

EXHIBIT 2-A – FINAL INVALIDITY CLAIM CHART
FOR U.S. PATENT NO. 11,530,454

Reference: Patel, A., et al., *Measurement of Nucleic Acid Variants Using Highly-Multiplexed Error-Suppressed Deep Sequencing*, Patent Application WO 2013/138510 AI (Sept. 19, 2013) (“Patel”)

Reference: Bashashati, A. et al., *Distinct evolutionary trajectories of primarily high-grade serous ovarian cancers revealed through spatial mutational profiling*, J. Pathol. 231: 21–34 (June 7, 2013) (including its supplementary material (including the appendix, Figures S1-S7, and Tables S1-S6) (“Bashashati”))

A POSA would be motivated to modify Patel in view of Bashashati to perform whole exome sequencing on a tumor biopsy sample of the subject to identify the plurality of tumor-specific mutations (as taught by Bashashati) that are then assayed in the cfDNA as taught by Patel. Patel teaches that “ctDNA can be distinguished from normal background DNA based on the presence of tumor-specific mutations.” Patel at 1. Bashashati discloses identification of target loci spanning tumor-specific mutations using whole exome sequencing. *See, e.g.*, Bashashati supplementary app’x at 5–6; Bashashati at 22. Because Patel discloses identifying ctDNA based on tumor-specific mutations and Bashashati discloses identifying tumor specific mutations by sequencing tumor biopsies, a POSA would be motivated to apply the multiplex PCR and ultrasensitive error-suppressed deep sequencing methods of Patel to a tumor-informed assay of cfDNA, as taught by Bashashati. A POSA would have a reasonable expectation of success in doing so because whole exome sequencing was within the general skill and knowledge of a POSA.

A POSA would be motivated to combine Patel with the whole exome sequencing approach disclosed by Bashashati at the time the ’454 patent was filed because Bashashati teaches the advantages of whole exome sequencing to identify SNV mutations for tracking in patient plasma samples, and a POSA would have understood that to monitor and track cancer effectively requires identifying the various categories of cancer-related mutations. A POSA would have had a reasonable expectation of success because whole genome sequencing and whole exome are well known technologies that can be used to identify variations in cancer samples. *See, e.g.*, Forshew at 9-10.

Additionally, Patel discloses a method comprising identification of SNVs in plasma, followed by confirming the mutations in tumor tissue. *See, e.g.*, Patel at 33. In view of Patel, Bashashati, and the knowledge of a POSA, it would have been obvious to modify this method to identify mutations first from a tumor tissue sample, followed by tracking those mutations in patient plasma. A POSA would have been motivated to do so because of the known benefits of using whole genome sequencing to identify mutations, discussed above. Further, a POSA would have reasonably expected to succeed because “[a]dvances in massively parallel sequencing make new approaches possible,” such as “whole-genome or whole-exome sequencing” to identify SNV mutations to track in plasma.

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See, e.g., Forshew at 9. A POSA would have known how to perform whole genome or whole exome sequencing to identify genomic or exomic regions of interest.

Also, a POSA would reasonably have expected to succeed because the prior art such as U.S. Patent Application Publication No. 2012/0270212 (“Diehn”) teach the use of whole exome sequencing or whole genome sequencing to identify mutations in tumor tissue. *See, e.g.,* Diehn at [0270]–[0271] (“The method may comprise (a) obtaining a genotype of a tumor in a subject; (b) identifying genomic regions comprising one or more mutations based on the genotype of the tumor; and (c) producing a selector set comprising at least one genomic region. 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.”).

A POSA would be motivated to modify Bashashati in view of Patel to perform multiplex PCR from isolated cfDNA because Patel teaches improved multiplex methods that allow highly multiplexed PCR from cfDNA while avoiding spurious amplification products. *See, e.g.,* Patel at 72 (“The highly multiplexed Round 3 PCR in which multiple gene targets from multiple samples were amplified in a single tube, resulted in the specific production of amplicons of the expected size.”), 65–73 (disclosing a method involving sequencing amplification products from three rounds of multiplex PCR in a single reaction tube while avoiding spurious amplification products). A POSA would be motivated to combine the cfDNA amplification reactions of Bashashati into a multiplex PCR as taught by Patel because Patel teaches that their multiplex assay allows one to “detect minute amounts of ctDNA and address the current deficiencies to assay ctDNA.” Patel at 1. A POSA would have a reasonable expectation of success because Patel teaches that their method “resulted in the specific production of amplicons of the expected size” (Patel at 72), that the background error level was below 0.015% for several mutation types (Patel at Table 3), and that known mutations were detected even at the lowest allele fractions tested (1 in 10,000, or 0.01%) (Patel at 36, Fig. 6).

A POSA would have been motivated to modify the methods of Bashashati in view of Patel to amplify at least 100 target loci at the time the ‘454 patent was filed because this would have increased efficiency and obtained more amplicons in a single operation. In addition, a POSA would reasonably have expected to succeed because prior art references such as Forshew discloses that more loci would “increase [] the robustness for tumor monitoring by compensating for effects of sampling noise or mutational drift.” Forshew at 10. A POSA would have a reasonable expectation of success to do so also because multiplex amplification was well known in the art. *See, e.g.,* Forshew at 3, 6 (Table 2), 9; Forshew supplementary materials at 2, 2 (Fig. 1B), 9 (Table S1), 17–18 (Table S6).

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Claim	To the extent the preamble is construed to be limiting, Patel and Bashashati disclose 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. <i>See, e.g.,</i> Patel at 1, 2, 10, 14, 16, 28 [Example 1]. For example, Patel discloses methods that have “been optimized for measurement of small amounts of mutant circulating tumor DNA (ctDNA) in a background of normal (wild-type) cell-free DNA in the plasma or serum of a patient having cancer.” Patel at 16. As another example, Patel discloses a method for identifying and quantifying nucleic acid variants, “particularly those available in low abundance or those obscured by an abundance of wild-type sequences” as in plasma, including by tagging and amplifying low abundance template nucleic acids from circulating tumor DNA, and by performing multiplex PCR with primers specific to mutation-prone regions associated with cancer. Patel at 2. As another example, Patel discloses that the terms “sequence variant” or “mutation” may refer to “any variation in a nucleic acid sequence including . . . single point-mutations . . . and single-nucleotide polymorphisms (SNPs).” Patel at 10. As another example, Patel discloses and attempts to solve the problem that “mutant ctDNA is usually only present in small amounts [in plasma], having been previously reported to comprise an average of 0.2% of total plasma DNA.” Patel at 1. As another example, Patel discloses that “unique somatic mutations can be used to distinguish tumor-derived DNA from normal background DNA in plasma” and describes “a new class of highly specific DNA-based cancer biomarkers . . . with clinical applications that may complement those of conventional serum protein markers” and “methods that specifically and sensitively measure rare mutant DNA molecules that are shed into blood from cancer cells.” Patel at 14.

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Claim	
	As another example, Patel discloses “application of a deep sequencing approach in which 3 mutation hotspot regions were analyzed from multiple plasma samples.” Patel at 28, Example 1. In this application, Patel teaches that “[t]housands of clonal sequence reads from each plasma sample were compared to reference sequences in order to identify and quantify variants.” Patel at 35, Example 1. <i>See, e.g.,</i> Bashashati at 22, 25; Bashashati supplementary app’x at 7. For example, Bashashati discloses that “[n]ext-generation sequencing technology has enabled the study and quantification of clonal evolution and intratumoural diversity in cancer, including inference from distributions of digital allelic representation of mutations in single samples, along with serial and regional comparisons of mutation profiles.” Bashashati at 22. Bashashati also discloses that “sequencing of cell-free circulating tumour DNA (ctDNA) extracted from plasma has been demonstrated to be an effective non-invasive tool for monitoring tumour burden.” <i>Id.</i> As another example, Bashashati discloses using plasma samples from a subset of patients “to identify the presence of clonally dominant and subclonal mutations through deep quenching of ctDNA.” Bashashati at 22; <i>see also id.</i> (“A total of 1349 somatic mutations in all samples were confirmed with deep amplicon resequencing (median 5079-fold coverage; see supplementary material, Table S2).”); Bashashati at 25 (“Mutational events in the ancestral clone are detectable in cell-free circulating tumour DNA from plasma.”); <i>id.</i> (“Plasma samples collected pre-operatively and pre-anaesthesia were available for four cases from which ctDNA was extracted and subjected to targeted deep-sequencing of validated somatic mutations.”). As another example, Bashashati teaches a method in which “[c]irculating tumour DNA was extracted from 1-2 ml of plasma” and then subjected to deep amplicon sequencing to validate candidate SNVs identified from initial sequencing of primary ovarian cancer samples. Bashashati supplementary app’x at 7.
1[a]. performing whole exome sequencing or whole genome sequencing on a	Patel and Bashashati teach performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations. <i>See, e.g.,</i> Patel at 16, 28, 33, 34.

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Claim	Patel teaches that its methods can be applied to, for example, “frozen tumor specimens and tumor specimens that have been formalin-fixed and paraffin-embedded.” Patel at 16; <i>see also, e.g.</i>, Patel at 28 (“Formalin-fixed, paraffin-embedded tumor specimens were obtained for all patients with non-squamous histology whose tumors had not already been tested for mutations by a clinical laboratory, and for whom sufficient tissue was available in the block after standard pathology evaluation.”). Patel also teaches “Analysis of cell line DNA” to simulate cfDNA (<i>i.e.</i>, because mutations are only present at a low frequency) and “Confirmation of mutations in tumor tissue” to identify a plurality of tumor-specific SNV mutation that should be identified in cell free DNA. “Genomic DNA was purified from human cancer cell lines using the same method used for purifying plasma DNA, after suspending cells in 0.2 ml of phosphate-buffered saline. The following cell lines were used: A549 (having a KRAS Gly12Ser mutation), H1957 (having an EGFR Leu858Arg mutation), and YUSAC (having a BRAF Val600Glu mutation). Cells were passed in culture for no more than 6 months after being thawed from original stocks. Because cell lines were used only for analysis of short regions of genomic DNA, authentication of lines by our laboratory was limited to sequencing of those regions. To test the performance of the deep sequencing method for a particular gene, DNA derived from cells known to be either mutant or wild-type with respect to that gene was mixed in various ratios between 10,000:1 and 1:10,000. Cell line DNA samples were then amplified and sequenced according to the same methods that were used for plasma DNA.” Patel at 33. “Genomic DNA was isolated from paraffin-embedded tumor tissue samples using the QuickExtract™ FFPE DNA Extraction Kit (Epicentre Biotechnologies, Madison, WI). Mutation hotspot regions of <i>KRAS</i>, <i>BRAF</i>, and <i>EGFR</i> were amplified using the same PCR primers that were used in the first round of PCR described above. Sanger sequencing was performed on gel-purified amplicons, and mutations were identified from chromatograms using Mutation Surveyor software (SoftGenetics LLC, State College, PA).” <i>See, e.g.</i>, Patel at 34. Bashashati also discloses performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations.

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Claim	
	<i>See, e.g.</i>, Bashashati supplementary app'x at 5–6, Bashashati at 22. For example, Bashashati discloses that “Genomic DNA was extracted from frozen samples using the Gentra Puregene kit (Qiagen) and quantified using a Qubit fluorometer (Invitrogen). Exome capture was achieved through solution hybrid selection with the Human All Exon kit SureSelect Target Enrichment System (Agilent) version 1 for Illumina Genome Analyzer paired-end sequencing (Gnirke et al., 2009) . . . Using the resulting tumour and normal pair of BAM files, somatic SNVs were called by JointSNVMix algorithm (Roth et al., 2012), sorted by marginal posterior probability and further run through the mutationSeq algorithm (Ding et al., 2012) to find high confidence candidate SNVs.” Bashashati supplementary app'x at 6. As another example, Bashashati discloses a method in which “the exomes of 25 samples (19 tumour, six normal; Table 1) were sequenced to a median exonic coverage of 70.7-fold across different samples (see supplementary material, Table S1 and Figure S1).” Bashashati at 22. As another example, Bashashati discloses a method in which “multiple tissue samples were obtained from primary ovarian tumor and metastatic sites where adequate tumor volume permitted,” teaching that “[g]enomic DNA was extracted from frozen samples” and that “[e]xome capture was achieved through solution hybrid selection with the Human All Exon kit SureSelect Target Enrichment System (Agilent) version 1 for Illumina Genome Analyzer paired-end sequencing.”). Bashashati supplementary app'x at 5–6. A POSA would understand that this method discloses whole exome sequencing because Bashashati is using the “Human All Exon kit.” As another example, Bashashati teaches that “[c]andidate SNVs and indels were subjected to deep sequencing using Illumina and Ion Torrent platforms” in order to validate mutations. Bashashati supplementary app'x at 6.
1[b]. performing targeted multiplex amplification to amplify 10 to 500 target loci each	Patel and Bashashati disclose 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.

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Claim 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	<i>See, e.g.</i>, Patel at 40, 52, 53, Table 9, 65, 66, 68, 70, 72. For example, Patel discloses an application of its methods in which “40 different genomic target regions were analyzed,” 38 of which “were prone to developing somatic mutations.” Patel at 40, Example 2. Patel discloses amplifying these 40 targets together in a multiplexed pre-amplification PCR step. Patel at 52 (“The purified primer-extension products were eluted directly into a cocktail of buffer nucleotides, and primers that was used to carry out the multiplexed pre-amplification reaction.”); Patel at 53, Table 9 (listing “40 forward primers and the single universal reverse primer (ExtV2Rev) used for the pre-amplification reaction”). As another example, Patel alternatively discloses the method of the above example, but wherein “the final amplification was performed in a single tube using universal PCR primers.” Patel at 65, Example 3. Patel discloses that, in this alternative example, “the same 40 genomic target regions were analyzed.” Patel; <i>see also</i> Patel at 70 (“In contrast to the final PCRs in Example 2 which were performed separately for each genomic target site, the final PCR in the present Example was performed in a combined reaction volume for all genomic targets and for all samples.”); Patel at 72 (“The highly multiplexed Round 3 PCR in which multiple gene targets from multiple samples were amplified in a single tube, resulted in the specific production of amplicons of the expected size.”). Patel also discloses obtaining amplicons having a length of 50-150 bases. <i>See, e.g.</i>, Patel at Fig. 4B (showing 75bp overlapping reads through a hotspot region). <i>See also, e.g.</i>, Bashashati supplementary Table S2at cols. T, U; Bashashati supplementary app’x at 6, 7. For example, Bashashati teaches a method in which “[v]alidations of candidate single nucleotide variants (SNVs) identified from the initial sequencing of the primary ovarian cancer samples were achieved using a deep amplicon sequencing approach.” Bashashati supplementary app’x at 7. In this method, candidate SNVs “were fed into the LIFE Technology AmpliSeq custom designed pipeline, after conversion to hg19 coordinates, and three multiplex primers pools were generated.” <i>Id.</i>

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Claim	As another example, Bashashati teaches amplification of target loci from cell-free DNA isolated from a plasma sample. First, Bashashati states that “[c]irculating tumour DNA was extracted from 1-2 ml of plasma.” Bashashati supplementary app’x at 7. Then, Bashashati teaches amplifying circulating tumor DNAQ by the method described in the Supplementary Appendix in the section titled “Deep amplicon sequencing using the Illumina MiSeq platform for cell-free DNA in the plasma,” which refers back to the section titled “Deep amplicon sequencing using the Illumina GAII platform.” <i>Id.</i> These sections teach the use of targeted PCR to amplify target loci encompassing different SNVs. Bashashati supplementary app’x at 6 (“Primer Paris were designed to place the variant position within 75bps of either end of the amplicon and to be between 50-300bp in length.”). Bashashati also discloses obtaining amplicons having a length of 50-150 bases. <i>See, e.g.,</i> Bashashati supplementary app’x at 6. For example, Bashashati discloses a method in which “[p]rimer pairs were designed to place the variant position within 75bps of either end of the amplicon and to be between 50-300bp in length.” Bashashati supplementary app’x at 6. A POSA would thus understand that the primer pairs could be designed to place the variant position with 75bps of both ends of the amplicon, leading to an amplicon no longer than 149 base pairs. In view of Bashashati, it would have been obvious to a POSA to amplify the target loci together in the same reaction volume. <i>See, e.g.,</i> Bashashati supplementary app’x at 6, 7. For example, Bashashati teaches a method in which “[v]alidations of candidate single nucleotide variants (SNVs) identified from the initial sequencing of the primary ovarian cancer samples were achieved using a deep amplicon sequencing approach.” Bashashati supplementary app’x at 7. In this method, candidate SNVs “were fed into the LIFE Technology AmpliSeq custom designed pipeline, after conversion to hg19 coordinates, and three multiplex primers pools were generated.” <i>Id.</i> Even though the targeted amplification of SNVs from ctDNA was conducted using single primer pairs per reaction, Bashashati thus teaches a POSA that SNVs can be amplified in multiplex reactions.

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Claim	
1[c]. sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads,	Patel and Bashashati disclose 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. The Court construed the term “sequencing the amplicons” to have its plain and ordinary meaning. Doc. 280 at 10. The Court construed the term “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” to require that the detecting and sequencing are part of the same step. <i>Id.</i> at 11. In addition, Natera has failed to supplement its response to NeoGenomics’ Interrogatory No. 14 to properly address NeoGenomics’ Initial Invalidity Contentions served on February 14, 2024 and the Court’s Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court’s claim construction ruling. Under Natera’s apparent interpretation of the Court’s adopted construction, Patel and Bashashati discloses 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. <i>See, e.g.,</i> Patel at 54, 57, 63, 70, 71, 72. For example, Patel discloses sequencing the amplicons produced from multiplex PCR reactions. Patel at 54, Example 2 (“After purification, products of the pre-amplification reaction were subjected to further amplification by PCR in separate tubes (one tube for each of the 40 target gene regions).”); Patel at 57 (disclosing “Preparation of DNA for next-generation sequencing” and “Next-generation sequencing” using the results of the separate PCR amplification of individual gene targets); Patel at 63 (“Sequencing of the 40 pooled amplicons from multiple barcoded samples on the Illumina HiSeq 2000 platform further confirmed that all intended gene segments were amplified.”). As another example, Patel discloses sequencing the amplicons produced in a multiplex PCR amplification. <i>See, e.g.,</i> Patel at 65-73; Patel at 70, Example 3 (“[T]he final PCR in the present Example was performed in a

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Claim	combined reaction volume for all genomic targets and for all samples.”); Patel at 71 (“The product of the Round 3 [multiplex] PCR was purified on a 2% agarose gel,” and then “[n]ext generation sequencing was performed . . . using the Illumina HiSeq 2000 platform.”); Patel at 72 (“The gel-extracted [multiplex] PCR products were subjected to next-generation sequencing using an Illumina MiSeq instrument.”); Patel at 72-73 (“After processing of the sequence data using the computer algorithm described above, control mutant oligonucleotides that were spiked into the plasma DNA were identified and quantified. Importantly, they were readily distinguished from the vast majority of errors introduced during amplification, processing, or sequencing.”). <i>See, e.g.</i>, Bashashati supplementary app’x at 6–7; Bashashati at 25, 26 (Fig. 2); Bashashati supplementary Table S2 at col. T. For example, Bashashati discloses a method in which “[s]omatic mutation predictions were subjected to rigorous validation by targeted deep amplicon sequencing in the tumour and normal DNA templates” and in which cell-free DNA in the plasma was subjected to deep amplicon sequencing using the Illumina MiSeq platform. Bashashati supplementary app’x at 6–7. As another example, Bashashati explains that “[p]lasma samples collected pre-operatively and pre-anaesthesia were available for four cases from which ctDNA was extracted and subjected to targeted deep-sequencing of validated somatic mutations (see supplementary material, Appendix).” Bashashati at 25. Bashashati then “assayed 72 mutations in case 1, 49 in case 2, 63 in case 4 and 131 in case 5” and found that, “[a]cross the four cases, 215/312 (69%) mutations contained sequencing coverage and 38/215 (18%) were detectable with mutation allele frequency above statistical background noise.” Bashashati at 25; <i>see also</i> Bashashati supplementary Table S2 at col. T (anything with RefCount.plasma). Bashashati presents these findings in Figure 2:

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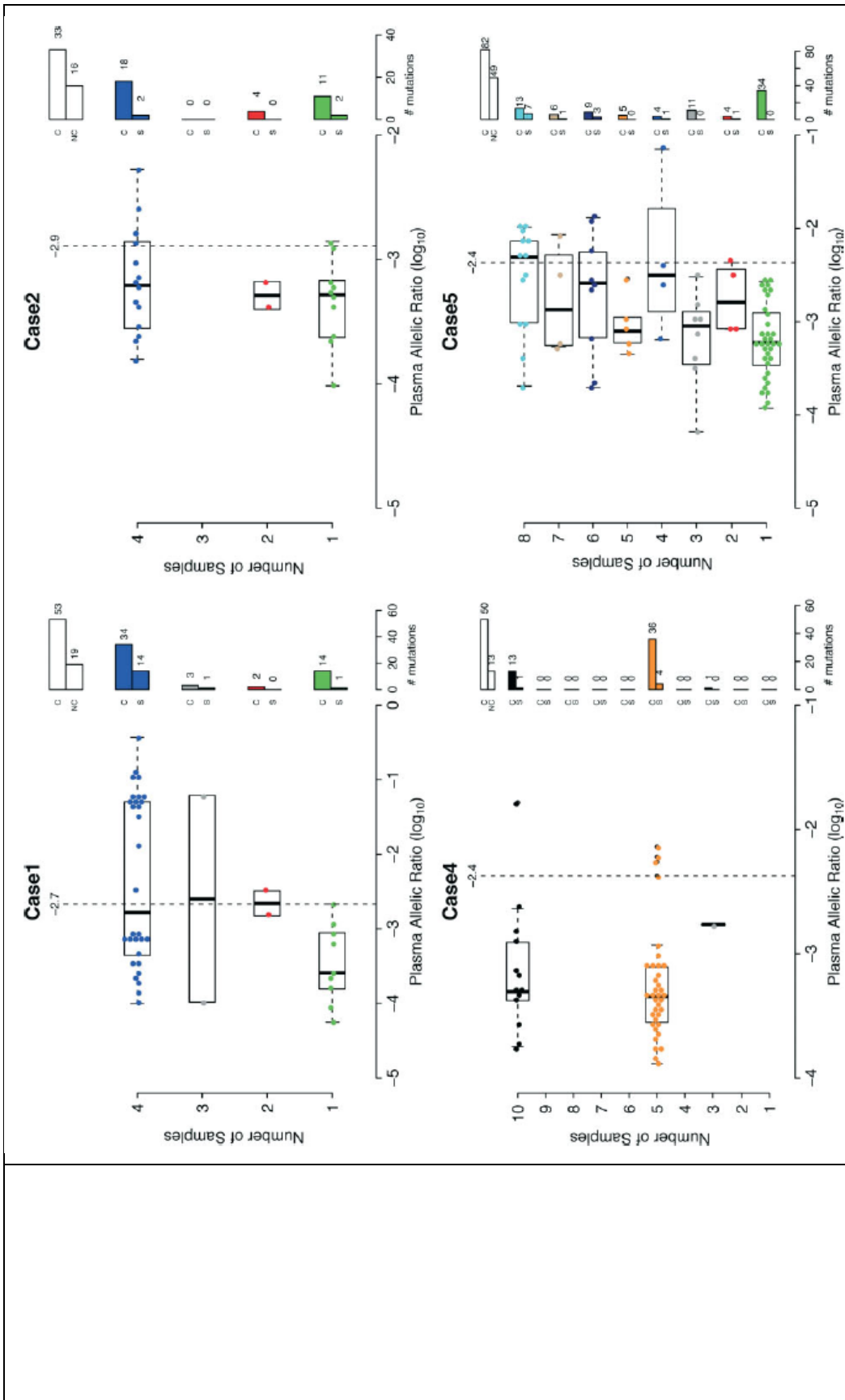


Figure 2. Deep sequencing results of cell-free circulating tumour DNA from the plasma in cases 1, 2, 4 and 5. The distribution of plasma variant allelic ratio (log₁₀ scale) is separated by the number of tumour samples in which the mutation was originally discovered. Cases 4 and 5 both also include the fallopian tube when counting the number of tumour samples. The number of mutations based on positions with coverage (C), no coverage (NC) and those with coverage that were significantly (S) detected, based on the binomial exact test (adjusted $p < 0.05$), are shown. The minimum variant allelic ratio detectable using the exact test for each patient is denoted by the vertical dashed line.

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Claim 1[d]. wherein the sequencing has a depth of read of at least 50,000 per target locus.	Patel and Bashashati disclose the sequencing has a depth of read of at least 50,000 per target locus. <i>See, e.g.,</i> Patel at 72 (“The total number of raw clonal paired-end sequences was 20,511,389. After application of the various filters described above, the remaining sequences numbered 11,184,975. The 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.”). <i>See, e.g.,</i> Bashashati supplementary Table S2 at cols. T, U; Bashashati at 25 (“Plasma samples collected pre-operatively and pre-anaesthesia were available for four cases from which ctDNA was extracted and subjected to targeted deep-sequencing of validated somatic mutations (see supplementary material, Appendix). We assayed 72 mutations in case 1, 49 in case 2, 63 in case 4 and 131 in case 5.”); Bashashati supplementary app’x at 6 (“Candidate SNVs and indels were subjected to deep sequencing using Illumina and Ion Torrent platforms.”).
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.	Patel and Bashashati disclose targeted multiplex amplification of 20 to 50 target loci each encompassing a different tumor-specific SNV mutation. <i>See, e.g.,</i> Patel at 40, Example 2 (“In this example, 40 different genomic target regions were analyzed.”); Patel at 65, Example 3 (“In this example, the same 40 genomic target regions were analyzed as in Example 2.”).
10. The method of claim 1, wherein the method further comprises designing PCR primers or	Patel and Bashashati disclose designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample. <i>See, e.g.,</i> Patel at 8 (“Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified.”); Patel at 29 (“Purified plasma DNA was

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hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample.	then subjected to 2 rounds of amplification by PCR (in triplicate) using primers designed to amplify short DNA segments that included codons 12 and 13 of <i>KRAS</i>, codon 858 of <i>EGFR</i>, and codon 600 of <i>BRAF</i>."); Patel at 35 ("By designing PCR primers that flank very short regions (<50 bp) surrounding these mutation hotspots, adequate amplification of highly fragmented plasma DNA could be ensured and greater sequence depth could be achieved."). <i>See, e.g.</i>, Bashashati supplementary app'x at 6 ("Primer pairs were designed to place the variant position within 75bps of either end of the amplicon and to be between 50-300bp in length."); Bashashati supplementary app'x at 7 ("The primers were designed to generate 100bp amplicons to accommodate the sequencing of DNA from formalin-fixed paraffin-embedded (FFPE) tissues."); Bashashati supplementary app'x at 7 ("DNA primers were synthesized in 96-well plates at a 25nmol scale with standard desalting (IDT Coralville, IA USA).").
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.	Patel and Bashashati disclose detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. <i>See, e.g.</i>, Patel at 14 ("Detection of circulating tumor DNA (ctDNA) has several applications including, but not limited to, detecting presence of a malignancy, informing a prognosis, assessing treatment efficacy, tracking changes in tumor mutation status, and monitoring for disease recurrence or progression."); Patel at 37 (disclosing "Monitoring ctDNA levels in cancer patients"); Patel at 37-39, Table 4 (showing clinical characteristics and mutation findings in various patients); Patel at 40 ("In two cases, the ctDNA level decreased upon treatment with radiation and/or systemic therapy. Aggressive progression of metastatic disease in a different patient was accompanied by a substantial rise in ctDNA. In another two cases, ctDNA levels increased shortly after initiating treatment, perhaps because more tumor DNA was released into the bloodstream as cancer cells were being killed."). <i>See, e.g.</i>, Bashashati at 22 ("[T]he divergence of clonal cell populations confers a heterogeneous genomic tapestry with implications for clinical endpoints, including the acquisition of metastatic potential."); Bashashati at 27 ("The FT lesion in case 5 harbored seven mutations that were not present in all samples, indicating that the clones comprising case 5h were unlikely to have been ancestral to the tumours . . . We

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	suggest that case 5h is therefore more likely to be a metastatic implant in the FT rather than a precursor or early lesion.”); Bashashati at 22 (“In case 6, we compared a primary sample to a recurrence obtained 42 months later, following multiple chemotherapeutic regimens (total 21 cycles) (see supplementary material, Figure S7).”).
14[pre]. 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:	Patel and Bashashati disclose 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. See claim 1[pre] above.
14[a]. performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;	Patel and Bashashati teach performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations. See claim 1[a] above.

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U.S. Patent No. 11,530,454	
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14[b]. performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons, wherein the target loci are amplified together in the same reaction volume. See claim 1[b] above.	Patel and Bashashati disclose 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.
14[c]. sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads. The Court construed the term “sequencing the amplicons” to have its plain and ordinary meaning. Doc. 280 at 10. The Court construed the term “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” to require that the detecting and sequencing are part of the same step. <i>Id.</i> at 11. In addition, Natera has failed to supplement its response to NeoGenomics’ Interrogatory No. 14 to properly address NeoGenomics’ Initial Invalidity Contentions served on February 14, 2024 and the Court’s Claim Construction Order issued on May	Patel and Bashashati disclose 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. The Court construed the term “sequencing the amplicons” to have its plain and ordinary meaning. Doc. 280 at 10. The Court construed the term “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” to require that the detecting and sequencing are part of the same step. <i>Id.</i> at 11. In addition, Natera has failed to supplement its response to NeoGenomics’ Interrogatory No. 14 to properly address NeoGenomics’ Initial Invalidity Contentions served on February 14, 2024 and the Court’s Claim Construction Order issued on May

EXHIBIT 2-A: NEOGENOMICS' FINAL INVALIDITY CONTENTIONS CLAIM CHARTS
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from the sequence reads,	15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Under Natera's apparent interpretation of the Court's adopted construction, Patel and Bashashati disclose 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. See claim 1[c] above.
14[d]. 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.	Patel and Bashashati disclose 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. See, e.g., Patel at 1, 3-4, Fig. 4B, 36, ("Mutant DNA could be accurately and reproducibly measured in a linear manner over approximately 8 orders of magnitude and down to levels as low as 1 in 10,000 molecules."), Fig. 6 (showing accurate and reproducible detection of SNVs down to levels as low as 1 in 10,000 molecules) (Fig. 1 illustrating an example of error-suppressed deep sequencing involving multiplex PCR of target loci). Patel further states that it describes "[m]ethods and compositions... that provide a multiplex assay to detect minute amounts of ctDNA." Patel at 1. As an example, to achieve error-suppressed sequencing, Patel discloses using primers comprising "a barcode and/or a molecular lineage tag (MLT)." See, e.g., Patel at 2. Patel discloses that MLT sequences can be used to "trace the lineage of molecules from initial copying through amplification, processing, and sequencing" and "can be used to distinguish sequences that arise from polymerase misincorporations or sequencer errors from sequences that are derived from true mutant template molecules." Patel at 13. See also Patel at 18-19 (describing the benefits and use of MLTs to distinguish variant sequences arising from true mutant template molecules versus those arising from misincorporation errors).

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Claim	As another example, Patel discloses overlapping paired-end reads covering a mutation hotspot from the forward and reverse strands of each clone “yields highly accurate base-calls, permitting detection and quantification of rare mutations with greater sensitivity.” Patel at 3-4, Fig. 4B. As another example, Patel discloses that sequencing reads from opposite strands “provide sequence redundancy via overlap in the mutation-prone region” and that “[this] clonal redundancy allowed sequences resulting from sequencer errors to be identified and discarded, permitting greater sensitivity for detection of rare sequence variants.” Patel at 7, Fig. 14. Patel at Fig. 15 further explains how analysis of variant sequences can be performed. <i>See also</i> Patel at 10-11 (explaining the benefits of clonal overlapping paired-end sequencing to greatly reduce the background of sequencer-generated errors and allow rare mutant molecules to be detected with greater sensitivity). A POSA would understand that Patel could be combined with the tumor-specific known SNVs described in Diaz to obtain overlapping reads for the SNV of interest, similarly to the method for obtaining overlapping reads for a mutation region. As another example, Patel discloses that: “Figs. 6A-6C indicates the performance of error-suppressed deep sequencing. Measurements of DNA extracted from mutant and wild-type cancer cell lines mixed in various ratios ranging from 1:10,000 to 10,000:1 show a high degree of accuracy and reproducibility.” Patel at 4, Figs. 6A-6C. As another example, Patel discloses how sensitivity of sequencing is typically limited by the inherent error rate of the sequencer “since incorrectly read bases might be mistaken for true mutant DNA copies.” Patel at 17-18. Patel further discloses how the error-suppressed PCR methods address sequencing errors, including by using “clonal overlapping paired-end sequencing to achieve sequence redundancy in mutation-prone regions, thereby allowing base calls to be made with much greater confidence.” <i>Id.</i> As another example, Patel explains that its methods for error-suppressed sequencing allow for “distinguishing nucleotide misincorporation errors that can be introduced during DNA copying, amplification, or processing.” Patel at 18. As another example, Patel discloses that when ultra-deep sequencing is performed, “[in] principle, because large numbers of sequences can be obtained for each target site and for each sample, rare variants can be detected and measured.” Patel at 27. However, Patel discloses that “the error rate of the sequencer can limit

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	the sensitivity of detection because such errors might be mistaken as true variants.” <i>Id.</i> Patel discloses a solution for improved sensitivity of detection for rare SNVs because “errors introduced into the DNA during amplification or processing can be distinguished from true template-derived mutant sequences by analyzing the distribution of molecular lineage tags (MLTs) associated with variant sequences.” <i>Id.</i> As another example, Patel discloses that its method of error-suppressed sequencing was able to reduce background levels of spurious mutation counts to levels below 0.015%, including (for some SNVs), several-fold or even ten-fold below 0.015% (0.00015). See Patel at 36, Table 3: Table 3: Background level of spurious mutation counts obtained from known wild-type samples. <table><tr><th>Mutation Type</th><th>Fraction of mutant : wild-type counts (from 3 replicate PCRs)</th></tr><tr><td>BRAF Val600Glu</td><td>0.00013</td></tr><tr><td>EGFR Leu858Arg</td><td>0.000047</td></tr><tr><td>KRAS Gly12Ser</td><td>0.00029</td></tr><tr><td>KRAS Gly12Val</td><td>0.00018</td></tr><tr><td>KRAS Gly12Arg</td><td>0.000057</td></tr><tr><td>KRAS Gly12Asp</td><td>0.00015</td></tr><tr><td>KRAS Gly12Ala</td><td>0.000014</td></tr><tr><td>KRAS Gly12Cys</td><td>0.00025</td></tr><tr><td>KRAS Gly13Ser</td><td>0.00015</td></tr><tr><td>KRAS Gly13Val</td><td>0.00029</td></tr><tr><td>KRAS Gly13Arg</td><td>0.000050</td></tr><tr><td>KRAS Gly13Asp</td><td>0.000044</td></tr><tr><td>KRAS Gly13Ala</td><td>0.000058</td></tr><tr><td>KRAS Gly13Cys</td><td>0.00049</td></tr><tr><td>Median Value</td><td>0.00014</td></tr><tr><td>Interquartile range</td><td>0.000052 to 0.00023</td></tr></table>	Mutation Type	Fraction of mutant : wild-type counts (from 3 replicate PCRs)	BRAF Val600Glu	0.00013	EGFR Leu858Arg	0.000047	KRAS Gly12Ser	0.00029	KRAS Gly12Val	0.00018	KRAS Gly12Arg	0.000057	KRAS Gly12Asp	0.00015	KRAS Gly12Ala	0.000014	KRAS Gly12Cys	0.00025	KRAS Gly13Ser	0.00015	KRAS Gly13Val	0.00029	KRAS Gly13Arg	0.000050	KRAS Gly13Asp	0.000044	KRAS Gly13Ala	0.000058	KRAS Gly13Cys	0.00049	Median Value	0.00014	Interquartile range	0.000052 to 0.00023
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Claim	
	As another example, Patel discloses that its method was able to detect SNVs down to levels as low as 1 in 10,000 molecules (0.01% VAF), while also being able to “simultaneously quantify more than one mutation from a given sample” using targeted multiplex amplification and sequencing. <i>See</i> Patel at 36. <i>See also, e.g.,</i> Bashashati supplementary Table S2 at cols. U and T (AltCount.Plasma and RefCount.plasma will give VAF, which is less than 0.015% in some cases). Under Natera's apparent interpretation of the claim, the combined teachings of Bashashati and Patel also teach the 0.015% limitation because Patel teaches background error rates for SNVs that are below 0.015%, and Bashashati also teaches detection of SNVs in less than 0.015% of the sequence reads for a particular locus. <i>See</i> Patel Table 3 (teaching background error rates below 0.00015 or 0.015%); Bashashati at Table S2, cols. U and T (showing that SNV reads (AltCount.Plasma) divided by total reads (RefCount.plasma plus AltCount.Plasma) is less than 0.015% in some cases). <i>See also, e.g.,</i> Metzker 2/11/2025 Dep. Tr. at 92:4-93:4, 112:1-114:5, 116:12-24, 125:9-20.
15. The method of claim 14, wherein the sequencing has a depth of read of at least 50,000 per target locus.	Patel and Bashashati disclose that the sequencing has a depth of read of at least 50,000 per target locus. <i>See</i> claim 1[d] above.
22. The method of claim 14, wherein the targeted multiplex amplification amplifies 20 to 50 target loci each	Patel and Bashashati disclose targeted multiplex amplification of 20 to 50 target loci each encompassing a different tumor-specific SNV mutation. <i>See</i> claim 8 above.

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encompassing a different tumor-specific SNV mutation.	
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.	Patel and Bashashati disclose designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample. See claim 10 above.
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.	Patel and Bashashati disclose detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. See claim 12 above.
28. The method of claim 14, wherein the method is	Patel and Bashashati disclose a method that 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.

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Claim capable of detecting an SNV mutation that is present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus.	See claims 1[d] and 14[d] above.