

EXHIBIT 1-C: NEOGENOMICS' FINAL INVALIDITY CONTENTIONS CLAIM CHARTS
FOR U.S. PATENT NO. 11,319,596
(PATEL AND BARSHASHATI)

EXHIBIT 1-C — FINAL INVALIDITY CLAIM CHART
FOR U.S. PATENT NO. 11,319,596

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

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)

To the extent Natera asserts that Bashashati or Patel does not disclose any limitation of Asserted Claims 1, 11, 12, and 18, it would have been obvious to combine Bashashati and Patel. A POSA would have considered the disclosures of the Bashashati and Patel because they both concern the same subject matter, namely amplification and/or sequencing of DNA for the purpose of identifying mutations therein, and particularly detection of rare SNVs in a wild-type background.

A POSA would be motivated to combine Bashashati and Patel in order to achieve improved detection of SNVs by increasing sequencing depth and improving the ability to distinguish true SNVs from sporadic errors, as suggested by Bashashati's methods as “inform[ing] future design of personalized therapeutic solutions.” See, e.g., Bashashati at Abstract (“High-grade serous ovarian cancer (HGSC) is characterized by poor outcome, often attributed to the emergence of treatment-resistant subclones . . . Our results revealed that HGSCs exhibit highly individual evolutionary trajectories and diverse genomic tapestries prior to therapy, exposing an essential biological characteristic to inform future design of personalized therapeutic solutions.”); *id.* at 32 (“[A]s proof of concept, examination of plasma ctDNA suggested that representative mutations in the ancestral clone and mutations present in subclones could be detected in ctDNA, although sensitivity to detect mutations varied from patient to patient. This implies that clinical sensitivity analyses will need to be undertaken to establish the generalizability of ctDNA analysis to clonally diverse tumors.”); *id.* at 26 Fig. 2 (noting the “minimum variant allelic ratio detectable using the exact test for each patient”).

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

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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 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 a reasonable expectation of success in combining Bashashati and Patel because Bashashati discloses that “the proportion of mutations detected in ctDNA was non-uniformly distributed across the four cases. In particular, cases 1 and 5 exhibited higher sensitivity (16/53 mutations and 13/82 mutations, respectively) than cases 2 and 4, suggestive of heterogeneous rates of shedding tumour DNA into the circulation.” Bashashati at 25. Thus, Bashashati teaches that detecting known SNVs using a tumor-informed approach allows SNV detection with greater sensitivity. Patel teaches methods wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having the SNV locus is detected from the sequence reads.

A POSA would also be motivated to combine Bashashati with Patel because both references teach methods for detecting minimal residual disease. *See, e.g.,* Bashashati at 22, Patel at 14 (describing embodiments for detecting cancer recurrence by targeting “unique somatic mutations can be used to distinguish tumor-derived DNA from normal background DNA in plasma.” Like Bashashati, Patel addresses the “need for a method that is able to detect and quantify rare variant sequences to detect cancers in situations where the amount of DNA in a given sample is limited.” Patel at 1. Patel further states that it describes “[m]ethods and compositions ... that provide a multiplex assay to detect minute amounts of ctDNA.” *Id.*

A POSA would also be motivated to combine Bashashati with Patel because Patel provides a method involving higher read depth, fidelity, and improved algorithms for analyzing cfDNA. A POSA would also have a reasonable expectation of success because both Bashashati and Patel applied their methods to cfDNA, and Patel teaches that its error-suppressed sequencing method could accurately

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and reproducibly detect mutations down to levels as low as 1 in 10,000 (0.01%), including while targeting multiple SNVs simultaneously. See Patel at 36, Fig. 6, Table 3.

A POSA would be motivated to assay cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points in view of the knowledge of a POSA, Bashashati and/or Patel in order to track cancer mutations over time and monitor for cancer recurrence. A POSA would also have a reasonable expectation of success because the Bashashati discloses assaying cell-free DNA and assaying cell-free DNA obtained at multiple time points would be a simple modification with predictable results.

Furthermore, a POSA would have a reasonable expectation of success because, as Natera has admitted, multiplex amplification of 50 to 100 target loci in a single reaction for detection of mutations in the amplified DNA using high-throughput sequencing was known in the prior art. See, e.g., United States Patent Application Publication No. 2012/0270212 (published Oct. 25, 2012) (Rabinowitz) 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”).

A POSA would be motivated to combine Bashashati and Patel to perform multiplex amplification to amplify 10 to 500 target loci in order to detect additional tumor-specific mutations without requiring additional reactions. A POSA would have a reasonable expectation of success because Tim Forsheo et al., *Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA*, 4 Sci. Transl., Med. 136 (May 30, 2012) (Forsheo) demonstrated successful multiplex amplification of 10 to 50 different loci from cfDNA in a single reaction, and Rabinowitz disclosed that multiplexed PCR reactions involving pools of up to about 50 or 100 primers were conventional as of Natera’s asserted priority date.

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Claim	To the extent the preamble is construed to be limiting, Bashashati and Patel disclose a method for preparing biological samples useful for monitoring the progression of cancer in a subject. See, e.g., Bashashati at Abstract, 22, 25, 32; Bashashati supplementary app’x at 7.

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Claim monitoring the progression of cancer in a subject, the method comprising:	As an 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 sequencing 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).”; <i>id.</i> 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 discloses testing a sample from two patients whose status is noted as “recurrent disease,” thus indicating that Bashashati discloses a method for preparing biological samples useful for monitoring the progression of cancer in a subject. Bashashati at 23, Table 1; <i>see also</i> Bashashati supplementary app’x at 3 (“Cases of high-grade serous carcinoma . . . where material was available over different time periods (e.g., at primary surgery and at recurrence) were chosen for this analysis”). As another example, Bashashati discloses, “In case 6, samples were obtained at primary surgery and after 42 months, with 21 cycles of multi-agent chemotherapy in between.” Bashashati at 32; <i>see also id.</i> (“Moreover, studies of evolution in cancer through analysis of serial biopsies (eg through pre- and post-treatment samples, or comparisons of primary and metastatic samples), will need to account for evidence of divergent profiles simply due to regional sampling bias.”); <i>see also id.</i> (“Finally, as proof of concept, examination of plasma ctDNA suggested that representative mutations in the ancestral clone and mutations present in subclones could be detected in ctDNA, although sensitivity to detect mutations varied from patient to patient.”).

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Claim	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. To the extent the preamble is construed to be limiting, Patel also discloses a method for preparing biological samples useful for monitoring the progression of cancer in a subject. <i>See, e.g.</i>, Patel at pages 1, 2, 4, 10, 14, 16, 28 [Example 1], Figure 7A-C 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....” <i>Id.</i> 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. <i>Id.</i> at 1. 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).” <i>Id.</i> 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.” <i>Id.</i> 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

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	markers” and “methods that specifically and sensitively measure rare mutant DNA molecules that are shed into blood from cancer cells.” <i>Id.</i> at 14. As another example, Patel discloses “application of a deep sequencing approach in which 3 mutation hotspot regions were analyzed from multiple plasma samples.” <i>id.</i> 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.” <i>Id.</i> at 35, Example 1. As another example, Patel discloses that “Figs. 7A-C show the changes in ctDNA levels with treatment or disease progression. Measurements of mutant ctDNA from patients with NSCLC are shown at various times in relation to therapeutic interventions and disease status.” <i>Id.</i> at 4; Figures 7A-C.
1(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;	Bashashati and Patel disclose 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. <i>See, e.g.,</i> Bashashati supplementary app’x at 5–6; Bashashati at Abstract, 22, 24 (Figure 1). 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 performing “exome sequencing, copy number analysis, targeted amplicon deep sequencing, and gene expression profiling on 31 spatially and temporally separated HGSC tumour specimens (six patients), including ovarian masses, distant metastases and fallopian tube lesions.” Bashashati at Abstract.

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Claim	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 “[c]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. 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. <i>Id.</i> at 6. As another example, Bashashati discloses “[i]ntratumoural mutational profiles of HGS ovarian cancer” obtained from sequencing tumor biopsy samples and identifying tumor-specific SNV mutations. <i>See</i> Bashashati at 24, Figure 1 (“point mutations are shown in blue, indels in green”). <i>See also, e.g.,</i> Patel at 16, 28, 33, 34. 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., id.</i> at 28, Example 1 (“Formalinfixed, paraffin-embedded tumor specimens were obtained for all patients with nonsquamous 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.

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	As another example, Patel teaches “Analysis of cell line 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. As another example, Patel teaches “Confirmation of mutations in tumor tissue. 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 KRAS, BRAF, and EGFR 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).” See, e.g., <i>id.</i> at 34.
1(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	Bashashati and Patel disclose evaluating results of the sequencing on the tumor biopsy sample to determine a plurality of target loci specific to the subject, wherein each target locus spans a tumor-specific mutation of the identified plurality of tumor-specific mutations. See, e.g., Bashashati at 22, 23, Figure 1; Bashashati supplementary app'x at 6; Bashashati supplementary material, Tables S2-S7. For example, Bashashati discloses, “Six women (Table 1) with histologically diagnosed high-grade serous cancer were included in this study (see supplementary material, Appendix). Tissue was obtained from a total of 31 tumour sites (range 3–10 samples/patient, including two fallopian tube lesions) with matched normal DNA for each patient (Table 1). In addition, for a subset of cases (four patients), plasma (pre-operative and pre-anaesthetic) was used to identify the presence of clonally dominant and subclonal mutations through deep

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Claim plurality of tumor-specific mutations; and	sequencing of ctDNA.” Bashashati at 22. Bashashati also discloses that the “19 tumour samples were used as index discovery samples to identify sets of mutations for interrogation by deep amplicon sequencing (median > 5,000 coverage) to: (a) confirm predicted mutations; (b) determine the presence/absence of mutations in specific samples; and (c) infer clonal diversity estimates both within and between samples from the same patient.” <i>Id.</i> Bashashati discloses that a “total of 1349 somatic mutations in all samples were confirmed with deep amplicon resequencing (median 5079-fold coverage; see supplementary material, Table S2), of which 404 were unique mutations (334 non-synonymous, 46 synonymous and 24 small indels), ranging from 31 to 137 unique mutations per case. A panel of shared, identical mutations, indicative of a single ancestral clonal origin, was evident, with 55, 30, 16, 15, 19 and 32 mutations in cases 1–6, respectively. All cases except case 1 harboured a mutation in TP53, with the identical mutation present in all samples from that individual (see supplementary material, Table S2), supporting a monoclonal origin with a TP53 mutation in the ancestral clone.” <i>Id.</i> Bashashati also discloses that “[n]on-synonymous mutations in known cancer genes not present in all samples included PDGFRB and SH3GL1 in case 2 (see supplementary material, Figure S3) and PIK3CA, CTNNB1 and RBM15 in case 4 (Figure 1B). Case 5c harboured 54 (of 102 total) mutations that were not found in the other left ovary samples, which contained 66, 33 and 45 total mutations, respectively.” Bashashati at 22-23. Bashashati discloses the intratumoral mutational profile for each case in supplementary Figures S2-S7, thus indicating the determination of 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. As another example, Bashashati discloses that “[s]omatic mutation predictions were subjected to rigorous validation by targeted deep amplicon sequencing in the tumour and normal DNA templates,” and that “[p]rimer pairs were designed to place the variant position within 75bps of either end of the amplicon and to be between 50-300bp in length.” Bashashati supplementary app’x at 6. This section of the supplementary appendix teaches the design of PCR primers to amplify target loci encompassing different SNVs that were identified after sequencing tumor samples. <i>See also, 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. As another example, Patel teaches “Analysis of cell line 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. As another example, Patel teaches “Confirmation of mutations in tumor tissue. 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 KRAS, BRAF, and EGFR 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.
1(c). assaying cell-free DNA isolated	Bashashati and Patel disclose assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points.

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Claim from a plurality of biological samples obtained from the subject at different time points, wherein the assaying comprises:	See, e.g., Bashashati at 22-23, 25, 32 Figure 1; Bashashati supplementary app'x at 6, 7; Bashashati supplementary material, Figures S2, S3, S4, S5; Bashashati supplementary spreadsheet at cols. T, U. For example, Bashashati discloses, “Six women (Table 1) with histologically diagnosed high-grade serous cancer were included in this study (see supplementary material, Appendix). Tissue was obtained from a total of 31 tumour sites (range 3–10 samples/patient, including two fallopian tube lesions) with matched normal DNA for each patient (Table 1). In addition, for a subset of cases (four patients), plasma (pre-operative and pre-anaesthetic) was used to identify the presence of clonally dominant and subclonal mutations through deep sequencing of ctDNA.” Bashashati at 22. As another example, Bashashati teaches amplification of target loci from cell-free DNA isolated from plasma samples. 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 Pairs were designed to place the variant position within 75bps of either end of the amplicon and to be between 50-300bp in length.”). As another example, Bashashati teaches 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.” Bashashati at 25 (“Mutational events in ancestral clone are detectable in cell-free circulating tumour DNA from plasma”); see <i>also</i> Bashashati supplementary material, Tables S2, S3, S4 S5 (intratumoral mutational profiles for cases wherein ctDNA was extracted from plasma). As another example, Bashashati discloses that, in one case, “we compared a primary sample to a recurrence obtained 42 months later[.]” Bashashati at 22. Bashashati discloses that “as proof of concept, examination of

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	plasma in ctDNA suggested that representative mutations in the ancestral clone and mutations present in subclones could be detected in ctDNA[.]” <i>Id.</i> at 32. It would have been obvious in view of Bashashati and the knowledge of a POSA to obtain biological samples from the subject at different time points. This is because Bashashati’s method teaches that “[c]ases of high-grade serous carcinoma where more than one sample were collected in different anatomic locations (e.g., different locations within the ovary, omentum) or where material was available over different time periods (e.g., at primary surgery and at recurrence)[.]” Bashashati supplementary app’x at 3. Bashashati’s method collected tumor samples over different time periods, and plasma samples from different anatomic locations. <i>Id.</i> at 3–5. It would have been obvious to also collect plasma samples over different time periods because Bashashati’s method teaches collecting tumor samples from different time periods. <i>See id.</i>; <i>see also</i> Bashashati at 23, Table 1 (disclosing testing a sample from two patients whose status is noted as “recurrent disease,” thus indicating that Bashashati discloses assaying biological samples obtained from the subjects at different time points). Patel also discloses assaying cell-free DNA isolated from a plurality of biological samples obtained from the subject at different time points. <i>See, e.g.,</i> 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”); <i>id.</i> at 4 (“Figs. 7A-C 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”); <i>id.</i> at Figs. 7A-7C; <i>id.</i> at 5 (“Figs. 8A-C show the ctDNA levels in patients with fewer than 3 time-points”); Figs. 8A-8C.
1(c)[1]. performing targeted multiplex PCR amplification to amplify the plurality of target	Bashashati and Patel disclose 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. <i>See, e.g.,</i> Bashashati supplementary spreadsheet at cols. T, U; Bashashati supplementary app’x at 6-7.

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Claim 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	As another example, Bashashati teaches targeted PCR 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. <i>Id.</i> at 6-7 (“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.”). Patel discloses performing targeted multiplex PCR amplification to amplify a plurality of loci from cell-free DNA. <i>See, e.g.,</i> Patel at 2, 3, 6, 12, 40, 52, 53, Table 9, 65, 66, 68, 70, 72, Fig. 4A, Fig. 4B. 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 (Ext V2Rev) used for the pre-amplification reaction”). As another example, Patel describes various embodiments of error-suppressed PCR that are compatible with multiplex PCR. <i>See</i> Patel at 6. Patel also describes “cell-free DNA purified from plasma undergoing two rounds of amplification by PCR. The first round amplifies mutation hotspot regions of several genes from a given sample in a single tube.” <i>See</i> Patel at 3; Figs. 4A, 4B. 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

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Claim	discloses that, in this alternative example, “the same 40 genomic target regions were analyzed[.]” <i>Id.</i>; <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.”). As another example, Patel discloses “measuring nucleic acid variants by tagging and amplifying low abundance template nucleic acids in a multiplexed primer extension or PCR.” Patel at 2. As another example, Patel discloses: “When multiple different target sequences are to be tagged and copied from a given sample, a mixture of several different target-specific primers are used in a single reaction volume[.]” Patel at 12. Patel discloses methods for multiplex amplification that avoid or remove spurious amplification products prior to sequencing. <i>See, e.g.</i>, Patel at 3 (describing stringent purification to remove unextended primers, spurious extension products, and template DNA). As another example, Patel discloses that Fig. 17 shows amplified products within the expected size range, with very little spurious product migrating at a different size. Patel at 7, Fig. 17. <i>See also</i> Patel at 20-21 (explaining that the provided methods minimize and/or remove spurious amplification products), 25 (disclosing that purification to remove unwanted products and excess primers allows greater specificity of polymerization in subsequent steps). Patel also discloses assaying cfDNA for tumor-specific mutations. As an example, Patel discloses: “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. Since unique somatic mutations can be used to distinguish tumor- derived DNA from normal background DNA in plasma, a new class of highly specific DNA-based cancer biomarkers are described with clinical applications that may complement those of conventional serum protein markers. In an embodiment, methods include screening ctDNA for presence of

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Patel and Bashashati	
Claim	
1(c)[2]. performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads,	tumor-specific, somatic mutations. In such embodiments, false-positive results are very rare since it would be very unlikely to find cancer-related mutations in the plasma DNA of a healthy individual.” Patel at 14. Bashashati and Patel disclose performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads. <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-anesthesia 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[.]” <i>Id.</i>; <i>see also</i> Bashashati supplementary Table S2 at col. T (anything with RefCountPlasma). Bashashati presents these findings in Figure 2:

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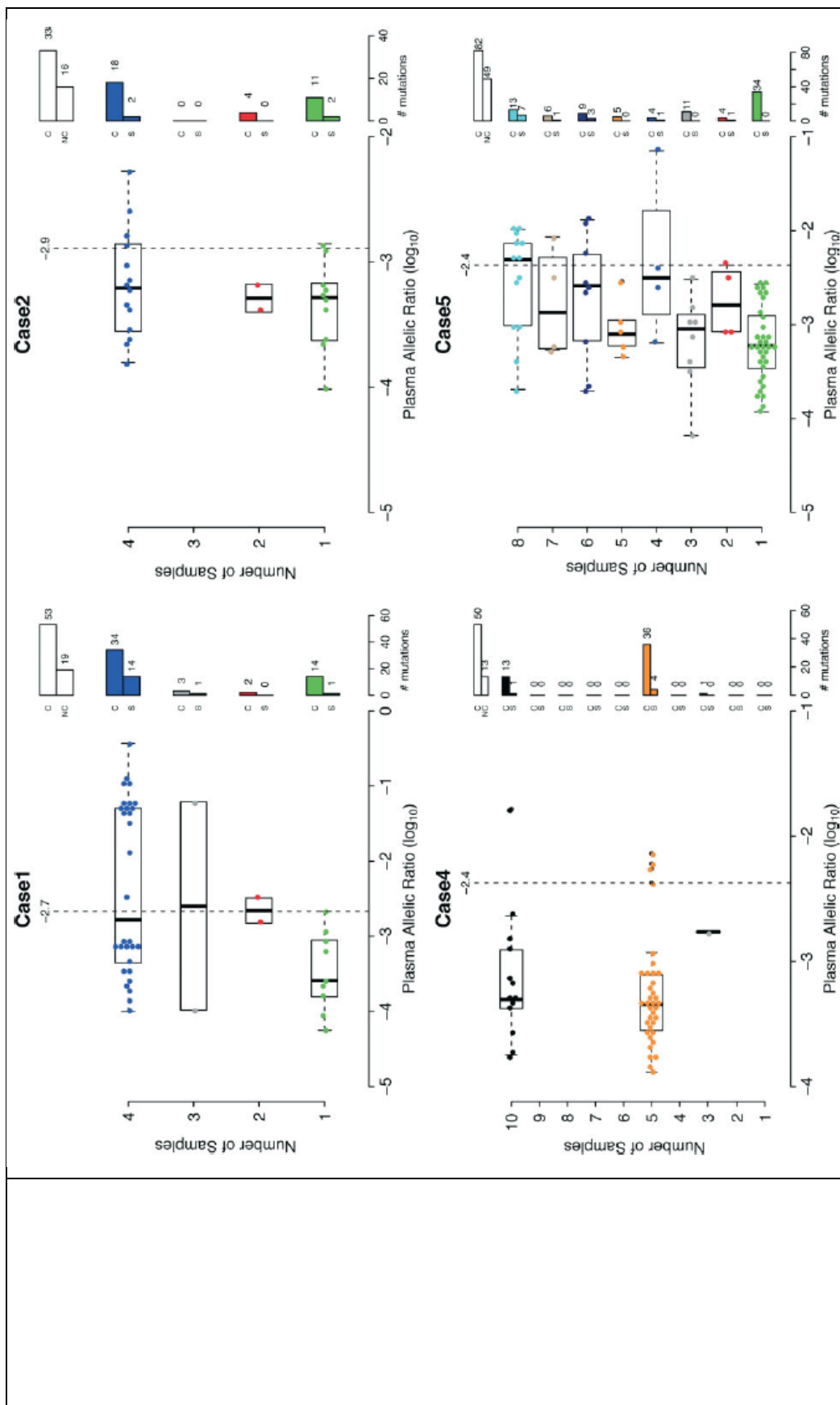


Figure 2. Deep sequencing results of cell-free circulating tumour DNA from the plasma in cases 1, 2, 4 and 5. The distribution of plasma variant allelic ratio ($\log_{10}$ scale) is separated by the number of tumour samples in which the mutation was originally discovered. Cases 4 and 5 both also include 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	
	Patel also discloses performing high-throughput sequencing of the amplified DNA comprising the plurality of target loci to obtain sequence reads. <i>See, e.g.</i>, Patel at 11, 17, 54, 57, 63, 70, 71, 72. For example, Patel discloses sequencing the amplicons produced in single-plex 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. Patel at 70, Example 3 (“[T]he final PCR in the present Example was performed in a combined reaction volume for all genomic targets and for all samples.”); <i>id.</i> 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.”); <i>id.</i> at 72 (“The gel-extracted [multiplex] PCR products were subjected to next-generation sequencing using an Illumina MiSeq instrument.”); <i>id.</i> 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.”).
1(c)[3]. wherein an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA having	Bashashati and Patel disclose 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. Under Natera’s apparent interpretation of “detect,” Bashashati discloses 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

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Claim	the SNV locus is detected from the sequence reads sequence reads. <i>See, e.g.</i>, Bashashati supplementary Table S2 at cols. U and T (AltCountPlasma divided by RefCountPlasma is less than 0.015% in some cases). Patel also discloses a method of error-suppressed sequencing 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. <i>See, e.g.</i>, 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).” <i>See, e.g.</i>, Patel at 2. Patel discloses that MLT sequences can be used to “track 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. <i>See also</i> 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). 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>

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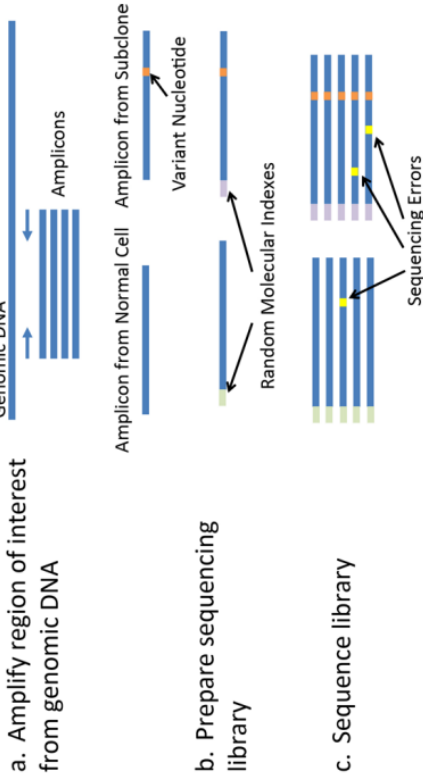
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Claim	Patel at 10-11 (explaining the benefits of clonal overlapping paired-end sequencing to greatly reduce the background of sequencer-generated errors and allow rare mutant molecules to be detected with greater sensitivity). A POSA would understand 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-C 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> at 18. 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 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>

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Patel and Bashashati																																			
Claim	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> 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. See Patel at 36.	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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KRAS Gly12Arg	0.000057																																		
KRAS Gly12Asp	0.00015																																		
KRAS Gly12Ala	0.000014																																		
KRAS Gly12Cys	0.00025																																		
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Median Value	0.00014																																		
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Supplementary Figure 1. Error-corrected sequencing workflow. Schematic depiction of library preparation and bioinformatics analysis for generating read families and error-corrected consensus sequences.

Library Preparation and Sequencing



Bioinformatics Analysis

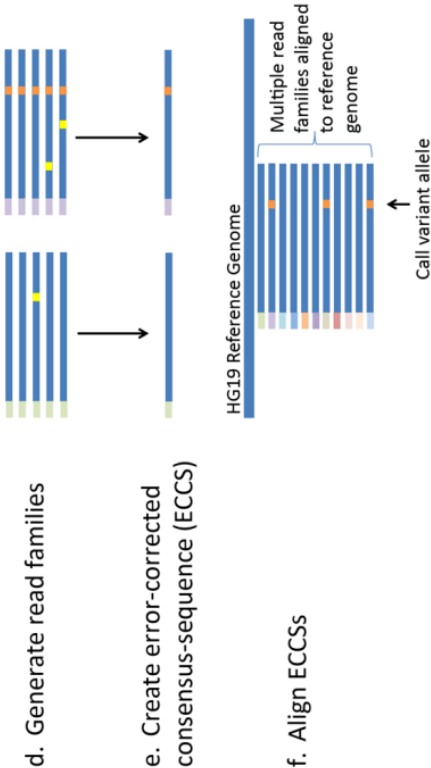


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Claim	
	Under Natera's interpretation of the claim, the combined teachings of Bashashati and Patel teach detection of an SNV because "once you have performed Step 1A and Step 1B and the first part of Step 1C and then you sequence, you will necessarily have identified the SNV if it exists." <i>See</i> Metzker 2/11/2025 Dep. Tr. at 29:1-12. 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 supplementary Table S2, cols. U and T (showing that SNV reads (AltCount.Plasma) divided by total reads (RefCountPlasma plus AltCountPlasma) 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.
11. The method of claim 1, wherein amplifying comprises targeted multiplex PCR amplification of 10 to 50 of the target loci.	Bashashati and Patel disclose performing targeted multiplex PCR amplification of 10 to 50 of the target loci. <i>See</i> limitation 1[c](1) above; <i>see also, e.g.,</i> Bashashati at 25; Bashashati supplementary Table S2 at cols. T, U; Bashashati supplementary app'x at 6, 7. For example, Bashashati teaches 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" and that "[w]e assayed . . . 49 [mutations] in case 2." Bashashati at 25; <i>see also</i> Bashashati supplementary Table S2 at cols. T, U; Bashashati at 25 ("We noted that the proportion of mutations detected in ctDNA was non-uniformly distributed across the four cases. In particular, cases 1 and 5 exhibited higher sensitivity (16/53 and 13/82 mutations, respectively) than cases 2 and 4[.]"). Patel also discloses perform targeted multiplex PCR amplification of 10 to 50 of the target loci. <i>See, e.g.,</i> Patel at 16 (describing embodiments that "can evaluate mutations in many genes simultaneously from a given sample"), Patel at 40-65 (Example 2, describing multiplex amplification of 40 target loci with MLTs for error-suppression).

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Claim	Bashashati and Patel disclose detecting recurrence and/or metastases of the cancer from the tumor- specific mutations detected in the cell-free DNA. <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[.]”); <i>id.</i> 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 suggest that case 5h is therefore more likely to be a metastatic implant in the FT rather than a precursor or early lesion.”); <i>id.</i> 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).”). Patel also discloses wherein the method further comprises detecting recurrence and/or metastases of the cancer from the tumor- specific mutations detected in the cell-free DNA. As an example, Patel discloses: “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. Since unique somatic mutations can be used to distinguish tumor- derived DNA from normal background DNA in plasma, a new class of highly specific DNA-based cancer biomarkers are described with clinical applications that may complement those of conventional serum protein markers. In an embodiment, methods include screening ctDNA for presence of tumor-specific, somatic mutations. In such embodiments, false-positive results are very rare since it would be very unlikely to find cancer-related mutations in the plasma DNA of a healthy individual.” Patel at 14. As another example, Patel teaches “For patients with detectable plasma DNA mutations, changes in measured ctDNA levels were followed in the context of therapeutic interventions or disease progression. To determine the absolute concentration of mutant KRAS DNA fragments in a plasma sample, the total concentration of KRAS fragments was measured by real-time PCR and then multiplied by the fraction of mutant molecules determined by deep sequencing. The median concentration among samples with detectable mutations was 5,694 mutant KRAS molecules per mL (IQR: 2,655 to 25,123). Time-courses of mutant ctDNA measurements

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Claim	for patients who had 3 or more samples collected are shown in Fig. 7 (data for patients with fewer measurements are shown in Fig. 8). 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.” Patel at 40.

Figure 7 A

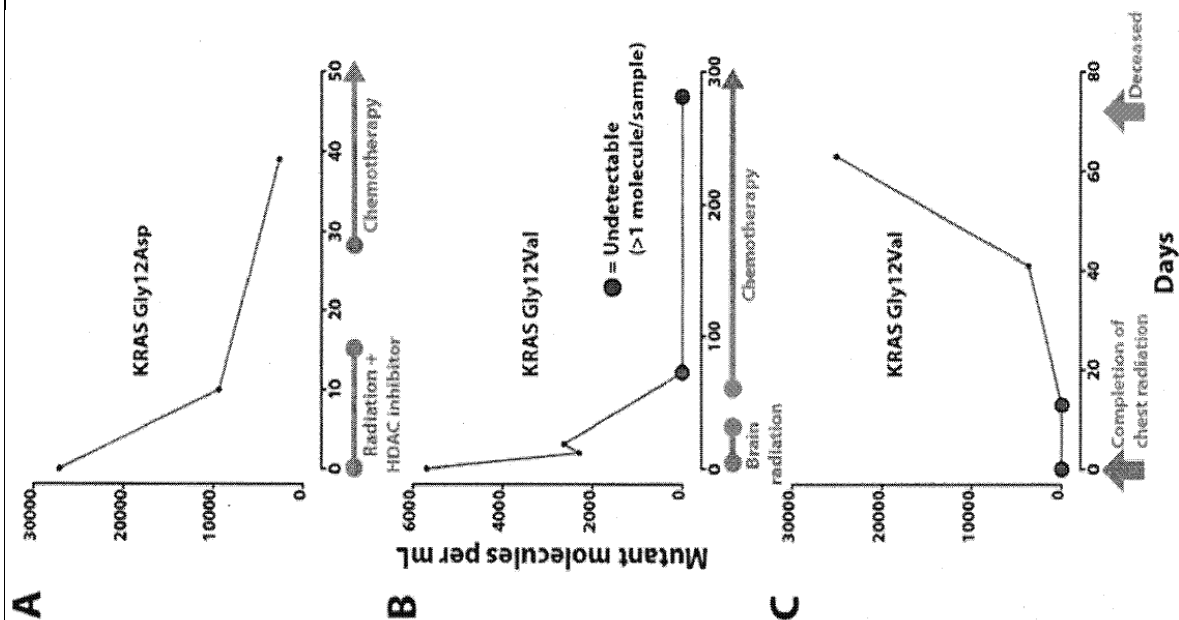


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Claim	

U.S. Patent No. 11,319,596	Patent and Bashashati	Figure 8 A Graph A: KRAS Gly12Val <table border="1"> <thead> <tr> <th>Time (Days)</th> <th>Mutant molecules per mL</th> <th>Notes</th> </tr> </thead> <tbody> <tr> <td>-10</td> <td>~100,000</td> <td></td> </tr> <tr> <td>-5</td> <td>~100,000</td> <td></td> </tr> <tr> <td>0</td> <td>~250,000</td> <td>Single plasma sample collected</td> </tr> <tr> <td>10</td> <td>~100,000</td> <td>Deceased</td> </tr> </tbody> </table> B Graph B: KRAS Gly12Cys <table border="1"> <thead> <tr> <th>Time (Days)</th> <th>Mutant molecules per mL</th> <th>Notes</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>~120,000</td> <td></td> </tr> <tr> <td>10</td> <td>~100,000</td> <td></td> </tr> <tr> <td>20</td> <td>~100,000</td> <td></td> </tr> <tr> <td>30</td> <td>~100,000</td> <td>Hospitalized</td> </tr> </tbody> </table> C Graph C: KRAS Gly13Asp <table border="1"> <thead> <tr> <th>Time (Days)</th> <th>Mutant molecules per mL</th> <th>Notes</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>~1,000</td> <td>Stereotactic brain radiosurgery</td> </tr> <tr> <td>10</td> <td>~4,000</td> <td></td> </tr> <tr> <td>20</td> <td>~5,000</td> <td>Deceased</td> </tr> </tbody> </table>	Time (Days)	Mutant molecules per mL	Notes	-10	~100,000		-5	~100,000		0	~250,000	Single plasma sample collected	10	~100,000	Deceased	Time (Days)	Mutant molecules per mL	Notes	0	~120,000		10	~100,000		20	~100,000		30	~100,000	Hospitalized	Time (Days)	Mutant molecules per mL	Notes	0	~1,000	Stereotactic brain radiosurgery	10	~4,000		20	~5,000	Deceased
Time (Days)	Mutant molecules per mL	Notes																																										
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Claim 18. The method of claim 1, wherein step (a) comprises performing whole exome sequencing on a tumor biopsy sample of the subject to identify the plurality of tumor-specific mutations.	Bashashati and Patel disclose performing whole exome sequencing on a tumor biopsy sample of the subject to identify the plurality of tumor-specific mutations. <i>See, e.g.,</i> Bashashati supplementary app'x at 5–6, 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. A POSA would be motivated to modify the method of Bashashati to use whole-exome sequencing (WES) rather than whole-genome sequencing (WGS) to identify the tumor-specific mutations in the tumor biopsy

EXHIBIT 1-C: NEOGENOMICS' FINAL INVALIDITY CONTENTIONS CLAIM CHARTS
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Claim	sample because WES is cheaper than WGS. Also, a POSA would reasonably have expected to succeed because the prior art such as Diehn taught a POSA how to perform whole genome or whole exome sequencing to identify genomic or exomic regions of interest. <i>See, e.g.,</i> 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.”).