

EXHIBIT 2-C: INIVATA'S PRELIMINARY INVALIDITY CONTENTIONS CLAIM CHARTS
FOR U.S. PATENT NO. 11,530,454
(FORSHEW)

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

References: Forshew, T. et al., *Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA* 4(136):136RA68 (May 2012) (Forshew)

This chart hereby incorporates by reference all prior art references contained within the “General Knowledge of a POSA” section of Inivata’s Invalidity Contentions cover pleading.

U.S. Patent No. 11,530,454	
Claim	Prior Art
1[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:	To the extent the preamble is construed to be limiting, Forshew discloses 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>, Forshew at Abstract (“Plasma of cancer patients contains cell-free tumor DNA that carries information on tumor mutations and tumor burden” and demonstrates “use of [tagged-amplicon deep sequencing (TAm-Seq)] to noninvasively identify the origin of metastatic relapse in a patient with multiple primary tumors.”); Forshew at 9 (“Whole-genome sequencing of tumor material was used to identify tumor mutations in a patient with metastatic breast cancer undergoing two phases of chemotherapy. Ten mutations were selected, and short amplicons (<120 bp) were designed to cover the mutation loci . . . Serial plasma samples were collected over the the course of 497 days, both before and after treatment. We performed TAm-Seq in duplicate, using DNA from 0.08 ml of plasma per amplification, and tracked dynamics of all mutations in parallel.”); Forshew at 4 (“We applied TAm-Seq to directly identify mutations in plasma of cancer patients.”); Forshew supplemental materials at 1 (“Dilution of plasma DNA samples to determine allele quantification accuracy”); Forshew supplemental materials at 17–19, Tables S6–S7 (identification of tumor mutations that are single nucleotide changes or substitutions in a genome, <i>i.e.</i>, SNVs).
1[a]. performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of	Forshew 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. <i>See, e.g.</i>, Forshew at 9 (performing “[w]hole-genome sequencing of tumor material [] to identify tumor mutations in a patient with metastatic breast cancer undergoing two phases of chemotherapy.”); Forshew supplemental materials at 19, Table S7 (identifying mutations that are single nucleotide changes (<i>i.e.</i>, SNVs)); Forshew at 3

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tumor-specific SNV mutations;	(disclosing the use of “TAm-Seq to sequence DNA extracted from 47 formalin-fixed, paraffin-embedded (FFPE) tumor specimens of ovarian cancers (table S3)” and that these samples “were also sequenced for <i>TP53</i> by Sanger sequencing”). Because <i>TP53</i> is a tumor suppressor gene associated with cancer, a POSA would have understood that Sanger sequencing to identify mutations in <i>TP53</i> constitutes identification of tumor-specific SNV mutations. Forsheiw further discloses that “43 single-base substitutions were called (table S3)” and “[t]hese matched 100% of mutations identified by Sanger sequencing and included three additional mutations at low [allele frequencies] that were below detection thresholds of Sanger sequencing (fig. S2).” Forsheiw at 3. Forsheiw identifies 40 single-base substitutions (<i>i.e.</i>, SNVs) in the ovarian tumor sample. Forsheiw supplemental materials at 13–14, Table S3. These “single-base substitutions” are tumor-specific SNVs, and a POSA would have understood that such tumor-specific SNVs could be identified in the same manner by performing whole exome sequencing or whole genome sequencing because the whole exome sequences and whole genome sequences include the SNVs.
1[b]. performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different tumor-specific SNV mutation from cell-free DNA isolated from a plasma sample of the subject or DNA derived therefrom to obtain amplicons having a length of 50-150 bases, wherein the target loci are amplified together in the same reaction volume; and	Forsheiw discloses 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. <i>See, e.g.</i>, Forsheiw at 9 (using “[w]hole-genome sequencing of tumor material . . . to identify tumor mutations in a patient with metastatic breast cancer undergoing two phases of chemotherapy,” wherein “[t]en mutations were selected, and short amplicons (<120 bp) were designed to cover the mutation loci (table S7)” and performing targeted multiplex amplification that amplifies the ten different tumor-specific SNV mutations in cfDNA from a plasma sample by performing “TAm-Seq in duplicate, using DNA from 0.08 ml of plasma per amplification, and track[ing] dynamics of all mutations in parallel”); Forsheiw at 3 (“We therefore applied a two-step amplification process: a limited-cycle preamplification step where all primer sets were used together to capture the starting molecules present in the template, followed by individual amplification to purify and select for intended targets. (Fig. 1B) (Supplementary Methods).”); Forsheiw supplemental materials at 2 (“Preamplification reactions were carried out in 10- µl reaction volumes containing 50 nM of each forward and reverse target-specific primer . . . Reactions were subjected to 15 cycles of amplification.”); Forsheiw at 2, Fig. 1B (depicting the targeted multiplex

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	amplification, wherein “[m]ultiple regions were amplified in parallel. An initial preamplification step was performed for 15 cycles using a pool of the target-specific primer pairs...”). Forshew further discloses that “[p]lasma of cancer patients contains cell-free tumor DNA that carries information on tumor mutations and tumor burden” and teaches the use of TAM-Seq to monitor cfDNA. Forshew at Abstract; <i>see also</i> Forshew at 9 (“Serial plasma samples were collected over the course of 497 days, both before and after treatment. We performed TAM-Seq in duplicate, using DNA from 0.08 ml of plasma per amplification, and tracked dynamics of all mutations in parallel.”). Forshew discloses amplicons that fall within the recited range of 50–150 bases. <i>See</i> Forshew at 9 (“[S]hort amplicons (<120 bp) were designed to cover the mutation loci.”); Forshew supplemental materials at 19, Table S7 (listing genomic locations of amplicons with a start location and a stop location, thus demonstrating amplicons with lengths of more than 50 bases). Forshew also discloses performing TAM-Seq with a degree of multiplex greater than ten. <i>See</i> Forshew Fig 1A, Forshew supplemental materials at 9, Table S1 (disclosing performing TAM-Seq with 48 sequence-specific primer pairs to amplify 5995 baes of genomic sequence). Forshew discloses performing multiplex amplification with a combination of “each forward and reverse target-specific primer” in the same volume. Forshew supplemental materials at 2; <i>see also id.</i> at 17-18 (Table S6); Forshew at 6 (Table 2).
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,	Under Natera’s apparent construction of this limitation, Forshew 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> Forshew at 2, Fig. 1B; Forshew at 3 (pooling and sequencing DNA that has been amplified through the TAM-Seq method and explaining that “[w]e performed 100-base single-end sequencing (GAIIx sequencer, Illumina), with an additional 10 cycles using the barcode sequencing primer, generating ~30 million reads per lane.”); Forshew at 9 (“[W]e demonstrate noninvasive identification of mutant alleles in plasma, at AFs as low as 2%, by targeted deep sequencing of circulating DNA.”).

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	Forsheiw further discloses analyzing the sequence data to detect tumor-specific SNV mutations by using a bioinformatics pipeline. <i>See</i> Forsheiw supplemental materials at 4 (“De-multiplexing and alignment,” “Mutation identification,” and “Mutation detection”); <i>see also</i> Forsheiw at 5, Table 1 (identifying tumor-specific SNV mutations in plasma samples from seven ovarian cancer patients); Forsheiw supplemental materials at 19, Table S7 (“[S]ubsequent data analysis was performed using the CASAVA 1.8 analysis pipeline (Illumina) to identify somatic mutations.”). To the extent this limitation is construed to require sequencing amplicons from the multiplex PCR step, such a modification of Forsheiw would have been obvious to a person of ordinary skill in the art. Forsheiw teaches that the multiplex PCR step was designed to avoid unwanted side products. <i>See</i> Forsheiw at 3 (“The final concentration of each primer in the preamplification reaction was 50 nM, reducing the potential for interprimer interactions, and 15 cycles of long-extension (4 min) polymerase chain reaction (PCR) were used to remain in the exponential phase of amplification.”); Forsheiw supplemental materials at 2 (“Primer designs were screened against the hg19 reference genome to prevent cross-product generation, and were screened to prevent primer-dimer interactions and formation of intramolecular secondary structure.”). Accordingly, a POSA would have understood that it was not necessary to perform single-plex PCR to improve purity. Further, it would have been obvious to a POSA to incorporate sample barcodes and sequencing adapters into the sequence-specific primers used in the targeted multiplex amplification such that they would be incorporated into the amplicons resulting from the multiplex amplification step. A POSA would have been motivated to do so because omitting the single-plex PCR step would simplify the overall process by requiring only a single amplification step. Additionally, a POSA would have had a reasonable expectation of success for two reasons. First, Forsheiw already teaches multiplex PCR conditions that avoid unwanted side products and preserve relative abundance of mutations, such that a single-plex PCR used to enhance purity would not be required. Second, it is well-established in the art that targeted multiplex amplification using a single amplification reaction step can be used to attach sequencing platform-specific barcodes prior to sequencing. <i>See, e.g., Hoffman et al., DNA bar coding and pyrosequencing to identify rare HIV drug resistance mutations</i>, <i>Nucleic Acids Research</i>, 2007, Vol. 35, No. 13, at 3 (teaching that “[g]enomic DNA samples of interest are amplified using primers that include 5’ extension providing binding sites for the 454A and B primers” that are required for sequencing); <i>id.</i> at 4, Figure 2 (showing the single amplification step, reproduced below).

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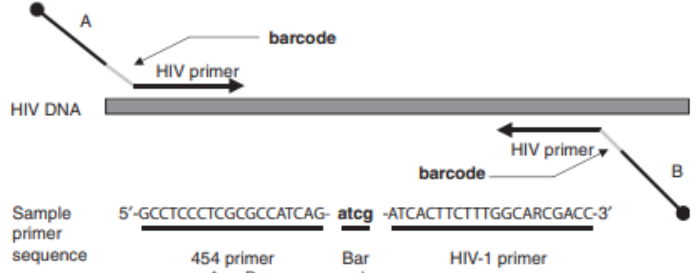
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Claim	Prior Art
	 Figure 2. The DNA bar coding system. Primers are shown diagrammatically, illustrating the placement of the DNA bar code between the 454 primers (A and B) and the HIV-complementary sequences. An example of a primer sequence is shown at the bottom. DNA 5' ends in the primers are shown as the black balls.
1[d]. wherein the sequencing has a depth of read of at least 50,000 per target locus.	The limitation “wherein the sequencing has a depth of read of at least 50,000 per target locus” is obvious in view of Forshew alone or in combination with the knowledge of a POSA. Forshew discloses a sequencing depth of at least 15,400, and an additional depth of at least 50,000 would have been obvious to a POSA in view of the express teaching of Forshew. For example, Forshew acknowledges that a “current limitation of Tam-Seq” was its “detection limit compared to assays that target individual loci” which were able “to detect two to three orders of magnitude lower frequencies.” Forshew at 10; <i>see also</i> Forshew at 9. Forshew teaches that higher read depth can overcome this limitation: “[h]igher read depth or fidelity, additional replicates, or improved algorithms could allow for enhanced mutation detection without change to protocols.” Forshew at 10. In addition to Forshew’s express teaching of a higher read depth, a POSA would have understood that using “additional replicates” is also using “higher read depth” because depth of read is a measure of the number of times a particular sequence is read or sequence reads that map to a given genetic locus, and one can increase the number of such reads by using “additional replicates.” Accordingly, Forshew teaches both the motivation and reasonable expectation of success to increase the read depth to at least 50,000 per target locus.

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2. The method of claim 1, wherein the cell-free DNA comprises circulating tumor DNA.	Forsheiw discloses that the cell-free DNA comprises circulating tumor DNA. <i>See, e.g.</i>, Forsheiw at Abstract (“Plasma of cancer patients contains cell-free tumor DNA that carries information on tumor mutations and tumor burden.”); <i>see also, e.g.</i>, Forsheiw at 4 (“For all seven patients, TP53 tumor mutations were identified in the circulating DNA at frequencies of 4% to 44% (Table 1).”).
3. The method of claim 1, wherein the SNV mutations comprise one or more clonal SNV mutations.	Forsheiw discloses that the SNV mutations comprise one or more clonal SNV mutations, either alone or in combination with the knowledge of a POSA. It is well established in the art that cancer cells are assumed to originate from monoclonal compositions. <i>See, e.g.</i>, Bai et al., <i>Detection and Clinical Significance of Intratumoral EGFR Mutations Heterogeneity in Chinese Patients with Advanced Non-Small Cell Lung Cancer</i>, PLOS ONE, February 2013, Vol. 8, Iss. 2, at 1–2 (“Although all malignant cells are thought to be derived from a common precursor cell, acquired genetic instability gives rise to subsequent generations expressing unique characteristics.”); Bashashati at 22 (“Rooted in the clonal evolution model of tumour progression, cancers are assumed to originate from a monoclonal composition (the ancestral clone), with subsequent evolution leading to selective expansions of genomically distinct subclones with distinct phenotypic properties.”). Accordingly, a POSA would have understood clonal SNV mutations to be mutations present in the ancestral clone of the tumor cells. Forsheiw discloses and attempts to solve the problem that “changes within [a] cancer can alter the mutational spectrum of the disease and its responsiveness to therapies, which may necessitate repeat biopsies.” <i>See, e.g.</i>, Forsheiw at 1. A POSA would have understood this to refer to the challenge of monitoring mutations that occur as cancer progresses, and Forsheiw therefore teaches that tumors will provide clonal and subclonal DNA. Forsheiw at 1. This challenge necessarily implies the existence of both clonal and subclonal DNA within a tumor. Forsheiw also discusses tracking “multiple mutations” that “can provide insight into clonal evolution and, at the same time, increases the robustness for tumor monitoring by compensating for effects of sampling noise or mutational drift.” Forsheiw at 10. Forsheiw also cites to prior work discussing “mutation profiles in different metastatic clones,” which “can differ significantly from each other or from the parent primary tumor (12, 13).” Forsheiw at 1.

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	In addition, ForsheW discloses a clonal SNV mutation. ForsheW at Fig. 4(A). Because tumor cells from omental mass, left ovary and right ovary all have the TP53 mutation, a POSA would understand that mutation to be a clonal SNV mutation.
4. The method of claim 1, wherein the SNV mutations comprise one or more subclonal SNV mutations.	ForsheW discloses that the SNV mutations comprise one or more subclonal SNV mutations. <i>See, e.g.</i>, ForsheW at 1 (disclosing and attempting to solve the problem that “changes within [a] cancer can alter the mutational spectrum of the disease and its responsiveness to therapies, which may necessitate repeat biopsies.”). A POSA would have understood this to refer to the challenge of monitoring mutations that occur as cancer progresses, and ForsheW therefore teaches that tumors will provide clonal and subclonal DNA. ForsheW at 1. This challenge necessarily implies the existence of both clonal and subclonal DNA within a tumor. <i>See, e.g.</i>, ForsheW at 7, Fig. 4A (disclosing a subclonal SNV mutation by identifying a particular <i>EGFR</i> mutation present in only two regions from the omental mass, unlike the <i>TP53</i> mutation that is present in all eight regions of the tissues sampled); ForsheW at 10 (“Tracking multiple mutations can provide insight into clonal evolution...”). A POSA would have understood this to mean subclonal SNV mutations because it is well established in the art that cancers originate from a monoclonal composition, diverging into genomically distinct subclones. <i>See, e.g.</i>, Bai at 1 (“[M]onoclonal cancer cells gradually become heterogeneous owing to clonal evolution and genetic/epigenetic instability.”); Bashashati at 22 (explaining that “cancers are assumed to originate from a monoclonal composition (the ancestral clone), with subsequent evolution leading to selective expansions of genomically distinct subclones with distinct phenotypic properties” and citing numerous articles dating to 1976).
5. The method of claim 1, wherein the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations.	ForsheW discloses that the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations. <i>See</i> claims 3 and 4 above. <i>See, e.g.</i>, ForsheW at 7, Fig. 4A, 9, 10.

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6. The method of claim 1, wherein the tumor sample of the subject is a tumor tissue sample.	Forsheiw discloses that the tumor sample of the subject is a tumor tissue sample. <i>See, e.g.</i>, Forsheiw at 9 (“Whole-genome sequencing of tumor material was used to identify tumor mutations in a patient with metastatic breast cancer undergoing two phases of chemotherapy.”); Forsheiw at 10 (“Between 3 and 20 sections were macrodissected depending on the tissue sample’s size. DNA from FFPE sections was extracted with QIAamp DNA FFPE Tissue Kit (Qiagen) according to the manufacturer’s instructions.”).
7. The method of claim 1, wherein the method further comprises determining clonal heterogeneity of the tumor sample.	Forsheiw discloses that the method further comprises determining clonal heterogeneity of the tumor sample. <i>See, e.g.</i>, Forsheiw at 10 (“Tracking multiple mutations can provide insight into clonal evolution and, at the same time, increases the robustness for tumor monitoring by compensating for effects of sampling noise or mutational drift.”); Forsheiw at 1 (explaining that “mutation profiles in different metastatic clones can differ significantly from each other or from the parent tumor (12, 13).”); Forsheiw at 7, Fig. 4A (determining the heterogeneity of tumor samples from an ovarian cancer patient at relapse and disclosing that the <i>TP53</i> mutation could be in the left and right ovaries and omental mass at different frequencies). Forsheiw also teaches determining allele frequency in the ovarian cancer formalin-fixed, paraffin-embedded (FFPE) tissue samples, <i>see</i> Forsheiw at 4, Figure 2:
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.	Forsheiw discloses that the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation. <i>See, e.g.</i>, Forsheiw at 2 (performing targeted multiplex amplification to amplify 20 and 48 target loci); Forsheiw supplemental materials at 9–10, Table S1 (performing TAm-Seq with 48 sequence-specific primer pairs to amplify 5995 bases of genomic sequence covering coding regions of <i>TP53</i> and <i>PTEN</i>, as well as selected regions in <i>EGFR</i>, <i>BRAF</i>, <i>KRAS</i>, and <i>PIK3CA</i> by overlapping short amplicons); Forsheiw at 6 (Table 2); Forsheiw supplemental materials at 17-18 (Table S6); Forsheiw at 10 (disclosing that more loci would “increase[] the robustness for tumor monitoring by compensating for effects of sampling noise or mutational drift”).
9. The method of claim 1, wherein the targeted multiplex amplification amplifies 50 to 100 target loci each encompassing a	Forsheiw discloses that the targeted multiplex amplification amplifies 50 to 100 target loci each encompassing a different tumor-specific SNV mutation. <i>See, e.g.</i>, Forsheiw at 2, Fig. 1A (using TAm-Seq with 48 sequence-specific primer pairs to amplify 5995 bases of genomic sequence covering coding regions of <i>TP53</i> and <i>PTEN</i>, and selected regions in <i>ECFR</i>, <i>BRAF</i>, <i>KRAS</i>, and <i>PIK3CA</i> by overlapping short amplicons); Forsheiw supplemental materials at 9–10, Table S1; Forsheiw at 6 (Table

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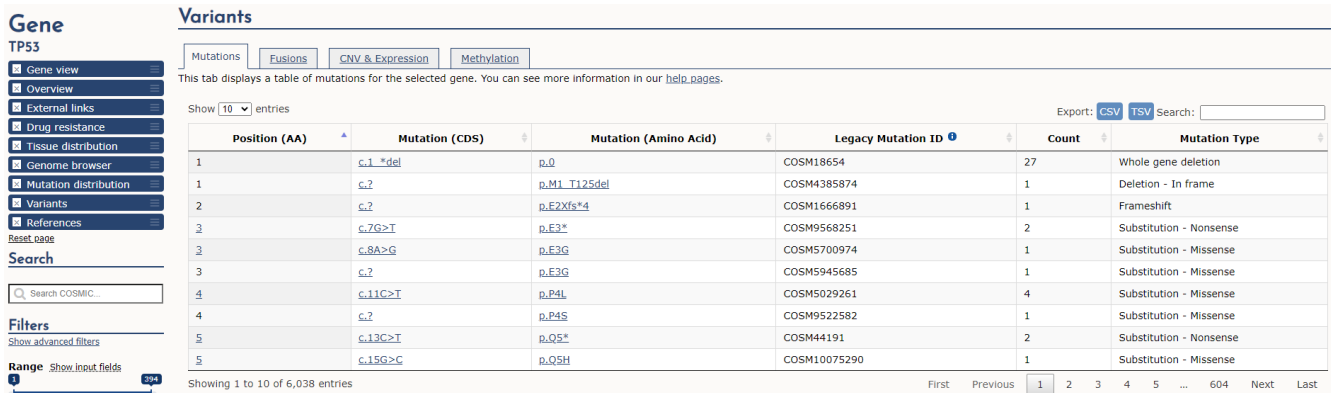
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different tumor-specific SNV mutation.	2); Forsheiw supplemental materials at 17-18 (Table S6); Forsheiw at 10 (disclosing that more loci would “increase[] the robustness for tumor monitoring by compensating for effects of sampling noise or mutational drift”). For the same reasons as Claim 8, Forsheiw renders obvious amplifying higher numbers of target loci. Furthermore, under Natera’s apparent construction of the claim term “target loci,” for example, the Catalogue of Somatic Mutations In Cancer (“COSMIC”) database identifies hundreds to thousands of variants within each of the genes disclosed in Forsheiw. For example, COSMIC identifies 6,038 different variants in the TP53 gene.  https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53 As another example, COSMIC identifies 3,275 different variants in the PTEN gene.

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Claim	Prior Art																																																																		
	Gene PTEN Gene view Overview External links Drug resistance Tissue distribution Genome browser Mutation distribution Variants References Reset page Search Search COSMIC Filters Show advanced filters Range Show input fields 1 100200300404 Variants Mutations Fusions CNV & Expression Methylation This tab displays a table of mutations for the selected gene. You can see more information in our help pages. Show 10 entries <table><tr><th>Position (AA)</th><th>Mutation (CDS)</th><th>Mutation (Amino Acid)</th><th>Legacy Mutation ID</th><th>Count</th><th>Mutation Type</th></tr><tr><td>1</td><td>c.1_*del</td><td>p.Q</td><td>COSM14078</td><td>212</td><td>Whole gene deletion</td></tr><tr><td>1</td><td>c.1_209del209</td><td>p.M11fs*3</td><td>COSM9869413</td><td>1</td><td>Deletion - Frameshift</td></tr><tr><td>1</td><td>c.1_801del801</td><td>p.M1_K267del</td><td>COSM9947920</td><td>1</td><td>Deletion - In frame</td></tr><tr><td>1</td><td>c.2T>A</td><td>p.M1?</td><td>COSM3967198</td><td>1</td><td>Frameshift</td></tr><tr><td>1</td><td>c.2T>C</td><td>p.M1?</td><td>COSM53241</td><td>4</td><td>Frameshift</td></tr><tr><td>1</td><td>c.2T>G</td><td>p.M1?</td><td>COSM9631222</td><td>9</td><td>Frameshift</td></tr><tr><td>1</td><td>c.2_13delinsGGTTCITT...</td><td>p.M1?</td><td>COSM36011</td><td>1</td><td>Complex - frameshift</td></tr><tr><td>1</td><td>c.3G>T</td><td>p.M1?</td><td>COSM5347009</td><td>1</td><td>Frameshift</td></tr><tr><td>1</td><td>c.2</td><td>p.?</td><td>COSM5779</td><td>3</td><td>Unknown</td></tr><tr><td>2</td><td>c.5C>T</td><td>p.T2I</td><td>COSM25087</td><td>1</td><td>Substitution - Missense</td></tr></table> Showing 1 to 10 of 3,275 entries First Previous 1 2 3 4 5 ... 328 Next Last https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=PTEN See also https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=EGFR https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=BRAF https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=KRAS https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=PIK3CA For example, U.S. Patent No. 11,519,035—which is related to the '454 patent and which Natera is asserting in a parallel district court proceeding—admits a “general belief in the art [] that multiplexing PCR for sequencing is limited to about 100 assays in the same well.” ’035 patent at 48:25-29. Thus, the related ’035 patent admits that a POSA would have been aware of how to perform multiplex PCR up to 100 assays in a single well, and would have been able to do so as a matter of routine.	Position (AA)	Mutation (CDS)	Mutation (Amino Acid)	Legacy Mutation ID	Count	Mutation Type	1	c.1_*del	p.Q	COSM14078	212	Whole gene deletion	1	c.1_209del209	p.M11fs*3	COSM9869413	1	Deletion - Frameshift	1	c.1_801del801	p.M1_K267del	COSM9947920	1	Deletion - In frame	1	c.2T>A	p.M1?	COSM3967198	1	Frameshift	1	c.2T>C	p.M1?	COSM53241	4	Frameshift	1	c.2T>G	p.M1?	COSM9631222	9	Frameshift	1	c.2_13delinsGGTTCITT...	p.M1?	COSM36011	1	Complex - frameshift	1	c.3G>T	p.M1?	COSM5347009	1	Frameshift	1	c.2	p.?	COSM5779	3	Unknown	2	c.5C>T	p.T2I	COSM25087	1	Substitution - Missense
Position (AA)	Mutation (CDS)	Mutation (Amino Acid)	Legacy Mutation ID	Count	Mutation Type																																																														
1	c.1_*del	p.Q	COSM14078	212	Whole gene deletion																																																														
1	c.1_209del209	p.M11fs*3	COSM9869413	1	Deletion - Frameshift																																																														
1	c.1_801del801	p.M1_K267del	COSM9947920	1	Deletion - In frame																																																														
1	c.2T>A	p.M1?	COSM3967198	1	Frameshift																																																														
1	c.2T>C	p.M1?	COSM53241	4	Frameshift																																																														
1	c.2T>G	p.M1?	COSM9631222	9	Frameshift																																																														
1	c.2_13delinsGGTTCITT...	p.M1?	COSM36011	1	Complex - frameshift																																																														
1	c.3G>T	p.M1?	COSM5347009	1	Frameshift																																																														
1	c.2	p.?	COSM5779	3	Unknown																																																														
2	c.5C>T	p.T2I	COSM25087	1	Substitution - Missense																																																														
10. The method of claim 1, wherein the method further comprises designing PCR primers or hybrid capture probes targeting the plurality of	Forsheiw discloses that the method further comprises designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample. See, e.g., Forsheiw supplemental materials at 2 (“Target-specific primers were designed with universal primer sequences (termed CS1 and CS2) appended at the 5’-end (table S1).”); Forsheiw at 2, Fig. 1 (“Primers were designed to amplify regions of interest in overlapping short amplicons.”); Forsheiw supplemental materials at 2																																																																		

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SNV mutations identified in the tumor sample.	(Primer design for Tam-Seq); Forshaw at 2 (Figure 1B showing the “initial preamplification step was performed for 15 cycles using a pool of the target-specific primer pairs...”).
11. The method of claim 1, wherein the method further comprises performing barcoding PCR prior to the sequencing.	Forshaw discloses that the method further comprises performing barcoding PCR prior to the sequencing. <i>See, e.g.,</i> Forshaw at 2, Fig. 1 (“[S]ample-specific barcodes were attached to the harvested amplicons in a further PCR.”); Forshaw at 3 (“Sequencing adaptors were separately attached at either end and the products [then] mixed together, such that single-end sequencing generated separate sets of forward and reverse reads.”); Forshaw supplemental materials at 5, Figure S1; Forshaw supplemental materials at 11, Table S2 (“Platform-specific adaptors and barcodes are attached through PCR following the single-plex amplification step.”).
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.	Forshaw discloses that the method further comprises detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. <i>See, e.g.,</i> Forshaw at Abstract (“We demonstrated use of TAM-Seq to noninvasively identify the origin of metastatic relapse in a patient with multiple primary tumors . . . We further used TAM-Seq to monitor tumor dynamics, and tracked 10 concomitant mutations in plasma of a metastatic breast cancer patient over 16 months.”); Forshaw at 4 (“In one plasma sample collected from an ovarian cancer patient at relapse, we also identified a de novo mutation in the tyrosine kinase domain of <i>EGFR</i> (exon 21), at AF of 6% (patient 27, Table 1).”); Forshaw at 10 (“a method that measures multiple mutations in parallel [when looking for recurrent or residual tumor] can have a low likelihood of a false-negative result even if the detection rate for each mutation is less than 50%.”).
13. The method of claim 1, wherein the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer.	Forshaw discloses the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer. <i>See, e.g.,</i> Forshaw at 9 (“Whole-genome sequencing of tumor material was used to identify tumor mutations in a patient with metastatic breast cancer undergoing two phases of chemotherapy.”).
14[pre]. A method for preparing a plasma sample of a subject having cancer or suspected of having	To the extent the preamble is construed to be limiting, Forshaw discloses 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. <i>See claim 1[pre] above.</i>

EXHIBIT 2-C: INVATA'S PRELIMINARY INVALIDITY CONTENTIONS CLAIM CHARTS
FOR U.S. PATENT NO. 11,530,454
(FORSHEW)

U.S. Patent No. 11,530,454	
Claim	Prior Art
cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:	
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;	ForsheW 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. <i>See claim 1[a] above.</i>
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; and	ForsheW discloses 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. <i>See claim 1[b] above.</i>

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U.S. Patent No. 11,530,454	
Claim	Prior Art
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,	Forsheiw 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 claim 1[c] above.</i>
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.	In view of Forsheiw alone or in combination and the knowledge of a POSA, it would have been obvious to detect an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus. Obtaining greater sensitivity by increasing sequencing depth was well-known in the art and a POSA would have had a reasonable expectation of achieving a sensitivity of 0.015% because this had already been accomplished before the ‘454 patent’s priority date. <i>See claim 1[d] above.</i> <i>See, e.g., Forsheiw at 3 (“Background frequencies of nonreference reads were ~0.1% (median, 0.03%; mean, 0.2%; in keeping with Q30 quality threshold applied)”); Forsheiw supplemental materials at 7, Fig. S3 (showing frequencies that could be detected with different confidence levels about the background read rate, including frequencies of 0.015% and below); Forsheiw at 9 (“Enhanced detection down to 1 mutant allele in 10,000 or more wild-type alleles can be obtained...”).</i> As to the degree of sensitivity, Forsheiw specifically teaches the importance of increasing sensitivity and how to improve sensitivity of the TAM-Seq assay, including by increasing sequencing depth and fidelity. Forsheiw at 10.
15. The method of claim 14, wherein the sequencing has a depth of read of at least 50,000 per target locus.	In view of Forsheiw, in combination with Bentley, Narayan, and the knowledge of a POSA, it would have been obvious to perform sequencing at a depth of read of at least 50,000 per target locus. <i>See claim 1[d] above.</i>

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U.S. Patent No. 11,530,454	
Claim	Prior Art
16. The method of claim 14, wherein the cell-free DNA comprises circulating tumor DNA.	ForsheW discloses that the cell-free DNA comprises circulating tumor DNA. <i>See claim 2 above.</i>
17. The method of claim 14, wherein the SNV mutations comprise one or more clonal SNV mutations.	ForsheW discloses that the SNV mutations comprise one or more clonal SNV mutations. <i>See claim 3 above.</i>
18. The method of claim 14, wherein the SNV mutations comprise one or more subclonal SNV mutations.	ForsheW discloses that the SNV mutations comprise one or more subclonal SNV mutations. <i>See claim 4 above.</i>
19. The method of claim 14, wherein the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations.	ForsheW discloses that the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations. <i>See claim 5 above.</i>
20. The method of claim 14, wherein the tumor sample of the subject is a tumor tissue sample.	ForsheW discloses that the tumor sample of the subject is a tumor tissue sample. <i>See claim 6 above.</i>

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(FORSHEW)

U.S. Patent No. 11,530,454	
Claim	Prior Art
21. The method of claim 14, wherein the method further comprises determining clonal heterogeneity of the tumor sample.	ForsheW discloses that the method further comprises determining clonal heterogeneity of the tumor sample. <i>See claim 7 above.</i>
22. The method of claim 14, wherein the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation.	ForsheW discloses that the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation. <i>See claim 8 above.</i>
23. The method of claim 14, wherein the targeted multiplex amplification amplifies 50 to 100 target loci each encompassing a different tumor-specific SNV mutation.	ForsheW discloses that the targeted multiplex amplification amplifies 50 to 100 target loci each encompassing a different tumor-specific SNV mutation. <i>See claim 9 above.</i>
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.	ForsheW discloses that the method further comprises designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample. <i>See claim 10 above.</i>

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(FORSHEW)

U.S. Patent No. 11,530,454	
Claim	Prior Art
25. The method of claim 14, wherein the method further comprises performing barcoding PCR prior to the sequencing.	ForsheW discloses that the method further comprises performing barcoding PCR prior to the sequencing. <i>See claim 11 above.</i>
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.	ForsheW discloses that the method further comprises detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. <i>See claim 12 above.</i>
27. The method of claim 14, wherein the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer.	ForsheW discloses that the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer. <i>See claim 13 above.</i>
28. The method of claim 14, wherein the method is capable of detecting an SNV mutation that is present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus.	In view of ForsheW alone or in combination with the knowledge of a POSA, it would be obvious to detect an SNV mutation that is present in 0.005% to 0.015% of the cell-free DNA comprising the SNV locus. <i>See claims 1[d] and 14[d] above.</i>