimed priority date would recognize that the inventors were not in possession of the claimed methods for detection of an SNV present at less than or equal to 0.015%.

The '596 patent does not demonstrate detection of an SNV present in less than or equal to 0.015% of the cell-free DNA having the SNV locus for any SNV, much less across the full claimed range. At best, Example 6 of the '596 specification describes a mean LOD of 0.24% and a median LOD of 0.09% for DNA in a plasma sample. '596 patent at 158:54–58. The specification does not enable or demonstrate possession of a method for detecting an SNV present at any level of sensitivity, or even a method wherein an SNV is detected that is present below a mean LOD of 0.24% and a median LOD of 0.09%. The lowest LOD for sequencing single nucleotide changes mentioned in the specification is 0.005%. *Id.* at 93:9–12. Similarly,

the lowest level at which an SNV is detected (or is capable of being detected) mentioned in the specification is 0.005%, *Id.* at 93:18–33, but the claimed range includes detecting SNVs present at even lower levels. The specification provides no support for any higher sensitivity or lower limit of detection than the recited threshold. A POSA reviewing the specification of the '596 patent would readily recognize that the inventors were not in possession of the claimed methods for sequencing at any level of sensitivity or at any limit of detection. A POSA would understand that the inventors were not in possession of the full scope of the claimed subject matter.

Example 14 of the '596 specification similarly shows that the inventors were not in possession of the full scope of the claimed subject matter. First, Example 14 is not an embodiment of the asserted claims of the '454 patent. Second, Fig. 59 illustrates “a plot of Limit of Detection (LOD) vs. Depth of Read (DOR) for detection of SNV Transition and Transversion mutations in a 84-plex PCR reaction at 15 PCR cycles,” showing that a LOD lower than 0.01% was not achieved for *any* SNV at read depths lower than 100,000. *Id.* at 42:15–18, Fig. 59. Figs. 60 and 61 similarly show that the LOD for SNVs was often greater than 0.01 or even 0.1 (meaning that an SNV present at lower frequencies could not be detected). *See id.* at 42:19–26, Figs. 60–61. Furthermore, Figs. 59–61 show that a LOD lower than 0.01% was not achieved for *any* SNV that was a transition mutation, regardless of read depth or number of PCR cycles. *Id.* at Figs. 59–61. Nor does the specification describe how to predict whether a given SNV could be detected when present at less than or equal to 0.015%.

Furthermore, the data in the '596 patent specification show that detection was not achieved of any SNV present below about 0.002%—a level that is within the claimed range of less than or equal to 0.015%. *See, e.g., id.* at Figs. 59–61. An annotated version of FIG. 61 is reproduced below, for example, highlighting a range of approximately 0.002% and below. The

specification does not show that the inventors were able to detect any SNVs in this range. Therefore, the '596 patent claims a range at which an SNV is detected, but the '596 patent specification fails to show possession or enablement of the full scope of the claimed range.

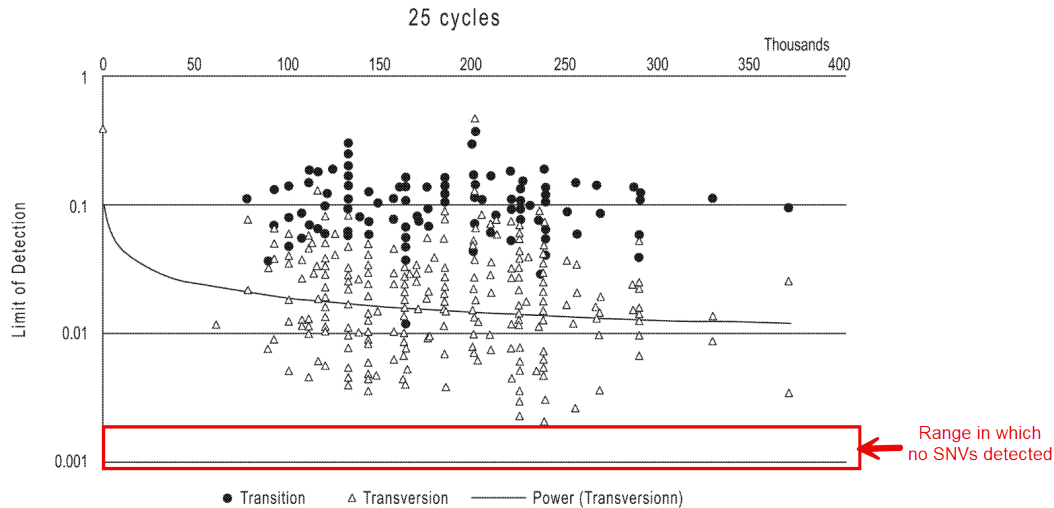


FIG. 61

Second, the '596 specification does not disclose any clinical sensitivities for detection of an SNV when present at less than or equal to 0.015%. And Natera's own admissions show that Natera was not in possession of the claimed method wherein an SNV is detected when present at less than or equal to 0.015%. For example, in a post-filing publication titled *A personalized, tumor-informed approach to detect molecular residual disease with high sensitivity and specificity*, Natera stated that key improvements with Signatera since the TRACERx study included that, "[a]t the single SNV level, detection at 0.01% VAF improved from <5% to 30%." 2019_08_27_NAT-801944 at 5 (2019), www.natera.com/wp-content/uploads/2020/11/Oncology-Clinical-A-personalized-tumor-informed-approach-to-detect-molecular-residual-disease-SGN_SR_WP.pdf. The TRACERx study itself was not published until years after Natera's asserted filing date. Christopher Abbosh et al., *Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution*, 545 Nature 446 (2017) ("Abbosh"). But the '596 does not cabin the

functional limitation requiring detection of an SNV present at less than or equal to 0.015% of the cfDNA to a limited level of clinical sensitivity or reproducibility (e.g., that the method only achieves detection of an SNV present at 0.01% less than 5% of the time). The '596 patent claims set a high bar for written description by claiming any method comprising performing the active steps recited by the claims "wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads," including methods that are highly reproducible, without demonstrating possession of the full scope of the claims. Simply put, the '596 patent claims a "problem to be solved while claiming all solutions to it," covering any method "later actually invented and determined to fall within the claim's functional boundaries," *Ariad*, 598 F.3d at 1353, which fails to satisfy the written description requirement.

Because the claims of the '596 patent recite detection of an SNV across an unbounded range of less than or equal to 0.015% and at any level of clinical sensitivity or reproducibility, and the specification does not describe performing the claimed methods to detect an SNV across the full claimed range of sensitivity, a POSA would understand that the inventors were not in possession of the full scope of the claimed subject matter. Nor are the claims limited to detecting transversion rather than transition mutations. Example 14 shows that transition mutations could not be detected at levels lower than 0.01% and that the inventors were not in possession of a method for detecting these mutations below a level of 0.015%.

A POSA reading the specification at the time of Natera's claimed priority date likewise would be unable to perform the claimed method reproducibly or to detect an SNV present at less than or equal to 0.015%, or for any type of SNV mutation, or with any amount of input cfDNA, without undue experimentation. For example, Bernhard Zimmerman, a named inventor of the

'596 patent, acknowledges “the analytical sensitivity . . . is different at any different [cfDNA] input.” Zimmerman 7/12/2024 30(b)(6) Dep. Tr. at 24:20-23. Natera specifically performed “sensitivity assessment” based on several different amounts of input cfDNA “at 60 nanogram input, 30 nanogram input” and “10 nanogram input.” *Id.* at 25:1-5. Indeed, improved techniques for each step of the claimed methods would be necessary to sequence a particular tumor-specific SNV mutation present at less than 0.005% of the DNA molecules in a patient sample, and it would require undue experimentation for a POSA to determine how, if it is even possible, to achieve detection at such levels. But the '596 patent does not disclose any such improved techniques. Additionally, as Natera admitted in “A personalized, tumor-informed approach to detect molecular residual disease with high sensitivity and specificity,” improved techniques were required to detect an SNV present at 0.01% even 30% of the time. *A personalized, tumor-informed approach to detect molecular residual disease with high sensitivity and specificity*, 2019_08_27_NAT-801944 at 5 (2019), www.natera.com/wp-content/uploads/2020/11/Oncology-Clinical-A-personalized-tumor-informed-approach-to-detect-molecular-residual-disease-SGN_SR_WP.pdf. Natera further stated that “[t]he limit of detection for Signatera, measured in VAF, is 0.01%” (*id.* at 1), but the claims encompass detection of SNVs present well below 0.01%.

c. “performing targeted multiplex PCR amplification . . . from the isolated cell-free DNA”

Asserted Claim 1 of the '596 patent requires “performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA.” This limitation was added to pending claim 1 on June 1, 2022, during prosecution of the application that issued as the '596 patent. The limitation “wherein an SNV

mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected” was added in the same amendment. The specification of the ’596 patent lacks adequate disclosure to support this claim limitation. The ’596 patent specification does not describe this limitation, much less in combination with the requirements that the method can detect “an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA”—which Natera added to claim 1 via the same amendment. Therefore this limitation is new matter, and the ’596 patent claims are invalid for lack of written description. In addition, the ’596 patent specification does not enable claim 1 and its dependents. For example, nowhere does the ’596 patent describe targeted multiplex PCR amplification from “the isolated cell-free DNA.” A POSA would understand that the inventors were not in possession of the claimed subject matter.

The claim language in the ’454 patent—belonging to the same patent family and sharing the same specification as the ’596 patent—recites two categories of DNA on which the recited targeted multiplex amplification can be performed. The first is isolated cell-free DNA and the second is DNA “derived []from” such isolated cell-free DNA. The specification only describes using the second category of DNA for performing targeted multiplex amplification. Nowhere does the specification describe targeted multiplex PCR amplification from the isolated cell-free DNA. A POSA would understand that the inventors were not in possession of the full scope of the claimed subject matter.

Much less does the ’596 patent provide sufficient written description or enablement support for “performing targeted multiplex PCR amplification to amplify the plurality of target loci together in the same reaction volume from the isolated cell-free DNA using primers specific to the plurality of target loci for the individual subject; and performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence

reads, wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads,” as required by Asserted Claim 1 of the ’596 patent.

To the extent Natera contends any of the data in the specification provide support for the claims, the Examples describing how cfDNA was prepared prior to multiplex amplification state that the input for multiplex PCR was an “amplified and purified Natera plasma library,” not isolated cfDNA. *See, e.g.,* ’596 patent at 164:57-59 (“[t]he input for mPCR was 2.5 uL of amplified and purified Natera plasma library at an input amount of approximately 600 ng.”). The input DNA for multiplex PCR differed from isolated cfDNA in multiple respects. For example, Natera has admitted that the Natera plasma library preparation process alters characteristics such as DNA fragment size distribution that could affect assay results. *See A personalized, tumor-informed approach to detect molecular residual disease with high sensitivity and specificity*, 2019_08_27_NAT-801944 at 5 (2019), www.natera.com/wp-content/uploads/2020/11/Oncology-Clinical-A-personalized-tumor-informed-approach-to-detect-molecular-residual-disease-SGN_SR_WP.pdf. The Natera amplified and purified plasma library largely contains amplicons derived from cfDNA. Natera has not shown that any “isolated cell-free DNA” as claimed in the ’596 patent remains in the Natera amplified and purified plasma library, much less that any remaining cfDNA in the amplified library would be present in sufficient quantities to perform the claimed method. As another example, Abbosh explains that Natera’s cfDNA library preparation involved cfDNA end-repair and A-tailing, followed by ligation of custom adaptors, amplification, and purification using Ampure beads, yielding a single peak around 250 bp. Abbosh at Methods (“cfDNA library preparation”). The size distribution of the amplified and purified libraries as a single peak around 250 bp differs

substantially from much broader size distribution of isolated cfDNA. *See, e.g., Sabine Jahr et al., DNA Fragments in the Blood Plasma of Cancer Patients: Quantitations and Evidence for Their Origin from Apoptotic and Necrotic Cells*, 61 *Cancer Research* 1659, 1663 (Feb. 15, 2001) (“Jahr”) (describing ladder-like pattern for size distribution in isolated cfDNA). Given the differences between the Natera amplified and purified plasma library used as input for the targeted multiplex PCR, a POSA would understand the inventors were not in possession of the claimed subject matter.

Likewise, a POSA reading the specification at the time of Natera’s claimed priority date would be unable to perform targeted multiplex PCR amplification to amplify the plurality of target loci from “the isolated cell-free DNA,” without undue experimentation, much less while achieving the claimed requirement that an SNV present at less than or equal to 0.015% is detected. Accordingly, claim 1 of the ’596 patent and its dependent claims are invalid for lack of written description and enablement.

d. Combination of Steps in Asserted Claims

The specification of the ’596 patent does not disclose adequate support for the particular claimed combination of steps recited in the Asserted Claims. Take claim 1 as an example: it claims a method of performing tumor-informed assay by which (a) a tumor biopsy sample is sequenced, (b) results of the sequencing are evaluated to determine a plurality of target loci spanning tumor-specific mutations, and (c) cell-free DNA isolated from the biological samples is assayed, which includes performing targeted multiplex PCR amplification and high-throughput sequencing to detect an SNV mutation present in less than or equal to 0.015% of the cell-free DNA. Nowhere in the text of the ’596 patent is there a description of the combination of claim 1’s elements as a whole. Even if Natera attempts to identify working examples of different

aspects of the claimed method in the patent's specification, the '596 patent specification does not include any disclosures that conveys to an artisan of ordinary skill that the inventors had invented a combination of those different aspects. Accordingly, the '596 patent's written description fails to sufficiently demonstrate that the inventors possessed the full scope of the claimed methods. *See, e.g., Natera, Inc. v. Caredx, Inc.*, No. 20-38-CFC-CJB, 2025 WL 592760, at *4-6 (D. Del. Feb. 24, 2025).

The only example of a tumor-informed assay in the '596 patent is in Example 13, which describes sequencing using an Illumina HiSeq2000 or Ion Torrent PGM® sequencing instrument. '596 Patent at 168:38-43; *see also id.* at 169:1-5. The error rate of Illumina HiSeq2000 instruments was known to be at least 0.1%, and the error rate for Ion Torrent sequencing instruments was known to be about 1%. Glenn, *Field guide to next-generation DNA sequencers*, MOLECULAR ECOLOGY RES. 11:759–769 (May 2011) (“Glenn (2011)”) at Table 3. According to Dr. Metzker, regardless of read depth, sequencing does not enable detection of mutations that fall below the sequencing workflow error rate (*see, e.g.,* Dkt. 145 ¶¶ 94-102, section titled “Ultra-Deep Sequencing Does Not Enable Detection of Rare Genetic Variants that Fall Below the Sequencing Workflow Error Rate”). FIG. 53B shows that the lowest variant allele fraction (VAF) of an SNV that Example 13's tumor-informed assay could detect in a plasma sample in the plasma using Natera's version of was 0.15% (around the error rate of an Illumina HiSeq instrument). *See* FIG. 53B (“VAF Plasma” column); *see also id.* at 169:13-16, 41:66. The Asserted Claims, however, require detecting an SNV that is present in less than or equal to 0.015% of the cell-free DNA having that SNV locus. And to the extent Natera contends that Forshew did not enable detection of an SNV present in less than or equal to 0.015% of the cfDNA because of error rates in the method, Natera's post-filing publication of pilot study

results for Signatera showed that, commensurate with Forshew's disclosure (*see* Figure S3), only a small subset of SNV loci demonstrated local error rates at or below 0.015%. Jamal-Hanjani et al., *Detection of ubiquitous and heterogeneous mutations in cell-free DNA from patients with early-stage non-small-cell lung cancer*, *Annals of Oncology* 27: 862–867 (2016) at Figure 2.

The only sequencing platforms disclosed throughout the specification are the “Illumina HiSeq or MiSeq system or an Ion Torrent PGM or Proton system,” ('596 patent at 169:39-41), the ILLUMINA IIGAX system (*id.* at 96:39, 152:64), and the Illumina HiSeq 2500 (*id.* at 164:13). All of these sequencing instruments have error rates much higher than 0.015%: at least 0.1% for the Illumina HiSeq2000, MiSeq, and IIGAx instruments (*see* Glenn at Table 3), and around 1% for the Ion Torrent instruments (*id.*). The error rate for Illumina HiSeq2500 instruments was similarly around 0.1%. *See* Stoler and Nekrutenko, *Sequencing error profiles of Illumina sequencing instruments*, *NAR Genomics and Bioinformatics*, 3:1 (2021) at Table 1.

2. '454 Patent

a. Combination of Steps in Asserted Claims

The Asserted Claims of the '454 patent are invalid for lack of written description and enablement. As an initial matter, the '454 patent specification does not disclose adequate support for the particular claimed combination of steps. Many of the individual concepts recited in the claims, such as multiplex amplification, molecular barcodes, and sequencing amplicons were known in the art and were routine and conventional as of Natera's claimed priority date. To the extent there is anything inventive in the Asserted Claims, it must reside in the combination of recited elements. But none of the embodiments or other disclosures demonstrate that the inventors possessed, or that the specification enables, the combination of all the elements in the manner claimed. For instance, none of the embodiments disclose an example that

discusses sequencing a tumor sample and then obtaining a depth of read of at least 50,000 per target locus for 10-500 target loci in circulating tumor DNA, as recited by claims 1 and 15, much less in the context of a tumor-informed assay. Nor do any of the embodiments disclose the combination of a method “capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus” in the context of a tumor-informed assay. Even if Natera tries to identify working examples of different pieces in the patent’s specification, the ’596 patent’s specification does not include any disclosures that convey to an artisan of ordinary skill that the inventors had invented a combination of those different pieces. Accordingly, the ’454 patent’s written description fails to sufficiently demonstrate that the inventors possessed the full scope of the claimed method. *See, e.g., Natera*, 2025 WL 592760, at *4-6.

In addition, the specification of the ’454 patent does not teach the purported invention in a manner that would have enabled the skilled scientists to practice the claims in its full scope without undue experimentation as of the assumed priority date of April 21, 2015. Indeed, the data in the patent shows that Natera was unable to achieve “a depth of read of at least 50,000 per target locus” of the “10 to 500 target loci.”

As an initial matter, the ’454 patent includes a 50,000 read depth appearing in a laundry list of possible depths of read, with no indication of how many loci can be sequenced at the listed depths, or whether such loci could first be identified by whole genome or whole exome sequencing of tumor DNA and then be monitored in a patients ctDNA.

In 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. Typically, the sequencing run is a high throughput sequencing run. The mean or median values generated for the on-test samples, in illustrative embodiments are weighted by depth of reads.

'454 patent at 68:33-42 (emphasis added).

In addition, none of the examples of the '454 patent describe at least 50,000 depth of read for each of 10-500 target loci. For example, Figures 1 - 13 describe depths of reads of 500, 1000, and 3000. *Id.* at 37:27-38:26. Figures 20-25 illustrate 100, 200, 300, 400, and 500 SNPs with 1000-5000 depth of read for each SNP. All of these are much lower than the claimed depth of read of “at least 50,000 per target locus.”

The closest the '454 patent comes to describing 10-500 loci sequenced at a depth of 50,000 per locus is in Figures 55-58, which “show results for the four 84-plex SNV PCR primer pools.” *Id.* at 169:56-57. The '454 patent observes “improved DOR efficiency with increasing cycles from 15 to 20 to 25” in Figures 55-58. *Id.* at 169:57-58. Although some of the loci are sequenced above a depth of read of 50,000, many are not. As highlighted below, for each of the 84-plex pools, a portion of the target loci are always below a depth of read of 50,000—and therefore do not describe or disclose the claimed requirement of “at least 50,000 *per target locus*.”

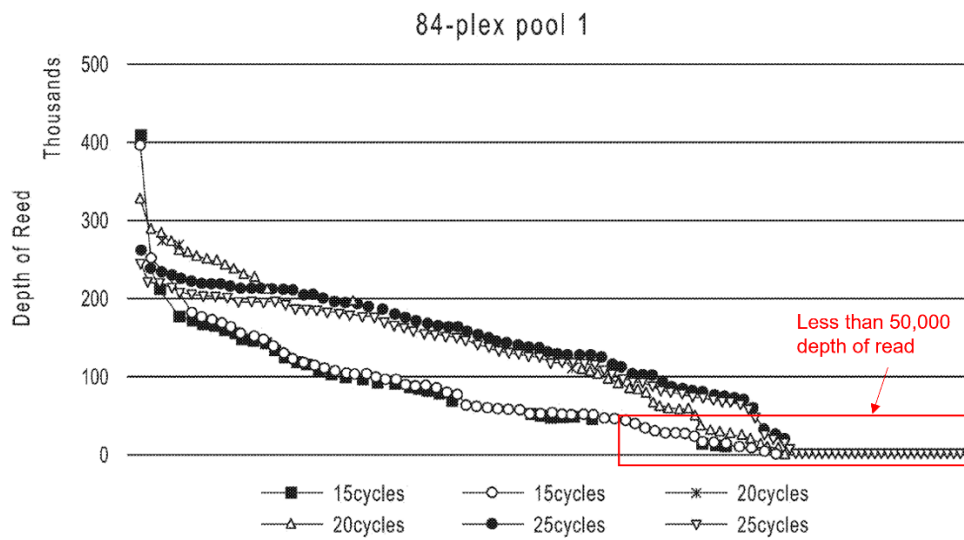


FIG. 55

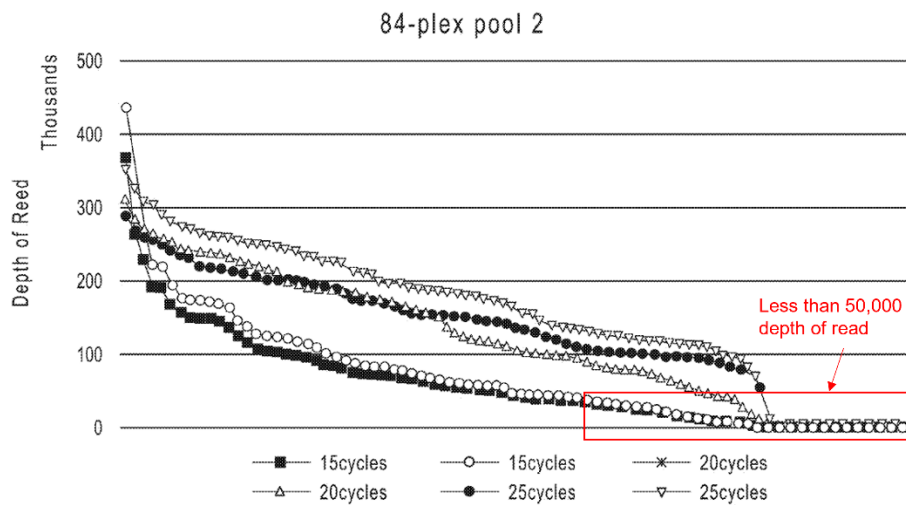


FIG. 56

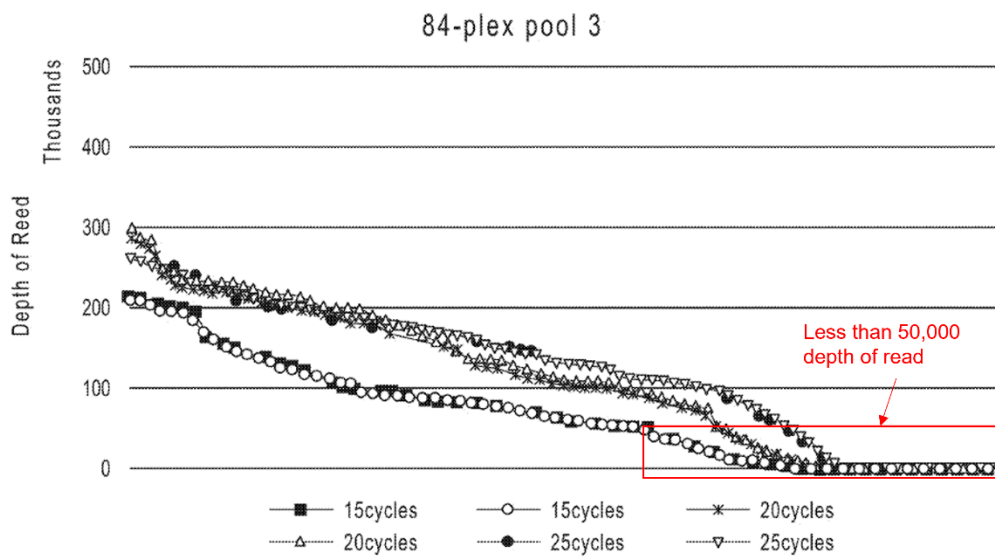


FIG. 57

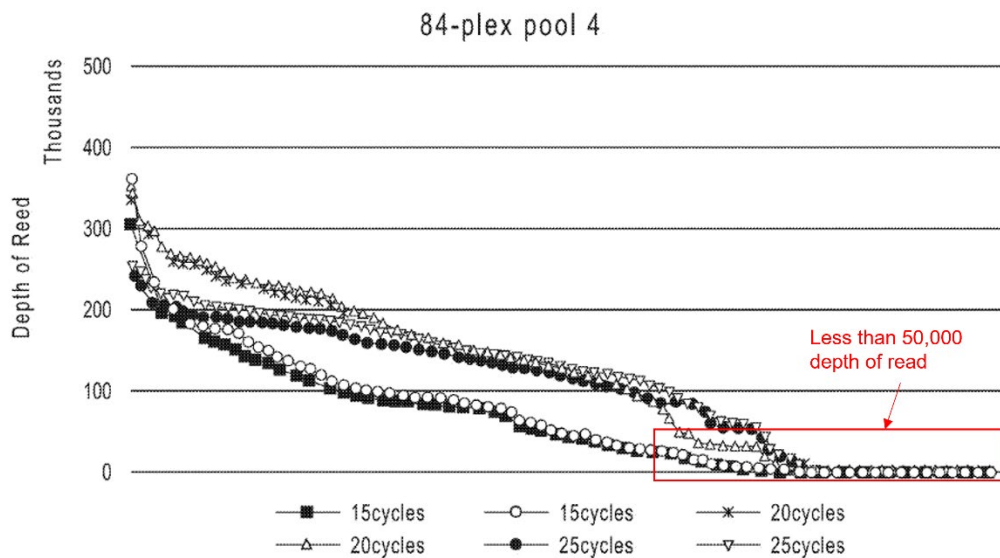


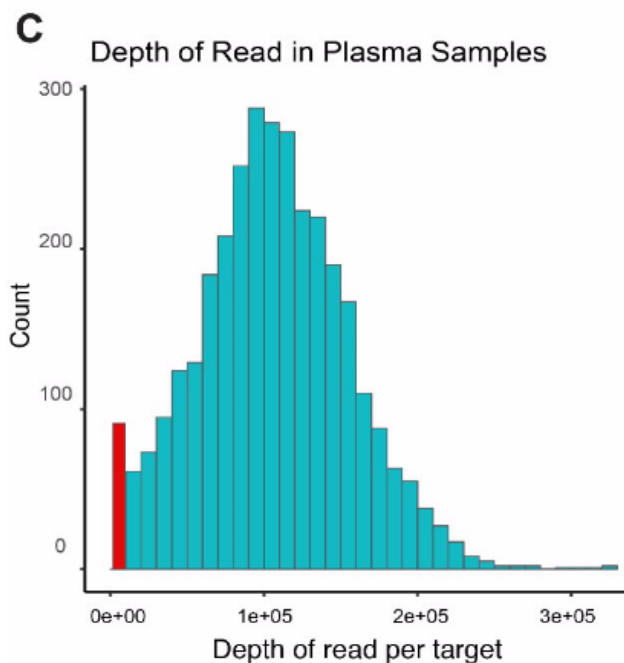
FIG. 58

Accordingly, the full scope of the claim includes sequencing 500 loci at a depth of read of 50,000 per target locus, but nothing in the '454 specification suggests that the inventors even contemplated such an analysis.

The '454 patent does not enable the full scope of the claims, which claims "10 to 500

target loci” with “a depth of read of at least 50,000 per target locus.” ’454 patent at claims 1 and 15. As demonstrated by Figures 55-58 above, the inventors could not achieve a depth of read of 50,000 per locus for 84 loci, let alone 500 loci as recited by claims 1 and 15.

Indeed, Natera’s own Signatera product is not able to achieve “a depth of read of at least 50,000 per target locus” as claimed by the ’454 patent—despite its assertion that Signatera practices the ’454 patent. Natera’s 5/3/2024 response to NeoGenomics’ Interrogatory No. 8. This is shown by Natera’s publication on Signatera: Coombes, et al., *Personalized Detection of Circulating Tumor DNA Antedates Breast Cancer Metastatic Recurrence*, 25 CLINICAL CANCER RSCH. 4255-63 (2019) (“Coombes”) (NEOGEN00281016) and supplemental materials thereof (“Coombes Supp.”) (NEOGEN00280991). Coombes reports testing data using Signatera. Coombes at 4257 (NEOGEN00281018). Coombes includes depth of read per target data for Signatera. Coombes Supp. at 16, Figure S1C (NEOGEN00281006), which is reproduced below. The data presented in Figure S1C indicates not all targets reach a depth of read at least 50,000.



Coombes Supp. at 16, Figure S1C (NEOGEN00281006).

Natera identifies Bernhard Zimmerman, a named inventor of the '454 patent as someone "with knowledge of Signatera" when it contends Signatera practices the '454 patent. Natera's 5/3/2024 response to NeoGenomics' Interrogatory No. 8. Nonetheless, Dr. Zimmerman acknowledges "not all of the targets reach a depth of read of 50,000" in Signatera during his deposition. Zimmerman 7/12/2024 30(b)(6) Dep. Tr. at 150:10-13.

To the extent Natera asserts the RaDaR sequences up to 48 target loci in the amplicons obtained from a single reaction volume to a depth of read of 50,000 per target locus, RaDaR uses an Illumina sequencer model NovaSeq series that was not even released until several years after Natera's asserted priority date of the '454 patent. *See, e.g.,* Susanne Flach et al., *Liquid Biopsy for Minimal Residual Disease Detection in Head and Neck Squamous Cell Carcinoma (LIONESS)-a personalized circulating tumour DNA analysis in head and neck squamous cell carcinoma*, 126 Br. J. Cancer 1186 (2022) (NEOGEN00014529 at -530) ("RaDaR™ libraries were sequenced using the Nova-Seq 6000 system (Illumina Inc., San Diego, USA)."; *see also A Decade in Sequencing*, Illumina (2024), <https://www.illumina.com/science/technology/next-generation-sequencing/illumina-sequencing-history/decade-in-sequencing.html> (captured July 31, 2024) (NEOGEN00283160) (Illumina sequencer instrument launch webpage showing NovaSeq launched in 2017). The '454 patent does not enable, as of its filing date, sequencing depths achieved using improved sequencers that were not available until years later.

In addition, the '454 patent recites a method of using whole genome or exome sequencing to identify tumor-specific SNVs, and the method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus. *E.g.,* '454 patent, claim 14. Example 13 is the only example of this method in the '454 patent, (*see id.* at 167:63-168:58), and the method is not shown to be capable of detecting SNVs

at the claimed percentage. The results of Natera's method are shown in Figure 53B and the percentage sensitivity for SNVs detected in cell free DNA are shown in the column labeled "VAF Plasma." *Id.* at 168:31-54, Fig. 53. The lowest percentage detected was 0.15%, which is an order of magnitude higher than the claims level of 0.015%. *Id.* As discussed in Section IV.A.1.d above, the error rates of the sequencing instruments described in the '454 patent were all known to be well above 0.015%. Thus, the specification does not provide any indication that the inventors were in possession of the method of claim 14 as of the priority date. Nor does the specification of the '454 patent describe any embodiment including the combination of limitations recited in claim 14.

The only example of a tumor-informed assay in the '454 patent is in Example 13, which describes sequencing using an Illumina HiSeq2000 or Ion Torrent PGM® sequencing instrument. '454 Patent at 168:14-16; *see also id.* at 168:45-46. The error rate of Illumina HiSeq2000 instruments was known to be at least 0.1%, and the error rate for Ion Torrent sequencing instruments was known to be about 1%. Glenn, *Field guide to next-generation DNA sequencers*, MOLECULAR ECOLOGY RES. 11:759–769 (May 2011) ("Glenn (2011)") at Table 3. According to Dr. Metzker, regardless of read depth, sequencing does not enable detection of mutations that fall below the sequencing workflow error rate (*see, e.g.*, Dkt. 145 ¶¶ 94-102, section titled "Ultra-Deep Sequencing Does Not Enable Detection of Rare Genetic Variants that Fall Below the Sequencing Workflow Error Rate"). FIG. 53B shows that the lowest variant allele fraction (VAF) of an SNV that Example 13's tumor-informed assay could detect in a plasma sample in the plasma using Natera's version of was 0.15% (around the error rate of an Illumina HiSeq instrument). *See* FIG. 53B ("VAF Plasma" column); *see also id.* at 168:35-36, 41:66. The Asserted Claims, however, require that the method is capable of detecting an SNV that is present

in less than or equal to 0.015% of the cell-free DNA having that SNV locus. And to the extent Natera contends that Forshew did not enable detection of an SNV present in less than or equal to 0.015% of the cfDNA because of error rates in the method, Natera's post-filing publication of pilot study results for Signatera showed that, commensurate with Forshew's disclosure (*see* Figure S3), only a small subset of SNV loci demonstrated local error rates at or below 0.015%. Jamal-Hanjani et al., *Detection of ubiquitous and heterogeneous mutations in cell-free DNA from patients with early-stage non-small-cell lung cancer*, *Annals of Oncology* 27: 862–867 (2016) at Figure 2.

Accordingly, a skilled scientist would not understand the inventors to possess the claimed invention and the Asserted Claims are therefore invalid because they do not meet the written description requirement. In addition, the data presented in the '454 patent shows it does not enable skilled scientists to reach the claimed depth of read requirement for each target locus at the time of the priority date of the '454 patent.

b. Failure to Claim an Essential Element of the Invention

The '454 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 for highly multiplexed PCR.

Claims 1 and 14 of the '454 patent 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." In particular, 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.

The '454 specification, however, shows that the inventors were only in possession of techniques involving target loci selection and primer design for highly multiplexed PCR in a single reaction volume. This is demonstrated by the specification's disclosure that "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." '454 patent at 108:19–22. Similarly, the specification discloses that "[o]ne way to identify the 'bad' primers is to look at the sequencing data of DNA that was amplified by targeted amplification; those primer dimers that are seen with greatest frequency can be removed to give a primer library that is significantly less likely to result in side product DNA." *Id.* at 106:1–6. If the inventors were in possession of a method that did not require target loci selection and primer design for highly multiplex amplification, they would not have explained that such techniques were "essential."

Further, the specification explains that removal of "problematic primers" is the element that "has unexpectedly enabled extremely high PCR multiplexing levels." *Id.* at 105:58–61. According to the specification, "[e]mpirical 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." *Id.* at 105:64–106:1⁶ In this way, the patentee characterized the particular primer design method as essential to practice the full scope of the claim.

Accordingly, and because the specification does not disclose a method for carrying out the claimed multiplex PCR without primer design approaches, the '454 patent lacks written description support for multiplex PCR amplification that does not utilize techniques for selection

⁶ The primer design methods disclosed in the '454 patent for highly multiplexed PCR from DNA derived from cell-free DNA were known in the prior art at the time of filing. *See* Rabinowitz at [0291]–[0298], as were the other variants of multiplex PCR disclosed in the '596 patent. *See, e.g., id.* at [0299]–[0329].

of primers to avoid primer side products. Therefore, a POSA would understand that the Asserted Claims fail to recite essential steps involving target loci selection and removal of primer pairs likely to form primer-dimers for targeted, highly multiplexed PCR amplification in a single reaction volume.

For at least these reasons, the Asserted Claims of the '454 patent lack an essential element of the invention and therefore lack adequate written description support. *See LizardTech, Inc. v. Earth Resource Mapping, Inc.*, 424 F.3d 1336 (Fed. Cir. 2005); *Tronzo v. Biomet, Inc.*, 156 F.3d 1154 (Fed. Cir. 1998).

c. Capability of Detecting an SNV Present at Less Than or Equal to 0.015%

Asserted Claim 14 of the '454 patent requires “detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads,” and that “the method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus.” The '454 patent does not provide adequate written description and enablement support for the full scope of claim 14 and its dependents. Nor does the claim specify any limit or requirements on the sensitivity of the method. Therefore, the patent claims a method that achieves a broad range of sensitivities, without disclosing or enabling a method that is capable of achieving the full scope of SNV detection required. “A ‘mere wish or plan’ for obtaining the claimed invention does not satisfy the written description requirement.” *Novozymes v. DuPont Nutrition Biosciences APS*, 723 F.3d 1336, 1344 (Fed. Cir. 2013); *see also, e.g., Medytox, Inc. v. Galderma S.A.*, 71 F.4th 990, 998 (Fed. Cir. 2023) (affirming decision that undue experimentation would be required to practice a claimed method

reciting a responder rate of at least 50% where the specification did not demonstrate any responder rates greater than 62%).

Claim 14 and related dependent claims therefore require support demonstrating that the inventors could perform sequencing and detection at all levels of sensitivity and for an SNV that is present in less than or equal to 0.015% of the cell free DNA having the locus for that SNV. But the specification does not demonstrate that the inventors were in possession of a method wherein an SNV is capable of being detected across the full claimed range. Nor does the specification enable the claims for a method that is capable of detecting an SNV across the full range of less than or equal to 0.015%. For example, the specification would have to demonstrate that the inventors could use the claimed method to detect tumor-specific SNV mutations at very low absolute and relative abundance, for example in a single molecule of cfDNA containing tumor-specific SNV mutations among many molecules of cfDNA not containing such mutations. A POSA reviewing the specification of the '454 patent at the time of Natera's claimed priority date would recognize that the inventors were not in possession of the claimed methods capable of detecting of an SNV present at less than or equal to 0.015%.

The '454 patent does not demonstrate detection of an SNV present in less than or equal to 0.015% of the cell-free DNA having the SNV locus for any SNV, much less across the full claimed range. At best, Example 6 of the '454 specification describes a mean LOD of 0.24% and a median LOD of 0.09% for DNA in a plasma sample. '454 patent at 158:32–36. The specification does not enable or demonstrate possession of a method capable of detecting an SNV present at any level of sensitivity, or even a method that is capable of detecting an SNV that is present below a mean LOD of 0.24% and a median LOD of 0.09%. The lowest LOD for sequencing single nucleotide changes mentioned in the specification is 0.005%. *Id.* at 92:61–66.

The specification provides no support for any higher sensitivity or lower limit of detection than the recited threshold. A POSA reviewing the specification of the '454 patent would readily recognize that the inventors were not in possession of the claimed methods for sequencing at any level of sensitivity or at any limit of detection. A POSA would understand that the inventors were not in possession of the full scope of the claimed subject matter.

Example 14 of the '454 specification similarly shows that the inventors were not in possession of the full scope of the claimed subject matter. First, Example 14 is not an embodiment of the asserted claims of the '454 patent. Second, Fig. 59 illustrates “a plot of Limit of Detection (LOD) vs. Depth of Read (DOR) for detection of SNV Transition and Transversion mutations in a 84-plex PCR reaction at 15 PCR cycles,” showing that a LOD lower than 0.01% was not achieved for *any* SNV at read depths lower than 100,000. *Id.* at 42:15–18, Fig. 59. Figs. 60 and 61 similarly show that the LOD for SNVs was often greater than 0.01 or even 0.1 (meaning that an SNV present at lower frequencies could not be detected). *See id.* at 42:19–26, Figs. 60–61. Furthermore, Figs. 59–61 show that a LOD lower than 0.01% was not achieved for *any* SNV that was a transition mutation, regardless of read depth or number of PCR cycles. *Id.* at Figs. 59–61. Nor does the specification describe how to predict whether a given SNV could be detected when present at less than or equal to 0.015%.

Furthermore, the data in the '454 patent specification show that detection was not achieved for any SNV present below about 0.002% within the claimed range of less than or equal to 0.015%. *See, e.g., id.* at Figs. 59–61. An annotated version of FIG. 61 is reproduced below, for example, highlighting a range of approximately 0.002% and below. The specification does not show that the inventors were able to detect any SNVs in this range. Therefore, the '454 patent claims a percentage range at which the method must be capable of detecting an SNV, but

the '454 patent specification fails to show possession or enablement of the full scope of the claimed range.

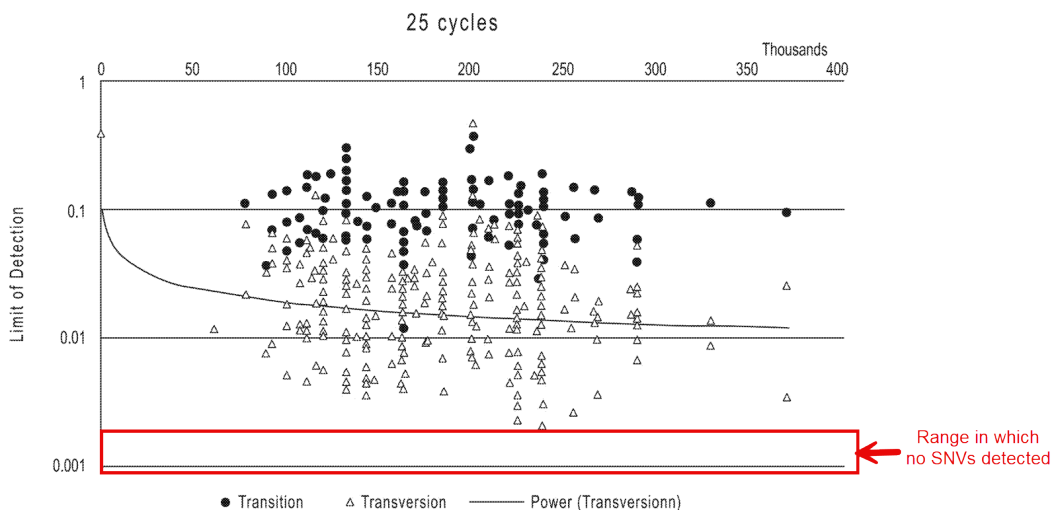


FIG. 61

Second, the '454 specification does not disclose any clinical sensitivities for detection of an SNV when present at less than or equal to 0.015%. And Natera's own admissions show that Natera was not in possession of the claimed method wherein an SNV is detected when present at less than or equal to 0.015%. For example, in a post-filing publication titled *A personalized, tumor-informed approach to detect molecular residual disease with high sensitivity and specificity*, Natera stated that key improvements with Signatera *since* the TRACERx study included that, "[a]t the single SNV level, detection at 0.01% VAF improved from <5% to 30%." 2019_08_27_NAT-801944 at 5 (2019), www.natera.com/wp-content/uploads/2020/11/Oncology-Clinical-A-personalized-tumor-informed-approach-to-detect-molecular-residual-disease-SGN_SR_WP.pdf. The TRACERx study itself was not published until years after Natera's asserted filing date. *See* Abbosh. But the '454 patent does not cabin the functional limitation requiring detection of an SNV present at less than or equal to 0.015% of the cfDNA to a limited level of clinical sensitivity or reproducibility (e.g., that the method is only

capable of achieving detection of an SNV present at 0.01% less than 5% of the time). The '454 patent claims set a high bar for written description by claiming any method comprising performing the active steps recited by the claims “wherein the method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus,” including methods that are highly reproducible, without demonstrating possession of the full scope of the claims. Simply put, the '454 patent claims a “problem to be solved while claiming all solutions to it,” covering any method “later actually invented and determined to fall within the claim’s functional boundaries,” *Ariad*, 598 F.3d at 1353, which fails to satisfy the written description requirement.

Because claim 14 of the '454 patent recites detection of an SNV across an unbounded range of less than or equal to 0.015% and at any level of clinical sensitivity or reproducibility, and the specification does not describe performing the claimed methods to detect an SNV across the full claimed range of sensitivity, a POSA would understand that the inventors were not in possession of the full scope of the claimed subject matter. Nor are the claims limited to detecting transversion rather than transition mutations. Example 14 shows that transition mutations could not be detected at levels lower than 0.01% and that the inventors were not in possession of a method for detecting these mutations below a level of 0.015%.

A POSA reading the specification at the time of Natera’s claimed priority date likewise would be unable to perform the claimed method at all levels of sensitivity to detect an SNV present at less than or equal to 0.015%, or for any type of SNV mutation, or with any amount of input cfDNA, without undue experimentation. For example, Bernhard Zimmerman, a named inventor of the '454 patent, acknowledges “the analytical sensitivity . . . is different at any different [cfDNA] input.” Zimmerman 7/12/2024 30(b)(6) Dep. Tr. at 24:20-23. Natera

specifically performed “sensitivity assessment” based on several different amounts of input cfDNA “at 60 nanogram input, 30 nanogram input” and “10 nanogram input.” *Id.* at 25:1-5. Indeed, improved techniques for each step of the claimed methods not disclosed in the ’454 patent would be necessary to sequence a particular tumor-specific SNV mutation present in less than 0.005% of the DNA molecules in a patient sample, and it would require undue experimentation for a POSA to determine how, if it is even possible, to achieve detection at such levels. Additionally, as Natera admitted in “A personalized, tumor-informed approach to detect molecular residual disease with high sensitivity and specificity,” improved techniques were required to detect an SNV present at 0.01% even 30% of the time. *A personalized, tumor-informed approach to detect molecular residual disease with high sensitivity and specificity*, 2019_08_27_NAT-801944 at 5 (2019), www.natera.com/wp-content/uploads/2020/11/Oncology-Clinical-A-personalized-tumor-informed-approach-to-detect-molecular-residual-disease-SGN_SR_WP.pdf. Natera further stated that “[the] limit of detection for Signatera, measured in VAF, is 0.01%” (*id.* at 1), but the claims encompass detection of SNVs present well below 0.01%.

d. “performing targeted multiplex amplification . . . from the cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom”

Asserted Claims 1 and 14 of the ’454 patent require “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.” The ’454 patent does not provide adequate written description and enablement support for the full scope of claims 1 and 14 or their dependents. The claim language recites two

categories of DNA on which the recited targeted multiplex amplification can be performed. The first is “cell-free DNA isolated from a plasma sample of the subject” and the second is DNA “derived []from” such isolated cell-free DNA. The ’454 patent only describes using the second category of DNA for performing targeted multiplex amplification. Nowhere does the ’454 patent describe targeted multiplex amplification from “the cell-free DNA isolated from a plasma sample of the subject.” A POSA would understand that the inventors were not in possession of the full scope of the claimed subject matter. The ’454 patent claims priority to a long list of prior applications, and the addition of the words “cell-free DNA isolated from a plasma sample of the subject” to the claims of the ’454 patent at the time its application was filed do not show that Natera’s inventors had conceived of or were in possession of performing targeted multiplex amplification on this class of DNA.

Much less does the ’454 patent provide sufficient written description or enablement support for “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,” as required by Asserted Claim 1 of the ’454 patent; or for “performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from the cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons, 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 method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus,” as required by Asserted Claim 14 of the ’454 patent.

To the extent Natera contends any of the data in the specification provide support for the claims, the Examples describing how cfDNA was prepared prior to multiplex amplification state that the input for multiplex PCR was an “amplified and purified Natera plasma library,” not cfDNA isolated from a plasma sample. *See, e.g.,* ’596 patent at 164:57-59 (“[t]he input for mPCR was 2.5 uL of amplified and purified Natera plasma library at an input amount of approximately 600 ng.”). The input DNA for multiplex PCR differed from isolated cfDNA in multiple respects. For example, Natera has admitted that the Natera plasma library preparation process alters characteristics such as DNA fragment size distribution that could affect assay results. *See A personalized, tumor-informed approach to detect molecular residual disease with high sensitivity and specificity*, 2019_08_27_NAT-801944 at 5 (2019), www.natera.com/wp-content/uploads/2020/11/Oncology-Clinical-A-personalized-tumor-informed-approach-to-detect-molecular-residual-disease-SGN_SR_WP.pdf. The Natera amplified and purified plasma library largely contains amplicons derived from cfDNA (i.e., “DNA derived therefrom” in ’454 patent claims 1 and 14). Natera has not shown that any “cell-free DNA isolated from a plasma sample of the subject” as claimed in the ’454 patent remains in the Natera amplified and purified plasma library, much less that any remaining cfDNA in the amplified library would be present in sufficient quantities to perform the claimed method. As another example, Abbosh explains that Natera’s cfDNA library preparation involved cfDNA end-repair and A-tailing, followed by ligation of custom adaptors, amplification, and purification using Ampure beads, yielding a

single peak around 250 bp. Abbosh at Methods (“cfDNA library preparation”). The size distribution of the amplified and purified libraries as a single peak around 250 bp differs substantially from much broader size distribution of isolated cfDNA. *See, e.g.,* Jahr at 1663 (describing ladder-like pattern for size distribution in isolated cfDNA). Given the differences between isolated cfDNA and the Natera amplified and purified plasma library used as input for the targeted multiplex PCR, a POSA would understand the inventors were not in possession of the claimed subject matter.

Likewise, a POSA reading the specification at the time the application for the ’454 patent was filed, or at any earlier time, would be unable to perform targeted multiplex amplification to amplify the plurality of target loci from “the cell-free DNA isolated from a plasma sample of the subject,” without undue experimentation, much less while achieving the claimed requirement of a depth of read of at least 50,000 per target locus or that an SNV present at less than or equal to 0.015% is detected. Accordingly, claims 1 and 14 of the ’454 patent and their dependent claims are invalid for lack of written description and enablement.

V. THE ASSERTED CLAIMS ARE INVALID UNDER 35 U.S.C. § 101.

The Asserted Patents are invalid under 35 U.S.C. § 101 for being directed to ineligible subject matter.

Under 35 U.S.C. § 101, “[l]aws of nature, natural phenomena, and abstract ideas are not patentable.” *Ass’n for Molecular Pathology v. Myriad Genetics, Inc.*, 569 U.S. 576, 589 (2013) (quoting *Mayo Collaborative Servs. v. Prometheus Lab’ys, Inc.*, 566 U.S. 66, 70 (2012)). Patent eligibility is assessed under the Alice/Mayo two-part test. First, it must be “determine[d] whether the claims at issue are directed to one of those patent-ineligible concepts.” *Alice Corp. Pty. Ltd. v. CLS Bank Int’l*, 573 U.S. 208, 217 (2014). If the claims are directed to an ineligible

concept, then the inquiry is whether any additional elements of the claim “transform the nature of the claim’ into a patent-eligible application.” *Id.* (quoting *Mayo*, 566 U.S. at 78).

The Asserted Claims of the ’596 and ’454 patents are invalid under 35 U.S.C. § 101. Under *Alice* step 1, claims 1, 11, 12, and 18 of the ’596 patent and claims 1, 8, 10, 12, 14, 15, 22, 24, 26, and 28 of the ’454 patent are directed to detecting a natural phenomenon: the presence in a blood (plasma) sample of DNA mutations associated with a cancerous tumor. *See, e.g.*, ’596 patent at claim 1 (“an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is **detected**”) (emphasis added); ’454 patent at claims 1 and 14 (“**detecting** one or more of the tumor-specific SNV mutations present in the cell-free DNA”) (emphasis added).

Under *Alice* step 2, the Asserted Claims recite conventional steps, i.e., they lack an inventive concept, to carry out the detection of the natural phenomenon. Natera acknowledges that each claimed step of the ’596 patent and ’454 patent is conventional. For example, the preamble of claim 1 in the ’596 patent recites a “method for preparing biological samples useful for monitoring the progression of cancer in a subject.” The preamble of claim 1 in the ’454 patent recites 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.” Such methods were well known and acknowledged as such by Natera. *See, e.g., CareDx, Inc. v. Natera, Inc.*, 563 F. Supp. 3d 329, 335 n.5 (D. Del. 2021) (“to obtain cfDNA samples ‘any technique known in the art may be used, e.g. a syringe or other vacuum suction device’”).

Claim 1 of the ’596 patent then recites steps (a) and (b) as “performing sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor-specific mutations” and

“evaluating results of the sequencing on the tumor biopsy sample to determine a plurality of target loci specific to the subject.” Claim 1 of the ’454 patent recites the first step as “performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutation.” The specification of the ’596 patent and ’454 patent acknowledges this step as a conventional operation that could even be performed on commercial equipment. *See, e.g.*, ’454 patent at 168:13-15 (“Each biopsied sample was assayed by whole exome sequencing (Illumina HiSeq2000; Illumina, San Diego, Calif.)”).

Next, step (c) of claim 1 of the ’596 patent performs “assaying cell-free DNA” by “targeted multiplex PCR amplification.” Similarly, the second step of claim 1 of the ’454 patent performs “multiplex amplification to amplify 10 to 500 target loci” in order “to obtain amplicons having a length of 50-150 bases.” The specification of the ’596 patent and ’454 patent acknowledges this step as well known. *See, e.g.*, ’454 patent at 97:52-65 and prior art references cited therein.

The final step of claim 1 of the ’596 patent and claim 1 of the ’454 patent performs “sequencing” to “detect[]” SNV mutations—a conventional operation that could be performed on commercially available Illumina sequencers. *See, e.g.*, ’454 patent at 96:18-22, 152:43-48, 163:52-59.

Accordingly, neither claim 1 of the ’596 patent or claim 1 of the ’454 patent adds an inventive concept to the detection of SNV tumor mutations, which is a natural phenomenon. In addition, both claims are representative such that a separate patentability analysis is not necessary for the remaining asserted claims. NeoGenomics hereby incorporates by reference all the materials submitted in support of its Motion to Dismiss (Dkt Nos. 51-54 and 135).

VI. THE ASSERTED PATENTS ARE INVALID DUE TO IMPROPER INVENTORSHIP

“A patent is invalid if more or less than the true inventors are named.” *Trovan, Ltd. v. Sokymat SA, Irori*, 299 F.3d 1292 (Fed. Cir. 2002). For purposes of determining the proper naming of inventors of a patent, as well as demarcating the exclusionary rights granted by a patent, the patented “invention” is defined by the subject matter set forth in the claims of the patent. “The claims of the patent provide the concise formal definition of the invention. They are the numbered paragraphs which “particularly [point] out and distinctly [claim] the subject matter which the applicant regards as his invention.” *Autogiro Co. of Am. v. United States*, 384 F.2d 391, 395 (Ct. Cl. 1967). The Supreme Court has ruled that the claimed invention consists of the entire subject matter specified in the individual claim, and that no part of the claim can be ignored in considering the scope of the invention. *Warner-Jenkinson Co., Inc. v. Hilton Davis Chem. Co.*, 520 U.S. 17, 29 (1997). The standard for inventorship requires a joint inventor to: “(1) contribute in some significant manner to the conception or reduction to practice of the invention, (2) make a contribution to the claimed invention that is not insignificant in quality, when that contribution is measured against the dimension of the full invention, and (3) do more than merely explain to the real inventors well-known concepts and/or the current state of the art.” *Pannu v. Iolab Corp.*, 155 F.3d 1344, 1351 (Fed. Cir. 1998).

The Asserted Patents are invalid due to failure to name the correct inventors of the subject matter being patented. The '596 and '454 patents list only Joshua Babiarz, Tudor Pompiliu Constantin, Lane A. Eubank, George Gemelos, Matthew Micah Hill, Huseyin Eser Kirkizlar, Matthew Rabinowitz, Onur Sakarya, Styrmir Sigurjonsson, and Bernhard Zimmerman from Natera as inventors. However, researchers at University College London (“UCL”)

including at least Drs. Mariam Jamal-Hanjani and Charles Swanton conceived of and contributed to aspects of the '596 and '454 patent claims and communicated that conception and contribution to named inventors on the '596 and '454 patents.

Drs. Jamal-Hanjani and Swanton developed and drafted the protocol for the TRACERx (TRacking Cancer Evolution through therapy (Rx)) study. The protocol is described in, among other things, *Jamal-Hanjani et al.*, Tracking Genomic Cancer Evolution for Precision Medicine: The Lung TRACERx Study 12:7 PLOS Biology 1 (July 2014) (NEOGEN00289346). As part of that study, they conceived of and performed multi-regional sampling of patient tumors, performed whole genome or whole exome sequencing on the different tumor samples, and identified SNV mutations in the tumor DNA that are clonal and subclonal. The protocol also described Dr. Jamal-Hanjani's and Dr. Swanton's conception of attempting to detect and track the clonal and subclonal tumor-specific SNV mutations in ctDNA.

Drs. Jamal-Hanjani and Swanton collaborated with Natera to implement their idea of detecting and tracking the clonal and subclonal tumor-specific SNV mutations they identified in ctDNA. At the time that collaboration began, Natera and the named inventors of the '596 and '454 patents were focused on developing tumor naïve panel tests or screening assays for detecting SNVs associated with cancer in ctDNA. Based on the work and ideas of Drs. Jamal-Hanjani and Swanton, Drs. Jamal-Hanjani and Swanton in collaboration with Natera scientists including the currently-named inventors performed what is identified as Example 13 of the '596 and '454 patents, in which clonal and subclonal tumor-specific SNV mutations identified by Drs. Jamal-Hanjani and Swanton were identified in ctDNA.

The '596 and '454 patents claim methods based on a tumor-informed assay by reciting a first 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” ('454 patent at claims 1 and 14) or “performing sequencing on a tumor biopsy sample of the subject to identify a plurality of tumor-specific mutations.” ('596 patent at claim 1) The claims then recite the steps of “performing targeted multiplex amplification” of the “cell-free DNA” and “sequencing” of the obtained amplicons in order to detect the SNV mutations. '454 patent at claims 1 and 14; '596 patent at claim 1. Researchers at UCL, including at least Drs. Mariam Jamal-Hanjani and Charles Swanton, conceived, reduced to practice, and communicated the tumor-informed aspect of the claims to the named inventors of the '596 and '454 patents. Natera, on the other hand, focused on a tumor-naïve approach before the collaboration with UCL researchers. For example, in a meeting related to the collaboration with Columbia University Medical Center dated June 19, 2014, Natera proposed “WGS or WES” in order “to determine concordance between mutational profile in primary tumors with mutation profile in cfDNA from plasma of patients.” NAT-NEO-00884440 at 11. The concordance determination between the tumor tissue sample and the cfDNA sample is not the tumor-informed method as claimed in the '596 and '454 patents.

In addition, certain aspects of the '596 and '454 patent claims, such as claims 4-9 of the '596 patent and claims 3-5, 7, 17-19, and 21 of the '454 patent, including the detection and determination of clonal and/or subclonal single nucleotide variant (“SNV”) mutations, were in fact conceived and communicated to Natera by researchers at University College London (“UCL”), such as Drs. Mariam Jamal-Hanjani and Charles Swanton as part of the TRACERx study.

The '596 patent claims methods of preparing a plasma sample for detecting “clonal SNV mutations” and/or “subclonal SNV mutations.” The '596 patent specifically claims clonal SNV mutations such as “CYFIP1, FAT1, MLLT4, RASA1, HERC4, JAK2, MSH2, MTOR, PLCG2, GARRG1, and TRIM67.” The '596 patent also specifically claims subclonal SNV mutations such as “CIC, KDM6A, NF1, and TRIM67.” Researchers at UCL, including at least Drs. Mariam Jamal-Hanjani and Charles Swanton, conceived, reduced to practice, and communicated these claimed methods to the named inventors of the '596 patent.

The '454 Patent claims methods of preparing a plasma sample for detecting “clonal SNV mutations” and/or “subclonal SNV mutations,” as well as “determining clonal heterogeneity of the tumor sample” present in “cell-free DNA isolated from a plasma sample.” Researchers at UCL, including at least Drs. Mariam Jamal-Hanjani and Charles Swanton, conceived, reduced to practice, and communicated these claimed methods to the named inventors of the '454 patent.

The sole support for foregoing claimed methods in both patents comes from Example 13 in the specification of the '454 and '596 patents. Example 13, however, describes a series of experiments that were conceived of not by the named inventors, but by researchers at UCL, including at least Drs. Mariam Jamal-Hanjani and Charles Swanton, who described the work that appears in Example 13 in Dr. Jamal-Hanjani's doctoral dissertation and contributed the research plan for the first experiment and actually performed at least part of the experiment leading up to the TRACERx study. This is clear from, for example, the figures and lists of genes referred to in Example 13 and claimed by the Asserted Patents, which are identical to those set forth in the doctoral dissertation of Mariam Jamal-Hanjani. *Compare* Mariam Jamal-Hanjani, *The role of intratumour heterogeneity and chromosomal instability in cancer* (Aug. 2015) (Ph.D. Thesis, University College London) (NEOGEN00017110 at 265-268 (Tables 22 and 23 and Figure 49))

with '454 Patent at Sheets 70, 73-75 (Figures 51A, 53A, 53B, and 54B) and '596 Patent at Sheets 70, 73-75 (Figures 51A, 53A, 53B, and 54B).

In addition, the claimed inventions of the '454 patent and '596 patent are “not patentably distinct from each other.” July 7, 2022 Non-Final Office Action during prosecution of '454 patent; Terminal Disclaimer to Obviate a Double Patenting Rejection Over a Prior Patent (“prior patent No. 11,319,596”) filed October 7, 2022 during prosecution of the '454 patent at 1. As a result, because UCL researchers such as Drs. Mariam Jamal-Hanjani and Charles Swanton conceived and reduced to practice the claimed invention in the '596 patent, they also conceived and reduced to practice the claimed invention in the '454 patent. Similarly, because UCL researchers such as Drs. Mariam Jamal-Hanjani and Charles Swanton conceived and reduced to practice the claimed invention in the '454 patent, they also conceived and reduced to practice the claimed invention in the '596 patent.

Accordingly, the Asserted Patents are invalid for improper inventorship by missing UCL inventors such as Dr. Mariam Jamal-Hanjani and Charles Swanton. NeoGenomics incorporates by reference its opposition brief to Natera’s preliminary injunction (Dkt. 107), Van Ness declaration ISO NeoGenomics’ opposition (Dkt. 116), Bernhard Zimmerman 10/4/2023 deposition transcript, Tim Forsheew 7/19/2024 deposition transcript, NeoGenomics’ opposition to Natera’s motion to dismiss NeoGenomics’ unenforceability counterclaim and strike related affirmative defenses (Dkt. 147), Dr. Niall Lennon’s opening expert report served on August 2, 2024, and NeoGenomics’ memorandum in support of its motion for issuance of letters of request for production of documents and to examine persons pursuant to Hague evidence convention (Dkt. 352). NeoGenomics incorporates by reference its responses and any supplements thereof to Natera’s interrogatory Nos. 5 and 8. NeoGenomics further incorporates by reference the

upcoming deposition transcript of Dr. Bernhard Zimmerman, deposition transcript of Dr. Mariam Jamal-Hanjani dated March 18, 2025 and deposition transcript of Dr. Charles Swanton dated March 19, 2025, and any other witness that addresses this topic.

ACCOMPANYING DOCUMENT PRODUCTION

Pursuant to Patent Local Rule 103.4(a), NeoGenomics is producing or has produced to Natera documents sufficient to show the operation of any aspects or elements of an Accused Instrumentality identified by Natera in its Preliminary Infringement Contentions. *See* NEOGEN00287705-NEOGEN00288025, NEOGEN00288957-NEOGEN00289009, and NEOGEN00304686-NEOGEN00311898.

Pursuant to Patent Local Rule 103.4(b), NeoGenomics is producing or has produced to Natera copies of the references mentioned above. *See* NEOGEN00288666–NEOGEN00288956 and NEOGEN00330114-NEOGEN00330568.

Pursuant to Patent Local Rule 103.6, NeoGenomics is producing or has produced to Natera additional documents in support of these Amended Final Invalidity Contentions. *See* NEOGEN00287705-NEOGEN00330568.

This the 14th day of March, 2025

/s/ Aaron G. Fountain
DARALYN J. DURIE*
MATTHEW I. KREEGER*
ANNIE A. LEE*
JACK WILLIAM HAISMAN*
MORRISON & FOERSTER LLP
425 Market Street
San Francisco, CA 94105
Tel: (415) 268-7000
Fax: (415) 268-7522
DDurie@mofo.com
MKreeger@mofo.com
AnnieLee@mofo.com
JHaisman@mofo.com

CHARLES CALHOUN PROVINE*
MORRISON & FOERSTER LLP
2100 L Street NW, Suite 900
Washington, DC 20037
Tel: (202) 887-1500
Fax: (202) 887-0763
CProvine@mofo.com

ERIC NIKOLAIDES*
MORRISON & FOERSTER LLP
250 West 55th St.
New York, NY 10019
Tel: (212) 336-4061
ENikolaides@mofo.com

AARON G. FOUNTAIN*
HUI ZHAO*
MORRISON & FOERSTER LLP
300 Colorado Street, Suite 1800
Austin, TX 78701
Tel: (737) 309-0700
AFountain@mofo.com
HZhao@mofo.com

MATTHEW R. STEPHENS*
MORRISON & FOERSTER LLP
12531 High Bluff Drive, Suite 100
San Diego, CA 92130
Tel: (858) 720-5100
Fax: (858) 720-5125
MStephens@mofo.com

JOHN F. MORROW, JR.
WOMBLE BOND DICKINSON (US) LLP
One West Fourth Street
Winston-Salem, NC 27101
Tel: 336-721-3584
Fax: 336-733-8429
Email: John.Morrow@wbd-us.com

Counsel for NeoGenomics Laboratories, Inc.
**Special Appearance under Local Civil Rule 83.1(d)*

CERTIFICATE OF SERVICE

I, the undersigned, hereby certify that on March 14, 2025, a true and correct copy of the foregoing document was served via electronic notice and electronic mail upon the following:

ANDREW R. SHORES
CARMELLE F. ALIPIO
ROBERT VAN ARNAM
WILLIAMS MULLEN
301 Fayetteville Street, Suite 1700
Raleigh, NC 27601
ashores@williamsmullen.com
calipio@williamsmullen.com
rvanarnam@williamsmullen.com

KEVIN ALEXANDER SMITH
TARA SRINIVASAN
QUINN EMANUEL URQUHART &
SULLIVAN LLP
50 California St., 22nd Floor
San Francisco, CA 94111
kevinsmith@quinnemanuel.com
tarasrinivasan@quinnemanuel.com

KEVIN P.B. JOHNSON
VICTORIA MAROULIS
ANDREW J BRAMHALL
QUINN EMANUEL URQUHART &
SULLIVAN LLP
555 Twin Dolphin Drive, 5th Floor
Redwood Shores, CA 94065
kevinjohnson@quinnemanuel.com
victoriamaroulis@quinnemanuel.com
andrewbramhall@quinnemanuel.com

SANDRA HABERNY
QUINN EMANUEL URQUHART &
SULLIVAN LLP
865 S. Figueroa Street, 10th Floor
Los Angeles, CA 90017
sandrahaberny@quinnemanuel.com
qe-natera-neogenomics@quinnemanuel.com

Attorneys for Natera, Inc.

/s/ Moe San

Moe San