EXHIBIT 1-A – FINAL INVALIDITY CLAIM CHART FOR U.S. PATENT NO. 11,519,035

Reference:
United States Patent Application Publication US 2004/0110153 A1 ("Dong")

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

To the extent Natera asserts that Dong does not disclose or teach one or more limitations of claims 1, 2, 12, 13, performing the methods as claimed in those claims would have been obvious to a person of ordinary skill in the art at the time of the alleged invention considering Dong in combination with the knowledge of a POSA and/or one or more of the following references:

- U.S. Patent 8,318,434 B2 ("Cuppens")
- International Patent Publication No. 2010/115154 ("May PCT")
- International Patent Publication No. 2011/142836 ("Pieprzyk")
- Shapero et al., *MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density DNA oligonucleotide arrays*, 32 NUCLEIC ACIDS RES. e101 (2004) ("Shapero")
- U.S. Patent Publication No. 2010/0120038 ("Mir")
- International Patent Publication WO 2010/126614 A2 ("Porreca")
- Parallel Preparation of Targeted Resequencing Libraries from 480 Genomics Regions Using Multiplex PCR on the Access Array™ System ("Kaper")
- U.S. Patent Publication No. 2012/0270739 ("Oliphant")
- International Patent Publication No. 2011/85491 ("Hansen")
- Parallel Production of Barcoded Amplicon Libraries for 454 Sequencing using Individual and Multiplex PCR in Nanoliter Volumes ("May Poster")

A POSA would have been motivated to combine Dong with one or more of the foregoing references because Dong expressly teaches using routine and conventional techniques known in the art to analyze fragmented sample DNA efficiently. For instance, a POSA would be motivated to utilize the methods of Dong for cell-free DNA diagnostic assays because Dong expressly invites individuals skilled in the art to perform further analysis of the amplified fragments. Dong at [0013] ("[a]s those skill in the art will appreciate, after amplification, the resulting sequences may be further analyzed using any known method including sequencing, HPLC, hybridization analysis, cloning, labeling, etc."). As another example, a POSA would be motivated to utilize the methods of Dong in combination with next generation sequencing techniques because Dong expressly invites

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individuals skilled in the art to perform further analysis of the amplified fragments. *Id.* As another example, a POSA would be motivated to utilize the methods of Dong for cell-free DNA analysis because Dong expressly teaches amplification and analysis of DNA fragments and a POSA understands that cell-free DNA exists in the blood stream as DNA fragments. Dong at [0087] (disclosing that the "genomic DNA sample of the current invention" "may be obtained from any biological or environmental source, including . . . . whole blood"); *id.* at [0004] (disclosing an illustrative embodiment wherein sample DNA is fragmented before analysis); *see*, *e.g.*, Chao Cheng, *Quantification of circulating cell-free DNA in the plasma of cancer patients during radiation therapy*, 100 Cancer Sci. 303, 303 (2009) (using qPCR to determine if the relative ratio of longer to shorter circulating cell free DNA fragments in the blood of cancer patients was altered through radiation therapy to build on previous studies showing that quantification of the integrity of DNA in plasma could be useful for the diagnosis of certain types of cancer).

Further, a POSA would reasonably expect to succeed in combining Dong with one or more of the foregoing references for the purposes described above because the techniques of amplifying and sequencing fragmented DNA, the utilization of universal tail adaptors, barcodes, and sequencing tags, and targeting SNP loci associated with cancer were all routine and conventional at the time of the inventions for DNA analysis and diagnostics.

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Claim	**Prior Art**
1[pre]. A method for amplifying and sequencing DNA, comprising:	To the extent the preamble is construed to be limiting, Dong discloses a method for amplifying and sequencing DNA. *See, e.g.,* Dong at [0001], [0002], [0004], [0007], [0013]-[0029], [0039]-[0041], [0056], [0083]-[0086], [0089], [0091], [0096], [0098], [0101], [0102], [0106]-[0110], [0112], [0113], [0117], [0138], [0143], [0144], [0146], Example 1, Example 2, Claim 1, Claim 2, Claim 3, Claim 4, Claim 5, Claim 6, Claim 7, Claim 8, Claim 9, Claim 10, Claim 11, Claim 12, Claim 13, Claim 14, Claim 15, Claim 16, Claim 17, Claim 18, Claim 19, Claim 20, Claim 21, Claim 22, Claim 23, Claim 24, Claim 25, Claim 26, Claim 27, Claim 28, Claim 29, Claim 30, Claim 31, Claim 32, Claim 33, Claim 34, Claim 35, Claim 36, Claim 37, Claim 38, Claim 39, Claim 40, Claim 41, Claim 42, Claim 43, Claim 44, Figures 1, 2, 3A, 3B, 4, 5, 6, 7, 8. For example, Dong discloses the field of invention as relating to "enrichment and amplification of sequences from a nucleic acid." Dong at [0001]. Dong discloses methods including amplifying DNA. For example, Dong discloses a first method of the invention as a method "for reducing the complexity of a first nucleic acid sample to produce a second nucleic acid sample wherein the second nucleic acid sample comprises a plurality of target sequences." Dong at [0004].

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Claim	**Prior Art**
	Dong discloses methods also including sequencing DNA. For example, Dong discloses that "after amplification, the result sequences may be further analyzed using any known method including sequencing, HPLC, hybridization analysis, cloning, labeling, etc." Dong at [0113].
1[a][i]. tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,	Dong discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See, e.g.*, Dong at [0004], [0005], [0007], [0010], [0013]-[0015], [0018], [0021]-[0027], [0035], [0056]-[0059], [0089], [0090], [0096]-[0098], [0106], [0108], [0113], [0138], [0146], Example 1, Example 2, Claim 1, Claim 2, Claim 3, Claim 4, Claim 5, Claim 6, Claim 7, Claim 8, Claim 9, Claim 10, Claim 11, Claim 12, Claim 13, Claim 14, Claim 15, Claim 16, Claim 17, Claim 18, Claim 19, Claim 20, Claim 20, Claim 21, Claim 22, Claim 23, Claim 24, Claim 25, Claim 26, Claim 27, Claim 28, Claim 29, Claim 30, Claim 31, Claim 32, Claim 33, Claim 34, Claim 35, Claim 36, Claim 37, Claim 38, Claim 39, Claim 41, Claim 41, Claim 42, Claim 43, Claim 44, Figures 1, 2, 3A, 3B, 6, 8. For example, Dong discloses methods that includes fragmenting a genomic sample and that those fragments are then "modified with one or more common sequences, for example, by ligation to common adaptor sequences, and the sample amplified with primers to the common adaptor sequences." Dong at [0096]. As another example, Dong discloses "ligating an adaptor to the [nucleic acid] fragments." Dong at [0018]. As another example, Dong discloses an embodiment wherein "genomic DNA is fragmented and adaptors are ligated to the ends" and "[t]he adapters comprise a common sequence." Dong at [0108]. As another example, Figure 3A in Dong discloses an embodiment wherein "[f]ragments are modified by the addition of a poly(A) tail, then a poly(T) primer with a common priming sequence is hybridized to the homopolymeric tail and extended to make cDNA. The cDNA is then modified by the addition of a poly(A) tail. The poly(U) primer with a second priming site is hybridized to the poly(A) tail of the cDNA and extended resulting in a double stranded fragment flanked by common priming sites." Dong at [0097]. As another example, Dong discloses an illustrative method wherein "[t]he ends of the [DNA] fragments may be modified by adding a first homopolymeric tail to the 3' end of the fragments using terminal transferase; and then the step of amplifying a subset of fragments may be by hybridizing a first primer to the first homopolymeric tail wherein the first primer comprises a first common priming site; extending the first primer; adding a second

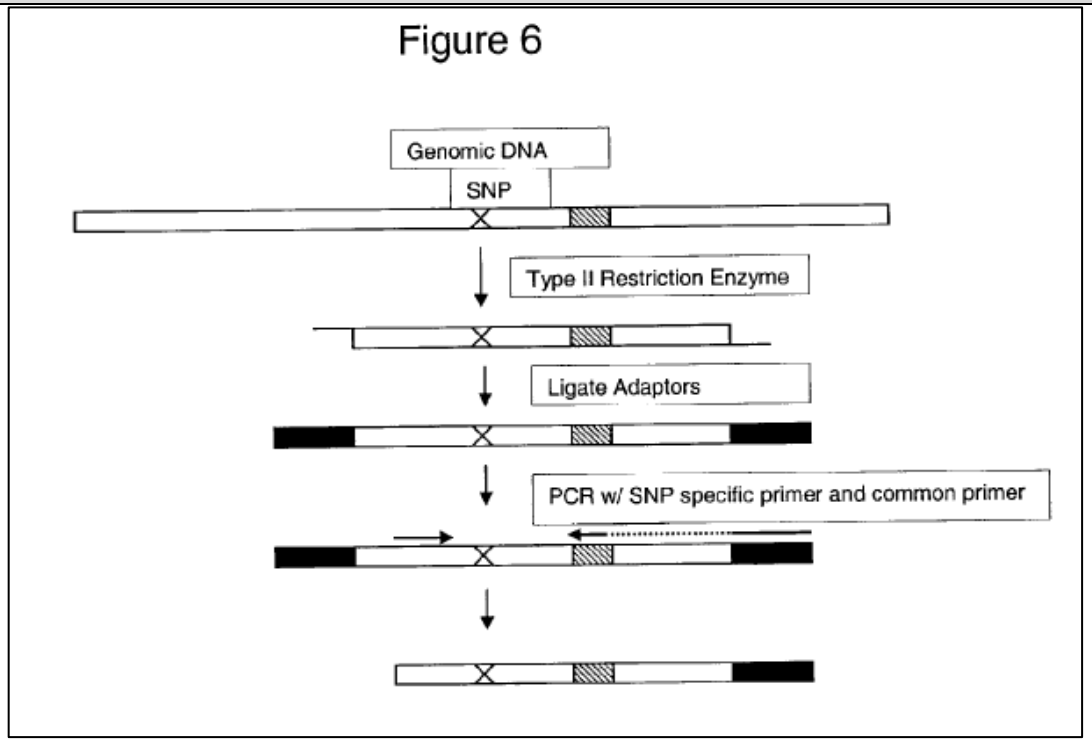
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Claim	**Prior Art**
	homopolymeric tail to the 3' end of the extended first primer; annealing a second primer to the second homopolymeric tail wherein the second primer comprises a second common priming site." Dong at [0014].
1[a][ii]. wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;	Dong discloses tagging cell free DNA isolated from a blood sample collected from a subject who is not a pregnant women. *See, e.g.*, Dong at [0087], [0117], [0122], [0126], [0129], [0133], [0135]. For example, Dong discloses that the "genomic DNA sample of the current invention" "may be obtained from any biological or environmental source, including plant, animal (including human), bacteria, fungi, or algae. Convenient suitable samples include whole blood, tissue, semen, saliva, tears, urine, fecal material, sweat, buccal skin and hair." Dong at [0087]. As another example, Dong discloses that "one can determine whether a blood sample from a suspect matches a blood or other tissue sample from a crime scene by determining whether the set of polymorphic forms occupying selected polymorphic sites is the same in the suspect and the sample." Dong at [0135]. As another example, Dong discloses a method to determine "relatedness between individuals" that includes analyzing genetic material from a child "for the occurrence of polymorphisms" and comparing that sample "to a similar analysis of the putative father's genetic material." Dong at [0133]. As another example, Dong discloses that the methods can be used "to make a more accurate diagnosis" including to "determine the specific type of cancer a patient has." Dong at [0126].
1[b][i]. amplifying the tagged products one or more times to generate final amplification products,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Dong meets this limitation. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Dong discloses amplifying the tagged products one or more times to generate final amplification products.

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Claim	**Prior Art**
	See, e.g., Dong at [0004], [0013]-[0029], [0083]-[0086], [0089], [0091], [0096], [0098], [0106], [0108]-[0111], [0113], [0117], [0138]-[0144], [0146], Example 1, Example 2, Claim 1, Claim 2, Claim 3, Claim 4, Claim 5, Claim 6, Claim 7, Claim 8, Claim 9, Claim 10, Claim 11, Claim 12, Claim 13, Claim 14, Claim 15, Claim 16, Claim 17, Claim 18, Claim 19, Claim 20, Claim 21, Claim 22, Claim 23, Claim 24, Claim 25, Claim 26, Claim 27, Claim 28, Claim 29, Claim 35, Claim 36, Claim 37, Claim 38, Claim 39, Claim 41, Claim 41, Claim 42, Claim 43, Claim 44, Figures 1, 2, 3A, 3B, 4, 5, 6, 8. For example, Dong discloses a method wherein "[t]he genomic sample may first be fragmented, the fragments modified with one or more common sequences, for example, by ligation to common adaptors sequences, and the sample amplified with primers to the common adaptor sequences." Dong at [0096]. As another example, Dong discloses an aspect of the invention wherein "a plurality of SNPs is genotyped by fragmenting a nucleic acid sample with a type IIs restriction enzyme" and "ligating an adaptor to the fragments." Dong further discloses "amplifying the adaptor ligated fragments using one target specific primer and a common primer." Dong at [0018]; *id.* at [0027] (explaining Figure 6).

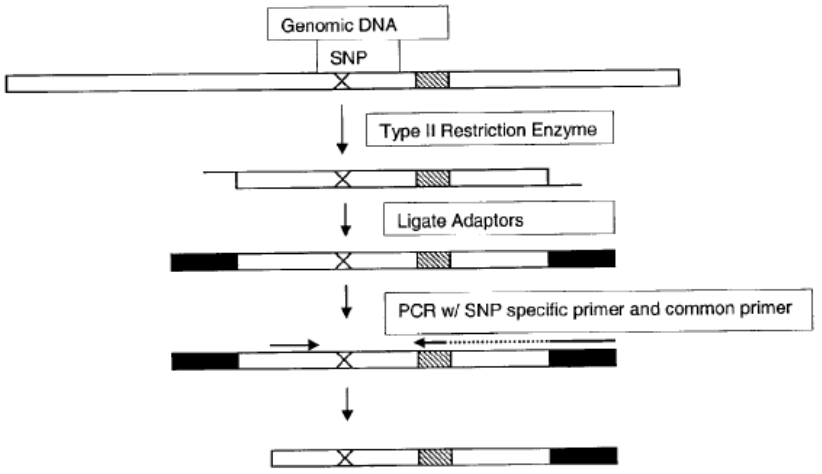
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Claim	**Prior Art**
	 As another example, Dong discloses "Amplification methods may be designed to amplify fragments that contain known SNPs and those amplified products may be analyzed to identify the genotype of a sample at one or more SNP locations." Dong at [0084]. As another example, Dong discloses "[w]hen interrogating genomes it is often useful to first reduce the complexity of the sample and analyze one or more subsets of the genome. Subsets can be defined by many characteristics of the fragments. In a preferred embodiment of the current invention, the subsets are defined by the presence of a polymorphic sequence. Collections of polymorphic sequences are targeted for amplification. In some embodiments a locus specific primer is used for each sequence to be amplified. Using a locus specific primer allows selection of the fragments and polymorphisms that will be amplified." Dong at [0086].

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Claim	**Prior Art**
	As another example, Dong discloses in Example 1 a "[s]emi-[s]pecific [a]mplification" wherein target sequences are amplified using tagged primers. Dong at [0138]-[0142]. Dong further discloses "Target sequences used were the human beta actin gene (X00351) and the Human GAPDH gene (M33179). One antisense primer was made for each target. The actin primer was complementary to sequence 1130-1111 and the GAPDH primer was complementary to sequence 1192-1173. Both primers are at least 80 nucleotides away from an intron site. A tag sequence was attached to each of the above primers the tag sequence was: ttaccctcactaaagggaga (SEQ ID N0:3). The common primers used were ST3U20: ggcacatcaattaccctcacuuuuuuuuuuuuuuuuuuuu (SEQ ID N0:4) and BT3: atcacacaattaccctcactaaagggaga (SEQ ID NO:S). The ST3U20 primer is used for copying tailed fragments and amplification and the BT3 primer is used for amplification and in vitro transcription." *Id.* at [0138]. To the extent Natera contends that Dong does not disclose or teach an amplification step that introduces a barcode and one or more sequencing tags, in view of the knowledge of a POSA at the time of the claimed inventions, it would have been obvious to do so. For instance, a POSA would be motivated to utilize the methods of Dong in combination with barcodes and sequencing tags because Dong discloses the simultaneous use of tag sequences and common primers. *See*, *e.g.,* Dong at [0138]-[0145]. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Dong also discloses this element for the same reasons.
1[b][ii]. wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Dong meets this limitation. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Dong discloses amplifying wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume.

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Claim	**Prior Art**
	See, e.g., Dong at [0004], [0005], [0007], [0008], [0012]-[0014], [0017]-[0029], [0039]-[0041], [0066], [072], [0083]-[0086], [0088], [0089], [0090], [0091], [0096], [0101], [0106]-[0110], [0117], [0138]-[0146], Example 1, Example 2 Claim 1, Claim 2, Claim 3, Claim 4, Claim 5, Claim 6, Claim 7, Claim 8, Claim 9, Claim 10, Claim 11, Claim 12, Claim 13, Claim 14, Claim 15, Claim 16, Claim 17, Claim 18, Claim 19, Claim 20, Claim 21, Claim 22, Claim 23, Claim 24, Claim 25, Claim 26, Claim 27, Claim 28, Claim 29,Claim 35, Claim 36, Claim 37, Claim 38, Claim 39, Figures 1, 2, 3A-B, 4, 5, 6, 8. For example, Dong discloses "a method for enriching a subject of target nucleic acids. Genomic DNA is digested with one or more Type IIs restriction enzymes and ligated to adaptors. Fragments are amplified with one primer that is specific for each target sequence to be amplified and a common primer to the adaptors." Dong at [0027] (describing Figure 6). Dong later discloses that "[t]he methods provide for highly parallel analysis of a large number of SNPs, for example, more than 1,000, 5,000, 10,000, or 50,000, that are spaced throughout the genome." *Id*. at [0084]. Figure 6 

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Claim	**Prior Art**
	As another example, Dong discloses that "target sequences may include regions of genomic DNA which are believed to contain one or more polymorphic sites . . .. The number of sequences to be interrogated can vary, but preferably are from 1, 10, 1000, or 1000 to 10,000, 100,000 or 1,000,000 target sequences." Dong at [0066]. As another example, Dong discloses "[w]hen interrogating genomes it is often useful to first reduce the complexity of the sample and analyze one or more subsets of the genome. Subsets can be defined by many characteristics of the fragments. In a preferred embodiment of the current invention, the subsets are defined by the presence of a polymorphic sequence. Collections of polymorphic sequences are targeted for amplification. In some embodiments a locus specific primer is used for each sequence to be amplified. Using a locus specific primer allows selection of the fragments and polymorphisms that will be amplified." Dong at [0086]. As another example, Dong discloses that "the methods of the presently claimed invention are used for SNP discovery and to genotype individuals. For example, any of the procedures describe above, alone or in combination, could be used to isolate the SNPs present in one or more specific regions of genomic DNA. Selection probes could be designed and manufactured to be used in combination with the methods of the invention to amplify only those fragments containing regions of interest, for example a region known to contain a SNP." Dong at [0117]. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Dong also discloses this element for the same reasons.
1[b][iii]. wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and	Dong discloses amplifying wherein one of the amplifying steps introduces one or more tags. *See, e.g.*, Dong at [0010], [0016], [0017], [0080], [0138]-[0142], Example 1, Claim 15, Claim 16, Claim 17, Claim 26, Claim 27, Claim 35, Figures 3A, 3B, 4, 5. For example, Dong discloses in Example 1 a "[s]emi-[s]pecific [a]mplification" wherein target sequences are amplified using tagged primers. Dong at [0138]-[0142]. Dong further discloses "Target sequences used were the human beta actin gene (X00351) and the Human GAPDH gene (M33179). One antisense primer was made for each target. The actin primer was complementary to sequence 1130-1111 and the GAPDH primer was complementary to

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Claim	**Prior Art**
	sequence 1192-1173. Both primers are at least 80 nucleotides away from an intron site. A tag sequence was attached to each of the above primers the tag sequence was: ttaccctcactaaagggaga (SEQ ID N0:3). The common primers used were ST3U20: ggcacatcaattaccctcacuuuuuuuuuuuuuuuuuuuu (SEQ ID N0:4) and BT3: atcacacaattaccctcactaaagggaga (SEQ ID NO:S). The ST3U20 primer is used for copying tailed fragments and amplification and the BT3 primer is used for amplification and in vitro transcription." *Id.* at [0138]. As another example, Dong discloses an illustrative embodiment wherein "[t]he sample is mixed with a plurality of tagged primers wherein each species of tagged primer comprises a unique tag sequence and a sequence that is complementary to a region immediately adjacent to a polymorphic base in a target sequence and the plurality of tagged primers is extended. The tagged primers may be extended by a single nucleotide that is complementary to the polymorphic base in the target sequence and the single nucleotide may comprise a detectable label. The detectable label may be a different label for each type of nucleotide." Dong at [0010]. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Dong also discloses this element for the same reasons.
1[c][i]. sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products,	Dong discloses sequencing the plurality of SNP loci on a nucleic acid sample by conducting massively parallel sequencing on the final amplification products. *See, e.g.,* Dong at [0018], [0084], [0113]. For example, Dong discloses that "[t]he methods provide for highly parallel analysis of a large number of SNPs, for example, more than 1,000, 5,000, 10,000 or 50,000 that are spaced throughout the genome." Dong at [0084]. As another example, Dong discloses that in another embodiment "a plurality of SNPs is genotyped." Dong at [0018].

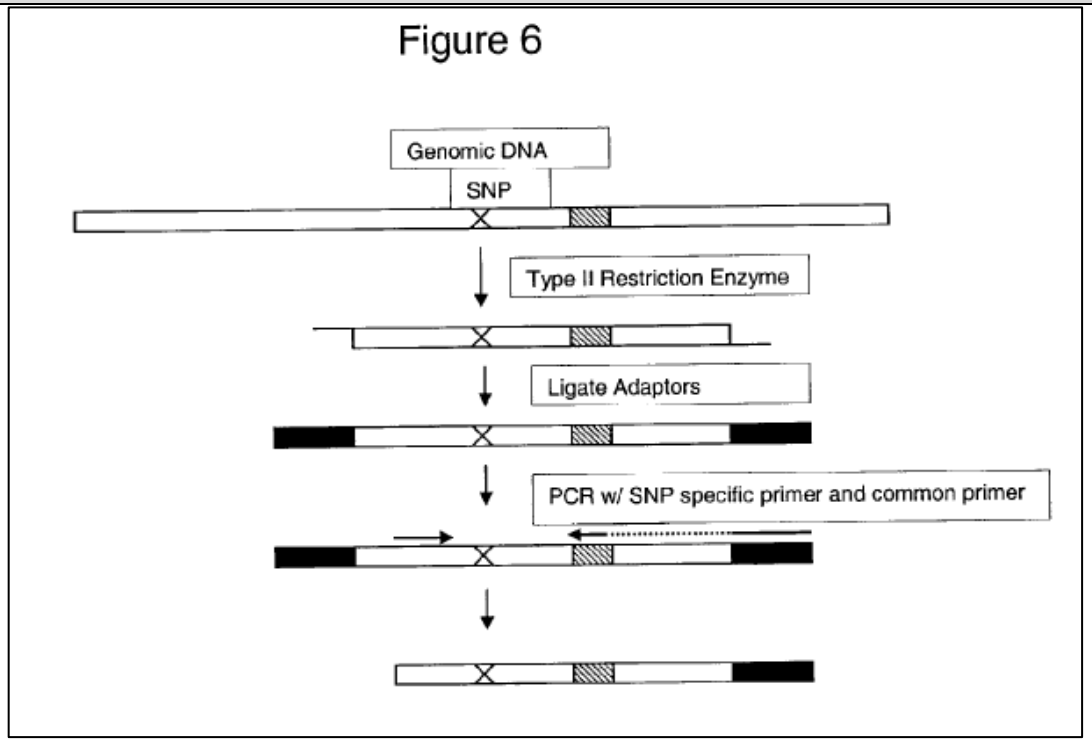
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Claim	**Prior Art**
	As another example, Dong discloses that "after amplification, the resulting sequences may be further analyzed using any known method, including sequencing, HPLC, hybridization analysis, cloning, labeling, etc." Dong at [0113].
1[c][ii]. wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.	The Court construed the term "SNP loci…associated with cancer" to mean "a single nucleotide that may differ between the genomes of two members of the same species and is associated with cancer. The usage of the term 'SNP' should not imply any limit on the frequency with which each variant occurs." Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Dong meets this limitation. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Dong discloses a plurality of SNP loci comprising 25-2,000 loci associated with cancer. *See, e.g.*, Dong at [0002], [0009], [0066], [0084], [0088], [0126]. For example, Dong discloses that "[t]he number of sequences to be interrogated can vary, but preferably are from 1, 10, 1000, or 1000 to 10,000, 100,000 or 1,000,000 target sequences." Dong at [0066]. Further, Dong discloses that "[t]he methods provide for highly parallel analysis of a large number of SNPs, for example, more than 1,000, 5,000, 10,000 or 50,000 that are spaced throughout the genome." *Id.* at [0084]. Moreover, Dong also discloses that genomic DNA can be analyzed to "identify, diagnose, or treat diseases like cancer, Alzheimer disease or alcoholism." *Id.* at [0002]. As another example, Dong discloses that the methods can be used "to make a more accurate diagnosis" including to "determine the specific type of cancer a patient has." Dong at [0126]. To the extent Natera asserts that any portion of this limitation is not disclosed under Natera's apparent interpretation of the Court's construction, it would have been obvious based on the knowledge of a POSA at the time of the alleged invention to look to the Catalogue of Somatic Mutations In Cancer ("COSMIC") database, which identifies hundreds to thousands of gene variants, including those that can be present in the same gene, to readily disclose a plurality of SNP loci comprising 25-2,000 loci associated with cancer. By way of example,

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	COSMIC identifies 6,038 different variants in the TP53 gene alone. *See, e.g.,* https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53. In addition, the '035 Patent admits a POSA's "general belief in the art is 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, would have understood that multiplex PCR up to 100 assays could readily be performed on cell free DNA, and would have been able to do so as a matter of routine.
2. The method of claim 1, wherein tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA.	Dong discloses tagging nucleic acids comprising ligating the one or more universal tail adapters to the cell free DNA. *See, e.g.*, Dong at [0018], [0027], [0056]-[0060], [0096], [0106], [0108], Claim 39, Figures 4, 5, 6. 8. For example, Dong discloses an aspect of the invention wherein "a plurality of SNPs is genotyped by fragmenting a nucleic acid sample with a type IIs restriction enzyme" and "ligating an adaptor to the fragments." Dong at [0018]; *id.* at [0027] (explaining Figure 6).

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Claim	**Prior Art**
	 As another example, Dong discloses that "[t]he genomic sample may first be fragmented, the fragments modified with one or more common sequences, for example, by ligation to common adaptor sequences, and the sample amplified with primers to the common adapter sequences." Dong at [0096]. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 27. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Dong also discloses this element for the same reasons as discussed below regarding claim 13.

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Claim	**Prior Art**
12. The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.	Dong discloses one or more universal tail adaptors comprising a first universal tail adaptor and a second universal tail adaptor. *See, e.g.*, Dong at [0056]-[0059], [0096]. For example, Dong discloses that "[t]he genomic sample may first be fragmented, the fragments modified with one or more common sequences, for example, by ligation to common adaptor sequences, and the sample amplified with primers to the common adaptor sequences." Dong at [0096].
13. The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.	In view of the knowledge of a POSA at the time of the claimed inventions, it would have been obvious to tag cell free DNA by amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor. For instance, a POSA would be motivated to utilize the methods of Dong in combination with tagging cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor because Dong discloses that, in some embodiments, fragments of interest can be "modified with one or more common sequences, for example, by ligation to common adaptor sequences and the sample amplified with primers to the common adaptor sequences." *See* Dong at [0096]. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Dong also discloses this element for the same reasons as discussed above regarding claim 2.

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FOR U.S. PATENT NO. 11,519,035

Reference:
U.S. Patent 8,318,434 B2 ("Cuppens")

To the extent Natera asserts that Cuppens does not disclose or teach one or more limitations of Claims 1, 2, 12, or 13, performing the methods as claimed in those claims would have been obvious to a person of ordinary skill in the art at the time of the alleged invention considering Cuppens in combination with the knowledge of a POSA and/or one or more of the following references:

- International Patent Publication No. 2011/142836 ("Pieprzyk")
- U.S. Patent Publication No. 2010/0120038 ("Mir")
- International Patent Publication No. 2010/115154 ("May PCT")
- International Patent Publication No. 2007/018601("Makarov")
- Shapero et al., *MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density DNA oligonucleotide arrays*, 32 NUCLEIC ACIDS RES. e101 (2004) ("Shapero")
- International Patent Publication WO 2010/126614 A2 ("Porreca")
- U.S. Patent Application Publication US 2004/0110153 A1 ("Dong")
- International Patent Publication No. 2011/85491 ("Hansen")

A POSA would have been motivated to combine Cuppens with one or more of the foregoing references because Cuppens expressly teaches using routine and conventional techniques known in the art to analyze fragmented sample DNA efficiently. For instance, a POSA would be motivated to utilize the methods of Cuppens for cell-free DNA diagnostic assays because Cuppens discloses that "[t]he new generation sequencing technology, such as parallel pyrosequencing in high-density picoliter reactors, allows the simultaneous sequencing of extreme high number of 25-500 bp fragments and is currently mainly used of total genomic sequencing," but "when using adapted sample preparation methods and protocols these sequencing technologies can also be used in other sequencing applications such as the resequencing of specific nucleic acid molecules in biological samples" for clinical diagnostics." Cuppens at 2:36-45. As another example, a POSA would have been motivated to utilize ligation to add one or more universal tail adaptors at least because Cuppens expressly teaches ligating adaptors to genomic DNA followed by amplification with "with two universal primers against these adaptors." Cuppens at 22:54-59. A POSA likewise would have been motivated to combine Cuppens with one or more of the foregoing references to analyze loci associated with cancer because Cuppens expressly teaches that "mutations/variations in the human genome are involved in most diseases . . . such as cancer" and "there is a need for robust assays in which different DNA regions, that harbor the different mutations/variations, are analyzed together." Cuppens at 1:32-47.

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Further, a POSA would reasonably expect to succeed in combining Cuppens with one or more of the foregoing references for the purposes described above because the techniques of amplifying and sequencing fragmented DNA, ligating universal tail adaptors, and targeting SNP loci associated with cancer were all routine and conventional at the time for DNA analysis and diagnostics.

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Claim	**Prior Art**
1[pre]. A method for amplifying and sequencing DNA, comprising:	To the extent the preamble is construed to be limiting, Cuppens discloses a method for amplifying and sequencing DNA. *See*, *e.g.*, Cuppens at 1:21-2:59, 3:14-5:24, 5:36-6:43, 7:20-9:39, 9:63-10:19, 11:27-51, 12:10-39, 14:9-17, 14:54-15:35, 15:40-16:20, 16:23-17:43, 18:7-56, 19:54-65, 21:15-62, Example 1, Example 2, Example 3, Claim 1, Claim 3, Claim 5, Claim 6, Claim 8, Figures 1, 2, 3, 4a, 4b, 5a, 5b, 6, 7, 10. For example, Cuppens discloses the field of invention as relating "to tools and methods for use in genetic tests involving high performance sequencing techniques." Cuppens at 1:21-28. "More particularly, the invention relates to a robust multiplex PCR method wherein the respective primers for amplifying are physically isolated from one another." *Id*. As another example, Cuppens discloses that in a first use of the invention "it was found that a successful use of the sequencing technologies preferably requires the availability of robust multiplex PCR reactions of a large number of amplicons." Cuppens at 2:36-59. As another example, Cuppens discloses an object of invention that "provides a method for marking and subsequently verifying the origin and/or identity of a biological sample" wherein it is preferable that "the presence of the biological sample of the known nucleic acid sequences is detected by PCR amplification of the sequences and subsequently sequencing the amplification products." Cuppens at 4:46-5:4. Cuppens discloses methods for amplifying DNA. For example, Cuppens discloses that "in a second object, the present invention provides a method for carrying out PCR amplification of a DNA fragment." Cuppens at 3:14-30. Cuppens also discloses "the PCR method of the present invention is of particular interest for the amplification of a genomic DNA fragment or set of fragments for the purpose of sequencing a section of the genomic DNA." *Id.* Cuppens discloses methods also including sequencing DNA. For example, Cuppens discloses "[i]n a preferred embodiment, a method is provided for the simultaneous sequencing of multiple DNA fragments derived from different samples" wherein DNA is tagged, amplified, and sequenced. Cuppens at 18:32-56.

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1[a][i]. tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,	Under Natera's apparent construction, Cuppens discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See*, *e.g.*, Cuppens at 3:45-4:37, 5:25-6:7, 8:3-43, 8:60-67, 9:1-18, 12:10-39, 14:54-15:21, 16:34-18:6, 18:7-48, 22:54-64, Example 2, Claim 1, Figures 4a, 4b, 5a, 5b. For example, Cuppens discloses a general method for tagging a DNA sample wherein "[e]ach forward primer contains an amplicon-specific region (AS) and a universal forward adaptor (FA), [and] analogously the reverse primers contain an AS region and another universal reverse adaptor (RA)." Cuppens at 8:60-67. Cuppens discloses that, under this protocol, "[a]ll PCR fragments will then carry identical sequences at their ends." *Id.* Further, "[i]n this amplification step, a sample tag (ST) is attached to each amplicon." *Id.* at 9:1-18. As another example, Cuppens discloses that "adaptor tag primers can be used on any amplicon, irrespective of the genomic region, or gene, from which it was derived, if all first step PCR reactions are performed with adaptor-amplicon specific primers that contain the same 'universal' forward and reverse adaptors." Cuppens at 16:56-60. As another example, Cuppens discloses a sequencing assay wherein "total genomic DNA is randomly fragmented to 25-500 bp fragments," then the sample is tagged with universal adaptors and "[a]mpification is then performed with two universal primers against these adaptors, so that all fragments are amplified simultaneously." Cuppens at 22:54-64.
1[a][ii]. Wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;	Cuppens discloses tagging cell free DNA isolated from a blood sample collected from a subject who is not a pregnant women. *See*, *e.g.,* Cuppens at 1:33-48, 2:36-3:29, 10:50-57, 19:17-46, Figures 13, 14. For example, Cuppens discloses using complex "multiplex amplification reactions" to analyze different pathogenic DNA mutations together. Cuppens at 1:33-48. Cuppens also discloses that "[m]utations/variations in the human genome are involved in most diseases" including "acquired diseases such as cancer." *Id*. As another example, Cuppens discloses, "The present invention is based on the finding that when using adapted sample preparation methods and protocols these sequencing technologies can also be used in other sequencing applications such as the resequencing of specific nucleic acid fragments in biological samples, as used in clinical diagnostics." Cuppens at 2:40-45.

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	As another example, Cuppens discloses quality control strategies that include, among other things, attaching a patient's barcode paper to the blood collection tube and utilizing blood collector tubes "in which sample-ID-barcode are already present." Cuppens at 10:50-57. Further, a POSA understands that cell-free DNA exists in the blood stream as DNA fragments. *See, e.g.*, Chao Cheng, *Quantification of circulating cell-free DNA in the plasma of cancer patients during radiation therapy*, 100 Cancer Sci. 303, 303 (2009) (using qPCR to determine if the relative ratio of longer to shorter circulating cell free DNA fragments in the blood of cancer patients was altered through radiation therapy to build on previous studies showing that quantification of the integrity of DNA in plasma could be useful for the diagnosis of certain types of cancer).
1[b][i]. amplifying the tagged products one or more times to generate final amplification products,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Cuppens meets this limitation. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Cuppens discloses amplifying the tagged products one or more times to generate final amplification products. *See*, *e.g.,* Cuppens at 1:44-48, 3:45-4:14, 5:1-24, 5:36-55, 6:8-19, 6:20-34, 7:61-6, 7:22-39, 7:44-46, 8:3-9:18, 9:28-29, 9:35-39, 9:45-62, 10:32-34, 11:13-19, 12:18-22, 16:44-55, 16:61-17:26, 18:7-57, 21:15-54, 22:65-23:5, Example 2, Claim 1, Claim 5, Claim 6, Claim 7, Claim 8, Claim 9, Figures 2, 4a, 4b, 5a, 5b, 6, 7, 9. For example, Cuppens discloses that "[a]s more and more mutations/variations are found to be involved in disease, there is a need for robust assays in which different DNA regions, that harbor different mutations/variations, are analyzed together. This may be achieved through (more complex) multiple amplification reactions." Cuppens at 1:44-48. As another example, Cuppens discloses a method of amplification wherein "isolated PCR reactors will be generated containing the 2 amplicon specific primers in a tube, so that different amplicon specific KNEX-DNA-crystals, which were prepared separately in a bulk solution, can combined in a single multiplex PCR reaction. Cuppens at 12:18-22.

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	As another example, Cuppens discloses that "multiplex amplification in a "single, or limited number of, PCR reaction(s) would render amplicon-sequence more convenient and cheaper." Cuppens at 22:65-23:5. Further, "[i]n combination with sample tags, different samples can be pooled (FIG. 4*b*) and in combination with molecular barcodes the complete process can be quality assured (FIG. 5*a*)." *Id.* Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Cuppens also discloses this element for the same reasons.
1[b][ii]. wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Cuppens meets this limitation. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Cuppens discloses amplifying wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume. *See*, *e.g.,* Cuppens at 1:39-41, 1:44-48, 1:57-59, 2:6-10, 2:48-59, 3:14-30, 3:45-4:3, 5:36-55, 5:56-61, 7:54-60, 7:63-8:43, 8:40-43, 9:1-25, 12:18-22, 12:36-39, 15:2-21, 15:61-67, 18:32-57, 21:15-58, 22:65-23:2, Example 1, Example 2, Claim 1, Claim 6, Figures 2, 3, 4a, 4b, 5a, 5b. For example, Cuppens discloses that "[a]s more and more mutations/variations are found to be involved in disease, there is a need for robust assays in which different DNA regions, that harbor different mutations/variations, are analyzed together. This may be achieved through (more complex) multiple amplification reactions." Cuppens at 1:44-48.

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	As another example, Cuppens discloses a method of amplification wherein "isolated PCR reactors will be generated containing the 2 amplicon specific primers in a tube, so that different amplicon specific KNEX-DNA-crystals, which were prepared separately in a bulk solution, can combined in a single multiplex PCR reaction. Cuppens at 12:18-22. As another example, Cuppens discloses "[i]n a robust multiplex PCR reaction, 5-15 amplicons can be combined." Cuppens at 2:6-10. As another example, Cuppens discloses in Example 2 that "[m]ultiplex amplification of all these amplicons in a single, or limited number of, PCR reaction(s) would thus tender amplicon-sequencing more convenient and cheaper." Cuppens at 22:65-67. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Cuppens also discloses this element for the same reasons.
1[b][iii]. Wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and	Cuppens discloses amplifying wherein one of the amplifying steps introduces a barcode and one or more sequencing tags. *See*, *e.g.,* Cuppens at 6:34-43, 8:3-54, 9:1-18, 9:45-63, 10:32-57, 11:13-26, 19:24-20:11, 20:22-42, 23:2-5, Claim 9, Figures 4b, 5a, 9, 11, 12, 13. For example, Cuppens discloses "the use of sample-ID-barcodes (marker nucleic acids) makes the complete genetic test quality-assured." Cuppens at 10:46-57. As another example, Cuppens discloses introducing "A and B adaptors [that] will allow further manipulation, such as a water-in-oil-emulsion-PCR and pyrosequencing in a GS-FLX sequencing protocol." Cuppens at 9:1-18. As another example, Figure 5a of Cuppens discloses "use of molecular barcode or marker nucleic acid molecules in multiplex parallel sequencing genetic tests." Cuppens at 8:44-47.

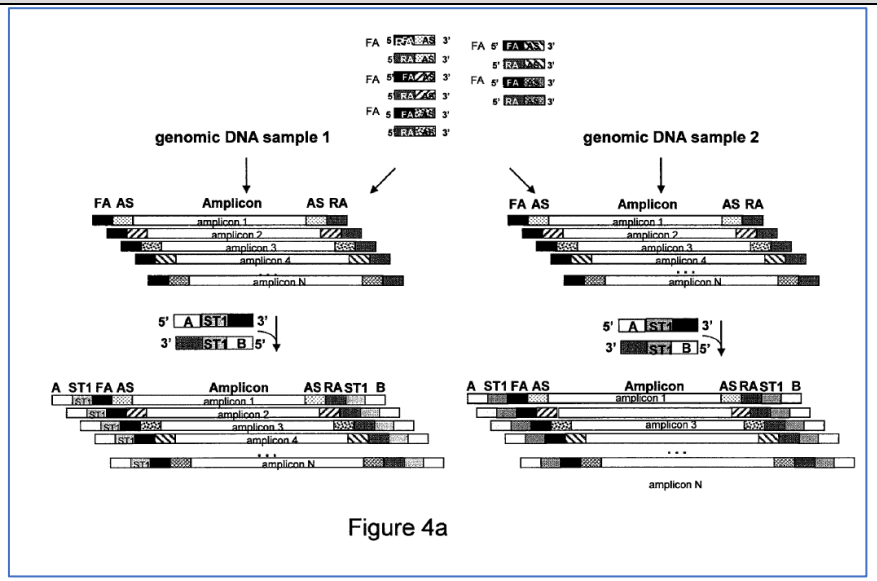
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	As another example, Cuppens discloses introducing sample tags to amplicons through PCR primers as well as using primers to add adaptors for, *e.g.,* "pyrosequencing in a GS-FLX sequencing protocol." Cuppens at 8:37-39. As another example, Cuppens discloses that combining a molecular barcode "with a sample tag will provide an extra control of the process, especially they become covalently linked, since the correct combination of molecular bar code and sample tag should be found at the end of the process which ensures that complete process is quality assured." Cuppens at 20:22-32. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Cuppens also discloses this element for the same reasons.
1[c][i]. sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products,	Cuppens discloses sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products. *See*, *e.g.,* Cuppens at 2:36-48, 8:37-47, 9:1-18, 9:47-62, 11:20-26, 12:36-39, 15:65-67, 20:38-40, 22:50-23:5, 23:61-24:9, Example 2, Claim 6, Figure 5a. For example, Cuppens discloses a method wherein "the number of amplicons that can be multiplexed may exceed 10-100 amplicons." Cuppens at 12:36-39. Further, Cuppens discloses that "this step can be easily transferred to any other gene, and even large or multiple genes." *Id*. As another example, Cuppens discloses that in Example 2 "[p]arallel pyrosequencing in high-density picoliter reactors by the Genome Sequencer . . . allows the determination of millions of nucleotides in a single run hours (2)." Cuppens at 22:50-53. As another example, Figure 5a of Cuppens "shows the use of molecular bar code or marker nucleic acid molecules in multiplex parallel sequencing genetic tests." Cuppens at 8:44-47.
1[c][ii]. Wherein the plurality of SNP loci	The Court construed the term "SNP loci…associated with cancer" to mean "a single nucleotide that may differ between the genomes of two members of the same species and is associated with cancer. The usage of the term

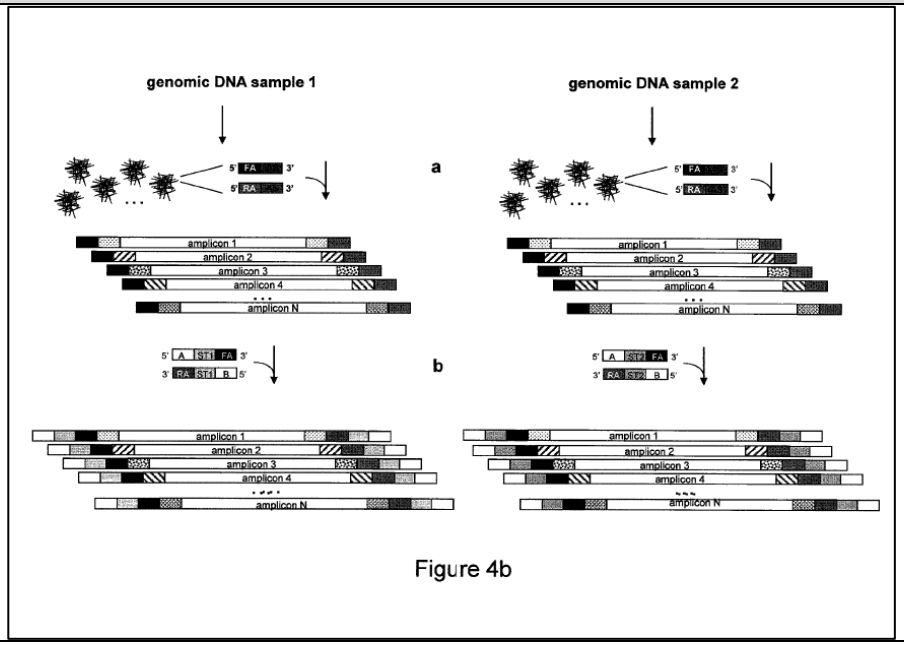
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comprises 25-2,000 loci associated with cancer.	'SNP' should not imply any limit on the frequency with which each variant occurs." Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Cuppens meets this limitation. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Cuppens discloses a plurality of SNP loci comprising 25-2,000 loci associated with cancer. *See*, *e.g.,* Cuppens at 1:33-48, 12:36-39, 7:63-8:43, Figure 4a, Figure 4b, Figure 5a, Figure 5b. For example, Cuppens discloses a method wherein "the number of amplicons that can be multiplexed may exceed 10-100 amplicons." Cuppens at 12:36-39. Further, Cuppens discloses that "this step can be easily transferred to any other gene, and even large or multiple genes." *Id*. As another example, Cuppens discloses using complex "multiplex amplification reactions" to analyze different pathogenic DNA mutations together. Cuppens at 1:33-48. Cuppens also discloses that "[m]utations/variations in the human genome are involved in most diseases" including "acquired diseases such as cancer." *Id*. As another example, Figures 4a, 4b, 5a, and 5b show use of multiplex amplification and/or sequencing of multiple SNP loci

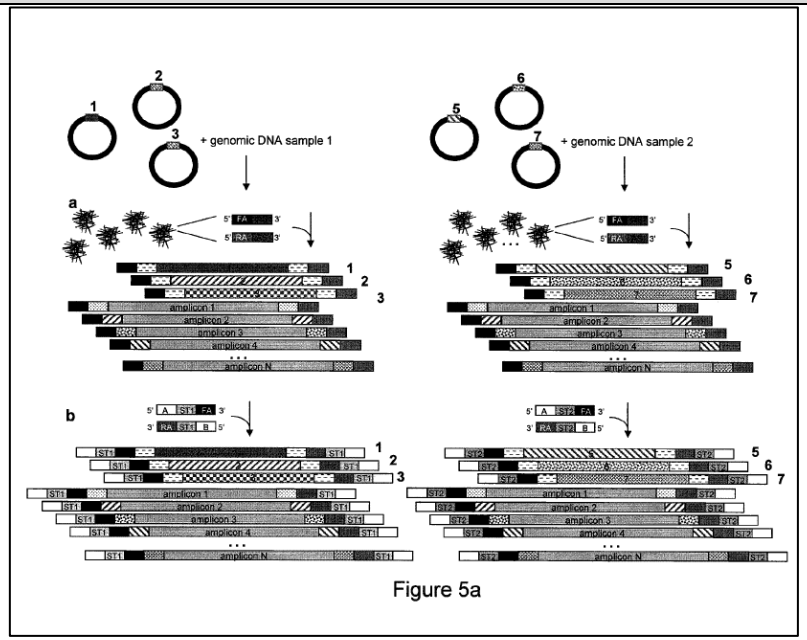
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	 Figure 4a

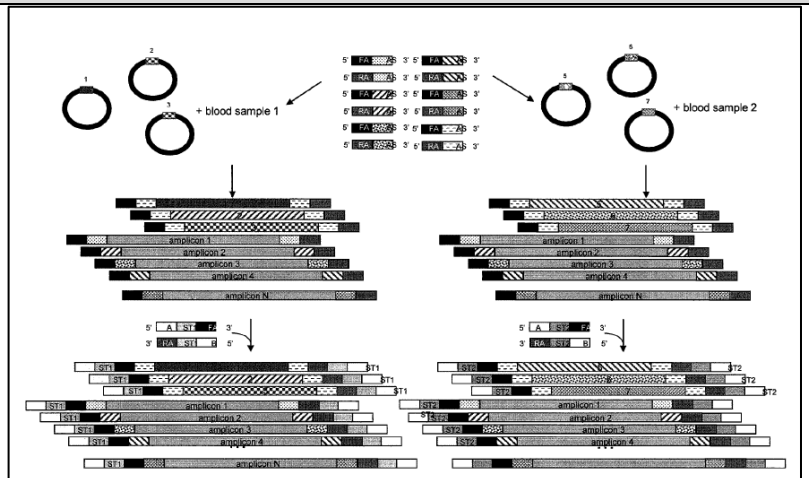
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	 Figure 4b

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	 Figure 5a

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	 Figure 5b To the extent Natera asserts that any portion of this limitation is not disclosed under Natera's apparent interpretation of the Court's construction, it would have been obvious based on the knowledge of a POSA at the time of the alleged invention to look to the Catalogue of Somatic Mutations In Cancer ("COSMIC") database, which identifies hundreds to thousands of gene variants, including those that can be present in the same gene, to readily disclose a plurality of SNP loci comprising 25-2,000 loci associated with cancer. By way of example, COSMIC identifies 6,038 different variants in the TP53 gene alone. *See*, *e.g.*, https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53. In addition, the '035 Patent admits a POSA's "general belief in the art is 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, would have understood that multiplex PCR up to 100 assays could readily be performed on cell free DNA, and would have been able to do so as a matter of routine.

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2. The method of claim 1, wherein tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA.	Cuppens discloses tagging the cell free DNA comprising ligating the one or more universal tail adaptors to the cell free DNA. *See, e.g.*, Cuppens at 22:54-64. For example, Cuppens discloses an illustrative example of a sequencing assay wherein genomic DNA was fragmented, ligated to adaptors, and then amplified with "two universal primers against these adaptors so that all fragments are amplified simultaneously." Cuppens at 22:54-64. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 27. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Cuppens also discloses this element for the same reasons as discussed below regarding claim 13. In addition, to the extent Cuppens does not specifically teach tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA, this limitation is taught by Dong (Ex. 1-A), Porreca (Ex. 1-F), Hoyal, and Wessler. A POSA would be motivated to combine Cuppens with Dong, Porreca, Hoyal, or Wessler because Cuppens expressly teaches using routine and conventional techniques known in the art to analyze sample circulating tumor DNA efficiently, and the references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end. For example, Hoyal teaches "a need for improved extraction methods capable of capturing small nucleic acid molecules"—cell-free DNA—because "the ability to successfully analyze short, fragmented DNA in the presence of more abundant, longer DNA ha[d] remained elusive." Hoyal at 1. As another example, Hoyal teaches "detecting and monitoring extracellular mutant oncogenes or tumor-associated DNA found in the plasma or serum fraction of blood by using DNA extraction with enrichment for mutant DNA," i.e., detecting cell-free tumor DNA. Hoyal at 20. Hoyal solves these problems through adapter-mediated ligation followed by universal pre-amplification before target-specific amplification. Hoyal at Figure 3.

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	For example, Wessler teaches methods for detecting polymorphisms in DNA. Wessler at 3:6-4:41. As another example, Wessler teaches methods for "investigation of genome structure . . . by the use of in vitro methods that detect variation in DNA sequence in the genomes between members of a species" Wessler at 2:26-29. Wessler reaches this goal through Wessler's ligation-mediated preamplification followed by two target-specific rounds of PCR-mediated amplification. Wessler at Figure 1. There is a reasonable expectation of success because the techniques disclosed in Cuppens and the cited references, including multiplex PCR, barcoding DNA fragments, adding sequencing tags, using multiple universal tail adapters, and performing massively parallel sequencing were all routine and conventional at the time for DNA analysis and diagnostics. Furthermore, the methods and techniques disclosed in the cited references are readily compatible with the methods of Cuppens. For example, Hoyal's method of tagging cell-free tumor DNA with universal adapters then performing universal preamplification of tagged products through PCR using primers against the adapters is readily compatible with Cuppens' PCR-based tagging methods. For example, Wessler's method of ligating double-stranded universal adaptors to target DNA then performing two rounds of target-specific amplification is readily compatible with Cuppens' PCR-based tagging methods.
12. The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.	Cuppens discloses one or more universal tail adaptors comprising a first universal tail adaptor and a second universal tail adaptor. *See, e.g.*, Cuppens at 3:45-4:37, 5:25-6:7, 8:60-67, 9:1-18, 12:10-39, 14:54-15:21, 16:34-18:6, 18:7-48, Example 2, Claim 1, Figures 4a, 4b, 5a, 5b. For example, Cuppens discloses a general method for tagging DNA wherein "[e]ach forward primer contains an amplicon-specific region (AS) and a universal forward adaptor (FA), analogously the reverse primers contain an AS region and another universal reverse adaptor (RA)". Cuppens at 8:60-67. "All PCR fragments will then carry identical sequences at their ends" *Id.* As another example, Cuppens discloses that "adaptor tag primers can be used on any amplicon, irrespective of the genomic region, or gene, from which it was derived, if all first step PCR reactions are performed with adaptor-amplicon specific primers that contain the same 'universal' forward and reverse adaptors." Cuppens at 16:56-17:23.

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13. The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.	Under Natera's apparent construction, Cuppens discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See, e.g.*, Cuppens at 3:45-4:37, 5:25-6:7, 8:60-67, 9:1-18, 12:10-39, 14:54-15:21, 16:34-18:6, 18:7-48, 22:54-64, Example 2, Claim 1, Figures 4a, 4b, 5a, 5b. For example, Cuppens discloses a general method for tagging DNA wherein "[e]ach forward primer contains an amplicon-specific region (AS) and a universal forward adaptor (FA), analogously the reverse primers contain an AS region and another universal reverse adaptor (RA)". Cuppens at 8:60-67. As another example, Cuppens discloses that "adaptor tag primers can be used on any amplicon, irrespective of the genomic region, or gene, from which it was derived, if all first step PCR reactions are performed with adaptor-amplicon specific primers that contain the same 'universal' forward and reverse adaptors." Cuppens at 16:56-17:23. As another example, Cuppens discloses a sequencing assay wherein "total genomic DNA is randomly fragmented to 25-500 bp fragments," then the sample is tagged with universal adaptors and "[a]mpification is then performed with two universal primers against these adaptors., so that all fragments are amplified simultaneously. Cuppens at 22:54-64. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Cuppens also discloses this element for the same reasons as discussed above regarding claim 2.

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EXHIBIT 1-C – FINAL INVALIDITY CLAIM CHART FOR U.S. PATENT NO. 11,519,035

Reference:
International Patent Publication No. 2010/115154 ("May PCT")

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

To the extent Natera asserts that May PCT does not disclose or teach one or more limitations of asserted claims 1, 2, 12, or 13, performing the methods as claimed would have been obvious to a person of ordinary skill in the art at the time of the alleged invention considering May PCT in combination with the knowledge of a POSA and/or one or more of the following references:

- International Patent Publication No. 2007/018601("Makarov")
- International Patent Publication WO 2010/126614 A2 ("Porreca")
- Shapero et al., *MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density DNA oligonucleotide arrays*, 32 NUCLEIC ACIDS RES. e101 (2004) ("Shapero")
- U.S. Patent Application Publication US 2004/0110153 A1 ("Dong")

For example, a POSA would have been motivated to combine May PCT with Makarov because, while May PCT discloses ligation of adaptors to fragmented DNA and amplification of random-sequence inserts using said adaptors in the context of DNA sequencing of the targets, Makarov discloses ligating "a universal adapter to one or both DNA ends" prior to PCR. Makarov at [0006]; see also id. at [0022] ("[P]reparation of DNA libraries for subsequent amplification and analysis involves ligation of adaptor sequences to DNA ends to introduce a priming stie for the PCR-mediated whole genome amplification and ligation-mediated PCR."); id. at Fig. 1A (disclosing the typical current adaptor attachment procedure in six steps) (reproduced below); id. at [0005] ("The usefulness of adapted DNA molecules is illustrated by but not limited to several examples, such as ligation-mediated locus-specific PCR, ligation-mediated whole genome amplification . . .").

A POSA would reasonably expect to succeed in combining May PCT with Makarov for the purposes of ligating one or more universal tail adapters to the cell free DNA because May PCT explains that its methods "can be substituted for ligation, to introduce adapter sequences." May PCT at [0112].

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Further, Porreca also discloses that "adapters may be ligated onto the ends of the molecules of interest. Adapters often contain PCR primer sites (for amplification or emulsion PCR) and/or sequencing primer sites. In addition, barcodes may be included, for example, to uniquely identify individual samples (e.g., patient samples) that may be mixed together. (See, e.g., USPTO Publication Number US 2007/0020640 Al (McCloskey et al.)." Porreca at 43:15–20. See also id. at FIG. 4C; id. at 43:23–25 (teaching that attachment of sequence tags by ligation is a "flexible method" because molecules "can be ligated to any blunt-ended nucleic acids"). For the same reasons as above, a POSA would have been motivated to combine May PCT with Porreca and would have reasonably expected to succeed at doing so.

Additionally, a POSA would have been motivated to combine May PCT with Shapero because Shapero expressly teaches a method of tagging DNA comprising ligating the one or more universal tail adapters, and a POSA would recognize that ligation-based DNA tagging methods are interchangeable with PCR-based DNA tagging methods. For example, Shapero discloses a method that includes ligation of a universal adaptor (A2) to fragmented genomic DNA. Shapero at 4, Figure 1. Further, a POSA would reasonably expect to succeed in combining May PCT with Shapero because Shapero's MARA method is a generally applicable approach to locus-specific DNA target preparation for highly multiplexed SNP genotyping, and has been known in the art for nearly 20 years.

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Claim	**Prior Art**
1[pre]. A method for amplifying and sequencing DNA, comprising:	To the extent the preamble is construed to be limiting, May PCT discloses a method for amplifying and sequencing DNA. *See, e.g.,* May PCT at [0002], [0011], [0026], [0121], [0243], [0262], Example 1, Example 5, Claim 1, Claim 12, Claim 13, Claim 14, Claim 15, Claim 17, Claim 29, Claim 30, Claim 39, Claim 41. For example, May PCT discloses the field of invention as relating "generally to the area of high-throughput assays for detection and/or sequencing of particular target nucleic acids" and that the invention "provides amplification methods in which nucleotide tag(s) and a barcode nucleotide sequence are added to target nucleotide sequences. May PCT at [0002]. May PCT discloses methods including amplifying DNA. For example, May PCT discloses a method "for amplifying, tagging, and barcoding a plurality of target nucleic acids in a plurality of samples" and provides that each "amplification mixture is subjected to amplification to produce a plurality of target amplicons, wherein each target amplicon includes a tagged target nucleotide sequence, with first and/or second

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	nucleotide tags flanking the target nucleotide sequence, and at least one barcode nucleotide sequence at the 5' or 3' end of the target amplicon." *Id.* at [0011]. As another example, May PCT discloses a method wherein "[g]enomic DNA samples . . . were amplified for 25 cycles 7900HT Fast Real-Time PCR System." *Id.* at [0243] (Example 1); *see also id.* at [0262] (Example 5) ("Sets of primer pairs were designed to amplify specific regions from the ECFR and MET genes."). May PCT discloses methods also including sequencing DNA. For example, May PCT discloses that "the methods herein can, optionally, include subjecting at least one target amplicon to DNA sequencing." *Id.* at [0026]; *see also id.* at [0121] ("The methods of the invention can include subjecting at least one target amplicon to DNA sequencing using any available DNA sequencing method. In particular embodiments, a plurality of target amplicons is sequenced using a high throughput sequencing method."); *id.* at Claim 39 ("The method of claim 37 or 38, additionally comprising subjecting at least one target amplicon to DNA sequencing."). As another example, May PCT discloses a method wherein "amplicons generated from PCR amplification were purified . . . and then re-amplified . . . , followed by Sanger sequencing with either 454 tail primers, which showed that the 3-primer method generated an amplicon having the correct sequence." *Id.* at [0234] (Example 1); *see also id.* at [0271] (Example 5) ("The purified product pool was subjected to emulsion PCR followed by pyrosequencing on a 454 FLX sequencer (Roche Analytical Sciences) according to manufacturer's instructions.").
1[a][i]. tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,	May PCT discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See, e.g.,* May PCT at [0002], [0011], [0018], [0050], [0055], [0103], [0107], [0132], [0247], [0248], [0273], Figure 15A, Figure 15B, Figure 18, Example 3, Example 4, Example 5, Example 6, Claim 1, Claim 2, Claim 3, Claim 6, Claim 9, Claim 13, Claim 17. For example, May PCT discloses that "the present invention provides amplification methods in which nucleotide tag(s) and a barcode nucleotide sequence are added to target nucleotide sequences." May PCT at

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Claim	**Prior Art**
	[0055]. Further, May PCT discloses that "[n]ucleic acids can be extracted or amplified from cells, bodily fluids (e.g., blood, a blood fraction, urine, etc.)" and that "[i]llustrative samples include samples of plasma, serum . . . [or] samples of tears, saliva, blood cells, stem cells, or tumors. For example, samples of fetal DNA can be obtained from an embryo or from maternal blood." *Id.* at [0132]. As another example, May PCT discloses tagging target DNA sequences. *See, e.g., id.* at Figure 18. As another example, May PCT discloses that in certain embodiments "amplification can be performed separately for each sample, wherein the same set of forward and reverse primers is used for each sample and the set of forward and reverse primers has at least one nucleotide tag that is common to all primers in the set" and that a "different barcode primer can be used for each sample, wherein the bar code primers have different barcode nucleotide sequences, but the same tag-specific portion that can anneal to the common nucleotide tag." *Id.* at [0103]. May PCT also discloses that "the present invention provides amplification methods in which nucleotide tag(s) and a barcode nucleotide sequence are added to target nucleotide sequences." *Id.* at [0002]; *see also id.* at [0011] ("In certain embodiments, the invention provides a method for amplifying, tagging, and barcoding a plurality of target nucleic acids in a plurality of samples."); *id.* at [0018] (disclosing that in "other embodiments, the invention provides a method in which barcoding is, optionally, omitted and the target nucleotide sequences are tagged after amplification" and that the "target nucleotide sequences are then tagged (e.g., by ligation of nucleotide tags unto one or both ends of the target nucleotide sequences) to produce a plurality of target amplicons"). As another example, May PCT discloses an example of a multi-primer reaction set-up using 4 outer primers with different combinations of primer binding site and nucleotide tags." *See id.* at [0050], Figs. 15A and 15B, Example 5. As another example, May PCT discloses a method "for amplifying a plurality of target nucleotides in which barcoding is, optionally, omitted and the target nucleotide sequences are tagged after amplification" in which

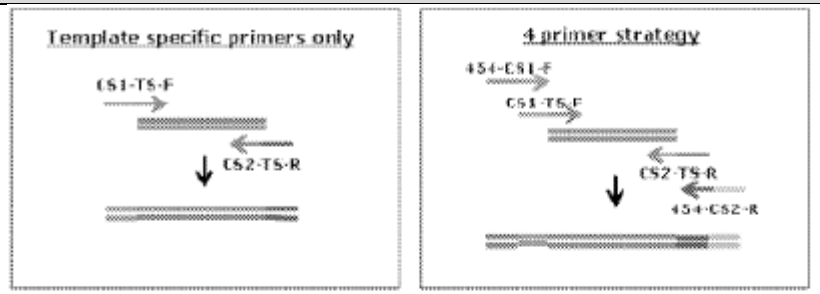
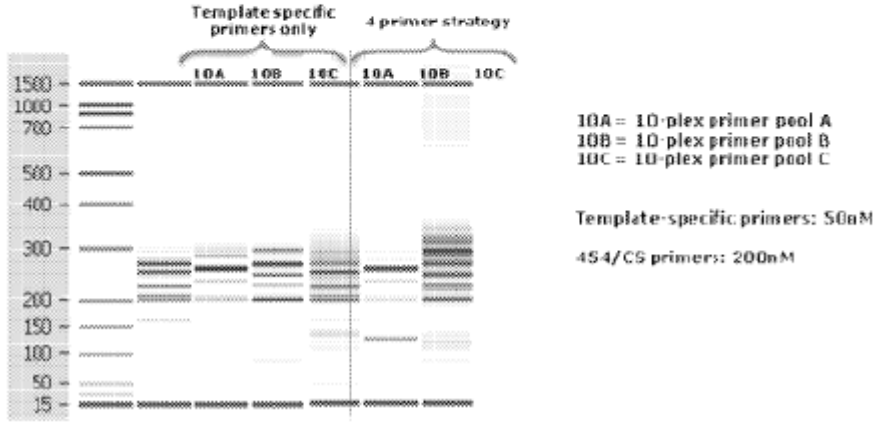
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Claim	**Prior Art**
	the "target nucleotide sequences are then tagged to produce a plurality of target amplicons, each including first and/or second nucleotide tags flanking the target nucleotide sequence." *Id.* at [0107]. *See also id.* at [0247] (Example 3); [0248] (Example 4) ("In addition to the target-specific regions, the primers were designed to contain additional tag sequences at the 5' end."); [0273] (Example 6) ("Sequences designed for a 4-primer tagging scheme to be used on the Illumina Genome Analyzer II are shown in Tables 13 and 14.").
1[a][ii]. wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;	May PCT discloses tagging cell free DNA isolated from a blood sample collected from a subject who is not a pregnant women. *See, e.g.,* May PCT at [0132], [0135], [0235], [0236], Example 4. For example, May PCT discloses that "[n]ucleic acids can be extracted or amplified from cells, bodily fluids (e.g., blood, a blood fraction, urine, etc.)" and that "[i]llustrative samples include samples of plasma" and "serum." May PCT at [0132]. As another example, May PCT discloses that "The targets can include, for example, nucleic acids associated with pathogens, such as viruses, bacteria, protozoa, or fungi; RNAs, e.g., those for which over-or under-expression is indicative of disease, those that are expressed in a tissue-or developmental-specific manner; or those that are induced by particular stimuli; genomic DNA, which can be analyzed for specific polymorphisms (such as SNPs), alleles, or haplotypes, e.g., in genotyping. Of particular interest are genomic DNAs that are altered (e.g., amplified, deleted, and/or mutated) in genetic diseases or other pathologies; sequences that are associated with desirable or undesirable traits; and/or sequences that uniquely identify an individual (e.g., in forensic or paternity determinations)." May PCT at [0135]. As another example, May PCT discloses that "these methods can also be employed in determinations DNA or RNA copy number. Determinations of aberrant DNA copy number in genomic DNA is useful, for example, in the diagnosis and/or prognosis of genetic defects and diseases, such as cancer." May PCT at [0235].

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Claim	**Prior Art**
	As another example, May PCT discloses that "Target-specific primers were designed for 48 genomic regions associated with prostate cancer. In addition to the target-specific regions, the primers were designed to contain additional tag sequences at the 5' end." May PCT at [0248].
1[b][i]. amplifying the tagged products one or more times to generate final amplification products,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, May PCT discloses amplifying the tagged products one or more times to generate final amplification products. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.,* May PCT at [0011], [0102], [0100], [0103], [0115], [0123], [0125], [0276], Example 1, Example 4, Example 5, Example 8, Figure 18, Claim 1, Claim 12, Claim 13, Claim 14, Claim 15, Claim 17, Claim 29, Claim 32, Claim 41, Claim 42, Claim 43, and Claim 44. For example, May PCT discloses the results of PCR reactions of three pools of 10 sets of PCR primers when the PCR reactions were run for template-specific primers only and in 4-primer mode; these results generally show tagging target DNA sequences and amplification of tagged products. May PCT at [0276] (Example 8); *id.* at Figure 18.

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Claim	**Prior Art**
	  As another example, May PCT discloses "an amplification method for introducing a plurality (e.g., at least three) of selected nucleotide sequences into one or more target nucleic acid(s)," which "entails amplifying a plurality of target nucleic acids, typically, in a plurality of samples." *Id.* at [0100]. As another example, May PCT discloses that each "amplification mixture is subjected to amplification to produce a plurality of target amplicons, wherein each target amplicon includes a tagged target nucleotide sequence, with first and/or second nucleotide tags flanking the target nucleotide sequence, with first and/or

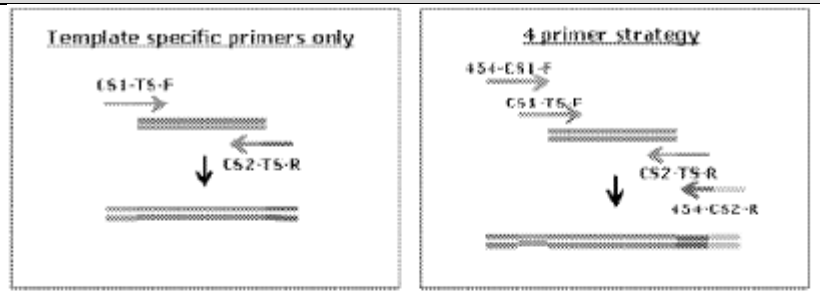
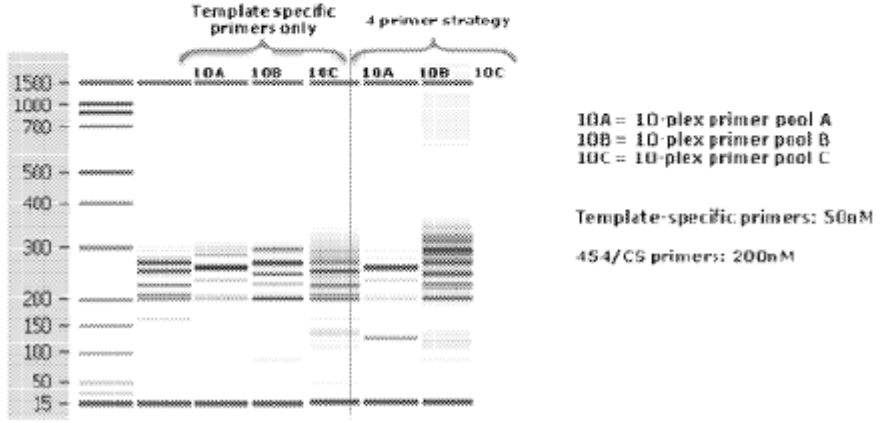
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Claim	**Prior Art**
	second nucleotide tags flanking the target nucleotide sequence, and at least one barcode nucleotide sequence at the 5' or 3' end of the target amplicon." *Id.* at [0011]. As another example, May PCT discloses that each "amplification mixture can be subjected to amplification to produce target amplicons comprising tagged target nucleotide sequences, each comprising first and second nucleotide tags flanking the target nucleotide sequence, and at least one barcode nucleotide sequence at the 5' or 3' end of the target amplicon (relative to one strand of the target amplicon)." *Id.* at [0102]. As another example, May PCT discloses a method wherein "the amplification produces a set of . . . amplicons from each sample that bear sample-specific barcodes." *Id.* at [0103]. As another example, May PCT discloses that in "certain embodiments, two of the forward primer, reverse primer, and barcode primer include at least one additional primer binding site (i.e., such that the amplicon produced upon amplification includes the nucleotide tag sequences, the barcode nucleotide sequence and the two additional binding sites)." *Id.* at [0115]. As another example, May PCT discloses embodiments in which "[a]mplification products produced in each well would be labeled uniquely with the combinations of barcode sequences loaded into these wells." *Id.* at [0123]. As another example, May PCT discloses a method wherein "digital amplification can be carried out after preamplification of sample nucleic acids." *Id.* at [0125]. *See also* May PCT at [0243] (Example 1) ("Genomic DNA samples . . . were amplified for 25 cycles . . ."); *id.* at [0248]–[0261] (Example 4); *id.* at [0262] (Example 5) ("Sets of primer pairs were designed to amplify specific regions from the EGFR and MET genes."). Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. See Natera's Preliminary Infringement

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Claim	**Prior Art**
	Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, May PCT also discloses this element for the same reasons.
1[b][ii]. wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, May PCT discloses amplifying wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.,* May PCT at [0055], [0119], [0135], [0210], [0235], [0276], Example 8, Figure 18. For example, May PCT discloses the results of PCR reactions of three pools of 10 sets of PCR primers when the PCR reactions were run for template-specific primers only and in 4-primer mode; these results generally show tagging target DNA sequences and amplification of tagged products. May PCT at [0276] (Example 8); *id.* at Figure 18.

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Claim	**Prior Art**
	  As another example, May PCT discloses that targets of its method can include "genomic DNA, which can be analyzed for specific polymorphisms (such as SNPs), alleles, or haplotypes, e.g., in genotyping. Of particular interest are genomic DNAs that are altered (e.g., amplified, deleted, and/or mutated) in genetic diseases or other pathologies." May PCT at [0135]. As another example, May PCT discloses methods that "are applicable to any technique aimed at detecting the presence or amount of one or more target nucleic acids in a nucleic acid sample. Thus, for example,

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Claim	**Prior Art**
	these methods are applicable to identifying the presence of particular polymorphisms (such as SNPs). . ." May PCT at [0235]. May PCT also discloses carrying out targeted amplification in a single reaction volume. For example, May PCT discloses a method wherein "multiplex detection is carried out in individual amplification mixture, e.g., in individual reaction chambers of a microfluidic device, which can be used to further increase the number of samples and/or targets that can be analyzed in a single assay or to carry out comparative methods, such as comparative genomic hybridization (CGH). In various embodiments, up to 2, 3, 4, 5, 6, 7, 8, 9, 10, 50, 100, 500, 1000, 5000, 10000 or more amplification reactions are carried out in each individual reaction chamber." May PCT at [0210]. As another example, May PCT discloses that "[t]aging and/or barcoding target nucleotide sequences can increase the number of samples that can be analyzed for one or multiple targets in a single assay, while minimizing increases in assay cost." May PCT at [0055]; *see also id.* at [0119] ("[M]ore than four primers can be combined in a single reaction to append different combinations of additional primer binding sites, barcode sequences, and nucleotide tags."). Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. See Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, May PCT also discloses this element for the same reasons.
1[b][iii]. wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and	May PCT discloses amplifying wherein one of the amplifying steps introduces a barcode and one or more sequencing tags. *See, e.g.,* May PCT at [0002], [0011], [0013], [0014], [0015], [0016], [0017], [0055], [0100], [0105], [0119], Claim 1, Claim 6, Claim 8, Claim 13, Claim 32.

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U.S. Patent No. 11,519,035	
Claim	**Prior Art**
	For example, May PCT discloses that "the present invention provides amplification methods in which nucleotide tag(s) and, optionally, a barcode nucleotide sequence are added to target nucleotide sequences." May PCT at [0002]; *see also id.* at [0055]; *id.* at [0011] (disclosing that "[e]ach amplification mixture includes: . . . at least one barcode primer including a barcode nucleotide sequence and a first and/or second nucleotide tag-specific portion, wherein the barcode primer is in excess of the forward and/or reverse primer(s)" and that "[e]ach amplification mixture is subjected to amplification to produce a plurality of target amplicons, wherein each target amplicon includes a tagged target nucleotide sequence, with first and/or second nucleotide tags flanking the target nucleotide sequence, and at least one barcode nucleotide sequence at the 5' or 3' end of the target amplicon."). As another example, May PCT discloses numerous embodiments of the "tagging/barcode method." *See* May PCT at [0013]–[0017]. As another example, May PCT discloses that the "nucleotide tag for the forward primer can have a sequence that is the same as, or different from, the nucleotide tag for the reverse primer" and that the "third primer is a barcode primer comprising a barcode nucleotide sequence and a first and/or second nucleotide tag-specific portion." May PCT at [0100]. As another example, May PCT discloses that "amplification is generally carried out for at least three cycles to introduce the first and second nucleotide tags and the barcode nucleotide sequence." May PCT at [0105]. As another example, May PCT discloses that "more than four primers can be combined in a single reaction to append different combinations of additional primer binding sites, barcode sequences, and nucleotide tags." May PCT at [0119]. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. See Natera's Preliminary Infringement

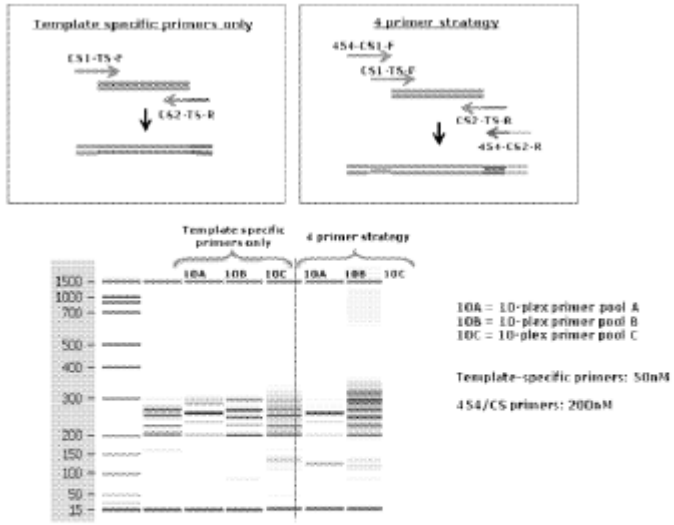
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U.S. Patent No. 11,519,035	
Claim	**Prior Art**
	Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, May PCT also discloses this element for the same reasons.
1[c][i]. sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products,	May PCT discloses sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products. *See, e.g.*, May PCT at [0259], Example 4, Figure 14A, Figure 14B, Claim 39. For example, May PCT discloses an embodiment wherein targeted sequences associated with prostate cancer were amplified using the 4-primer method, followed by "pyrosequencing on a 454 FLX sequencer (Roche Analytical Sciences) according to manufacturer's instructions. The sequence file output by the sequencer was then analyzed for the presence of barcoded PCR products." May PCT at [0259], Example 4. May PCT discloses the results of this sequencing in Figures 14A and 14B. As another example, May PCT claims a method "additionally comprising subjecting at least one target amplicon to DNA sequencing." May PCT at Claim 39.
1[c][ii]. wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.	The Court construed the term "SNP loci…associated with cancer" to mean "a single nucleotide that may differ between the genomes of two members of the same species and is associated with cancer. The usage of the term 'SNP' should not imply any limit on the frequency with which each variant occurs." Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, May PCT discloses a plurality of SNP loci comprising 25-2,000 loci associated with cancer. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.*, May PCT at [0210], [0235], [0248]; Table 9; Example 8.

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Claim	**Prior Art**
	For example, May PCT discloses methods that are "applicable to identifying the presence of particular polymorphisms (such as SNPs), alleles, or haplotypes, or chromosomal abnormalities, such as amplifications, deletions, or aneuploidy." May PCT at [0235]. Further, May PCT discloses that "up to 2, 3, 4, 5, 6, 7, 8, 9, 10, 50, 100, 500, 1000, 5000, 10000 or more amplification reactions are carried out in each individual reaction chamber," each corresponding to a locus associated with cancer. *Id.* at [0210]. As another example, May PCT discloses that "[t]arget-specific primers were designed for 48 genomic regions associated with prostate cancer," also demonstrating disclosure of 25–2,000 loci associated with cancer. May PCT at [0248]. As another example, May PCT discloses performing three multiplex PCR reactions with ten primer pairs each, wherein each of the resulting amplicons is in a common cancer gene (*see* May PCT at 95–99, Table 9) such that most if not all amplicons contain more than 25 "loci associated with cancer." May PCT at [0276] (Example 8). May PCT discloses the results of these PCR reactions in Figure 18:

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Claim	**Prior Art**
	 ***Fig. 18*** To the extent Natera asserts that any portion of this limitation is not disclosed under Natera's apparent interpretation of the Court's construction, it would have been obvious based on the knowledge of a POSA at the time of the alleged invention to look to the Catalogue of Somatic Mutations In Cancer ("COSMIC") database, which identifies hundreds to thousands of gene variants, including those that can be present in the same gene, to readily disclose a plurality of SNP loci comprising 25-2,000 loci associated with cancer. By way of example, COSMIC identifies 6,038 different variants in the TP53 gene alone. *See*, *e.g.,* https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53. In addition, the '035 Patent admits a POSA's "general belief in the art is 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

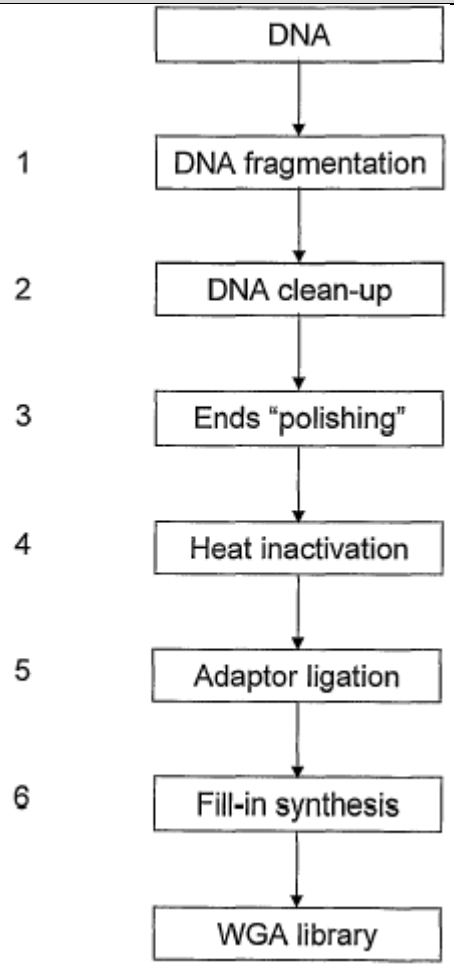
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	patent admits that a POSA would have been aware of how to perform multiplex PCR up to 100 assays in a single well, would have understood that multiplex PCR up to 100 assays could readily be performed on cell free DNA, and would have been able to do so as a matter of routine.
2. The method of claim 1, wherein tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA.	May PCT discloses tagging the cell free DNA comprising ligating the one or more universal tail adapters to the cell free DNA. *See, e.g.*, May PCT at [0018], [0081], [0085] [0112]. For example, May PCT discloses that "Each amplification mixture is subjected to amplification to produce a plurality of target nucleotide sequences. The target nucleotide sequences are then tagged (e.g., by ligation of nucleotide tags unto one or both ends of the target nucleotide sequences) to produce a plurality of target amplicons." May PCT at [0018]. As another example, May PCT discloses that "[n]ucleotide tags can also be added by an "encoding ligation" reaction that can comprise a ligation reaction in which at least one primer comprises a target specific portion and nucleotide tag located on the 5' end of the target-specific portion, and a second primer that comprises a target-specific portion only or a target-specific portion and a nucleotide tag located on the 5' end of the target specific portion. Illustrative encoding ligation reactions are described, for example, in U.S. Patent Publication No. 2005/0260640, which is hereby incorporated by reference in its entirety, and in particular for ligation reactions." May PCT at [0081] As another example, May PCT discloses that ligation of adaptors to fragmented DNA and amplification of random-sequence inserts using said adapters. May PCT at [0112]. To the extent Natera asserts that May PCT does not teach tagging the cell free DNA comprising ligating the one or more universal tail adapters to the cell free DNA, performing the method as claimed would have been obvious to a person of ordinary skill in the art at the time of the alleged invention considering May PCT in combination with the knowledge of a POSA, Makarov, Shapero, Porreca, Dong, and/or Oliphant.

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	For example, a POSA would have been motivated to combine May PCT with Makarov because, while May PCT discloses ligation of adaptors to fragmented DNA and amplification of random-sequence inserts using said adaptors in the context of DNA sequencing of the targets, Makarov discloses ligating "a universal adapter to one or both DNA ends" prior to PCR. Makarov at [0006]; *see also id.* at [0022] ("[P]reparation of DNA libraries for subsequent amplification and analysis involves ligation of adaptor sequences to DNA ends to introduce a priming stie for the PCR-mediated whole genome amplification and ligation-mediated PCR."); *id.* at Fig. 1A (disclosing the typical current adaptor attachment procedure in six steps) (reproduced below); *id.* at [0005] ("The usefulness of adapted DNA molecules is illustrated by but not limited to several examples, such as ligation-mediated locus-specific PCR, ligation-mediated whole genome amplification . . ."). For example, a POSA would recognize that Makarov's universal adapter ligation methods would allow for universal amplification of DNA from a given sample, which would provide the advantage of increasing target DNA abundance in preparation for use with the methods of May PCT. Further, a POSA would reasonably expect to succeed in combining May PCT with Makarov for the purposes of ligating one or more universal tail adapters to the cell free DNA because May PCT explains that its methods "can be substituted for ligation, to introduce adapter sequences." May PCT at [0112].

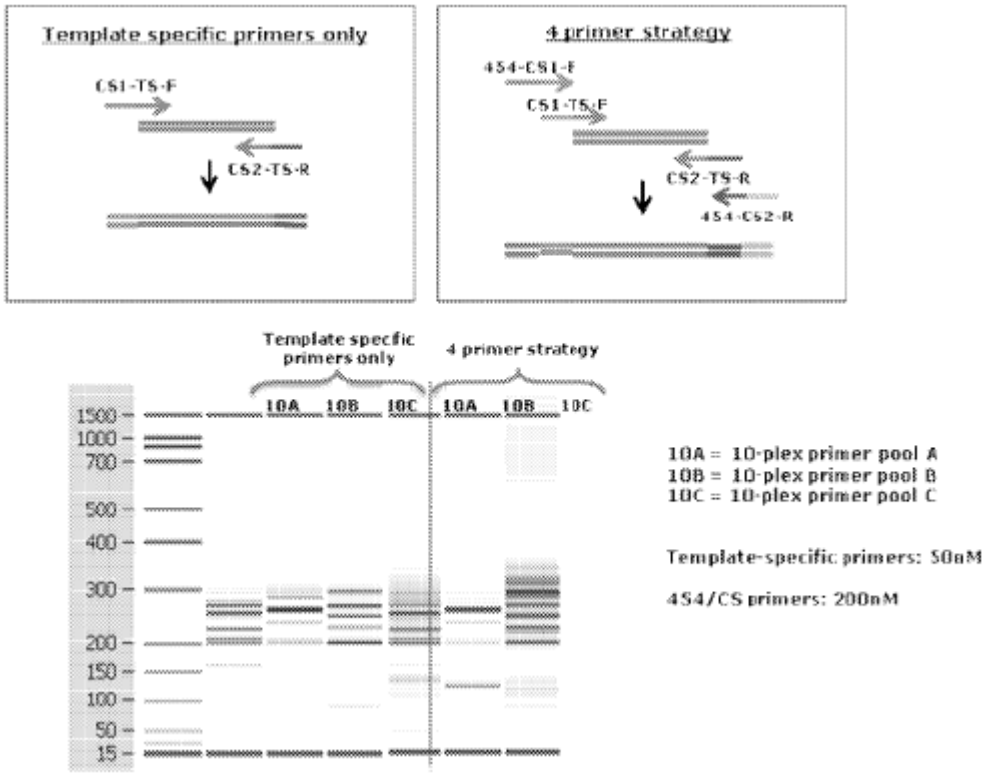
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	 Further, Porreca also discloses that "adapters may be ligated onto the ends of the molecules of interest. Adapters often contain PCR primer sites (for amplification or emulsion PCR) and/or sequencing primer sites. In addition, barcodes may be included, for example, to uniquely identify individual samples (e.g.,

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	patient samples) that may be mixed together. (See, e.g., USPTO Publication Number US 2007/0020640 Al (McCloskey et al.)." Porreca at 43:15-20. See also *id.* at FIG. 4C; *id.* at 43:23–25 (teaching that attachment of sequence tags by ligation is a "flexible method" because molecules "can be ligated to any blunt-ended nucleic acids"). For the same reasons as above, a POSA would have been motivated to combine May PCT with Porreca and would have reasonably expected to succeed at doing so. Additionally, a POSA would have been motivated to combine May PCT with Shapero because Shapero expressly teaches a method of tagging DNA comprising ligating the one or more universal tail adapters. For example, Shapero discloses a method that includes ligation of a universal adaptor (A2) to fragmented genomic DNA. Shapero at 4, Figure 1. Further, a POSA would reasonably expect to succeed in combining May PCT with Shapero because Shapero's MARA method is a generally applicable approach to locus-specific DNA target preparation for highly multiplexed SNP genotyping, and has been known in the art for nearly 20 years.
12. The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.	May PCT discloses one or more universal tail adaptors comprising a first universal tail adaptor and a second universal tail adaptor. *See, e.g.*, May PCT at [0103], Figure 18; *see also* Claim 2 above. For example, May PCT discloses that "amplification can be performed separately for each sample, wherein the same set of forward and reverse primers has at least one nucleotide tag that is common to all primers in the set." May PCT at [0103]. As another example, May PCT discloses tagging of target sequences with primers comprising universal sequences CS1 and CS2 (i.e., a first and second universal tail adaptor). *Id.* at Figure 18.

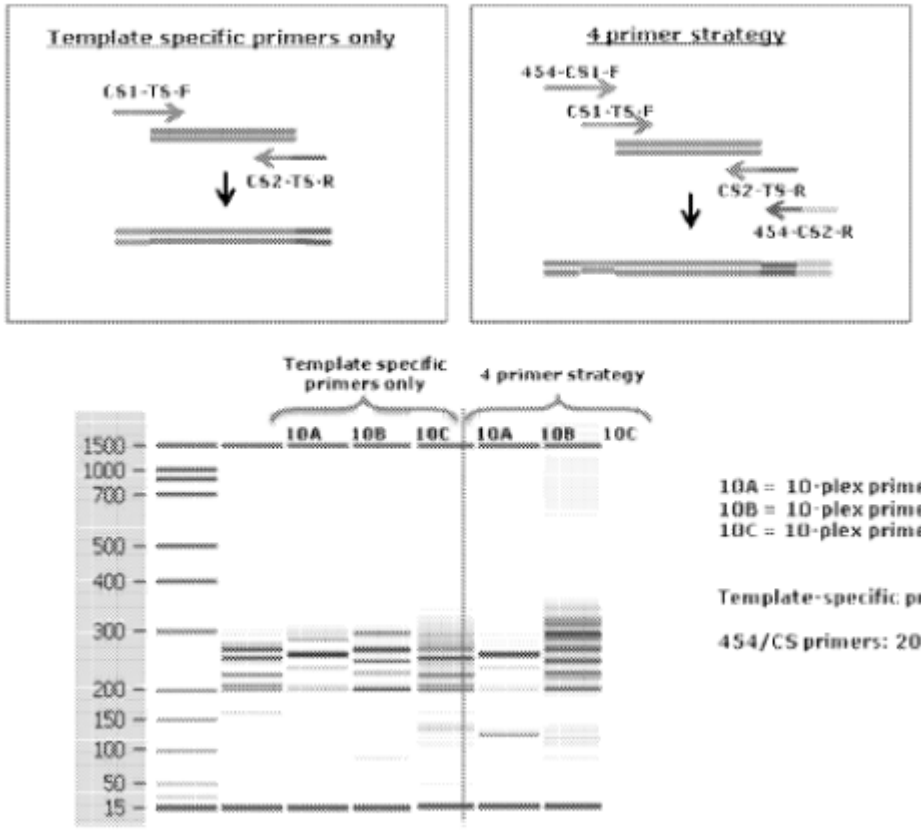
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13. The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first	May PCT discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See, e.g.,* May PCT at [0103], Figure 18; *see also* Claim 2 above. For example, May PCT discloses that "amplification can be performed separately for each sample, wherein the same set of forward and reverse primers I sued for each sample and the set of forward and reverse primers has at least one nucleotide tag that is common to all primers in the set" and that a "different barcode

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universal tail adaptor and a second primer comprising the second universal tail adaptor.	primer can be used for each sample, wherein the bar code primers have different barcode nucleotide sequences, but the same tag-specific portion that can anneal to the common nucleotide tag." May PCT at [0103]. As another example, May PCT discloses tagging of target sequences with primers comprising universal sequences CS1 and CS2 (i.e., a first and second universal tail adaptor). *Id.* at Figure 18.

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EXHIBIT 1-D – FINAL INVALIDITY CLAIM CHART FOR U.S. PATENT NO. 11,519,035

Reference:
International Patent Publication WO 2011/142836 A2 ("Pieprzyk")

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

To the extent Natera asserts that Pieprzyk does not disclose or teach one or more limitations of Claims 1, 2, 12 or 13, performing the methods as claimed in those claims would have been obvious to a person of ordinary skill in the art at the time of the alleged invention considering Pieprzyk in combination with the knowledge of a POSA and/or one or more of the following references:

- International Patent Publication No. 2007/018601("Makarov")
- International Patent Publication WO 2010/126614 A2 ("Porreca")
- Shapero et al., *MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density DNA oligonucleotide arrays*, 32 NUCLEIC ACIDS RES. e101 (2004) ("Shapero")
- U.S. Patent Application Publication US 2004/0110153 A1 ("Dong")
- International Publication No. WO 2010/115154 ("May PCT")

A POSA would have been motivated to combine Pieprzyk with one or more of the foregoing references because Pieprzyk expressly teaches its "amplification-based methods for detection of genotype, mutations, and/or aneuploidy" have "broad applicability." Pieprzyk at Abstract. Pieprzyk also invites the use of other methods by describing "general approaches for increasing the accuracy and/or precision." Pieprzyk at [106]-[110]. For example, Pieprzyk discloses "Assay methods employing small amplicons are useful for applications aimed at determining copy number in samples containing fragmented nucleic acids, as is the case, e.g., for cell-free fetal DNA in a maternal bodily fluid (e.g., plasma), cell-free DNA in the bodily fluid (e.g., plasma) of subjects with cancer, or DNA from formalin-fixed paraffin-embedded tissue." Pieprzyk at [0111]. As another example, Pieprzyk discloses, "Any method of detection and/or quantification of nucleic acids can be used in the invention to detect amplification products." Pieprzyk at [0158]. Further, a POSA would reasonably expect to succeed in combining Pieprzyk with one or more of the foregoing references because methods for combining the methods described were known in the art and would not have required undue experimentation.

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1[pre]. A method for amplifying and sequencing DNA, comprising:	To the extent the preamble is construed to be limiting, Pieprzyk discloses a method for amplifying and sequencing DNA. *See, e.g.,* Pieprzyk at pages [0002], [0004], [0030], [0091], [0185-0188], [0200], [0206], [0207], [0223], Example 6, Example 7, Example 8, Example 9, Example 10, Example 12, Example 13, Example 14, Claim 27, Figures 3C, 4A, 4B, 5B, 8A, 8B, 9, 10, 11. For example, Pieprzyk discloses the field of invention as relating "generally to the area of detecting genotype and/or aneuploidy." Pieprzyk at [0002]. Pieprzyk discloses methods including amplifying DNA. For example, Pieprzyk discloses a first method of the invention "is a method for detecting and/or quantifying one or more target amplicon(s) produced by amplification, wherein the detecting and/or quantifying is carried out during amplification or after an amplification endpoint has been reached." *Id.* at [0004]. Pieprzyk discloses methods also including sequencing DNA. For example, Pieprzyk discloses in Example 10 "Nexgen sequencing detection of fetal aneuploidy with amplicon tagging: A: by pre-PCR; B: by ligation." *Id.* at [0030]. As another example, Pieprzyk discloses a method where a sample is subjected to "preamplification using primers capable of amplifying a plurality of target nucleic acids to produce a plurality of target amplicons . . . After preamplification, the number of copies of target amplicons . . . are determined by any suitable method, including, e.g., amplification, digital amplification, or DNA sequencing." Pieprzyk at [0091]. As another example, Pieprzyk discloses, "[t]he methods of the invention can include subjecting at least one target amplicon to DNA sequencing using any available DNA sequencing method. In particular embodiments, a plurality of target amplicons is sequenced using a high throughput sequencing method." Pieprzyk at [0187]. As another example, "Embodiments using a microfluidic device that provides for recovery of reaction products provide a system suitable for PCR sample preparation that features reduced cost, time, and labor in the preparation of amplicon libraries from an input DNA template." Pieprzyk at [0207]. Furthermore, to the extent Natera asserts that Pieprzyk does not disclose or teach a method for amplifying and sequencing DNA, a POSA would be motivated to combine Pieprzyk with May PCT because Pieprzyk expressly teaches using routine and conventional techniques known in the art to amplify and sequence DNA, and the

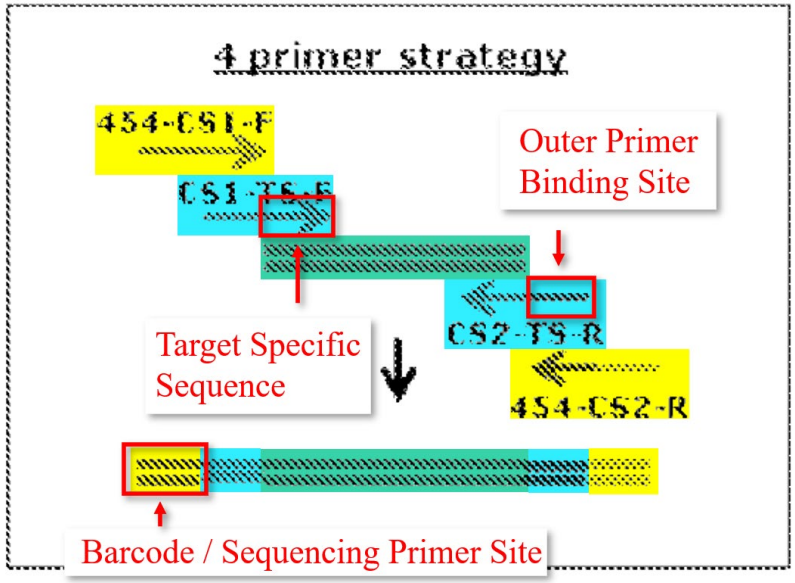
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	references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end, and thus are readily interchangeable with each other. For example, Pieprzyk teaches methods for analyzing "cell-free DNA in the bodily fluid (e.g., plasma) of subjects with cancer." Pieprzyk at [0110]. Pieprzyk's methods can be applied to 50 to 1,000 "or more amplification reactions...carried out in each individual reaction chamber." (*Id.* at [0218]) and can "identify[] the presence of particular polymorphisms (such as SNPs)." (Id., [0221].) And Pieprzyk's methods can "facilitate the detection and or quantification of target sequences in…tumor DNA in plasma from cancer patients." (*Id.* at [0116].) Similarly, May PCT discloses analyzing "[n]ucleic acids [] extracted or amplified from…blood [or] a blood fraction," including via "multiplex detection…[in an] individual amplification mixture." (*Id.* at [0132], *see also* [0210].) Like Pieprzyk, May PCT also teaches sequencing the amplified DNA via massively parallel sequencing. (*Id.* at [0121].) May PCT further discloses this claim limitation in the examples cited in Claim 1[pre] of Exhibit 1-C, which are hereby incorporated by reference in their entireties.
1[a][i]. tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,	Under Natera's apparent construction, Pieprzyk discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See, e.g.,* Pieprzyk at [0015], [0033], [0060], [0064], [0095], [0111], [0113], [0177], [0459], Example 2, Example 6, Example 7, Example 13, Claims 32-33. For example, Pieprzyk discloses that "the preamplification step can be used to introduce a nucleotide tag" and that "at least one primer of each primer pair employed for preamplification can include a nucleotide tag, which becomes incorporated into the preamplified nucleic acids." Pieprzyk at [0113]. Pieprzyk discloses that the nucleotide tag "can include any desired sequence, e.g., one that encodes an item of information about the target and/or internal control sequence and/or one that includes a primer binding site and/or a probe binding site" and can include a "universal tag and/or a common tag." *Id.* Pieprzyk discloses "For ease of discussion, the term 'preamplification' is used below to describe amplification performed prior to digital amplification and the products of this amplification are termed 'amplicons.'" Pieprzyk at [0177].

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	Pieprzyk also discloses that "[a]mplification according to the present teachings encompasses any means by which at least a part of at least one target nucleic acid is reproduced, typically in a template-dependent manner, including without limitation, a broad range of techniques for amplifying nucleic acid sequences, either linearly or exponentially. Illustrative means for performing an amplifying step include . . . PCR, . . . and the like, including multiplex versions and combinations thereof, for example but not limited to, OLA/PCR, PCR/OLA, LDR/PCR, PCR/PCR/LDR, PCR/LDR, LCR/PCR, PCR/LCR (also known as combined chain reaction—CCR), and the like." *Id.* at [0064]. Pieprzyk then discloses additional sources containing descriptions of such techniques. *Id.* (citing papers). As another example, Pieprzyk discloses methods that include "introducing one or more nucleotide tag(s) into the target amplicons" during preamplification, where "at least one primer of each primer pair employed for preamplification can include a nucleotide tag." Pieprzyk at [0015]. Pieprzyk discloses that "[u]seful nucleotide tags include, e.g., a universal tag and a chromosome-specific nucleotide tag." *Id. See also id.* at [0095]. As another example, Pieprzyk discloses methods that "introduce a barcode into an amplicon" by employing a "barcode primer…in an amplification reaction" wherein "the term 'barcode' refers to a specific nucleotide sequence that encodes information about an amplicon . . ." Pieprzyk at [0060]. Pieprzyk discloses tagging of isolated cell free DNA. For example, Pieprzyk discloses that "[a]ssay methods employing small amplicons are useful for applications aimed at determining copy number in samples containing fragmented nucleic acids, as is the case, e.g., for cell-free fetal DNA in a maternal bodily fluid (e.g., plama), cell-free DNA in the bodily fluid (e.g., plasma) of subjects with cancer, or DNA from formalin-fixed paraffin-embedded tissue." Pieprzyk at [0111]. *See also, e.g.*, Pieprzyk at [0003]. Furthermore, to the extent Natera asserts that Pieprzyk does not disclose or teach tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products, a POSA would find this limitation obvious in view of Pieprzyk combined with May PCT. For example, May PCT teaches the same four-primer strategy as Pieprzyk. For instance, May PCT teaches inner primers with universal "nucleotide tag[s]" and outer primers including additional sequences. May PCT at [0011]. As another example, May PCT adds to the methods of Pieprzyk outer "barcode" primers that can contain both a "barcode nucleotide sequence" and "additional primer binding sites…capable of being bound by DNA sequencing

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	primers." May PCT at [0115]. May PCT illustrates its approach by depicting outer primers (yellow), inner target-specific primers (blue), a target sequence (green), and an amplification product (multicolor) in its Figure 18:  (May PCT at Fig.18 (cropped and annotated).) A POSA would be motivated to combine Pieprzyk with May PCT because Pieprzyk expressly teaches using routine and conventional techniques known in the art to amplify and sequence DNA, and the references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end, and thus are readily interchangeable with each other. Furthermore, a POSA would have been motivated to combine Pieprzyk with May PCT, since doing so would have been a simple substitution of one known element (May's primers) for another (Pieprzyk's primers) between two references. Furthermore, a POSA would recognize the benefit of May PCT's method, since placing barcodes and sequencing tags on outer primers rather than on inner primers would shorten and thereby simplify the design of the inner, target-specific primers. Furthermore, a POSA would understand that short primers also are less prone to dimerization than long ones. Furthermore, a POSA would recognize that May PCT's primer configuration is

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	economical: separating barcodes from targeting primers reduces the number of unique primers needed by orders of magnitude. A POSA would have reasonably expected the combination to succeed. Both Pieprzyk and May PCT already teach using four primers to tag and amplify DNA. Furthermore, May PCT discloses that its 4-primers works for multiplex reactions like those in Pieprzyk. *See, e.g.,* May PCT at [0210], [0276], and Fig.18. Thus, a POSA would recognize this combination as trivial. May PCT further discloses this claim limitation in the examples cited in Claim 1[a][i] of Exhibit 1-C, which are hereby incorporated by reference in their entireties.
1[a][ii]. wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;	Pieprzyk discloses tagging cell free DNA isolated from a blood sample collected from a subject who is not a pregnant women. *See, e.g.,* Pieprzyk at [0003], [0111], [0116], [0138], [0221], [0222], [0387], [0388], [0497]. As an example, Pieprzyk discloses tagging methods that can be combined with the methods described to "further facilitate the detection and or quantification of target sequences in samples having mixed length nucleic acids (e.g. fetal DNA in maternal plasma or tumor DNA in plasma from cancer patients." Pieprzyk at [0116]. As another example, Pieprzyk discloses methods to address difficulties with detecting genotype and/or aneuploidy in fetal DNA "due to the presence of cell-free maternal DNA at a much higher percentage than the fetal DNA," and then discloses that "[s]imilar difficulties exist with respect to the detection of cell-free tumor DNA in bodily fluids from cancer patients." Pieprzyk at [0003]. As another example, Pieprzyk discloses "the methods described herein can be used with essentially any nucleic acid sample comprising long and short nucleic acids (nucleic acid molecules)." Pieprzyk at [0138]. Pieprzyk discloses embodiments where the nucleic acid is derived form a maternal biological sample, and then also discloses embodiments where the nucleic acids are "derived from a biological sample from a mammal (*e.g.*, a human or non-human mammal) having, suspected of having, or at risk for, a pathology or congenital disorder characterized by a nucleic acid abnormality (*e.g.*, aneuploidy, fragmentation, amplification, deletion, single-nucleotide polymorphism, translocation, chromosomal rearrangement or resorting, *etc.*)", embodiments where nucleic acid is "derived from a biological sample from a mammal (*e.g.*, a human or non-human mammal) having, suspected of having, or at risk

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	for a cancer," and embodiments where "the short nucleic acid fragments comprise tumor or metastatic cell DNA, and the long nucleic acids comprise normal DNA. *Id.* As another example, Pieprzyk discloses, "The methods of the invention are applicable to any technique aimed at detecting the presence or amount of one or more target nucleic acids in a nucleic acid sample. Thus, for example, these methods are applicable to identifying the presence of particular polymorphisms (such as SNPs), alleles, or haplotypes, or chromosomal abnormalities, such as amplifications, deletions, or aneuploidy. The methods may be employed in genotyping, which can be carried out in a number of contexts, including diagnosis of genetic diseases or disorders, pharmacogenomics (personalized medicine), quality control in agriculture (e.g., for seeds or livestock), the study and management of populations of plants or animals (e.g., in aquaculture or fisheries management or in the determination of population diversity), or paternity or forensic identifications. The methods of the invention can be applied in the identification of sequences indicative of particular conditions or organisms in biological or environmental samples. For example, the methods can be used in assays to identify pathogens, such as viruses, bacteria, and fungi). The methods can also be used in studies aimed at characterizing environments or microenvironments, e.g., characterizing the microbial species in the human gut." Pieprzyk at [0221]. Furthermore, to the extent Natera asserts that Pieprzyk does not disclose or teach isolated cell-free DNA that is isolated from a blood sample collected from a subject who is not a pregnant women, this limitation would have been obvious. A POSA would have been motivated to apply the combined method to non-pregnant females because many humans with cancer are not pregnant. Furthermore, a POSA would have reasonably expected success in doing so because Pieprzyk's method would work equally on blood samples from pregnant women and non-pregnant persons alike.
1[b][i]. amplifying the tagged products one or more times to generate final amplification products,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Pieprzyk discloses amplifying the tagged products one or more times to generate final amplification products. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). Specifically, Natera's Response to NeoGenomics Laboratories, Inc.'s Interrogatory No. 14 does not dispute that Pieprzyk discloses "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification". NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling.

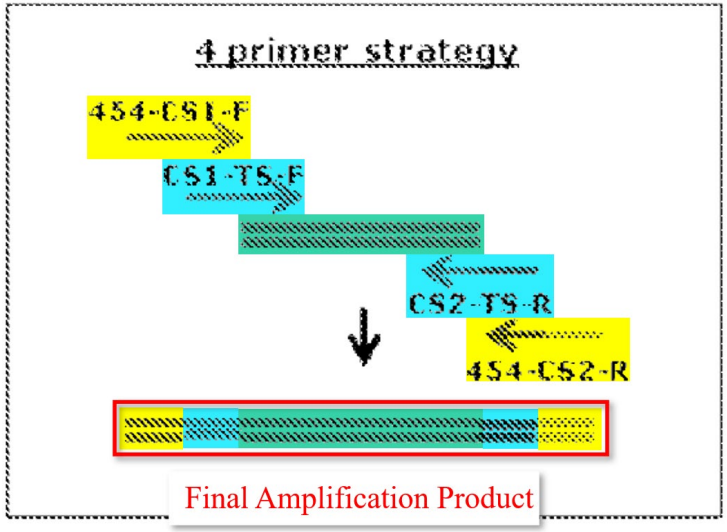
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	See, e.g., Pieprzyk at [0013], [0016], [0044], [0109], [0013], [0014], [0015], [0031], [0064], [0069], [0091], [0094], [0095], [0096], [0109], [0111-0115], [0174], [0177-0179], Example 4, Example 7, Example 11, Example 14, Claim 28, For example, Pieprzyk discloses that "the preamplification step can be used to introduce a nucleotide tag" and that "at least one primer of each primer pair employed for preamplification can include a nucleotide tag, which becomes incorporated into the preamplified nucleic acids." Pieprzyk at [0113]. Pieprzyk discloses that the nucleotide tag "can include any desired sequence, e.g., one that encodes an item of information about the target and/or internal control sequence and/or one that includes a primer binding site and/or a probe binding site" and can include a "universal tag and/or a common tag." *Id.* Pieprzyk discloses "For ease of discussion, the term 'preamplification' is used below to describe amplification performed prior to digital amplification and the products of this amplification are termed 'amplicons.'" Pieprzyk at [0177]. Pieprzyk also discloses that "[a]mplification according to the present teachings encompasses any means by which at least a part of at least one target nucleic acid is reproduced, typically in a template-dependent manner, including without limitation, a broad range of techniques for amplifying nucleic acid sequences, either linearly or exponentially. Illustrative means for performing an amplifying step include . . . PCR, . . . and the like, including multiplex versions and combinations thereof, for example but not limited to, OLA/PCR, PCR/OLA, LDR/PCR, PCR/PCR/LDR, PCR/LDR, LCR/PCR, PCR/LCR (also known as combined chain reaction—CCR), and the like." *Id.* at [0064]. Pieprzyk then discloses additional sources containing descriptions of such techniques. *Id.* (citing papers). As another example, Pieprzyk discloses methods that include "introducing one or more nucleotide tag(s) into the target amplicons" during preamplification, where "at least one primer of each primer pair employed for preamplification can include a nucleotide tag." Pieprzyk at [0015]. Pieprzyk discloses that "[u]seful nucleotide tags include, e.g., a universal tag and a chromosome-specific nucleotide tag." *Id. See also id.* at [0095].

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	As another example, Pieprzyk discloses methods that "introduce a barcode into an amplicon" by employing a "barcode primer…in an amplification reaction" wherein "the term 'barcode' refers to a specific nucleotide sequence that encodes information about an amplicon . . ." Pieprzyk at [0060]. For example, Pieprzyk discloses illustrative embodiments where a sample is "preamplified, and then each preamplified aliquot is divided into further aliquots and subjected to amplification." Pieprzyk at [0109]. As discussed above, Pieprzyk discloses "For ease of discussion, the term 'preamplification' is used below to describe amplification performed prior to digital amplification and the products of this amplification are termed 'amplicons.'" Pieprzyk at [0177]. Furthermore, Pieprzyk discloses that "the preamplification step can be used to introduce a nucleotide tag" and that "at least one primer of each primer pair employed for preamplification can include a nucleotide tag, which becomes incorporated into the preamplified nucleic acids." Pieprzyk at [0113]. As another example, Pieprzyk discloses, "In certain embodiments, digital amplification can be carried out after preamplification of sample nucleic acids." Pieprzyk at [0177]. Pieprzyk then discloses "For ease of discussion, the term 'preamplification' is used below to describe amplification performed prior to digital amplification and the products of this amplification are termed 'amplicons.'" *Id.* As another example, Pieprzyk discloses a method that entails "subjecting a sample to preamplification using primers capable of amplifying a plurality of target nucleic acids to produce a plurality of target amplicons." Pieprzyk at [0013]. Pieprzyk then discloses some embodiments where "the number of copies of target amplicons derived from the first chromosome and the number of copies of target amplicons derived from the second chromosome are determined by a method that includes amplification." *Id.* Pieprzyk discloses that "[i]n variations of such embodiments, the amplification comprises digital amplification." *Id.* As another example, Pieprzyk discloses a method where, "[a]fter preamplification, the number of copies of target amplicons derived from the first chromosome and the number of copies of target amplicons derived from the second chromosome are determined by any suitable method, including, e.g., amplification, digital amplification, or DNA sequencing." Pieprzyk at [0091]. Furthermore, to the extent Natera asserts that Pieprzyk does not disclose or teach amplifying the tagged products one or more times to generate final amplification products, a POSA would find this limitation obvious in view of Pieprzyk combined with May PCT.

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	For example, May PCT, similar to Pieprzyk, discloses methods to tag cell-free DNA via amplification. May PCT discloses using inner primers to generate tagged products by amplifying DNA and the outer primers, then amplify those tagged products. May PCT at [0011], *see also, e.g.,* [0115]. Furthermore, May PCT discloses the products of those later amplification cycles as the final amplification products. May PCT at [0121], *see also, e.g.,* [0164].  (May PCT at Fig.18 (cropped and annotated).) A POSA would be motivated to combine Pieprzyk with May PCT because Pieprzyk expressly teaches using routine and conventional techniques known in the art to amplify and sequence DNA, and the references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end, and thus are readily interchangeable with each other. Furthermore, a POSA would have been motivated to combine Pieprzyk with May PCT, since doing so would have been a simple substitution of one known element (May's primers) for another (Pieprzyk's primers) between two references. Furthermore, a POSA would recognize the benefit of May PCT's method, since placing barcodes and sequencing tags on outer primers rather than on inner primers would shorten and thereby simplify the design of the

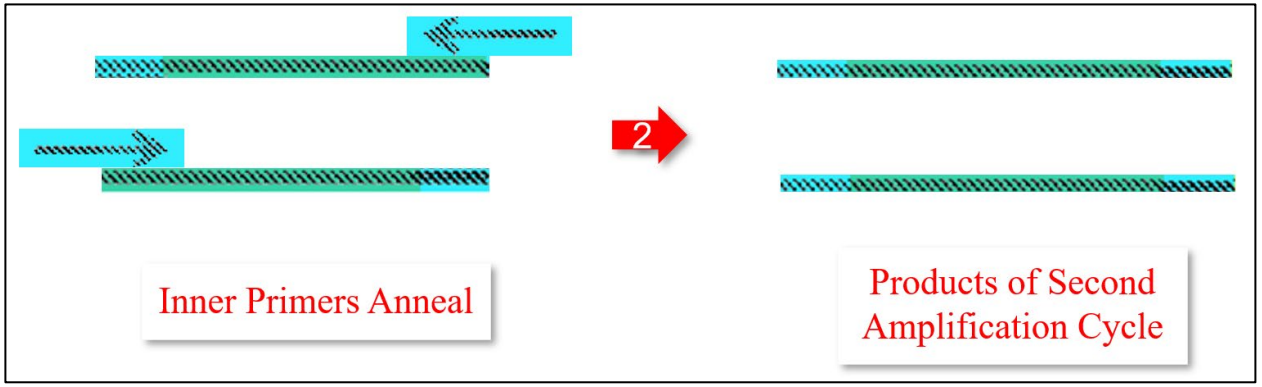
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	inner, target-specific primers. Furthermore, a POSA would understand that short primers also are less prone to dimerization than long ones. Furthermore, a POSA would recognize that May PCT's primer configuration is economical: separating barcodes from targeting primers reduces the number of unique primers needed by orders of magnitude. A POSA would have reasonably expected the combination to succeed. Both Pieprzyk and May PCT teach using four primers to tag and amplify DNA. Furthermore, May PCT discloses that its 4-primers works for multiplex reactions like those in Pieprzyk. *See, e.g.,* May PCT at [0210], [0276], and Fig.18. Thus, a POSA would recognize this combination as trivial. May PCT further discloses this claim limitation in the examples cited in Claim 1[b][i] of Exhibit 1-C, which are hereby incorporated by reference in their entireties. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Pieprzyk also discloses this element for the same reasons.
1[b][ii]. wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Pieprzyk discloses amplifying wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). Specifically, Natera's Response to NeoGenomics Laboratories, Inc.'s Interrogatory No. 14 does not dispute that Pieprzyk discloses "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification". NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling.

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	See, e.g., Pieprzyk at [0004], [0005], [0009], [0010], [0011], [0012], [0032], [0033], [0058], [0071], [0081], [0086], [0087], [0088], [0090], [0114], [0115], [0133], Example 3, Figures 3A-3C, Claim 1, Claim 2, Claim 11, Claim 16. For example, Pieprzyk discloses "introduc[ing] one or more nucleotide tags during preamplification" where "one or more primers include a target-specific portion and a nucleotide tag." Pieprzyk at [0114]. As another example, Pieprzyk discloses, "if common nucleotide tags are employed, common tag-specific primers can be used to produce amplicons for detection." Pieprzyk at [0115]. As another example, Pieprzyk discloses, a "first method of the invention is a method for detecting and/or quantifying one or more target amplicon(s) produced by amplification, wherein the detecting and/or quantifying is carried out during amplification or after an amplification endpoint has been reached" using "at least one target-specific primer pair." Pieprzyk at [0004]. As another example, Pieprzyk discloses certain embodiments comprising "adding additional quantities the same or different target-specific primer pairs to the reaction mixture and performing one or more amplification cycles to preferentially amplify the tagged target sequences." Pieprzyk at [0133]. As another example, Pieprzyk discloses, "the unique combination of 100 different tag primers on the 5' primer and 100 different tag primers on the 3' primer generates 10,000 combinatorial variants representing 10,000 specific primer sets." Pieprzyk at [0033]. Pieprzyk then discloses, "the unique combination of 100 different tag primers on the 5' primer and 100 different tag primers on the 3' primer permits 10,000 combinations, representing 10,000 specific RNAs or genes to be targeted." *Id.* As another example, Pieprzyk discloses analyzing target nucleic acids "for specific polymorphisms (such as SNPs)". Pieprzyk at [0151]. As another example, Pieprzyk discloses that its "methods of invention are applicable to any technique aimed at detecting the presence or amount of one or more target nucleic acids…[such as] identifying the presence of particular polymorphisms (such as SNPs), alleles, or haplotypes, or chromosomal abnormalities, such as amplifications, deletions, or aneuploidy." Pieprzyk at [0221].

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	Furthermore, to the extent Natera asserts that Pieprzyk does not disclose or teach one of the amplification steps comprising targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume, a POSA would find this limitation obvious in view of Pieprzyk combined with May PCT. For example, May PCT teaches using its method for "identifying the presence of particular polymorphisms (such as SNPs)." May PCT at [0235]. As another example, May PCT teaches analyzing "[n]ucleic acids [] extracted or amplified from…blood [or] a blood fraction," including via "multiplex detection…[in an] individual amplification mixture." May PCT at [0132], [0210]. As another example, the combined method of Pieprzyk and May PCT includes using inner primers to perform target-specific amplification in the second amplification cycle of the PCR reaction with the inner target-specific primers. May PCT at [0011]. That second cycle is illustrated below:  In the combined method of Pieprzyk and May PCT, the inner primers amplify the plurality of SNP loci disclosed in Pieprzyk in a single reaction chamber. A POSA would be motivated to combine Pieprzyk with May PCT because Pieprzyk expressly teaches using routine and conventional techniques known in the art to amplify and sequence DNA, and the references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end, and thus are readily interchangeable with each other.

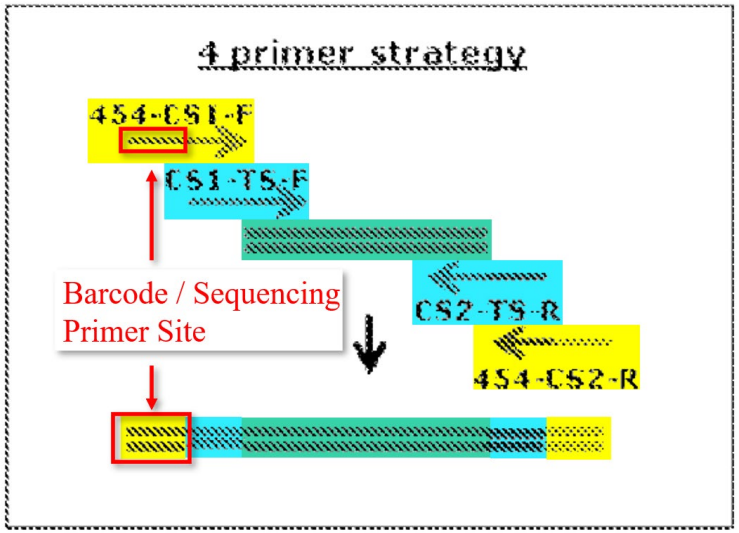
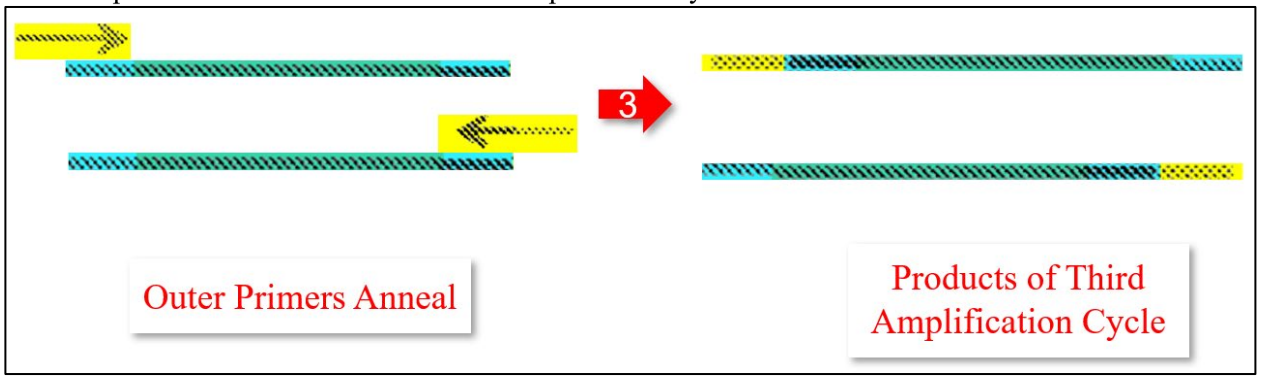
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	Furthermore, a POSA would have been motivated to combine Pieprzyk with May PCT, since doing so would have been a simple substitution of one known element (May's primers) for another (Pieprzyk's primers) between two references. Furthermore, a POSA would recognize the benefit of May PCT's method, since placing barcodes and sequencing tags on outer primers rather than on inner primers would shorten and thereby simplify the design of the inner, target-specific primers. Furthermore, a POSA would understand that short primers also are less prone to dimerization than long ones. Furthermore, a POSA would recognize that May PCT's primer configuration is economical: separating barcodes from targeting primers reduces the number of unique primers needed by orders of magnitude. A POSA would have reasonably expected the combination to succeed. Both Pieprzyk and May PCT teach using four primers to tag and amplify DNA. Furthermore, May PCT discloses that its 4-primers works for multiplex reactions like those in Pieprzyk. *See, e.g.,* May PCT at [0210], [0276], and Fig.18. Thus, a POSA would recognize this combination as trivial. May PCT further discloses this claim limitation in the examples cited in Claim 1[b][ii] of Exhibit 1-C, which are hereby incorporated by reference in their entireties. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Pieprzyk also discloses this element for the same reasons.
1[b][iii]. wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and	Pieprzyk discloses amplifying wherein one of the amplifying steps introduces a barcode and one or more sequencing tags. *See, e.g.,* Pieprzyk at [0060], [0306], [0018], [0099], [0208], Example 3, Example 10, Claim 48, Claim 49. As an example, Pieprzyk describes two methods of amplicon tagging with barcoding "by pre-PCR (see Figure 8A) and by Ligation (see Figure 8D), both using primers tailed with the barcodes common sequencing tails. This multiplex is performed and then run on the sequencer." Pieprzyk at [0508].

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	As another example, Pieprzyk describes the term "barcode" as referring to "a specific nucleotide sequence that encodes information about an amplicon produce during preamplification or amplification." Pieprzyk at [0060]. Pieprzyk discloses, "To introduce a barcode into an amplicon, 'barcode primer' that includes the barcode nucleotide sequence can be employed in an amplification reaction. For example, a different barcode primer can be employed to amplify one or more target sequences from each of a number of different samples, such that the barcode nucleotide sequence indicates the sample origin of the resulting amplicons." *Id.* As another example, Pieprzyk discloses in Example 3 an access array protocol "for producing PCR products with Sequencing tags and sample barcode" Pieprzyk at [0306]. As another example, Pieprzyk discloses sequencing primers that "additionally include a barcode nucleotide sequence between the primer binding site for the forward DNA sequencing primer and the first and second target nucleotide sequences, respectively. Alternatively, or in addition, the first and second DNA sequencing primers can additionally include a barcode nucleotide sequence between the first and second target nucleotide sequences, respectively, and the primer binding site for the reverse DNA sequencing primer." Pieprzyk at [0018]. Furthermore, to the extent Natera asserts that Pieprzyk does not disclose or teach one of the amplifying steps introducing a barcode and one or more sequencing tags, a POSA would find this limitation obvious in view of Pieprzyk combined with May PCT. For example, May PCT teaches inner primers with universal "nucleotide tag[s]" and outer primers including additional sequences. May PCT at [0011]. As another example, May PCT teaches outer "barcode" primers that can contain both a "barcode nucleotide sequence" and "additional primer binding sites…capable of being bound by DNA sequencing primers." May PCT at [0115]. As another example, in the combined method of Pieprzyk and May PCT, the outer primers introduce barcodes and sequencing tags beginning in the third amplification cycle of the PCR reaction because they include barcode sequences and sequencing tags and, thus, so do their amplification products. May PCT at [0011], [0115].

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	 (May PCT at Fig.18 (cropped and annotated).) The below graphic illustrates how a POSA would recognize some of the amplifications that occur in the third amplification cycle:  A POSA would be motivated to combine Pieprzyk with May PCT because Pieprzyk expressly teaches using routine and conventional techniques known in the art to amplify and sequence DNA, and the references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose

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	overlapping methods and techniques directed to the same or similar analytical end, and thus are readily interchangeable with each other. Furthermore, a POSA would have been motivated to combine Pieprzyk with May PCT, since doing so would have been a simple substitution of one known element (May's primers) for another (Pieprzyk's primers) between two references. Furthermore, a POSA would recognize the benefit of May PCT's method, since placing barcodes and sequencing tags on outer primers rather than on inner primers would shorten and thereby simplify the design of the inner, target-specific primers. Furthermore, a POSA would understand that short primers also are less prone to dimerization than long ones. Furthermore, a POSA would recognize that May PCT's primer configuration is economical: separating barcodes from targeting primers reduces the number of unique primers needed by orders of magnitude. A POSA would have reasonably expected the combination to succeed. Both Pieprzyk and May PCT teach using four primers to tag and amplify DNA. Furthermore, May PCT discloses that its 4-primers works for multiplex reactions like those in Pieprzyk. *See, e.g.,* May PCT at [0210], [0276], and Fig.18. Thus, a POSA would recognize this combination as trivial. May PCT further discloses this claim limitation in the examples cited in Claim 1[b][iii] of Exhibit 1-C, which are hereby incorporated by reference in their entireties. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Pieprzyk also discloses this element for the same reasons.
1[c][i]. sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on	Pieprzyk discloses sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products. *See, e.g.*, Pieprzyk at [0091], [0143], [0185-0188], [0206]-[0208], [0223], Example 10, Claim 27.

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the final amplification products,	For example, Pieprzyk discloses "subjecting at least one target amplicon to DNA sequencing using any available DNA sequencing method. In particular embodiments, a plurality of target amplicons is sequenced using a high throughput sequencing method." Pieprzyk at [0187]. As another example, Pieprzyk discloses use of emPCR and sources describing use of emPCR for sequencing. Pieprzyk at [0187] (citing papers). As another example, Pieprzyk discloses "[m]any current DNA sequencing technologies [that] rely on 'sequencing by synthesis.' These techniques entail library creation, massively parallel PCR amplification of library molecules, and sequencing." Pieprzyk at [0185]. As another example, Pieprzyk discloses a "typical use case" for embodiments where "first amplification will be used to generate libraries for next-generation sequencing." Pieprzyk at [0207]. Furthermore, to the extent Natera asserts that Pieprzyk does not disclose or teach sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products, a POSA would find this limitation obvious in view of Pieprzyk combined with May PCT. For example, May PCT teaches "subjecting at least one target amplicon to DNA sequencing using any available DNA sequencing method." May PCT at [0121]. As another example, May PCT teaches sequencing "a plurality of target amplicons. . . using a high throughput sequencing method." *Id.* As another example, May PCT teaches emPCR as a DNA isolation method for use with massively parallel sequencing. *Id.* As another example, May PCT teaches "library creation, massively parallel PCR amplification of library molecules, and sequencing." May PCT at [0112]. A POSA would be motivated to combine Pieprzyk with May PCT because Pieprzyk expressly teaches using routine and conventional techniques known in the art to amplify and sequence DNA, and the references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end, and thus are readily interchangeable with each other. Furthermore, a POSA would have been motivated to combine Pieprzyk with May PCT, since doing so would have been a simple substitution of one known element (May's primers) for another (Pieprzyk's primers) between two

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	references. Furthermore, a POSA would recognize the benefit of May PCT's method, since placing barcodes and sequencing tags on outer primers rather than on inner primers would shorten and thereby simplify the design of the inner, target-specific primers. Furthermore, a POSA would understand that short primers also are less prone to dimerization than long ones. Furthermore, a POSA would recognize that May PCT's primer configuration is economical: separating barcodes from targeting primers reduces the number of unique primers needed by orders of magnitude. A POSA would have reasonably expected the combination to succeed. Both Pieprzyk and May PCT teach using four primers to tag and amplify DNA. Furthermore, May PCT discloses that its 4-primers works for multiplex reactions like those in Pieprzyk. *See, e.g.,* May PCT at [0210], [0276], and Fig.18. Thus, a POSA would recognize this combination as trivial. May PCT further discloses this claim limitation in the examples cited in Claim 1[c][i] of Exhibit 1-C, which are hereby incorporated by reference in their entireties.
1[c][ii]. wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.	The Court construed the term "SNP loci…associated with cancer" to mean "a single nucleotide that may differ between the genomes of two members of the same species and is associated with cancer. The usage of the term 'SNP' should not imply any limit on the frequency with which each variant occurs." Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Pieprzyk discloses a plurality of SNP loci comprising 25-2,000 loci associated with cancer. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). Specifically, Natera's Response to NeoGenomics Laboratories, Inc.'s Interrogatory No. 14 does not dispute that Pieprzyk discloses "SNP loci…associated with cancer". NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.*, Pieprzyk at [0110], [0013], [0016], [0033], [0138], [0221], [0546], [0550], [0552]. For example, Pieprzyk discloses "illustrative embodiments" where "2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000 or more target and/or internal control sequences on a chromosome of interest are

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	analyzed." Pieprzyk at [0110]. Pieprzyk discloses, "In addition, any number of sequences falling within ranges bounded by any of these values can be analyzed." *Id.* As another example, Pieprzyk discloses, "the unique combination of 100 different tag primers on the 5' primer and 100 different tag primers on the 3' primer generates 10,000 combinatorial variants representing 10,000 specific primer sets." Pieprzyk at [0033]. Pieprzyk then discloses, "the unique combination of 100 different tag primers on the 5' primer and 100 different tag primers on the 3' primer permits 10,000 combinations, representing 10,000 specific RNAs or genes to be targeted." *Id.* As another example, Pieprzyk discloses that its "methods of invention are applicable to any technique aimed at detecting the presence or amount of one or more target nucleic acids. . . [such as] identifying the presence of particular polymorphisms (such as SNPs), alleles, or haplotypes, or chromosomal abnormalities, such as amplifications, deletions, or aneuploidy." Pieprzyk at [0221]. Pieprzyk further discloses that its methods "may be employed in genotyping, which can be carried out in a number of contexts, including diagnosis of genetic diseases or disorders. . ." *Id.* As another example, Pieprzyk discloses application of its methods to "nucleic acids [] derived from a biological sample from a mammal (*e.g.,* a human or non-human mammal) having, suspected of having, or at risk for a cancer." Pieprzyk at [0138]. To the extent Natera asserts that any portion of this limitation is not disclosed under Natera's apparent interpretation of the Court's construction, it would have been obvious based on the knowledge of a POSA at the time of the alleged invention to look to the Catalogue of Somatic Mutations In Cancer ("COSMIC") database, which identifies hundreds to thousands of gene variants, including those that can be present in the same gene, to readily disclose a plurality of SNP loci comprising 25-2,000 loci associated with cancer. By way of example, COSMIC identifies 6,038 different variants in the TP53 gene alone. *See*, *e.g.,* https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53. Furthermore, to the extent Natera asserts that Pieprzyk does not disclose or teach any portion of this limitation under Natera's apparent interpretation of the Court's construction, it would have been obvious to a POSA. Disclosures in Pieprzyk would have motivated a POSA to conduct multiplex amplification in a single reaction volume on cell-free tumor DNA. In the context of detecting fetal cell-free DNA, Pieprzyk teaches that "[p]lasma contains a limited concentration of target nucleic acids (…SNPS…etc.)." Pieprzyk at [0379]. There,

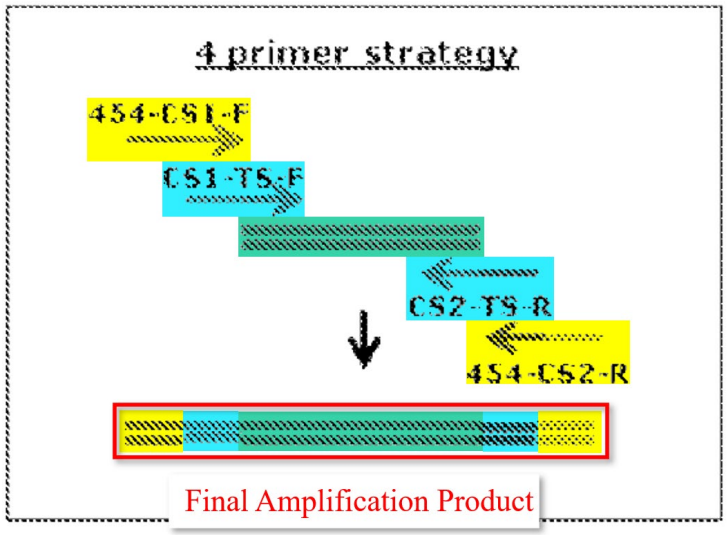
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	"[m]ultiplexing will be necessary" because the alternative, "[d]ividing the sample[,] will reduce the number of target[s] per assay and increase sampling error." *Id.* Pieprzyk also teaches that "[s]imilar challenges exist for mutation detection by mutation dosage in…cancer, and therefore this approach can be used in these contexts." *Id.* at [0387]. Finally, Pieprzyk proposes analyzing between "30" and "1000 or more target and/or internal control sequences," which falls within the claimed bounds of 25-2,000 SNP loci under Natera's apparent interpretation of the Court's construction. *Id.* at [0110]. In addition, the '035 Patent admits a POSA's "general belief in the art is 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, would have understood that multiplex PCR up to 100 assays could readily be performed on cell free DNA, and would have been able to do so as a matter of routine.
2. The method of claim 1, wherein tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA.	Pieprzyk discloses tagging the cell free DNA comprising ligating the one or more universal tail adapters to the cell free DNA. *See, e.g.*, Pieprzyk at [0011], [0012], [0026], [0030], [0031], [0032], [0033], [0058], [0165], [0339], [0358], [0360], [0490], [0496], [0502], Example 4, Fig. 4A, Fig. 8B, Example 10, Example 11, Example 12, Example 13. For example, Pieprzyk discloses, "In some embodiments, the method entails using whole blood (or other unfractionated bodily fluid [sic] and generating a sequencing library (e.g. as described with plasma by Quake and Lo independently in PNAS ~2008 by blunt-ending DNA fragment and blunt-end ligation of sequencing adapters), while at the same time enriching for short fragments. If proceeding to sequencing, the sequencing method may further bias in favor of shorter (including fetal) fragments and/or the sequencing library can be size-separated." Pieprzyk at [0101]. As another example, Pieprzyk discloses, "Library creation starts with conversion of sample nucleic acids to appropriately sized fragments, ligation of adaptor sequences onto the ends of the fragments, and selection for molecules properly appended with adaptors. The presence of the adaptor sequences on the ends of the library molecules enables amplification of random-sequence inserts." Pieprzyk at [0185]. As another example, Pieprzyk discloses illustrative encoding ligation reactions in U.S. Patent Publication No. 2005/0260640, which Pieprzyk incorporates by reference "in particular for ligation reactions." Pieprzyk at [0058].

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Claim	**Prior Art**
	Pieprzyk describes "encoding reaction" as "produc[ing] a 'tagged target nucleotide sequence,' which includes a nucleotide tag linked to a target nucleotide sequence." *Id.* at [0059]. As another example, Pieprzyk discloses a method "by next generation sequencers as published by both Quake and Lo utiliz[ing] the standard sequencing prep method (fragmentation, ligation of adapters and emulsion or bridge PCR)." Pieprzyk at [0502]. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 27. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Pieprzyk also discloses this element for the same reasons as discussed below regarding claim 13.
12. The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.	Pieprzyk discloses one or more universal tail adaptors comprising a first universal tail adaptor and a second universal tail adaptor. *See, e.g.*, Pieprzyk at [0388]-[0404], [0483], [0459], [0033], [0068], [0069], [0095], Example 13, Fig. 11A-B. For example, Example 7 of Pieprzyk discloses a PCR-based pre-amplification step comprising primers with universal tags. Pieprzyk at [0388]-[0404]. Pieprzyk discloses "[u]sing universal tags for preamp, universal or common tags (common = same tag for a group of targets, e.g. per each chromosome." Pieprzyk at [0399]. As another example, Pieprzyk discloses that "the preamplification step can be used to introduce a nucleotide tag" and that "at least one primer of each primer pair employed for preamplification can include a nucleotide tag, which becomes incorporated into the preamplified nucleic acids." Pieprzyk at [0113]. Pieprzyk discloses that the nucleotide tag "can include any desired sequence, e.g., one that encodes an item of information about the target and/or internal control sequence and/or one that includes a primer binding site and/or a probe binding site" and can include a "universal tag and/or a common tag." *Id.* As another example, Pieprzyk discloses "[u]niversal target tag sequences of forward primers are underlined." Pieprzyk at [0459].

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Claim	**Prior Art**
	Furthermore, to the extent Natera asserts that Pieprzyk does not disclose or teach one or more universal tail adaptors comprising a first universal tail adaptor and a second universal tail adaptor, a POSA would find this limitation obvious in view of Pieprzyk combined with May PCT. For example, May PCT, similar to Pieprzyk, discloses methods to tag cell-free DNA via amplification. May PCT discloses using inner primers to generate tagged products by amplifying DNA and the outer primers, then amplify those tagged products. May PCT at [0011], *see also, e.g.,* [0115]. Furthermore, May PCT discloses the products of those later amplification cycles as the final amplification products. May PCT at [0121], *see also, e.g.,* [0164]. Finally, as illustrated below, May PCT discloses using two inner primers, which comprise first and second universal tail adapters.  May PCT at Fig.18 (cropped and annotated). A POSA would be motivated to combine Pieprzyk with May PCT because Pieprzyk expressly teaches using routine and conventional techniques known in the art to amplify and sequence DNA, and the references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end, and thus are readily interchangeable with each other.

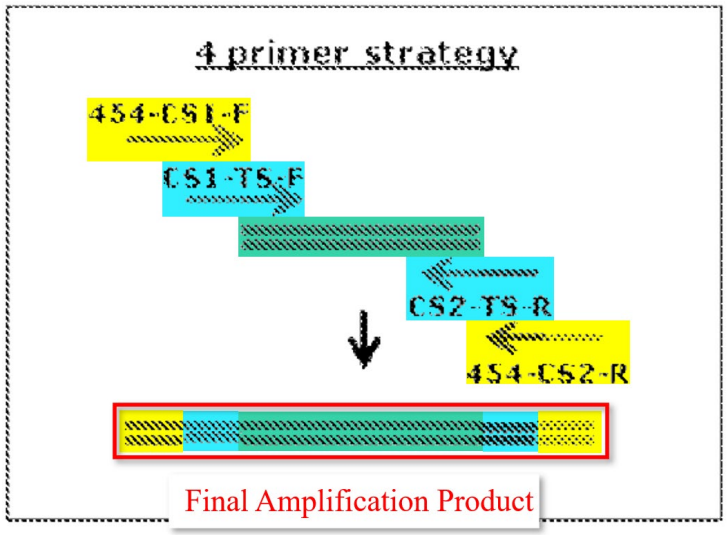
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Claim	**Prior Art**
	Furthermore, a POSA would have been motivated to combine Pieprzyk with May PCT, since doing so would have been a simple substitution of one known element (May's primers) for another (Pieprzyk's primers) between two references. Furthermore, a POSA would recognize the benefit of May PCT's method, since placing barcodes and sequencing tags on outer primers rather than on inner primers would shorten and thereby simplify the design of the inner, target-specific primers. Furthermore, a POSA would understand that short primers also are less prone to dimerization than long ones. Furthermore, a POSA would recognize that May PCT's primer configuration is economical: separating barcodes from targeting primers reduces the number of unique primers needed by orders of magnitude. A POSA would have reasonably expected the combination to succeed. Both Pieprzyk and May PCT teach using four primers to tag and amplify DNA. Furthermore, May PCT discloses that its 4-primers works for multiplex reactions like those in Pieprzyk. *See, e.g.,* May PCT at [0210], [0276], and Fig.18. Thus, a POSA would recognize this combination as trivial. May PCT further discloses this claim limitation in the examples cited in Claim 12 of Exhibit 1-C, which are hereby incorporated by reference in their entireties.
13. The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.	Pieprzyk discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See, e.g.,* Pieprzyk at [0015], [0033], [0095], [0379], [0387], [0388]-[0404], [0459], Example 2, Example 6, Example 7, Example 13, Claims 32-33. For example, Pieprzyk discloses that "the preamplification step can be used to introduce a nucleotide tag" and that "at least one primer of each primer pair employed for preamplification can include a nucleotide tag, which becomes incorporated into the preamplified nucleic acids." Pieprzyk at [0113]. Pieprzyk discloses that the nucleotide tag "can include any desired sequence, e.g., one that encodes an item of information about the target and/or internal control sequence and/or one that includes a primer binding site and/or a probe binding site" and can include a "universal tag and/or a common tag." *Id.*

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U.S. Patent No. 11,519,035	
Claim	**Prior Art**
	In Example 6, Pieprzyk discloses using pre-amplification to detect fetal aneuploidy from cfDNA including the tagging by amplification. Pieprzyk at [0379]-[0387]. For instance, Pieprzyk discloses "a pre-amplification protocol which amplifies all targets of interest in the sample" which "allows analysis of all targets – in parallel, or together (e.g. introducing common tags in the pre-amp process)." Pieprzyk at [0381]. As another example, Pieprzyk discloses "5' tagging can be a pert [sic] of pre-amplification and actually also be used as a stand-alone procedure to combine multiple targets." *Id.* at [0386]. Pieprzyk discloses "For ease of discussion, the term 'preamplification' is used below to describe amplification performed prior to digital amplification and the products of this amplification are termed 'amplicons.'" Pieprzyk at [0177]. Pieprzyk also discloses that "[a]mplification according to the present teachings encompasses any means by which at least a part of at least one target nucleic acid is reproduced, typically in a template-dependent manner, including without limitation, a broad range of techniques for amplifying nucleic acid sequences, either linearly or exponentially. Illustrative means for performing an amplifying step include . . . PCR, . . . and the like, including multiplex versions and combinations thereof, for example but not limited to, OLA/PCR, PCR/OLA, LDR/PCR, PCR/PCR/LDR, PCR/LDR, LCR/PCR, PCR/LCR (also known as combined chain reaction—CCR), and the like." *Id.* at [0064]. Pieprzyk then discloses additional sources containing descriptions of such techniques. *Id.* (citing papers). As another example, Pieprzyk discloses methods that include "introducing one or more nucleotide tag(s) into the target amplicons" during preamplification, where "at least one primer of each primer pair employed for preamplification can include a nucleotide tag." Pieprzyk at [0015]. Pieprzyk discloses that "[u]seful nucleotide tags include, e.g., a universal tag and a chromosome-specific nucleotide tag." *Id. See also id.* at [0095]. Pieprzyk discloses tagging of isolated cell free DNA. For example, Pieprzyk discloses that "[a]ssay methods employing small amplicons are useful for applications aimed at determining copy number in samples containing fragmented nucleic acids, as is the case, e.g., for cell-free fetal DNA in a maternal bodily fluid (e.g., plama), cell-free DNA in the bodily fluid (e.g., plasma) of subjects with cancer, or DNA from formalin-fixed paraffin-embedded tissue." Pieprzyk at [0111]. *See also, e.g.*, Pieprzyk at [0003]. Furthermore, to the extent Natera asserts that Pieprzyk does not disclose or teach this limitation under Natera's apparent construction, a POSA would find this limitation obvious in view of Pieprzyk combined with May PCT.

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Claim	**Prior Art**
	For example, May PCT, similar to Pieprzyk, discloses methods to tag cell-free DNA via amplification. May PCT discloses using inner primers to generate tagged products by amplifying DNA and the outer primers, then amplify those tagged products. May PCT at [0011], *see also, e.g.,* [0115]. Furthermore, May PCT discloses the products of those later amplification cycles as the final amplification products. May PCT at [0121], *see also, e.g.,* [0164]. Finally, as illustrated below, May PCT discloses using a first primer comprising a first universal tail adapter and a second primer comprising a second universal tail adapter.  May PCT at Fig.18 (cropped and annotated). A POSA would be motivated to combine Pieprzyk with May PCT because Pieprzyk expressly teaches using routine and conventional techniques known in the art to amplify and sequence DNA, and the references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end, and thus are readily interchangeable with each other. Furthermore, a POSA would have been motivated to combine Pieprzyk with May PCT, since doing so would have been a simple substitution of one known element (May's primers) for another (Pieprzyk's primers) between two

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Claim	**Prior Art**
	references. Furthermore, a POSA would recognize the benefit of May PCT's method, since placing barcodes and sequencing tags on outer primers rather than on inner primers would shorten and thereby simplify the design of the inner, target-specific primers. Furthermore, a POSA would understand that short primers also are less prone to dimerization than long ones. Furthermore, a POSA would recognize that May PCT's primer configuration is economical: separating barcodes from targeting primers reduces the number of unique primers needed by orders of magnitude. A POSA would have reasonably expected the combination to succeed. Both Pieprzyk and May PCT teach using four primers to tag and amplify DNA. Furthermore, May PCT discloses that its 4-primers works for multiplex reactions like those in Pieprzyk. *See, e.g.,* May PCT at [0210], [0276], and Fig.18. Thus, a POSA would recognize this combination as trivial. May PCT further discloses this claim limitation in the examples cited in Claim 13 of Exhibit 1-C, which are hereby incorporated by reference in their entireties. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Pieprzyk also discloses this element for the same reasons as discussed above regarding claim 2.

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EXHIBIT 1-E – FINAL INVALIDITY CLAIM CHART FOR U.S. PATENT NO. 11,519,035

Reference:
U.S. Patent Publication No. 2010/0120038 A1 ("Mir")

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

To the extent Natera asserts that Mir does not disclose or teach one or more limitations of Claims 1, 2, 12 or 13, performing the methods as claimed in those claims would have been obvious to a person of ordinary skill in the art at the time of the alleged invention considering Mir in combination with the knowledge of a POSA and/or one or more of the following references:

- International Patent Publication No. 2007/018601("Makarov")
- Shapero et al., *MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density DNA oligonucleotide arrays*, 32 NUCLEIC ACIDS RES. e101 (2004) ("Shapero")
- International Patent Publication WO 2010/126614 A2 ("Porreca")
- U.S. Patent Application Publication US 2004/0110153 A1 ("Dong")

A POSA would have been motivated to combine Mir with one or more of the foregoing references because Mir expressly teaches using routine and conventional techniques known in the art to analyze fragmented sample DNA efficiently. For example, Mir expressly invites the use of its method with other routine and conventional techniques. Mir at [0143] ("Any method of detection and/or quantification of nucleic acids can be used in the invention to detect amplification products"). As another example, Mir teaches methods comprising "ligation of adaptor sequences onto the ends of [DNA] fragments" (Mir at [0160]) as well as "restriction fragment length polymorphism[s] (RLFPs)" (Mir at [0083]), and a POSA would recognize cell-free DNA as a form of fragmented DNA. As another example, a POSA would be motivated to utilize the methods of Mir for cell free tumor DNA assays because it was well known at the time that cancer DNA can take the form of cell free DNA and Mir expressly teaches that its methods "can also be employed to determine DNA or RNA (*e.g.,* mRNA, miRNA) copy number" and "[d]etermination of aberrant DNA copy number in genomic DNA is useful, for example, in the diagnosis and/or prognosis of genetic defects and diseases, such as cancer." Mir at [0176]. As another example, a POSA would be motivated to utilize ligation to add one or more universal tail adaptors at least because Mir expressly teaches the use of universal tail adaptors, and it was well known in the art at the time of the invention that such adaptors could be added through ligation.

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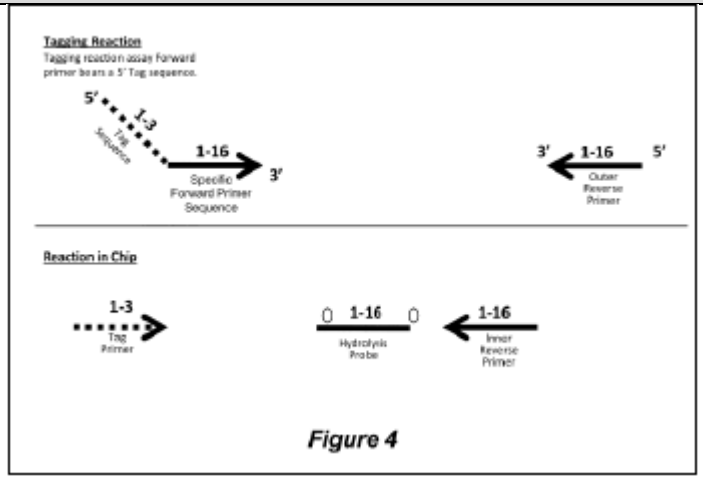
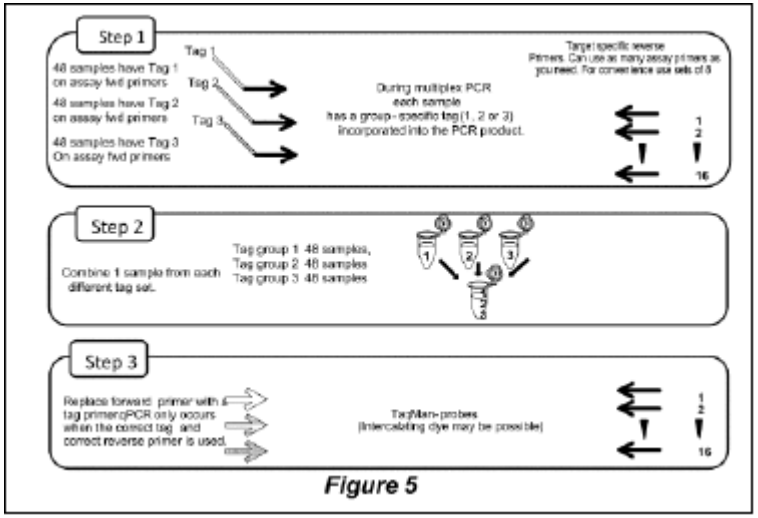
Further, a POSA would reasonably expect to succeed in combining Mir with one or more of the foregoing references for the purposes described above because the techniques of isolating, tagging, amplifying, and sequencing cell free DNA as well as using ligation to tag cell free DNA with universal tail adaptors were all well known in the art at the time of the invention.

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Claim	**Prior Art**
1[pre]. A method for amplifying and sequencing DNA, comprising:	To the extent the preamble is construed to be limiting, Mir discloses a method for amplifying and sequencing DNA. *See, e.g.,* Mir at [0005]-[0026], [0029]-[0033], [0035], [0036], [0062], [0069], [0070]-[0072], [0086]-[0094], [0095]-[0122], [124], [0140]-[0141], [0143], [0149], [0150], [0154], [0155], [0159]-[0163], [0169]-[0173], [0177], [0183]-[0188], [0190]-[0198] Example 1, Example 2, Example 3, Claim 1, Claim 2, Claim 3, Claim 4, Claim 5, Claim 6, Claim 7, Claim 8, Claim 9, Claim 10, Claim 17, Claim 23, Claim 24, Claim 25, Claim 26, Claim 27, Claim 28, Claim 29, Claim 30, Claim 31, Claim 32, Claim 33, Claim 34, Claim 35, Claim 36, Claim 37, Claim 38, Claim 39, Claim 40, Claim 41, Claim 42, Claim 43, Claim 44, Claim 45, Claim 46, Figures 2, 3, 4, 5, 6, 8. For example, Mir discloses an illustrative example of a method "for detecting a plurality of target nucleic acids" which entails an amplification step "whereby the tagged target nucleotide sequences are amplified using at least one primer that anneals to at least one nucleotide tag." Mir at [0086]. As another example, Mir discloses that "[i]f desired, tagged nucleotide sequences generates as described herein may be analyzed by DNA sequencing." Mir at [0160]. As another example, Mir discloses an assay method "which detects a plurality of nucleic acids" in a sample wherein "[t]he sample is subjected to preamplification to produce an assay mixture, wherein any preamplification product produced for a particular target incorporates a unique combination of forward and reverse set-specific nucleotide tags." Mir at [0021]. As another example, Mir discloses that "[t]he presence or amount of the amplification product of a unique primer pair can be detected using any means capable of detecting the presence of a target nucleotide sequencing in combination with a particular sample-specific tag." Mir at [0093]. Mir further discloses that

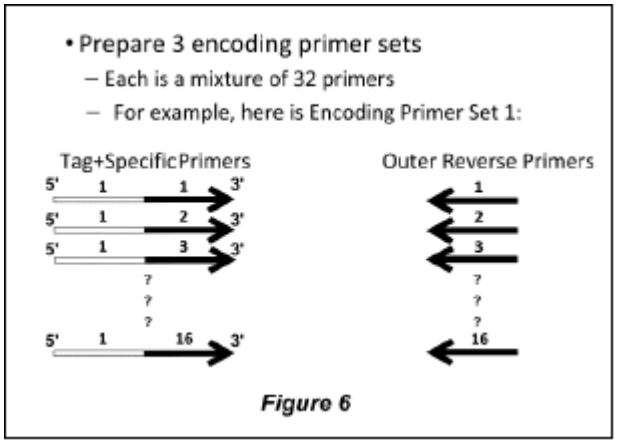
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Claim	**Prior Art**
	one such method is sequencing. Mir at [0160] ("[i]f desired, tagged nucleotide sequences generates as described herein may be analyzed by DNA sequencing.")
1[a][i]. tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,	Under Natera's apparent construction, Mir discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See, e.g.,* Mir at [0008]-[0012], [0021]-[0023], [0031], [0032], [0064], [0066]-[0068], [0089]-[0096], [0097-102], [0106]-[0112], [0115]-[0117], [0159], [0162], [0178], [0190]-[0197], Example 2, Example 3, Claim 1, Claim 2, Claim 3, Claim 4, Claim 9, Claim 10, Claim 11, Claim 12, Claim 23, Claim 24, Claim 25, Claim 26, Claim 27, Claim 28, Claim 29, Claim 30, Claim 31, Claim 32, Claim 33, Claim 34, Claim 35, Claim 36, Claim 37, Claim 38, Claim 39, Claim 40, Claim 41, Claim 42, Claim 43, Claim 44, Claim 45, Claim 46, Figures 4, 5, 6. For example, Figures 4, 5, and 6 of Mir disclose a multistep protocol involving tagging, pooling samples, and qPCR. Mir at Figures 4-6. Figure 4 discloses a "tagging reaction" wherein the "[f]orward primer bears a 5' Tag sequence" followed by a "[s]pecific [f]orward [p]rimer [s]equence." *Id* at Figure 4. Step 1 of the protocol comprises target specific amplification and tagging with multiplex PCR wherein the forward primer comprises a universal adaptor and the reverse primer is target specific. *Id.* at Figures 4-5.

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Claim	**Prior Art**
	 *Figure 4*  *Figure 5* As another example, Mir discloses an exemplary protocol for "carrying out an assay method of invention to quantify the expression of 16 cDNAs in 36 serially diluted samples" wherein "[d]uring the tagging step

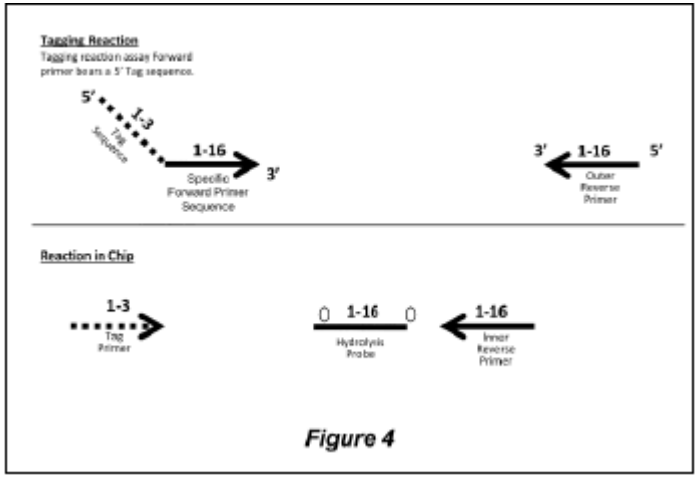
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Claim	**Prior Art**
	(Fig. 6), sample was divided into 3 separate groups with 48 tag-specific reactions containing differing dilution series. Three sets of 16 forward primers for amplifying 16 cDNAs were synthesized. Each set bears a different tag sequence appended to the 5' end of the forward primers. Each 16 forward primers with the same 5' Tag were pooled together and mixed with 16 gene specific 3' reverse primers to make a multiplex tagging primer mix, one for each Tag. See FIG. 6." Mir at [01941-F0195] (describing Example 3); *id.* at Figure 6.  *Figure 6* As another example, Mir discloses an exemplary embodiment that entails "separately subjecting each of the S samples to preamplification using a distinct set of forward and reverse preamplification primers for each sample to produce preamplified samples." Mir at [0095]. Mir further discloses that, for this example, "all forward preamplification primers or all reverse preamplification primers in a given set comprise a sample-specific nucleotide tag, which can, but need not, be a common sample-specific nucleotide tag. The sample specific nucleotide tag is generally 5' of the target-specific nucleotide sequence in the primer." *Id.* As another example, Mir discloses an illustrative embodiment wherein "all forward preamplification primers or all reverse preamplification primers in a given set comprise a common sample-specific nucleotide tag." Mir at [0010]. Mir further discloses that "[t]he nucleotide sequence of the sample-specific nucleotide tag encodes sample identify. All tagged target nucleotide sequences produced from a given sample can be

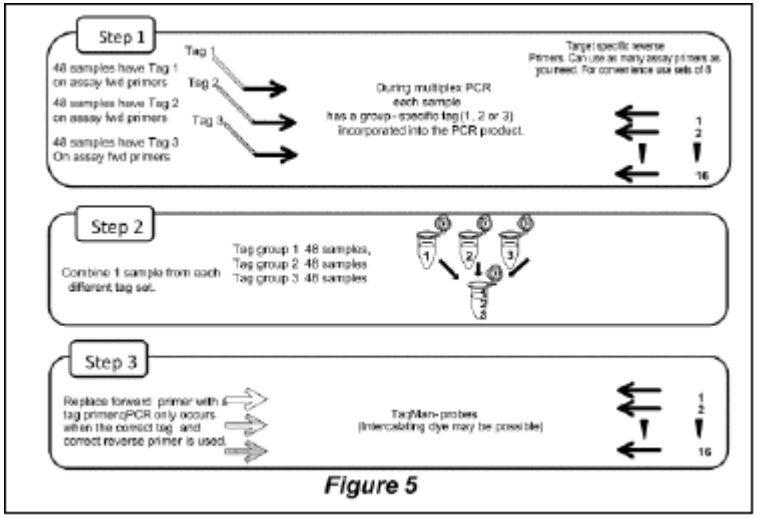
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Claim	**Prior Art**
	tagged with a common sample-specific nucleotide tag, *i.e.,* one that has the same nucleotide sequence." *Id.* at [0094].
1[a][ii]. Wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;	Mir discloses tagging cell free DNA isolated from a blood sample collected from a subject who is not a pregnant women. *See, e.g.,* Mir at [0047], [0122], [0176]. For example, Mir discloses that "[p]reparations of nucleic acids ("samples") can be obtained from biological sources and prepared using conventional methods known in the art." Mir at [0122]. Mir further discloses "[n]ucleic acids can be extracted or amplified from cells, bodily fluids (e.g., blood, a blood fraction, using, etc.), or tissue samples by any variety of standard techniques. Exemplary samples include samples of plasma, serum, spinal fluid, lymph fluid, peritoneal fluid, pleural fluid, oral fluid, and external sections of the skin; samples from the respiratory, intestinal genital, and urinary tracts; samples of tears, saliva, blood cells, stem cells, or tumors. Nucleic acids of interest can be isolated using methods well known in the art, with the choice of a specific method depending on the source, the nature of nucleic acid, and similar factors. The sample nucleic acids need not be in pure form, but are typically sufficiently pure to allow the amplification steps of the methods of the invention to be performed." *Id.* As another example, Mir discloses that "determinations of aberrant DNA copy number in genomic DNA is useful, for example, in the diagnosis and/or prognosis of genetic defects and diseases, such as cancer." Mir at [0176]. As another example, Mir discloses that "[t]he nucleic acid(s) can be derived . . . form a biological source, such as through isolation from any specific that produces nucleic acid." Mir at [0047].
1[b][i]. amplifying the tagged products one or more times to generate final	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Mir discloses amplifying the tagged products one or more times to generate final amplification products. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity

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Claim	**Prior Art**
amplification products,	Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.,* [0005]-[0015], [0021]-[0023], [0031], [0086], [0087], [0090]-[0092], [0095], [0096], [0098] - [0101], [0106]-[0108], [0110]-[0112], [0115]-[0117], [0159], [0160], [0161], [0162], Example 1, Example 2, Example 3, Claim 1, Claim 2, Claim 3, Claim 4, Claim 5, Claim 6, Claim 7, Claim 8, Claim 9, Claim 10, Claim 11, Claim 12, Claim 13, Claim 23, Claim 24, Claim 25, Claim 26, Claim 27, Claim 28, Claim 29, Claim 30, Claim 31, Claim 32, Claim 33, Claim 34, Claim 35, Claim 36, Claim 37, Claim 38, Claim 39, Claim 40, Claim 41, Claim 42, Claim 43, Claim 44, Claim 45, Claim 46, Figures 4, 5, 6, 7, 8, 9. For example, Figures 4, 5, and 6 of Mir disclose a multistep protocol involving tagging, pooling samples, and qPCR. Mir at Figures 4-6. Figure 4 discloses a "tagging reaction" wherein the "[f]orward primer bears a 5' Tag sequence" followed by a "[s]pecific [f]orward [p]rimer [s]equence." *Id* at Figure 4.  ***Figure 4***

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Claim	**Prior Art**
	For example, Step 1 of the protocol disclosed in Figure 5 comprises target specific amplification and tagging with multiplex PCR wherein the forward primer comprises a universal adaptor and the reverse primer is target specific. Mir at Figure 5.  *Figure 5* As another example, Mir discloses "[d]etection of the tagged target nucleotide sequences can be carried out by separately subjecting each aliquot to amplification using the same set of T different amplification primer pairs for each aliquot, each primer pair Including *[sic]* a first primer that anneals to the first nucleotide tag and a second primer that anneals to the second nucleotide tag in each tagged target nucleotide sequence." Mir at [0117]. As another example, Mir discloses that "preamplification can be carried out for 2-20 cycles to introduce the sample-specific or set-specific nucleotide tags." Mir at [0159].

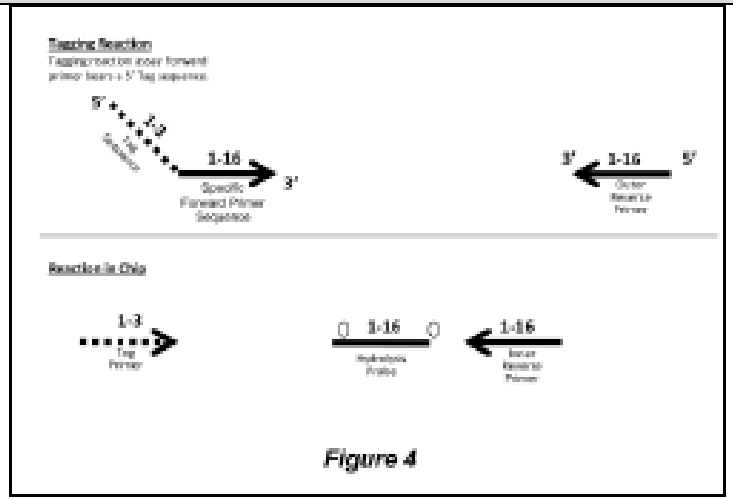
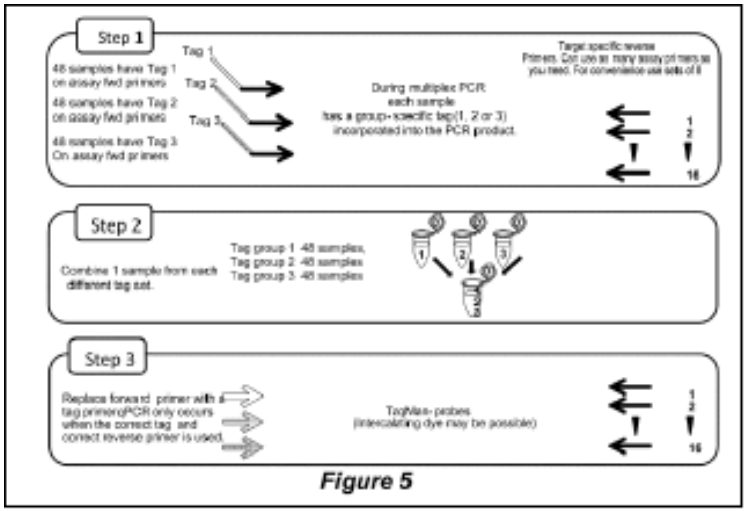
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U.S. Patent No. 11,519,035	
Claim	**Prior Art**
	As another example, Mir discloses an illustrative embodiment that entails "[a]n amplification step" "whereby the tagged target nucleotide sequences are amplified using at least one primer that anneals to at least one nucleotide tag." Mir at [0086]. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Mir also discloses this element for the same reasons.
1[b][ii]. Wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Mir discloses amplifying wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.,* Mir at [0005]-[0023], [0030]-[0033], [0086]-[0121], [0125], [0143], [0159], [0160], [0162], [0163], [0175], Example 1, Example 2, Example 3, Example 4, Claim 1, Claim 2, Claim 3, Claim 4, Claim 5, Claim 6, Claim 7, Claim 8, Claim 9, Claim 10, Claim 11, Claim 12, Claim 13, Claim 26, Claim 27, Claim 28, Claim 29, Claim 30, Claim 31, Claim 32, Claim 33, Claim 34, Claim 35, Claim 36, Claim 37, Claim 38, Claim 39, Claim 40, Claim 41, Claim 42, Claim 43, Claim 44, Claim 45, Claim 46, Figures 3, 4, 5, 6, 7, 8, 9.

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	For example, Mir discloses that "[t]he methods of the invention are applicable to any technique aimed at detecting the presence or amount of one or more target nucleic acids in a sample. Thus, for example, these methods are applicable to identifying the presence of particular polymorphisms (such as SNPs)." Mir at [0175]. As another example, Figure 3 of Mir discloses data from a genotyping study wherein "16 SNP loci were genotyped using tagging and, for comparison, standardized methodologies." Mir at [0030] (describing Figure 3). Mir further discloses in Figure 4 an "illustrative setup for analyzing multiple target nucleic acids in multiple samples" wherein the "[t]aging reactions employ an assay-specific tagged primer and an outer reverse primer." *Id.* at [0031] (describing Figure 4). Mir discloses in Figure 5 that this method comprises targeted amplification. *Id.* at Figure 5; *id.* at [0032] (describing Figure 5). Truth (not Tagged) % Call Rate, n=3 Assay | % Call Rate | SD SNP01 | 98.4 | 1.3 SNP02 | 97.8 | 0.0 SNP03 | 97.8 | 0.0 SNP04 | 97.1 | 1.3 SNP05 | 97.1 | 1.3 SNP06 | 97.8 | 0.0 SNP07 | 97.8 | 0.0 SNP08 | 97.8 | 0.0 SNP09 | 97.8 | 0.0 SNP10 | 98.8 | 1.3 SNP11 | 97.1 | 1.3 SNP12 | 97.8 | 0.0 SNP13 | 98.7 | 0.0 SNP14 | 98.7 | 0.0 SNP15 | 97.8 | 0.0 SNP16 | 97.8 | 0.0 Average | 97.4 | 0.4 Std Genotyping (no tagging) Invalids, no calls removed Mean Call rate = 97.4% Test (Tagged) % Call Rate by Tag Assay | 1 | 2 | 3 SNP01 | 100 | 100 | 97.9 SNP02 | 100 | 100 | 97.9 SNP03 | 100 | 100 | 97.9 SNP04 | 100 | 100 | 97.9 SNP05 | 100 | 100 | 100 SNP06 | 100 | 100 | 100 SNP07 | 100 | 100 | 100 SNP08 | 100 | 100 | 98 SNP09 | 100 | 100 | 100 SNP10 | 100 | 100 | 97.9 SNP11 | 100 | 100 | 97.9 SNP12 | 100 | 100 | 97.9 SNP13 | 100 | 100 | 97.9 SNP14 | 100 | 100 | 100 SNP15 | 100 | 100 | 100 SNP16 | 100 | 100 | 100 Average | 100 | 100 | 98.8 Tagged Genotyping Same DNA, Same assays Mean call rate 98.8 - 100% *Figure 3*

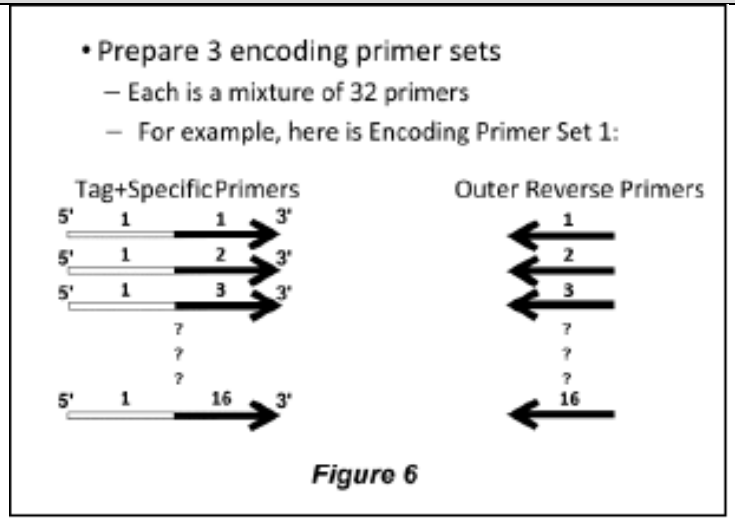
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Claim	**Prior Art**
	 *Figure 4*  *Figure 5*

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	As another example, Mir discloses an assay method wherein each sample "is separately subjected to an encoding reaction that produces a set of T tagged target nucleotide sequences, each tagged target nucleotide sequence comprising a first nucleotide tag linked to a target nucleotide sequence, which is linked to a second nucleotide tag. For each of these S samples (i.e., each of the samples to be combined), the tagged target nucleotide sequences are mixed to form an assay mixture. The assay mixture, or aliquots thereof, is subjected to amplification using S x T unique pairs of amplification primers, wherein each amplification primer pair comprises: a forward or a reverse amplification pruner that anneals to a first nucleotide tag; and a reverse or a forward amplification primer, respectively, that anneals to a second nucleotide tag." Mir at [0013]-[0015]. As another example, Mir discloses an exemplary protocol for "carrying out an assay method of invention to quantify the expression of 16 cDNAs in 36 serially diluted samples" wherein "[d]uring the tagging step (Fig. 6), sample was divided into 3 separate groups with 48 tag-specific reactions containing differing dilution series. Three sets of 16 forward primers for amplifying 16 cDNAs were synthesized. Each set bears a different tag sequence appended to the 5' end of the forward primers. Each 16 forward primers with the same 5' Tag were pooled together and mixed with 16 gene specific 3' reverse primers to make a multiplex tagging primer mix, one for each Tag. See FIG. 6." Mir at [0194]-[0195] (describing Example 3); *id.* at Figure 6.

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	 ***Figure 6*** Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Mir also discloses this element for the same reasons.
1[b][iii]. Wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and	Mir discloses amplifying wherein one of the amplifying steps introduces a barcode and one or more sequencing tags. *See, e.g.,* Mir at [0021]-[0023], [0067], [0094], [0106]-[0108], [0110]-[0112], [0159], [0160], [0161], Claim 13. For example, Mir discloses that "[i]f desires, tagged target nucleotide sequences generated as described herein may be analyzed by DNA sequencing. Many current DNA sequencing techniques rely on 'sequencing by synthesis.' These techniques entail library creation, massively parallel PCR amplification of

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	library molecules, and sequencing. Library creation starts with conversion of sample nucleic acids to appropriately sized fragments, ligation of adaptor sequences onto the ends of the fragments, and selection for molecules properly appended with adaptors. The presence of the adaptor sequences on the ends of the library molecules enables amplification of random-sequence inserts. The above-described methods for tagging nucleotide sequences can be substituted for ligation, to introduce adaptor sequences." Mir at [0160]. As another example, Mir discloses an illustrative embodiment wherein "[t]he sample is subjected to preamplification to produce an assay mixture, wherein any preamplification product produced for a particular target incorporates a unique combination of forward and reverse set-specific nucleotide tags. The assay mixture, or aliquots thereof, to amplification using amplification primers wherein each amplification primer pair comprises: a forward amplification primer that anneals to the forward set-specific nucleotide tag; and a reverse amplification primer that anneals to the reverse set-specific nucleotide tag. For each unique primer pair, the presence or amount of an amplification product in the amplification mixture, or aliquot thereof, is determined. The presence of an amplification product indicates the presence of a particular target nucleic acid in the sample." Mir at [0021]–[0023]. As another example, Mir discloses an illustrative "dual tagging assay method" wherein "each forward preamplification primer includes a different target-specific nucleotide sequence and a set-specific nucleotide tag." Mir at [0106]. As another example, Mir discloses that "[i]n various embodiments, employing preamplification, the number of preamplification cycles is sufficient to add one or more nucleotide tags to the target nucleotide sequences, so that the relative copy numbers of the tagged target nucleotide sequences is substantially representative of the relative copy numbers of the target nucleic acids in the sample. For example, preamplification can be carried out for 2-20 cycles to introduce the sample-specific or set-specific nucleotide tags." Mir at [0159]. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement

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	Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Mir also discloses this element for the same reasons.
1[c][i]. sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products,	Mir discloses sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products. *See, e.g.,* Mir at [0088], [0113], [0141], [0159], [0160], [0161], [0175], [0177]. For example, Mir discloses that "[i]f desires, tagged target nucleotide sequences generated as described herein may be analyzed by DNA sequencing. Many current DNA sequencing techniques rely on 'sequencing by synthesis.' These techniques entail library creation, massively parallel PCR amplification of library molecules, and sequencing. Library creation starts with conversion of sample nucleic acids to appropriately sized fragments, ligation of adaptor sequences onto the ends of the fragments, and selection for molecules properly appended with adaptors. The presence of the adaptor sequences on the ends of the library molecules enables amplification of random-sequence inserts. The above-described methods for tagging nucleotide sequences can be substituted for ligation, to introduce adaptor sequences." Mir at [0160]. Mir further discloses that "[i]n particular embodiments, the number of library DNA molecules produced in the massively parallel PCR step is low enough that the chance of two molecules associating with the same substrate, e. g. the same bead (in 454 DNA sequencing) or the same surface patch (in Solexa DNA sequencing) is low, but high enough so that the yield of amplified sequences is sufficient to provide a high throughput in automated sequencing. After suitable adaptor sequences are introduced, as discussed above, digital PCR can be employed to calibrate the number of library DNA molecules prior to sequencing by synthesis." *Id.* at [0161]. As another example, Mir discloses "[i]n various embodiments, employing preamplification, the number of preamplification cycles is sufficient to add one or more nucleotide tags to the target nucleotide sequences, so that the relative copy numbers of the tagged target nucleotide sequences is substantially representative of the relative copy numbers of the target nucleic acids in the sample. For example, preamplification can be carried out for 2-20 cycles to introduce the sample-specific or set-specific nucleotide tags." Mir at [0159].

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	As another example, Mir discloses "The methods of the invention are applicable to any technique aimed at detecting the presence or amount of one or more target nucleic acids in a nucleic acid sample. Thus, for example, these methods are applicable to identifying the presence of particular polymorphisms (such as SNPs), alleles, or haplotypes, or chromosomal abnormalities, such as amplifications, deletions, or aneuploidy." Mir at [0175].
1[c][ii]. Wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.	The Court construed the term "SNP loci…associated with cancer" to mean "a single nucleotide that may differ between the genomes of two members of the same species and is associated with cancer. The usage of the term 'SNP' should not imply any limit on the frequency with which each variant occurs." Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Mir discloses a plurality of SNP loci comprising 25-2,000 loci associated with cancer. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.,* Mir at [0088], [0094], [0109], [0113], [0116], [0121], [0122], [0175], [0176], [0183]-[0188], [0195], [0196], Example 1, Claim 11, Claim 12, Figures 5, 6. For example, Mir discloses that under its "dual tagging assay method" "where X+Y primers are prepared, XxY targets can be analyzed in a single assay. For example, if X and Y are each 100, only 200 primers need be synthesized to detect 10,000 target nucleic acids." Mir at [0109]. As another example, Mir discloses "methods [] applicable to identifying the presence of particular polymorphisms (such as SNPs)." Mir at [0175]. As another example, Mir discloses "in particular embodiments the encoding reaction can produce up to RxT (e.g., 96x96=9216) different tagged target nucleotide sequences, thus permitting the assay of RxT (e.g., 9216)." Mir at [0116]. Mir further discloses that "in particular embodiments, this modular approach is extremely flexible and can be easily expanded by additional targets (e.g., an additional 96 targets)." *Id.* at

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	[0121]. Mir further discloses methods useful "in the diagnosis and/or prognosis of genetic defects and diseases, such as cancer." *Id.* at [0176]. As another example, Mir discloses methods of sample analysis, wherein "exemplary samples include . . . samples of tears, saliva, blood cells, stem cells, or tumors." Mir at [0122]. To the extent Natera asserts that any portion of this limitation is not disclosed under Natera's apparent interpretation of the Court's construction, it would have been obvious based on the knowledge of a POSA at the time of the alleged invention to look to the Catalogue of Somatic Mutations In Cancer ("COSMIC") database, which identifies hundreds to thousands of gene variants, including those that can be present in the same gene, to readily disclose a plurality of SNP loci comprising 25-2,000 loci associated with cancer. By way of example, COSMIC identifies 6,038 different variants in the TP53 gene alone. *See, e.g.,* https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53. In addition, the '035 Patent admits a POSA's "general belief in the art is 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.
2. The method of claim 1, wherein tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA.	Mir discloses tagging the cell free DNA comprising ligating the one or more universal tail adaptors to the cell free DNA. *See, e.g.,* Mir at [0062], [0160]. For example, Mir discloses that "These techniques entail library creation, massively parallel PCR amplification of library molecules, and sequencing. Library creation starts with conversion of sample nucleic acids to appropriately sized fragments, ligation of adaptor sequences onto the ends of the fragments, and selection for molecules properly appended with adaptors. The presence of the adaptor sequences on the ends of the library molecules enables amplification of random-sequence inserts. The above-described

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	methods for tagging nucleotide sequences can be substituted for ligation, to introduce adaptor sequences." Mir at [0160]. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 27. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Mir also discloses this element for the same reasons as discussed below regarding claim 13.
12. The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.	Mir discloses one or more universal tail adaptors comprising a first universal tail adaptor and a second universal tail adaptor. *See, e.g.,* Mir at [0005]-[0012], [0021]-[0023], [0064], [0066]-[0068], [0089]-[0096], [0101], [0106]-[0112], [0117], [0159], [0190]-[0197], Example 2, 3, Claim 1, Claim 2, Claim 3, Claim 4, Claim 9, Claim 10, Claim 23, Claim 24, Claim 25, Claim 26, Claim 27, Claim 28, Claim 29, Claim 30, Claim 31, Claim 32, Claim 33, Claim 34, Claim 35, Claim 36, Claim 37, Claim 38, Claim 39, Claim 40, Claim 41, Claim 42, Claim 43, Claim 44, Claim 45, Claim 46, Figures 4, 5, 6. For example, Mir discloses an illustrative method "which detects a plurality of target nucleic acids (i.e., T target nucleic acids) in a sample. The method entails providing T forward preamplification primers to a sample, wherein each forward preamplification primer comprises a different target specific nucleotide sequence and a set-specific nucleotide tag, wherein X different forward set-specific nucleotide tags are employed, and X is an integer that is greater than 1 and less than T, whereby T/X primers comprise the same forward set-specific nucleotide tag. Also provided to the sample are T reverse preamplification primers, wherein each reverse preamplification primer comprises a different target-specific nucleotide sequence and a reverse set-specific nucleotide tag, wherein Y different reverse set-specific nucleotide tags are employed, and Y is an integer that is greater than I and less than T, whereby T/Y primers comprise the same reverse set specific nucleotide tag. The sample is subjected to preamplification to produce an assay mixture, wherein any preamplification product produced for a particular target incorporates a unique combination of forward

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	and reverse set-specific nucleotide tags. The assay mixture, or aliquots thereof, to amplification using amplification primers wherein each amplification primer pair comprises: a forward amplification primer that anneals to the forward set-specific nucleotide tag; and a reverse amplification primer that anneals to the reverse set-specific nucleotide tag. For each unique primer pair, the presence or amount of an amplification product in the amplification mixture, or aliquot thereof, is determined. The presence of an amplification product indicates the presence of a particular target nucleic acid in the sample." Mir at [0021]-[0023]. As another example, Mir discloses an illustrative "dual tagging assay" which entails "entails providing T forward preamplification primers to a sample, wherein T is the number of targets to be detected. Each forward preamplification primer includes a different target-specific nucleotide sequence and a set-specific nucleotide tag. The set-specific nucleotide tag is generally 5' of the target-specific nucleotide tag. X different forward set-specific nucleotide tags are employed, and X is an integer that is greater than I and less than T. Thus, T/X primers comprise the same forward set specific nucleotide tag. Also provided to the sample are T reverse preamplification primers. Each reverse preamplification primer includes a different target-specific nucleotide sequence and a reverse set-specific nucleotide tag. The set-specific nucleotide tag is generally 5' of the target-specific nucleotide tag. Y different reverse set-specific nucleotide tags are employed, and Y is an integer that is greater than I and less than T. Thus, T/Y primers comprise the same reverse set-specific nucleotide tag." Mir at [0106]-[0107].
13. The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the	Under Natera's apparent construction, Mir discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See, e.g.,* Mir at [0005]-[0012], [0021]-[0023], [0031], [0032], [0064], [0066]-[0068], [0089]-[0096], [0097-102 [0106]-[0112], [0115]-[0117], [0159], [0162], [0178], [0190]-[0197], Example 2, Example 3, Claim 1, Claim 2, Claim 3, Claim 4, Claim 9, Claim 10, Claim 11, Claim 12, Claim 23, Claim 24, Claim 25, Claim 26, Claim 27, Claim 28, Claim 29, Claim 30, Claim 31, Claim 32, Claim 33, Claim 34, Claim 35, Claim 36, Claim 37, Claim 38, Claim 39, Claim 40, Claim 41, Claim 42, Claim 43, Claim 44, Claim 45, Claim 46, Figures 4, 5, 6.

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second universal tail adaptor.	For example, Mir discloses an illustrative embodiment wherein "[t]he sample is subjected to preamplification to produce an assay mixture, wherein any preamplification product produced for a particular target incorporates a unique combination of forward and reverse set-specific nucleotide tags. The assay mixture, or aliquots thereof, to amplification using amplification primers wherein each amplification primer pair comprises: a forward amplification primer that anneals to the forward set-specific nucleotide tag; and a reverse amplification primer that anneals to the reverse set-specific nucleotide tag. For each unique primer pair, the presence or amount of an amplification product in the amplification mixture, or aliquot thereof, is determined. The presence of an amplification product indicates the presence of a particular target nucleic acid in the sample." Mir at [0021]–[0023]. As another example, Mir discloses an illustrative "dual tagging assay" which entails "entails providing T forward preamplification primers to a sample, wherein T is the number of targets to be detected. Each forward preamplification primer includes a different target-specific nucleotide sequence and a set-specific nucleotide tag. The set-specific nucleotide tag is generally 5' of the target-specific nucleotide tag. X different forward set-specific nucleotide tags are employed, and X is an integer that is greater than I and less than T. Thus, T/X primers comprise the same forward set specific nucleotide tag. Also provided to the sample are T reverse preamplification primers. Each reverse preamplification primer includes a different target-specific nucleotide sequence and a reverse set-specific nucleotide tag. The set-specific nucleotide tag is generally 5' of the target-specific nucleotide tag. Y different reverse set-specific nucleotide tags are employed, and Y is an integer that is greater than I and less than T. Thus, T/Y primers comprise the same reverse set-specific nucleotide tag." Mir at [0106]-[0107]. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Mir also discloses this element for the same reasons as discussed above regarding claim 2.

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EXHIBIT 1-F – FINAL INVALIDITY CLAIM CHART FOR U.S. PATENT NO. 11,519,035

Reference:
International Patent Publication WO 2010/126614 A2 ("Porreca")

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

To the extent Natera asserts that Porreca does not disclose or teach one or more limitations of Claims 1, 2, 12, or 13, performing the methods as claimed in those claims would have been obvious to a person of ordinary skill in the art at the time of the alleged invention considering Porreca in combination with the knowledge of a POSA and/or one or more of the following references:

- International Patent Publication No. 2007/18601 ("Makarov")
- International Patent Publication No. 2011/85491 ("Hansen")
- International Patent Publication No. 2011/142836 ("Pieprzyk")
- International Publication No. WO 2007/140417 ("Hoyal")
- U.S. Patent No. 6,420,117 ("Wessler")

A POSA would have been motivated to combine Porreca with one or more of the foregoing references because it would have been obvious to a POSA to apply Porreca's method to analyze cell-free DNA from any type of sample. For example, Porreca discloses detection of mutations in "maternally-circulating fetal DNA," and a POSA would understand that methods to detect mutations in maternally-circulating fetal DNA would be readily applicable to circulating tumor DNA. Porreca at 7:9. Furthermore, Porreca discloses more generally that "aspects of the invention are not limited" with respect to sample (Porreca at 4:7), and states generally that "the disclosure provides methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood." Porreca at 4:2-7.

Further, a POSA would reasonably expect to succeed in applying Porreca's methods to analyze cell-free DNA as disclosed in one or more of the foregoing references without undue experimentation.

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1[pre]. A method for amplifying and	To the extent the preamble is construed to be limiting, Porreca discloses a method for amplifying and sequencing DNA.

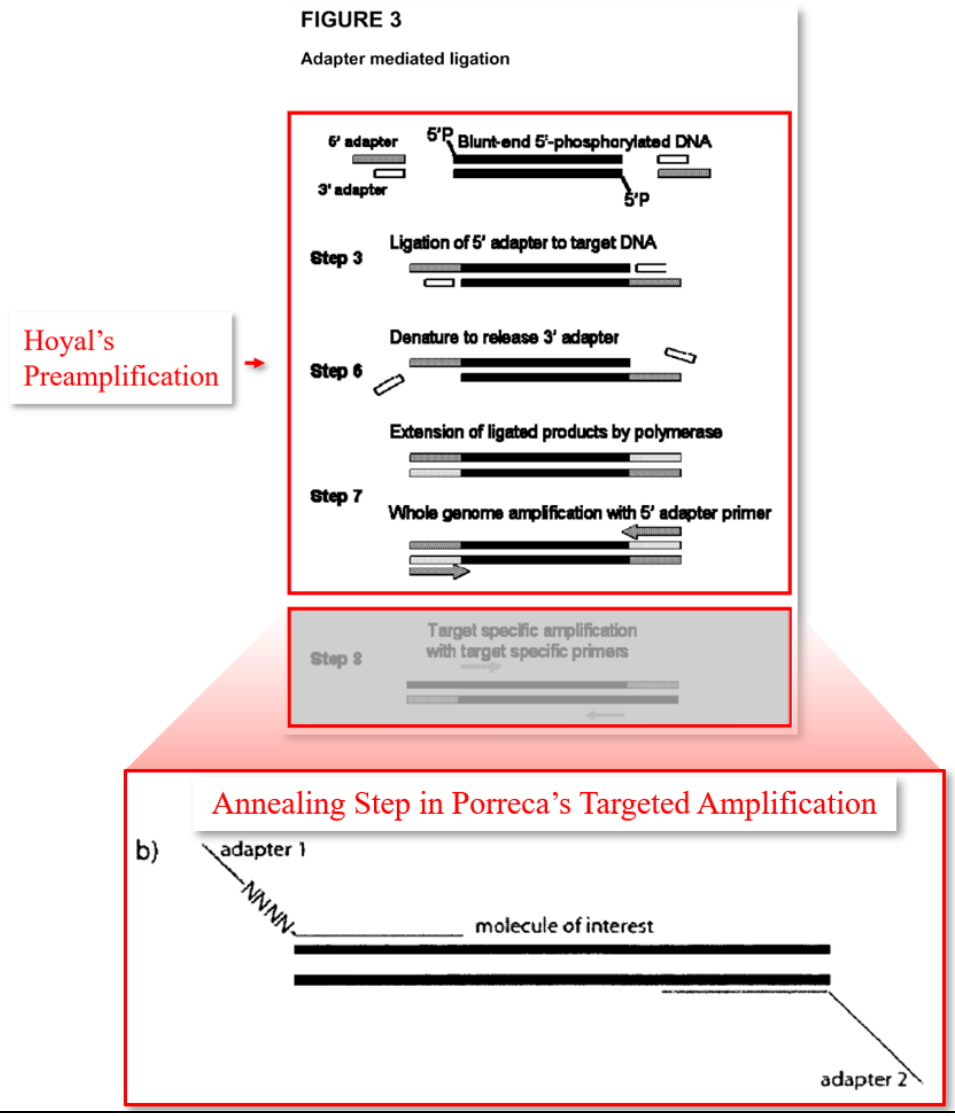
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sequencing DNA, comprising:	*See, e.g.,* Porreca at Abstract, 3:13-15, 2:12-16, 10:10-12, 13:6-8, 4:15-20, 8:5-17, 22:1-13, FIGS. 4A, 4B, 4C, FIG. 9. For example, Porreca discloses: "In some embodiments, the use of a single detection modality (e.g., sequencing) to assay multiple types of genetic lesions (e.g., point mutations, insertions/deletions, length polymorphisms) is advantageous in the clinical setting." Porreca at 3:13-15. As another example, Porreca discloses that "[i]n some configurations, predetermined preparative steps and/or nucleic acid sequence analysis techniques are used in multiplex analyses for a plurality of genetic loci in a plurality of samples." Porreca at Abstract. As another example, Porreca discloses: "According to aspects of the invention, certain types of genetic information can be under-represented or over-represented in a genetic analysis due to a combination of stochastic variation and systematic bias in any of the preparative stages (e.g., capture, amplification, etc.), determining stages (e.g., allele-specific detection, sequencing, etc.)." Porreca at 2:12-16. As another example, Porreca discloses that "[i]n some embodiments, the genotyping comprises determining the sequence of a target nucleic acid (e.g., a polymorphic sequence) at one or more (both) alleles of a genome (a diploid genome) of a subject." Porreca at 10:10-12. As another example, Porreca discloses: "Aspects of the invention may be combined with one or more sequence-based assays (e.g., SNP detection assays), for example in a multiplex format, to determine the genotype of one or more regions of a subject." Porreca at 13:6-8. As another example, Porreca discloses: "Accordingly, in some embodiments, aspects of the methods disclosed herein relate to genotyping a polymorphism of a target nucleic acid. In some embodiments, the genotyping may comprise determining that one or more alleles of the target nucleic acid are heterozygous or homozygous. In further embodiments, the genotyping may comprise determining the sequence of a polymorphism and comparing that sequence to a control sequence that is indicative of a disease risk." Porreca at 4:15-20. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach a method for amplifying and sequencing DNA, a POSA would find this limitation obvious in view of Porreca combined with Hoyal.

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	For example, Hoyal teaches "detecting and monitoring extracellular mutant oncogenes or tumor-associated DNA found in the plasma or serum fraction of blood by using DNA extraction with enrichment for mutant DNA," i.e., detecting cell-free tumor DNA. Hoyal at 20. As another example, Hoyal teaches a preamplification step involving "[a]dapter-mediated ligation." *Id.* at 27. Hoyal's adapter-mediated ligation ligates "a non-genome specific adapter to the blunt ends" of DNA in a sample, extends the ligated adapter, performs "whole genome amplification" with primers specific to the ligated adapter, and performs "specific amplification of a nucleic acid region of interest for further analysis." *Id.* at 27-28. A POSA would be motivated to combine Porreca with Hoyal because Porreca expressly teaches using routine and conventional techniques known in the art to amplify and sequence DNA, and the references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end, and thus are readily interchangeable with each other. Furthermore, a POSA would be motivated to combine Porreca and Hoyal because Porreca and Hoyal are analogous art because both are in the same field as the '035 patent or, at the very least, are reasonably pertinent to the problem the '035 patent sought to solve. Both references teach analyzing cell-free DNA derived from blood. (E.g., Porreca at 4:3-5 ("methods for analyzing multiple genetic loci…from a patient sample, such as a blood"); Hoyal at 10, 20 ("monitoring…tumor-associated DNA found in the plasma or serum fraction of blood by using DNA extraction with enrichment for mutant DNA,"). A POSA would have found it obvious to modify Porreca's method by adding Hoyal's isolation and adapter-mediated ligation preamplification steps to Porreca's method before Porreca's targeted amplification step. The following graphic showing Hoyal's Figure 3 (top) and Porreca's Figure 4b (bottom) illustrates how the combined method's amplifications would flow together by depicting Hoyal's steps 3, 6 and 7 before Porreca's targeted amplification step:

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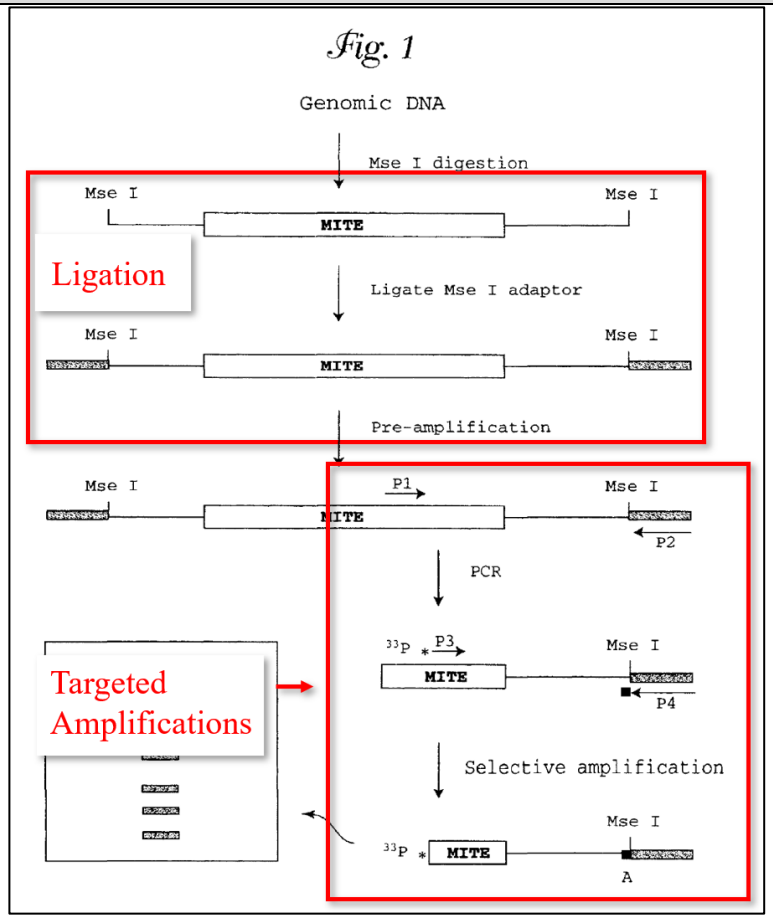
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Claim	**Prior Art**
	Porreca at Fig.4b; Hoyal at Fig.3 (combined & annotated). Teachings from both Porreca and Hoyal would have motivated a POSA to combine these methods. Porreca states that some methods, like its targeted amplification, can produce a skewed pool of amplicons that "does not adequately represent the input abundance distribution." Porreca at 67:27-28. This flaw "reduce[s] the sensitivity of the sequencing method" by increasing the risk that "potentially the 'mutant' allele was not the one observed." *Id.* at 67:33-68:2. Porreca calls this flaw "acute" in the context of monitoring cancer. *Id.*, 68:2-7. Hoyal's teachings provided a solution. Hoyal at ¶227. Like Porreca, Hoyal acknowledges that there was "a need for improved extraction methods capable of capturing small nucleic acid molecules"—cell-free DNA—because "the ability to successfully analyze short, fragmented DNA in the presence of more abundant, longer DNA ha[d] remained elusive." Hoyal at 1-2, 20. Hoyal solved that problem with isolation followed by universal preamplification. By isolating and then pre-amplifying the cell-free tumor DNA, Hoyal's method increased the initial number of copies of all target sequences and thereby improved the odds that a later, targeted amplification and sequencing could detect sequences with low prevalence. *Id.*, 7. A POSA thus would have been motivated to add a pre-amplification step to Porreca's method to achieve that benefit. A POSA would have had a reasonable expectation of success in adding Hoyal's preamplification steps to Porreca's method. For example, Porreca already discloses applying its method to nucleic acids from blood. Porreca at 8:5-8, 18:31-32, 19:3-4, 22:1-4, 67:20-21. As another example, Porreca already discloses ligating adapters to cell-free DNA to provide primer sites for non-targeted amplification. Porreca at 8:5-17, 43:17, 43:23-29; *see also id.*, 8:28-31. As another example, Porreca already discloses using primers for targeted amplification. *Id.* at 8:5-7, 43:29-33, Fig.4b; *see also id.*, 8:28-31. A POSA would reasonably have expected success in sequentially performing these routine steps, as in the combined method of Porreca and Hoyal. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach a method for amplifying and sequencing DNA, a POSA would find this limitation obvious in view of Porreca combined with Wessler. For example, Wessler discloses methods of "detecting at least one polymorphism between the nucleic acid fragments of two individuals." Wessler at Abstract. As another example, Wessler describes a targeted amplification involving selectively amplifying part of a target sequence ("MITE") via a first primer that anneals to MITE and a second primer complementary to part of a ligated sequencing adapter. *Id.* at 10:63-11:7, 11:45-60, 12:5-15; see also *id.,* 3:36-42. Then, in a second, optional targeted amplification, Wessler uses third and fourth

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	primers to amplify part of the earlier amplification product. (Id., 10:63-11:7, 11:45-60, 12:16-37; see also id., 3:36-42.) A POSA would be motivated to combine Porreca with Wessler because Porreca expressly teaches using routine and conventional techniques known in the art to amplify and sequence DNA, and the references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end, and thus are readily interchangeable with each other. Furthermore, a POSA would be motivated to combine Porreca with Wessler because Wessler is analogous art because it is in the same field as the '035 patent or, at the very least, is reasonably pertinent to the problem the '035 patent sought to solve. Wessler teaches methods for detecting polymorphisms in DNA. Wessler at 3:6-4:41. Although Wessler applies its methods to plant genomes, similar polymorphisms exist in humans and Wessler's methods are not limited to plants. Wessler at 2:1-4, 3:6-4:41, 29:22-30:4. And even if otherwise, by the critical date a POSA would have considered methods developed in the plant genomic context when devising methods of characterizing human genomes. Thus, as set forth below, it would have been obvious to combine Porreca and Wessler and then extend that combination to cell-free tumor DNA. Furthermore, a POSA would have been motivated to modify Porreca's method by performing Wessler's ligation and one or two targeted amplifications in lieu of Porreca's targeted amplification. The following graphic from Wessler shows its ligation and targeted amplification steps:

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	 Wessler at Fig.1 (annotated). Teachings from Porreca and Wessler would have motivated a POSA to combine these methods. First, a POSA would recognize that using two targeted amplifications as described in Wessler would improve performance. Porreca's single targeted amplification can sometimes produce a skewed pool of amplicons that limits the method's accuracy. Porreca at 67:27-68:7. A POSA would recognize that Wessler's ligation followed by preamplification

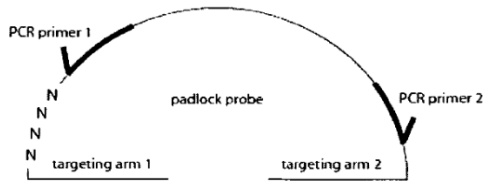
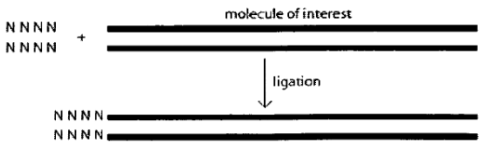
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Claim	**Prior Art**
	provides a solution. Wessler at Fig. 1. A POSA would recognize that, by preamplifying isolated, cell-free DNA, the combined method of Porreca and Wessler would increase the initial numbers of copies of all target sequences and thereby improve the odds that a later targeted amplification and sequencing could detect sequences with low initial prevalence. Second, a POSA also would have recognized that Wessler's second, optional amplification offered improved specificity over Porreca's method. A POSA would recognize that amplifying a first sequence and then amplifying a second sequence within the first sequence was a well-known method of reducing the abundance of non-specific or off-target amplification products compared to a single PCR reaction. Third, a POSA would understand that Wessler's two targeted amplifications would significantly reduce the number of possible off-target interactions between primers. Specifically, a POSA would recognize that Each targeted amplification uses half as many target-specific primers as in Porreca's amplifications. For example, a POSA would reason that a reaction with 100 pairs of unique forward and reverse primers involves 10,000 possible interactions between forward and reverse primers, yet a reaction with 100 unique forward primers and one reverse primer, however, involves only 100 such interactions. A POSA also would have reasonably expected the combination to succeed. By the critical date, adapting Porreca's method according to Wessler was within a POSA's skill. Furthermore, Porreca teaches its methods can "be implemented as described herein using alternative techniques and/or protocols that are available to one o[f] ordinary skill in the art." Porreca at 87:31-33, 88:1-2. Further still, a POSA would understand that the modification of Porreca's method with that of Wessler would have required nothing more than applying known ligation and amplification methods to isolated, cell-free DNA.
1[a][i]. tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,	Under Natera's apparent construction of this limitation, Porreca discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See, e.g.,* Porreca at 8:5-17, 8:32-9:6, 22:1-13, 27:1-3, 29:25-28, 29:28-29, 29:32-30:1, 60:18-24, 60:32-33, 61:4-5, 70:24-29, 4:2-7, 4:8, 7:9, 25:3-5, 26:3-6, 26:21-25, 31:23:26, 43:29-33, 75:4-11, FIGS. 4A, 4B, 4C, FIG. 9, claim 47, claim 48.

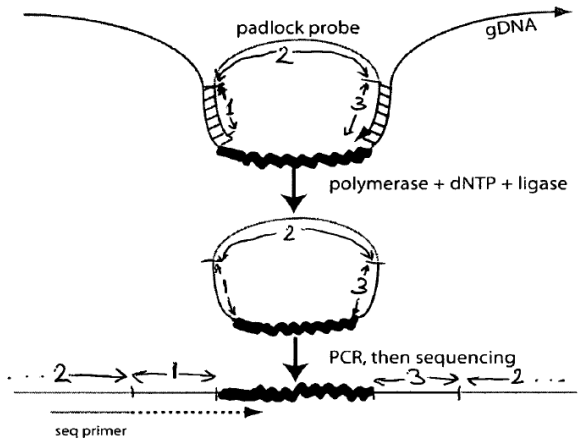
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Claim	**Prior Art**
	For example, Porreca discloses that the nucleic acids tagged in their methods can be circulating DNA from a blood sample of any patient. *See, e.g.,* Porreca at 4:2-7 ("In some embodiments, the disclosure provides methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample, or other sample (e.g., other biological fluid or tissue sample such as a biopsy sample) as aspects of the invention are not limited in this respect."). As another example, Porreca discloses tagging the isolated cell free DNA with a molecular inversion probe comprising a "constant" (universal) region: "In some embodiments, all probes are composed of three regions: 1) a 20 bp 'targeting arm' comprised of sequence which hybridizes immediately upstream from the sub-target, 2) a 30 bp 'constant region' comprised of sequence used as a pair of amplification priming sites, and 3) a second 20 bp 'targeting arm' comprised of sequence which hybridizes immediately downstream from the sub-target. Targeting arm sequences will be different for each capture probe in a set, while constant region sequence will be the same for all probes in the set, allowing all captured targets to be amplified with a single set of primers." Porreca at 75:4-11. As another example, Porreca discloses embodiments wherein each different molecular inversion probe comprises the same primer-binding sequence (a universal sequence). *See, e.g.*, Porreca at claim 47. As another example, Porreca discloses tagging the isolated cell free DNA with a molecular inversion probe comprising "a differentiator tag sequence and, optionally, a primer sequence." Porreca at 8:32-9:6; *see also, e.g.*, FIG. 4A. The primer sequence is a universal sequence. *See* Porreca at 75:4-11. Porreca further discloses that "one or more intervening sequences (e.g., sequence between the first and second targeting arms on a MIP capture probe), identifier or tag sequences, or other probe sequences that are not designed to hybridize to a target sequence (e.g., a genomic target sequence) should be designed to avoid excessive complementarity (to avoid cross-hybridization) to target sequences or other sequences (e.g., other genomic sequences) that may be in a biological sample." Porreca at 70:24-29. As another example, Porreca discloses that "FIGs. 4A, B, and C depict various non-limiting methods" for combining a tag sequence and target sequences." Porreca at 27:1-3.

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	FIG. 4A  FIG. 4B  FIG. 4C  Porreca at FIGS. 4A-4C. As another example, Porreca discloses: "In some embodiments, samples from different subjects are tagged with subject-specific (e.g., patient-specific) tags (e.g., unique sequence tags) so that the information from each product can be assigned to an identified subject. In some embodiments, each of the different capture probes used for each patient sample have a common patient-specific tag." Porreca at 29:25-29. As another example, Porreca discloses: "In some embodiments, the capture probes do not have patient-specific tags, but the captured products from each subject may be amplified using one or a pair of amplification primers that

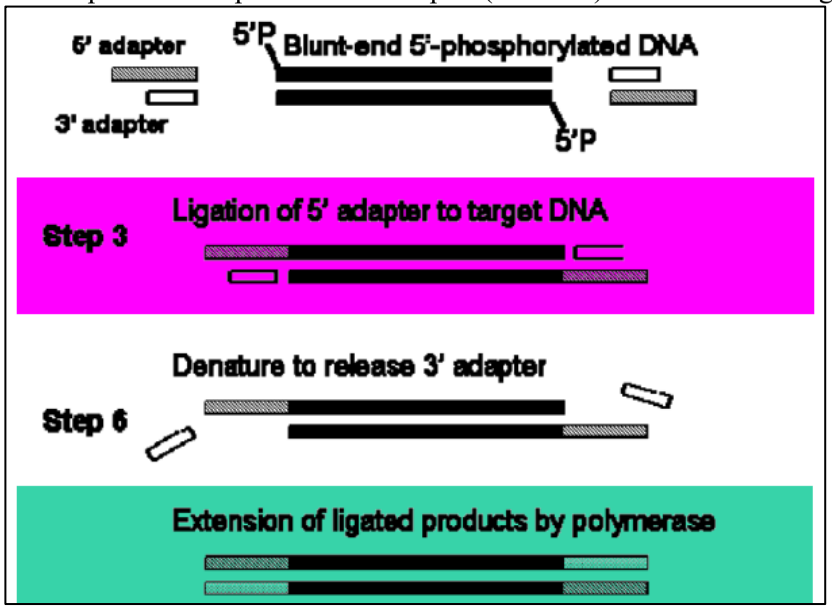
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Claim	**Prior Art**
	are labeled with a patient-specific tag." Porreca at 29:29-32. Porreca discloses embodiments with a "common patient-specific tag." Porreca at 29:29. As another example, Porreca discloses that "[o]ther techniques for associating a patient-specific tag with the captured product from a single patient sample may be used as aspects of the invention are not limited in this respect." Porreca at 29:32-30:1. Porreca discloses embodiments with a "common patient-specific tag." Porreca at 29:29. As another example, Porreca provides a non- limiting scheme of padlock (MIP) capture of a region of target DNA in FIG. 9. *See* Porreca at FIG. 9, 60:32-33. Porreca further explains that "[a]fter the padlock is circularized and amplified, it can be end-sequenced to obtain the sequence of the unique sequence, which represents the repetitive region of interest." Porreca at 61:4-5. Figure 9  Porreca at FIG. 9.

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	As another example, Porreca discloses a method wherein a "primer structure is arranged with the common adaptor sequence followed by the random differentiator tag sequence followed by the PCR priming sequence (in 5' to 3' order). A differentiator tag sequence and adaptor sequence (which may contain the sequencing primer site) are incorporated as tags." Porreca at 43:29-33. As another example, Porreca discloses: "In amplification-based (e.g., PCR-based or LCR-based, etc.) preparative methods, genomic loci (target nucleic acids) are isolated directly by means of a polymerase chain reaction or ligase chain reaction (or other amplification method) that selectively amplifies each locus using one or more oligonucleotide primers. It is to be understood that primers will be sufficiently complementary to the target sequence to hybridize with and prime amplification of the target nucleic acid. Any one of a variety of art known methods may be utilized for primer design and synthesis. One or more of the primers may be perfectly complementary to the target sequence. Degenerate primers may also be used. Primers may also include additional nucleic acids that are not complementary to target sequences but that facilitate downstream applications, including for example restriction sites and differentiator tag sequences. Amplification-based methods include amplification of a single target nucleic acid and multiplex amplification (amplification of multiple target nucleic acids in parallel)." Porreca at 8:5-17; *see also* Porreca at 22:1-13. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach tagging isolated cell free DNA, it would have been obvious to a POSA. For example, Porreca teaches that its method is suitable for the "detection of mutations in maternally-circulating fetal DNA," meaning cell-free fetal DNA. Porreca at 7:5-8. Furthermore, Porreca teaches that its method is useful for detecting cancer. See, e.g., Porreca at 18:32-33 ("[i]n some embodiments, the biological sample is a…tumor sample, circulating tumor cells"), 29:5-6 ("monitoring tumor progression/regression [or] analyzing circulating tumor cells"), 25:3-7 ("detection of cancer mutations"), 4:23-33, 7:5-10.) Furthermore, Porreca teaches that its method is "useful for any application where sensitivity is important." Id., 25:3-7; see also id., 5:1-3, 20:16-17, 25:3-7, 25:28-29, 26:18-25, 28:7-10, 30:9-10, 87:31-88:4. These disclosures would have motivated a POSA to apply the combined method to analyze cell-free DNA, in particular SNP loci associated with cancer in cell-free DNA. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products, a POSA would find this limitation obvious in view of Porreca combined with Hoyal.

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	For example, the combined method of Porreca and Hoyal as described in Claim 1[pre] satisfies this limitation in Hoyal's preamplification step. In the preamplification step of Hoyal, DNA is tagged by ligating adapter complexes made up of a 5' adapter and a 3' adapter (lavender) and then extending the ligated products (green):  Hoyal at Fig.3 (cropped and annotated), 16, 27-28. The adapter complexes of Hoyal comprise universal tail adapters because their constituent adapters contain sequences used for non-targeted "whole genome amplification." *Id.* at 16, 27-28. Alternatively, the 5' adapters of Hoyal themselves comprise universal tail adapters. In either scenario, the green highlighted DNA is a tagged product. Finally, as discussed above for Claim 1[pre], a POSA would have found it obvious to apply the combined method to isolated cell-free DNA. For at least the reasons explained above in Claim 1[pre], a POSA would have been motivated to combine Porreca with Hoyal, with reasonable expectation of success in doing so.

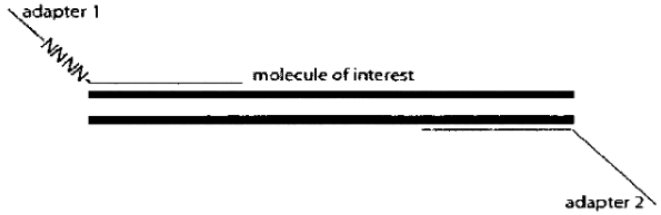
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Claim	**Prior Art**
	Furthermore, to the extent Natera asserts that Porreca does not disclose or teach tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products, a POSA would find this limitation obvious in view of Porreca combined with Wessler. For example, the combined method of Porreca and Wessler as described in Claim 1[pre] performs tagging of isolated, cell-free tumor DNA during Wessler's ligation step. Wessler at 10:53-62, Fig.1. Furthermore, the ligated adapters of Wessler comprise universal tail adapters because they contain sequences used for non-target specific amplification. (Ex.1010, 12:5-36, Fig.1.) Ligating the adapters generates amplicons tagged with the adapters. Wessler at Fig.1. For at least the reasons explained above in Claim 1[pre], a POSA would have been motivated to combine Porreca with Wessler, with reasonable expectation of success in doing so.
1[a][ii]. wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;	Porreca discloses tagging cell free DNA isolated from a blood sample collected from a subject who is not a pregnant women. *See, e.g.,* Porreca at 4:2-7, 4:8, 7:9, 25:3-5, 26:3-6, 26:21-25, 31:23:26, claim 48. For example, Porreca discloses that the nucleic acids can be from a blood sample of any patient. *See, e.g.,* Porreca at 4:2-7 ("In some embodiments, the disclosure provides methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample, or other sample (e.g., other biological fluid or tissue sample such as a biopsy sample) as aspects of the invention are not limited in this respect."). As another example, Porreca discloses that "[o]ther samples may include tumor tissue or circulating tumor cells." Porreca at 4:8. As another example, Porreca discloses that "the methods disclosed herein are useful for any application where sensitivity is important" including detection of cancer mutations. Porreca at 25:3-5. As another example, Porreca discloses that "the disclosure provides methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1 or 2) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample." Porreca at 26:3-6.

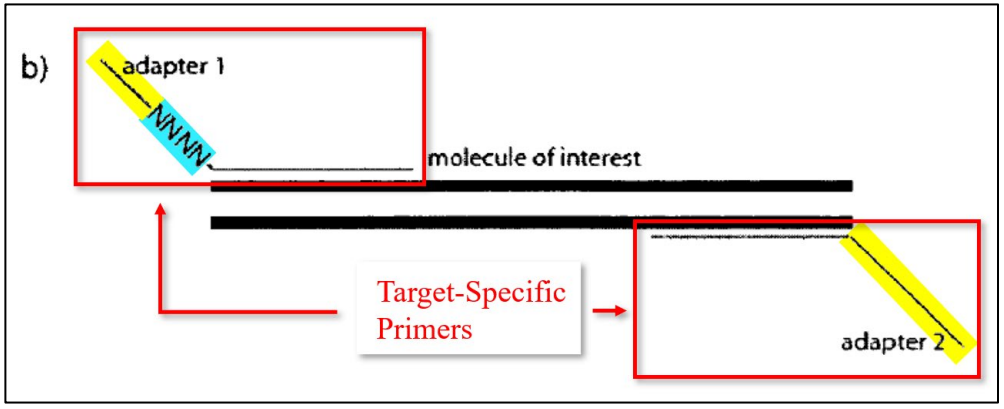
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Claim	**Prior Art**
	As another example, Porreca discloses that "aspects of the invention may be used in connection with detection, in tumor tissue or circulating tumor cells, of mutations in cellular pathways that cause cancer or predict efficacy of treatment regimens, or with detection and identification of pathogenic organisms in the environment or a sample obtained from a subject, e.g., a human subject." Porreca at 26:21-25. As another example, Porreca discloses: "In some embodiments, the disclosure provides methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample." Porreca at 31:23:26. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach tagging isolated cell free DNA, it would have been obvious to a POSA. For example, Porreca teaches that its method is suitable for the "detection of mutations in maternally-circulating fetal DNA," meaning cell-free fetal DNA. Porreca at 7:5-8. Furthermore, Porreca teaches that its method is useful for detecting cancer. See, e.g., Porreca at 18:32-33 ("[i]n some embodiments, the biological sample is a…tumor sample, circulating tumor cells"), 29:5-6 ("monitoring tumor progression/regression [or] analyzing circulating tumor cells"), 25:3-7 ("detection of cancer mutations"), 4:23-33, 7:5-10.) Furthermore, Porreca teaches that its method is "useful for any application where sensitivity is important." Id., 25:3-7; see also id., 5:1-3, 20:16-17, 25:3-7, 25:28-29, 26:18-25, 28:7-10, 30:9-10, 87:31-88:4. These disclosures would have motivated a POSA to apply Porreca to analyze cell-free DNA, in particular SNP loci associated with cancer in cell-free DNA.
1[b][i]. amplifying the tagged products one or more times to generate final amplification products,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Porreca discloses amplifying the tagged products one or more times to generate final amplification products. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.,* Porreca at 7:20-23, 8:5-17, 22:1-13.

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	For example, Porreca discloses: "In amplification-based (e.g., PCR-based or LCR-based, etc.) preparative methods, genomic loci (target nucleic acids) are isolated directly by means of a polymerase chain reaction or ligase chain reaction (or other amplification method) that selectively amplifies each locus using one or more oligonucleotide primers. It is to be understood that primers will be sufficiently complementary to the target sequence to hybridize with and prime amplification of the target nucleic acid. Any one of a variety of art known methods may be utilized for primer design and synthesis. One or more of the primers may be perfectly complementary to the target sequence. Degenerate primers may also be used. Primers may also include additional nucleic acids that are not complementary to target sequences but that facilitate downstream applications, including for example restriction sites and differentiator tag sequences. Amplification-based methods include amplification of a single target nucleic acid and multiplex amplification (amplification of multiple target nucleic acids in parallel)." Porreca at 8:5-17; *see also* Porreca at 22:1-13. As another example, Porreca discloses: "In some embodiments, the isolated nucleic acids are products of a circularization selection-based preparative method, e.g., molecular inversion probe capture products. In other embodiments, the isolated nucleic acids are products of an amplification-based preparative methods." 7:20-23. As another example, Porreca discloses annealing target specific primers comprising sequencing primer sites and differentiator tag sequences for tagging and amplification in a single PCR process. Porreca at Figure 4b and 8:5-8. **FIG. 4B** 

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	Furthermore, to the extent Natera asserts that Porreca does not disclose or teach amplifying the tagged products one or more times to generate final amplification products, a POSA would find this limitation obvious in view of Porreca combined with Hoyal. For example, the combined method of Porreca and Hoyal as described in Claim 1[pre] comprises two amplification steps. First, the tagged products from Hoyal's ligation steps undergo "[w]hole genome amplification":  Hoyal at Fig.3 (cropped and annotated), 27-28. Second, the products of that first amplification undergo targeted amplification with Porreca's primers:  Porreca at Fig.4b (annotated), 8:5-8, 8:15-17, 8:28-31, 43:29-33. A POSA would understand that the products of both amplification steps—target sequences tagged with Porreca's target-specific primers—are final amplification products.

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	For at least the reasons explained above in Claim 1[pre], a POSA would have been motivated to combine Porreca with Hoyal to arrive at the limitation of claim 1[b][i], with reasonable expectation of success in doing so. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach amplifying the tagged products one or more times to generate final amplification products, a POSA would find this limitation obvious in view of Porreca combined with Wessler. For example, as described in claim 1[pre] the combined method of Porreca and Wessler comprises two targeted amplification steps. First, the tagged products from Wessler's ligation undergo targeted amplification using one of Porreca's target-specific primers and one of Wessler's adapter primers. Second (and optionally), the process repeats. A POSA would understand that the products of these amplification steps are final amplification products. For at least the reasons explained above in Claim 1[pre], a POSA would have been motivated to combine Porreca with Wessler to arrive at the limitation of claim 1[b][i], with reasonable expectation of success in doing so. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Porreca also discloses this element for the same reasons.
1[b][ii]. wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Porreca discloses amplifying wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling.

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	See, e.g., Porreca at 3:27-32, 5:20-25, 19:3-4, 8:15-17, 22:11-13, 28:7-10, 31:17-19, 45:9-14, 58:18-20, 70:19-21. For example, Porreca discloses: "It should be appreciated that in some embodiments, aspects of the invention relate to multiplex diagnostic methods. In some embodiments, multiplex diagnostic methods comprise capturing a plurality of genetic loci in parallel (e.g., one or more genetic loci from Table 1). In some embodiments, the genetic loci possess one or more polymorphisms (e.g., one or more polymorphisms from Table 2) the genotypes of which correspond to disease causing alleles." Porreca at 3:27-32. As another example, Porreca discloses: "In other embodiments, methods are provided for increasing reproducibility (e.g., by reducing the effect of polymorphisms on target nucleic acid capture) in the detection of a plurality of genetic loci in parallel. In further embodiments, methods are provided for reducing the effect of probe synthesis and/or probe amplification variability on the analysis of a plurality of genetic loci in parallel." Porreca at 5:20-25. As another example, Porreca discloses that "[i]n some embodiments, nucleic acids from a biological sample are analyzed in multiplex reactions". Porreca at 19:3-4. As another example, Porreca discloses that "[a]mplification-based methods include amplification of a single target nucleic acid and multiplex amplification (amplification of multiple target nucleic acids in parallel)." Porreca at 8:15-17. As another example, Porreca discloses that "PCR based methods may include amplification of a single target nucleic acid and multiplex amplification (amplification of multiple target nucleic acids in parallel)." 22:11-13. As another example, Porreca discloses that "[m]ethods of the invention may be used for any type of mutation, for example a single base change (e.g., insertion, deletion, transversion or transition, etc.), a multiple base insertion, deletion, duplication, inversion, and/or any other change or combination thereof." Porreca at 28:7-10. As another example, Porreca discloses that "[i]n some embodiments, genetic loci possess one or more polymorphisms… the genotypes of which correspond to disease causing alleles." Porreca at 31:17-19. As another example, Porreca discloses: "In some aspects, the disclosure provides methods for analyzing a plurality of target sequences which are genetic loci or portions of genetic loci (e.g., a genetic locus of Table 1). The genetic

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	loci may be analyzed by sequencing to obtain a genotype at one or more polymorphisms (e.g., SNPs). Exemplary polymorphisms are disclosed in Table 2. The skilled artisan will appreciate that other polymorphisms are known in the art and may be identified, for example, by querying the Entrez Single Nucleotide Polymorphism database." Porreca at 45:9-14. Porreca discloses that the polymorphisms can be in disease-associated genes. Porreca at 50:1-2. For example, Porreca discloses that "[t]he polymorphisms can be associated with diseases or predisposition to diseases (e.g., certain polymorphic alleles are recessive alleles associated with a disease or condition)." Porreca at 58:18-20. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach one of the amplification steps comprising targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume, Porreca renders it obvious. A POSA would have been motivated by the foregoing disclosures to selectively amplify sequences containing SNP loci associated with cancer in a single reaction volume because doing so is more efficient than amplifying them separately. Porreca at 29:15-18. A POSA would have reasonably expected the combined amplification to succeed because multiplexing up to "100 assays in the same well" was well-known to a POSA at the time. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach one of the amplification steps comprising targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume, a POSA would find this limitation obvious in view of Porreca combined with Hoyal. For example, in the combined method of Porreca and Hoyal as described in Claim 1[pre], the second amplification step is a "targeted amplification" because it selectively amplifies the cell-free DNA with target-specific primers. Porreca at 8:5-8, 43:29-33, Fig.4b. As another example, the targeted amplification of the combined method of Porreca and Hoyal amplifies a plurality of SNP loci in a single reaction volume. Porreca discloses that its "PCR based methods may include…multiplex amplification (amplification of multiple target nucleic acids in parallel)." Porreca at 22:11-13. Porreca also discloses its invention is "particularly useful for analyzing multiple loci in a multiplex assay" and for the "detection of cancer mutations," which Natera apparently interprets the Court's construction as being SNPs. Id. at 10:22-23; 25:3-7. Furthermore, Porreca discloses that its "[m]ultiplex amplification strategies disclosed herein may be used analytically, as in detection of SNPs." Id. at 37:24-26; see also id., 13:9-10, 28:7-10, 29:15-18, 45:11-12. A POSA

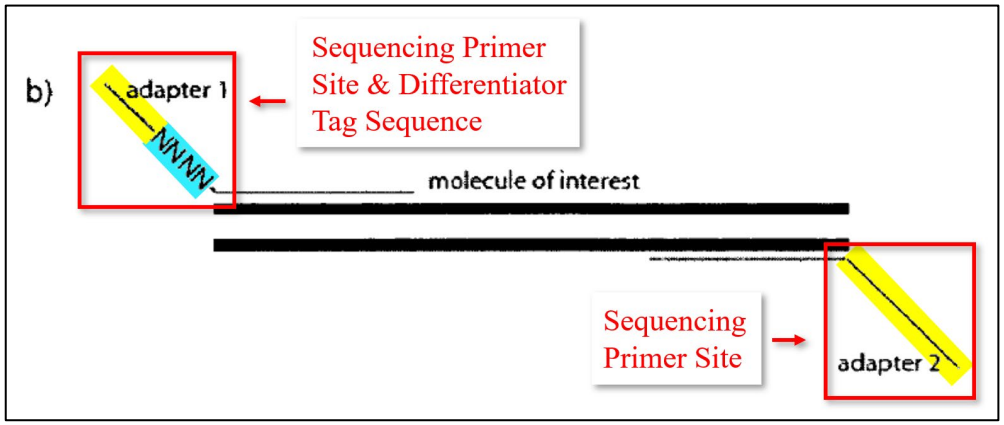
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	would have known that "multiplex amplification" meant amplifying multiple sequences in a single reaction volume. *E.g.* Porreca at 8:15-17; 29:23-25. For at least the reasons explained above in Claim 1[pre], a POSA would have been motivated to combine Porreca with Hoyal to arrive at the limitation of claim 1[b][ii], with reasonable expectation of success in doing so. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach one of the amplification steps comprising targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume, a POSA would find this limitation obvious in view of Porreca combined with Wessler. For example, the combined method of Porreca and Wessler described in Claim 1[pre] targeted amplifications of the combined method of Porreca and Wessler as described in Claim 1[pre] are "targeted" because they selectively amplify the cell-free DNA with target-specific primers. E.g., Porreca at 8:5-8, 43:29-33, Fig.4b. Second, those amplifications would amplify a plurality of SNP loci in a single reaction volume because, as explained above, Porreca teaches those elements or renders them obvious. A POSA would have been motivated by the foregoing disclosures to selectively amplify sequences containing SNP loci associated with cancer in a single reaction volume because doing so is more efficient than amplifying them separately. Porreca at 29:15-18. A POSA would have reasonably expected the combined amplification to succeed because multiplexing up to "100 assays in the same well" was well-known to a POSA at the time. For at least the reasons explained above in Claim 1[pre], a POSA would have been motivated to combine Porreca with Wessler to arrive at the limitation of claim 1[b][ii], with reasonable expectation of success in doing so. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Porreca also discloses this element for the same reasons.
1[b][iii]. wherein one of the amplifying steps	Under Natera's apparent construction of this limitation, Porreca discloses amplifying wherein one of the amplifying steps introduces a barcode and one or more sequencing tags.

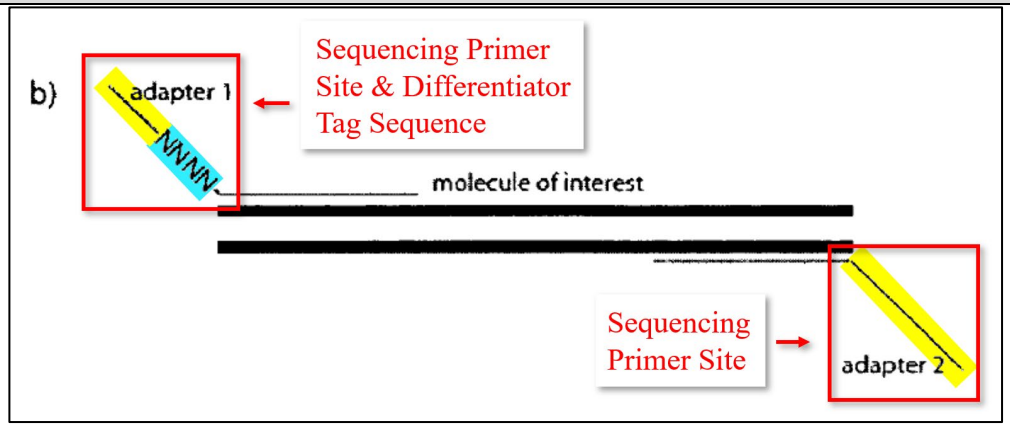
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introduces a barcode and one or more sequencing tags; and	*See, e.g.,* Porreca at 29:28-29, 43:21-34, 8:5-17; 22:1-13. For example, Porreca discloses: "In amplification-based (e.g., PCR-based or LCR-based, etc.) preparative methods, genomic loci (target nucleic acids) are isolated directly by means of a polymerase chain reaction or ligase chain reaction (or other amplification method) that selectively amplifies each locus using one or more oligonucleotide primers. It is to be understood that primers will be sufficiently complementary to the target sequence to hybridize with and prime amplification of the target nucleic acid. Any one of a variety of art known methods may be utilized for primer design and synthesis. One or more of the primers may be perfectly complementary to the target sequence. Degenerate primers may also be used. Primers may also include additional nucleic acids that are not complementary to target sequences but that facilitate downstream applications, including for example restriction sites and differentiator tag sequences. Amplification-based methods include amplification of a single target nucleic acid and multiplex amplification (amplification of multiple target nucleic acids in parallel)." Porreca at 8:5-17; *see also* Porreca at 22:1-13. As another example, Porreca discloses incorporation of differentiator tag sequences (barcodes) and sequencing primers (sequencing tags). *See* Porreca at 43:21-34. Porreca discloses that the differentiator tag sequence can be added in an amplification step using a PCR primer. *Id.* For example, one "method is to incorporate the differentiator tag sequence into a PCR primer, such that the primer structure is arranged with the common adaptor sequence followed by the random differentiator tag sequence followed by the PCR priming sequence (in 5' to 3' order). A differentiator tag sequence and adaptor sequence (which may contain the sequencing primer site) are incorporated as tags." *Id.* As another example, Porreca discloses: "In some embodiments, the capture probes do not have patient-specific tags, but the captured products from each subject may be amplified using one or a pair of amplification primers that are labeled with a patient-specific tag." Porreca at 29:28-29. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach one of the amplifying steps introducing a barcode and one or more sequencing tags, a POSA would find this limitation obvious in view of Porreca combined with Hoyal. For example, the targeted amplification step of Porreca and Hoyal's combined method, as described in Claim 1[pre], introduces a sequencing adapter and differentiator tag, which correspond to the claimed sequencing tag and

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	barcode, respectively. Porreca at 8:5-8, 43:29-33. Porreca's Figure 4b, below, depicts its target-specific primers having two sequencing primer sites (yellow) and one differentiator tag (blue):  Porreca at Fig.4b (annotated). A POSA would understand that, through amplification, the barcode and sequencing tags are incorporated into final amplification products. For at least the reasons explained above in Claim 1[pre], a POSA would have been motivated to combine Porreca with Hoyal to arrive at the limitation of claim 1[b][iii], with reasonable expectation of success in doing so. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach one of the amplifying steps introducing a barcode and one or more sequencing tags, a POSA would find this limitation obvious in view of Porreca combined with Wessler. For example, first and optional second amplification steps of the combined method of Porreca and Wessler as described in Claim 1[pre] introduce sequencing adapter and differentiator tags, which correspond to the claimed sequencing tag and barcode, respectively. Porreca at 8:5-8, 43:29-33. The left-hand portion of Porreca's Figure 4b depicts a sequencing adapter (yellow) and a barcode (blue):

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	 Porreca at Fig.4b (annotated). A POSA would understand that, through amplification, the barcode and sequencing tags are incorporated into final amplification products. For at least the reasons explained above in Claim 1[pre], a POSA would have been motivated to combine Porreca with Wessler to arrive at the limitation of claim 1[b][iii], with reasonable expectation of success in doing so. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Porreca also discloses this element for the same reasons.
1[c][i]. sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products,	Porreca discloses sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products. *See, e.g.*, Porreca at 23:5-7, 34:9-13, 37:6-8, 37:24-26, 38:8-19 For example, Porreca discloses that "[n]ext-generation sequencing may be used to quantitatively measure the abundance of each isolated target nucleic acid obtained from a certain preparative method." Porreca at 23:5-7.

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	As another example, Porreca discloses that "where analysis of a target involves sequencing, e.g., with a next-generation sequencer, the target length should typically match the sequencing read-length so that shotgun library construction is not necessary." Porreca at 34:9-13. As another example, Porreca states that provided are "methods to simplify downstream workflows when multiplex amplification by MIPs is used as a preparative step for analysis by nucleic acid sequencing." Porreca at 37:6-8. As another example, Porreca discloses that "[m]ultiplex amplification strategies disclosed herein may be used analytically, as in detection of SNPs, or preparatively, often for next-generation sequencing or other sequencing techniques." Porreca at 37:24-26. As another example, Porreca discloses: "There are currently many genomics assays that utilize next-generation (polony- based) sequencing to generate data, including genome resequencing, RNA-seq for gene expression, bisulphite sequencing for methylation, and Immune-seq, among others. In order to make quantitative measurements (including genotype calling), these methods utilize the counts of sequencing reads of a given genomic locus as a proxy for the representation of that sequence in the original sample of nucleic acids. The majority of these techniques require a preparative step to construct a high-complexity library of DNA molecules that is representative of a sample of interest. This may include chemical or biochemical treatment of the DNA (e.g., bisulphite treatment), capture of a specific subset of the genome (e.g., padlock probe capture, solution hybridization), and a variety of amplification techniques (e.g., polymerase chain reaction, whole genome amplification, rolling circle amplification)." Porreca at 38:8-19. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products, it would have been obvious to a POSA to use Porreca's massively parallel sequencing to sequence the final amplification products. A POSA would have been motivated to use massively parallel sequencing because next generation sequencing was in widespread use by POSAs as of the earliest relevant date, including for cell-free DNA. A POSA would have known that, unlike prior Sanger sequencing, such massively parallel sequencing was accessible and easy-to-use. Furthermore, a POSA would have known that Illumina's HiSeq and MiSeq massively parallel sequencers were widely available before its drafting. Given the method's widespread use and ready availability, a POSA would also

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	have found it trivial to perform massively parallel sequencing on the final amplification products by the critical date.
1[c][ii]. wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.	The Court construed the term "SNP loci…associated with cancer" to mean "a single nucleotide that may differ between the genomes of two members of the same species and is associated with cancer. The usage of the term 'SNP' should not imply any limit on the frequency with which each variant occurs." Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Porreca discloses a plurality of SNP loci comprising 25-2,000 loci associated with cancer. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.*, Porreca at 29:15-21, 29:21-25, 32:14-23, 30:9-11, 32:3-5, 58:3-5, 69:9-11. For example, Porreca discloses: "Certain embodiments may be particularly useful where multiple reactions (e.g., multiple loci and/or multiple patient samples) are being processed. For example, 10-25, 25-50, 50-75, 75-100 or more loci may be evaluated for each subject out of any number of subject samples that may be processed in parallel (e.g., 1-25, 25-50, 50-100, 100-500, 500-1,000, 1 ,000-2,500, 2,500-5,000 or more or intermediate numbers of patient samples)... It should be appreciated that different embodiments of the invention may involve conducting two or more target capture reactions and/or two or more patient sample analyses in parallel in a single multiplex reaction." Porreca at 29:15-21. As another example, Porreca discloses that "in some embodiments a plurality of capture reactions (e.g., using different capture probes for different target loci) may be performed in a single multiplex reaction on a single patient sample. In some embodiments, a plurality of captured nucleic acids from each one of a plurality of patient samples may be combined in a single multiplex analysis reaction." Porreca at 29:21-25. As another example, Porreca discloses: "In some embodiments, following probe hybridization, polymerase fill-in and ligation reactions are performed to convert the hybridized probe to a covalently-closed, circular molecule containing the desired target. PCR or rolling circle amplification plus exonuclease digestion of non-circularized material is performed to isolate and amplify the circular targets from the starting nucleic acid pool. Since one of the

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	main benefits of the method is the potential for a high degree of multiplexing, generally thousands of targets are captured in a single reaction containing thousands of probes." Porreca at 60:18-24. As another example, Porreca discloses: "Molecular inversion probe technology is used to detect or amplify particular nucleic acid sequences in potentially complex mixtures. Use of molecular inversion probes has been demonstrated for detection of single nucleotide polymorphisms (Hardenbol et al. 2005 Genome Res 15:269-75) and for preparative amplification of large sets of exons (Porreca et al. 2007 Nat Methods 4:931 -6, Krishnakumar et al. 2008 Proc Natl Acad Sci USA 105:9296-301). One of the main benefits of the method is in its capacity for a high degree of multiplexing, because generally thousands of targets may be captured in a single reaction containing thousands of probes. However, challenges associated with, for example, amplification efficiency (See, e.g., Turner EH, et al., Nat Methods. 2009 Apr 6: 1-2.) have limited the practical utility of the method in research and diagnostic settings." Porreca at 32:14-23. Porreca discloses that the SNP loci can be associated with diseases, such as cancer. For example, Porreca discloses: "An embodiment of the invention combines MIPs plus hybridization enrichment, plus optionally extra MIPs targeted to specific known, common disease-associated loci, e.g., to detect the presence of a polymorphism in a target nucleic acid." Porreca at 69:9-11. As another example, Porreca discloses methods suitable for "monitoring tumor progression/regression [or] analyzing circulating tumor cells" and "detection of cancer mutations. Porreca at 29:5-6 and 25:3-7. As another example, Porreca discloses that "[e]mbodiments of the invention may be used for any of a number of different settings: reproductive settings, disease screening, identifying subjects having cancer, identifying subjects having increased risk for a disease." Porreca at 30:9-11. As another example, Porreca discloses that "it should be appreciated that any other diseases may be studied and/or risk factors for diseases or disorders including, but not limited to allergies, responsiveness to treatment, cancer tumor profiling for treatment and prognosis." Porreca at 32:3-5. As another example, Porreca discloses applications of the method for analyzing length polymorphisms, including in cancer, "which has been associated with microsatellite instability (MSI) involving an increase or decrease in the genomic copy number of nucleic acid repeats at one or more microsatellite loci (e.g., BAT-25 and/or BAT-26)." Porreca at 58:3-5.

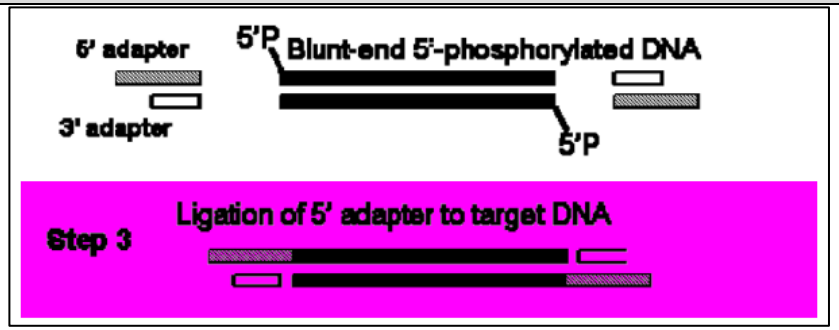
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	To the extent Natera asserts that any portion of this limitation is not disclosed under Natera's apparent interpretation of the Court's construction, it would have been obvious based on the knowledge of a POSA at the time of the alleged invention to look to the Catalogue of Somatic Mutations In Cancer ("COSMIC") database, which identifies hundreds to thousands of gene variants, including those that can be present in the same gene, to readily disclose a plurality of SNP loci comprising 25-2,000 loci associated with cancer. By way of example, COSMIC identifies 6,038 different variants in the TP53 gene alone. *See*, *e.g.*, https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach applying its methods to analyze a plurality of SNP loci comprises 25-2,000 loci associated with cancer, it would have been obvious to a POSA to use Porreca's methods to do so. For example, Porreca discloses analyzing (including sequencing) "10-25, 25-50, 50-75, 75-100 or more loci…for each subject." Porreca at 29:10-18; *see also id.*, 12:25-26, 25:28-31, 31:15-19. Furthermore, Porreca discloses that "[e]mbodiments of [its] invention may be used for any of a number of different settings," including "identifying subjects having cancer." *Id.*, 30:9-18. Further still, Porreca recites that its "multiplex diagnostic methods comprise capturing a plurality of genetic loci in parallel," and gives specific examples of genes containing SNP loci associated with cancer. *Id.*, 31:15-21, 45:16-49:33 (ATRX/RAD54, FANCC.) As another example, Porreca teaches that its method is suitable for the "detection of mutations in maternally-circulating fetal DNA," meaning cell-free fetal DNA. Porreca at 7:5-8. Furthermore, Porreca teaches that its method is useful for detecting cancer. See, e.g., Porreca at 18:32-33 ("[i]n some embodiments, the biological sample is a…tumor sample, circulating tumor cells"), 29:5-6 ("monitoring tumor progression/regression [or] analyzing circulating tumor cells"), 25:3-7 ("detection of cancer mutations"), 4:23-33, 7:5-10.) Furthermore, Porreca teaches that its method is "useful for any application where sensitivity is important." Id., 25:3-7; see also id., 5:1-3, 20:16-17, 25:3-7, 25:28-29, 26:18-25, 28:7-10, 30:9-10, 87:31-88:4. These disclosures would have motivated a POSA to apply Porreca to analyze cell-free DNA, in particular SNP loci associated with cancer in cell-free DNA. In addition, the '035 Patent admits a POSA's "general belief in the art is 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, would have

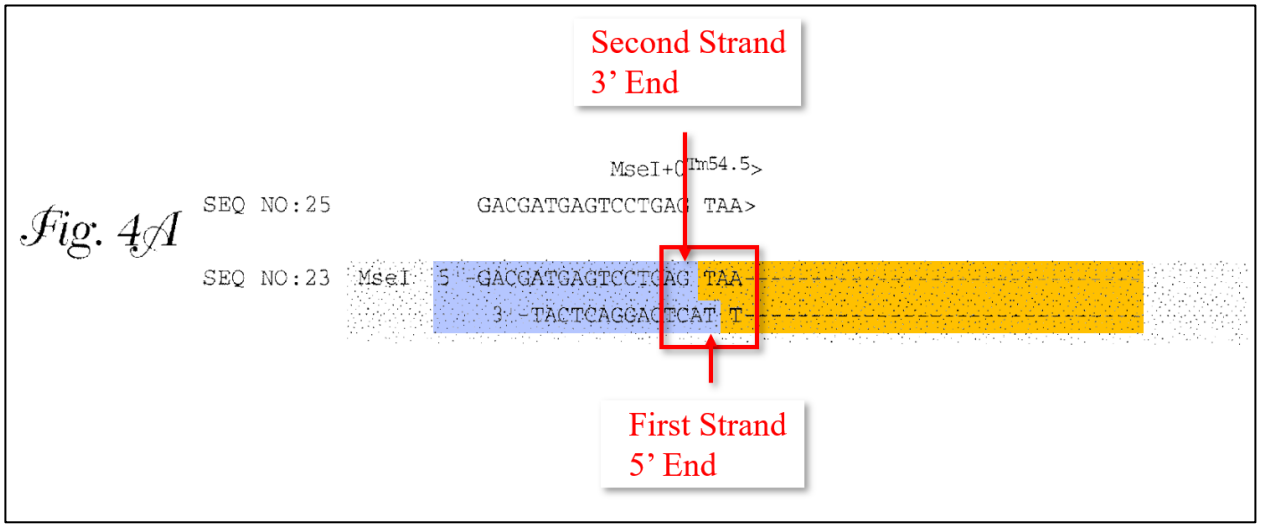
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	understood that multiplex PCR up to 100 assays could readily be performed on cell free DNA, and would have been able to do so as a matter of routine.
2. The method of claim 1, wherein tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA.	Porreca discloses tagging the cell free DNA comprising ligating the one or more universal tail adapters to the cell free DNA. *See, e.g.*, Porreca at 43:15-20, 43:23-25, 75:4-33, FIG. 4A, FIG. 4B, FIG. 9. For example, Porreca discloses that "adapters may be ligated onto the ends of the molecules of interest. Adapters often contain PCR primer sites (for amplification or emulsion PCR) and/or sequencing primer sites. In addition, barcodes may be included, for example, to uniquely identify individual samples (e.g., patient samples) that may be mixed together. (See, e.g., USPTO Publication Number US 2007/0020640 Al (McCloskey et al.)." Porreca at 43:15-20. *See also* Porreca at FIG. 4C. As another example, Porreca teaches that attachment of sequence tags by ligation is "a flexible method" because molecules "can be ligated to any blunt-ended nucleic acids." Porreca at 43:23-25. As another example, Porreca discloses that attaching the MIP probe comprising a constant sequence to a target DNA comprises a fill-in and ligation reaction. Porreca at 75:4-33; *see also* Porreca at FIG. 4A, FIG. 9. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach tagging the cell free DNA comprising ligating the one or more universal tail adaptors to the cell free DNA, a POSA would find this limitation obvious in view of Porreca combined with Hoyal. For example, the combined method of Porreca and Hoyal as described in Claim 1[pre] tags cell-free DNA by ligating universal tail adapters to it. Hoyal at 16, 27-28, Fig.3. Those adapter complexes are double-stranded:

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	 Hoyal at Fig.3 (cropped and annotated), 27-28. For example, a POSA would recognize that each adapter complex has two ends, and each end includes a 5' portion of one strand and a 3' portion of a second strand (that is how double-stranded DNA is structured). Furthermore, the first blunt ends of the adapter complexes are ligated to the cell-free DNA via a covalent link between the 5' adapters and the target DNA. Hoyal at 16, 27-28, Fig.3. As another example, a POSA would have understood it to be obvious to use (and ligate) a double-stranded adapter with two strands of equal length in place of Hoyal's single-stranded 5' adapter. This is because a POSA would know that DNA either is double-stranded or single-stranded and, left with two choices, would understand that each of these choices is obvious. A POSA also would have been motivated to do so because the POSA would recognize that this configuration would simplify the combined method by obviating the extension reaction. A POSA would have reasonably expected success in ligating double-stranded DNA sequences because doing so would have required nothing more than including a 5' phosphate group on the '3 adapter strand's 5' prime end, which was well-known and trivial to a POSA by the critical date. For at least the reasons explained above in Claim 1[pre], a POSA would have been motivated to combine Porreca with Hoyal to arrive at the limitation of claim 2, with reasonable expectation of success in doing so. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach tagging the cell free DNA comprising ligating the one or more universal tail adaptors to the cell free DNA, a POSA would find this limitation obvious in view of Porreca combined with Wessler.

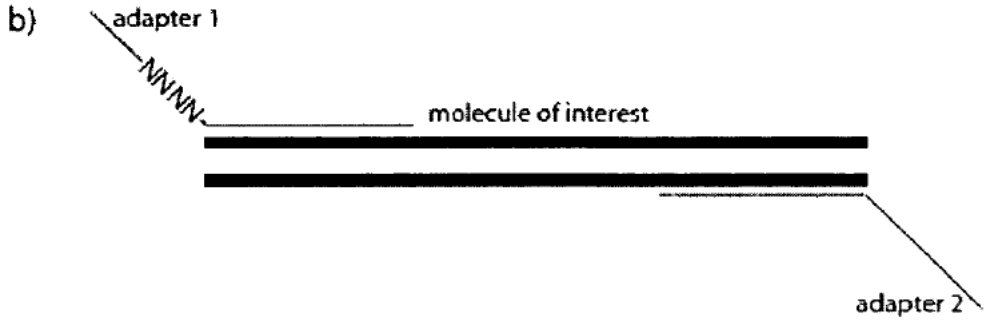
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Claim	**Prior Art**
	For example, as explained above at Claim 1[pre], the combined method of Porreca and Wessler tags cell-free DNA by ligating universal tail adapters to it. Wessler teaches that its adapters may comprise a double-stranded "SEQ ID No:23." Wessler at 6:28-34; 10:59-62, 13:9-14. Those adapters include a first strand and a second strand, wherein a first end of each adapter includes a 5' portion of the first strand and a 3' portion of the second strand that are ligated to the target, cell-free DNA. *Id.*, Fig.4A. Wessler depicts one of these adapters (navy) ligated to one end of target DNA (gold) with a first end in Figure 4A:  *Id.* at Fig.4A (annotated) For at least the reasons explained above in Claim 1[pre], a POSA would have been motivated to combine Porreca with Wessler to arrive at the limitation of claim 2, with reasonable expectation of success in doing so. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 27. To the extent Natera

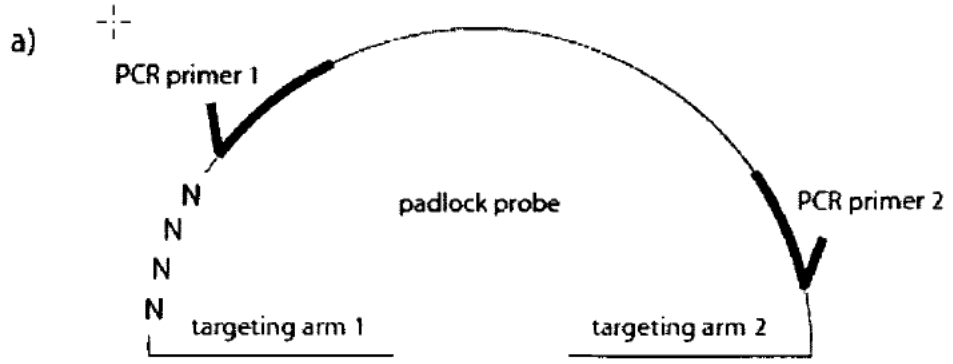
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Claim	**Prior Art**
	argues tagging by ligation is equivalent to tagging by amplification, Porreca also discloses this element for the same reasons as discussed below regarding claim 13.
12. The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.	Porreca discloses one or more universal tail adaptors comprising a first universal tail adaptor and a second universal tail adaptor. *See, e.g.*, Porreca at 8:5-17, 22:1-13, 29:28-29, 43:15-20, 75:4-11, FIG. 4A, FIG. 4B. For example, Porreca discloses multiple universal tail adaptors: "adapters may be ligated onto the ends of the molecules of interest" and said adapters often contain universal sequences such as "PCR primer sites (for amplification or emulsion PCR) and/or sequencing primer sites." Porreca at 43:15-20. As another example, Porreca discloses that the captured products from each subject may be amplified using one or a "pair of amplification primers that are labeled with a patient-specific tag." Porreca at 29:28-29. As another FIG. 4B of Porreca discloses using a pair of amplification primers comprising "adapter 1" and "adapter 2" to tag the molecule of interest:

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Claim	**Prior Art**
	 Porreca at FIG. 4B. As another example, Porreca discloses that a molecular inversion probe used to tag a molecule of interest comprises "a 30 bp 'constant region' comprised of sequence used as a pair of amplification priming sites." Porreca at 75:4-11 (emphasis provided). As another example, Porreca FIG. 4A shows how a molecular inversion probe used to tag a molecule of interest comprises a first common PCR priming site (PCR primer 1) and a second common PCR priming site (PCT primer 2):

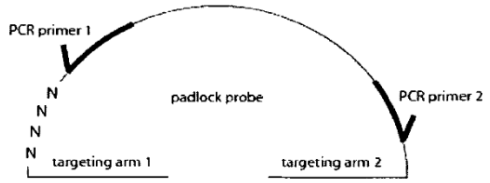
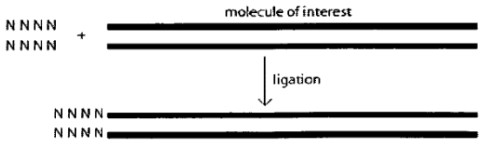
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Claim	**Prior Art**
	 Porreca at FIG. 4A. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach one or more universal tail adaptors comprising a first universal tail adaptor and a second universal tail adaptor, a POSA would find this limitation obvious in view of Porreca combined with Hoyal. For example, the combined method of Porreca and Hoyal as described in Claim 1[pre] tags with two universal tail adapters in both the ligation and, alternatively, the targeted amplification steps. For at least the reasons explained above in Claim 1[pre], a POSA would have been motivated to combine Porreca with Hoyal to arrive at the limitation of claim 12, with reasonable expectation of success in doing so. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach one or more universal tail adaptors comprising a first universal tail adaptor and a second universal tail adaptor, a POSA would find this limitation obvious in view of Porreca combined with Wessler. For example, the combined method of Porreca and Wessler as described in Claim 1[pre] tags isolated cell-free DNA with multiple universal tail adapters across the ligation and targeted amplification steps. For at least the reasons explained above in Claim 1[pre], a POSA would have been motivated to combine Porreca with Wessler to arrive at the limitation of claim 12, with reasonable expectation of success in doing so.

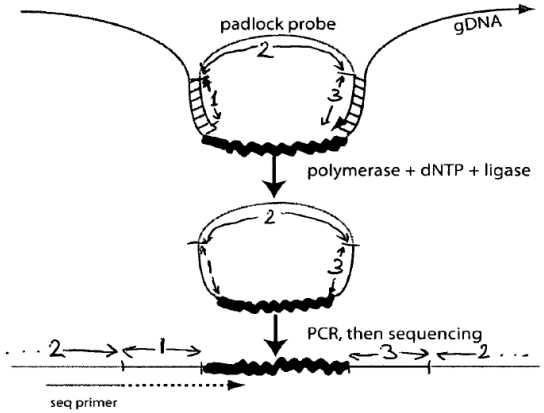
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Claim	**Prior Art**
13. The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.	Under Natera's apparent construction of this limitation, Porreca discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See, e.g.,* Porreca at 8:5-17, 8:32-9:6, 22:1-13, 27:1-3, 29:25-28, 29:28-29, 29:32-30:1, 60:18-24, 60:32-33, 61:4-5, 70:24-29, 4:2-7, 4:8, 7:9, 25:3-5, 26:3-6, 26:21-25, 31:23:26, 43:29-33, 75:4-11, FIGS. 4A, 4B, 4C, FIG. 9, claim 47, claim 48. For example, Porreca discloses that the nucleic acids tagged in their methods can be circulating DNA from a blood sample of any patient. *See, e.g.,* Porreca at 4:2-7 ("In some embodiments, the disclosure provides methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample, or other sample (e.g., other biological fluid or tissue sample such as a biopsy sample) as aspects of the invention are not limited in this respect."). As another example, Porreca discloses tagging the isolated cell free DNA with a molecular inversion probe comprising a "constant" (universal) region: "In some embodiments, all probes are composed of three regions: 1) a 20 bp 'targeting arm' comprised of sequence which hybridizes immediately upstream from the sub-target, 2) a 30 bp 'constant region' comprised of sequence used as a pair of amplification priming sites, and 3) a second 20 bp 'targeting arm' comprised of sequence which hybridizes immediately downstream from the sub-target. Targeting arm sequences will be different for each capture probe in a set, while constant region sequence will be the same for all probes in the set, allowing all captured targets to be amplified with a single set of primers." Porreca at 75:4-11. As another example, Porreca discloses embodiments wherein each different molecular inversion probe comprises the same primer-binding sequence (a universal sequence). *See, e.g.*, Porreca at claim 47. As another example, Porreca discloses tagging the isolated cell free DNA with a molecular inversion probe comprising "a differentiator tag sequence and, optionally, a primer sequence." Porreca at 8:32-9:6; *see also, e.g.*, FIG. 4A. The primer sequence is a universal sequence. *See* Porreca at 75:4-11. Porreca further discloses that "one or more intervening sequences (e.g., sequence between the first and second targeting arms on a MIP capture probe), identifier or tag sequences, or other probe sequences that are not designed to hybridize to a target sequence (e.g., a genomic target sequence) should be designed to avoid excessive complementarity (to avoid cross-hybridization) to target sequences or other sequences (e.g., other genomic sequences) that may be in a biological sample." Porreca at 70:24-29.

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Claim	**Prior Art**
	As another example, Porreca discloses that "FIGs. 4A, B, and C depict various non-limiting methods" for combining a tag sequence and target sequences." Porreca at 27:1-3. **FIG. 4A**  **FIG. 4B**  **FIG. 4C**  Porreca at FIGS. 4A-4C. As another example, Porreca discloses: "In some embodiments, samples from different subjects are tagged with subject-specific (e.g., patient-specific) tags (e.g., unique sequence tags) so that the information from each product can be assigned to an identified subject. In some embodiments, each of the different capture probes used for each patient sample have a common patient-specific tag." Porreca at 29:25-29.

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Claim	**Prior Art**
	As another example, Porreca discloses: "In some embodiments, the capture probes do not have patient-specific tags, but the captured products from each subject may be amplified using one or a pair of amplification primers that are labeled with a patient-specific tag." Porreca at 29:29-32. Porreca discloses embodiments with a "common patient-specific tag." Porreca at 29:29. As another example, Porreca discloses that "[o]ther techniques for associating a patient-specific tag with the captured product from a single patient sample may be used as aspects of the invention are not limited in this respect." Porreca at 29:32-30:1. Porreca discloses embodiments with a "common patient-specific tag." Porreca at 29:29. As another example, Porreca provides a non- limiting scheme of padlock (MIP) capture of a region of target DNA in FIG. 9. *See* Porreca at FIG. 9, 60:32-33. Porreca further explains that "[a]fter the padlock is circularized and amplified, it can be end-sequenced to obtain the sequence of the unique sequence, which represents the repetitive region of interest." Porreca at 61:4-5. Figure 9  Porreca at FIG. 9.

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Claim	**Prior Art**
	As another example, Porreca discloses a method wherein a "primer structure is arranged with the common adaptor sequence followed by the random differentiator tag sequence followed by the PCR priming sequence (in 5' to 3' order). A differentiator tag sequence and adaptor sequence (which may contain the sequencing primer site) are incorporated as tags." Porreca at 43:29-33. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach this limitation, a POSA would find this limitation obvious in view of Porreca combined with Hoyal. For example, in the combined method of Porreca and Hoyal as described in Claim 1[pre] the first amplification cycle of the targeted amplification tags the cell-free DNA with two, target specific-primers, which a POSA would understand comprise universal tail adapters. For at least the reasons explained above in Claim 1[pre], a POSA would have been motivated to combine Porreca with Hoyal to arrive at the limitation of claim 13, with reasonable expectation of success in doing so. Furthermore, to the extent Natera asserts that Porreca does not disclose or teach this limitation, a POSA would find this limitation obvious in view of Porreca combined with Wessler. For example, in the combined method of Porreca and Wessler as described in Claim 1[pre], the first and second target-specific primers comprise universal tail adapters. For at least the reasons explained above in Claim 1[pre], a POSA would have been motivated to combine Porreca with Wessler to arrive at the limitation of claim 13, with reasonable expectation of success in doing so. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Porreca also discloses this element for the same reasons as discussed above regarding claim 2.

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EXHIBIT 1-G – FINAL INVALIDITY CLAIM CHART FOR U.S. PATENT NO. 11,519,035

Reference:
International Patent Publication No. 2007/018601("Makarov")

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

To the extent Natera asserts that Makarov does not disclose or teach one or more limitations of claims 1, 2, 12 and 13, performing the methods as claimed in those claims would have been obvious to a person of ordinary skill in the art at the time of the alleged invention considering Makarov in combination with the knowledge of a POSA and/or one or more of the following references:

- U.S. Patent 8,318,434 B2 ("Cuppens")
- International Patent Publication No. 2010/115154 ("May PCT")
- International Patent Publication No. 2011/142836 ("Pieprzyk")
- Shapero et al., *MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density DNA oligonucleotide arrays*, 32 NUCLEIC ACIDS RES. e101 (2004) ("Shapero")
- U.S. Patent Publication No. 2010/0120038 ("Mir")
- International Patent Publication No. 2010/126614 ("Porreca")
- Parallel Preparation of Targeted Resequencing Libraries from 480 Genomics Regions Using Multiplex PCR on the Access Array™ System ("Kaper")
- U.S. Patent Publication No. 2012/0270739 ("Oliphant")
- International Patent Publication No. 2011/85491 ("Hansen")
- Parallel Production of Barcoded Amplicon Libraries for 454 Sequencing using Individual an Multiplex PCR in Nanoliter Volumes ("May Poster")

A POSA would have been motivated to combine Makarov with one or more of the foregoing references because Makarov expressly teaches using routine and conventional techniques known in the art to analyze sample DNA efficiently. For instance, Makarov discloses different types of multiplex PCR reactions that were known at the time and could be used to amplify several regions "in one polymerase chain reaction carried out by one thermostable DNA polymerase." Makarov at [0014]. As another example, a POSA would have been motivated to introduce a barcode and one or more sequencing tags by amplification at least because Makarov expressly teaches "utiliz[ing] synthetic nucleic acid components, such as single stranded or double stranded oligonucleotides that function as PCR or sequencing primers, ligation adaptors, or fluorescent probes, for

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example." Makarov at [0004]. A POSA likewise would have been motivated to use more than one universal tail adapter and massively parallel sequencing because such technologies were well known in the art at the time of the invention and commonly used to detect mutations in sample DNA. A POSA also would have been motivated to combine Makarov with one or more of the foregoing references to analyze loci associated with cancer because Makarov expressly teaches analyzing DNA samples "in cancer diagnostics." Makarov at [0229].

Further, a POSA would reasonably expect to succeed in combining Makarov with one or more of the foregoing references for the purposes described above because the techniques of multiplex PCR, barcoding, adding a sequencing tag, using multiple universal tail adapters, and performing massively parallel sequencing were all routine and conventional at the time for DNA analysis and diagnostics.

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Claim	**Prior Art**
1[pre]. A method for amplifying and sequencing DNA, comprising:	To the extent the preamble is construed to be limiting, Makarov discloses a method for amplifying and sequencing DNA. *See, e.g.,* Makarov at Abstract, [0006], [0009], [0020], [0022], [0030], [0033], [0056], [0059], Figure 1A, Figure 1B, Figure 6A, Figure 19A, Figure 30, Figure 31, Figure 34. For example, Makarov discloses, "The present invention overcomes deficiencies in the art by providing novel methods and compositions for amplification of a plurality of double stranded DNA molecules by incorporating at least one sequence onto the ends of the DNA molecules. However, the invention avoids the disadvantages of other methods, such as the generation of primer dimers in polymerase chain reaction, for example." Makarov at [0019]. Makarov further discloses, "In particular, the present invention allows for the amplification of molecules having at least one double stranded region by using adaptors that avoid the limitations of some adaptor molecules, such as those having the propensity to form adaptor dimers." Makarov at [0020]. As another example, Makarov discloses, "The present invention concerns preparation of DNA molecules, such as a library, using a stem-loop oligonucleotide. In particular embodiments, the invention employs a single reaction mixture and conditions. In particular, at least part of the inverted palindrome is removed during the preparation of the molecules to facilitate amplification of the molecules. Thus, in specific embodiments, the DNA molecules are suitable for amplification and are not hindered by the presence of the palindrome." Makarov, Abstract. As another example, Makarov discloses, "the present invention is directed to a system and method for preparing a collection of molecules, particularly molecules suitable for amplification, such as amplification utilizing known sequences on the molecules." Makarov at [0059].

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Claim	**Prior Art**
	As another example, Makarov discloses, "Libraries generated by DNA fragmentation and addition of a universal adaptor to one or both DNA ends were used to amplify (by PCR) and sequence DNA regions adjacent to a previously established DNA sequence (see U.S. Patent No. 6,777,187 and references therein, for example, all of which are incorporated by reference herein in their entirety)." Makarov at [0006]. As another example, Makarov discloses the method "can be used for *in vitro* as well as for *in vivo* nucleic acid amplification and/or detection." Makarov at [0033]. As another example, Makarov discloses "[s]tem-loop (also referred to as hairpin) oligonucleotides have been used in several applications for analysis and detection of nucleic acids. These applications include molecular beacons, step-loop PCR primers, and stem-loop DNA probes, immobilized on microarrays (Broude, 2002)." Makarov at [0009]. As another example, Makarov discloses, "preparation of DNA libraries for subsequent amplification and analysis involves ligation of adaptor sequences to DNA ends to introduce a priming site for the PCR-mediated whole genome amplification and ligation-mediated PCR, a promoter element for the transcription-mediated DNA amplification, and/or cohesive ends to facilitate DNA integration into a vector molecule for cloning." Makarov at [0022]. As another example, Makarov discloses "combinations of enzymes and stem-loop oligonucleotides are mixed together to produce Enz-O-Mix kits for high throughput DNA processing and diagnostic applications." Makarov at [0056].
1[a][i]. tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,	Under Natera's apparent construction, Makarov discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See, e.g.,* Makarov at [0046]-[0048], [0006], [0095], [0096], [0099], [0103], [0107], [0126], [0132], [0161], [0162], [0180], [0032], [0036], [0158], [0162], [0253]-[0259], [0174], [0206], [0007], Figure 3, Figure 6A, Figure 9, Figure 8, Figure 10A, Figure 10B, Figure 12, Figure 14, Figure 15, Figure 32, Example 5. Makarov discloses "Ligation-mediated PCR amplification is achieved by using a locus-specific primer (or several nested primers) and a universal primer complementary to the adaptor sequence." Makarov at [0006].

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Claim	**Prior Art**
	As another example, Makarov discloses a process that "results in the library of DNA fragments with universal sequence A at the ends and an inverted repeat attached only to the 5' end of DNA." Makarov at [0096]. As another example, Makarov discloses "[h]eating and replication generates DNA molecules with the universal sequence A and no inverted repeats at the ends (shown by dash lines) that can be successfully amplified by PCR." Makarov at [0097]. As another example, Makarov discloses tagging with one or more universal tail adaptors. For example, Makarov discloses embodiments where " a library generated by a one-step process is further amplified by PCR using universal primer complementary to the sequence introduced by attachment of a stem-loop oligonucleotide," where "library generation and PCR amplification are combined into one integrated, closed-tube process in a thermocycler," and where "an integrated, closed-tube library preparation/amplification process is supported by a universal PCR primer included in the original Enz-O-Mix." Makarov at [0046]-[0048]. Makarov further discloses applying its methods to cell-free DNA. For instance, Makarov discloses "The Enz-O-Mix library/amplification has no limitations on the size and nature of DNA in the reaction. The method can be applied to high molecular weight DNA, such as is isolated from tissues or cell culture, for example, as well as highly degraded DNA, such as cell-free DNA from blood and urine and/or DNA extracted from formalin-fixed, paraffin-embedded tissues, for example." Makarov at [0032]. As another example, Makarov discloses that "the methods of the invention can be easily applied to any type of fragmented double stranded DNA including but not limited to, for example, free DNA isolated from plasma, serum, and/or urine; apoptotic DNA from cells and/or tissues; DNA fragmented enzymatically *in vitro* (for example, by DNase I and/or restriction endonuclease); and/or DNA fragmented by mechanical forces (hydro-shear, sonication, nebulization, *etc.*)." Makarov at [0036]. Makarov further discloses specific adjustments that can be made for cell-free DNA. For example, Makarov discloses, that "[i]n the case of fragmented DNA (for example, cell-free DNA from blood and/or urine) the reaction does not require a fragmentation endonuclease (Enz-O-Mix 2, FIG. 19A); all other components may be the same as described above." Makarov at [0158].

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Claim	**Prior Art**
	Makarov further discloses specific examples involving cell-free DNA. For instance, Example 5 describes a "simplified protocol combining preparation of methylome libraries from cell-free urine DNA and cleavage with methylation-sensitive restriction enzymes in one step." Makarov at [0253]-[0259].
1[a][ii]. wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;	Makarov discloses tagging cell free DNA isolated from a blood sample collected from a subject who is not a pregnant women. *See, e.g.,* Makarov at [0032], [0036], [0158], [0253]-[0259], [0229], Example 5. For instance, Makarov discloses "The Enz-O-Mix library/amplification has no limitations on the size and nature of DNA in the reaction. The method can be applied to high molecular weight DNA, such as is isolated from tissues or cell culture, for example, as well as highly degraded DNA, such as cell-free DNA from blood and urine and/or DNA extracted from formalin-fixed, paraffin-embedded tissues, for example." Makarov at [0032]. As another example, Makarov discloses that "the methods of the invention can be easily applied to any type of fragmented double stranded DNA including but not limited to, for example, free DNA isolated from plasma, serum, and/or urine; apoptotic DNA from cells and/or tissues; DNA fragmented enzymatically *in vitro* (for example, by DNase I and/or restriction endonuclease); and/or DNA fragmented by mechanical forces (hydro-shear, sonication, nebulization, *etc.*)." Makarov at [0036]. Makarov further discloses specific adjustments that can be made for cell-free DNA. For example, Makarov discloses, that "[i]n the case of fragmented DNA (for example, cell-free DNA from blood and/or urine) the reaction does not require a fragmentation endonuclease (Enz-O-Mix 2, FIG. 19A); all other components may be the same as described above." Makarov at [0158]. Makarov further discloses specific examples involving cell-free DNA. For instance, Example 5 describes a "simplified protocol combining preparation of methylome libraries from cell-free urine DNA and cleavage with methylation-sensitive restriction enzymes in one step." Makarov at [0253]-[0259]. For instance, Makarov discloses methods "to maintain and generate necessary quantities of valuable but limited DNA samples for gene associated studies, mutation and microsatellite instability detection in cancer diagnostics, etc." Makarov at [0229].

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Claim	**Prior Art**
1[b][i]. amplifying the tagged products one or more times to generate final amplification products,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Makarov discloses amplifying the tagged products one or more times to generate final amplification products. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.,* Makarov at [0046]-[0048], [0006], [0097], [0107], [0162], [0164], [0169], [0180], [0239], [0246], [0250]. Makarov discloses amplifying DNA containing a universal primer. For example, Makarov discloses embodiments where " a library generated by a one-step process is further amplified by PCR using universal primer complementary to the sequence introduced by attachment of a stem-loop oligonucleotide," where "library generation and PCR amplification are combined into one integrated, closed-tube process in a thermocycler," and where "an integrated, closed-tube library preparation/amplification process is supported by a universal PCR primer included in the original Enz-O-Mix." Makarov at [0046]-[0048]. As another example, Makarov discloses, "Ligation-mediate PCR is achieved by using a locus-specific primer (or several nested primers) and a universal primer complementary to the adaptor sequence." Makarov at [0006]. As another example, Makarov discloses "[h]eating and replication generates DNA molecules with the universal sequence A and no inverted repeats at the ends (shown by dash lines) that can be successfully amplified by PCR." Makarov at [0097]. As another example, Makarov discloses a process that "results in the library of DNA fragments with universal sequence A at the ends and very short inverted repeat attached to the ends of DNA fragments. Amplification by PCR is possible, because the melting temperature for terminal hairpins is low enough to prevent folding, self-priming and formation of long hairpin molecules, as shown on FIG. 7." Makarov at [0107]. As another example, Makarov discloses an exemplary reaction that produces "a library of DNA fragments with universal sequence or sequences attached to the ends of DNA fragments so that it can be amplified by PCR (PCR WGA Library)." Makarov at [0180].

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Claim	**Prior Art**
	Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Makarov also discloses this element for the same reasons.
1[b][ii]. wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Makarov discloses amplifying wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.,* Makarov at [0006], [0059], [0229], Figure 38. For example, Makarov discloses, "Ligation-mediated PCR amplification is achieved by using a locus-specific primer (or several nested primers) and a universal primer complementary to the adaptor sequence." Makarov at [0006]. As another example, Makarov discloses, "the present invention is directed to a system and method for preparing a collection of molecules, particularly molecules suitable for amplification, such as amplification utilizing known sequences on the molecules." Makarov at [0059]. As another example, Makarov discloses, "DNA amplification and re-amplification can be used as an in vitro 'immortalization' process to maintain and generate necessary quantities of valuable but limited DNA samples for gene associated studies, mutation and microsatellite instability detection in cancer diagnostics, etc." Makarov at [0229].

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	As another example, Figure 38 discloses adding "two locus-specific . . . stem-loop oligonucleotides A and B" and PCR buffer before performing PCR amplification. Makarov at Figure 38. To the extent Natera contends Makarov does not disclose targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume, performing the method as claimed would have been obvious to a POSA at the time of the invention in combination with the knowledge of a POSA. Makarov expressly discloses the multiplex PCR was well known in the art. For example, in the Background section, Makarov discloses processing of DNA sometimes involves reactions that are "multiplexed to combine in one reaction the analysis of several DNA or RNA targets, and the nucleic acid processing or analysis may involve multiplexing of two or three enzymatic processes in one reaction." Makarov at [0004]. As another example, Makarov discloses generally, "Several types of multiplexed reactions that involve DNA or RNA are described to date. The multiplexed reactions can be divided into two major categories: reactions where two or more DNA/RNA sequences are amplified or detected simultaneously in one enzymatic process, and reactions where several enzymatic processes occur simultaneously with one DNA or RNA template." Makarov at [0013]. As another example, Makarov discloses, "There is description of multiplexed amplification and detection of several nucleic acid sequences using three-enzyme TMA and NASBA methods (van Deursen *et al.*, 1999). As discussed above, Makarov discloses tagging before performing amplification using a universal primer. A POSA at the time of the invention would have known that universal amplification could easily be replaced with or performed in addition to targeted amplification without undue experimentation. Accordingly, a POSA would have been motivated to perform the step of targeted amplification after tagging as claimed and would reasonably expect to succeed in doing so. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Makarov also discloses this element for the same reasons.

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Claim	**Prior Art**
1[b][iii]. wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and	In view of the knowledge of a POSA at the time of the claimed invention, it would have been obvious to amplify the DNA as claimed wherein one of the amplifying steps introduces a barcode and one or more sequencing tags. Introduction of an oligonucleotide sequence (such as a barcode or sequencing tag) by amplification was routine and conventional at the time. Moreover, Makarov expressly teaches in the Background section, "DNA and RNA enzymatic reactions frequently utilize synthetic nucleic acid components, such as single stranded or double stranded oligonucleotides that function as PCR or sequencing primers, ligation adaptors, or fluorescent probes, for example." Makarov at [0004]. As another example, Makarov teaches, "Ligation-mediated PCR amplification is achieved by using a locus-specific primer (or several nested primers) and a universal primer complementary to the adaptor sequence." Makarov at [0006]. As another example, Makarov teaches "a one-step process is further amplified by PCR using universal primer complementary to the sequence introduced by attachment of a stem-loop oligonucleotide" and "an integrated, closed-tube library preparation/amplification process is supported by a universal PCR primer included in the original Enz-O-Mix." Makarov at [0046], [0048]. As another example, Makarov teaches stem-loop oligonucleotides that can be modified. For example, Makarov discloses, "Stem-loop (also referred to as hairpin) oligonucleotides have been used in several applications for analysis and detection of nucleic acids. These applications include molecular beacons, stem-loop PCR primers, and stem-loop DNA probes, immobilized on microarrays (Broude, 2002)." Accordingly, a POSA would have been motivated to introduce a barcode and one or more sequencing tags as claimed and would reasonably expect to succeed in doing so. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Makarov also discloses this element for the same reasons.

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Claim	**Prior Art**
1[c][i]. sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products,	Makarov discloses sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products. *See, e.g.,* Makarov at [0006], [0033], [0022], [0056], [0204]. For example, Makarov discloses, "DNA cloning is a powerful tool for whole genome DNA sequencing and analysis of cDNA libraries, for example. In a standard process, DNA is randomly fragmented, repaired, attached to linkers, and then integrated into a vector. This embodiment describes the Enz-O-Mix reagents for the one-step preparation of DNA fragments with sticky ends that is easy to clone." Makarov at [0204]. As another example, Makarov discloses, "Libraries generated by DNA fragmentation and addition of a universal adaptor to one or both DNA ends were used to amplify (by PCR) and sequence DNA regions adjacent to a previously established DNA sequence (see U.S. Patent No. 6,777,187 and references therein, for example, all of which are incorporated by reference herein in their entirety)." Makarov at [0006]. As another example, Makarov discloses the method "can be used for *in vitro* as well as for *in vivo* nucleic acid amplification and/or detection." Makarov at [0033]. As another example, Makarov discloses, "preparation of DNA libraries for subsequent amplification and analysis involves ligation of adaptor sequences to DNA ends to introduce a priming site for the PCR-mediated whole genome amplification and ligation-mediated PCR, a promoter element for the transcription-mediated DNA amplification, and/or cohesive ends to facilitate DNA integration into a vector molecule for cloning." Makarov at [0022]. As another example, Makarov discloses "combinations of enzymes and stem-loop oligonucleotides are mixed together to produce Enz-O-Mix kits for high throughput DNA processing and diagnostic applications." Makarov at [0056]. To the extent Natera contends Makarov does not disclose conducting massively parallel sequencing on the final amplification products as claimed, doing so would have been obvious to a POSA at the time of the invention in combination with the knowledge of a POSA. Massively parallel sequencing technologies were well known in the art at the time and it was routine and conventional to perform amplification in preparation for massively parallel sequencing. And as discussed above, Makarov expressly teaches performing sequencing after amplification.

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Claim	**Prior Art**
	Accordingly, a POSA would have been motivated to perform sequencing after amplification as claimed and would reasonably expect to succeed in doing so.
1[c][ii]. wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.	The Court construed the term "SNP loci…associated with cancer" to mean "a single nucleotide that may differ between the genomes of two members of the same species and is associated with cancer. The usage of the term 'SNP' should not imply any limit on the frequency with which each variant occurs." Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Makarov meets this limitation. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. In view of the knowledge of a POSA at the time of the claimed invention, it would have been obvious to perform the claimed method on a plurality of SNP loci comprising 25-2,000 loci associated with cancer. Makarov discloses its methods for analyzing loci associated with cancer. For instance, Makarov discloses methods "to maintain and generate necessary quantities of valuable but limited DNA samples for gene associated studies, mutation and microsatellite instability detection in cancer diagnostics, etc." Makarov at [0229]. Further, Makarov expressly teaches multiplex PCR was well known in the art. For example, Makarov discloses generally, "Several types of multiplexed reactions that involve DNA or RNA are described to date. The multiplexed reactions can be divided into two major categories: reactions where two or more DNA/RNA sequences are amplified or detected simultaneously in one enzymatic process, and reactions where several enzymatic processes occur simultaneously with one DNA or RNA template." Makarov at [0013]. Makarov further discloses, "Multiplex PCR and RT-PCR are examples of the first category of multiplexed reactions (Mackay et al., 2000). In this case, several genomic or cDNA regions are amplified in one polymerase chain reaction carried out by one thermostable DNA polymerase. Whole genome or whole transcriptome amplification is another example of highly multiplexed DNA/RNA amplification reactions carried out by methophilic (Phi 29) or thermophilic (Taq) DNA polymerase (Sambrook et al., 1989)." Makarov at [0014]. As another example, in the Background section, Makarov discloses processing of DNA sometimes involves reactions that are "multiplexed to combine in one reaction the analysis of several DNA or RNA targets, and the

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Claim	**Prior Art**
	nucleic acid processing or analysis may involve multiplexing of two or three enzymatic processes in one reaction." Makarov at [0004]. As another example, Makarov discloses, "There is description of multiplexed amplification and detection of several nucleic acid sequences using three-enzyme TMA and NASBA methods (van Deursen *et al.*, 1999). Makarov at [0018]. Accordingly, a POSA would have been motivated to perform the claimed method on SNP loci comprising 25-2,000 loci associated with cancer and would reasonably expect to succeed in doing so. To the extent Natera asserts that any portion of this limitation is not disclosed under Natera's apparent interpretation of the Court's construction, it would have been obvious based on the knowledge of a POSA at the time of the alleged invention to look to the Catalogue of Somatic Mutations In Cancer ("COSMIC") database, which identifies hundreds to thousands of gene variants, including those that can be present in the same gene, to readily disclose a plurality of SNP loci comprising 25-2,000 loci associated with cancer. By way of example, COSMIC identifies 6,038 different variants in the TP53 gene alone. *See*, *e.g.,* https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53. In addition, the '035 Patent admits a POSA's "general belief in the art is 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, would have understood that multiplex PCR up to 100 assays could readily be performed on cell free DNA, and would have been able to do so as a matter of routine.
2. The method of claim 1, wherein tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA.	Makarov discloses tagging wherein tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA. *See, e.g.,* [0006], [0005], [0046]-[0048], [0020], [0022], [0004], [0061], Figure 1A, Figure 1B, Figure 3, Figure 6A, Figure 8, Figure 9, Figure 10A, Figure 10B, Figure 11A, Figure 14, Figure 30. Makarov discloses "Libraries generated by DNA fragmentation and addition of a universal adaptor to one or both DNA ends were used to amplify (by PCR) and sequence DNA regions adjacent to a previously established DNA sequence (see U.S. Patent No. 6,777,187 and references therein, for example, all of which are incorporated by

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Claim	**Prior Art**
	reference herein in their entirety). The adaptor can be ligated to the 5' end, the 3' end, or both strands of DNA. The adaptor can have a 3' or 5' overhang, depending on the structure of the overhang generated by restriction enzyme digestion of DNA. It can also have a blunt end, especially in the cases where DNA ends are 'polishes' after enzymatic, mechanical, or chemical DNA fragmentation. Ligation-mediated PCR amplification is achieved by using a locus-specific primer (or several nested primers) and a universal primer complementary to the adaptor sequence." Makarov at [0006]. As another example, Makarov discloses "Supplementing DNA ends with additional short polynucleotide sequences, referred to as an adaptor or linker, is used in many areas of molecular biology. The usefulness of adapted DNA molecules is illustrated by but not limited to several examples, such as ligation-mediated locus-specific PCR, ligation-mediated whole genome amplification, adaptor-mediated DNA cloning, DNA affinity tagging, DNA labeling, *etc*." Makarov at [0005]. As another example, Makarov discloses embodiments where " a library generated by a one-step process is further amplified by PCR using universal primer complementary to the sequence introduced by attachment of a stem-loop oligonucleotide," where "library generation and PCR amplification are combined into one integrated, closed-tube process in a thermocycler," and where "an integrated, closed-tube library preparation/amplification process is supported by a universal PCR primer included in the original Enz-O-Mix." Makarov at [0046]-[0048]. As another example, Makarov discloses, "In certain aspects, the present invention provides an inert oligonucleotide for attachment to a double stranded molecule such that it renders the oligonucleotide-ligated molecule capable of being modified, such as amplified, for example by polymerase chain reaction." Makarov at [0020]. As another example, Makarov discloses the invention "provides novel conditions for modification of DNA molecules with the adaptors, and subsequent amplification." Makarov at [0022]. Makarov further discloses, "For example, preparation of DNA libraries for subsequent amplification and analysis involves ligation of adaptor sequences to DNA ends to introduce a priming site for the PCR-mediated whole genome amplification and ligation-mediated PCR, ..." Makarov at [0022]. As another example, Makarov discloses, "DNA and RNA enzymatic reactions frequently utilize synthetic nucleic acid components, such as single stranded or double stranded oligonucleotides that function as PCR or sequencing primers, ligation adaptors, or fluorescent probes, for example." Makarov at [0004].

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Claim	**Prior Art**
	Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 27. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Makarov also discloses this element for the same reasons as discussed below regarding claim 13.
12. The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.	In view of the knowledge of a POSA at the time of the claimed invention, it would have been obvious to perform the claimed method wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor. Makarov discloses tagging with a universal tail adapter. For example, Makarov discloses "Ligation-mediated PCR amplification is achieved by using a locus-specific primer (or several nested primers) and a universal primer complementary to the adaptor sequence." Makarov at [0006]. *See also, e.g.*, Makarov at [0096], [0097]. Moreover, Makarov expressly teaches in the Background section, "DNA and RNA enzymatic reactions frequently utilize synthetic nucleic acid components, such as single stranded or double stranded oligonucleotides that function as PCR or sequencing primers, ligation adaptors, or fluorescent probes, for example." Makarov at [0004]. Tagging using more than one adapter was routine and conventional at the time of the claimed invention. Accordingly, a POSA would have been motivated to tag with one or more universal tail adaptors and would reasonably expect to succeed in doing so.
13. The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.	Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Makarov also discloses this element for the same reasons as discussed above regarding claim 2.

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EXHIBIT 1-H – FINAL INVALIDITY CLAIM CHART FOR U.S. PATENT NO. 11,519,035

Reference:
United States Patent Pub. No. 2013/0178371 A1 ("Oliphant")

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

To the extent Natera asserts that Oliphant does not disclose or teach one or more limitations of claims 1, 2, 12 and 13, performing the methods as claimed in those claims would have been obvious to a person of ordinary skill in the art at the time of the alleged invention considering Oliphant in combination with the knowledge of a POSA and/or one or more of the following references:

- International Patent Publication No. 2010/115154 ("May PCT")
- International Patent Publication No. 2011/142836 ("Pieprzyk")
- International Patent Publication No. 2007/018601("Makarov")
- International Patent Publication WO 2010/126614 A2 ("Porreca")
- Shapero et al., *MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density DNA oligonucleotide arrays*, 32 NUCLEIC ACIDS RES. e101 (2004) ("Shapero")
- U.S. Patent Application Publication US 2004/0110153 A1 ("Dong")
  International Patent Publication No. 2011/85491 ("Hansen")

A POSA would have been motivated to combine Oliphant with one or more of the foregoing references because Oliphant expressly teaches using routine and conventional techniques known in the art to analyze sample cfDNA using massively parallel sequencing. For instance, Oliphant discloses multiplex targeted amplification that adds universal sequence, followed by a universal amplification. *See, e.g.*, Oliphant at [0111], at [0080]-[0081]. For example, to the extent Natera asserts that Oliphant does not disclose any aspect of claims that require the isolated cell free DNA isolated from a blood sample come from a subject who is not a pregnant women or to sequence a plurality of SNP loci associated with cancer, a POSA would have been motivated to combine the teachings of Oliphant with the teachings of one or more of the foregoing references because Oliphant expressly teaches assaying "cell free DNA" and a "wide number of pathologies, including pathologies . . . caused by somatic mutations (e.g. cancer)." Oliphant at [0012], [0003]. As another example, a POSA would have been motivated to combine Oliphant with the ligation methods of one or more of the foregoing references because Oliphant expressly teaches using "adaptor-ligation and single primer PCR" in a preferred embodiment and cites other references for descriptions of "ligation reactions for detection of nucleic acid sequences." Oliphant at [00094], [000113].

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Further, a POSA would reasonably expect to succeed in combining Oliphant with one or more of the foregoing references for the purposes described above because the techniques of multiplex PCR, barcoding, adding a sequencing tag, using multiple universal tail adapters, and performing massively parallel sequencing were all routine and conventional at the time for DNA analysis and diagnostics.

U.S. Patent Pub. No. 2012/0270739	
Claim	**Prior Art**
1[pre]. A method for amplifying and sequencing DNA, comprising:	To the extent the preamble is construed to be limiting, Oliphant discloses a method for amplifying and sequencing DNA. *See*, *e.g.*, Oliphant at [0011]. For example, Oliphant discloses, "The present invention provides assay systems and related methods for determining genetic abnormalities in maternal samples comprising genomic material (e.g., cell-free DNA) in egg donor pregnancies." Oliphant at [0011]. Written description support for this disclosure is supported by at least the following in U.S. Provisional Pat. App. No. 61/436,135, to which Oliphant Claims priority: [0001], [00194].
1[a][i]. tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,	Under Natera's apparent construction of this limitation, Oliphant discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See*, *e.g.*, Oliphant at [0111]. For example, Oliphant discloses, "In another aspect of the invention, nucleic acid regions are selectively amplified during the nucleic acid region detection technique without any prior amplification. In a multiplexed assay system, this is preferably done through use of universal amplification of the various nucleic acid regions to be analyzed using the assay systems of the invention. Universal primer sequences are added to the selectively amplified nucleic acid regions, either during or following selective amplification, so that they may be further amplified in a single universal amplification reaction." *See*, *e.g.*, Oliphant at [0111]. Written description support for this disclosure is supported by at least the following in U.S. Provisional Pat. App. No. 61/436,135, to which Oliphant Claims priority: [00013].

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Claim	**Prior Art**
1[a][ii]. wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;	Oliphant discloses tagging cell free DNA isolated from a blood sample collected from a subject who is not a pregnant women. For example, Oliphant discloses, "non-invasive methods of screening for genetic abnormalities, including aneuploidies, in mixed samples comprising normal and putative abnormal DNA. The present invention addresses this need." *See*, *e.g.*, Oliphant at [0111]. To the extent Natera asserts that any portion of the limitation requiring isolated cell free DNA isolated from a blood sample collected from a subject who is not a pregnant women is not disclosed by Oliphant, it would have been obvious to a POSA at the time of the alleged invention considering Oliphant in combination with the knowledge of a POSA. Written description support for this disclosure is supported by at least the following in U.S. Provisional Pat. App. No. 61/436,135, to which Oliphant Claims priority: Abstract; [00012].
1[b][i]. amplifying the tagged products one or more times to generate final amplification products,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Oliphant discloses amplifying the tagged products one or more times to generate final amplification products. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). Specifically, Natera's Response to NeoGenomics Laboratories, Inc.'s Interrogatory No. 14 does not dispute that Oliphant discloses "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification". NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. See, *e.g.,* [0063], [0073], and [0111]. For example, Oliphant discloses, "In another aspect of the invention, nucleic acid regions are selectively amplified during the nucleic acid region detection technique without any prior amplification. In a multiplexed assay system, this is preferably done through use of universal amplification of the various nucleic acid regions to be analyzed using the assay systems of the invention. Universal primer sequences are added to the selectively

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U.S. Patent Pub. No. 2012/0270739	
Claim	**Prior Art**
	amplified nucleic acid regions, either during or following selective amplification, so that they may be further amplified in a single universal amplification reaction." *See*, *e.g.*, Oliphant at [0111]. For example, Oliphant discloses, "The term 'selective amplification', 'selectively amplify' and the like refers to an amplification procedure that depends in whole or in part on hybridization of an oligo to a sequence in a selected genomic region. In certain selective amplifications, the primers used for amplification are complementary to a selected genomic region. In other selective amplifications, the primers used for amplification are universal primers, but they only result in a product if a region of the nucleic acid used for amplification is complementary to a genomic region of interest." Oliphant at [0063]. As another example, Oliphant, discloses, "At step 103, two or more polymorphic nucleic acid regions are interrogated in the sell-free [sic] DNA from the sample. Interrogation may comprise sequencing of selected polymorphic nucleic acids followed by selective amplification of those nucleic acid regions, increasing the copy number of the selected nucleic acid regions in a manner that allows preservation of the relative quantity of the selected nucleic acid regions in the initial sample." Oliphant at [0073]. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Oliphant also discloses this element for the same reasons. Written description support for this disclosure is supported by at least the following in U.S. Provisional Pat. App. No. 61/436,135, to which Oliphant Claims priority: [00014].
1[b][ii]. wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Oliphant discloses amplifying wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). Specifically, Natera's Response to NeoGenomics

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Claim	**Prior Art**
(SNP) loci in a single reaction volume,	Laboratories, Inc.'s Interrogatory No. 14 does not dispute that Oliphant discloses "amplifying the tagged products . . . wherein one of the5mplifycation steps comprises targeted amplification". NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. See, *e.g.,* [0080]-[0081]. For example, Oliphant discloses, "In one preferred aspect, the percent fetal cell-free DNA in a maternal sample can be quantified using multiplexed SNP detection without using prior knowledge of the maternal or paternal genotype. In this aspect, two or more selected polymorphic nucleic acid regions with a known SNP in each region are used. In a preferred aspect, the selected polymorphic nucleic acid regions are located on an autosomal chromosome that is unlikely to be aneuploidy, e.g., Chroma-somes 1-12. The selected polymorphic nucleic acid regions from the maternal are amplified. In a preferred aspect, the amplification is universal. In a preferred embodiment, the selected polymorphic nucleic acid regions are amplified in one reaction in one vessel." Oliphant at [0080]-[0081]. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Oliphant also discloses this element for the same reasons. Written description support for this disclosure is supported by at least the following in U.S. Provisional Pat. App. No. 61/436,135, to which Oliphant Claims priority: [00013], [00014], [00018], [[00148].
1[b][iii]. Wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and	Oliphant discloses amplifying wherein one of the amplifying steps introduces a barcode and one or more sequencing tags. For example, Oliphant discloses, "amplification techniques for labeling and amplifying a nucleic acid using an adapter comprising a unique primer and a barcode." Oliphant at [0126]. As another example, Oliphant discloses that an "Assay product was generated from each mixed sample, and products from the mixed samples were pooled and used as template for cluster amplification on a single lane of a

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Claim	**Prior Art**
	TruSeq v2 flow slide (Illumina, San Diego, Calif.). The slide was processed on an Illumina HiSeq 2000 to generate an average of 1.18M raw sequencing reads/sample. An average of 1.15M reads had fewer than 3 mismatches with expected assay structures resulting in an average of854 reads/locus/sample." Oliphant at [0126]. To the extent Natera asserts that any portion of the limitation requiring introduction of a barcode is not disclosed by Oliphant, it would have been obvious to a POSA at the time of the alleged invention considering Oliphant in combination with the knowledge of a POSA that preparation of the Illumina library would including adding barcode and sequencing tags by adaptor ligation followed by PCR. *See, e.g.*, Oliphant at [0126]. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Oliphant also discloses this element for the same reasons. Written description support for this disclosure is supported by at least the following in U.S. Provisional Pat. App. No. 61/436,135, to which Oliphant Claims priority: [00114], [00188], [00189].
1[c][i]. sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products,	Oliphant discloses sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products. For example, Oliphant discloses, "In another example, massively parallel sequencing of nucleic acids (e.g., DNA fragments randomly selected from the sample) is used to determine the sequence of the nucleic acids in the maternal sample to determine selected frequency of the nucleic acids within the maternal sample. Exemplary techniques for performing massively parallel sequencing of nucleic acid sequences include those disclosed in U.S. Pat. Nos. 8,318,430, 8,296,076 and 8,195,415, and in U.S. applications US20090029377, 20090087847, 20100112590, 20110312503, 20110319272, 20110246083, 20110318734, 20120003635, 20120003636, 20120003637, 20120190559, and 20120208708." Oliphant at [0095]; [0096] ("In more specific aspects, the samples used for massively parallel sequencing of nucleic acids are enriched for polymorphic regions. Exemplary techniques for performing enrichment include those disclosed in, e.g., WO2011091063, WO2011091046 and US Pat Appln No. 20110230358. Briefly, a portion of a maternal sample comprising cell free DNA is amplified to

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Claim	**Prior Art**
	augment the number of copies of the one or more polymorphic sequences in the sample, and the amplified portions of nucleic acids are then added back to the original sample for sequencing."). Written description support for this disclosure is supported by at least the following in U.S. Provisional Pat. App. No. 61/436,135, to which Oliphant Claims priority: [00188], [00189].
1[c][ii]. Wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.	The Court construed the term "SNP loci…associated with cancer" to mean "a single nucleotide that may differ between the genomes of two members of the same species and is associated with cancer. The usage of the term 'SNP' should not imply any limit on the frequency with which each variant occurs." Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Oliphant discloses a plurality of SNP loci comprising 25-2,000 loci associated with cancer. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). Specifically, Natera's Response to NeoGenomics Laboratories, Inc.'s Interrogatory No. 14 does not dispute that Oliphant discloses "SNP loci…associated with cancer". NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. See, *e.g.,* [0169], [0187]-[0188], and Example 5. For example, Oliphant discloses, "To assess percent contribution of each individual in the mixed samples, assays were designed against a set of 192 SNP-containing loci on chromosomes 1 through 12, where two middle oligos differing by one base were used to query each SNP. SNPs were optimized for minor allele frequency in the HapMap 3 dataset. Duan, et al., Bioinformation, 3(3):139-41 (2008); Epub 2008 Nov. 9. Oligonucleotides were synthesized by IDT (Integrated DNA Technologies, Coralville, Iowa) and pooled together to create a single multiplexed assay pool. Assay product was generated from each mixed sample, and products from 8 mixed samples were pooled and used as template for cluster amplification on a single lane of a TruSeq v2 flow slide (Illumina, San Diego, Calif.). The slide was processed on an Illumina HiSeq 2000 to generate an average of 1.18M raw sequencing reads/sample. An average of 1.15M reads had fewer than 3 mismatches with expected assay structures resulting in an average of 854 reads/locus/sample." *See* Oliphant at [0187]-[0188], Example 5.

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Claim	**Prior Art**
	As another example, Oliphant discloses, "Given the multiplexed nature of the assay systems of the invention, in certain aspects it may be beneficial to utilize the assay to detect other nucleic acids that could pose a risk to the health of the subject(s) or otherwise impact on clinical decisions about the treatment or prognostic outcome for a subject. Such nucleic acids could include but are not limited to indicators of disease or risk such as maternal alleles, polymorphisms, or somatic mutations known to present a risk for maternal or fetal health. Such indicators include, but are not limited to, genes associated with Rh status; mutations or polymorphisms associated with diseases such as diabetes, hyperlipidemia, hypercholesterolemia, blood disorders such as sickle cell anemia, hemophilia or thalassemia, cardiac conditions, etc.; exogenous nucleic acids associated with active or latent infections; somatic mutations or copy number variations associated with autoimmune disorders or malignancies (e.g., breast cancer), or any other health issue that may impact on the subject, and in particular on the clinical options that may be available in the treatment and/or prevention of health risks in a subject based on the outcome of the assay results." Oliphant at [0169]. Written description support for this disclosure is supported by at least the following in U.S. Provisional Pat. App. No. 61/436,135, to which Oliphant Claims priority: [00003], [00189]. To the extent Natera asserts that any portion of this limitation is not disclosed under Natera's apparent interpretation of the Court's construction, it would have been obvious based on the knowledge of a POSA at the time of the alleged invention to look to the Catalogue of Somatic Mutations In Cancer ("COSMIC") database, which identifies hundreds to thousands of gene variants, including those that can be present in the same gene, to readily disclose a plurality of SNP loci comprising 25-2,000 loci associated with cancer. By way of example, COSMIC identifies 6,038 different variants in the TP53 gene alone. *See*, *e.g.*, https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53. In addition, the '035 Patent admits a POSA's "general belief in the art is 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, would have understood that multiplex PCR up to 100 assays could readily be performed on cell free DNA, and would have been able to do so as a matter of routine.

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Claim	**Prior Art**
2. The method of claim 1, wherein tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA.	Oliphant discloses tagging the cell free DNA comprising ligating the one or more universal tail adaptors to the cell free DNA. For example, Oliphant discloses, "In some aspects DNA is amplified by multiplex locus-specific PCR. In a preferred aspect the DNA is amplified using adaptor-ligation and single primer PCR." *See*, *e.g.*, Oliphant at [0105]. Written description support for this disclosure is supported by at least the following in U.S. Provisional Pat. App. No. 61/436,135, to which Oliphant Claims priority: [00094], [00113].
12. The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.	Under Natera's apparent construction of this limitation, Oliphant discloses one or more universal tail adaptors comprising a first universal tail adaptor and a second universal tail adaptor. For example, Oliphant discloses "In certain aspects, the nucleic acid regions are amplified using universal amplification methods. Universal amplification can be done, for example, after an initial selective amplification or enrichment step from the maternal sample from an egg donor pregnancy. The use of universal amplification allows multiple nucleic acid regions to be amplified using a single or limited number of amplification primers, and is especially useful in amplifying multiple selected nucleic acid regions in a single reaction. This allows the simultaneous processing of multiple nucleic acid regions from a single or multiple samples." *See*, *e.g.*, Oliphant at [0023]; [0137] ("In aspects of the invention using indices, the selective amplification primers are preferably designed so that indices comprising identifying information are coded at one or both ends of the primer. Alternatively, the indices and universal amplification sequences can be added to the selectively amplified nucleic acids following initial selective amplification."). As another example, Oliphant discloses "primer pairs," which function as the claimed first and second universal tail adaptors. *See* Oliphant at [0107] ("In certain aspects, the selective amplification uses one or a few rounds of amplification with primer pairs comprising nucleic acids complementary to the selected nucleic acids."). Written description support for this disclosure is supported by at least the following in U.S. Provisional Pat. App. No. 61/436,135, to which Oliphant Claims priority: [00014], [00188].
13. The method of claim 12, wherein tagging the	Oliphant discloses that tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.

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Claim	**Prior Art**
cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.	*See, e.g.*, Claim 1[a][i]. Written description support for this disclosure is supported by at least the following in U.S. Provisional Pat. App. No. 61/436,135, to which Oliphant Claims priority: [00014].

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EXHIBIT 1-I – FINAL INVALIDITY CLAIM CHART FOR U.S. PATENT NO. 11,519,035

Reference:

International Patent Publication WO 2011/085491 ("Hansen")

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

To the extent Natera asserts that Hansen does not disclose or teach one or more limitations of Claims 1, 2, 12 or 13, performing the methods as claimed in those claims would have been obvious to a person of ordinary skill in the art at the time of the alleged invention considering Hansen in combination with the knowledge of a POSA and/or one or more of the following references:

- International Patent Publication No. 2007/018601("Makarov")
- Shapero et al., *MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density DNA oligonucleotide arrays*, 32 NUCLEIC ACIDS RES. e101 (2004) ("Shapero")
- International Patent Publication WO 2010/126614 A2 ("Porreca")
- U.S. Patent Application Publication US 2004/0110153 A1 ("Dong")
- International Patent Publication WO 2007/140417 ("Hoyal")
- U.S. Patent No. 6,420,117 ("Wessler")

A POSA would have been motivated to combine Hansen with one or more of the foregoing references because Hansen expressly teaches using "methods known to persons skilled in the art" to design primer pairs based on the desired target. A POSA would have been motivated to use the methods disclosed in Hansen to analyze 25-2,000 loci associated with cancer because Hansen teaches, "Plasma and serum DNA from cancer patients has been shown to contain large quantities of tumor DNA (Chen et al. 1996; Nawroz et al. 1996; Anker et al. 1997)," and using "a number of target primer pairs that may be equal to the number of target sites." Hansen at 2, 23. Moreover, as discussed below, it would have been obvious to use prior art methods to introduce a barcode and one or more sequencing tags by amplification as claimed by the '035 patent because barcoding and adding sequencing tags were known techniques in the art at the time of the claimed invention.

Further, a POSA would reasonably expect to succeed in combining Hansen with one or more of the foregoing references because methods for combining the methods described were known in the art and would not have required undue experimentation.

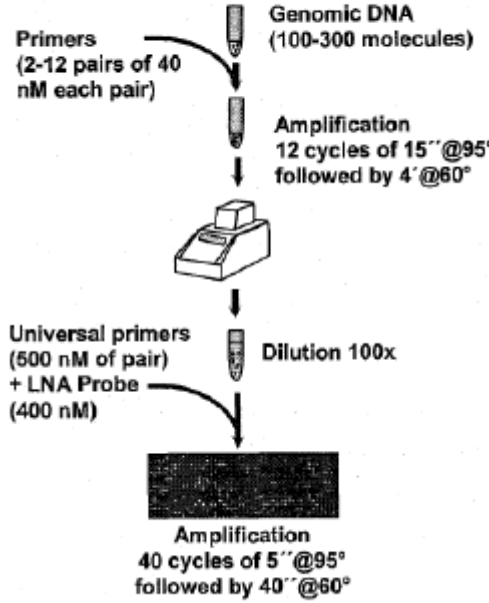
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Claim	**Prior Art**
1[pre]. A method for amplifying and sequencing DNA, comprising:	To the extent the preamble is construed to be limiting, Hansen discloses a method for amplifying and sequencing DNA. Hansen discloses amplifying the tagged products one or more times to generate final amplification products. *See, e.g.,* Hansen at 7, 8, 15, 17, 18, 30, 45, Figure 3. For example, Hansen defines "pre-amplification" as referring to "a DNA replication step performed on a biological sample with target and reference pre-amplification primer pairs prior to distributing the biological sample into discrete subsamples for digital amplification." Hansen at 30. For example, Hansen discloses a method including performing digital amplification of the tagged products: "In accordance with another embodiment, there is provided a method of detecting the relative amount of target source to reference source in a biological sample, the method including: (a) providing a subset of target pre-amplification primer pairs, wherein each target pre-amplification primer pair is specific to at least one target site, and a subset of reference pre-amplification primer pairs, wherein each reference preamplification primer pair is specific to at least one reference site, and wherein each target pre-amplification primer pair has a first universal non-specific primer sequence and wherein each reference pre-amplification primer pair has a second universal non-specific primer sequence; (b) performing a pre-amplification reaction on the biological sample with the first and second subsets to synthesize the target molecules and the reference molecules, wherein the biological sample includes additional target and reference molecules performing digital amplification with the target primer subset and the reference primer subset." Hansen at 7. *See also id.* at 8 (same). As another example, Hansen discloses, "Alternatively, the design of primers may include both the gene specific sequences of interest together with universal primer sequences (for example, TABLE 3). Alternatively, the design of primers may include gene specific sequences of interest together with universal primer sequences along with a probe sequence as described herein. Digital amplification may occur via digital PCR (dPCR) or rolling circle amplification." Hansen at 15.

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Claim	**Prior Art**
	As another example, Hansen discloses, "3) Performing a first multiplex digital amplification reaction on the pre- amplification reaction product, wherein the first multiplex digital amplification reaction includes i) one or more universal primers designed to hybridize to the universal primer binding sites incorporated into the pre-amplification primers; and ii) a hybridization probe which binds to the common internal probe target sequence from the target chromosome amplified products; 4) Performing a second multiplex digital amplification on the maternal biological sample, wherein the second multiplex digital amplification includes i) one or more universal primers designed to hybridize to the universal primer binding sites incorporated into the pre-amplification primers; and ii) a hybridization probe which binds to common internal probe sequence from the non-target chromosome amplified products." Hansen at 17. As another example, Hansen discloses, "Further, and with reference to Figure 3 herein, this Figure demonstrates a digital PCR analysis of two-step amplification protocol using primers with gene specific regions and flanking universal primer sequences. Part (A) of Figure 3 demonstrates a schematic diagram for a potential multiplex digital PCR, which includes a pre-amplification step. The pre-amplification occurs with a plurality of PCR primer pairs, and each primer pair can amplify a PCR product comprising a common internal probe sequence." Hansen at 46.

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Claim	**Prior Art**
	FIGURE 3 A 
l[a][i]. tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,	Under Natera's apparent construction, Hansen discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See, e.g.,* Hansen at 7, 8, 10, 14, 15, 16, 17, 18, 22, 23, 28, 30, 31, Figure 3.

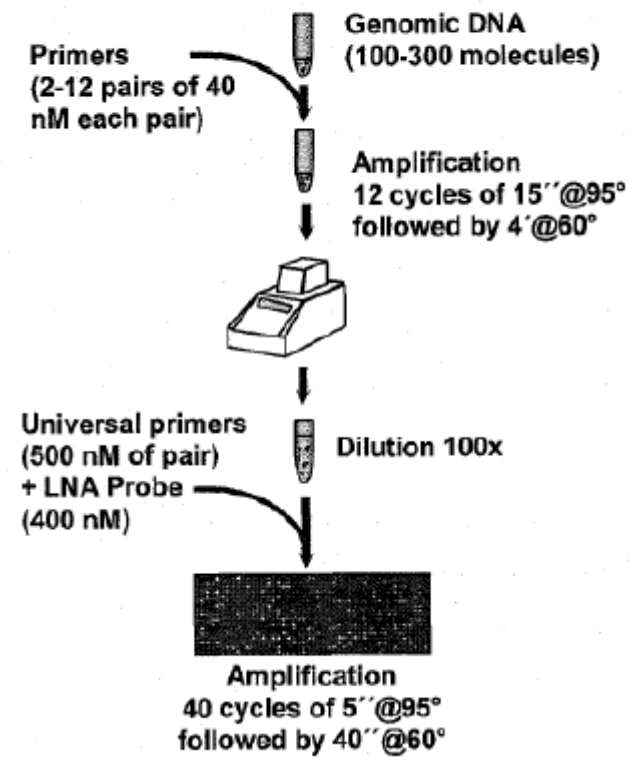
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Claim	**Prior Art**
	Hansen discloses a pre-amplification step that "may be performed using pre-amplification primers designed to introduce the sequences on a target or reference molecule to which a target primer or reference primer may anneal. Such sequences may comprise universal non-specific primer sequences." Hansen at 30. Hansen defines "pre-amplification" as referring to "a DNA replication step performed on a biological sample with target and reference pre-amplification primer pairs prior to distributing the biological sample into discrete subsamples for digital amplification. Pre-amplification may be performed with a limited number of cycles so as to avoid biased replication of individual target molecules. In one aspect, a pre-amplification step may serve to synthesize target and reference molecules that are more amenable to amplification by target and reference primers. In another aspect, a pre-amplification step may be performed using pre-amplification primers designed to introduce the sequences on a target or reference molecule to which a target primer or reference primer may anneal Such sequences may comprise universal non-specific primer sequences. In yet another aspect, a pre-amplification step may be performed with pre-amplification primers designed to introduce a probe target sequence or a probe reference sequence to a target molecule or reference molecule." Hansen at 30. For example, Hansen discloses a method including: "(a) providing a subset of target pre-amplification primer pairs, wherein each target pre-amplification primer pair is specific to at least one target site, and a subset of reference pre- amplification primer pairs, wherein each reference preamplification primer pair is specific to at least one reference site, and wherein each target pre-amplification primer pair has a first universal non-specific primer sequence and wherein each reference pre-amplification primer pair has a second universal non-specific primer sequence; (b) performing a pre-amplification reaction on the biological sample with the first and second subsets to synthesize the target molecules and the reference molecules, wherein the biological sample includes additional target and reference molecules." Hansen at 7. *See also id.* at 8 (same). Hansen defines "universal non-specific primer sequence" as follows: "As used herein, the term "universal non-specific primer sequence" refers to the sequence of a target primer or reference primer that may be used to amplify all target or reference molecules, respectively. Accordingly, a "universal target primer" or "universal reference primer" as used herein refers to a target primer or reference primer that may be used to

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Claim	**Prior Art**
	amplify all target or reference molecules, respectively. An example of universal primers are as follows: GTTGTAAAACGACGGCCAGT (universal forward primer, SEQ ID NO: 17) and CACAGGAAACAGCTATGACC (universal reverse primer, SEQ ID NO: 18)." Hansen at 23. Table 3 provides a list of primer sequences "compris[ing] a sequence-specific sequence, non-underlined, as well as a universal primer target sequence, underlined." Hansen at 46. As another example, Figure 3 shows "a digital PCR analysis of two-step amplification protocol using primers with gene specific regions and flanking universal primer sequences." Hansen at 14

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Claim	**Prior Art**
	FIGURE 3  As another example, Hansen discloses, "The universal primer pair may be SEQ ID NO: 17 and SEQ ID: 18." Hansen at 10.

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Claim	**Prior Art**
	As another example, Hansen discloses, "Alternatively, the design of primers may include both the gene specific sequences of interest together with universal primer sequences (for example, TABLE 3). Alternatively, the design of primers may include gene specific sequences of interest together with universal primer sequences along with a probe sequence as described herein." Hansen at 15. As another example, Hansen discloses, "Where the target molecule is such an amplified copy, the target molecule may comprise a universal non-specific primer sequence that is introduced through a pre-amplification reaction." Hansen at 22. As another example, Hansen discloses, "A target primer may be common to all target sites (i.e., a universal target primer). The sequence to which a target primer anneals may be introduced to a target molecule during a pre-amplification step with pre-amplification primers (e.g., as for the addition of a universal non-specific primer sequence)." Hansen at 22.
l[a][ii]. wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;	Hansen discloses tagging cell free DNA isolated from a blood sample collected from a subject who is not a pregnant women. *See, e.g.,* Hansen at 1, 2, 36. For example, Hansen discloses in the Background section that the occurrence of "[c]hromosomal abnormalities and imbalances are responsible for a significant portion of genetic disorders . . . The occurrence after birth may also result in serious pathologies, including cancer." Hansen at 1. As another example, Hansen discloses, "The use of DNA derived from various biological sources, including plasma or serum, for molecular genotyping and diagnosis has been in development for more than a decade (Boland 1996). Plasma and serum DNA from cancer patients has been shown to contain large quantities of tumor DNA (Chen et al. 1996; Nawroz et al. 1996; Anker et al. 1997). Lo et al. (1997) demonstrated that fetal DNA is present in maternal plasma and serum, and that detection of fetal DNA sequences was possible with just 10 ml of boiled plasma and serum. These observations suggested that plasma or serum DNA, or DNA from other biological samples including urine or feces, may be a useful

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Claim	**Prior Art**
	source of material for the noninvasive and early diagnosis of various disorders associated with chromosomal abnormalities." Hansen at 2. As another example, Hansen discloses, "The disclose[d] methods may further be used for the non-invasive detection or surveillance of a chromosomal abnormality that may be indicative [of] a pathology, such as cancer or a pre-cancerous condition, in a subject. For example, the disclosed methods may be used to detect the presence of cells in the body of a subject which comprise a gain or loss of a defined target source, or portion thereof.... Overrepresentation, or under-representation, of the target source relative to the reference source(s) would be indicative of the presence of cells in the body which carry a chromosomal abnormality associated with the target source Accordingly, the disclosed methods may further be used to monitor the progression of a pathological condition, or evaluate the efficacy treatments for the condition." Hansen at 36.
l[b][i]. amplifying the tagged products one or more times to generate final amplification products,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Hansen discloses amplifying the tagged products one or more times to generate final amplification products. To the extent Natera argues Hansen does not meet this limitation, Natera's position is inconsistent with the Court's claim construction order. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). Specifically, Natera's Response to NeoGenomics Laboratories, Inc.'s Interrogatory No. 14 does not dispute that Hansen discloses "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification". NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.,* Hansen at 7, 8, 15, 17, 18, 23, 30, 45, Figure 3. For example, Hansen discloses a method including: "(a) providing a subset of target pre-amplification primer pairs, wherein each target pre-amplification primer pair is specific to at least one target site, and a subset of reference pre- amplification primer pairs, wherein each reference preamplification primer pair is

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Claim	**Prior Art**
	specific to at least one reference site, and wherein each target pre-amplification primer pair has a first universal non-specific primer sequence and wherein each reference pre-amplification primer pair has a second universal non-specific primer sequence; (b) performing a pre-amplification reaction on the biological sample with the first and second subsets to synthesize the target molecules and the reference molecules, wherein the biological sample includes additional target and reference molecules." Hansen at 7. *See also id.* at 8 (same). Hansen defines "universal non-specific primer sequence" as follows: "As used herein, the term "universal non-specific primer sequence" refers to the sequence of a target primer or reference primer that may be used to amplify all target or reference molecules, respectively. Accordingly, a "universal target primer" or "universal reference primer" as used herein refers to a target primer or reference primer that may be used to amplify all target or reference molecules, respectively. An example of universal primers are as follows: GTTGTAAAACGACGGCCAGT (universal forward primer, SEQ ID NO: 17) and CACAGGAAACAGCTATGACC (universal reverse primer, SEQ ID NO: 18)." Hansen at 23. As another example, Hansen discloses, "Alternatively, the design of primers may include both the gene specific sequences of interest together with universal primer sequences (for example, TABLE 3). Alternatively, the design of primers may include gene specific sequences of interest together with universal primer sequences along with a probe sequence as described herein. Digital amplification may occur via digital PCR (dPCR) or rolling circle amplification." Hansen at 15. For example, Hansen defines "pre-amplification" as referring to "a DNA replication step performed on a biological sample with target and reference pre-amplification primer pairs prior to distributing the biological sample into discrete subsamples for digital amplification." Hansen at 30. For example, Hansen discloses a method including performing digital amplification of the tagged products: "In accordance with another embodiment, there is provided a method of detecting the relative amount of target source to reference source in a biological sample, the method including: (a) providing a subset of target pre-amplification primer pairs, wherein each target pre-amplification primer pair is specific to at least

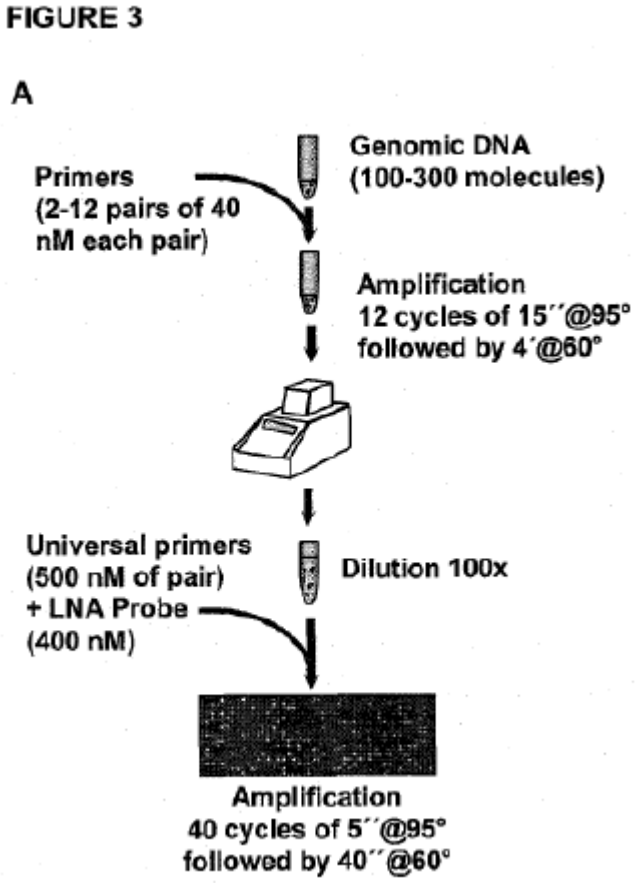
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Claim	**Prior Art**
	one target site, and a subset of reference pre-amplification primer pairs, wherein each reference preamplification primer pair is specific to at least one reference site, and wherein each target pre-amplification primer pair has a first universal non-specific primer sequence and wherein each reference pre-amplification primer pair has a second universal non-specific primer sequence; (b) performing a pre-amplification reaction on the biological sample with the first and second subsets to synthesize the target molecules and the reference molecules, wherein the biological sample includes additional target and reference molecules performing digital amplification with the target primer subset and the reference primer subset." Hansen at 7. *See also id.* at 8 (same). As another example, Hansen discloses, "Alternatively, the design of primers may include both the gene specific sequences of interest together with universal primer sequences (for example, TABLE 3). Alternatively, the design of primers may include gene specific sequences of interest together with universal primer sequences along with a probe sequence as described herein. Digital amplification may occur via digital PCR (dPCR) or rolling circle amplification." Hansen at 15. As another example, Hansen discloses, "3) Performing a first multiplex digital amplification reaction on the pre- amplification reaction product, wherein the first multiplex digital amplification reaction includes i) one or more universal primers designed to hybridize to the universal primer binding sites incorporated into the pre-amplification primers; and ii) a hybridization probe which binds to the common internal probe target sequence from the target chromosome amplified products; 4) Performing a second multiplex digital amplification on the maternal biological sample, wherein the second multiplex digital amplification includes i) one or more universal primers designed to hybridize to the universal primer binding sites incorporated into the pre-amplification primers; and ii) a hybridization probe which binds to common internal probe sequence from the non-target chromosome amplified products." Hansen at 17. As another example, Hansen discloses, "Further, and with reference to Figure 3 herein, this Figure demonstrates a digital PCR analysis of two-step amplification protocol using primers with gene specific regions and flanking universal primer sequences. Part (A) of Figure 3 demonstrates a schematic diagram for a potential multiplex digital PCR, which includes a pre-amplification step. The pre-amplification occurs

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	with a plurality of PCR primer pairs, and each primer pair can amplify a PCR product comprising a common internal probe sequence." Hansen at 46.

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Claim	**Prior Art**
	 Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products

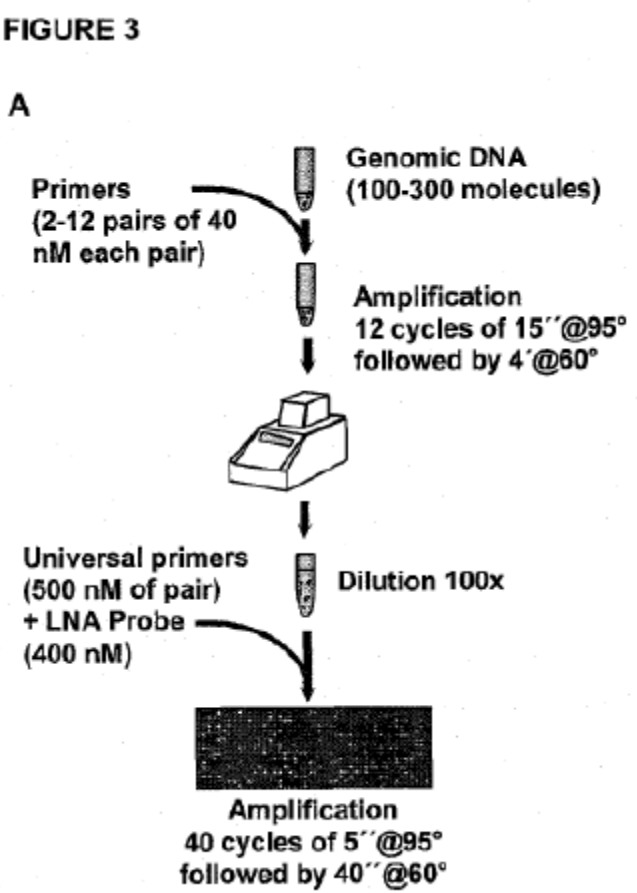
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Claim	**Prior Art**
	perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Hansen also discloses this element for the same reasons.
l[b][ii]. wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Hansen discloses amplifying wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume. To the extent Natera argues Hansen does not meet this limitation, Natera's position is inconsistent with the Court's claim construction order. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). Specifically, Natera's Response to NeoGenomics Laboratories, Inc.'s Interrogatory No. 14 does not dispute that Hansen discloses "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification". NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.,* Hansen at 7, 8, 14, 15, 22, 30, 46, Figure 3. Hansen discloses a pre-amplification step that "may be performed using pre-amplification primers designed to introduce the sequences on a target or reference molecule to which a target primer or reference primer may anneal. Such sequences may comprise universal non-specific primer sequences." Hansen at 30. Hansen defines "pre-amplification" as referring to "a DNA replication step performed on a biological sample with target and reference pre-amplification primer pairs prior to distributing the biological sample into discrete subsamples for digital amplification. Pre-amplification may be performed with a limited number of cycles so as to avoid biased replication of individual target molecules. In one aspect, a

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	pre-amplification step may serve to synthesize target and reference molecules that are more amenable to amplification by target and reference primers. In another aspect, a pre-amplification step may be performed using pre-amplification primers designed to introduce the sequences on a target or reference molecule to which a target primer or reference primer may anneal. Such sequences may comprise universal non-specific primer sequences. In yet another aspect, a pre-amplification step may be performed with pre-amplification primers designed to introduce a probe target sequence or a probe reference sequence to a target molecule or reference molecule." Hansen at 30. For example, Hansen discloses a method including: "(a) providing a subset of target pre-amplification primer pairs, wherein each target pre-amplification primer pair is specific to at least one target site, and a subset of reference pre- amplification primer pairs, wherein each reference preamplification primer pair is specific to at least one reference site, and wherein each target pre-amplification primer pair has a first universal non-specific primer sequence and wherein each reference pre-amplification primer pair has a second universal non-specific primer sequence; (b) performing a pre-amplification reaction on the biological sample with the first and second subsets to synthesize the target molecules and the reference molecules, wherein the biologic.al sample includes additional target and reference molecules." Hansen at 7. *See also id.* at 8 (same). Table 3 provides a list of primer sequences "compris[ing] a sequence-specific sequence, non-underlined, as well as a universal primer target sequence, underlined." Hansen at 46. As another example, Figure 3 shows "a digital PCR analysis of two-step amplification protocol using primers with gene specific regions and flanking universal primer sequences." Hansen at 14

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Claim	**Prior Art**
	FIGURE 3  As another example, Hansen discloses, "Alternatively, the design of primers may include both the gene specific sequences of interest together with universal primer sequences (for example, TABLE 3).

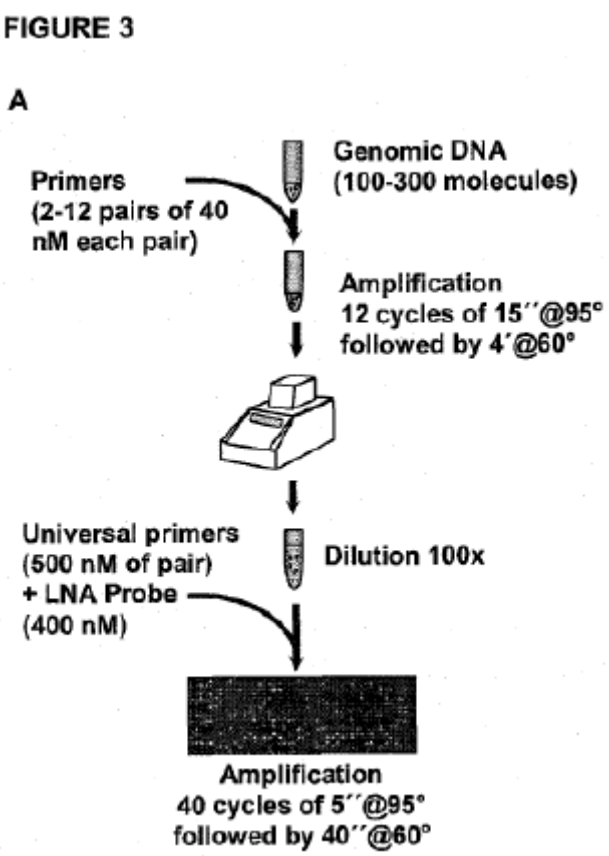
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Claim	**Prior Art**
	Alternatively, the design of primers may include gene specific sequences of interest together with universal primer sequences along with a probe sequence as described herein." Hansen at 15. As another example, Hansen discloses, "A target primer may be common to all target sit.es (i.e., a universal target primer). The sequence to which a target primer anneals may be introduced to a target molecule during a pre-amplification step with pre-amplification primers (e.g., as for the addition of a universal non-specific primer sequence)." Hansen at 22. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Hansen also discloses this element for the same reasons.
1[b][iii]. wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and	To the extent Natera contends Hansen does not disclose introducing a barcode and one or more sequencing tags by amplification as claimed by the '035 patent, doing so would have been obvious to a POSA because barcoding and adding sequencing tags were known techniques in the art at the time of the claimed invention, and accordingly could have been used with a reasonable expectation of success and without undue experimentation. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Hansen also discloses this element for the same reasons.
1[c][i]. sequencing the plurality of SNP loci on the cell free DNA by conducting	Hansen discloses sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products.

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Claim	**Prior Art**
massively parallel sequencing on the final amplification products,	For example, Hansen discloses "US 20090029377 (Lo. et al.) rejects probe-based detection altogether and discloses the sequencing of digital amplification products to identify the chromosomal origin of the target molecule." Hansen at 5-6. To the extent Natera contends Hansen does not disclose sequencing as claimed by the '035 patent, sequencing the plurality of SNP loci by massively parallel sequencing would have been obvious to a POSA because sequencing techniques and technologies were known in the art at the time of the claimed invention, and accordingly could have been used with a reasonable expectation of success and without undue experimentation.
l[c][ii]. wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.	The Court construed the term "SNP loci…associated with cancer" to mean "a single nucleotide that may differ between the genomes of two members of the same species and is associated with cancer. The usage of the term 'SNP' should not imply any limit on the frequency with which each variant occurs." Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Hansen discloses a plurality of SNP loci comprising 25-2,000 loci associated with cancer. To the extent Natera argues Hansen does not meet this limitation, Natera's position is inconsistent with the Court's claim construction order. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). Specifically, Natera's Response to NeoGenomics Laboratories, Inc.'s Interrogatory No. 14 does not dispute that Hansen discloses "SNP loci…associated with cancer". NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. For example, figure 3 discloses using "2-12 pairs" of primers in a pre-amplification step.

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Claim	**Prior Art**
	FIGURE 3  As another example, Table 3 discloses a table of 16 primer sequences each "compri[sing] a sequence-specific sequence, non-underlined, as well as a universal primer target sequence, underlined." Hansen at 46. *See also id.* at 11.

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Claim	**Prior Art**
	To the extent Natera contends Hansen does not disclose a plurality of 25-2,000 loci, it would have been obvious to a POSA to use the claimed methods to analyze the claimed number of loci. For instance, Hansen expressly discloses, "alternative primer pairs design strategies are described herein and are known to persons of skill in the art." Hansen at 11. As another example, Hansen discloses, "a person of skill in the art would be able to design primer pairs based on the desired target source or reference source using methods known to persons skilled in the art." Hansen at 15. As another example, Hansen discloses, "In one aspect, the subset of target primers includes a single pair of target primers that is common to all target molecules. In another aspect, the subset includes a number of target primer pairs that may be equal to the number of target sites." Hansen at 23. To the extent Natera asserts that any portion of this limitation is not disclosed under Natera's apparent interpretation of the Court's construction, it would have been obvious based on the knowledge of a POSA at the time of the alleged invention to look to the Catalogue of Somatic Mutations In Cancer ("COSMIC") database, which identifies hundreds to thousands of gene variants, including those that can be present in the same gene, to readily disclose a plurality of SNP loci comprising 25-2,000 loci associated with cancer. By way of example, COSMIC identifies 6,038 different variants in the TP53 gene alone. *See, e.g.,* https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53. In addition, the '035 Patent admits a POSA's "general belief in the art is 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, would have understood that multiplex PCR up to 100 assays could readily be performed on cell free DNA, and would have been able to do so as a matter of routine.
2. The method of claim 1, wherein tagging the cell free DNA comprises ligating the one or more universal tail	Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 27. To the extent Natera argues tagging by ligation is equivalent to tagging by

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Claim	**Prior Art**
adaptors to the cell free DNA.	amplification, Hansen also discloses this element for the same reasons as discussed below regarding claim 13. In addition, to the extent Hansen does not specifically teach tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA, this limitation is taught by Dong (Ex. 1-A), Porreca (Ex. 1-F), Hoyal (Ex. 1-M), and Wessler (Ex. 1-M). A POSA would be motivated to combine Hansen with Dong, Porreca, Hoyal, or Wessler because Hansen expressly teaches using routine and conventional techniques known in the art to analyze sample circulating tumor DNA efficiently, and the references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end, and thus are readily interchangeable with each other. For example, Hoyal teaches "a need for improved extraction methods capable of capturing small nucleic acid molecules"—cell-free DNA—because "the ability to successfully analyze short, fragmented DNA in the presence of more abundant, longer DNA ha[d] remained elusive." Hoyal at 1. As another example, Hoyal teaches "detecting and monitoring extracellular mutant oncogenes or tumor-associated DNA found in the plasma or serum fraction of blood by using DNA extraction with enrichment for mutant DNA," i.e., detecting cell-free tumor DNA. Hoyal at 20. Hoyal solves these problems through adapter-mediated ligation followed by universal pre-amplification before target-specific amplification. Hoyal at Figure 3. For example, Wessler teaches methods for detecting polymorphisms in DNA. Wessler at 3:6-4:41. As another example, Wessler teaches methods for "investigation of genome structure . . . by the use of in vitro methods that detect variation in DNA sequence in the genomes between members of a species" Wessler at 2:26-29. Wessler reaches this goal through Wessler's ligation-mediated preamplification followed by two target-specific rounds of PCR-mediated amplification. Wessler at Figure 1.

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	There is a reasonable expectation of success because the techniques disclosed in Hansen and the cited references, including multiplex PCR, barcoding DNA fragments, adding sequencing tags, using multiple universal tail adapters, and performing massively parallel sequencing were all routine and conventional at the time for DNA analysis and diagnostics. Furthermore, the methods and techniques disclosed in the cited references are readily compatible with the methods of Hansen. For example, Hoyal's method of tagging cell-free tumor DNA with universal adapters then performing universal preamplification of tagged products through PCR using primers against the adapters is readily compatible with Forshew's PCR-based tagging methods. For example, Wessler's method of ligating double-stranded universal adaptors to target DNA then performing two rounds of target-specific amplification is readily compatible with Forshew's PCR-based tagging methods.
12. The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.	Hansen discloses one or more universal tail adaptors comprising a first universal tail adaptor and a second universal tail adaptor. For example, Hansen discloses a method including: "(a) providing a subset of target pre-amplification primer pairs, wherein each target pre-amplification primer pair is specific to at least one target site, and a subset of reference pre-amplification primer pairs, wherein each reference preamplification primer pair is specific to at least one reference site, and wherein each target pre-amplification primer pair has a first universal non-specific primer sequence and wherein each reference pre-amplification primer pair has a second universal non-specific primer sequence; (b) performing a pre-amplification reaction on the biological sample with the first and second subsets to synthesize the target molecules and the reference molecules, wherein the biological sample includes additional target and reference molecules." Hansen at 7. *See also id.* at 8 (same). As another example, Hansen discloses, "The universal primer pair may be SEQ ID NO: 17 and SEQ ID: 18." Hansen at 10.

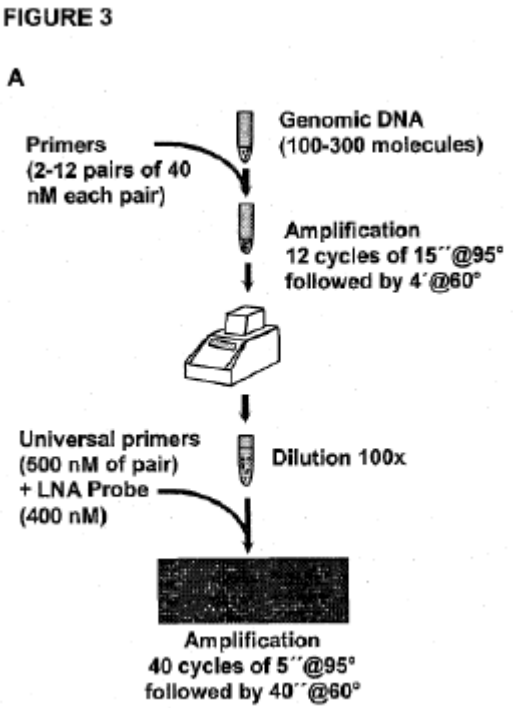
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Claim	**Prior Art**
13. The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.	Under Natera's apparent construction, Hansen discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See, e.g.,* Hansen at 7, 8, 10, 14, 15, 16, 17, 18, 22, 23, 28, 30, 31, Figure 3. Hansen discloses a pre-amplification step that "may be performed using pre-amplification primers designed to introduce the sequences on a target or reference molecule to which a target primer or reference primer may anneal. Such sequences may comprise universal non-specific primer sequences." Hansen at 30. Hansen defines "pre-amplification" as referring to "a DNA replication step performed on a biological sample with target and reference pre-amplification primer pairs prior to distributing the biological sample into discrete subsamples for digital amplification. Pre-amplification may be performed with a limited number of cycles so as to avoid biased replication of individual target molecules. In one aspect, a pre-amplification step may serve to synthesize target and reference molecules that are more amenable to amplification by target and reference primers. In another aspect, a pre-amplification step may be performed using pre-amplification primers designed to introduce the sequences on a target or reference molecule to which a target primer or reference primer may anneal Such sequences may comprise universal non-specific primer sequences. In yet another aspect, a pre-amplification step may be performed with pre-amplification primers designed to introduce a probe target sequence or a probe reference sequence to a target molecule or reference molecule." Hansen at 30. For example, Hansen discloses a method including: "(a) providing a subset of target pre-amplification primer pairs, wherein each target pre-amplification primer pair is specific to at least one target site, and a subset of reference pre- amplification primer pairs, wherein each reference preamplification primer pair is specific to at least one reference site, and wherein each target pre-amplification primer pair has a first universal non-specific primer sequence and wherein each reference pre-amplification primer pair has a second universal non-specific primer sequence; (b) performing a pre-amplification reaction on the biological sample with the first and second subsets to synthesize the target molecules and the reference molecules, wherein the biological sample includes additional target and reference molecules." Hansen at 7. *See also id.* at 8 (same).

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	Hansen defines "universal non-specific primer sequence" as follows: "As used herein, the term "universal non-specific primer sequence" refers to the sequence of a target primer or reference primer that may be used to amplify all target or reference molecules, respectively. Accordingly, a "universal target primer" or "universal reference primer" as used herein refers to a target primer or reference primer that may be used to amplify all target or reference molecules, respectively. An example of universal primers are as follows: GTTGTAAAACGACGGCCAGT (universal forward primer, SEQ ID NO: 17) and CACAGGAAACAGCTATGACC (universal reverse primer, SEQ ID NO: 18)." Hansen at 23. Table 3 provides a list of primer sequences "compris[ing] a sequence-specific sequence, non-underlined, as well as a universal primer target sequence, underlined." Hansen at 46. As another example, Figure 3 shows "a digital PCR analysis of two-step amplification protocol using primers with gene specific regions and flanking universal primer sequences." Hansen at 14

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Claim	**Prior Art**
	FIGURE 3  As another example, Hansen discloses, "The universal primer pair may be SEQ ID NO: 17 and SEQ ID: 18." Hansen at 10. As another example, Hansen discloses, "Alternatively, the design of primers may include both the gene specific sequences of interest together with universal primer sequences (for example, TABLE 3). Alternatively, the design of primers may include gene specific sequences of interest together with universal primer sequences along with a probe sequence as described herein." Hansen at 15.

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Claim	**Prior Art**
	As another example, Hansen discloses, "Where the target molecule is such an amplified copy, the target molecule may comprise a universal non-specific primer sequence that is introduced through a pre-amplification reaction." Hansen at 22. As another example, Hansen discloses, "A target primer may be common to all target sites (i.e., a universal target primer). The sequence to which a target primer anneals may be introduced to a target molecule during a pre- amplification step with pre-amplification primers (e.g., as for the addition of a universal non-specific primer sequence)." Hansen at 22. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary infringement Contentions at 22. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Hansen also discloses this element for the same reasons as discussed above regarding claim 2.

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EXHIBIT 1-J – FINAL INVALIDITY CLAIM CHART FOR U.S. PATENT NO. 11,519,035

Reference:
Shapero, M. et al., *MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density DNA oligonucleotide arrays* Nucleic Acids Research, Vol. 32 No. 22 (Dec. 15, 2004) (Shapero)

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

A POSA would have been motivated to combine Shapero's express teaching of a method of tagging DNA comprising ligating or amplifying the one or more universal tail adapters with other references such as Pieprzyk, May PCT, Cuppens, Makarov, Kaper, May Poster, Dong, Mir, Oliphant, and Hansen. Further, a POSA would reasonably expect to succeed in combining Shapero with other references that disclose methods of targeted amplification and sequencing because Shapero's MARA method was a generally applicable approach to locus-specific DNA target preparation for highly multiplexed SNP genotyping.

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1[pre]. A method for amplifying and sequencing DNA, comprising:	To the extent the preamble is construed to be limiting, Shapero discloses a method for amplifying DNA. *See, e.g.,* Shapero at Abstract, 2, 3, 4, Figure 1, Figure 2. For example, Figure 1 discloses, "(v) The first cycle of secondary PCR allows primer A2 to convert full-length extension products (which contain an A2 primer annealing site) into double-stranded DNA, while partial extension products lacking the A2 primer annealing site are not converted into a double-stranded product. (vi) Subsequent cycles of secondary PCR with universal A2 and A2 primers amplify all full-length products derived from the first round locus-specific reaction."

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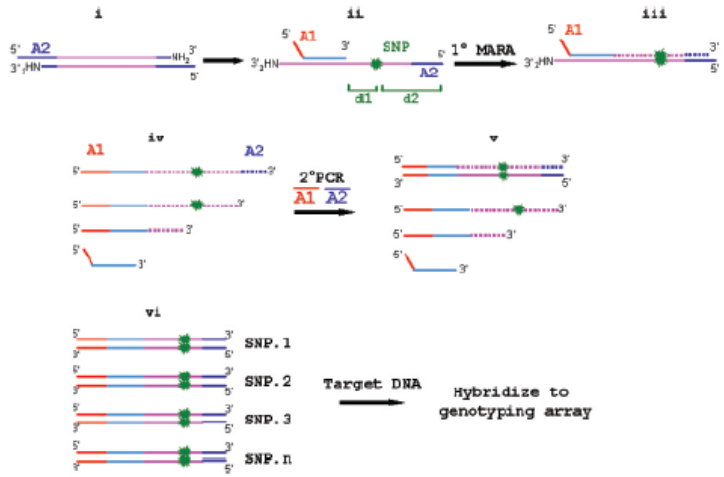
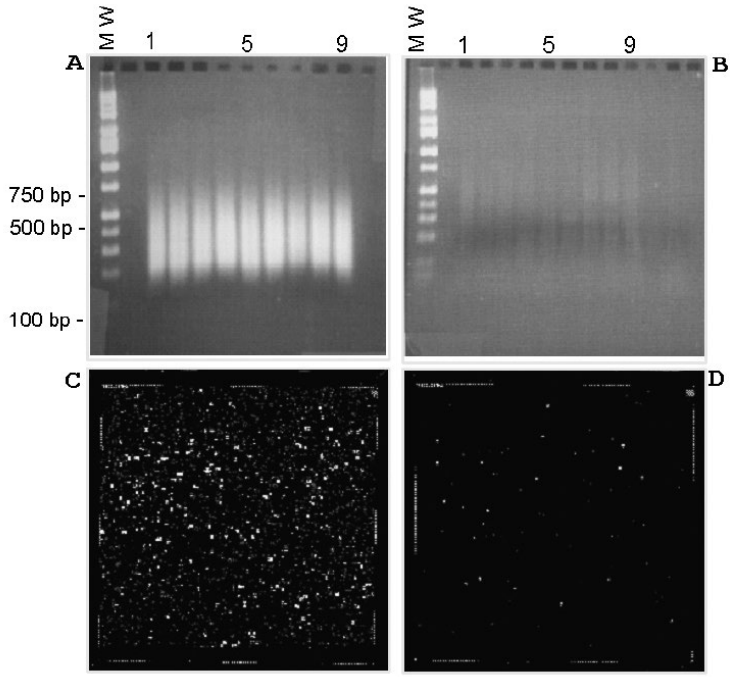


Figure 1. MARA (i) Human genomic DNA (pink) is digested to completion with a restriction enzyme and ligated to adapters (blue). The 3′ amino block helps to prevent background genomic DNA amplification. (ii) A MARA forward primer (containing common sequence A1) is shown annealing to the bottom strand of DNA upstream of a SNP (green symbol). (iii) The primary MARA reaction involves multiple cycles of primer annealing and extension, which can lead to a full-length extension product (dashed line) that has copied the adapter sequence (A2) from the genomic DNA template. (iv) At the completion of cycling, the reaction may contain unused primers as well as a mixture of both full-length extension products and truncated extension products that do not contain the copied A2 adapter. An enrichment step was used in the MARA 250-plex reactions following the primary reaction. One cycle of PCR with primer A2 converts full-length amplification products (which contain an A2 primer annealing site) into double-strand DNA, while incomplete products lacking the A2 primer annealing site are not converted into a double-stranded product. Exonuclease I digestion removes single strand products, while double-strand products are resistant to digestion. This step enriches for full-length products generated by the primary reaction, but cannot discriminate between products derived from specific and non-specific primer–target DNA annealing (v) The first cycle of secondary PCR allows primer A2 to convert full-length extension products (which contain an A2 primer annealing site) into double-stranded DNA, while partial extension products lacking the A2 primer annealing site are not converted into a double-stranded product. (vi) Subsequent cycles of secondary PCR with universal A1 and A2 primers amplify all full-length products derived from the first round locus-specific reaction. Target DNA is prepared for hybridization by enzymatic fragmentation with DNase I and end-labeling with TdT and biotin-ddATP.

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Claim	**Prior Art**
	As another example, Shapero discloses that "[f]ollowing a primary reaction in which locus-specific products are generated, a secondary universal amplification is carried out using a generic primer pair corresponding to the oligonucleotide and genomic DNA adapter sequences." Shapero at Abstract. As another example, Shapero describes "the development of a target preparation method for highly multiplexed SNP genotyping termed MARA (Multiplexed Anchored Runoff Amplification." Shapero at 2. As another example, Shapero discloses that "[s]ubsequent cycles of amplification are carried out over a range of annealing temperatures" and that a "secondary amplification step is then carried out using an aliquot of the primary MARA reaction as template." Shapero at 3. As another example, Shapero discloses that "[o]ne cycle of PCR with primer A2 converts full-length amplification products (which contain an A2 primer annealing site) into double-strand DNA" and that the "first cycle of secondary PCR allows primer A2 to convert full-length extension products (which contain an A2 primer annealing site) into double-stranded DNA, while partial extension products lacking the A2 primer annealing site are not converted into a double-stranded product." Shapero at 4, Figure 1. As another example, Shapero discloses, "Representative secondary universal amplification products are shown in Figure 2A." Shapero at 4, Figure 2.

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	 **Figure 2.** (**A**) 250-plex 2° PCR products separated on a 2%, 1×TBE agarose gel. (**B**) Negative control (no locus-specific primers) 2° PCR products separated on a 2%, 1×TBE agarose gel. (**C**) p502 chip image using 250 primer-plex 2° PCR products as target derived from input genomic DNA NA10830 (D) p502 chip image using negative control 2° PCR products as target derived from input genomic DNA NA10830.
1[a][i]. tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,	Shapero discloses tagging DNA with one or more universal tail adaptors to generate tagged products. *See, e.g.,* Shapero at Abstract, 1, 3, 4, Figure 1.

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	For example, Shapero discloses in Figure 1 the steps of MARA, which includes a step where, "Human genomic DNA (pink) is digested to completion with a restriction enzyme and ligated to adapters (blue)." Figure 1 shows that the ligated adapter is universal adapter A2. Figure 1 also discloses tagging with a second universal adaptor by a PCR step using a single primer comprising universal adaptor A1 and a target-specific region to generate tagged products comprising universal adaptors A1 and A2 and the target SNP locus.

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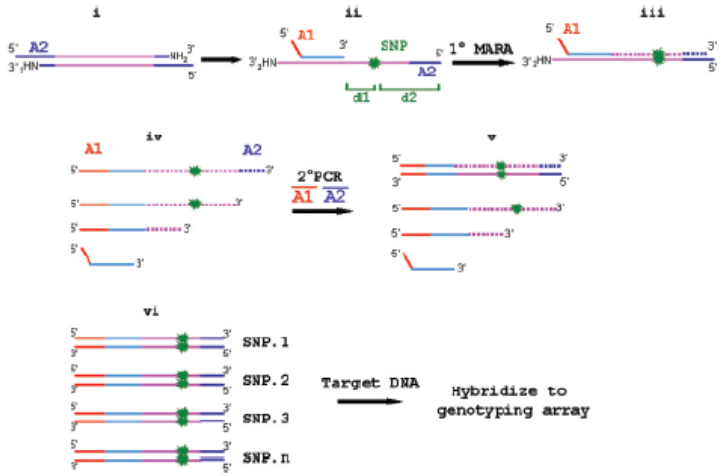


Figure 1. MARA (i) Human genomic DNA (pink) is digested to completion with a restriction enzyme and ligated to adapters (blue). The 3′ amino block helps to prevent background genomic DNA amplification. (ii) A MARA forward primer (containing common sequence A1) is shown annealing to the bottom strand of DNA upstream of a SNP (green symbol). (iii) The primary MARA reaction involves multiple cycles of primer annealing and extension, which can lead to a full-length extension product (dashed line) that has copied the adapter sequence (A2) from the genomic DNA template. (iv) At the completion of cycling, the reaction may contain unused primers as well as a mixture of both full-length extension products and truncated extension products that do not contain the copied A2 adapter. An enrichment step was used in the MARA 250-plex reactions following the primary reaction. One cycle of PCR with primer A2 converts full-length amplification products (which contain an A2 primer annealing site) into double-strand DNA, while incomplete products lacking the A2 primer annealing site are not converted into a double-stranded product. Exonuclease I digestion removes single strand products, while double-strand products are resistant to digestion. This step enriches for full-length products generated by the primary reaction, but cannot discriminate between products derived from specific and non-specific primer–target DNA annealing (v) The first cycle of secondary PCR allows primer A2 to convert full-length extension products (which contain an A2 primer annealing site) into double-stranded DNA, while partial extension products lacking the A2 primer annealing site are not converted into a double-stranded product. (vi) Subsequent cycles of secondary PCR with universal A1 and A2 primers amplify all full-length products derived from the first round locus-specific reaction. Target DNA is prepared for hybridization by enzymatic fragmentation with DNase I and end-labeling with TdT and biotin-ddATP.

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	As another example, "[u]niversal primers are used in the secondary amplification that anneal to common sites present in all full-length amplification products." Shapero at 3. As another example, Shapero discloses that a "number of alternative approaches to standard multiplex PCR have been described over the years," including "the use of universal primer sequences added to the ends of locus-specific sequences." Shapero at 1. As another example, Shapero discloses a method wherein following "a primary reaction in which locus-specific products are generated, a secondary universal amplification is carried out using a generic primer pair corresponding to the oligonucleotide and genomic DNA adapter sequences." Shapero at Abstract.
1[a][ii]. wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;	In view of the knowledge of a POSA at the time of the claimed invention, it would have been obvious to perform the claimed method on cell-free DNA isolated from a blood sample collected from a subject who is not a pregnant woman. For instance, a POSA would be motivated to utilize the methods of Shapero for cell-free tumor DNA assays because Shapero teaches that its MARA method "improve[s] the throughput of SNP genotyping. . .by allowing levels of multiplexing during target generation that far exceed the capacity of traditional multiplex PCR" and the POSA would therefore expect to be able to increase the capacity of multiplex PCR using cell-free DNA samples. Shapero at 1. As another example, Shapero teaches that its method of detecting SNPs may help "identify the underlying genetic basis of complex diseases" such as cancers. *Id.* Furthermore, a POSA would be motivated to combine Shapero with Pieprzyk, Makarov, and Mir, because Shapero expressly teaches using routine and conventional techniques known in the art to analyze target DNA using ligation of one or more universal tail adapters followed by amplification, and the references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end, and thus are readily interchangeable with each other. For example, a POSA would recognize that ligation-based DNA tagging methods and PCR-based DNA tagging methods are readily interchangeable as known and predictable solutions that would be obvious to select or modify as a matter of design choice, ordinary skill or common sense. Accordingly it would be obvious to a POSA to combine the methods of the foregoing references to analyze a sample of interest such as cell-free DNA. For example, Pieprzyk teaches "method[s] for detecting and/or quantifying one or more target amplicon(s) produced by amplification, wherein the detecting and/or quantifying is carried out during amplification or after an amplification endpoint has been reached." Pieprzyk at [0004]. As another example, Pieprzyk teaches "library

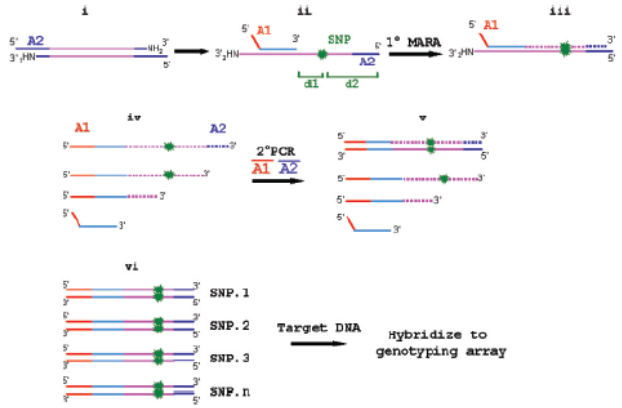
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	creation starts with conversion of sample nucleic acids to appropriately sized fragments, ligation of adaptor sequences onto the ends of the fragments, and selection for molecules properly appended with adaptors. The presence of the adaptor sequences on the ends of the library molecules enables amplification of random-sequence inserts." *Id.* at [0185]. Pieprzyk employs these methods for "identifying the presence of particular polymorphisms (such as SNPs), alleles, or haplotypes, or chromosomal abnormalities, such as amplifications, deletions, or aneuploidy" (*Id.* at [0221]) on "target sequences in samples having mixed length nucleic acids (e.g. fetal DNA in maternal plasma or tumor DNA in plasma from cancer patients." *Id.* at [0116]. For example, Makarov teaches "methods and compositions for amplification of a plurality of double stranded DNA molecules by incorporating at least one sequence onto the ends of the DNA molecules." Makarov at [0019]. As another example, Makarov teaches "amplification of molecules having at least one double stranded region by using adaptors that avoid the limitations of some adaptor molecules, such as those having the propensity to form adaptor dimers." *Id.* at [0020]. As another example, Makarov teaches "Ligation-mediated PCR amplification is achieved by using a locus-specific primer (or several nested primers) and a universal primer complementary to the adaptor sequence." *Id.* at [0006]. Makarov teaches that "the methods of the invention can be easily applied to any type of fragmented double stranded DNA including but not limited to, for example, free DNA isolated from plasma, serum, and/or urine; apoptotic DNA from cells and/or tissues; DNA fragmented enzymatically *in vitro* (for example, by DNase I and/or restriction endonuclease); and/or DNA fragmented by mechanical forces (hydro-shear, sonication, nebulization, *etc.*)." *Id.* at [0036]. For example, Mir teaches methods "for detecting a plurality of target nucleic acids" which entails an amplification step "whereby the tagged target nucleotide sequences are amplified using at least one primer that anneals to at least one nucleotide tag." Mir at [0086]. As another example, Mir teaches "Library creation starts with conversion of sample nucleic acids to appropriately sized fragments, ligation of adaptor sequences onto the ends of the fragments, and selection for molecules properly appended with adaptors. The presence of the adaptor sequences on the ends of the library molecules enables amplification of random-sequence inserts. The above-described methods for tagging nucleotide sequences can be substituted for ligation, to introduce adaptor sequences." *Id.* at [0160]. Mir employs these methods on ""[n]ucleic acids can be extracted or amplified from cells, bodily fluids (e.g., blood, a blood fraction, using, etc.), or tissue samples by any variety of standard techniques. Exemplary samples include samples of plasma, serum, spinal fluid, lymph fluid, peritoneal fluid, pleural fluid, oral fluid, and external sections of the skin; samples from the respiratory, intestinal genital, and urinary tracts; samples of tears, saliva, blood cells, stem cells, or tumors." *Id.* at [0122].

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	There is a reasonable expectation of success in combining Shapero with one or more of the foregoing references for the purposes described above because the techniques of isolating, tagging, amplifying, and sequencing cell free DNA as well as using ligation to tag cell free DNA with universal tail adaptors were all well known in the art at the time of the invention, and the one or more foregoing references disclose or teach using said well-known techniques to analyze isolated cell-free DNA from a blood sample collected from a subject who is not a pregnant woman.
1[b][i]. amplifying the tagged products one or more times to generate final amplification products,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Shapero discloses amplifying the tagged products one or more times to generate final amplification products. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). Specifically, Natera's Response to NeoGenomics Laboratories, Inc.'s Interrogatory No. 14 does not dispute that Shapero discloses "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification". NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.,* Shapero at 4, Figure 1, Figure 2. For example, Figure 1 discloses multiple steps of PCR to generate amplification products comprising the A1 and A2 universal tail adaptors. For example, Figure 1 discloses "a MARA forward primer (containing common sequence A1)…annealing to the bottom strand of DNA upstream of a SNP" wherein the MARA forward primer is used in a "primary MARA reaction involve[ing] multiple cycles of primer annealing and extension…" Shapero at Figure 1.

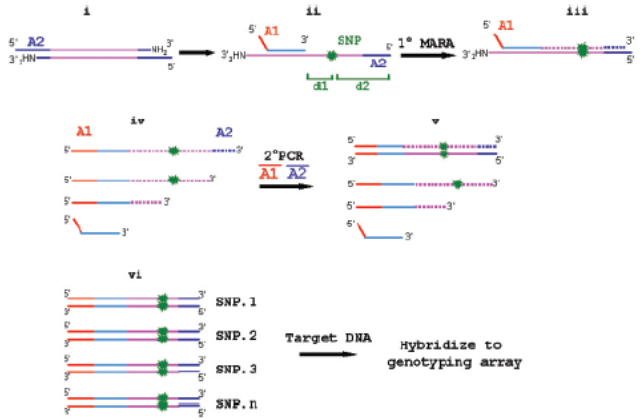
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	 **Figure 1.** MARA (i) Human genomic DNA (pink) is digested to completion with a restriction enzyme and ligated to adapters (blue). The 3′ amino block helps to prevent background genomic DNA amplification. (ii) A MARA forward primer (containing common sequence A1) is shown annealing to the bottom strand of DNA upstream of a SNP (green symbol). (iii) The primary MARA reaction involves multiple cycles of primer annealing and extension, which can lead to a full-length extension product (dashed line) that has copied the adapter sequence (A2) from the genomic DNA template. (iv) At the completion of cycling, the reaction may contain unused primers as well as a mixture of both full-length extension products and truncated extension products that do not contain the copied A2 adapter. An enrichment step was used in the MARA 250-plex reactions following the primary reaction. One cycle of PCR with primer A2 converts full-length amplification products (which contain an A2 primer annealing site) into double-strand DNA, while incomplete products lacking the A2 primer annealing site are not converted into a double-stranded product. Exonuclease I digestion removes single strand products, while double-strand products are resistant to digestion. This step enriches for full-length products generated by the primary reaction, but cannot discriminate between products derived from specific and non-specific primer–target DNA annealing (v) The first cycle of secondary PCR allows primer A2 to convert full-length extension products (which contain an A2 primer annealing site) into double-stranded DNA, while partial extension products lacking the A2 primer annealing site are not converted into a double-stranded product. (vi) Subsequent cycles of secondary PCR with universal A1 and A2 primers amplify all full-length products derived from the first round locus-specific reaction. Target DNA is prepared for hybridization by enzymatic fragmentation with DNase I and end-labeling with TdT and biotin-ddATP.

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1[b][ii]. wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Shapero discloses amplifying wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). Specifically, Natera's Response to NeoGenomics Laboratories, Inc.'s Interrogatory No. 14 does not dispute that Shapero discloses "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification". NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.,* Shapero at 4, 5, 7, 8, Figure 1. For example, Shapero discloses, "In this report, we describe the development of a target preparation method for highly multiplexed SNP genotyping termed MARA (Multiplexed Anchored Runoff Amplification) that is combined with allele discrimination based on hybridization of DNA target to high-density oligonucleotide arrays. We have evaluated MARA reactions at primer multiplex levels between 250- and 4300-plex." Shapero at 2. For example, Shapero discloses a first PCR reaction in a single reaction volume that involves targeted amplification of the region comprising a SNP of interest. Shapero at 4, Figure 1.

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	 **Figure 1.** MARA (i) Human genomic DNA (pink) is digested to completion with a restriction enzyme and ligated to adapters (blue). The 3′ amino block helps to prevent background genomic DNA amplification. (ii) A MARA forward primer (containing common sequence A1) is shown annealing to the bottom strand of DNA upstream of a SNP (green symbol). (iii) The primary MARA reaction involves multiple cycles of primer annealing and extension, which can lead to a full-length extension product (dashed line) that has copied the adapter sequence (A2) from the genomic DNA template. (iv) At the completion of cycling, the reaction may contain unused primers as well as a mixture of both full-length extension products and truncated extension products that do not contain the copied A2 adapter. An enrichment step was used in the MARA 250-plex reactions following the primary reaction. One cycle of PCR with primer A2 converts full-length amplification products (which contain an A2 primer annealing site) into double-strand DNA, while incomplete products lacking the A2 primer annealing site are not converted into a double-stranded product. Exonuclease I digestion removes single strand products, while double-strand products are resistant to digestion. This step enriches for full-length products generated by the primary reaction, but cannot discriminate between products derived from specific and non-specific primer–target DNA annealing (v) The first cycle of secondary PCR allows primer A2 to convert full-length extension products (which contain an A2 primer annealing site) into double-stranded DNA, while partial extension products lacking the A2 primer annealing site are not converted into a double-stranded product. (vi) Subsequent cycles of secondary PCR with universal A1 and A2 primers amplify all full-length products derived from the first round locus-specific reaction. Target DNA is prepared for hybridization by enzymatic fragmentation with DNase I and end-labeling with TdT and biotin-ddATP.

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	As another example, Shapero discloses that in order to test MARA, "250 WGSA EcoRI SNPs from this array were chosen" and that a "total of 250 oligonucleotide primers were synthesized and combined, and then used in individual MARA reactions in conjunction with MseI digested, adapter-ligated genomic DNA (20 ng per reaction) from nine CEPH individuals." Shapero at 4. As another example, Shapero discloses a method wherein the "multiplexing level was increased about 250 primer-plex by the addition of 500 primers to the original MARA 250-plex reactions." Shapero at 5. As another example, Shapero discloses that "a new array was designed containing 8936 SNPs" in order to "assess higher levels of multiplexing." Shapero at 5. As another example, Shapero discloses that "[m]ultiplex reactions were carried out at 4265 primer-plex as well as using a random pooling of oligonucleotides into four separate groups of 1113-, 1140-, 1056-, and 1056-plex [these reactions are referred to as ~1100 primer-plex (x4) (Table 2)]." Shapero at 5. As another example, Shapero discloses a "highly multiplexed DNA target preparation method termed MARA. The key finding of this report is that multiplexing of over 4000 independent primers showed a subset of ~75% of the input total which demonstrated high SNP call rates and concordance across multiple individuals." Shapero at 7. As another example, Shapero discloses "multiplex levels for SNP genotyping that are higher than typically achieved through standard multiplex PCR." Shapero at 8.
1[b][iii]. wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and	To the extent Natera contends Shapero does not disclose introducing a barcode and one or more sequencing tags by amplification as claimed by the '035 patent, doing so would have been obvious to a POSA because barcoding and adding sequencing tags were known techniques in the art at the time of the claimed invention, and accordingly could have been used with a reasonable expectation of success and without undue experimentation. Furthermore, a POSA would be motivated to combine Shapero with Pieprzyk and May PCT to achieve this element of the claimed invention because Shapero expressly teaches using routine and conventional techniques known in the art to analyze target DNA through tagging and PCR-based amplification, and the references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end.

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	For example, Pieprzyk teaches "method[s] for detecting and/or quantifying one or more target amplicon(s) produced by amplification, wherein the detecting and/or quantifying is carried out during amplification or after an amplification endpoint has been reached." Pieprzyk at [0004]. As another example, Pieprzyk teaches preamplification step can be used to introduce a nucleotide tag" and that "at least one primer of each primer pair employed for preamplification can include a nucleotide tag, which becomes incorporated into the preamplified nucleic acids." *Id.* at [0113]. Pieprzyk employs these methods for "for producing PCR products with Sequencing tags and sample barcode" *Id.* at [0306]. For example, May PCT teaches its field of invention as relating "generally to the area of high-throughput assays for detection and/or sequencing of particular target nucleic acids" and that the invention "provides amplification methods in which nucleotide tag(s) and a barcode nucleotide sequence are added to target nucleotide sequences. May PCT at [0002]. As another example, May PCT teaches that "[n]ucleotide tags can also be added by an "encoding ligation" reaction that can comprise a ligation reaction in which at least one primer comprises a target specific portion and nucleotide tag located on the 5' end of the target-specific portion, and a second primer that comprises a target-specific portion only or a target-specific portion and a nucleotide tag located on the 5' end of the target specific portion. Illustrative encoding ligation reactions are described, for example, in U.S. Patent Publication No. 2005/0260640, which is hereby incorporated by reference in its entirety, and in particular for ligation reactions." May PCT at [0081]. There is a reasonable expectation of success in combining Shapero with one or more of the foregoing references for the purposes described above because the techniques of isolating, tagging, amplifying, and sequencing cell free DNA as well as using ligation to tag cell free DNA with universal tail adaptors were all well known in the art at the time of the invention. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. See Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Shapero also discloses this element for the same reasons.
1[c][i]. sequencing the plurality of SNP loci on the cell free DNA by	To the extent Natera contends Shapero does not disclose introducing a barcode and one or more sequencing tags by amplification as claimed by the '035 patent, doing so would have been obvious to a POSA because massively

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conducting massively parallel sequencing on the final amplification products,	parallel sequencing was a known technique in the art at the time of the claimed invention, and accordingly could have been used with a reasonable expectation of success and without undue experimentation. Furthermore, a POSA would be motivated to combine Shapero with Pieprzyk and May PCT to achieve this element of the claimed invention because Shapero expressly teaches using routine and conventional techniques known in the art to analyze target DNA through tagging and PCR-based amplification, and the references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end, and thus are readily interchangeable with each other. For example, a POSA would recognize that ligation-based DNA tagging methods and PCR-based DNA tagging methods are interchangeable as known and predictable solutions that woulld be obvious to select or modify as a matter of design choice, ordinary skill or common sense. Accordingly it would be obvious to a POSA to combine the methods of the foregoing references to sequence SNP loci on cell-free DNA. For example, Pieprzyk teaches "method[s] for detecting and/or quantifying one or more target amplicon(s) produced by amplification, wherein the detecting and/or quantifying is carried out during amplification or after an amplification endpoint has been reached." Pieprzyk at [0004]. As another example, Pieprzyk teaches "Library creation starts with conversion of sample nucleic acids to appropriately sized fragments, ligation of adaptor sequences onto the ends of the fragments, and selection for molecules properly appended with adaptors. The presence of the adaptor sequences on the ends of the library molecules enables amplification of random-sequence inserts." *Id.* at [0185]. As another example, Pieprzyk teaches analyzing target nucleic acids "for specific polymorphisms (such as SNPs)". *Id.* at [0151]. Pieprzyk teaches massively parallel sequencing as one approach to analyze SNP loci on target nucleic acids prepared through its methods. *Id.* at [0185] and [0207]. For example, May PCT teaches its field of invention as relating "generally to the area of high-throughput assays for detection and/or sequencing of particular target nucleic acids" and that the invention "provides amplification methods in which nucleotide tag(s) and a barcode nucleotide sequence are added to target nucleotide sequences. May PCT at [0002]. As another example, May PCT teaches ligation of adaptors to fragmented DNA and amplification of random-sequence inserts using said adapters. *Id.* at [0112]. As another example, May PCT teaches that targets of its method can include "genomic DNA, which can be analyzed for specific polymorphisms (such as SNPs), alleles, or haplotypes, e.g., in genotyping. Of particular interest are genomic DNAs that are altered (e.g., amplified, deleted, and/or mutated) in genetic diseases or other pathologies." *Id.* at [0135]. May PCT teaches massively parallel sequencing as one approach to analyze SNP loci on target nucleic acids prepared through its methods. *Id.* at [0259], Figures 14A and 14B, and Claim 39.

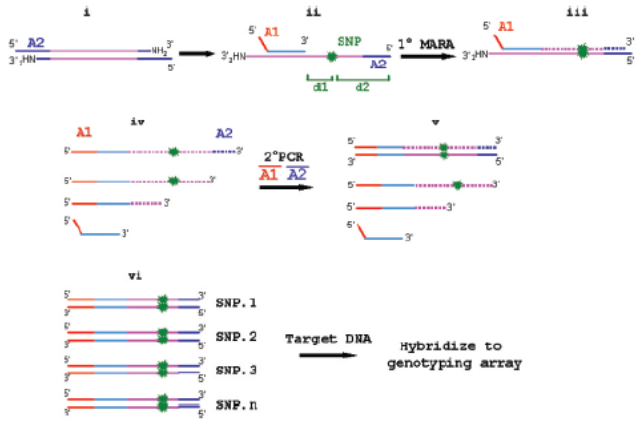
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	There is a reasonable expectation of success in combining Shapero with one or more of the foregoing references for the purposes described above because the techniques of isolating, tagging, amplifying, and sequencing cell free DNA as well as using ligation to tag cell free DNA with universal tail adaptors were all well known in the art at the time of the invention.
1[c][ii]. wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.	The Court construed the term "SNP loci…associated with cancer" to mean "a single nucleotide that may differ between the genomes of two members of the same species and is associated with cancer. The usage of the term 'SNP' should not imply any limit on the frequency with which each variant occurs." Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Shapero discloses wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). Specifically, Natera's Response to NeoGenomics Laboratories, Inc.'s Interrogatory No. 14 does not dispute that Shapero discloses or renders obvious "SNP loci…associated with cancer". NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.*, Shapero at Abstract, 1, 4, 5. For example, Shapero discloses, "We have developed a locus-specific DNA target preparation method for highly multiplexed single nucleotide polymorphism (SNP) genotyping called MARA (Multiplexed Anchored Runoff Amplification). . . . Initial multiplex reactions containing either 250 primers or 750 primers across nine DNA samples demonstrated . . . We have also evaluated > 1000- and 4000-primer plex MARA to genotype SNPs from human chromosome 21." Shapero at Abstract. As another example, Shapero discloses "Although multiple sources of genetic variation occur among individuals, single nucleotide polymorphisms (SNPs) are emerging as the marker of choice for genetic association studies due to their abundance, stability and relative ease of scoring." Shapero at 1. As another example, Shapero discloses, "In this report, we describe the development of a target preparation method for highly multiplexed SNP genotyping termed MARA (Multiplexed Anchored Runoff Amplification)

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	that is combined with allele discrimination based on hybridization of DNA target to high-density oligonucleotide arrays. We have evaluated MARA reactions at primer multiplex levels between 250- and 4300-plex. At levels of multiplexing over 1000-plex, >75% of the input oligonucleotides generate DNA target such that the targeted SNPs show a call rate and concordancy of >95 and 99%, respectively, across a panel of DNA samples. These results suggest that MARA may serve as a target preparation method for highly multiplexed locus specific SNP genotyping that far exceeds the capacity of standard traditional multiplex PCR." Shapero at 2. As another example, Shapero discloses, "Primary MARA 250- and 750-plex reactions were carried out in a thermal cycler in a total volume of 25 ml containing 20 ng adapter-ligated genomic DNA, 250 mM of each dNTP (dATP, dCTP, dGTP and dTTP), 1· GeneAmp PCR Gold Buffer (15 mM Tris–HCl, pH 8.0, 50 mM KCl), 2.5 mM MgCl2, 1.25 U AmpliTaq Gold (Applied Biosystems) and 1.25 U Perfect Match PCR Enhancer (Stratagene). The final concentration of each locus-specific primer was 0.16 mM(250- plex) and 0.05 mM (750-plex). Primary MARA 4365-plex reactions were carried out in a total volume of 60 ml containing 60 ng adapter-ligated genomic DNA, 250 mM of each dNTP (dATP, dCTP, dGTP and dTTP), 1· GeneAmp PCR Gold Buffer (15 mM Tris–HCl, pH 8.0, 50 mM KCl), 2.5 mM MgCl2, 0.025 mM of each locus-specific oligonucleotide, 1.25 U AmpliTaq Gold (Applied Biosystems) and 1.25 U Perfect Match PCR Enhancer (Stratagene). Primary MARA ~1100-plex (x4) reactions were performed in four separate reactions as described for 4365-plex, and subsequently reactions were pooled prior to the secondary PCR. Conditions for primary MARA amplifications were as follows: 1 cycle of 95°C for 7 min, followed by 18 cycles of 95°C for 20 s, 68°C for 2.5 min (-0.5°C per cycle) and 72°C for 60 s. The annealing and elongation times were increased to 5 and 2 min, respectively, per cycle for 4365-plex MARA. An enrichment step was carried out only on the 250-plex reactions following the primary reaction." Shapero at 3. As another example, Shapero discloses, "Secondary amplification reactions for MARA 250- and 750- plex were carried out in a total volume of 100 ml containing 250 mM of each dNTP (dATP, dCTP, dGTP and dTTP), 1· GeneAmp PCR Gold Buffer (15 mM Tris–HCl, pH 8.0, 50 mM KCl), 2.5 mM MgCl2, 0.8 mM T3 primer, 0.8 mM T7 primer and 5 U AmpliTaq Gold (Applied Biosystems). Secondary reactions used an aliquot of the primary reactions as template: 1 ml (250-plex) or 1 ml of 1:125 dilution (750- plex). Conditions for secondary amplifications were as follows: 1 cycle of 95°C for 7 min, followed by 35 cycles of 95°C for 20 s, 55°C for 30 s and 72°C for 30 s, followed by 72°C for 7 min. Negative control reactions were carried out as described above, except that no locus-specific primers were used during the primary MARA reaction." Shapero at 3. As another example, Shapero discloses, "MARA is a method designed to generate locus-specific DNA target sequences in a highly multiplexed manner. An overview is shown in Figure 1." Shapero at 3.

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Claim	**Prior Art**
	 **Figure 1.** MARA (i) Human genomic DNA (pink) is digested to completion with a restriction enzyme and ligated to adapters (blue). The 3′ amino block helps to prevent background genomic DNA amplification. (ii) A MARA forward primer (containing common sequence A1) is shown annealing to the bottom strand of DNA upstream of a SNP (green symbol). (iii) The primary MARA reaction involves multiple cycles of primer annealing and extension, which can lead to a full-length extension product (dashed line) that has copied the adapter sequence (A2) from the genomic DNA template. (iv) At the completion of cycling, the reaction may contain unused primers as well as a mixture of both full-length extension products and truncated extension products that do not contain the copied A2 adapter. An enrichment step was used in the MARA 250-plex reactions following the primary reaction. One cycle of PCR with primer A2 converts full-length amplification products (which contain an A2 primer annealing site) into double-strand DNA, while incomplete products lacking the A2 primer annealing site are not converted into a double-stranded product. Exonuclease I digestion removes single strand products, while double-strand products are resistant to digestion. This step enriches for full-length products generated by the primary reaction, but cannot discriminate between products derived from specific and non-specific primer–target DNA annealing (v) The first cycle of secondary PCR allows primer A2 to convert full-length extension products (which contain an A2 primer annealing site) into double-stranded DNA, while partial extension products lacking the A2 primer annealing site are not converted into a double-stranded product. (vi) Subsequent cycles of secondary PCR with universal A1 and A2 primers amplify all full-length products derived from the first round locus-specific reaction. Target DNA is prepared for hybridization by enzymatic fragmentation with DNase I and end-labeling with TdT and biotin-ddATP.

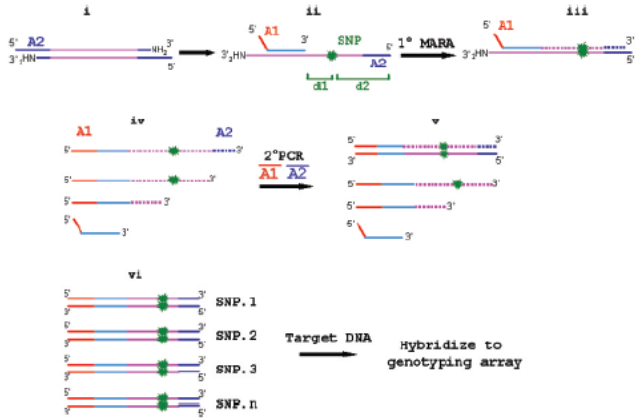
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Claim	**Prior Art**
	For example, Shapero discloses that in order to test MARA, "250 WGSA EcoRI SNPs from this array were chosen" and that a "total of 250 oligonucleotide primers were synthesized and combined, and then used in individual MARA reactions in conjunction with MseI digested, adapter-ligated genomic DNA." Shapero at 4. As another example, Shapero discloses that a "small number of primers target amplicons containing more than one SNP tiled on the array, resulting in 28 additional SNPs to the original pool of 250 SNPs (278 total SNPs)." Shapero at 4. As another example, Shapero discloses the addition "of 500 primers to the original MARA 250-plex reactions; 250 (set A) of these additional primers targeted SNPs tiled on the p502 array, while the remaining 250 primers (set B) were designed for MARA and are compatible with MseI restriction enzyme digested genomic DNA but did not target p502-specific SNPs." Shapero at 5. As another example, Shapero discloses that "a new array was designed containing 8936 SNPs from human chromosome 21, of which 4198 are defined as haplotype-defining SNPs." Shapero at 5. As another example, Shapero discloses a method wherein the "multiplexing level was increased about 250 primer-plex by the addition of 500 primers to the original MARA 250-plex reactions." Shapero at 5. As another example, Shapero discloses that "a new array was designed containing 8936 SNPs" in order to "assess higher levels of multiplexing." Shapero at 5. As another example, Shapero discloses that "[m]ultiplex reactions were carried out at 4265 primer-plex as well as using a random pooling of oligonucleotides into four separate groups of 1113-, 1140-, 1056-, and 1056-plex [these reactions are referred to as ~1100 primer-plex (x4) (Table 2)]." Shapero at 5. As another example, Shapero discloses a "highly multiplexed DNA target preparation method termed MARA. The key finding of this report is that multiplexing of over 4000 independent primers showed a subset of ~75% of the input total which demonstrated high SNP call rates and concordance across multiple individuals." Shapero at 7. As another example, Shapero discloses "multiplex levels for SNP genotyping that are higher than typically achieved through standard multiplex PCR." Shapero at 8.

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Claim	**Prior Art**
	To the extent Natera asserts that any portion of this limitation is not disclosed under Natera's apparent interpretation of the Court's construction, it would have been obvious based on the disclosure of Shapero and the knowledge of a POSA. Shapero discloses, for example, that "common-allele SNPs that are expected to provide the framework for new genome-wide studies designed to identify the underlying genetic basis of complex diseases" which may include diseases such as cancer (Shapero at 1). Presented with this and other disclosure in Shapero, a POSA would be motivated to search for SNP loci associated with diseases such as cancer as potential targets for the methods disclosed in Shapero. A POSA at the time of the alleged invention would readily know to look to the Catalogue of Somatic Mutations In Cancer ("COSMIC") database, which identifies hundreds to thousands of gene variants, including those that can be present in the same gene, to readily disclose a plurality of SNP loci comprising 25-2,000 loci associated with cancer. By way of example, COSMIC identifies 6,038 different variants in the TP53 gene alone. *See*, *e.g.,* https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53. In addition, the '035 Patent admits a POSA's "general belief in the art is 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, would have understood that multiplex PCR up to 100 assays could readily be performed on cell free DNA, and would have been able to do so as a matter of routine.
2. The method of claim 1, wherein tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA.	Shapero discloses tagging the DNA comprising ligating the one or more universal tail adapters. *See, e.g.*, Shapero at Abstract, 1, 3, 4, 5, 8, Figure 1. For example, Shapero discloses the ligation of one universal adaptor (A2) to fragmented genomic DNA. Shapero at 4, Figure 1.

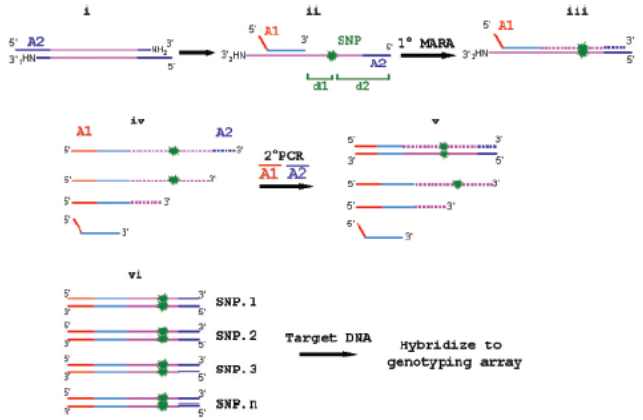
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U.S. Patent No. 11,519,035	
Claim	**Prior Art**
	 **Figure 1.** MARA (i) Human genomic DNA (pink) is digested to completion with a restriction enzyme and ligated to adapters (blue). The 3′ amino block helps to prevent background genomic DNA amplification. (ii) A MARA forward primer (containing common sequence A1) is shown annealing to the bottom strand of DNA upstream of a SNP (green symbol). (iii) The primary MARA reaction involves multiple cycles of primer annealing and extension, which can lead to a full-length extension product (dashed line) that has copied the adapter sequence (A2) from the genomic DNA template. (iv) At the completion of cycling, the reaction may contain unused primers as well as a mixture of both full-length extension products and truncated extension products that do not contain the copied A2 adapter. An enrichment step was used in the MARA 250-plex reactions following the primary reaction. One cycle of PCR with primer A2 converts full-length amplification products (which contain an A2 primer annealing site) into double-strand DNA, while incomplete products lacking the A2 primer annealing site are not converted into a double-stranded product. Exonuclease I digestion removes single strand products, while double-strand products are resistant to digestion. This step enriches for full-length products generated by the primary reaction, but cannot discriminate between products derived from specific and non-specific primer–target DNA annealing (v) The first cycle of secondary PCR allows primer A2 to convert full-length extension products (which contain an A2 primer annealing site) into double-stranded DNA, while partial extension products lacking the A2 primer annealing site are not converted into a double-stranded product. (vi) Subsequent cycles of secondary PCR with universal A1 and A2 primers amplify all full-length products derived from the first round locus-specific reaction. Target DNA is prepared for hybridization by enzymatic fragmentation with DNase I and end-labeling with TdT and biotin-ddATP.

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Claim	**Prior Art**
	As another example, Shapero discloses that a "number of alternative approaches to standard multiplex PCR have been described over the years," including "the use of universal primer sequences added to the ends of locus-specific sequences." Shapero at 1. As another example, Shapero discloses, "Adaptors were ligated to genomic DNA in 20 ul reactions containing 100 ng of restriction enzyme digested genomic DNA . . . Primary MARA 250- and 750-plex reactions were carried out in a thermal cycler in a total volume of 25 ul containing 20 ng adapter-ligated genomic DNA . . . Primary MARA 4365-plex reactions were carried out in a total volume of 60 ul containing 60 ng adapter-ligated genomic DNA" Shapero at 2. As another example, Shapero discloses, "After the primer anneals to the target and is elongated during the first cycle, the adapter sequences ligated to the genomic DNA are incorporated into the new extension product provided that elongation is complete through the end of the restriction fragment." Shapero at 3. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Shapero also discloses this element for the same reasons as discussed above regarding claim 13.
12. The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.	Shapero discloses one or more universal tail adaptors comprising a first universal tail adaptor and a second universal tail adaptor. *See, e.g.*, Shapero at Abstract, 1, 4, Figure 1. For example, Figure 1 discloses tagging with universal tail adaptors A1 and A2.

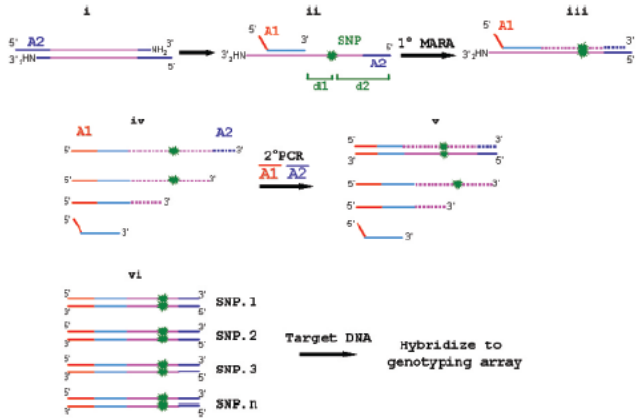
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U.S. Patent No. 11,519,035	
Claim	**Prior Art**
	 **Figure 1.** MARA (i) Human genomic DNA (pink) is digested to completion with a restriction enzyme and ligated to adapters (blue). The 3′ amino block helps to prevent background genomic DNA amplification. (ii) A MARA forward primer (containing common sequence A1) is shown annealing to the bottom strand of DNA upstream of a SNP (green symbol). (iii) The primary MARA reaction involves multiple cycles of primer annealing and extension, which can lead to a full-length extension product (dashed line) that has copied the adapter sequence (A2) from the genomic DNA template. (iv) At the completion of cycling, the reaction may contain unused primers as well as a mixture of both full-length extension products and truncated extension products that do not contain the copied A2 adapter. An enrichment step was used in the MARA 250-plex reactions following the primary reaction. One cycle of PCR with primer A2 converts full-length amplification products (which contain an A2 primer annealing site) into double-strand DNA, while incomplete products lacking the A2 primer annealing site are not converted into a double-stranded product. Exonuclease I digestion removes single strand products, while double-strand products are resistant to digestion. This step enriches for full-length products generated by the primary reaction, but cannot discriminate between products derived from specific and non-specific primer–target DNA annealing (v) The first cycle of secondary PCR allows primer A2 to convert full-length extension products (which contain an A2 primer annealing site) into double-stranded DNA, while partial extension products lacking the A2 primer annealing site are not converted into a double-stranded product. (vi) Subsequent cycles of secondary PCR with universal A1 and A2 primers amplify all full-length products derived from the first round locus-specific reaction. Target DNA is prepared for hybridization by enzymatic fragmentation with DNase I and end-labeling with TdT and biotin-ddATP.

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Claim	**Prior Art**
	As another example, Shapero discloses tagging with two universal adapters: "The approach uses a single primer per SNP in conjunction with restriction enzyme digested, adapter-ligated human genomic DNA. Each primer is composed of common sequence at the 50 end followed by locus-specific sequence at the 30 end. Following a primary reaction in which locus-specific products are generated, a secondary universal amplification is carried out using a generic primer pair corresponding to the oligonucleotide and genomic DNA adapter sequences." Shapero at Abstract.
13. The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.	Shapero discloses tagging wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor. *See, e.g.,* Shapero at 3, 4, Figure 1. For example, Figure 1 discloses, "A MARA forward primer (containing common sequence A1) is shown annealing to the bottom strand of DNA upstream of a SNP (green symbol).

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Claim	**Prior Art**
	 **Figure 1.** MARA (i) Human genomic DNA (pink) is digested to completion with a restriction enzyme and ligated to adapters (blue). The 3′ amino block helps to prevent background genomic DNA amplification. (ii) A MARA forward primer (containing common sequence A1) is shown annealing to the bottom strand of DNA upstream of a SNP (green symbol). (iii) The primary MARA reaction involves multiple cycles of primer annealing and extension, which can lead to a full-length extension product (dashed line) that has copied the adapter sequence (A2) from the genomic DNA template. (iv) At the completion of cycling, the reaction may contain unused primers as well as a mixture of both full-length extension products and truncated extension products that do not contain the copied A2 adapter. An enrichment step was used in the MARA 250-plex reactions following the primary reaction. One cycle of PCR with primer A2 converts full-length amplification products (which contain an A2 primer annealing site) into double-strand DNA, while incomplete products lacking the A2 primer annealing site are not converted into a double-stranded product. Exonuclease I digestion removes single strand products, while double-strand products are resistant to digestion. This step enriches for full-length products generated by the primary reaction, but cannot discriminate between products derived from specific and non-specific primer–target DNA annealing (v) The first cycle of secondary PCR allows primer A2 to convert full-length extension products (which contain an A2 primer annealing site) into double-stranded DNA, while partial extension products lacking the A2 primer annealing site are not converted into a double-stranded product. (vi) Subsequent cycles of secondary PCR with universal A1 and A2 primers amplify all full-length products derived from the first round locus-specific reaction. Target DNA is prepared for hybridization by enzymatic fragmentation with DNase I and end-labeling with TdT and biotin-ddATP.

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Claim	**Prior Art**
	As another example, Shapero discloses, "A secondary amplification step is then carried out using an aliquot of the primary MARA rection as template. Universal primers are used in the secondary amplification that anneal to common sites present in all full-length amplification products and thus secondary amplification is independent of the sequence of the individual locus-specific amplicons generated in the primary MARA reactions." Shapero at 3. As another example, Shapero discloses, "Following a primary reaction in which locus-specific products are generated, a secondary universal amplification is carried out using a generic primer pair corresponding to the oligonucleotide and genomic DNA adapter sequences." Shapero at Abstract. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Shapero also discloses this element for the same reasons as discussed above regarding claim 2.

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EXHIBIT 1-K – FINAL INVALIDITY CLAIM CHART FOR U.S. PATENT NO. 11,519,035

Reference:
Parallel Preparation of Targeted Resequencing Libraries from 480 Genomics Regions Using Multiplex PCR on the Access Array™ System ("Kaper")

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

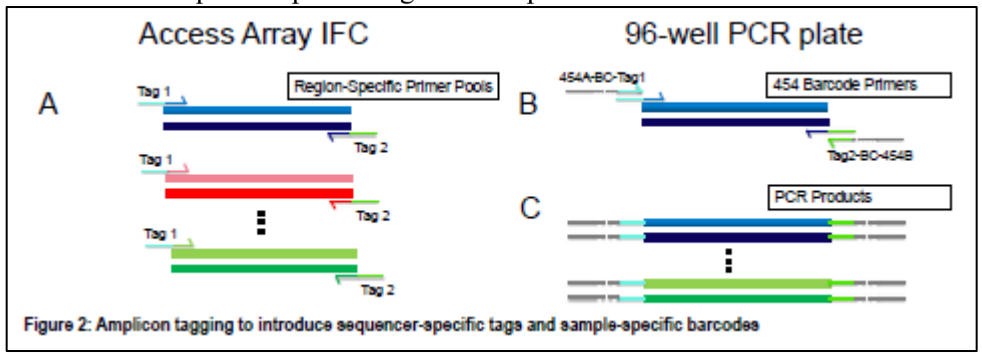
To the extent Natera asserts that Kaper does not disclose or teach one or more limitations of Claims 1, 2, 12, or 13, performing the methods as claimed in those claims would have been obvious to a person of ordinary skill in the art at the time of the alleged invention considering Kaper in combination with the knowledge of a POSA and/or one or more of the following references:

- International Patent Publication No. 2011/142836 ("Pieprzyk")
- International Patent Publication No. 2011/85491 ("Hansen")
- International Patent Publication No. 2007/018601("Makarov")
- Shapero et al., *MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density DNA oligonucleotide arrays*, 32 NUCLEIC ACIDS RES. e101 (2004) ("Shapero")
- International Patent Publication WO 2010/126614 A2 ("Porreca")
- U.S. Patent Application Publication US 2004/0110153 A1 ("Dong")
- International Patent Publication WO 2007/140417 ("Hoyal")
- U.S. Patent No. 6,420,117 ("Wessler")

A POSA would have been motivated to combine Kaper with one or more of the foregoing references because Kaper expressly teaches using routine and conventional techniques known in the art to analyze fragmented sample DNA efficiently. For instance, a POSA would be motivated to utilize the methods of Kaper for cell free tumor DNA assays because Kaper expressly emphasizes the utility of investigating "genetic variants or somatic mutations underlying tumorigenesis"and it was well known at the time that cancer DNA can take the form of cell free DNA. Further, Kaper provides an express invitation to perform its methods on samples with low amounts of DNA by disclosing that the Access Array system can be used with "only 50 ng input DNA per sample." A POSA would accordingly have understood that the methods of Kaper could be used with other types of DNA and was particularly suited for samples with low amounts of DNA such as cell free tumor DNA. As another example, a POSA would be motivated to utilize ligation to add one or more universal tail adaptors at least because Kaper expressly teaches the use of universal tail adaptors, and it was well known in the art at the time of the invention that such adaptors could be added through ligation.

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Further, a POSA would reasonably expect to succeed in combining Kaper with one or more of the foregoing references for the purposes described above because the techniques of isolating, tagging, amplifying, and sequencing cell free DNA as well as using ligation to tag cell free DNA with universal tail adaptors were all well known in the art at the time of the invention.

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Claim	**Prior Art**
1[pre]. A method for amplifying and sequencing DNA, comprising:	To the extent the preamble is construed to be limiting, Kaper discloses a method for amplifying and sequencing DNA. For example, Kaper discloses a "novel microfluidic platform," "the Access Array™ system, [that] combines 48 samples with 48 primer sets resulting in 2,304 simultaneously occurring PCR amplifications requiring as little as 50ng DNA per sample. PCR products generated on the Access Array system can be used for sequencing on all next-generation sequencing platforms, including 454 GS FLX and Illumina GAII." As another example, Figure 2 discloses "an amplicon-tagging method (Figure 2) that enables direct attachment of sample-specific barcodes and sequence-specific tags to PCR products."  Figure 2: Amplicon tagging to introduce sequencer-specific tags and sample-specific barcodes As another example, Kaper discloses an "initial proof of principle experiment" wherein "48 Primer sets covering the EGFR and MET exons, were designed and validated by PCR ([Figure 3] A). The 48 regions were amplified from a HapMap control sample (normalized to 50ng/ul) using 5 pools of 9 to 10 primer pairs each, following the strategy outlined above (Figure 2). The remaining 43 assay inlets on the Access Array IFC were filled with buffer. A comparison was made to singleplex, 2-step PCR and our singleplex, 1 step amplicon tagging strategy. Final PCR products ([Figure 3] C) were analyzed on an Agilent BioAnalyzer 2100 and amplicon representation was assessed

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Claim	**Prior Art**
	with a nested Q-PCR. The PCR products were subsequently run on a 454 FLX sequencer. Representation of each PCR product was even, with 90% represented within 2-fold of average coverage ([Figure 3] D)."

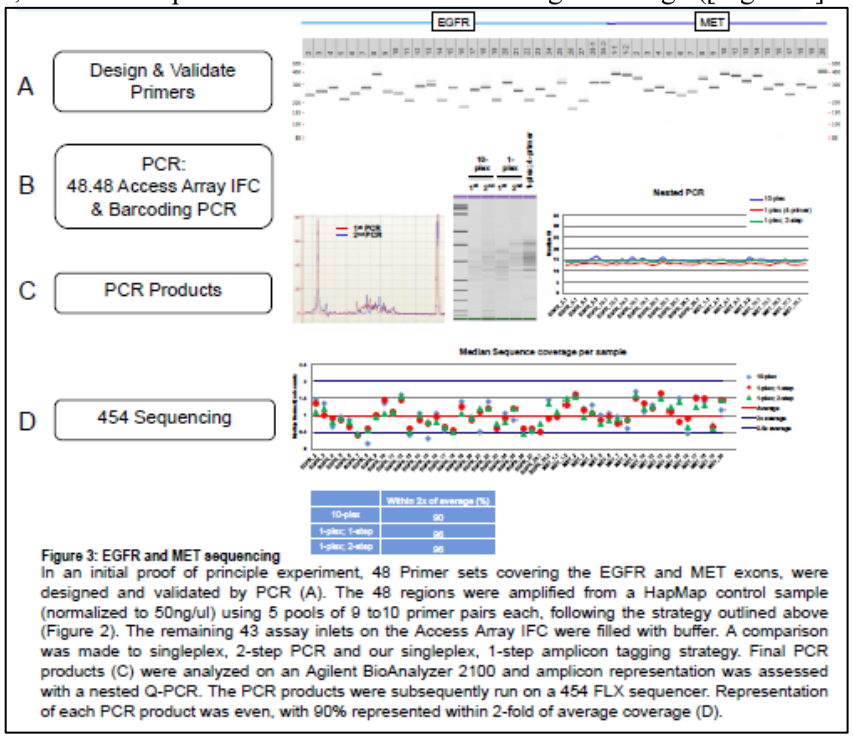
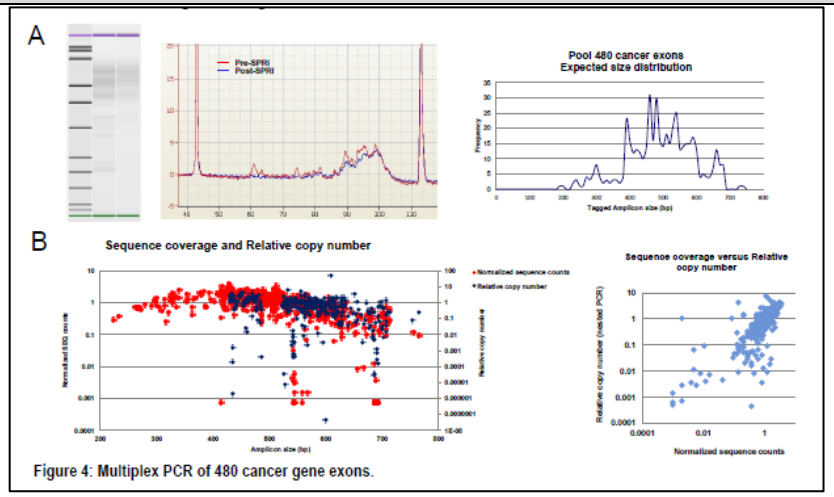


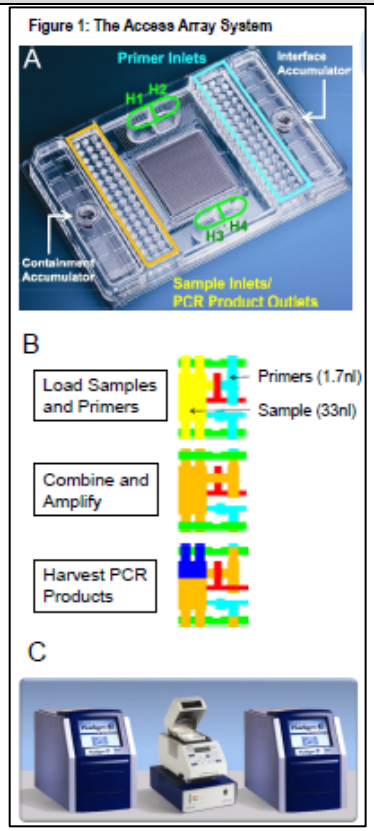
Figure 3: EGFR and MET sequencing
In an initial proof of principle experiment, 48 Primer sets covering the EGFR and MET exons, were designed and validated by PCR (A). The 48 regions were amplified from a HapMap control sample (normalized to 50ng/ul) using 5 pools of 9 to10 primer pairs each, following the strategy outlined above (Figure 2). The remaining 43 assay inlets on the Access Array IFC were filled with buffer. A comparison was made to singleplex, 2-step PCR and our singleplex, 1-step amplicon tagging strategy. Final PCR products (C) were analyzed on an Agilent BioAnalyzer 2100 and amplicon representation was assessed with a nested Q-PCR. The PCR products were subsequently run on a 454 FLX sequencer. Representation of each PCR product was even, with 90% represented within 2-fold of average coverage (D).

As another example, Kaper discloses an experiment that "multiplexed amplifications of a set of commonly mutated cancer gene exons across 48 genomic DNA samples." Kaper further discloses, "[p]rimer pairs were grouped in sets of 10 based on their expected PCR product sizes, resulting in 48 primer pools of 10 primer pairs each. Multiplex PCR was carried out following the strategy outlined in Figure 2. The resulting products are 48 uniquely barcoded PCR product pools, each comprising 480 amplicons derived from one sample, that are ready for sequencing." Then, the "[h]arvested PCR product pools were analyzed on an Agilent Bio Analyzer 2100 (Figure 4A) and PCR product representation was assessed with a nested Q-PCR for a subset of amplicons (Figure 4B). The PCR products were subsequently run on a 454 FLX sequencer."

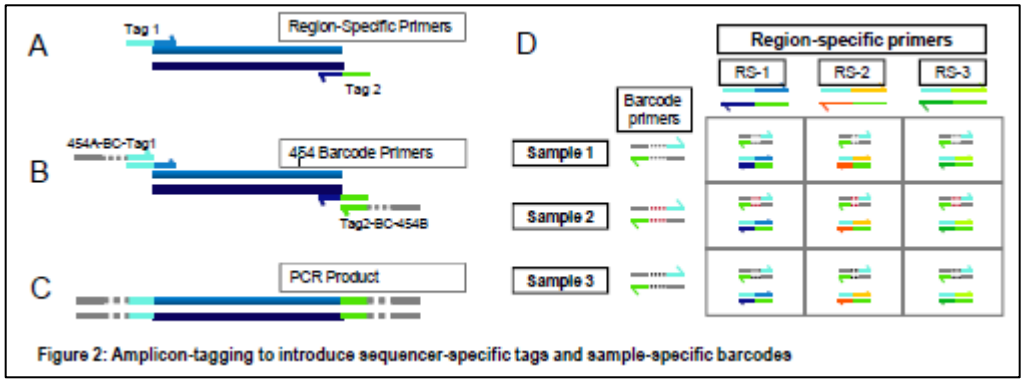
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Claim	**Prior Art**
	 Figure 4: Multiplex PCR of 480 cancer gene exons. As another example, Kaper discloses "a simple, easy-to-use method for PCR-based target enrichment from multiple samples in parallel using only 50ng human genomic DNA per sample for 480 PCR reactions." Kaper discloses developing "an amplicon tagging protocol that enables direct labeling of PCR products with sample-specific barcodes and sequencer-specific tags." Kaper discloses that "[d]ata from sequencing experiments demonstrates that amplicon representation within and between samples is highly uniform." As another example, Figure 1 discloses a "microfluidic chip that systematically combines 48 sample inputs with 48 primer inputs," then undergoes thermal cycling to "amplify regions of interest from the samples."

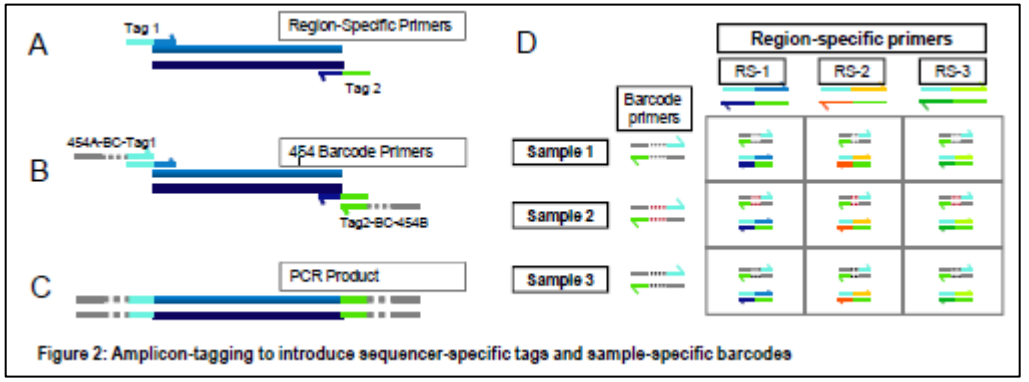
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Claim	**Prior Art**
	
1[a][i]. tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,	Under Natera's apparent construction, Kaper discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. For example, Kaper discloses an "amplicon tagging method (Figure 2) that enables direct attachment of sample - specific barcodes and sequencer-specific tags to PCR products generated in the Access Array. Region-specific primers are tagged at the 5' end with universal sequence tags (Figure 2A.). 48 pools of up to 10 Region-specific primer pairs are pooled and loaded into the assay inlets of the Access Array IFC. The 48.48 Access Array IFC then combines the region-specific primer pools with the samples in each reaction. After harvesting the PCR product

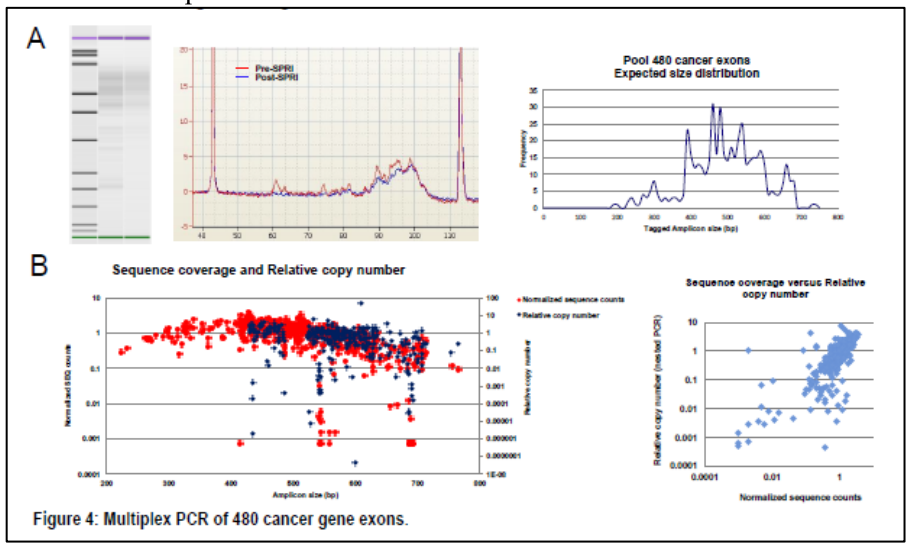
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	pools form the 48.48 Access Array IFC, a second PCR is carried out on a 96-well plate with barcode primers comprising 454 sequence tags, a barcode sequence, and the universal tags. (Figure 2B). The final result is a fully tagged, barcoded PCR product (Figure 2C)."  Figure 2: Amplicon-tagging to introduce sequencer-specific tags and sample-specific barcodes
1[a][ii]. wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;	In view of the knowledge of a POSA at the time of the claimed invention, it would have been obvious to perform the claimed method on cell-free DNA isolated from a blood sample collected from a subject who is not a pregnant woman. For instance, a POSA would be motivated to utilize the methods of Kaper for cell free tumor DNA assays because Kaper expressly emphasizes the utility of investigating "genetic variants or somatic mutations underlying tumorigenesis" and it was well known at the time that cancer DNA can take the form of cell free DNA. Further, Kaper provides an express invitation to perform its methods on samples with low amounts of DNA by disclosing that the Access Array system can be used with "only 50 ng input DNA per sample."
1[b][i]. amplifying the tagged products one or more times to generate final amplification products,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Kaper discloses amplifying the tagged products one or more times to generate final amplification products. Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling.

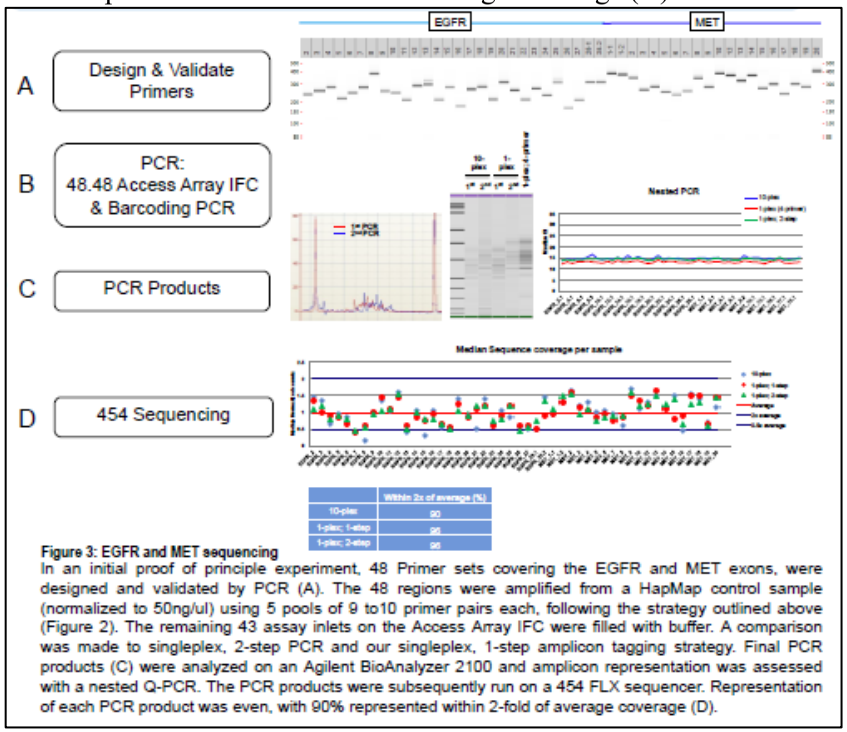
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	See, *e.g.,* Kaper at Figure 2A, Figure 2B, Figure 2C, Figure 3A, Figure 4A, Figure 4B For example, Kaper discloses amplifying tagged products one or more times to generate final amplification products. For example, Kaper discloses an "amplicon tagging method (Figure 2) that enables direct attachment of sample-specific barcodes and sequencer-specific tags to PCR products generated in the Access Array. Region-specific primers are tagged at the 5' end with universal sequence tags (Figure 2A.). 48 pools of up to 10 Region-specific primer pairs are pooled and loaded into the assay inlets of the Access Array IFC. The 48.48 Access Array IFC then combines the region-specific primer pools with the samples in each reaction. After harvesting the PCR product pools form the 48.48 Access Array IFC, a second PCR is carried out on a 96-well plate with barcode primers comprising 454 sequence tags, a barcode sequence, and the universal tags. (Figure 2B). The final result is a fully tagged, barcoded PCR product (Figure 2C)."  Figure 2: Amplicon-tagging to introduce sequencer-specific tags and sample-specific barcodes As another example, Kaper discloses an experiment that "multiplexed amplifications of a set of commonly mutated cancer gene exons across 48 genomic DNA samples." Kaper further discloses, "[p]rimer pairs were grouped in sets of 10 based on their expected PCR product sizes, resulting in 48 primer pools of 10 primer pairs each. Multiplex PCR was carried out following the strategy outlined in Figure 2. The resulting products are 48 uniquely barcoded PCR product pools, each comprising 480 amplicons derived from one sample, that are ready for sequencing." Then, the "[h]arvested PCR product pools were analyzed on an Agilent Bio Analyzer 2100 (Figure 4A) and PCR product

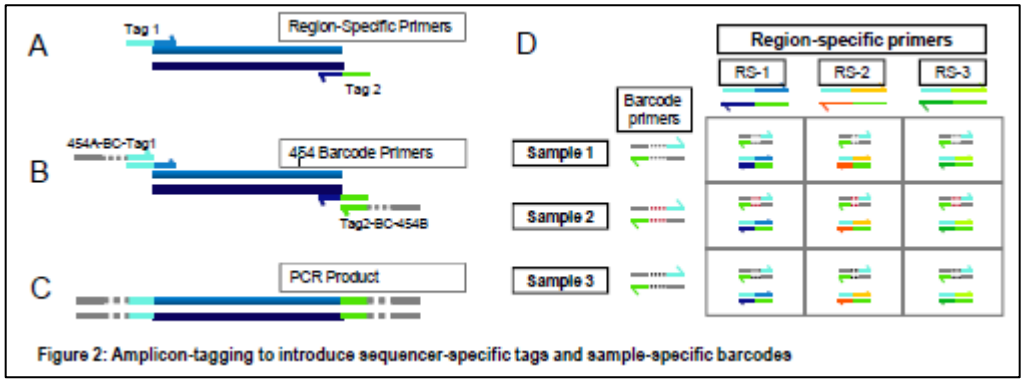
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	representation was assessed with a nested Q-PCR for a subset of amplicons (Figure 4B). The PCR products were subsequently run on a 454 FLX sequencer."  Figure 4: Multiplex PCR of 480 cancer gene exons. As another example, Kaper discloses that "when used in combination with primers designed for Amplicon tagging, the Access Array products sequencer-ready amplicon libraries that can be introduced to emulsion PCF for 454 sequencing." As another example, Kaper discloses that, through the Access Array, "we have developed an amplicon-tagging protocol that enables direct labeling of PCR products with sample-specific barcodes and sequencer-specific tags." As another example, Kaper discloses an "initial proof of principle experiment" wherein "48 Primer sets covering the EGFR and MET exons, were designed and validated by PCR ([Figure 3] A). The 48 regions were amplified from a HapMap control sample (normalized to 50ng/ul) using 5 pools of 9 to 10 primer pairs each, following the strategy outlined above (Figure 2). The remaining 43 assay inlets on the Access Array IFC were filled with buffer. A comparison was made to singleplex, 2-step PCR and our singleplex, 1 step amplicon tagging strategy. Final PCR products (C) were analyzed on an Agilent BioAnalyzer 2100 and amplicon representation was assessed with a

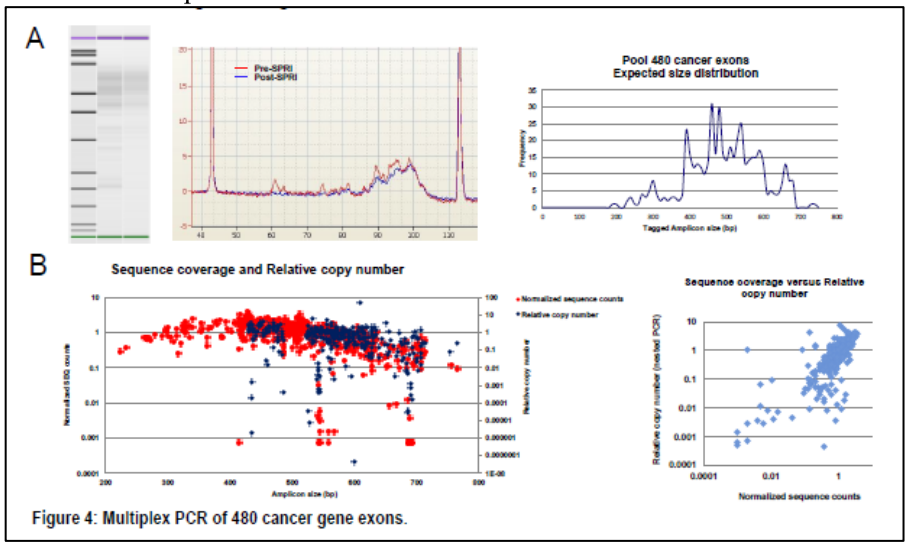
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	nested Q-PCR. The PCR products were subsequently run on a 454 FLX sequencer. Representation of each PCR product was even, with 90% represented within 2-fold of average coverage (D)."  **Figure 3: EGFR and MET sequencing** In an initial proof of principle experiment, 48 Primer sets covering the EGFR and MET exons, were designed and validated by PCR (A). The 48 regions were amplified from a HapMap control sample (normalized to 50ng/ul) using 5 pools of 9 to10 primer pairs each, following the strategy outlined above (Figure 2). The remaining 43 assay inlets on the Access Array IFC were filled with buffer. A comparison was made to singleplex, 2-step PCR and our singleplex, 1-step amplicon tagging strategy. Final PCR products (C) were analyzed on an Agilent BioAnalyzer 2100 and amplicon representation was assessed with a nested Q-PCR. The PCR products were subsequently run on a 454 FLX sequencer. Representation of each PCR product was even, with 90% represented within 2-fold of average coverage (D). Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Kaper also discloses this element for the same reasons.
1[b][ii]. wherein one of the amplification steps	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent

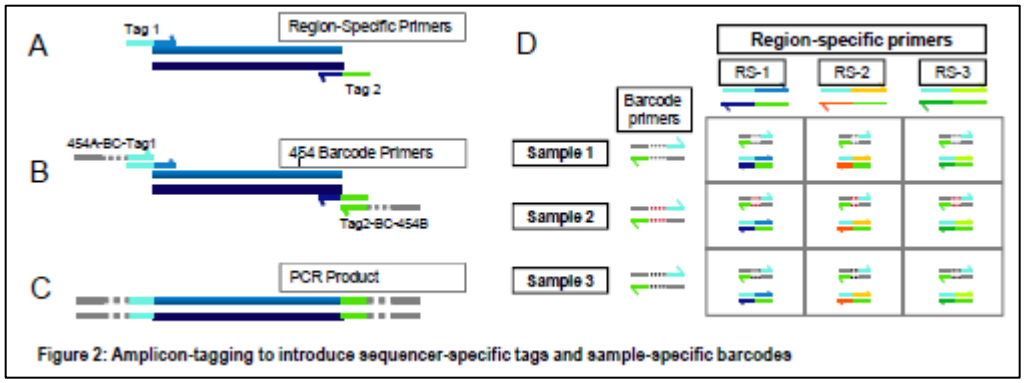
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comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume,	interpretation of the Court's adopted construction, Kaper discloses amplifying wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume. Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. For example, Kaper discloses an "amplicon tagging method (Figure 2) that enables direct attachment of sample-specific barcodes and sequencer-specific tags to PCR products generated in the Access Array. Region-specific primers are tagged at the 5' end with universal sequence tags (Figure 2A.). 48 pools of up to 10 Region-specific primer pairs are pooled and loaded into the assay inlets of the Access Array IFC. The 48.48 Access Array IFC then combines the region-specific primer pools with the samples in each reaction. After harvesting the PCR product pools form the 48.48 Access Array IFC, a second PCR is carried out on a 96-well plate with barcode primers comprising 454 sequence tags, a barcode sequence, and the universal tags. (Figure 2B). The final result is a fully tagged, barcoded PCR product (Figure 2C)."  Figure 2: Amplicon-tagging to introduce sequencer-specific tags and sample-specific barcodes

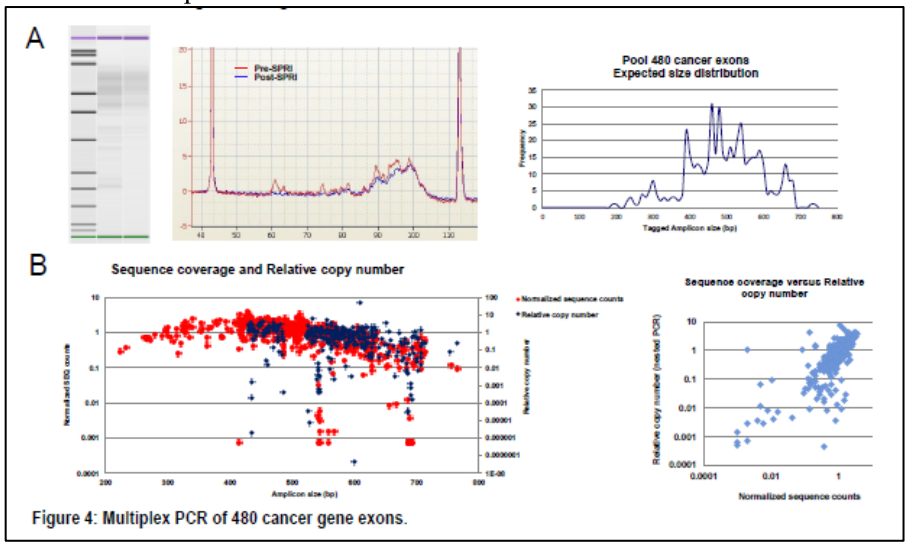
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	Kaper further discloses targeted amplification of a plurality of SNP loci in a single reaction volume. For example, Kaper discloses that the Access Array system "combines 48 samples with 48 primer sets resulting in 2,304 simultaneously occurring PCR amplifications requiring as little as 50ng DNA per sample." As another example, Kaper discloses an experiment that "multiplexed amplifications of a set of commonly mutated cancer gene exons across 48 genomic DNA samples." Kaper further discloses, "[p]rimer pairs were grouped in sets of 10 based on their expected PCR product sizes, resulting in 48 primer pools of 10 primer pairs each. Multiplex PCR was carried out following the strategy outlined in Figure 2. The resulting products are 48 uniquely barcoded PCR product pools, each comprising 480 amplicons derived from one sample, that are ready for sequencing." Then, the "[h]arvested PCR product pools were analyzed on an Agilent Bio Analyzer 2100 (Figure 4A) and PCR product representation was assessed with a nested Q-PCR for a subset of amplicons (Figure 4B). The PCR products were subsequently run on a 454 FLX sequencer."  Figure 4: Multiplex PCR of 480 cancer gene exons. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera

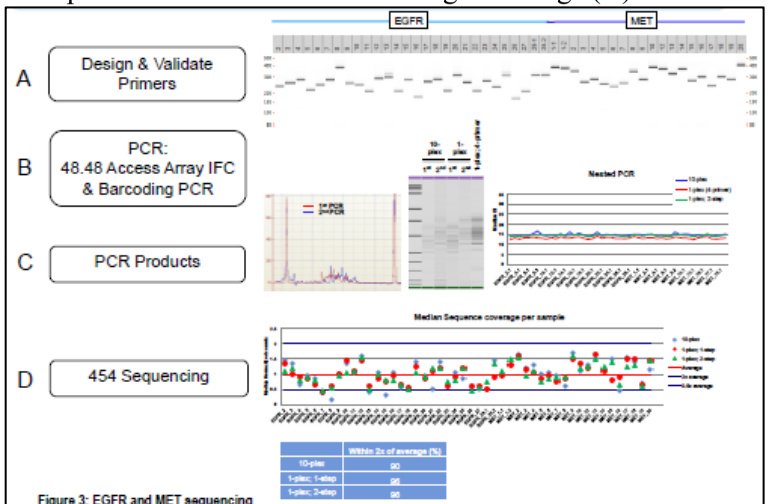
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	argues this element is infringed under the doctrine of equivalents, Kaper also discloses this element for the same reasons.
1[b][iii]. wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and	Kaper discloses amplifying wherein one of the amplifying steps introduces a barcode and one or more sequencing tags. For example, Kaper discloses an "amplicon tagging method (Figure 2) that enables direct attachment of sample-specific barcodes and sequencer-specific tags to PCR products generated in the Access Array. Region-specific primers are tagged at the 5' end with universal sequence tags (Figure 2A.). 48 pools of up to 10 Region-specific primer pairs are pooled and loaded into the assay inlets of the Access Array IFC. The 48.48 Access Array IFC then combines the region-specific primer pools with the samples in each reaction. After harvesting the PCR product pools form the 48.48 Access Array IFC, a second PCR is carried out on a 96-well plate with barcode primers comprising 454 sequence tags, a barcode sequence, and the universal tags. (Figure 2B). The final result is a fully tagged, barcoded PCR product (Figure 2C)."  Figure 2: Amplicon-tagging to introduce sequencer-specific tags and sample-specific barcodes As another example, Kaper discloses "an amplicon-tagging protocol that enables direct labeling of PCR products with sample-specific barcodes and sequencer-specific tags." As another example, Kaper discloses an experiment that "multiplexed amplifications of a set of commonly mutated cancer gene exons across 48 genomic DNA samples." Kaper further discloses, "[p]rimer pairs were grouped in sets of 10 based on their expected PCR product sizes, resulting in 48 primer pools of 10 primer pairs each. Multiplex

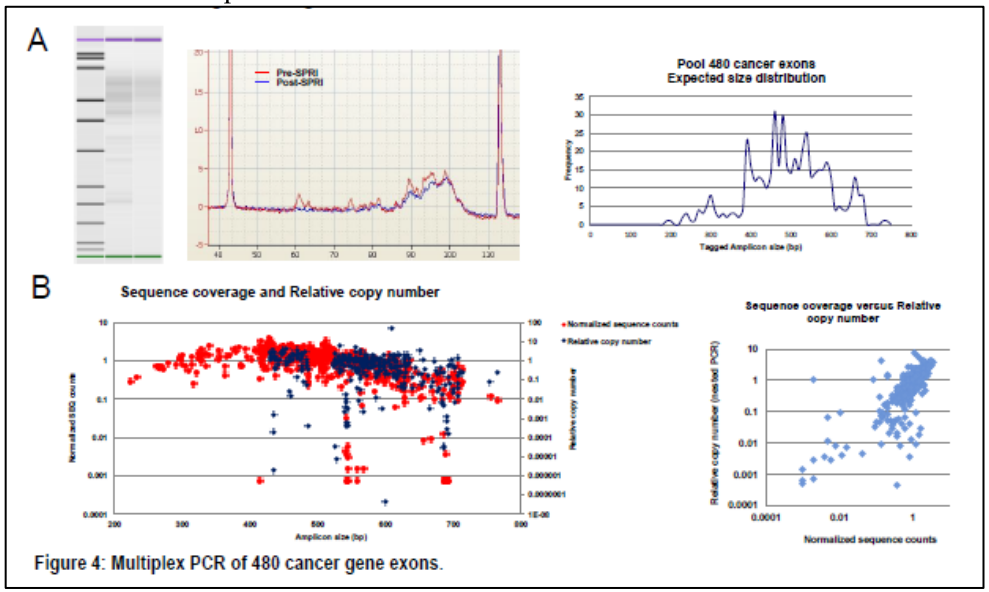
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	PCR was carried out following the strategy outlined in Figure 2. The resulting products are 48 uniquely barcoded PCR product pools, each comprising 480 amplicons derived from one sample, that are ready for sequencing." Then, the "[h]arvested PCR product pools were analyzed on an Agilent Bio Analyzer 2100 (Figure 4A) and PCR product representation was assessed with a nested Q-PCR for a subset of amplicons (Figure 4B). The PCR products were subsequently run on a 454 FLX sequencer."  Figure 4: Multiplex PCR of 480 cancer gene exons. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Kaper also discloses this element for the same reasons.

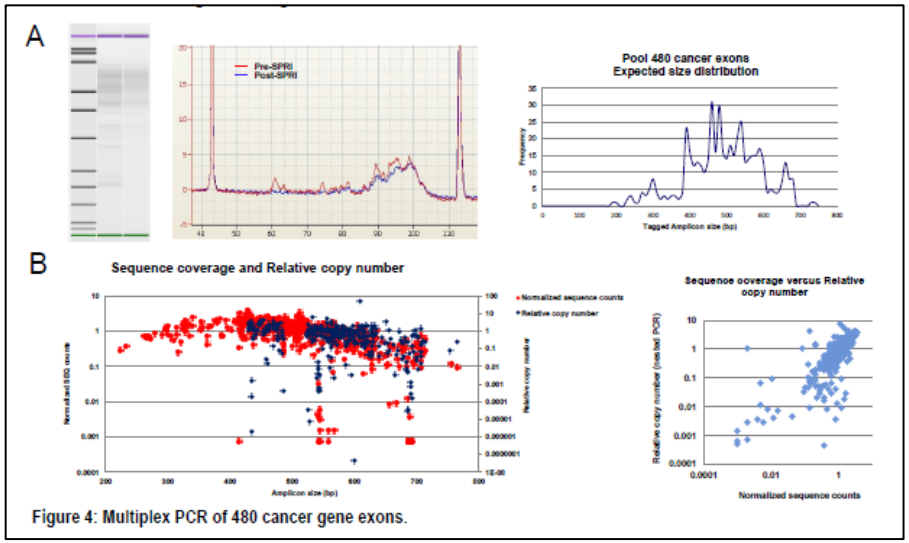
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1[c][i]. sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products,	Kaper discloses sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products. For example, Kaper discloses an "initial proof of principle experiment" wherein "48 Primer sets covering the EGFR and MET exons, were designed and validated by PCR ([Figure 3] A). The 48 regions were amplified from a HapMap control sample (normalized to 50ng/ul) using 5 pools of 9 to 10 primer pairs each, following the strategy outlined above (Figure 2). The remaining 43 assay inlets on the Access Array IFC were filled with buffer. A comparison was made to singleplex, 2-step PCR and our singleplex, 1 step amplicon tagging strategy. Final PCR products (C) were analyzed on an Agilent BioAnalyzer 2100 and amplicon representation was assessed with a nested Q-PCR. The PCR products were subsequently run on a 454 FLX sequencer. Representation of each PCR product was even, with 90% represented within 2-fold of average coverage (D)."  **Figure 3: EGFR and MET sequencing** In an initial proof of principle experiment, 48 Primer sets covering the EGFR and MET exons, were designed and validated by PCR (A). The 48 regions were amplified from a HapMap control sample (normalized to 50ng/ul) using 5 pools of 9 to10 primer pairs each, following the strategy outlined above (Figure 2). The remaining 43 assay inlets on the Access Array IFC were filled with buffer. A comparison was made to singleplex, 2-step PCR and our singleplex, 1-step amplicon tagging strategy. Final PCR products (C) were analyzed on an Agilent BioAnalyzer 2100 and amplicon representation was assessed with a nested Q-PCR. The PCR products were subsequently run on a 454 FLX sequencer. Representation of each PCR product was even, with 90% represented within 2-fold of average coverage (D). As another example, Kaper discloses an experiment that "multiplexed amplifications of a set of commonly mutated cancer gene exons across 48 genomic DNA samples." Kaper further discloses, "[p]rimer pairs were grouped in sets

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	of 10 based on their expected PCR product sizes, resulting in 48 primer pools of 10 primer pairs each. Multiplex PCR was carried out following the strategy outlined in Figure 2. The resulting products are 48 uniquely barcoded PCR product pools, each comprising 480 amplicons derived from one sample, that are ready for sequencing." Then, the "[h]arvested PCR product pools were analyzed on an Agilent Bio Analyzer 2100 (Figure 4A) and PCR product representation was assessed with a nested Q-PCR for a subset of amplicons (Figure 4B). The PCR products were subsequently run on a 454 FLX sequencer."  Figure 4: Multiplex PCR of 480 cancer gene exons. As another example, Kaper discloses that "when used in combination with primers designed for Amplicon tagging, the Access Array products sequencer-ready amplicon libraries that can be introduced to emulsion PCF for 454 sequencing."
1[c][ii]. wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.	The Court construed the term "SNP loci…associated with cancer" to mean "a single nucleotide that may differ between the genomes of two members of the same species and is associated with cancer. The usage of the term 'SNP' should not imply any limit on the frequency with which each variant occurs." Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Kaper discloses a plurality of SNP loci comprising 25-2,000 loci associated with cancer. In addition, Natera has failed to supplement its response to

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	NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. For example, Kaper discloses that the Access Array system "is a microfluidic chip that systematically combines 48 samples inputs with 48 primer inputs to make all possible 2304 combinations of samples and primers." Kaper also discloses an experiment that "multiplexed amplifications of a set of commonly mutated cancer gene exons across 48 genomic DNA samples." Kaper further discloses, "[p]rimer pairs were grouped in sets of 10 based on their expected PCR product sizes, resulting in 48 primer pools of 10 primer pairs each. Multiplex PCR was carried out following the strategy outlined in Figure 2. The resulting products are 48 uniquely barcoded PCR product pools, each comprising 480 amplicons derived from one sample, that are ready for sequencing." Then, the "[h]arvested PCR product pools were analyzed on an Agilent Bio Analyzer 2100 (Figure 4A) and PCR product representation was assessed with a nested Q-PCR for a subset of amplicons (Figure 4B). The PCR products were subsequently run on a 454 FLX sequencer."  Figure 4: Multiplex PCR of 480 cancer gene exons.

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	As another example, Kaper discloses that it "demonstrated the potential for multiplexing primer pairs within PCR reactions on the Access Array system" and that "[r]epresentation of each amplicon in the multiplex reaction is similar to that within individual PCR reactions." As another example, Kaper discloses that "[t]o increase coverage and throughput, PCR reactions can be multiplexed within Access Array IFC's generating up to 480 amplicons per sample." As another example, Kaper discloses "a need exists for multiplexed, targeted amplification methods that allow for the analysis of multiple genomic regions in large cohorts" because "it currently remains too expensive to routinely reseqeuence entire human genomes in order to discover genetic variants or somatic mutations underlying tumorigenesis." Under Natera's apparent interpretation of the Court's construction, Kaper's disclosure of "genetic variants or somatic mutations underlying tumorigenesis" would meet the limitation of "a single nucleotide that may differ between the genomes of two members of the same species and is associated with cancer. The usage of the term 'SNP' should not imply any limit on the frequency with which each variant occurs." To the extent Natera contends Kaper does not disclose 25-2,000 loci associated with cancer, it would have been obvious to a POSA at the time of the invention in combination with the knowledge of a POSA. For instance, a POSA would be motivated to utilize the methods of Kaper for cell free tumor DNA assays because Kaper expressly emphasizes the utility of investigating "genetic variants or somatic mutations underlying tumorigenesis" and it was well known at the time that cancer DNA can take the form of cell free DNA. Further, Kaper provides an express invitation to perform its methods on samples with low amounts of DNA by disclosing that the Access Array system can be used with "only 50 ng input DNA per sample." Additionally, a POSA would have understood that a single target region can contain multiple loci. To the extent Natera asserts that any portion of this limitation is not disclosed under Natera's apparent interpretation of the Court's construction, it would have been obvious based on the knowledge of a POSA at the time of the alleged invention to look to the Catalogue of Somatic Mutations In Cancer ("COSMIC") database, which identifies hundreds to thousands of gene variants, including those that can be present in the same gene, to readily disclose a plurality of SNP loci comprising 25-2,000 loci associated with cancer. By way of example, COSMIC identifies 6,038 different variants in the TP53 gene alone. *See*, *e.g.*, https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53.

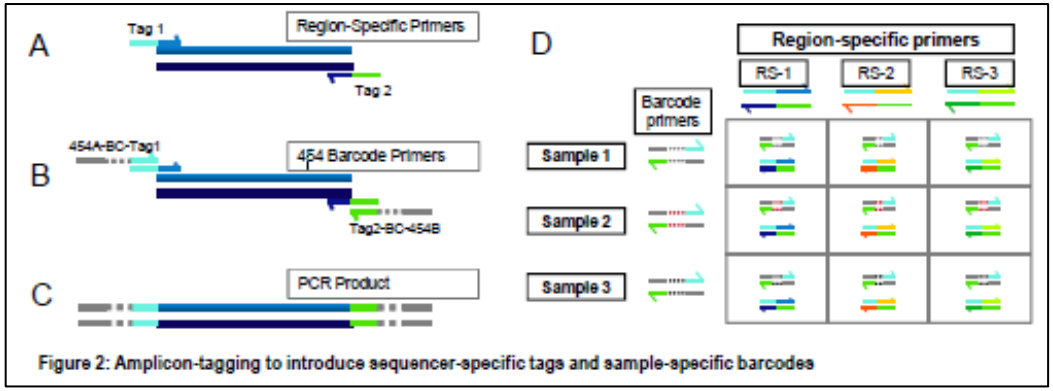
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	In addition, the '035 Patent admits a POSA's "general belief in the art is that multiplexing PCR for sequencing is limited to about 100 assays in the same well." '035 patent at 48:25-29. Thus, the related '035 patent admits that a POSA would have been aware of how to perform multiplex PCR up to 100 assays in a single well, would have understood that multiplex PCR up to 100 assays could readily be performed on cell free DNA, and would have been able to do so as a matter of routine.
2. The method of claim 1, wherein tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA.	Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 27. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Kaper also discloses this element for the same reasons as discussed below regarding claim 13. In addition, to the extent Kaper does not specifically teach tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA, this limitation is taught by Dong (Ex. 1-A), Porreca (Ex. 1-F), Hoyal (Ex. 1-M), and Wessler (Ex. 1-M). A POSA would be motivated to combine Kaper with Dong, Porreca, Hoyal, or Wessler because Kaper expressly teaches using routine and conventional techniques known in the art to analyze fragmented sample DNA efficiently, and the references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end. For example, Dong teaches "enrichment and amplification of sequences from a nucleic acid." Dong at [0001]. As another example, Dong teaches "methods [that] provide for highly parallel analysis of a large number of SNPs, for example, more than 1,000, 5,000, 10,000, or 50,000, that are spaced throughout the genome." *Id*. at [0084]. Dong solves these problems through ligation of common (universal) adaptors to fragmented DNA followed by targeted amplification using primers against the target sequence of interest and the common adaptors. Dong at [0027] and Figure 6. For example, Porreca teaches "predetermined preparative steps and/or nucleic acid sequence analysis techniques [] used in multiplex analyses for a plurality of genetic loci in a plurality of samples". Porreca at Abstract. As another example, Porreca teaches "methods. . . relat[ing] to genotyping a polymorphism of a target nucleic acid." Porreca

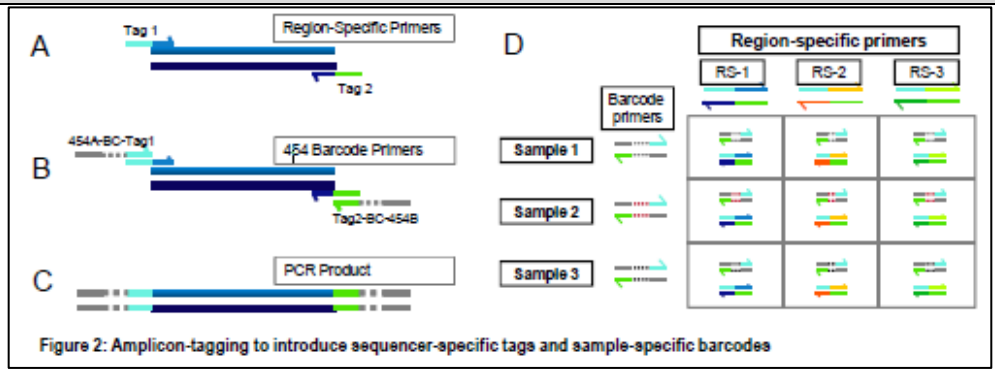
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	at 4:15-20. As another example, Porreca teaches "methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample, or other sample". Porreca at 4:2-7. Porreca solves these problems through ligation of adapters containing PCR primer sites onto the ends of the nucleic acid molecules of interest, followed by PCR-mediated amplification. Porreca at 43:15-20. *See also* Porreca at FIG. 4C. For example, Hoyal teaches "a need for improved extraction methods capable of capturing small nucleic acid molecules"—cell-free DNA—because "the ability to successfully analyze short, fragmented DNA in the presence of more abundant, longer DNA ha[d] remained elusive." Hoyal at 1. As another example, Hoyal teaches "detecting and monitoring extracellular mutant oncogenes or tumor-associated DNA found in the plasma or serum fraction of blood by using DNA extraction with enrichment for mutant DNA," i.e., detecting cell-free tumor DNA. Hoyal at 20. Hoyal solves these problems through adapter-mediated ligation followed by universal pre-amplification before target-specific amplification. Hoyal at Figure 3. For example, Wessler teaches methods for detecting polymorphisms in DNA. Wessler at 3:6-4:41. As another example, Wessler teaches methods for "investigation of genome structure . . . by the use of in vitro methods that detect variation in DNA sequence in the genomes between members of a species" Wessler at 2:26-29. Wessler reaches this goal through Wessler's ligation-mediated preamplification followed by two target-specific rounds of PCR-mediated amplification. Wessler at Figure 1. There is a reasonable expectation of success in combining Kaper with one or more of the foregoing references for the purposes described above because the techniques of isolating, tagging, amplifying, and sequencing cell free DNA as well as using ligation to tag cell free DNA with universal tail adaptors were all well known in the art at the time of the invention.
12. The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.	Kaper discloses one or more universal tail adaptors comprising a first universal tail adaptor and a second universal tail adaptor. For example, Kaper discloses an "amplicon tagging method (Figure 2) that enables direct attachment of sample-specific barcodes and sequencer-specific tags to PCR products generated in the Access Array. Region-specific primers are tagged at the 5' end with universal sequence tags (Figure 2A.). 48 pools of up to 10 Region-specific primer pairs are pooled and loaded into the assay inlets of the Access Array IFC. The 48.48 Access Array IFC then

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Claim	**Prior Art**
	combines the region-specific primer pools with the samples in each reaction. After harvesting the PCR product pools form the 48.48 Access Array IFC, a second PCR is carried out on a 96-well plate with barcode primers comprising 454 sequence tags, a barcode sequence, and the universal tags. (Figure 2B). The final result is a fully tagged, barcoded PCR product (Figure 2C)."  Figure 2: Amplicon-tagging to introduce sequencer-specific tags and sample-specific barcodes As another example, Kaper discloses that through the Access Array system and protocol "[w]e have developed an amplicon-tagging protocol that enables direct labeling of PCR products with sample-specific barcodes and sequencer-specific tags."
13. The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.	Under Natera's apparent construction, Kaper discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. For example, Kaper discloses an "amplicon tagging method (Figure 2) that enables direct attachment of sample-specific barcodes and sequencer-specific tags to PCR products generated in the Access Array. Region-specific primers are tagged at the 5' end with universal sequence tags (Figure 2A.). 48 pools of up to 10 Region-specific primer pairs are pooled and loaded into the assay inlets of the Access Array IFC. The 48.48 Access Array IFC then combines the region-specific primer pools with the samples in each reaction. After harvesting the PCR product pools form the 48.48 Access Array IFC, a second PCR is carried out on a 96-well plate with barcode primers comprising 454 sequence tags, a barcode sequence, and the universal tags. (Figure 2B). The final result is a fully tagged, barcoded PCR product (Figure 2C)."

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Claim	**Prior Art**
	 Figure 2: Amplicon-tagging to introduce sequencer-specific tags and sample-specific barcodes Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Kaper also discloses this element for the same reasons as discussed above regarding claim 2.

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EXHIBIT 1-L – FINAL INVALIDITY CLAIM CHART FOR U.S. PATENT NO. 11,519,035

Reference:
Parallel Production of Barcoded Amplicon Libraries for 454 Sequencing using Individual and Multiplex PCR in Nanoliter Volumes ("May Poster")

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

To the extent Natera asserts that May Poster does not disclose or teach one or more limitations of Claims 1, 2, or 13, performing the methods as claimed in those claims would have been obvious to a person of ordinary skill in the art at the time of the alleged invention considering May Poster in combination with the knowledge of a POSA and/or one or more of the following references:

- International Patent Publication No. 2011/142836 ("Pieprzyk")
- International Patent Publication No. 2007/018601("Makarov")
- Shapero et al., *MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density DNA oligonucleotide arrays*, 32 NUCLEIC ACIDS RES. e101 (2004) ("Shapero")
- International Patent Publication WO 2010/126614 A2 ("Porreca")
- U.S. Patent Application Publication US 2004/0110153 A1 ("Dong")
- International Patent Publication WO 2007/140417 ("Hoyal")
- U.S. Patent No. 6,420,117 ("Wessler")
- Shigematsu et al., *Clinical and Biological Features Associated with Epidermal Growth Factor Receptor Gene Mutations in Lung Cancers*, JOURNAL OF THE NATIONAL CANCER INSTITUTE, Vol. 97. No. 5 (2005) ("Shigematsu")
- Faoro et al., *MET Receptor Tyrosine Kinase*, SANTA MONICA SUPPLEMENT, Volume 4, Issue 11, Supplement 3 (November 2009) ("Faoro")

A POSA would have been motivated to combine May Poster with one or more of the foregoing references because May Poster expressly teaches using routine and conventional techniques known in the art to analyze fragmented sample DNA efficiently. For instance, a POSA would be motivated to utilize the methods of May Poster for cell free tumor DNA assays because May Poster discloses an experiment where 48 primer sets were "designed against regions of interest in prostate cancer" and it was well known at the time that cancer DNA can take the form of cell free DNA. Furthermore, it was well known to a POSA at the time that many cancer samples, including cancer tissue biopsies, are stored under conditions that lead to high levels of DNA fragmentation (*e.g.,* storage using formalin-fixed paraffin-embedding (FFPE)), and that methods that

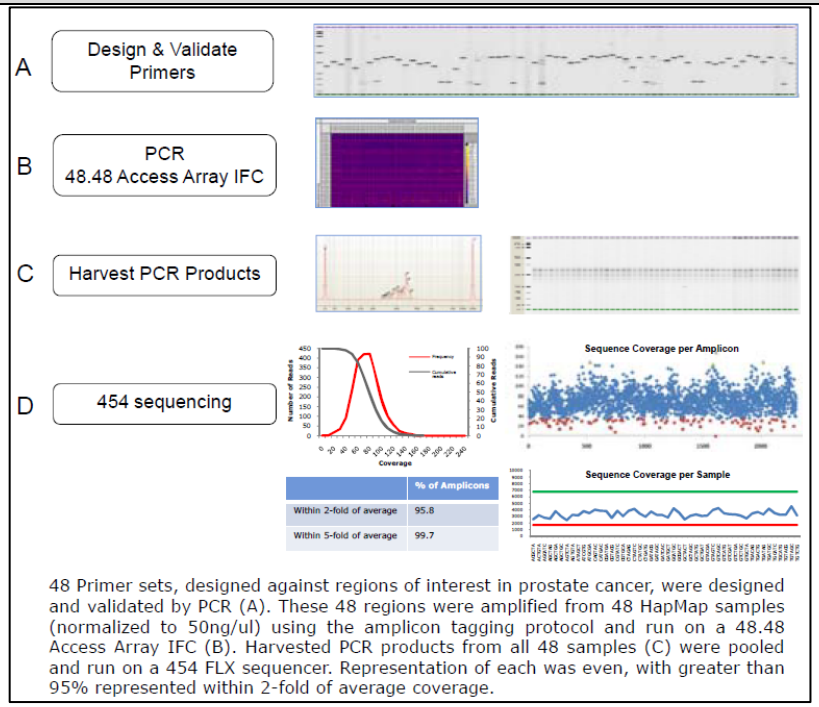
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work on samples with high levels of DNA fragmentation such as those disclosed in May Poster would work with cell free tumor DNA. Further still, May Poster provides an express invitation to perform its methods on samples with low amounts of DNA by disclosing that the Access Array system can be used with "only 50 ng input DNA per sample." A POSA would accordingly have understood that the methods of May Poster could be used with other types of DNA and was particularly suited for samples with low amounts of DNA such as cell free tumor DNA. As another example, a POSA would be motivated to utilize ligation to add one or more universal tail adaptors at least because May Poster expressly teaches the use of universal tail adaptors, and it was well known in the art at the time of the invention that such adaptors could be added through ligation.

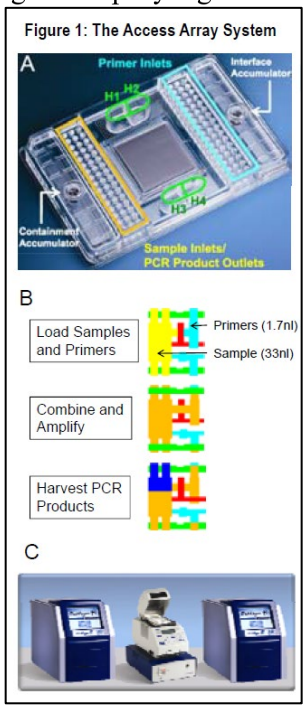
Further, a POSA would reasonably expect to succeed in combining May Poster with one or more of the foregoing references for the purposes described above because the techniques of isolating, tagging, amplifying, and sequencing cell free DNA as well as using ligation to tag cell free DNA with universal tail adaptors were all well known in the art at the time of the invention.

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Claim	**Prior Art**
1[pre]. A method for amplifying and sequencing DNA, comprising:	To the extent the preamble is construed to be limiting, May Poster discloses a method for amplifying and sequencing DNA. For example, May Poster discloses methods "to address the problems associated with scaling up PCR to the throughput required for second-generation sequencers. The Access Array system combines 48 samples systematically with 48 primer pairs to assemble 2304 PCR reactions using only 50ng input DNA per sample." As another example, May Poster discloses an experiment "designed against regions of interest in prostate cancer." That experiment utilized 48 primer sets and "[t]hese 48 regions were amplified from 48 HapMap samples (normalized to 50ng/ul) using the amplicon tagging protocol and run on a 48.48 Access Array IFC ([Figure 3]B). [Then] [h]arvested PCR products from all 48 samples ([Figure 3]C) were pooled and run on a 454 FLX sequencer."

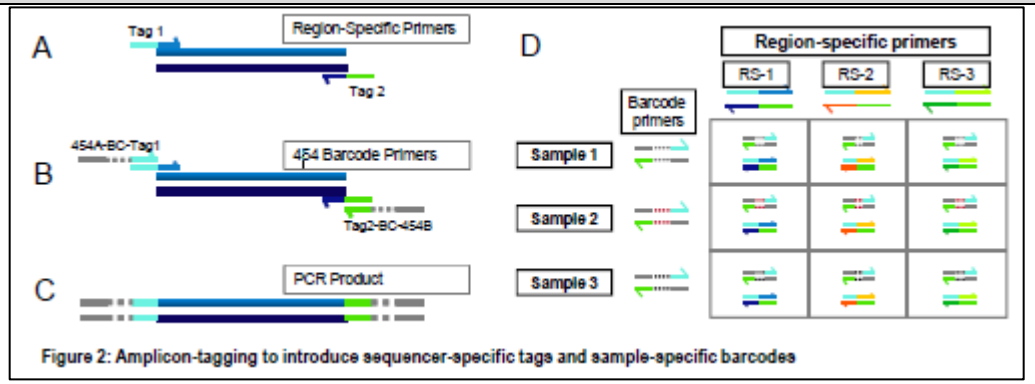
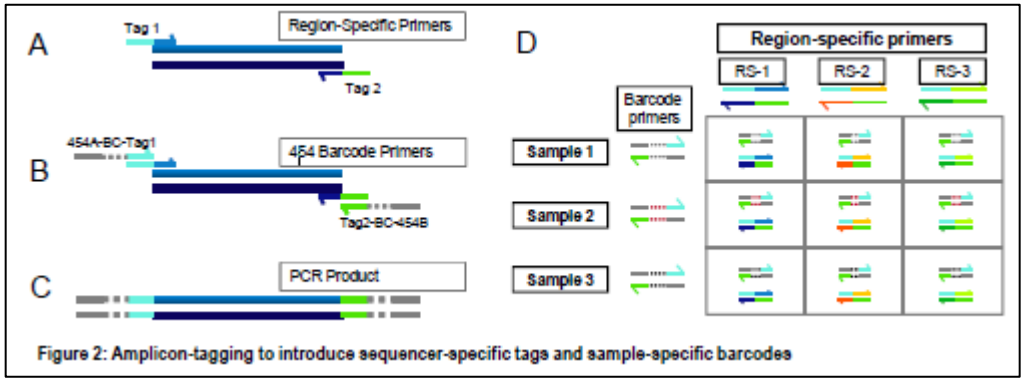
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U.S. Patent No. 11,519,035	
Claim	**Prior Art**
	 48 Primer sets, designed against regions of interest in prostate cancer, were designed and validated by PCR (A). These 48 regions were amplified from 48 HapMap samples (normalized to 50ng/ul) using the amplicon tagging protocol and run on a 48.48 Access Array IFC (B). Harvested PCR products from all 48 samples (C) were pooled and run on a 454 FLX sequencer. Representation of each was even, with greater than 95% represented within 2-fold of average coverage. As another example, May Poster discloses an experiment wherein "[m]ultiplex PCR was run in a 2-step reaction" and step 1 "involved amplification of the region-specific multiplex tools. Then, following step 2, the "[s]amples were sequenced directly on a 454 FLX Sequencer using Titanium Amplicon chemistry with no additional purification." As another example, May Poster discloses "a simple, easy-to-use method for PCR-based target enrichment from multiple samples in parallel using only 50ng human genomic DNA per sample for 48 PCR reactions." May Poster discloses developing "an amplicon-tagging protocol that enables direct labeling of PCR products with sample-specific barcodes and sequencer-specific tags." May Poster discloses that "[d]ata from sequencing experiments demonstrates that amplicon representation within and between samples is highly uniform."

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Claim	**Prior Art**
	As another example, Figure 1 discloses a "microfluidic chip that systematically combines 48 sample inputs with 48 primer inputs," then undergoes thermal cycling to "amplify regions of interest from the sample."  As another example, Figure 2 discloses "an amplicon-tagging method (Figure 2) that enables direct attachment of sample-specific barcodes and sequence-specific tags to PCR products."

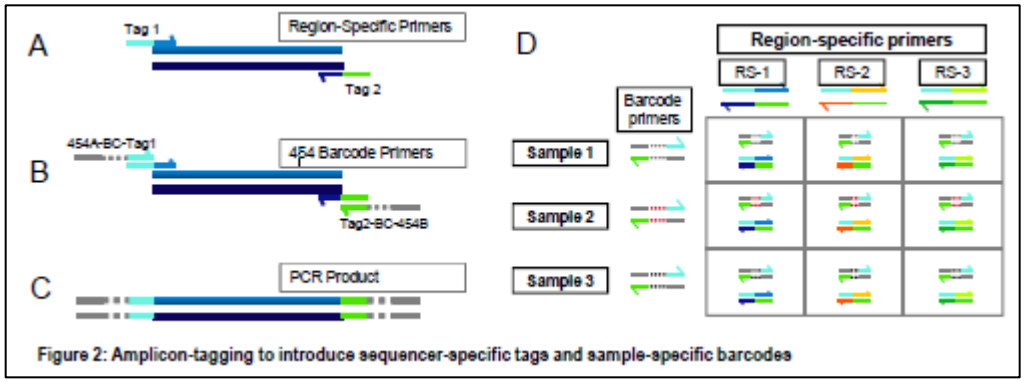
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U.S. Patent No. 11,519,035	
Claim	**Prior Art**
	 Figure 2: Amplicon-tagging to introduce sequencer-specific tags and sample-specific barcodes
1[a][i]. tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,	Under Natera's apparent construction, May Poster discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. For example, May Poster discloses an "amplicon tagging method (Figure 2) that enables direct attachment of sample -specific barcodes and sequencer-specific tags to PCR products generated in the Access Array. Region-specific primers are tagged at the 5' end with universal sequence tags (Figure 2A.). Barcode primers comprise 454 sequence tags, a barcode sequence, and the universal tags (Figure 2B). Barcode primers are added to the samples before loading on to the Access Array IFC. The Access Array IFC combines the region-specific primers, samples and barcodes in each reaction (Figure 2D) to generate a fully tagged, barcoded PCR product (Figure 2C) in each chamber."  Figure 2: Amplicon-tagging to introduce sequencer-specific tags and sample-specific barcodes

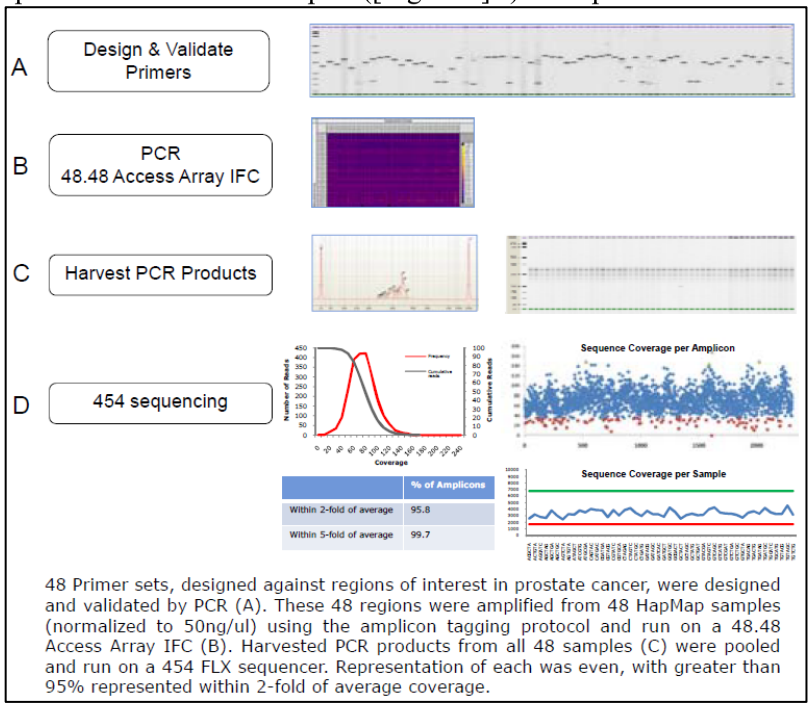
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U.S. Patent No. 11,519,035	
Claim	**Prior Art**
1[a][ii]. wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;	In view of the knowledge of a POSA at the time of the claimed invention, it would have been obvious to perform the claimed method on cell-free DNA isolated from a blood sample collected from a subject who is not a pregnant woman. For instance, a POSA would be motivated to utilize the methods of May Poster for cell free tumor DNA assays because May Poster discloses an experiment where 48 primer sets were "designed against regions of interest in prostate cancer" and it was well known at the time that cancer DNA can take the form of cell free DNA. Furthermore, it was well known to a POSA at the time that many cancer samples, including cancer tissue biopsies, are stored under conditions that lead to high levels of DNA fragmentation (*e.g.,* storage using formalin-fixed paraffin-embedding (FFPE)), and that methods that work on samples with high levels of DNA fragmentation such as those disclosed in May Poster would work with cell free tumor DNA. Further still, May Poster provides an express invitation to perform its methods on samples with low amounts of DNA by disclosing that the Access Array system can be used with "only 50 ng input DNA per sample."
1[b][i]. amplifying the tagged products one or more times to generate final amplification products,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, May Poster discloses amplifying the tagged products one or more times to generate final amplification products. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. See, *e.g.,* Figure 2A, Figure 2B, Figure 2C, Figure 2D, Figure 3B, and Figure 3C. For example, May Poster discloses an "amplicon tagging method (Figure 2) that enables direct attachment of sample-specific barcodes and sequencer-specific tags to PCR products generated in the Access Array. Specifically, individual steps disclosed in Figure 2A and 2B of the amplicon-tagging method are each tagging and amplifying steps that occur within the same PCR process and therefore meet the limitations of claim 1[b][i]. For example, Region-specific primers are tagged at the 5' end with universal sequence tags and amplified in a single PCR process (Figure 2A.).

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Claim	**Prior Art**
	As another example, barcode primers comprising 454 sequence tags, a barcode sequence, and the universal tags are introduced to the products of Figure 2A in a single PCR process (Figure 2B). As another example, May Poster discloses that, through the Access Array, "we have developed an amplicon-tagging protocol that enables direct labeling of PCR products with sample-specific barcodes and sequencer-specific tags." For example, May Poster discloses an "amplicon tagging method (Figure 2) that enables direct attachment of sample-specific barcodes and sequencer-specific tags to PCR products generated in the Access Array. Region-specific primers are tagged at the 5' end with universal sequence tags (Figure 2A.). Barcode primers comprise 454 sequence tags, a barcode sequence, and the universal tags (Figure 2B). Barcode primers are added to the samples before loading on to the Access Array IFC. The Access Array IFC combines the region-specific primers, samples and barcodes in each reaction (Figure 2D) to generate a fully tagged, barcoded PCR product (Figure 2C) in each chamber."  Figure 2: Amplicon-tagging to introduce sequencer-specific tags and sample-specific barcodes As another example, May Poster discloses that "when used in combination with primers designed for Amplicon tagging, the Access Array products sequencer-ready amplicon libraries that can be introduced to emulsion PCF for 454 sequencing."

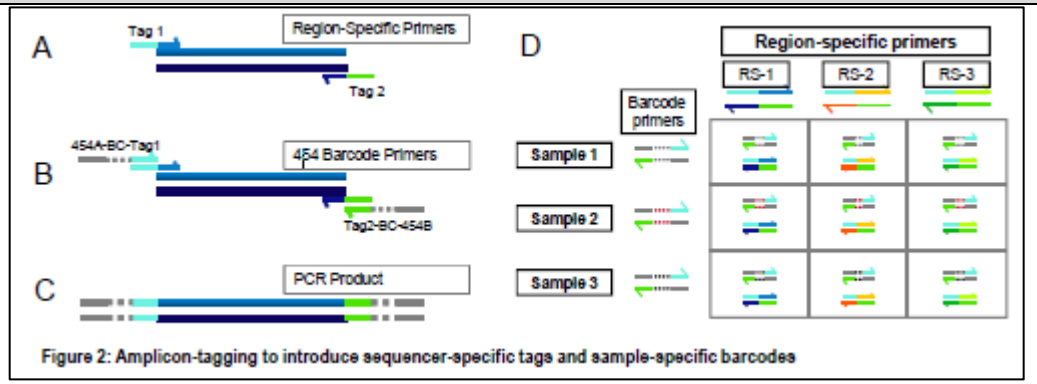
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U.S. Patent No. 11,519,035	
Claim	**Prior Art**
	As another example, May Poster discloses that, through the Access Array, "we have developed an amplicon-tagging protocol that enables direct labeling of PCR products with sample-specific barcodes and sequencer-specific tags." As another example, May Poster discloses an experiment "designed against regions of interest in prostate cancer." That experiment utilized 48 primer sets and "[t]hese 48 regions were amplified from 48 HapMap samples (normalized to 50ng/ul) using the amplicon tagging protocol and run on a 48.48 Access Array IFC ([Figure 3]B). [Then] [h]arvested PCR products from all 48 samples ([Figure 3]C) were pooled and run on a 454 FLX sequencer."  48 Primer sets, designed against regions of interest in prostate cancer, were designed and validated by PCR (A). These 48 regions were amplified from 48 HapMap samples (normalized to 50ng/ul) using the amplicon tagging protocol and run on a 48.48 Access Array IFC (B). Harvested PCR products from all 48 samples (C) were pooled and run on a 454 FLX sequencer. Representation of each was even, with greater than 95% represented within 2-fold of average coverage. As another example, May Poster discloses an Access Array system use-case wherein "[i]ndividual PCR reactions were run using the amplicon tagging protocol." Then [m]ultiplex PCR was run in a 2-step reaction."

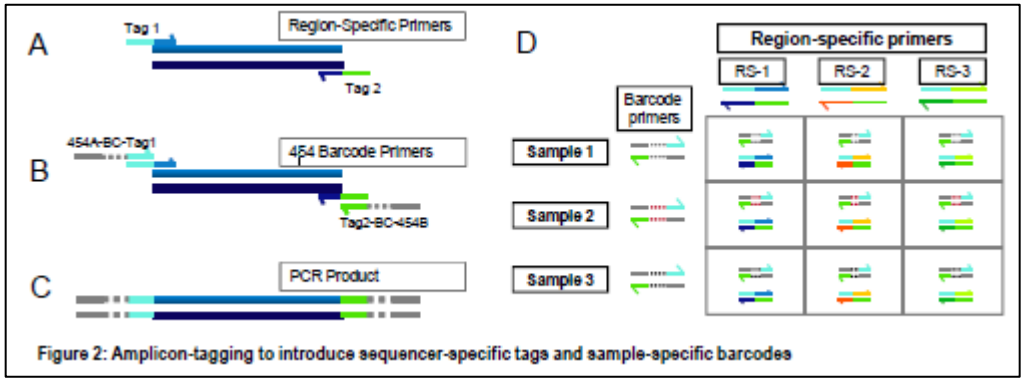
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U.S. Patent No. 11,519,035	
Claim	**Prior Art**
	Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, May Poster also discloses this element for the same reasons.
1[b][ii]. wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, May Poster discloses amplifying wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. See, *e.g.,* Figure 2A, Figure 2B, Figure 2C, Figure 2D, and Figure 4A. For example, May Poster discloses an "amplicon tagging method (Figure 2) that enables direct attachment of sample -specific barcodes and sequencer-specific tags to PCR products generated in the Access Array. Region-specific primers are tagged at the 5' end with universal sequence tags (Figure 2A.). Barcode primers comprise 454 sequence tags, a barcode sequence, and the universal tags (Figure 2B). Barcode primers are added to the samples before loading on to the Access Array IFC. The Access Array IFC combines the region-specific primers, samples and barcodes in each reaction (Figure 2D) to generate a fully tagged, barcoded PCR product (Figure 2C) in each chamber."

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U.S. Patent No. 11,519,035	
Claim	**Prior Art**
	 Figure 2: Amplicon-tagging to introduce sequencer-specific tags and sample-specific barcodes May Poster further discloses targeted amplification of a plurality of SNP loci in a single reaction volume. For example, May Poster discloses that the Access Array system is "a microfluidic chip that systematically combines 48 sample inputs with 48 primer inputs to make all possible 2304 combinations of samples and primers." As another example, May Poster discloses, "With multiplexing of primer pairs in each reaction, there is potential for increasing the number of regions enriched per sample, from the same quantity of input DNA and at the same cost per sample (around $7). Primers were designed to amplify the exons of the EGFR and MET genes (Figure 4A). These primer sets were then used to amplify regions from a standard human genomic DNA sample as 48 individual primer sets or in 5 groups of multiplex primers." As another example, May Poster discloses an experiment using the Access Array system wherein "[m]ultiplex PCR was run in a 2-step reaction" and step 1 "involved amplification of the region-specific multiplex tools, [and] the second step involved the addition of the outer primer pairs to harvested PCR products from the first step, followed by 10 cycles of PCR in a PCR tube to assembled the tagged, barcoded samples." Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, May Poster also discloses this element for the same reasons.

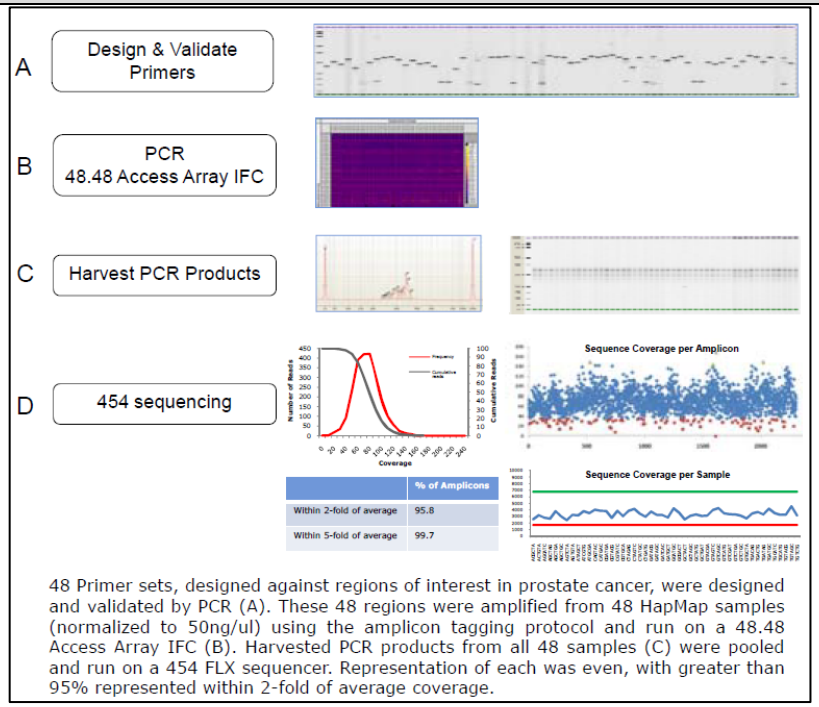
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U.S. Patent No. 11,519,035	
Claim	**Prior Art**
1[b][iii]. wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and	May Poster discloses amplifying wherein one of the amplifying steps introduces a barcode and one or more sequencing tags. For example, May Poster discloses an "amplicon tagging method (Figure 2) that enables direct attachment of sample -specific barcodes and sequencer-specific tags to PCR products generated in the Access Array. Region-specific primers are tagged at the 5' end with universal sequence tags (Figure 2A.). Barcode primers comprise 454 sequence tags, a barcode sequence, and the universal tags (Figure 2B). Barcode primers are added to the samples before loading on to the Access Array IFC. The Access Array IFC combines the region-specific primers, samples and barcodes in each reaction (Figure 2D) to generate a fully tagged, barcoded PCR product (Figure 2C) in each chamber."  Figure 2: Amplicon-tagging to introduce sequencer-specific tags and sample-specific barcodes As another example, May Poster discloses "a primer design strategy that incorporates sample-specific barcodes and 454 sequencing adaptors into each sample, [which] remov[es] the need for library preparation before sequencing." As another example, May Poster discloses an experiment wherein "[m]ultiplex PCR was run in a 2-step reaction" and step 1 "involved amplification of the region-specific multiplex tools, [and] the second step involved the addition of the outer primer pairs to harvested PCR products from the first step, followed by 10 cycles of PCR in a PCR tube to assembled the tagged, barcoded samples." As another example, May Poster discloses "an amplicon-tagging protocol that enables direct labeling of PCR products with sample-specific barcodes and sequencer-specific tags."

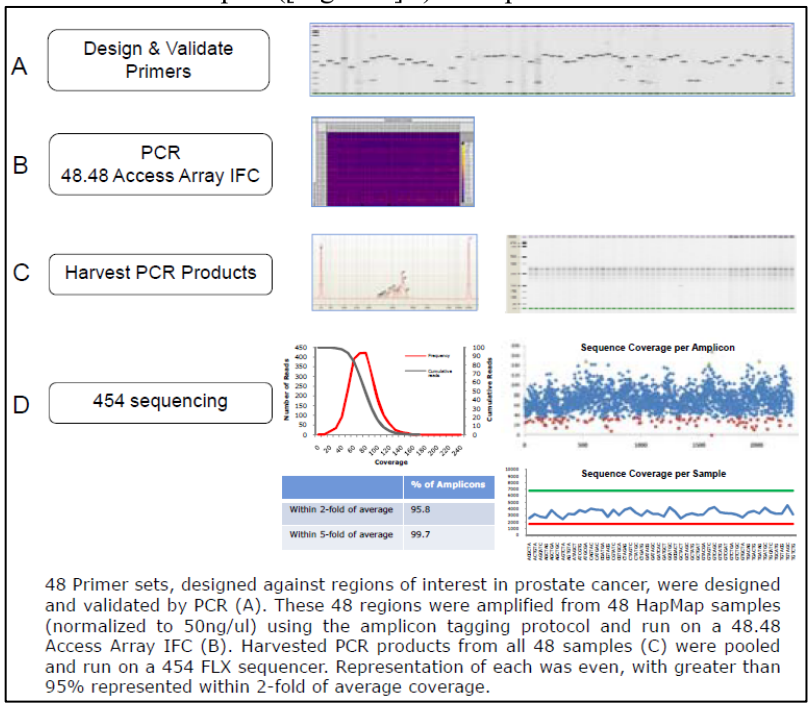
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U.S. Patent No. 11,519,035	
Claim	**Prior Art**
	Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, May Poster also discloses this element for the same reasons.
1[c][i]. sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products,	May Poster discloses sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products. For example, May Poster discloses an experiment "designed against regions of interest in prostate cancer." That experiment utilized 48 primer sets and "[t]hese 48 regions were amplified from 48 HapMap samples (normalized to 50ng/ul) using the amplicon tagging protocol and run on a 48.48 Access Array IFC ([Figure 3]B). [Then] [h]arvested PCR products from all 48 samples ([Figure 3]C) were pooled and run on a 454 FLX sequencer."

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Claim	**Prior Art**
	 48 Primer sets, designed against regions of interest in prostate cancer, were designed and validated by PCR (A). These 48 regions were amplified from 48 HapMap samples (normalized to 50ng/ul) using the amplicon tagging protocol and run on a 48.48 Access Array IFC (B). Harvested PCR products from all 48 samples (C) were pooled and run on a 454 FLX sequencer. Representation of each was even, with greater than 95% represented within 2-fold of average coverage. As another example, May Poster discloses an experiment wherein "[m]ultiplex PCR was run in a 2-step reaction" and step 1 "involved amplification of the region-specific multiplex tools. Then, following step 2, the "[s]amples were sequenced directly on a 454 FLX Sequencer using Titanium Amplicon chemistry with no additional purification."
1[c][ii]. wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.	The Court construed the term "SNP loci…associated with cancer" to mean "a single nucleotide that may differ between the genomes of two members of the same species and is associated with cancer. The usage of the term 'SNP' should not imply any limit on the frequency with which each variant occurs." Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, May Poster discloses a plurality of SNP loci comprising 25-2,000 loci associated with cancer. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics

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Claim	**Prior Art**
	reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. For example, May Poster discloses that the Access Array system "is a microfluidic chip that systematically combines 48 samples inputs with 48 primer inputs to make all possible 2304 combinations of samples and primers." May Poster also discloses an experiment "designed against regions of interest in prostate cancer." That experiment utilized 48 primer sets and "[t]hese 48 regions were amplified from 48 HapMap samples (normalized to 50ng/ul) using the amplicon tagging protocol and run on a 48.48 Access Array IFC ([Figure 3]B). [Then] [h]arvested PCR products from all 48 samples ([Figure 3]C) were pooled and run on a 454 FLX sequencer."  48 Primer sets, designed against regions of interest in prostate cancer, were designed and validated by PCR (A). These 48 regions were amplified from 48 HapMap samples (normalized to 50ng/ul) using the amplicon tagging protocol and run on a 48.48 Access Array IFC (B). Harvested PCR products from all 48 samples (C) were pooled and run on a 454 FLX sequencer. Representation of each was even, with greater than 95% represented within 2-fold of average coverage.

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	As another example, May Poster discloses that it "demonstrated the potential for multiplexing primer pairs within PCR reactions on the Access Array system" and that "[r]epresentation of each amplicon in the multiplex reaction is similar to that within individual PCR reactions." As another example, May Poster discloses, "With multiplexing of primer pairs in each reaction, there is potential for increasing the number of regions enriched per sample, from the same quantity of input DNA and at the same cost per sample (around $7). Primers were designed to amplify the exons of the EGFR and MET genes (Figure 4A). These primer sets were then used to amplify regions from a standard human genomic DNA sample as 48 individual primer sets or in 5 groups of multiplex primers." In view of the knowledge of a POSA at the time of the claimed invention, it would have been obvious that the EGFR and MET genes contained SNP loci associated with cancer. For example, Shigematsu discloses "a total of 134 EGFR TK domain mutations in 130 (21%) of the 617 NSCLCs [non-small cell lung cancers]" identified by "sequenc[ing] exons 18-21 of the EGFR TK domain from genomic DNA isolated from 617 non-small-cell lung cancers (NSCLCs) and 524 normal lung tissue samples from the same patients in Japan, Taiwan, the United States, and Australia." Shigematsu at Abstract. As another example, Faoro discloses that "MET can be overexpressed in a number of malignancies, sometimes mutated (germline mutations/single nucleotide polymorphisms [SNPs] or somatic mutations), or sometimes even amplified." Faoro at 1. Faoro also discloses that "missense mutations of *MET* have been reported in a variety of cancers" including "activating mutations of *MET* in hereditary papillary renal carcinomas", "somatic mutations…in some sporadic papillary renal carcinomas", "TK domain mutations…in other tumor types such as head and neck cancer and glioblastomas." *Id.* To the extent Natera contends May Poster does not disclose 25-2,000 loci associated with cancer, it would have been obvious to a POSA at the time of the invention in combination with the knowledge of a POSA. For instance, a POSA would be motivated to utilize the methods of May Poster for cell free tumor DNA assays because May Poster discloses an experiment where 48 primer sets were "designed against regions of interest in prostate cancer" and it was well known at the time that cancer DNA can take the form of cell free DNA. Furthermore, it was well known to a POSA at the time that many cancer samples, including cancer tissue biopsies, are stored under conditions that lead to high levels of DNA fragmentation (*e.g.,* storage using formalin-fixed paraffin-embedding (FFPE)), and that methods that work on samples with high levels of DNA fragmentation such as those disclosed in

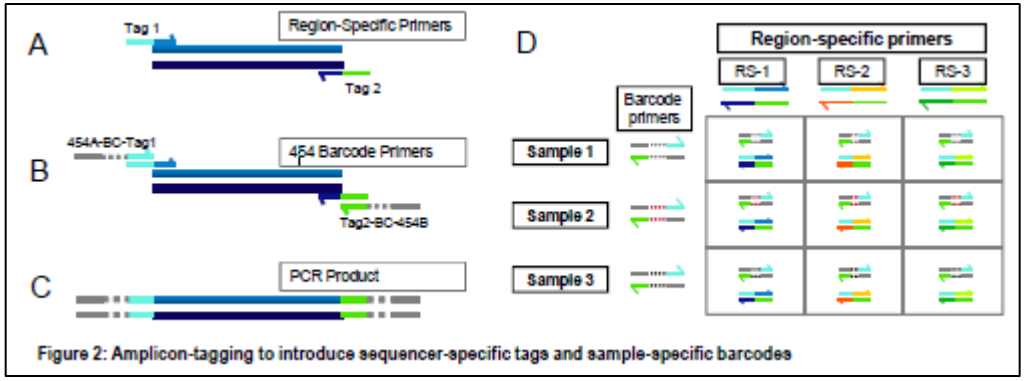
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	May Poster would work with cell free tumor DNA. Further still, May Poster provides an express invitation to perform its methods on samples with low amounts of DNA by disclosing that the Access Array system can be used with "only 50 ng input DNA per sample." Additionally, a POSA would have understood that a single target region can contain multiple loci. To the extent Natera asserts that any portion of this limitation is not disclosed under Natera's apparent interpretation of the Court's construction, it would have been obvious based on the knowledge of a POSA at the time of the alleged invention to look to the Catalogue of Somatic Mutations In Cancer ("COSMIC") database, which identifies hundreds to thousands of gene variants, including those that can be present in the same gene, to readily disclose a plurality of SNP loci comprising 25-2,000 loci associated with cancer. By way of example, COSMIC identifies 6,038 different variants in the TP53 gene alone. *See*, *e.g.*, https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53. In addition, the '035 Patent admits a POSA's "general belief in the art is 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, would have understood that multiplex PCR up to 100 assays could readily be performed on cell free DNA, and would have been able to do so as a matter of routine.
2. The method of claim 1, wherein tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA.	Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 27. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, May Poster also discloses this element for the same reasons as discussed below regarding claim 13. In addition, to the extent May Poster does not specifically teaches tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA, this limitation is taught by Dong (Ex. 1-A), Porreca (Ex. 1-F), Hoyal (Ex. 1-M), and Wessler (Ex. 1-M). A POSA would be motivated to combine May Poster with Dong, Porreca, Hoyal, or Wessler because May Poster expressly teaches using routine and conventional techniques known in the art to analyze fragmented sample DNA efficiently, and the references cited herein are reasonably within the same technological field, or at the very least

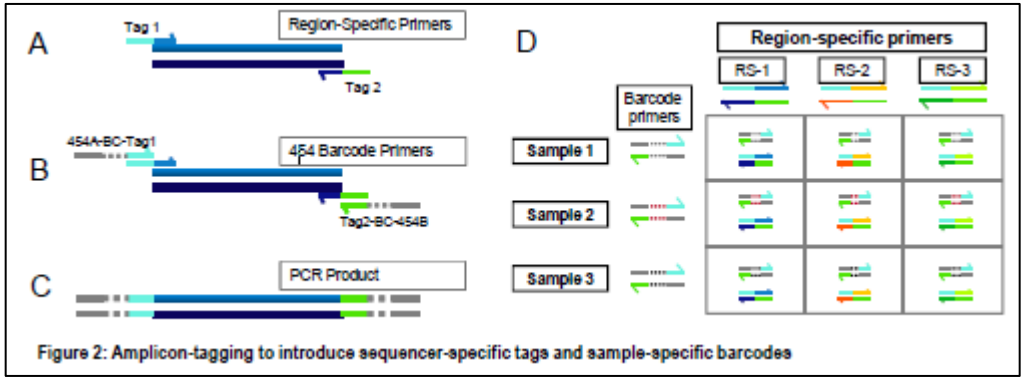
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	reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end. For example, Dong teaches "enrichment and amplification of sequences from a nucleic acid." Dong at [0001]. As another example, Dong teaches "methods [that] provide for highly parallel analysis of a large number of SNPs, for example, more than 1,000, 5,000, 10,000, or 50,000, that are spaced throughout the genome." *Id*. at [0084]. Dong solves these problems through ligation of common (universal) adaptors to fragmented DNA followed by targeted amplification using primers against the target sequence of interest and the common adpators. Dong at [0027] and Figure 6. For example, Porreca teaches "predetermined preparative steps and/or nucleic acid sequence analysis techniques [] used in multiplex analyses for a plurality of genetic loci in a plurality of samples". Porreca at Abstract. As another example, Porreca teaches "methods. . . relat[ing] to genotyping a polymorphism of a target nucleic acid." Porreca at 4:15-20. As another example, Porreca teaches "methods for analyzing multiple genetic loci (e.g., a plurality of target nucleic acids selected from Table 1) from a patient sample, such as a blood, pre-implantation embryo, chorionic villus or amniotic fluid sample, or other sample". Porreca at 4:2-7. Porreca solves these problems through ligation of adapters containing PCR primer sites onto the ends of the nucleic acid molecules of interest, followed by PCR-mediated amplification. Porreca at 43:15-20. *See also* Porreca at FIG. 4C. For example, Hoyal teaches "a need for improved extraction methods capable of capturing small nucleic acid molecules"—cell-free DNA—because "the ability to successfully analyze short, fragmented DNA in the presence of more abundant, longer DNA ha[d] remained elusive." Hoyal at 1. As another example, Hoyal teaches "detecting and monitoring extracellular mutant oncogenes or tumor-associated DNA found in the plasma or serum fraction of blood by using DNA extraction with enrichment for mutant DNA," i.e., detecting cell-free tumor DNA. Hoyal at 20. Hoyal solves these problems through adapter-mediated ligation followed by universal pre-amplification before target-specific amplification. Hoyal at Figure 3. For example, Wessler teaches methods for detecting polymorphisms in DNA. Wessler at 3:6-4:41. As another example, Wessler teaches methods for "investigation of genome structure . . . by the use of in vitro methods that detect variation in DNA sequence in the genomes between members of a species" Wessler at 2:26-29. Wessler reaches this goal through Wessler's ligation-mediated preamplification followed by two target-specific rounds of PCR-mediated amplification. Wessler at Figure 1.

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Claim	**Prior Art**
	There is a reasonable expectation of success in combining May Poster with one or more of the foregoing references for the purposes described above because the techniques of isolating, tagging, amplifying, and sequencing cell free DNA as well as using ligation to tag cell free DNA with universal tail adaptors were all well known in the art at the time of the invention and were known to be interchangeable and adjustable depending on the requirements of a given implementation.
12. The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.	May Poster discloses one or more universal tail adaptors comprising a first universal tail adaptor and a second universal tail adaptor. For example, May Poster discloses an "amplicon tagging method (Figure 2) that enables direct attachment of sample -specific barcodes and sequencer-specific tags to PCR products generated in the Access Array. Region-specific primers are tagged at the 5' end with universal sequence tags (Figure 2A.). Barcode primers comprise 454 sequence tags, a barcode sequence, and the universal tags (Figure 2B). Barcode primers are added to the samples before loading on to the Access Array IFC. The Access Array IFC combines the region-specific primers, samples and barcodes in each reaction (Figure 2D) to generate a fully tagged, barcoded PCR product (Figure 2C) in each chamber."  Figure 2: Amplicon-tagging to introduce sequencer-specific tags and sample-specific barcodes

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	As another example, May Poster discloses that through the Access Array system and protocol "[w]e have developed an amplicon-tagging protocol that enables direct labeling of PCR products with sample-specific barcodes and sequencer-specific tags."
13. The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.	Under Natera's apparent construction, May Poster discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. For example, May Poster discloses an "amplicon tagging method (Figure 2) that enables direct attachment of sample -specific barcodes and sequencer-specific tags to PCR products generated in the Access Array. Region-specific primers are tagged at the 5' end with universal sequence tags (Figure 2A.). Barcode primers comprise 454 sequence tags, a barcode sequence, and the universal tags (Figure 2B). Barcode primers are added to the samples before loading on to the Access Array IFC. The Access Array IFC combines the region-specific primers, samples and barcodes in each reaction (Figure 2D) to generate a fully tagged, barcoded PCR product (Figure 2C) in each chamber."  Figure 2: Amplicon-tagging to introduce sequencer-specific tags and sample-specific barcodes Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, May Poster also discloses this element for the same reasons as discussed above regarding claim 2.

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EXHIBIT 1-M – FINAL INVALIDITY CLAIM CHART FOR U.S. PATENT NO. 11,519,035

Reference:
Forshew, T. et al., *Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA* 4(136):36RA68 (May 2012) ("Forshew"); Forshew, T. et al., *Supplementary Materials for Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA* 4 (136):36RA68 (May 2012) (available at https://www.science.org/doi/suppl/10.1126/scitranslmed.3003726/suppl_file/4-136ra68_sm.pdf) ("Forshew Supp.")

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

To the extent Natera asserts that Forshew does not disclose or teach one or more limitations of claims 1, 2, 12 and 13, performing the methods as claimed in those claims would have been obvious to a person of ordinary skill in the art at the time of the alleged invention considering Forshew in combination with the knowledge of a POSA and/or one or more of the following references:

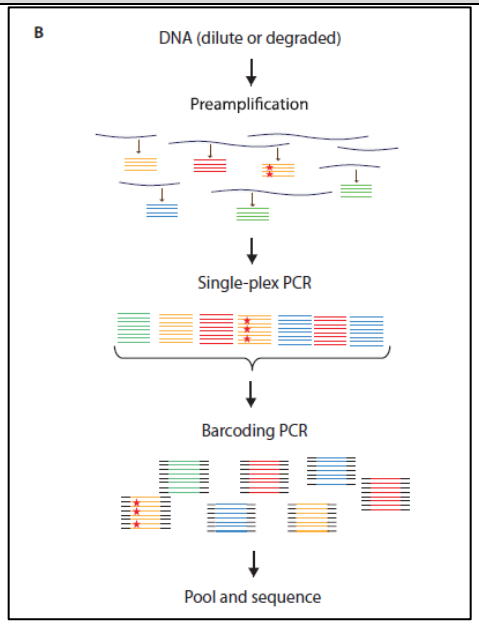
- U.S. Patent Application Publication US 2004/0110153 A1 ("Dong")
- U.S. Patent 8,318,434 B2 ("Cuppens")
- Shapero et al., *MARA: a novel approach for highly multiplexed locus-specific SNP genotyping using high-density DNA oligonucleotide arrays*, 32 NUCLEIC ACIDS RES. e101 (2004) ("Shapero")
- International Patent Publication No. 2010/126614 ("Porreca")
- U.S. Patent Publication No. 2012/0270739 ("Oliphant")
- International Patent Publication No. 2007/018601("Makarov")
- International Publication No. WO 2007/140417 ("Hoyal")
- U.S. Patent No. 6,420,117 ("Wessler")

A POSA would have been motivated to combine Forshew with one or more of the foregoing references because Forshew expressly teaches using routine and conventional techniques known in the art to analyze sample circulating tumor DNA efficiently. For example, a POSA would be motivated to utilize ligation to add one or more universal tail adaptors at least because Forshew expressly teaches the use of universal tail adaptors, and it was well known in the art at the time of the invention that such adaptors could be added through ligation, and thus a POSA would recognize ligation-based DNA tagging and PCR-based DNA tagging as interchangeable. Further, a POSA would reasonably expect to succeed in combining Forshew with one or more of the foregoing references for the purposes described above because the techniques of multiplex PCR, barcoding DNA fragments, adding sequencing tags, using multiple universal tail adapters, and performing massively parallel sequencing were all routine and conventional at the time for DNA analysis and diagnostics.

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1[pre]. A method for amplifying and sequencing DNA, comprising:	To the extent the preamble is construed to be limiting, Forshew discloses a method for amplifying and sequencing DNA. *See, e.g.,* Forshew at Abstract, 1-10, Figures 1-4, Tables 1-3; Forshew Supp. 1-19, Figures S1-4, Tables S1-7. For example, Forshew discloses "a tool for noninvasive mutation analysis on the basis of tagged-amplicon deep sequencing (TAm-Seq), which allows amplification and deep sequencing of genomic regions spanning thousands of bases from as little as individual copies of fragmented DNA." Forshew at 1. As another example, Figure 1B of Forshew discloses "tagged-amplicon sequencing (TAm-Seq)" workflow overview 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 to preserve representation of all alleles in the template material. The schematic diagram shows DNA molecules that carry mutations (red stars) being amplified alongside wild-type molecules. Regions of interest in the preamplified material were then selectively amplified in individual (single-plex) PCR, thus excluding nonspecific products. Finally, sequencing adaptors and sample-specific barcodes were attached to the harvested amplicons in a further PCR." Forshew at 2.

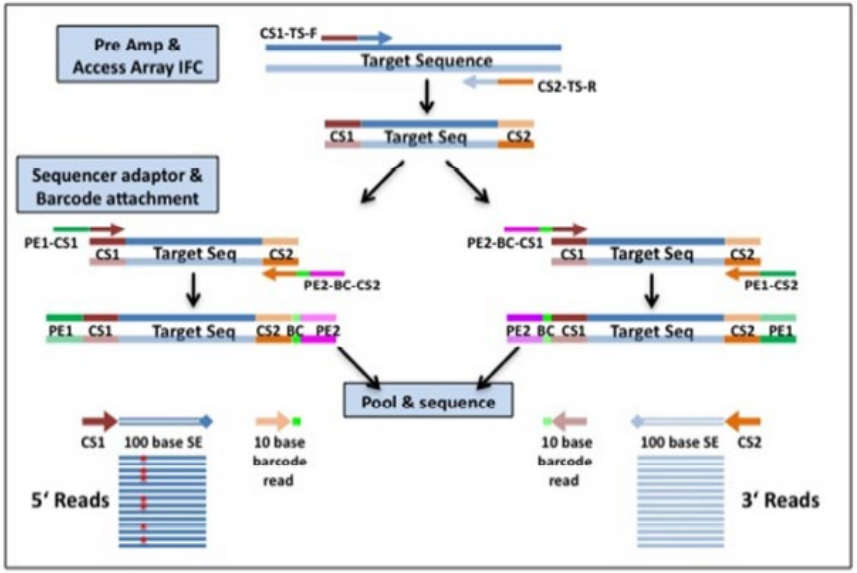
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Claim	**Prior Art**
	 As another example, Forshew discloses that the TAm-Seq method was validated "on a larger panel of plasma samples in which levels of tumor-specific mutations were measured in parallel using patient-specific digital PCR assays. DNA extracted from 62 additional plasma samples collected at different time points from 37 patients with advanced HGSOC was amplified in duplicate (table S6), using DNA present in ~0.15 ml of plasma per reaction (range, 0.06 to 0.2 ml). Amplicon libraries were tagged and pooled together for sequencing with libraries prepared from 24 control samples. This generated an average sequencing depth of 650 for 62 plasma samples, sufficient to detect mutations present at AFs of 1% to 2%." Forshew at 4. As another example, Forshew discloses an experiment wherein "[w]hole-genome sequencing of tumor material was used to identify tumor mutations in a patient with metastatic breast cancer undergoing two phases of chemotherapy. Ten mutations were selected, and short amplicons (<120 bp) were designed to cover the mutation loci (table S7). Serial plasma samples were collected over the course of 497 days, both before and after treatment. We performed TAm-Seq in duplicate, using DNA from 0.08 ml of plasma per amplification, and tracked dynamics of all mutations in parallel (Fig. 4D)." Forshew at 9.

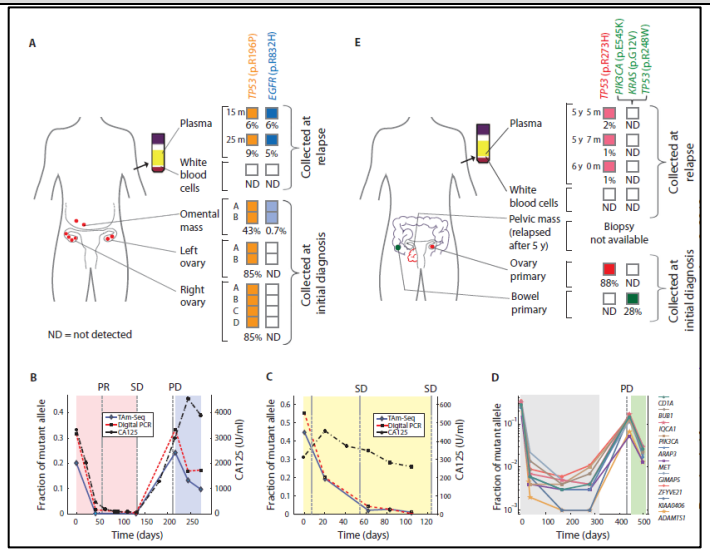
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Claim	**Prior Art**
	As another example, Forshew discloses that "[o]ur TAm-Seq method uses a combination of short amplicons, two-step amplification, sample barcodes, and high-throughput PCR. Because the amplicons are short, this method effectively amplifies even small amounts of fragmented DNA such as are present in circulating DNA. The two-step amplification permits extensive primer multiplexing that enables the amplification and sequencing of sizeable genomic regions by tiling short amplicons without loss of fidelity or efficiency. Duplicate sequencing of each sample is used to avoid false positives stemming from PCR errors. Sample barcodes and high-throughput PCR reduce the per-sample costs to a range where this may be widely applicable. Preparing TAm-Seq libraries for sequencing from 48 samples takes less than 24 hours and involves only few hours of hands-on time. New platforms for massively parallel sequencing allow for fast turnaround times, which make this approach practical in a clinical setting." Forshew at 9.
1[a][i]. tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products,	Under Natera's apparent construction, Forshew discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See, e.g.,* Forshew at Abstract, 1-5, 8, 9, 10, Figures 1, 3, Table 3; Forshew Supp. at 2, 3, 5, 9-10, 15 Figure S1, Tables S1, S4. For example, Forshew discloses that "[t]o amplify and sequence fragmented DNA, we designed primers to generate amplicons that tile regions of interest in short segments of about 150 to 200 bases (Fig. 1A and table S1), incorporating universal adaptors at 5′ ends (fig. S1)." Forshew at 2-3. As another example, Figure S1 of Forshew Supp. discloses that "Target-specific primers were synthesised with common adaptor sequences at their 5' ends (CS1 and CS2). These were used during the preamplification and single-plex stages to amplify the selected regions. Sequencing platform-specific adaptors and unique sample barcodes were attached during an additional round of PCR. The barcoding primers consist of Illumina sequencer adaptors (PE1 and PE2) at their 3' end, a unique 10 base barcode, and the common CS1 and CS2. Permutations of the universal tags and the sequencing adaptors enabled construction of bidirectional barcoded sequencing libraries. Using custom sequencing primers directed to the two tag sequences, sequence reads representing both strands of the amplicon pool were obtained from a single-end run. Both combinations were used in order to barcode and read amplicons in both directions. All samples were then pooled, cleaned, and then sequenced." Forshew Supp. at 5.

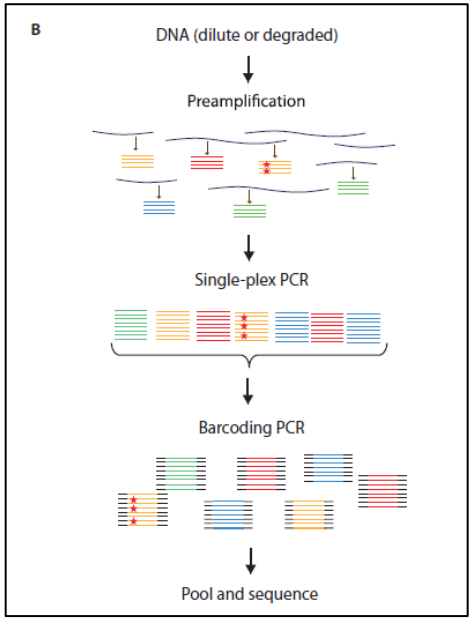
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Claim	**Prior Art**
	
1[a][ii]. wherein the isolated cell-free DNA is isolated from a blood sample collected from a subject who is not a pregnant women;	Under Natera's apparent construction, Forshew discloses tagging cell free DNA isolated from a blood sample collected from a subject who is not a pregnant women. *See, e.g.,* Forshew at 1-10, Figures 2-4, Tables 1-3; Forshew Supp. at 15, 17-18, Tables S4, S6. For example, Forshew discloses that "[w]e applied this technique for detection of both abundant and rare mutations in circulating DNA from blood plasma of ovarian and breast cancer patients." Forshew at 1-2. As another example, Forshew discloses that "[w]e applied TAm-Seq to directly identify mutations in plasma of cancer patients. Forshew at 4. As another example, Figure 4 of Forshew discloses "[c]linically relevant applications of plasma DNA sequencing using TAm-Seq.

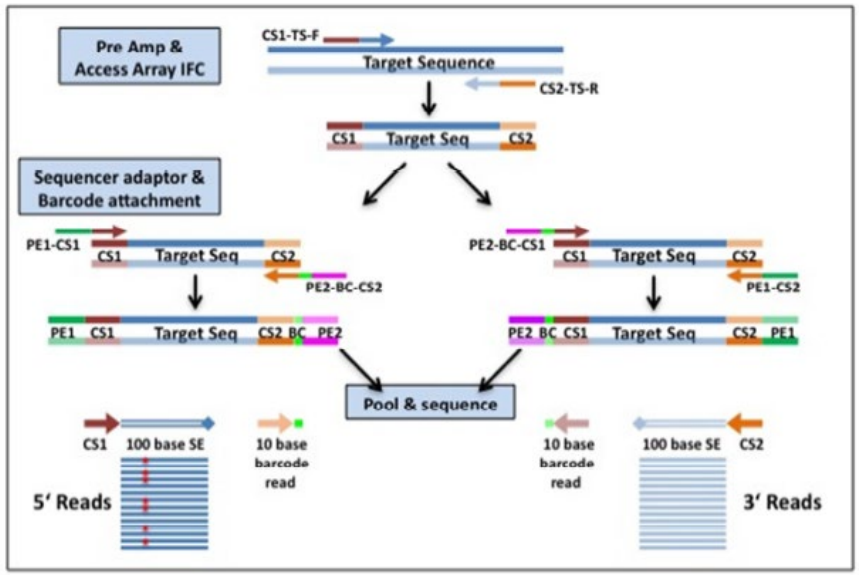
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Claim	**Prior Art**
	 Furthermore, to the extent Natera asserts that Forshew does not disclose or teach this limitation, it would have been obvious to use TAm-Seq on subjects who are not pregnant women. A POSA would have been motivated to apply Forshew's methods to humans who are not pregnant women because many humans with cancer are not pregnant. And the POSA would have reasonably expected success in doing so: after all, Forshew discloses that its method is suitable for monitoring for breast and ovarian cancers, and many non-pregnant people have breasts and ovaries. Forshew at 5-11.
1[b][i]. amplifying the tagged products one or more times to generate final amplification products,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Forshew discloses amplifying the tagged products one or more times to generate final amplification products. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling.

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Claim	**Prior Art**
	See, e.g., Forshew at 1-4, 9, Figure 1; Forshew Supp. at 2-3, 5, 11-12, Figure S1, Table S2. For example, Figure 1B of Forshew discloses an exemplar workflow overview 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 to preserve representation of all alleles in the template material. The schematic diagram shows DNA molecules that carry mutations (red stars) being amplified alongside wild-type molecules. Regions of interest in the preamplified material were then selectively amplified in individual (single-plex) PCR, thus excluding nonspecific products. Finally, sequencing adaptors and sample-specific barcodes were attached to the harvested amplicons in a further PCR." Forshew at 2.  As another example, Forshew discloses "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)." Forshew at 3.

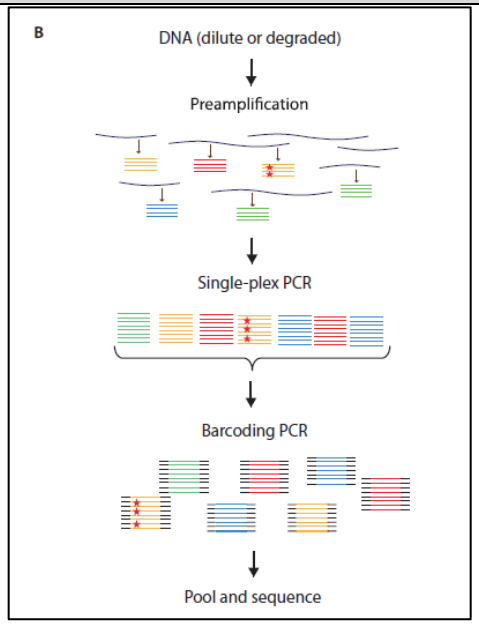
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Claim	**Prior Art**
	As another example, Figure S1 of Forshew Supp. discloses that "Target-specific primers were synthesised with common adaptor sequences at their 5' ends (CS1 and CS2). These were used during the preamplification and single-plex stages to amplify the selected regions. Sequencing platform-specific adaptors and unique sample barcodes were attached during an additional round of PCR. The barcoding primers consist of Illumina sequencer adaptors (PE1 and PE2) at their 3' end, a unique 10 base barcode, and the common CS1 and CS2. Permutations of the universal tags and the sequencing adaptors enabled construction of bidirectional barcoded sequencing libraries. Using custom sequencing primers directed to the two tag sequences, sequence reads representing both strands of the amplicon pool were obtained from a single-end run. Both combinations were used in order to barcode and read amplicons in both directions. All samples were then pooled, cleaned, and then sequenced." Forshew Supp. at 5. As another example, Figure S1 of Forshew Supp. discloses that "Sequencing platform-specific adaptors and unique sample barcodes were attached during an additional round of PCR." Forshew Supp. at 5.  Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or

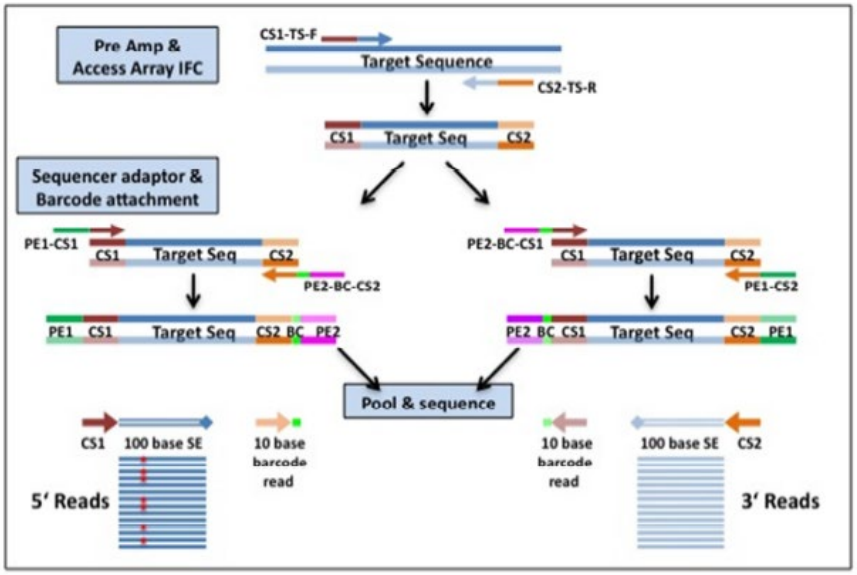
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Claim	**Prior Art**
	substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Forshew also discloses this element for the same reasons.
1[b][ii]. wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume,	The Court construed the term "amplifying the tagged products . . . wherein one of the amplification steps comprises targeted amplification" to have its plain and ordinary meaning. Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Forshew discloses amplifying wherein one of the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.,* Forshew at 2-4, 8, 9, Figure 1; Forshew Supp. at 3-5, 19, Figures S1, S3, Table S4. For example, Figure 1B of Forshew discloses an exemplar workflow overview 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 to preserve representation of all alleles in the template material. The schematic diagram shows DNA molecules that carry mutations (red stars) being amplified alongside wild-type molecules. Regions of interest in the preamplified material were then selectively amplified in individual (single-plex) PCR, thus excluding nonspecific products. Finally, sequencing adaptors and sample-specific barcodes were attached to the harvested amplicons in a further PCR." Forshew at 2.

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

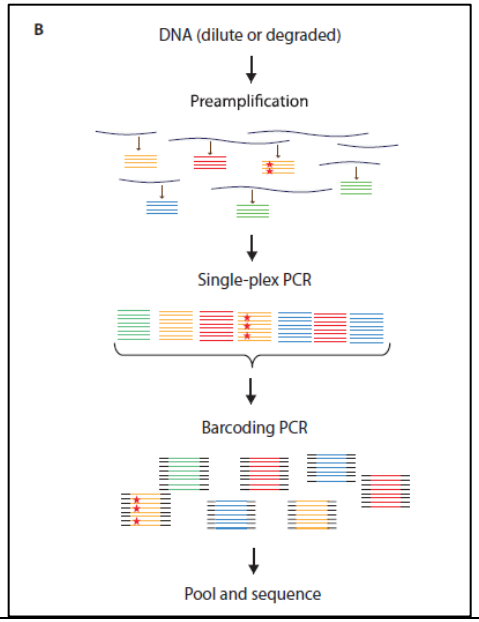
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	 As another example, Forshew discloses that an experiment wherein the authors "applied a two-step amplification process: a limited-cycle preamplification step where all primer sets were used together to capture the starting molecules present in the template, followed by individual amplification to purify and select for intended targets (Fig. 1B) (Supplementary Methods). 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. We used a microfluidic system (Access Array, Fluidigm) to perform parallel singleplex amplification from multiple preamplified samples using multiple primer sets. An additional PCR step attached sequencing adaptors (fig. S1) and tagged each sample by a unique molecular identifier or "barcode" (table S2). Sequencing adaptors were separately attached at either end and the products mixed together, such that single-end sequencing generated separate sets of forward and reverse reads. We 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. This produced an average read depth of 3250 for each of 96 barcoded samples for 48 amplicons read in two possible orientations." Forshew at 3.

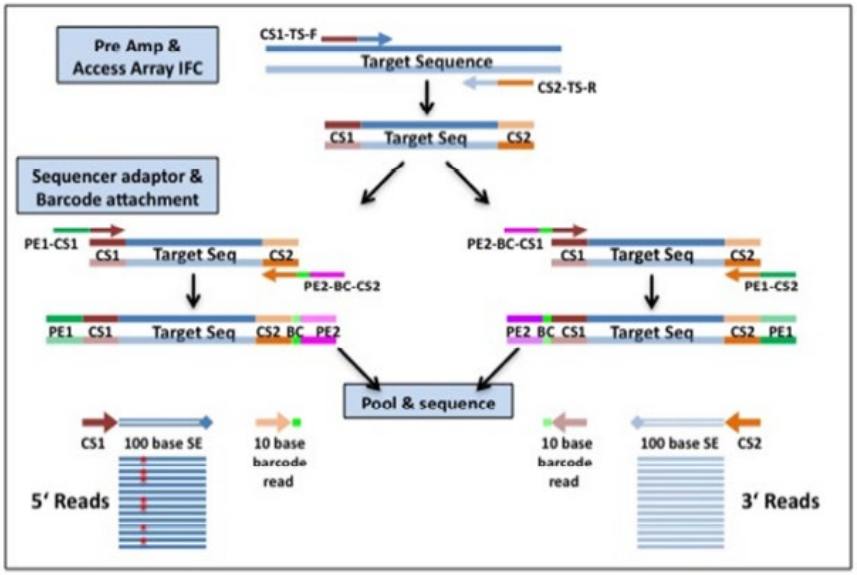
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	As another example, Forshew discloses an experiment wherein "DNA [was] extracted from 62 additional plasma samples collected at different time points from 37 patients with advanced HGSOC was amplified in duplicate (table S6), using DNA present in ~0.15 ml of plasma per reaction (range, 0.06 to 0.2 ml). Amplicon libraries were tagged and pooled together for sequencing with libraries prepared from 24 control samples. This generated an average sequencing depth of 650 for 62 plasma samples, sufficient to detect mutations present at AFs of 1% to 2%." Forshew at 4. As another example, Forshew discloses that "[w]e designed a set of 48 primer pairs to amplify 5995 bases of genomic sequence covering coding regions (exons and exon junctions) of TP53 and PTEN, and selected regions in EGFR, BRAF, KRAS, and PIK3CA (table S1) by overlapping short amplicons (Fig. 1A). The sequenced regions cover mutations that account for 38% of all point mutations in the COSMIC database (v55) (32). We used TAm-Seq to sequence DNA extracted from 47 formalin-fixed, paraffin-embedded (FFPE) tumor specimens of ovarian cancers (table S3), which were also sequenced for TP53 by Sanger sequencing (36) (Supplementary Methods). DNA extracted from FFPE samples is generally degraded and fragmented as a result of fixation and long-term ambient storage. We amplified DNA from each sample in duplicate, tagging each replicate with a different barcode. Using a single lane of sequencing, we generated 3.5 gigabases of data passing signal purity filters, producing mean read depth of 3200 above Q30 for each of the 9024 expected read groups (48 amplicons × 2 directions × 94 barcoded samples). Background frequencies of nonreference reads were ~0.1% (median, 0.03%; mean, 0.2%; in keeping with Q30 quality threshold applied), yet varied substantially between loci and base substitutions (Fig. 1C) and showed a clear bias toward purine/pyrimidine conservation (Fig. 1D). Sixty-six percent of loci had mean background rate of <0.1%, and 96% of loci had background rate of <0.6%." Forshew at 3. As another example, Forshew discloses that "[o]ur TAm-Seq method uses a combination of short amplicons, two-step amplification, sample barcodes, and high-throughput PCR. Because the amplicons are short, this method effectively amplifies even small amounts of fragmented DNA such as are present in circulating DNA. The two-step amplification permits extensive primer multiplexing that enables the amplification and sequencing of sizeable genomic regions by tiling short amplicons without loss of fidelity or efficiency." Forshew at 9. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera

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	argues this element is infringed under the doctrine of equivalents, Forshew also discloses this element for the same reasons.
1[b][iii]. wherein one of the amplifying steps introduces a barcode and one or more sequencing tags; and	Under Natera's apparent construction, Forshew discloses amplifying wherein one of the amplifying steps introduces a barcode and one or more sequencing tags. *See, e.g.,* Forshew at 2-4, 9, 10, Figure 1; Forshew Supp. at 2-5, 7, 11, Figures S1, S3, Table S2. For example, Figure 1B of Forshew discloses "tagged-amplicon sequencing (TAm-Seq)" workflow overview 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 to preserve representation of all alleles in the template material. The schematic diagram shows DNA molecules that carry mutations (red stars) being amplified alongside wild-type molecules. Regions of interest in the preamplified material were then selectively amplified in individual (single-plex) PCR, thus excluding nonspecific products. Finally, sequencing adaptors and sample-specific barcodes were attached to the harvested amplicons in a further PCR." Forshew at 2. 

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	As another example, Figure S1 of Forshew Supp. discloses that "Target-specific primers were synthesised with common adaptor sequences at their 5' ends (CS1 and CS2). These were used during the preamplification and single-plex stages to amplify the selected regions. Sequencing platform-specific adaptors and unique sample barcodes were attached during an additional round of PCR. The barcoding primers consist of Illumina sequencer adaptors (PE1 and PE2) at their 3' end, a unique 10 base barcode, and the common CS1 and CS2. Permutations of the universal tags and the sequencing adaptors enabled construction of bidirectional barcoded sequencing libraries. Using custom sequencing primers directed to the two tag sequences, sequence reads representing both strands of the amplicon pool were obtained from a single-end run. Both combinations were used in order to barcode and read amplicons in both directions. All samples were then pooled, cleaned, and then sequenced." Forshew Supp. at 5.  As another example, Table S2 of Forshew Supp. discloses "Unique sequencing barcodes. Platform-specific adaptors and barcodes are attached through PCR following the single-plex amplification step. The primers consisted of the PE1 and PE2 sequences for Illumina cluster generation, a 10-bp barcode, and the CS1 and CS2

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	adaptors, used in pairs: PE1-CS1 with PE2-BC-CS2, and PE1-CS2 with PE2-BC-CS1." Forshew Supp. at 11-12 (describing Table S2). Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues this element is infringed under the doctrine of equivalents, Forshew also discloses this element for the same reasons.
1[c][i]. sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products,	Under Natera's apparent construction, Forshew discloses sequencing the plurality of SNP loci on the cell free DNA by conducting massively parallel sequencing on the final amplification products. *See, e.g.,* Forshew at Abstract, 1-5, 7-10, Figures 1, 3, 4, Table 3; Forshew Supp. at 3-5, 15, 19, Figure S1, Tables S4, S7. For example, Forshew discloses that it's "two-step amplification permits extensive primer multiplexing that enables the amplification and sequencing of sizeable genomic regions by tiling short amplicons without loss of fidelity or efficiency. Duplicate sequencing of each sample is used to avoid false positives stemming from PCR errors. Sample barcodes and high-throughput PCR reduce the per-sample costs to a range where this may be widely applicable. Preparing TAm-Seq libraries for sequencing from 48 samples takes less than 24 hours and involves only few hours of hands-on time. New platforms for massively parallel sequencing allow for fast turnaround times, which make this approach practical in a clinical setting." Forshew at 9. As another example, Forshew discloses that an experiment wherein the authors "[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. This produced an average read depth of 3250 for each of 96 barcoded samples for 48 amplicons read in two possible orientations." Forshew at 3.
1[c][ii]. wherein the plurality of SNP loci comprises 25-2,000 loci associated with cancer.	The Court construed the term "SNP loci…associated with cancer" to mean "a single nucleotide that may differ between the genomes of two members of the same species and is associated with cancer. The usage of the term 'SNP' should not imply any limit on the frequency with which each variant occurs." Doc. 280 at 15. Under Natera's apparent interpretation of the Court's adopted construction, Forshew discloses amplifying wherein one of

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	the amplification steps comprises targeted amplification of a plurality of single nucleotide polymorphism (SNP) loci in a single reaction volume. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. *See, e.g.,* Forshew at 1-10, Figures 1, 3, 4, Table 3; Forshew Supp. at 2-4, 9-12, Tables S1, S2. For example, Forshew discloses "[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." Forshew at 9. As another example, Forshew Supp. discloses performing TAm-Seq with 48 sequence-specific primers. Forshew Supp. at 9 (showing Table S1). As another example, Forshew discloses that "[w]e designed a set of 48 primer pairs to amplify 5995 bases of genomic sequence covering coding regions (exons and exon junctions) of TP53 and PTEN, and selected regions in EGFR, BRAF, KRAS, and PIK3CA (table S1) by overlapping short amplicons (Fig. 1A). The sequenced regions cover mutations that account for 38% of all point mutations in the COSMIC database (v55) (32). We used TAm-Seq to sequence DNA extracted from 47 formalin-fixed, paraffin-embedded (FFPE) tumor specimens of ovarian cancers (table S3), which were also sequenced for TP53 by Sanger sequencing (36) (Supplementary Methods). DNA extracted from FFPE samples is generally degraded and fragmented as a result of fixation and long-term ambient storage. We amplified DNA from each sample in duplicate, tagging each replicate with a different barcode. Using a single lane of sequencing, we generated 3.5 gigabases of data passing signal purity filters, producing mean read depth of 3200 above Q30 for each of the 9024 expected read groups (48 amplicons × 2 directions × 94 barcoded samples). Background frequencies of nonreference reads were ~0.1% (median, 0.03%;

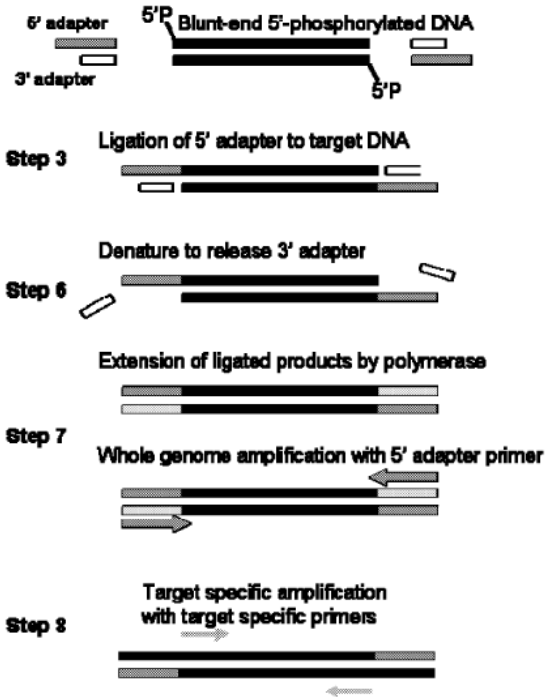
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	mean, 0.2%; in keeping with Q30 quality threshold applied), yet varied substantially between loci and base substitutions (Fig. 1C) and showed a clear bias toward purine/pyrimidine conservation (Fig. 1D). Sixty-six percent of loci had mean background rate of <0.1%, and 96% of loci had background rate of <0.6%." Forshew at 3. To the extent Natera asserts that any portion of this limitation is not disclosed under Natera's apparent interpretation of the Court's construction, it would have been obvious based on the knowledge of a POSA at the time of the alleged invention to look to the Catalogue of Somatic Mutations In Cancer ("COSMIC") database, which identifies hundreds to thousands of gene variants, including those that can be present in the same gene, to readily disclose a plurality of SNP loci comprising 25-2,000 loci associated with cancer. By way of example, COSMIC identifies 6,038 different variants in the TP53 gene alone. *See*, *e.g.,* https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53. In addition, the '035 Patent admits a POSA's "general belief in the art is 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, would have understood that multiplex PCR up to 100 assays could readily be performed on cell free DNA, and would have been able to do so as a matter of routine.
2. The method of claim 1, wherein tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA.	In view of the knowledge of a POSA at the time of the claimed inventions, it would have been obvious to tag cell free DNA by ligating the one or more universal tail adaptors to the cell free DNA. For instance, a POSA would be motivated to utilize the methods of Forshew in combination with ligation to add one or more universal tail adaptors at least because Forshew expressly teaches the use of universal tail adaptors, and it was well known in the art at the time of the invention that such adaptors could be added through ligation, and thus ligation-mediated DNA tagging and PCR-mediated DNA tagging are readily interchangeable with each other. Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 27. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Forshew also discloses this element for the same reasons as discussed below regarding claim 13.

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	In addition, to the extent Forshew does not specifically teach tagging the cell free DNA comprises ligating the one or more universal tail adaptors to the cell free DNA, this limitation is taught by Dong (Ex. 1-A), Porreca (Ex. 1-F), Hoyal, and Wessler. *See, e.g.,* Hoyal at 1, 2, 7, 16, 20, 27, 28, and Figure 3. For example, Hoyal discloses methods for isolating and amplifying DNA from human blood and plasma comprising a universal pre-amplification step involving "adapter-mediated ligation". Hoyal at 1, 27. Hoyal discloses adapter-mediated ligation as comprising ligation of "non-genome-specific adapter to the blunt ends" of DNA in a sample for use in "whole genome amplification." Hoyal at 27-28.

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	As another example, Hoyal discloses its adaptor-mediated ligation strategy, wherein DNA from a sample (*e.g.,* cell-free DNA) is ligated with 5' adapters (Step 3), extended, then subjected to whole genome amplification using universal sequences comprised in the 5' adapters (Step 7). Hoyal at Figure 3. **FIGURE 3** **Adapter mediated ligation**  *See, e.g.,* Wessler at Abstract, 3:36-42, 10:53-62, 10:63-11:7, 11:45-60, 12:5-15, 12:16-37, and Figure 1.

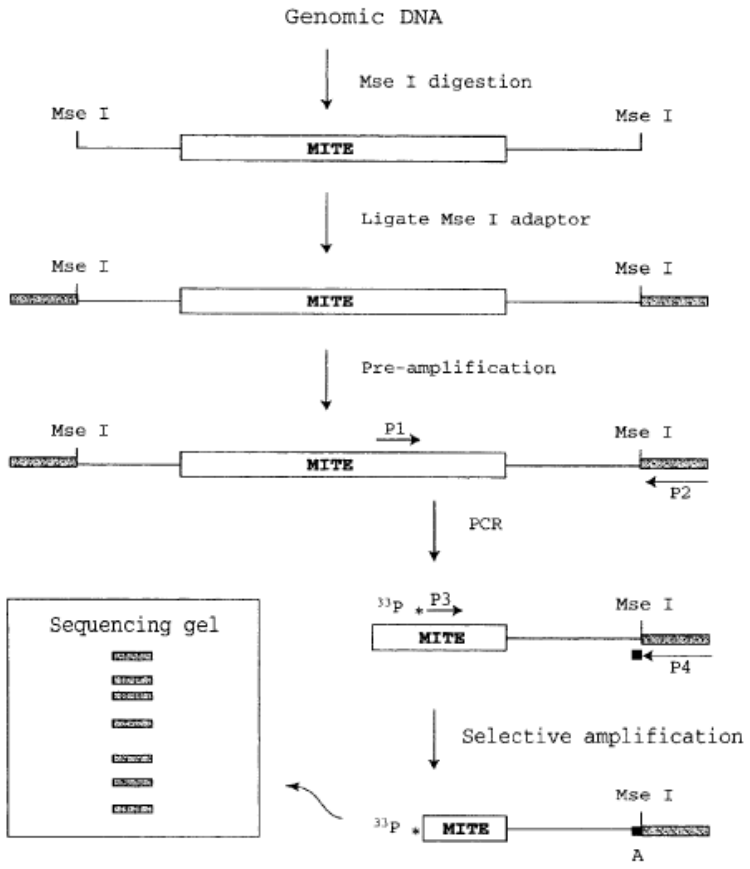
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	For example, Wessler discloses ligating double-stranded adapters onto target DNA, then performing targeted amplification involving selectively amplifying part of a target sequence ("MITE") via a first primer that anneals to

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	MITE and a second primers complementary to part of a ligated sequencing adapter. Wessler at 10:63-11:7, 11:45-60, 12:5-15, and Figure 1.

Fig. 1



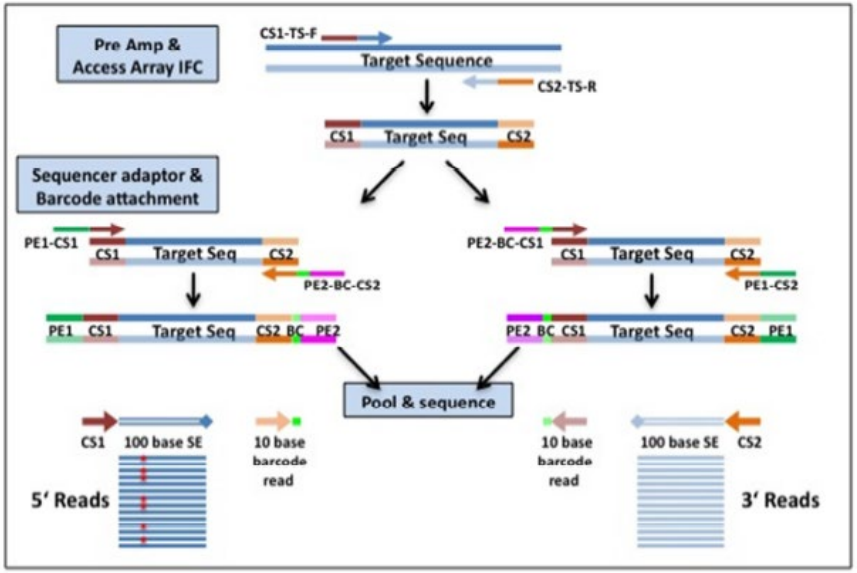
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	As another example, Wessler teaches a second, optional targeted amplification off of the ligated tagged molecule using third and fourth primers to amplify part of the earlier amplification product, wherein the third primer anneals to part of MITE and, the fourth primer contains a nucleotide sequence identical to a portion of the adapter's first strand. Wessler at 11:45-60, 12:16-37. A POSA would be motivated to combine Forshew with Dong, Porreca, Hoyal, or Wessler because Forshew expressly teaches using routine and conventional techniques known in the art to analyze sample circulating tumor DNA efficiently, and the references cited herein are reasonably within the same technological field, or at the very least reasonably pertinent to the field, and disclose overlapping methods and techniques directed to the same or similar analytical end, and thus are readily interchangeable with each other. For example, a POSA would recognize that ligation-based DNA tagging methods and PCR-based DNA tagging methods are readily interchangeable as known and predictable solutions that would be obvious to select or modify as a matter of design choice, ordinary skill or common sense. Accordingly, it would be obvious to a POSA to combine the methods of the foregoing references to analyze cell-free DNA. For example, Hoyal teaches "a need for improved extraction methods capable of capturing small nucleic acid molecules"—cell-free DNA—because "the ability to successfully analyze short, fragmented DNA in the presence of more abundant, longer DNA ha[d] remained elusive." Hoyal at 1. As another example, Hoyal teaches "detecting and monitoring extracellular mutant oncogenes or tumor-associated DNA found in the plasma or serum fraction of blood by using DNA extraction with enrichment for mutant DNA," i.e., detecting cell-free tumor DNA. Hoyal at 20. Hoyal solves these problems through adapter-mediated ligation followed by universal pre-amplification before target-specific amplification. Hoyal at Figure 3. For example, Wessler teaches methods for detecting polymorphisms in DNA. Wessler at 3:6-4:41. As another example, Wessler teaches methods for "investigation of genome structure . . . by the use of in vitro methods that detect variation in DNA sequence in the genomes between members of a species" Wessler at 2:26-29. Wessler reaches this goal through Wessler's ligation-mediated preamplification followed by two target-specific rounds of PCR-mediated amplification. Wessler at Figure 1. There is a reasonable expectation of success because the techniques disclosed in Forshew and the cited references, including multiplex PCR, barcoding DNA fragments, adding sequencing tags, using multiple universal tail adapters, and performing massively parallel sequencing were all routine and conventional at the time for DNA

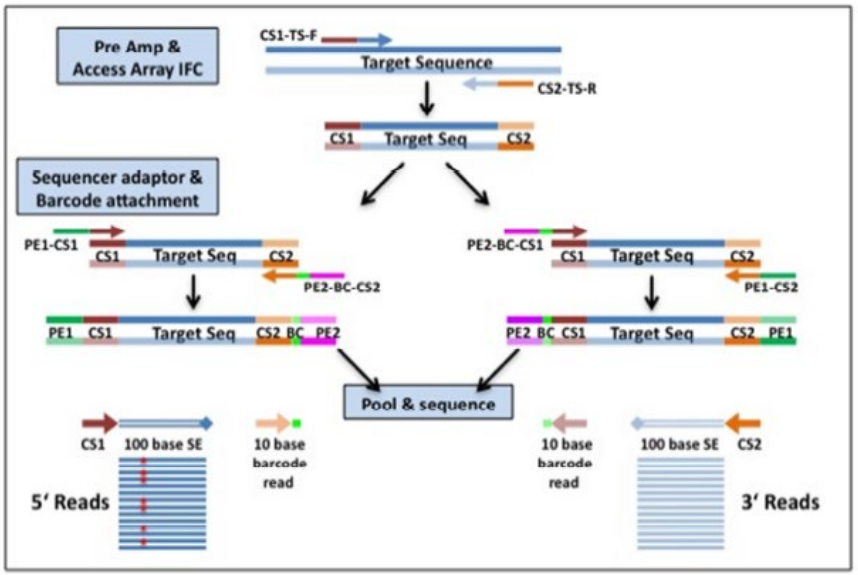
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	analysis and diagnostics. Furthermore, the methods and techniques disclosed in the cited references are readily compatible with the methods of Forshew. For example, Hoyal's method of tagging cell-free tumor DNA with universal adapters then performing universal preamplification of tagged products through PCR using primers against the adapters is readily compatible with Forshew's PCR-based tagging methods. For example, Wessler's method of ligating double-stranded universal adaptors to target DNA then performing two rounds of target-specific amplification is readily compatible with Forshew's PCR-based tagging methods.
12. The method of claim 1, wherein the one or more universal tail adaptors comprise a first universal tail adaptor and a second universal tail adaptor.	Under Natera's apparent construction, Forshew discloses one or more universal tail adaptors comprising a first universal tail adaptor and a second universal tail adaptor. *See, e.g.,* Forshew at Abstract, 2-4, Figure 1; Forshew Supp. at 2, 3, 5, 9-10, Figures S1, Table S1. For example, Forshew discloses that "[t]o amplify and sequence fragmented DNA, we designed primers to generate amplicons that tile regions of interest in short segments of about 150 to 200 bases (Fig. 1A and table S1), incorporating universal adaptors at 5′ ends (fig. S1)." Forshew at 2-3. As another example, Figure S1 of Forshew Supp. discloses that "Target-specific primers were synthesised with common adaptor sequences at their 5' ends (CS1 and CS2). These were used during the preamplification and single-plex stages to amplify the selected regions. Sequencing platform-specific adaptors and unique sample barcodes were attached during an additional round of PCR. The barcoding primers consist of Illumina sequencer adaptors (PE1 and PE2) at their 3' end, a unique 10 base barcode, and the common CS1 and CS2. Permutations of the universal tags and the sequencing adaptors enabled construction of bidirectional barcoded sequencing libraries. Using custom sequencing primers directed to the two tag sequences, sequence reads representing both strands of the amplicon pool were obtained from a single-end run. Both combinations were used in order to barcode and read amplicons in both directions. All samples were then pooled, cleaned, and then sequenced." Forshew Supp. at 5.

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13. The method of claim 12, wherein tagging the cell free DNA comprises amplifying the cell free DNA with a first primer comprising the first universal tail adaptor and a second primer comprising the second universal tail adaptor.	Under Natera's apparent construction, Forshew discloses tagging isolated cell free DNA with one or more universal tail adaptors to generate tagged products. *See, e.g.,* Forshew at Abstract, 1-5, 8, 9, 10, Figures 1, 3, Table 3; Forshew Supp. at 2, 3, 5, 9-10, 15 Figure S1, Table S1, S4. For example, Forshew discloses that "[t]o amplify and sequence fragmented DNA, we designed primers to generate amplicons that tile regions of interest in short segments of about 150 to 200 bases (Fig. 1A and table S1), incorporating universal adaptors at 5′ ends (fig. S1)." Forshew at 2-3. As another example, Figure S1 of Forshew Supp. discloses that "Target-specific primers were synthesised with common adaptor sequences at their 5' ends (CS1 and CS2). These were used during the preamplification and single-plex stages to amplify the selected regions. Sequencing platform-specific adaptors and unique sample barcodes were attached during an additional round of PCR. The barcoding primers consist of Illumina sequencer

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Claim	**Prior Art**
	adaptors (PE1 and PE2) at their 3' end, a unique 10 base barcode, and the common CS1 and CS2. Permutations of the universal tags and the sequencing adaptors enabled construction of bidirectional barcoded sequencing libraries. Using custom sequencing primers directed to the two tag sequences, sequence reads representing both strands of the amplicon pool were obtained from a single-end run. Both combinations were used in order to barcode and read amplicons in both directions. All samples were then pooled, cleaned, and then sequenced." Forshew Supp. at 5.  Natera contends this element is infringed under the doctrine of equivalents, because the differences, if any, between the claimed inventions and the Accused Products are insubstantial and the Accused Products perform the same or substantially the same functions, in the same or substantially the same ways, to achieve the same or substantially the same results as such elements. *See* Natera's Preliminary Infringement Contentions at 22. To the extent Natera argues tagging by ligation is equivalent to tagging by amplification, Forshew also discloses this element for the same reasons as discussed above regarding claim 2.

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

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

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

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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, Patel 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. *See, e.g.*, Patel at pages 1, 2, 10, 14, 16, 28 [Example 1] For example, Patel discloses methods that have "been optimized for measurement of small amounts of mutant circulating tumor DNA (ctDNA) in a background of normal (wild-type) cell-free DNA in the plasma or serum of a patient having cancer." Patel at 16. As another example, Patel discloses a method for identifying and quantifying nucleic acid variants, "particularly those available in low abundance or those obscured by an abundance of wild-type sequences" as in plasma, including by tagging and amplifying low abundance template nucleic acids from circulating tumor DNA, and by performing multiplex PCR with primers specific to mutation-prone regions associated with cancer. Patel at 2. As another example, Patel discloses that the terms "sequence variant" or "mutation" may refer to "any variation in a nucleic acid sequence including . . . single point-mutations . . . and single-nucleotide polymorphisms (SNPs)." Patel at 10.

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Claim	**Prior Art**
	As another example, Patel discloses and attempts to solve the problem that "mutant ctDNA is usually only present in small amounts [in plasma], having been previously reported to comprise an average of 0.2% of total plasma DNA." Patel. at 1. As another example, Patel discloses that "unique somatic mutations can be used to distinguish tumor-derived DNA from normal background DNA in plasma" and describes "a new class of highly specific DNA-based cancer biomarkers . . . with clinical applications that may complement those of conventional serum protein markers" and "methods that specifically and sensitively measure rare mutant DNA molecules that are shed into blood from cancer cells." Patel at 14. As another example, Patel discloses "application of a deep sequencing approach in which 3 mutation hotspot regions were analyzed from multiple plasma samples." Patel at 28, Example 1. In this application, Patel teaches that "[t]housands of clonal sequence reads from each plasma sample were compared to reference sequences in order to identify and quantify variants." Patel at 35, Example 1.
1[a]. performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;	In view of Patel, it would have been obvious to POSA to perform whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations. *See, e.g.*, 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; *see also, e.g.*, 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" 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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Claim	**Prior Art**
	"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 Va1600Glu mutation). Cells were passed in culture for no more than 6 months after being thawed from original stocks. Because cell lines were used only for analysis of short regions of genomic DNA, authentication of lines by our laboratory was limited to sequencing of those regions. To test the performance of the deep sequencing method for a particular gene, DNA derived from cells known to be either mutant or wild-type with respect to that gene was mixed in various ratios between 10,000:1 and 1:10,000. Cell line DNA samples were then amplified and sequenced according to the same methods that were used for plasma DNA." Patel at 33. "Genomic DNA was isolated from paraffin-embedded tumor tissue samples using the QuickExtractni 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.*, Patel at 34.
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	Patel 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, wherein the target loci are amplified in the same reaction volume. *See, e.g.,* Patel at 40, 52, 53, Table 9, 65, 66, 68, 70, 72 For example, Patel discloses an application of its methods in which "40 different genomic target regions were analyzed," 38 of which "were prone to developing somatic mutations." Patel at 40, Example 2. Patel discloses amplifying these 40 targets together in a multiplexed pre-amplification PCR step. Patel at 52 ("The purified primer-extension products were eluted directly into a cocktail of buffer nucleotides, and primers that was used to carry out the multiplexed pre-amplification reaction."); Patel at 53, Table 9 (listing

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Claim	**Prior Art**
the target loci are amplified together in the same reaction volume; and	"40 forward primers and the single universal reverse primer (ExtV2Rev) used for the pre-amplification reaction"). As another example, Patel alternatively discloses the method of the above example, but wherein "the final amplification was performed in a single tube using universal PCR primers." Patel at 65, Example 3. Patel discloses that, in this alternative example, "the same 40 genomic target regions were analyzed." Patel; *see also* Patel at 70 ("In contrast to the final PCRs in Example 2 which were performed separately for each genomic target site, the final PCR in the present Example was performed in a combined reaction volume for all genomic targets and for all samples."); Patel at 72 ("The highly multiplexed Round 3 PCR in which multiple gene targets from multiple samples were amplified in a single tube, resulted in the specific production of amplicons of the expected size."). Patel also discloses obtaining amplicons having a length of 50-150 bases. *See, e.g.*, Patel at Fig. 4B (showing 75bp overlapping reads through a hotspot region).
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,	The Court construed the term "sequencing the amplicons" to have its plain and ordinary meaning. Doc. 280 at 10. The Court construed the term "sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads" to require that the detecting and sequencing are part of the same step. *Id.* at 11. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Under Natera's apparent interpretation of the Court's adopted construction, Patel 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.

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Claim	**Prior Art**
	See, e.g., Patel at 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 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."); Patel at 71 ("The product of the Round 3 [multiplex] PCR was purified on a 2% agarose gel," and then "[n]ext generation sequencing was performed . . . using the Illumina HiSeq 2000 platform."); Patel at 72 ("The gel-extracted [multiplex] PCR products were subjected to next-generation sequencing using an Illumina MiSeq instrument"); Patel at 72 ("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[d]. wherein the sequencing has a depth of read of at least 50,000 per target locus.	Patel discloses the sequencing has a depth of read of at least 50,000 per target locus. *See, e.g.*, Patel at 72 ("The total number of raw clonal paired-end sequences was 20,511,389. After application of the various filters described above, the remaining sequences numbered 11,184,975. The 40 different gene target regions were fairly evenly represented among the filtered sequences. The median sequence count for the 40 gene-specific regions (all barcodes) was 166,867.").
2. The method of claim 1, wherein the cell-free DNA comprises circulating tumor DNA.	Patel discloses that the cell-free DNA comprises circulating tumor DNA. *See, e.g.*, Patel at 1 ("Tumor-derived DNA is released into the bloodstream from dying cancer cells in patients with various types of malignances. Such circulating tumor DNA (ctDNA) is showing excellent

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Claim	**Prior Art**
	promise as a non-invasive cancer biomarker."); Patel at 2 ("Low abundance template nucleic acids may be . . . circulating tumor DNA (ctDNA). . . "); Patel at 14 ("Tumor-derived DNA is released into the bloodstream from dying cancer cells in patients with various types of malignancies. Detection of circulating tumor DNA (ctDNA) has several applications . . ."); Patel at 16 ("[T]he methods describes herein have been optimized for measurement of small amounts of mutant circulating tumor DNA (ctDNA) in a background of normal (wild-type) cell-free DNA in the plasma or serum of a patient having cancer."); Patel at 37 ("A total of 117 samples were obtained from 30 patients (17 patients with adenocarcinoma, 9 with undifferentiated NSCLC, and 4 with squamous cell carcinoma. *KRAS*, Gly12Asp, Gly12Val, Gly12Cys, or Cly13Asp point-mutations were detectable in the plasma DNA of 6 patients."); Patel at 37 ("The presence or absence of *KRAS* mutations in all tested tumor samples was tested to be concordant with the findings in plasma: 5 patients had identical KRAS mutations in both tumor and plasma."); Patel at 37-39, Table 4 (listing the clinical characteristics and mutation findings for all enrolled patients); Patel at claim 8 ("The method of any one of claims 1 to 7, wherein the low abundance template nucleic acid is circulating tumor DNA (ctDNA).");
3. The method of claim 1, wherein the SNV mutations comprise one or more clonal SNV mutations.	Patel, alone or in combination with the knowledge of a POSA, discloses that the SNV mutations comprise one or more clonal SNV mutations. *See, e.g.*, Patel at 14 ("Detection of circulating tumor DNA (ctDNA) has several applications including, but not limited to, detecting presence of a malignancy, informing a prognosis, assessing treatment efficacy, tracking changes in tumor mutation status, and monitoring for disease recurrence or progression.") (emphasis added). A POSA would have understood "tracking changes in tumor mutation status" to refer to subclonal SNV mutations because it is well established in the art that cancers originate from a monoclonal composition, diverging into genomically distinct subclones. *See, e.g.*, 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 a subsequent evolution leading to selective expansions of genomically distinct subclones with distinct phenotypic properties" and citing numerous articles dating to 1976). It is well established in the art that cancer cells are assumed to originate from monoclonal compositions. *See, e.g.*, Bai et al., *Detection and Clinical Significance of Intratumoral EGFR Mutations Heterogeneity in*

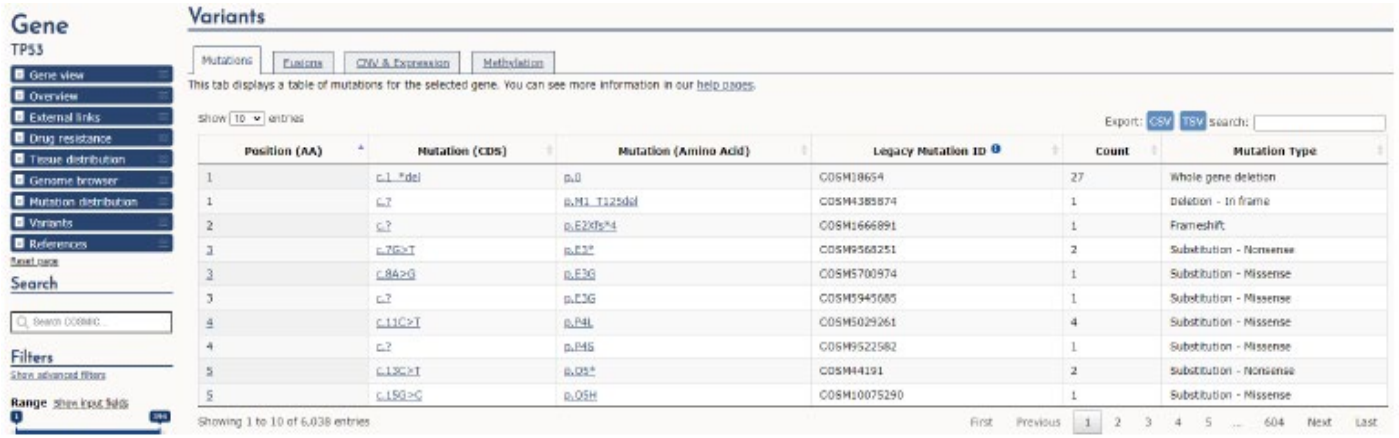
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Claim	**Prior Art**
	Chinese Patients with Advanced Non-Small Cell Lung Cancer, PLOS ONE, February 2013, Vol. 8, Iss. 2, at 1-2 ("Although all malignant cells are thought to be derived from a common precursor cell, acquired genetic instability gives rise to subsequent generations expressing unique characteristics."); Bashashati et al., *Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling*, J Pathol 2013; 231, 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, it would have been obvious to a POSA that the SNV mutations comprise one or more clonal SNV mutations.
4. The method of claim 1, wherein the SNV mutations comprise one or more subclonal SNV mutations.	Patel, alone or the knowledge of a POSA, discloses that the SNV mutations comprise one or more subclonal SNV mutations. *See, e.g.*, Patel at 14 ("Detection of circulating tumor DNA (ctDNA) has several applications including, but not limited to, detecting presence of a malignancy, informing a prognosis, assessing treatment efficacy, tracking changes in tumor mutation status, and monitoring for disease recurrence or progression.") (emphasis added). A POSA would have understood "tracking changes in tumor mutation status" to refer to subclonal SNV mutations because it is well established in the art that cancers originate from a monoclonal composition, diverging into genomically distinct subclones. *See, e.g.*, 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 a 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	Patel, alone or in combination with the knowledge of a POSA, discloses that the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations. *See* claims 3 and 4 above.

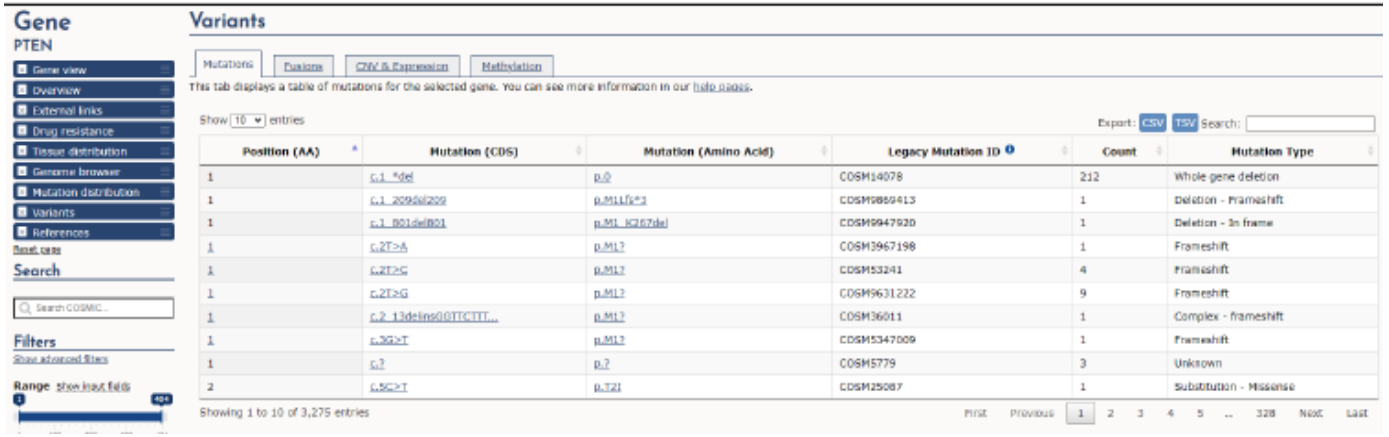
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Claim	**Prior Art**
more subclonal SNV mutations.	
6. The method of claim 1, wherein the tumor sample of the subject is a tumor tissue sample.	Patel discloses that the tumor sample of the subject is a tumor tissue sample. *See, e.g.*, Patel at 28 ("Formalin-fixed, 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.").
7. The method of claim 1, wherein the method further comprises determining clonal heterogeneity of the tumor sample.	Patel discloses determining clonal heterogeneity of the tumor sample. *See, e.g.*, claim 3 above; Patel at 14 ("Detection of circulating tumor DNA (ctDNA) has several applications including, but not limited to, . . . tracking changes in tumor mutation status."); Patel at 60-61 ("All clones belonging to a particular sample-specific bin and a particular gene segment bin were compared to the wild-type sequence in the amplicon target region. If a clonal sequence had perfect agreement between its two overlapping reads in the amplicon target region, but deviated from the wild-type sequence, then that clone was identified and counted as a 'consistent variant' . . . If a clonal sequence had deviations from the amplicon target reference sequence seen in either or both of the forward and reverse reads, but the two reads were not perfectly consistent with each other, then that clone was identified and counted as an 'inconsistent variant'."). To the extent Natera argues that Patel does not disclose determining clonal heterogeneity of the tumor sample, this limitation would have been obvious over Patel and/or the knowledge of a POSA.
8. The method of claim 1, wherein the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a	Patel discloses targeted multiplex amplification of 20 to 50 target loci each encompassing a different tumor-specific SNV mutation. *See, e.g.*, Patel at 40, Example 2 ("In this example, 40 different genomic target regions were analyzed."); Patel at 65, Example 3 ("In this example, the same 40 genomic target regions were analyzed as in Example 2.").

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Claim	**Prior Art**
different tumor-specific SNV mutation.	
9. The method of claim 1, wherein the targeted multiplex amplification amplifies 50 to 100 target loci each encompassing a different tumor-specific SNV mutation	In view of Patel, it would have been obvious to a POSA to amplify higher numbers of target loci. For example, the related '035 patent admits a "general belief in the art is 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. Furthermore, a POSA would have been motivated to perform multiplex amplification of 50 to 100 target loci in a single reaction, *e.g.,* in order to increase efficiency by amplifying more loci in a single reaction volume. Further, under Natera's apparently construction of the claim term "target loci," the analyzed genes disclosed by Patel—TP53, PTEN, EGFR, BRAF, KRAS, and PIK3CA—include thousands of variants. For example, a POSA would understand that the Catalog of Somatic Mutations in Cancer ("COSMIC") database identifies hundreds to thousands of variants within each of the genes disclosed in Patel. For example, COSMIC identifies 6,038 different variants of the TP53 gene. 

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Claim	**Prior Art**
	https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53 As another example, COSMIC identifies 3,275 different variants in the PTEN gene.  https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=PTEN *See also* https://cancer.sanger.ac.uk/cosmic/gene/analysis?1n=EGFR https://cancer.sanger.ac.uk/cosmic/gene/analysis?1n=BRAF https://cancer.sanger.ac.uk/cosmic/gene/analysis?1n=KRAS https://cancer.sanger.ac.uk/cosmic/gene/analysis?1n=PIK3CA
10. The method of claim 1, wherein the method further comprises designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations	Patel discloses designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample. *See, e.g.*, Patel at 8 ("Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified."); Patel at 29 ("Purified plasma DNA

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Claim	**Prior Art**
identified in the tumor sample.	was then subjected to 2 rounds of amplification by PCR (in triplicate) using primers designed to amplify short DNA segments that included condons 12 and 13 of *KRAS*, codon 858 of *EGRF*, and codon 600 of *BRAF*."); Patel at 35 ("By designing PCR primers that flank very short regions (<50 bp) surrounding these mutation hotspots, adequate amplification of highly fragmented plasma DNA could be ensured and greater sequence depth could be achieved.").
11. The method of claim 1, wherein the method further comprises performing barcoding PCR prior to the sequencing.	Patel discloses performing barcoding PCR prior to the sequencing. *See, e.g.*, Patel at 3 ("Use of primers synthesized from modular segments allowed early barcoding of targeted template DNA during the first round of PCR, enabling subsequent steps to be performed in a combined reaction volume."); Patel at 30 ("The nested primers were labeled with sample-specific barcode sequences to allow multiplexed sequencing of DNA from many samples."); Patel ("In order to build flexibility and scalability into the design of the deep sequencing scheme, barcoded oligonucleotides and gene-specific PCR primers were combined in a modular fashion, as illustrated in Fig. 9 . . . The sequences of the template and barcode-containing oligonucleotides are listed in Table 2.1; Patel at 31-32 (Table 2); *Patel* at 33 ("Barcoded PCR products from all samples were mixed to produce 3 separate pools" prior to ultra-deep sequencing); Patel at 35 ("Modular attachment of DNA barcode tags to the 5'-ends of the PCR primers allowed sequencing of up to 256 DNA samples in batch (Fig. 4A and Fig. 9); Patel at 46 ("As described previously, each set of gene-specific primers had a unique, sample-specific DNA sequence (a barcode) near the 5'-end of the primers that were incorporated in a modular fashion."); Patel at 57 ("An index read was also performed, and the length of the index read was increased from the standard 7cycles up to 13 cycles so that our longer custom barcodes and MLT sequences could be appropriately read."); 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."); Patel at 65 ("The reverse primers used in the first round of PCR were the modular barcoded mixtures of gene-specific primers as described above . . ."); Patel at 66 ("Forty different gene regions were targeted, and therefore a combination of 40 different biotinylated forward primers and 40 different modular barcoded gene-specific reverse primers were used in the Round 1 PCR for each sample."); Patel at 67 ("Since the PCR products now had sample-specific barcodes attached, the products of all reactions could be pooled together into a single tube.").

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Claim	**Prior Art**
12. The method of claim 1, wherein the method further comprises detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA.	Patel discloses detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. *See, e.g.*, Patel at 14 ("Detection of circulating tumor DNA (ctDNA) has several applications including, but not limited to, detecting presence of a malignancy, informing a prognosis, assessing treatment efficacy, tracking changes in tumor mutation status, and monitoring for disease recurrence or progression."); Patel at 37 (disclosing "Monitoring ctDNA levels in cancer patients"); Patel at 37-39, Table 4 (showing clinical characteristics and mutation findings in various patients); Patel at 40 ("In two cases, the ctDNA level decreased upon treatment with radiation and/or systemic therapy. Aggressive progression of metastatic disease in a different patient was accompanied by a substantial rise in ctDNA. In another two cases, ctDNA levels increased shortly after initiating treatment, perhaps because more tumor DNA was released into the bloodstream as cancer cells were being killed.").
13. The method of claim 1, wherein the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer.	Patel discloses that the cancer is lung cancer. *See, e.g.*, Patel at 28 ("[P]lasma samples were obtained from 30 patients with stage I-IV non-small cell lung cancer.").
14[pre]. A method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:	Patel 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. *See* claim 1[pre] above.

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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;	In view of Patel, it would have been obvious to a POSA to perform whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations. *See* Claim 1[a] above.
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	Patel 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. *See* claim 1[b] above.
14[c]. sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the	The Court construed the term "sequencing the amplicons" to have its plain and ordinary meaning. Doc. 280 at 10. The Court construed the term "sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads" to require that the detecting and sequencing are part of the same step. *Id.* at 11. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera

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Claim	**Prior Art**
cell-free DNA from the sequence reads,	supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Under Natera's apparent interpretation of the Court's adopted construction, Patel 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. *See* claim 1[c] above.
14[d]. wherein the method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus.	Patel discloses a method capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus. *See, e.g.*, Patel at 36, Fig. 6 ("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.").
15. The method of claim 14, wherein the sequencing has a depth of read of at least 50,000 per target locus.	Patel discloses that the sequencing has a depth of read of at least 50,000 per target locus. *See* claim 1[d] above.
16. The method of claim 14, wherein the cell-free DNA comprises circulating tumor DNA.	Patel discloses that the cell-free DNA comprises circulating tumor DNA. *See* claim 2 above.
17. The method of claim 14, wherein the SNV mutations comprise one or more clonal SNV mutations.	Patel, alone or in combination with the knowledge of a POSA, discloses that the SNV mutations comprise one or more clonal SNV mutations. *See* claim 3 above.

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Claim	**Prior Art**
18. The method of claim 14, wherein the SNV mutations comprise one or more subclonal SNV mutations.	Patel, alone or in combination with the knowledge of a POSA, discloses that the SNV mutations comprise one or more subclonal SNV mutations. *See* claim 4 above.
19. The method of claim 14, wherein the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations.	Patel, alone or in combination with the knowledge of a POSA, discloses that the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations. *See* claims 3 and 4 above.
20. The method of claim 14, wherein the tumor sample of the subject is a tumor tissue sample.	Patel discloses that the tumor sample of the subject is a tumor tissue sample. *See* claim 6 above.
21. The method of claim 14, wherein the method further comprises determining clonal heterogeneity of the tumor sample.	Patel discloses the clonal heterogeneity of the tumor sample. *See* claim 7 above.
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.	Patel discloses targeted multiplex amplification of 20 to 50 target loci each encompassing a different minor-specific SNV mutation. *See* claim 8 above.

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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.	In view of Patel, it would be obvious to a POSA to amplify higher numbers of target loci. *See* claim 9 above.
24. The method of claim 14, wherein the method further comprises designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample.	Patel discloses designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample. *See* claim 10 above.
25. The method of claim 14, wherein the method further comprises performing barcoding PCR prior to the sequencing.	Patel discloses performing barcoding PCR prior to the sequencing. *See* claim 11 above.
26. The method of claim 14, wherein the method further comprises detecting recurrence and/or metastases of the cancer from the SNV mutations	Patel discloses detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. *See* claim 12 above.

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Claim	**Prior Art**
detected in the cell-free DNA.	
27. The method of claim 14, wherein the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer.	Patel discloses that the cancer is lung cancer. *See* claim 13 above.
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 Patel, it would be obvious to a POSA to increase sensitivity by increasing sequencing depth and fidelity. *See* claims 9 and 14[d] above.

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

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 appendix and supplementary spreadsheet "Bashashati")

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

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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, Bashashati 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. *See, e.g.,* Bashashati at 22, 25; Bashashati supplementary app'x at 7. For example, Bashashati discloses that "[n]ext-generation sequencing technology has enabled the study and quantification of clonal evolution and intratumoural diversity in cancer, including inference from distributions of digital allelic representation of mutations in single samples, along with serial and regional comparisons of mutation profiles." Bashashati at 22. Bashashati also discloses that "sequencing of cell-free circulating tumour DNA (ctDNA) extracted from plasma has been demonstrated to be an effective non-invasive tool for monitoring tumour burden." Bashashati at 22. As another example, Bashashati discloses using plasma samples from a subset of patients "to identify the presence of clonally dominant and subclonal mutations through deep quenching of ctDNA." Bashashati at 22; *see also id.* ("A total of 1349 somatic mutations in all samples were confirmed with deep amplicon resequencing (median 5079-fold coverage; see supplementary material, Table S2)."); Bashashati at 25 ("Mutational events in the ancestral clone are detectable in cell-free circulating tumour DNA from plasma."); *id.* ("Plasma samples collected pre-operatively and pre-anaesthesia were available for four cases

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Claim	**Prior Art**
	from which ctDNA was extracted and subjected to targeted deep-sequencing of validated somatic mutations."). As another example, Bashashati teaches a method in which "[c]irculating tumour DNA was extracted from 1-2 ml of plasma" and then subjected to deep amplicon sequencing to validate candidate SNVs identified from initial sequencing of primary ovarian cancer samples. Bashashati supplementary app'x at 7.
1[a]. performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;	Bashashati 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. *See, e.g.,* 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.

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	A POSA would understand that this method discloses whole exome sequencing because Bashashati is using the "Human **All** Exon kit." As another example, Bashashati teaches that "[c]andidate SNVs and indels were subjected to deep sequencing using Illumina and Ion Torrent platforms" in order to validate mutations. Bashashati supplementary app'x at 6.
1[b]. performing targeted multiplex amplification to amplify 10 to 500 target loci each 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	Bashashati discloses, alone or in combination with the knowledge of a POSA, 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. *See, e.g.,* Bashashati supplementary spreadsheet at cols. T, U; Bashashati supplementary app'x at 6, 7. For example, in Case1a, Bashashati discloses 61 tumor-specific SNVs amplified in plasma. *See* Bashashati supplementary spreadsheet at cols. T, U. As another example, Bashashati teaches amplification of target loci from cell-free DNA isolated from a plasma sample. First, Bashashati states that "[c]irculating tumour DNA was extracted from 1-2 ml of plasma." Bashashati supplementary app'x at 7. Then, Bashashati teaches amplifying circulating tumor DNAQ by the method described in the Supplementary Appendix in the section titled "Deep amplicon sequencing using the Illumina MiSeq platform for cell-free DNA in the plasma," which refers back to the section titled "Deep amplicon sequencing using the Illumina GAII platform." *Id.* 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."). Bashashati also discloses obtaining amplicons having a length of 50-150 bases. *See, e.g.,* Bashashati supplementary app'x at 6.

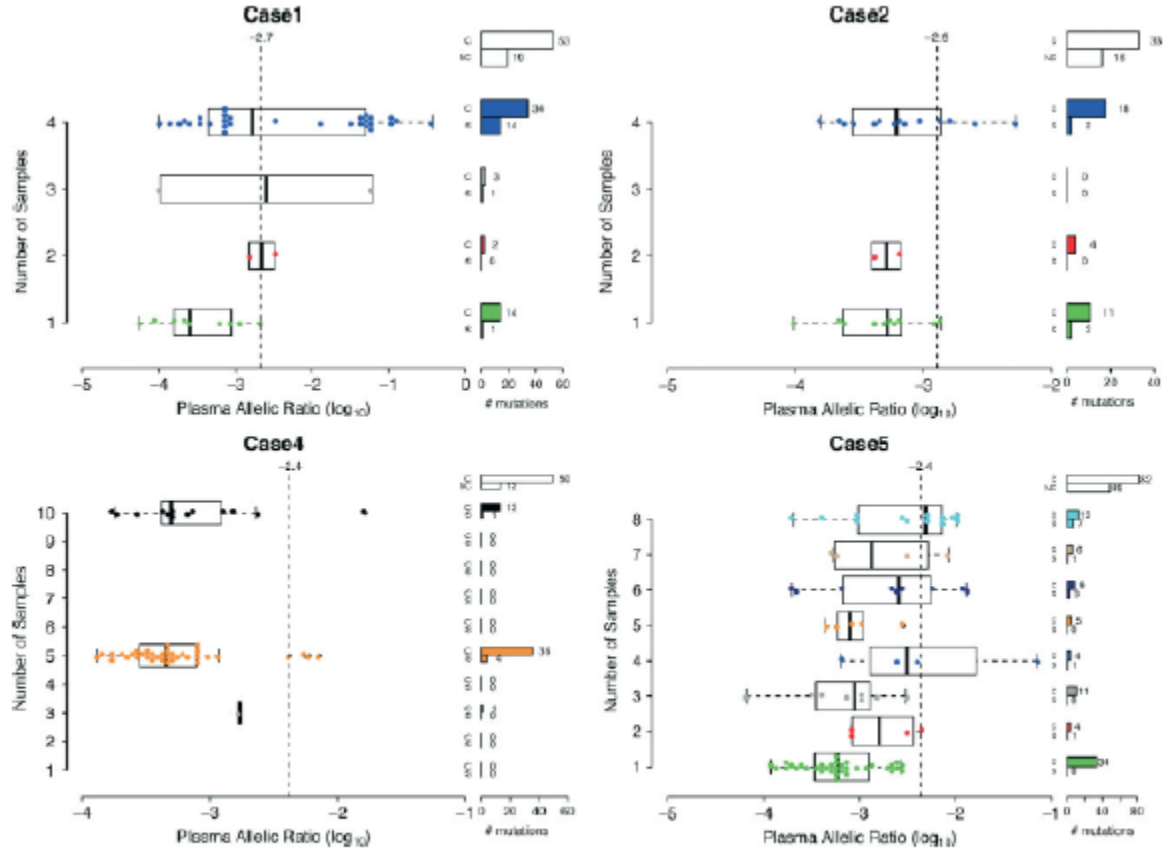
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Claim	**Prior Art**
	For example, Bashashati discloses a method in which "[p]rimer pairs were designed to place the variant position within 75bps of either end of the amplicon and to be between 50-300bp in length." Bashashati supplementary app'x at 6. A POSA would thus understand that the primer pairs could be designed to place the variant position with 75bps of both ends of the amplicon, leading to an amplicon no longer than 149 base pairs. In view of Bashashati, it would have been obvious to a POSA to amplify the target loci together in the same reaction volume. *See, e.g.,* Bashashati supplementary app'x at 6, 7. For example, Bashashati teaches a method in which "[v]alidations of candidate single nucleotide variants (SNVs) identified from the initial sequencing of the primary ovarian cancer samples were achieved using a deep amplicon sequencing approach." Bashashati supplementary app'x at 7. In this method, candidate SNVs "were fed into the LIFE Technology AmpliSeq custom designed pipeline, after conversion to hg19 coordinates, and three multiplex primers pools were generated." *Id.* Even though the targeted amplification of SNVs from ctDNA was conducted using single primer pairs per reaction, Bashashati thus teaches a POSA that SNVs can be amplified in multiplex reactions. A POSA would have been motivated to do so for the benefit of requiring fewer separate reactions, and would have had a reasonable expectation of successfully doing so because multiplex amplification was well known in the art. *See, e.g.,* Forshew at 3, 6 (Table 2), 9; Forshew supplementary materials at 2, 2 (Fig. 1B), 9 (Table S1), 17–18 (Table S6). In addition, any of the Multiplex PCR References provide motivations and a reasonable expectation of success to perform targeted multiplex amplification to amplify 10 to 500 target loci s recited in this claim limitation.
1[c]. sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in	The Court construed the term "sequencing the amplicons" to have its plain and ordinary meaning. Doc. 280 at 10. The Court construed the term "sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads" to require that the detecting and sequencing are part of the same step. *Id.* at 11. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on

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Claim	**Prior Art**
the cell-free DNA from the sequence reads,	May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Under Natera's apparent interpretation of the Court's adopted construction, Bashashati discloses sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads.. *See, e.g.,* Bashashati supplementary app'x at 6–7; Bashashati at 25, 26 (Fig. 2); Bashashati supplementary spreadsheet at col. T. For example, Bashashati discloses a method in which "[s]omatic mutation predictions were subjected to rigorous validation by targeted deep amplicon sequencing in the tumour and normal DNA templates" and in which cell-free DNA in the plasma was subjected to deep amplicon sequencing using the Illumina MiSeq platform. Bashashati supplementary app'x at 6–7. As another example, Bashashati explains that "[p]lasma samples collected pre-operatively and pre-anaesthesia were available for four cases from which ctDNA was extracted and subjected to targeted deep-sequencing of validated somatic mutations (see supplementary material, Appendix)." Bashashati at 25. Bashashati then "assayed 72 mutations in case 1, 49 in case 2, 63 in case 4 and 131 in case 5" and found that, "[a]cross the four cases, 215/312 (69%) mutations contained sequencing coverage and 38/215 (18%) were detectable with mutation allele frequency above statistical background noise." Bashashati at 25; *see also* Bashashati supplementary spreadsheet at col. T (anything with RefCount.plasma). Bashashati presents these findings in Figure 2:

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

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	The Court's Claim Construction Order states that "NeoGenomics is correct that the sequencing must be done on the amplicons obtained from the targeted multiplex amplification, not on some other set of amplicons obtained from some other source or process; the term "the" must mean something. Doc. 280 at 8. To the extent the Court's construction is interpreted to require sequencing the amplicons from the multiplex PCR step rather than sequencing only a subset of the amplicons from the multiplex PCR step, this would have been obvious in view of Bashashati. For example, Bashashati teaches constructing sequencing libraries "using the SPRIworks Fragment Library System I with no on-board size selection." Bashashati at 7.
1[d]. wherein the sequencing has a depth of read of at least 50,000 per target locus.	Bashashati discloses a sequencing depth of read of at least 50,000 per target locus. *See, e.g.,* Bashashati supplementary spreadsheet at cols. U, T (showing depth often in excess of 50,000, with a maximum depth of 969,361 and an average depth of 62,692).
2. The method of claim 1, wherein the cell-free DNA comprises circulating tumor DNA.	Bashashati discloses that cell-free DNA comprises circulating tumor DNA. *See, e.g.,* Bashahati at 22 ("[S]equencing of cell-free circulating tumour DNA (ctDNA) extracted from plasma has been demonstrated to be an effective non-invasive tool for monitoring tumour burden."); Bashashati at 25 ("Mutational events in the ancestral clone are detectable in cell-free circulating tumour DNA from plasma."); Bashashati at 26, Figure 2 (showing "[d]eep sequencing results of cell-free circulating tumour DNA from the plasma.").
3. The method of claim 1, wherein the SNV mutations comprise one or more clonal SNV mutations.	Bashashati discloses or renders obvious to a POSA that the SNV mutations comprise one or more clonal SNV mutations. *See, e.g.,* Bashashati at Abstract, 22, 24, 25, 28, 32, Fig. 1, Supplementary Appx at 10. *See* 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."); *see also id.*

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	("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.") Bashashanti also discloses, "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." Bashashati at 22.
4. The method of claim 1, wherein the SNV mutations comprise one or more subclonal SNV mutations.	Bashashati discloses or renders obvious to a POSA that the SNV mutations comprise one or more subclonal SNV mutations. *See* claim 3, above. *See also* Bashashati at 22, 25, 26, 27-32, Figs. 1-5; Supplementary App'x at 10. *See, e.g.,* Bashashati at 22 ("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 25 ("Mutational events in the ancestral clone are detectable in cell-free circulating tumour DNA from plasma."); Bashashati at 26 ("Observations of subclonal NF1 deletions reinforced evidence from the mutation data that important driver mutations may be acquired late in the evolutionary history of the tumour."); *see also, e.g.*, Bashashati at 25 ("Mutational event in the ancestral clone are detectable in cell-free circulating tumour DNA from plasma"); Bashashati at 25 ("The intratumoural variation in genomic architectures of HGSCs"); Bashashati at 27 ("The origins of fallopian tube lesions and reconstruction of HGSC evolutionary histories"); Bashashati at 29 ("Intra-sample clonal diversity revealed by cellular frequency analysis of mutational profiles"); Bashashati at 30 ("Diverse genomic landscapes associate with heterogeneous transcriptional profiles"); Bashashati at 31 ("Genomic aberrations impacting transcriptional networks").
5. The method of claim 1, wherein the SNV mutations comprise one or more clonal SNV mutations and one or	Bashashati discloses or renders obvious to a POSA that the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations.

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more subclonal SNV mutations.	*See* claims 3 and 4 above.
6. The method of claim 1, wherein the tumor sample of the subject is a tumor tissue sample.	Bashashati discloses that the tumor sample of the subject is a tumor tissue sample. *See, e.g.,* Bashashati at 22, Table 1 ("Tissue was obtained from a total of 31 tumour sites (range 3–10 samples/patient, including two fallopian tube lesions) with matched normal DAN for each patient (Table 1)."); Bashashati at 1 ("The 19 tumour samples were used as index discovery samples to identify sets of mutations for interrogation by deep amplicon sequencing . . .").
7. The method of claim 1, wherein the method further comprises determining clonal heterogeneity of the tumor sample.	Bashashati discloses or renders obvious to a POSA to determine clonal heterogeneity of the tumor sample. Bashashati teaches determining clonal heterogeneity of tumors in its disclosure of characterizing and monitoring tumor cells in cancer patients. *See, e.g.*, Bashashati at 28 ("Results from PIK3CA mutation sequencing suggested the [fallopian tube] lesion in case 4 was composed of heterogeneous subclones" with further analysis revealing "two cooexisting populations of nuclei in the [fallopian tube] lesion"); Bashashati at 27–28 (determining a fallopian tube lesion harbored mutations not present in all samples and determining phylogenetic relationships, and determining likely origin on particular tumor as metastatic implant). Bashashati also shows that a POSA would have appreciated the benefit of monitoring clonal heterogeneity of the tumor sample for improved treatment. *See* Bashashati at Abstract ("[W]e observed mixed cell populations in the early fallopian tube lesion . . . Our results revealed that [high-grade serous ovarian cancers] 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 and investigation of drug-resistance mechanisms."). *See also, e.g.,* Bashashati at 28 (analyzing a fallopian tube lesion and determining that it "was composed of heterogeneous subclones" with further analysis revealing "two coexisting populations" in the lesion.); Bashashati at 25 (tracking these different mutations in cfDNA using personalized NGS assays, thus monitoring clonal heterogeneity, and explaining that "Cases 1 and 5 were enriched for the presence of mutations in the plasma that were also present in all intrapatient tumour samples, and thus part of the

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	ancestral clone (Fisher exact test $p < 0.05$; Figure 2)"); *See, e.g.,* Bashashati at 22 ("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 25 ("Mutational events in the ancestral clone are detectable in cell-free circulating tumour DNA from plasma."); Bashashati at 26 ("Observations of subclonal NF1 deletions reinforced evidence from the mutation data that important driver mutations may be acquired late in the evolutionary history of the tumour."); *see also, e.g.*, Bashashati at 25 ("Mutational event in the ancestral clone are detectable in cell-free circulating tumour DNA from plasma"); Bashashati at 25 ("The intratumoural variation in genomic architectures of HGSCs"); Bashashati at 27 ("The origins of fallopian tube lesions and reconstruction of HGSC evolutionary histories"); Bashashati at 29 ("Intra-sample clonal diversity revealed by cellular frequency analysis of mutational profiles"); Bashashati at 30 ("Diverse genomic landscapes associate with heterogeneous transcriptional profiles"); Bashashati at 31 ("Genomic aberrations impacting transcriptional networks").
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.	Bashashati discloses, alone or in combination with the knowledge of a POSA, performing targeted multiplex amplification to amplify 20 to 50 target loci each encompassing a different tumor-specific SNV mutation. *See, e.g.,* Bashashati supplementary spreadsheet at cols. T, U (teaching amplification of 61 tumor-specific SNVs in Case 1a); Bashashati at 25 ("Plasma samples collected pre-operatively and pre-anaesthesia were available for four cases from which ctDNA was extracted and subjected to targeted deep-sequencing of validated somatic mutations (see supplementary material, Appendix). We assayed 72 mutations in case 1, 49 in case 2, 63 in case 4 and 131 in case 5."); Bashashati supplementary app'x at 6 ("Candidate SNVs and indels were subjected to deep sequencing using Illumina and Ion Torrent platforms."). *See also* claim 1[b] above.
9. The method of claim 1, wherein the targeted multiplex amplification	Bashashati discloses performing targeted multiplex amplification to amplify 50 to 100 target loci each encompassing a different tumor-specific SNV mutation.

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amplifies 50 to 100 target loci each encompassing a different tumor-specific SNV mutation.	*See, e.g.*, Bashashati at 25 ("Plasma samples collected pre-operatively and pre-anaesthesia were available for four cases from which ctDNA was extracted and subjected to targeted deep-sequencing of validated somatic mutations (see supplementary material, Appendix). We assayed 72 mutations in case 1, 49 in case 2, 63 in case 4 and 131 in case 5."); Bashashati supplementary app'x at 6 ("Candidate SNVs and indels were subjected to deep sequencing using Illumina and Ion Torrent platforms."). *See also* claim 1[b] above.
10. The method of claim 1, wherein the method further comprises designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample.	Bashashati discloses designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample. *See, e.g.,* Bashashati supplementary app'x at 6 ("Primer pairs were designed to place the variant position within 75bps of either end of the amplicon and to be between 50-300bp in length."); Bashashati supplementary app'x at 7 ("The primers were designed to generate 100bp amplicons to accommodate the sequencing of DNA from formalin-fixed paraffin-embedded (FFPE) tissues."); Bashashati supplementary app'x at 7 ("DNA primers were synthesized in 96-well plates at a 25nmol scale with standard desalting (IDT Coralville, IA USA).")
11. The method of claim 1, wherein the method further comprises performing barcoding PCR prior to the sequencing.	Bashashati discloses performing barcording PCR prior to the sequencing. *See, e.g.,* Bashashati supplementary app'x at 7 ("Adapter-ligated products were purified on Qiaquick spin columns (Qiagen, Valencia, CA, USA) and PCR-amplified using Phusion DNA polymerase (NEB, Ipswich, MA, USA) in 10 cycles using PE primer 1.0 (Illumina) and a custom multiplexing PCR Primer [5 CAAGCAGAAGACGGCATACGAGAT NNNNNNCGGTCTCGGCATTCCTGC TGAACCGCTCTTCCGATCT-3] where NNNNNN was replaced with unique fault tolerant hexamer barcodes for each template . . . Between the paired 75bp reads a third 7 base pair read was performed using the following custom sequencing primer [5-GATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCG] to sequence the hexamer barcode.").

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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.	Bashashati discloses detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. *See, e.g.,* Bashashati at 22 ("[T]he divergence of clonal cell populations confers a heterogeneous genomic tapestry with implications for clinical endpoints, including the acquisition of metastatic potential."); Bashashati at 27 ("The FT lesion in case 5 harbored seven mutations that were not present in all samples, indicating that the clones comprising case 5h were unlikely to have been ancestral to the tumours . . . We suggest that case 5h is therefore more likely to be a metastatic implant in the FT rather than a precursor or early lesion."); Bashashati at 22 ("In case 6, we compared a primary sample to a recurrence obtained 42 months later, following multiple chemotherapeutic regimens (total 21 cycles) (see supplementary material, Figure S7).").
13. The method of claim 1, wherein the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer.	Bashashati discloses or renders obvious wherein the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer. For example, the References cited by Bashashati disclose the implementation of related techniques for colorectal cancer and breast cancer. Bashashati at 33; Bashashati Supplementary Appendix at 12. To the extent that Bashashati does not disclose this limitation, it would have been obvious to a POSA to apply the techniques and methods of Bashashati to any of colorectal cancer, lung cancer, bladder cancer, or breast cancer based on the teachings of Bashashati and the knowledge of a POSA. For example, Bashashati teaches that clonal diversity affect the progression and treatment of "ovarian carcinoma and other primary cancers." Colorectal cancer, lung cancer, bladder cancers, and breast cancer are also examples of primary cancers. Accordingly, a POSA would be motivated to study these other cancers for the same reasons that Bashashati studied ovarian cancer. And a POSA would have a reasonable expectation of success analyzing other cancers because a POSA would understand that the techniques Bashashati used to analyze ovarian cancer would be equally applicable to other primary cancers.

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U.S. Patent No. 11,530,454	
Claim	**Prior Art**
14[pre]. A method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:	To the extent the preamble is construed to be limiting, Bashashati 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. *See* claim 1[pre] above.
14[a]. performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;	Bashashati 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. *See* claim 1[a] above.
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	Bashashati 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. *See* claim 1[b] above.

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together in the same reaction volume; and	
14[c]. sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads,	The Court construed the term "sequencing the amplicons" to have its plain and ordinary meaning. Doc. 280 at 10. The Court construed the term "sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads" to require that the detecting and sequencing are part of the same step. *Id.* at 11. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Under Natera's apparent interpretation of the Court's adopted construction, Bashashati discloses sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads. *See* claim 1[c] above.
14[d]. wherein the method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus.	Bashashati discloses a method capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus. *See, e.g.,* Bashashati supplementary spreadsheet at cols. U and T (AltCount.Plasma and RefCount.plasma will give VAF, which is less than 0.015% in some cases).
15. The method of claim 14, wherein the sequencing has a depth of read of at least 50,000 per target locus.	Bashashati discloses the sequencing has a depth of read of at least 50,000 per target locus. *See* claim 1[d] above.

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Claim	**Prior Art**
16. The method of claim 14, wherein the cell-free DNA comprises circulating tumor DNA.	Bashashati discloses that cell-free DNA comprises circulating tumor DNA. *See* claim 2 above.
17. The method of claim 14, wherein the SNV mutations comprise one or more clonal SNV mutations.	Bashashati discloses or renders obvious to a POSA that the SNV mutations comprise one or more clonal SNV mutations. *See* claim 3 above.
18. The method of claim 14, wherein the SNV mutations comprise one or more subclonal SNV mutations.	Bashashati discloses or renders obvious to a POSA that the SNV mutations comprise one or more subclonal SNV mutations. *See* claim 4 above.
19. The method of claim 14, wherein the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations.	Bashashati discloses or renders obvious to a POSA that the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations. *See* claim 5 above.
20. The method of claim 14, wherein the tumor sample of the subject is a tumor tissue sample.	Bashashati discloses that the tumor sample of the subject is a tumor tissue sample. *See* claim 6 above.
21. The method of claim 14, wherein the method further comprises determining	Bashashati discloses or renders obvious to a POSA that the method further comprises determining clonal heterogeneity of the tumor sample.

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Claim	**Prior Art**
clonal heterogeneity of the tumor sample.	*See* claim 7 above.
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.	Bashashati discloses, alone or in combination with the knowledge of a POSA, performing targeted multiplex amplification to amplify 20 to 50 target loci each encompassing a different tumor-specific SNV mutation. *See* claim 8 above.
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.	Bashashati discloses, alone or in combination with the knowledge of a POSA, performing targeted multiplex amplification to amplify 50 to 100 target loci each encompassing a different tumor-specific SNV mutation. *See* claim 9 above.
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.	Bashashati discloses designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample. *See* claim 10 above.
25. The method of claim 14, wherein the method further comprises performing	Bashashati discloses performing barcording PCR prior to the sequencing. *See* claim 11 above.

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Claim	**Prior Art**
barcoding PCR prior to the sequencing.	
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.	Bashashati discloses detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. *See* claim 12 above.
27. The method of claim 14, wherein the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer.	Bashashati discloses or renders obvious wherein the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer. *See* claim 13 above.
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.	Bashashati discloses a method capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus. *See* claim 14[d] above.

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EXHIBIT 2-C — FINAL 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):36RA68 (May 2012) (Forshew)

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

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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. *See, e.g.,* 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 7 ("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.e.,* SNVs).

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Claim	**Prior Art**
1[a]. performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;	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. *See, e.g.,* 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.e.,* SNVs)); Forshew at 3 (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 *TP53* by Sanger sequencing"). Because *TP53* is a tumor suppressor gene associated with cancer, a POSA would have understood that Sanger sequencing to identify mutations in *TP53* constitutes identification of tumor-specific SNV mutations. Forshew 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)." Forshew at 3. Forshew identifies 40 single-base substitutions (*i.e.*, SNVs) in the ovarian tumor sample. Forshew 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	Forshew 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. *See, e.g.,* Forshew 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

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Claim	**Prior Art**
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	(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"); Forshew 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)."); Forshew supplemental materials at 2 ("Preamplification methods 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."); Forshew at 2, Fig. 1B (depicting the targeted multiplex 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; *see also* 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. *See* 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. *See* 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; *see also id.* at 17-18 (Table S6); Forshew at 6 (Table 2).

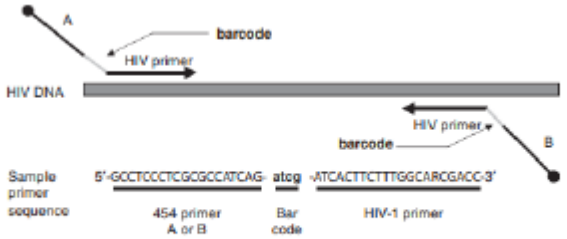
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Claim	**Prior Art**
1[c]. sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads,	The Court construed the term "sequencing the amplicons" to have its plain and ordinary meaning. Doc. 280 at 10. The Court construed the term "sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads" to require that the detecting and sequencing are part of the same step. *Id.* at 11. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Under Natera's apparent interpretation of the Court's adopted construction, 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. *See, e.g.,* 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."). Forshew further discloses analyzing the sequence data to detect tumor-specific SNV mutations by using a bioinformatics pipeline. *See* Forshew supplemental materials at 4 ("De-multiplexing and alignment," "Mutation identification," and "Mutation detection"); *see also* Forshew at 5, Table 1 (identifying tumor-specific SNV mutations in plasma samples from seven ovarian cancer patients); Forshew supplemental materials at 19, Table S7 ("[S]ubsequent data analysis was performed using the CASAVA 1.8 analysis pipeline (Illumina) to identify somatic mutations."). The Court's Claim Construction Order states that "NeoGenomics is correct that the sequencing must be done on the amplicons obtained from the targeted multiplex amplification, not on some other set of amplicons obtained from some other source or process; the term "the" must mean something. Doc. 280 at 8. To the extent the Court's construction is interpreted to require sequencing the amplicons from the

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	multiplex PCR step rather than sequencing only a subset of the amplicons from the multiplex PCR step, such a modification of Forshew would have been obvious to a person of ordinary skill in the art. Forshew teaches that the multiplex PCR step was designed to avoid unwanted side products. *See* Forshew 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."); Forshew 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, Forshew 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 amplification using a single amplification reaction step can be used to attach sequencing platform-specific barcodes prior to sequencing. *See, e.g.,* Hoffman et al., *DNA bar coding and pyrosequencing to identify rare HIV drug resistance mutations*, Nucleic Acids Research, 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); *id.* at 4, Figure 2 (showing the single amplification step, reproduced below).

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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, *e.g.*, because higher read depth could allow for enhanced detection of lower levels of mutation without change to protocols. *See* Forshew at 10. 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 order of magnitude lower frequencies." Forshew at 10; *see also* 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." For example, Forshew teaches: "Sequencing adaptors were separately attached at either end and the products mixed together, such that single-end sequencing generated separate sets of forward and reverse reads. We performed 100-base single-end sequencing (GAIIx sequencer, Illumina), with an additional 10 cycles using the

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	barcode sequencing primer, generating ~30 million reads per lane. This produced an average readdepth of 3250 for each of 96 barcoded samples for 48 amplicons read in two possible orientations." Forshew at 3. The read depth for a single replicate of a given locus would therefore be 6,500 per replicate, or 13,000 across the two replicates used for TAm-Seq. *See* Forshew at 3, 9, 7, FIG. 4D legend ("TAm-Seq results are reported as the mean frequency of duplicate analyses"), 4, FIG. 2 legend ("measured mutant AF (average of both repeats)"), Forshew Supplementary Information at 4, 17, 18. Accordingly, Forshew teaches both the motivation and reasonable expectation of success to increase the read depth to at least 50,000 per target locus. Further evidence of motivation and reasonable expectation of success to increase the read depth to at least 50,000 per target locus is found in NEOGEN00275278–NEOGEN00275334 and NEOGEN00275270–NEOGEN0027527. *See, e.g.*, NEOGEN00275326 (describing tables plotting the "number of reads for each barcode according to which block of 48 barcodes it belongs to") and NEOGEN00275329 (providing read counts of greater than 50,000 for some loci (e.g., in columns labeled FLD0200, FLD0217, FLD0233, FLD0234, FLD0235, FLD0236, FLD0237, FLD0238, and FLD0239)).
2. The method of claim 1, wherein the cell-free DNA comprises circulating tumor DNA.	Forshew discloses that the cell-free DNA comprises circulating tumor DNA. *See, e.g.,* Forshew at Abstract ("Plasma of cancer patients contains cell-free tumor DNA that carries information on tumor mutations and tumor burden."); *see also, e.g.*, Forshew 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	Forshew 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. *See, e.g.,* Bai et al., *Detection and Clinical Significance of Intratumoral EGFR*

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Claim	**Prior Art**
more clonal SNV mutations.	*Mutations Heterogeneity in Chinese Patients with Advanced Non-Small Cell Lung Cancer*, 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 et al., *Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling*, J Pathol 2013; 231, 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. Forshew 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." *See, e.g.*, Forshew at 1. 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. Forshew 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 draft." Forshew at 10. Forshew 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)." Forshew at 1. 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. Further evidence of motivation and reasonable expectation of success to detect clonal SNV mutations is found in NEOGEN00275297 ("I think by comparing two genes in the way you suggested we should

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	indeed be able to follow tumour evolution"). *See also id.* ("we are thinking of how this might be used to monitor the tumour heterogeneity and clonal selection during treatment").
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. *See, e.g.,* 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 subloncal DNA. Forshew at 1. This challenge necessarily implies the existence of both clonal and subclonal DNA within a tumor. *See, e.g.,* Forshew at 7, Fig. 4A (disclosing a subclonal SNV mutation by identifying a particular *EGFR* mutation present in only two regions from the omental mass, unlike the *TP53* 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. *See, e.g.,* 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 a subsequent evolution leading to selective expansions of genomically distinct subclones with distinct phenotypic properties" and citing numerous articles dating to 1976). Further evidence of motivation and reasonable expectation of success to detect subclonal SNV mutations is found in NEOGEN00275297 ("I think by comparing two genes in the way you suggested we should indeed be able to follow tumour evolution"). *See also id.* ("we are thinking of how this might be used to monitor the tumour heterogeneity and clonal selection during treatment").

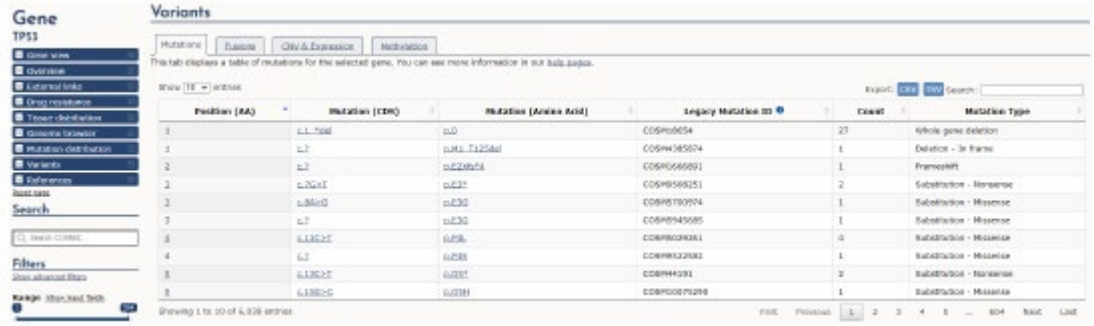
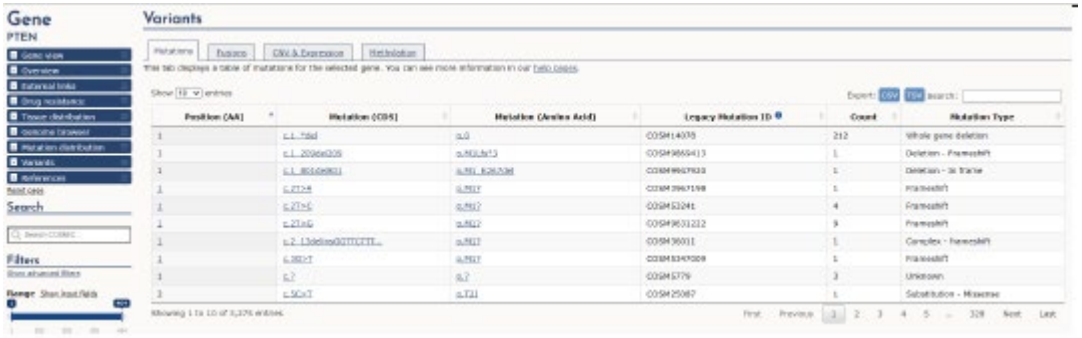
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5. The method of claim 1, wherein the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations.	Forshew discloses that the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations. *See* claims 3 and 4 above. *See, e.g.,* Forshew at 7, Fig. 4A, 9, 10. Further evidence of motivation and reasonable expectation of success to detect one or more clonal SNV mutations and one or more subclonal SNV mutations is found in NEOGEN00275297 ("I think by comparing two genes in the way you suggested we should indeed be able to follow tumour evolution"). *See also id.* ("we are thinking of how this might be used to monitor the tumour heterogeneity and clonal selection during treatment").
6. The method of claim 1, 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. *See, e.g.,* Forshew at 9 ("Whole-genome sequencing of tumor material was used to identify tumor mutations in a patient with breast cancer undergoing two phases of chemotherapy."); Forshew 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.	Forshew discloses that the method further comprises determining clonal heterogeneity of the tumor sample. *See, e.g.,* Forshew 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."); Forshew at 1 (explaining that "mutation profiles in different metastatic clones can differ significantly from each other or from the parent tumor."); Forshew at 7, Fig. 4A (determining the heterogeneity of tumor samples from an ovarian cancer patient at relapse and disclosing that the *TP53* mutation could be in the left and right ovaries and omental mass at different frequencies). Forshew also teaches determining allele frequency in the ovarian cancer formalin-fixed, paraffin-embedded (FFPE) tissue samples. *See, e.g.,* Forshew at 4, Figure 2. As another example, Forshew teaches detecting an *EGFR* mutation in plasma, which "was not found in the ovarian mass

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	removed by interval debulking surgery 15 months before the blood sample was collected." Forshew at 4. Forshew further discloses that "[the] EGFR mutation was detected in the two omental samples above the 0.99 confidence margin (fig. S3) at AF of 0.7%, but was not detected in the six ovarian samples (below the 0.8 confidence margin)." *Id.* Further evidence of motivation and reasonable expectation of success to determine clonal heterogeneity of the tumor sample is found in NEOGEN00275297 ("we are thinking of how this might be used to monitor the tumour heterogeneity and clonal selection during treatment").
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.	Forshew discloses that the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation. *See, e.g.,* Forshew at 2 (performing targeted multiplex amplification to amplify 20 and 48 target loci); Forshew 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 *TP53* and *PTEN*, as well as selected regions in *EGFR*, *BRAF*, *KRAS*, and *PIK3CA* by overlapping short amplicons); Forshew at 6 (Table 2); Forshew supplemental materials at 17-18 (Table S6); Forshew 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 at 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. *See, e.g.,* Forshew at 2, Fig. 1A (using TAm-Seq with 48 sequence-specific primer pairs to amplify 5995 bases of genomic sequence covering coding regions of *TP53* and *PTEN*, and selected regions in *EGFR*, *BRAF*, *KRAS*, and *PIK3CA* by overlapping short amplicons); Forshew supplemental materials at 9–10, Table S1; Forshew at 6 (Table 2); Forshew supplemental materials at 17-18 (Table S6); Forshew 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, Forshew renders obvious amplifying higher numbers of target loci.

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	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 Forshew. 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.  https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=PTEN

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	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 In addition, the related '035 patent admits a POSA's "general belief in the art is 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.
10. The method of claim 1, wherein the method further comprises designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample.	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. *See, e.g.,* Forshew supplemental materials at 2 ("Target-specific primers were designed with universal primer sequences (termed CS1 and CS2) appended at the 5'-end (table S1)."); Forshew at 2, Fig. 1 ("Primers were designed to amplify regions of interest in overlapping short amplicons."); Forshew supplemental materials at 2 (Primer design for Tam-Seq); Forshew at 2 (Figure 1B showing the "initial preamplification step was performed for 15 cycles using a poos of target-specific primer pairs.").
11. The method of claim 1, 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. *See, e.g.,* Forshew at 2, Fig. 1 ("[S]ample-specific barcodes were attached to the harvested amplicons in a further PCR."); Forshew 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."); Forshew supplemental materials at 5, Figure S1; Forshew supplemental materials at 11, Table S2 ("Platform-specific adaptors and barcodes are attached through PCR following the single-plex amplification step.").

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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.	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. *See, e.g.,* Forshew 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."); Forshew 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 *EGFR* (exon 21), at AF of 6% (patient 27, Table 1)."); Forshew 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.	Forshew discloses the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer. *See, e.g.,* 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.")
14[pre]. A method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:	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, the method comprising. *See* claim 1[pre] above.

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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. *See* claim 1[a] above.
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. *See* claim 1[b] above.
14[c]. sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the	The Court construed the term "sequencing the amplicons" to have its plain and ordinary meaning. Doc. 280 at 10. The Court construed the term "sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads" to require that the detecting and sequencing are part of the same step. *Id.* at 11. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right

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cell-free DNA from the sequence reads,	to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Under Natera's apparent interpretation of the Court's adopted construction, 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. *See* claim 1[c] above.
14[d]. wherein the method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus.	In view of Forshew 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. *See* claim 1[d] above. *See, e.g.,* Forshew at 3 ("Background frequencies of nonreference reads were ~0.1% (median, 0.03%; mean, 0.2%; in keeping with Q30 quality threshold applied)"); Forshew 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); Forshew at 9 ("Enhanced detection down to 1 mutant allele in 10,000 or more wild-type alleles can be obtained."). As to the degree of sensitivity, Forshew specifically teaches the importance of increasing sensitivity and how to improve sensitivity of the TAm-Seq assay, including by increasing sequencing depth and fidelity. Forshew at 10. Further evidence of motivation and reasonable expectation of success is found in NEOGEN00275278–NEOGEN00275334 and NEOGEN00275270–NEOGEN0027527. As an example, NEOGEN00275278 describes experiments performed by Forshew around March 2011 demonstrating successful detection of TP53 mutations in cell-free DNA. For example, NEOGEN00275278 states: "Last week we successfully identified the same mutation by sequencing cell free DNA from the blood directly. To do this we extracted the DNA from blood plasma at two different

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	treatment time points. We then pre amplified the DNA using primers to a number of genes including TP53. We then put this preamplified DNA on a Fluidigm Access Array before sequencing with an Illumina sequencer. We were able to sequence all of TP53 and PTEN and a number of other regions. Through this we identified the same mutation. In the first time point we found that 17% of this DNA was mutated (336/2014 reads). At the second time point this had dropped to 1% (21/2614 reads)." NEOGEN00275326 and NEOGEN00275329 provide further evidence of motivation and reasonable expectation of success for increased read depths. *See, e.g.*, NEOGEN00275326 (describing tables plotting the "number of reads for each barcode according to which block of 48 barcodes it belongs to") and NEOGEN00275329 (providing read counts of greater than 50,000 for some loci (e.g., in columns labeled FLD0200, FLD0233, FLD0234, FLD0235, FLD0236, FLD0237, FLD0238, and FLD0239). As Forshew specifically teaches, "[h]igher read depth or fidelity, additional replicates, or improved algorithms could allow for enhanced mutation detection without change to protocols." Forshew at 10. *See also* Forshew at 3 ("The minimal nonreference AFs that could be detected depend on the read depth and background rates of nonreference reads… for example, a minimum of 10 reads implies that sequencing depth of 5000 would be required to detect mutations at AF as low as 0.2%.").
15. The method of claim 14, 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. *See* claim 1[d] above.
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. *See* claim 2 above.

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Claim	**Prior Art**
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. *See* claim 3 above.
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. *See* claim 4 above.
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. *See* claim 5 above.
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. *See* claim 6 above.
21. The method of claim 14, wherein the method further comprises determining clonal	Forshew discloses that the method further comprises determining clonal heterogeneity of the tumor sample. *See* claim 7 above.

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heterogeneity of the tumor sample.	
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. *See* claim 8 above.
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. *See* claim 9 above.
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. *See* claim 10 above.

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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. *See* claim 11 above.
26. The method of claim 14, wherein the method further comprises detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA.	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. *See* claim 12 above.
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. *See* claim 13 above.
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. *See* claims 1[d] and 14[d] above.

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

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

Reference: Bai, H. et al., *Detection and Clinical Significance of Intratumoral EGFR Mutational Heterogeneity in Chinese Patients with Advanced Non-Small Cell Lung Cancer*, PLOS ONE Vol. 8 Iss. 2 (Feb. 2013) (Bai)

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 appendix and supplementary spreadsheet "Bashashati")

Reference: Forshew, T. et al., *Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA* 4(136):36RA68 (May 2012) (Forshew)

Reference: Page, K. et al., *Influence of Plasma Processing on Recovery and Analysis of Circulating Nucleic Acids*, PLOS ONE, Vol.8, Iss. 10 (Oct. 18, 2013) (Page)

Reference: Dawson, S. et al., *Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer*, N Engl J Med 368;13 (Mar. 28, 2013) (Dawson)

Reference: U.S. Patent No. 11,384,394 (issued Jul. 12, 2022) (Bartha)

Reference: U.S. Patent Application Publication No. 2016/0032396 (published Feb. 4, 2016) (Diehn)

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

Motivation to Combine

To the extent Natera asserts that Patel does not disclose any limitation of Asserted Claims 1-28, it would have been obvious to combine Patel with any of the other prior art mentioned herein. A POSA would have considered the disclosures of the aforementioned

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prior art because the prior art references all concern the same subject matter, namely amplification and/or sequencing of DNA for the purpose of identifying mutations therein.

A POSA would be motivated to combine Patel with the whole genome sequencing approach disclosed by Forshew at the time the '454 patent was filed because, as Forshew explains, "identification of rare mutations in tumor suppressor genes such as TP53, which are widely mutated in cancers but lack a well-defined hotspot region, remains an elusive goal." Forshew at 9. Additionally, Forshew discloses that "[s]creening of entire genes for mutations [is] useful for some applications" because "mutant alleles can reach a sizable fraction of DNA" in patients with advanced cancers. Id.

Further, a POSA would have a reasonable expectation of success to modify the methods of Patel with whole exome sequencing or whole genome sequencing as taught by prior art like Forshew because the prior art such as Forshew, Bartha, Diehn, and Dawson taught a POSA how to perform whole genome or whole exome sequencing to identify genomic or exomic regions of interest.

A POSA would have been motivated to combine Patel with Bashashati, Bai, and/or the knowledge of a POSA because a POSA would have understood that to monitor and track cancer effectively requires identifying the various categories of cancer-related mutations, including clonal mutations and subclonal mutations. A POSA would have had a reasonable expectation of successfully doing so because Patel, Bashashati, and Bai teach detection of clonal (and subclonal) mutations in tumor and cfDNA.

A POSA would be motivated to modify the method of Patel with the method of Forshew to determine clonal heterogeneity because it would be useful for monitoring and tracking cancer to gain insight into clonal evolution and compensate for mutational drift. A POSA would have a reasonable expectation of success because Forshew and Patel are directed to very similar methods of detecting and tracking cancer.

A POSA would have been motivate to combine Patel with Page. For example, Page teaches 207 amplicons amplified in a single reaction mixture. *See, e.g.*, Page at 2, 3. A POSA would be motivated to apply the method disclosed by Patel to amplify at least 100 target loci at the time the '454 patent was filed because this would have increased efficiency and obtained more amplicons in a single operation. In addition, there is reasonable expectation of success for a POSA to combine Patel with references such as Page because Patel discloses methods that "utilize next-generation, high-throughput DNA sequencing technologies to identify and quantify nucleic acid variants." Patel at 17.

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

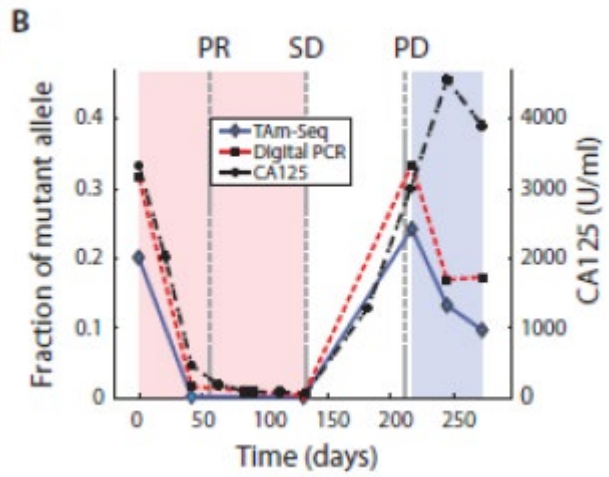
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	As another example, Patel discloses "application of a deep sequencing approach in which 3 mutation hotspot regions were analyzed from multiple plasma samples." Patel at 28, Example 1. In this application, Patel teaches that "[t]housands of clonal sequence reads from each plasma sample were compared to reference sequences in order to identify and quantify variants." Patel at 35, Example 1.
1[a]. performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;	In view of Patel, it would have been obvious to a POSA to perform whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations. *See, e.g.*, 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; *see also, e.g.*, 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.e.*, because mutations are only present at a low frequency) and "Confirmation of mutations in tumor tissue" to identify a plurality of tumor-specific SNV mutation that should be identified in cell free DNA. "Genomic DNA was purified from human cancer cell lines using the same method used for purifying plasma DNA, after suspending cells in 0.2 ml of phosphate-buffered saline. The following cell lines were used: A549 (having a KRAS Gly12Ser mutation), H1957 (having an EGFR Leu858Arg mutation), and YUSAC (having a BRAF Val600Glu mutation). Cells were passed in culture for no more than 6 months after being thawed from original stocks. Because cell lines were used only for analysis of short regions of genomic DNA, authentication of lines by our laboratory was limited to sequencing of those regions. To test the performance of the deep sequencing method for a particular gene, DNA derived from cells known to be either mutant or wild-type with respect to that gene was

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	mixed in various ratios between 10,000:1 and 1:10,000. Cell line DNA samples were then amplified and sequenced according to the same methods that were used for plasma DNA." Patel at 33. "Genomic DNA was isolated from paraffin-embedded tumor tissue samples using the QuickExtract™ FFPE DNA Extraction Kit (Epicentre Biotechnologies, Madison, WI). Mutation hotspot regions of 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.*, Patel at 34. To the extent Natera asserts that Patel does not disclose any portion of the limitations requiring whole exome sequencing or whole genome sequencing on a tumor sample, it would have been obvious to a POSA at the time of the alleged invention considering Patel in combination with the knowledge of a POSA and/or references such as Forshew, which teaches the advantages of whole exome sequencing or whole genome sequencing to identify SNV mutations for tracking in patient plasma samples. A POSA would have had a reasonable expectation of success because whole genome sequencing and whole exome are well known technologies that can be used to identify variations in cancer samples. *See, e.g.,* Forshew at 9, 10. A POSA would be motivated to modify the method of Patel with the whole genome sequencing approach disclosed by Forshew at the time the '454 patent was filed because, as Forshew explains, "identification of rare mutations in tumor suppressor genes such as *TP53*, which hare widely mutated in cancers but lack a well-defined hotspot region, remains an elusive goal." Forshew at 9. Additionally, Forshew discloses that "[s]creening of entire genes for mutations [is] useful for some applications" because "mutant alleles can reach a sizable fraction of DNA" in patients with advanced cancers. *Id.* *See also* Forshew at 7, Figure 4B (monitoring frequency of mutant DNA in plasma of an ovarian cancer patient over time using TAm-Seq and digital PCR, based on SNV mutations identified via whole genome sequencing of tumor material) (reproduced below).

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	 Further, a POSA would have a reasonable expectation of success to modify the methods of Patel with whole exome sequencing or whole genome sequencing as taught by prior art like Forshew. For example, Forshew teaches that performing whole-genome or whole-exome sequencing was well known in the art. *See, e.g.*, Forshew at 9 ("Advances in massively parallel sequencing make new approaches possible. These have largely focused on large-scale analyses, including whole-genome or whole-exome sequencing."). Additionally, Patel discloses a method comprising identification of SNVs in plasma, followed by confirming the mutations in tumor tissue. *See, e.g.*, Patel at 33. In view of Patel, Forshew, and/or the knowledge of a POSA, it would have been obvious to modify this method to identify mutations first from a tumor tissue sample, followed by tracking those mutations in patient plasma. A POSA would have been motivated to do so because of the known benefits of using whole genome sequencing to identify mutations, discussed above. Further, a POSA would have reasonably expected to succeed because "[a]dvances in massively parallel sequencing make new approaches possible," such as "whole

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	genome or whole-exome sequencing" to identify SNV mutations to track in plasma." *See, e.g.*, Forshew at 9. Also, a POSA would reasonably have expected to succeed because the prior art such as Forshew, Bartha, Diehn, and Dawson taught a POSA how to perform whole genome or whole exome sequencing to identify genomic or exomic regions of interest. *See, e.g.*, Forshew at 7, Figure 4B; Bartha at 78:54–79:23; 80:18–21 (claiming a method comprising a tumor-informed assay); 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."); Dawson at 1199 ("We used targeted or whole-genome sequencing to identify somatic genomic alterations and designed personalized assays to quantify circulating tumor DNA in serially collected plasma specimens.").
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	Patel 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, wherein the target loci are amplified in the same reaction volume. *See, e.g.*, Patel at 40, 52, 53, Table 9, 65, 66, 68, 70, 72. For example, Patel discloses an application of its methods in which "40 different genomic target regions were analyzed," 38 of which "were prone to developing somatic mutations." Patel at 40, Example 2. Patel discloses amplifying these 40 targets together in a multiplexed pre-amplification PCR step. Patel at 52 ("The purified primer-extension products were eluted directly into a cocktail of buffer nucleotides, and primers that was used to carry out the multiplexed pre-amplification reaction."); Patel at 53, Table 9 (listing "40 forward primers and the single universal reverse primer (ExtV2Rev) used for the pre-amplification reaction").

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amplified together in the same reaction volume; and	As another example, Patel alternatively discloses the method of the above example, but wherein "the final amplification was performed in a single tube using universal PCR primers." Patel at 65, Example 3. Patel discloses that, in this alternative example, "the same 40 genomic target regions were analyzed." Patel; *see also* Patel at 70 ("In contrast to the final PCRs in Example 2 which were performed separately for each genomic target site, the final PCR in the present Example was performed in a combined reaction volume for all genomic targets and for all samples."); Patel at 72 ("The highly multiplexed Round 3 PCR in which multiple gene targets from multiple samples were amplified in a single tube, resulted in the specific production of amplicons of the expected size."). Patel also discloses obtaining amplicons having a length of 50-150 bases. *See, e.g.*, Patel at Fig. 4B (showing 75bp overlapping reads through a hotspot region).
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,	The Court construed the term "sequencing the amplicons" to have its plain and ordinary meaning. Doc. 280 at 10. The Court construed the term "sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads" to require that the detecting and sequencing are part of the same step. *Id.* at 11. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Under Natera's apparent interpretation of the Court's adopted construction, Patel 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. *See, e.g.*, Patel at 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

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	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 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."); Patel at 71 ("The product of the Round 3 [multiplex] PCR was purified on a 2% agarose gel," and then "[n]ext generation sequencing was performed . . . using the Illumina HiSeq 2000 platform."); *Patel* at 72 ("The gel-extracted [multiplex] PCR products were subjected to next-generation sequencing using an Illumina MiSeq instrument."); Patel at 72 ("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[d]. wherein the sequencing has a depth of read of at least 50,000 per target locus.	Patel discloses the sequencing has a depth of read of at least 50,000 per target locus. *See, e.g.,* Patel at 72 ("The total number of raw clonal paired-end sequences was 20,511,389. After application of the various filters described above, the remaining sequences numbered 11,184,975. The 40 different gene target regions were fairly evenly represented among the filtered sequences. The median sequence count for the 40 gene-specific regions (all barcodes) was 166,867.").
2. The method of claim 1, wherein the cell-free DNA comprises circulating tumor DNA.	Patel discloses that the cell-free DNA comprises circulating tumor DNA. *See, e.g.*, Patel at 1 ("Tumor-derived DNA is released into the bloodstream from dying cancer cells in patients with various types of malignances. Such circulating tumor DNA (ctDNA) is showing excellent promise as a non-invasive cancer biomarker."); Patel at 2 ("Low abundance template nucleic acids may be . . . circulating tumor DNA (ctDNA). . . "); Patel at 14 ("Tumor-derived DNA is released into the bloodstream from dying cancer cells in patients with various types of malignancies. Detection

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	of circulating tumor DNA (ctDNA) has several applications . . ."); Patel at16 ("[T]he methods describes herein have been optimized for measurement of small amounts of mutant circulating tumor DNA (ctDNA) in a background of normal (wild-type) cell-free DNA in the plasma or serum of a patient having cancer."); Patel at 37 ("A total of 117 samples were obtained from 30 patients (17 patients with adenocarcinoma, 9 with undifferentiated NSCLC, and 4 with squamous cell carcinoma. *KRAS*, Gly12Asp, Gly12Val, Gly12Cys, or Cly13Asp point-mutations were detectable in the plasma DNA of 6 patients."); Patel at 37 ("The presence or absence of *KRAS* mutations in all tested tumor samples was tested to be concordant with the findings in plasma: 5 patients had identical *KRAS* mutations in both tumor and plasma."); Patel at 37–39, Table 4 (listing the clinical characteristics and mutation findings for all enrolled patients); Patel at claim 8 ("The method of any one of claims 1 to 7, wherein the low abundance template nucleic acid is circulating tumor DNA (ctDNA).");
3. The method of claim 1, wherein the SNV mutations comprise one or more clonal SNV mutations.	In view of Patel, Bashashati, Bai, Forshew, and/or the knowledge of a POSA, it would have been obvious that the SNV mutations comprise one or more clonal SNV mutations. *See, e.g.*, Patel at 14 ("Detection of circulating tumor DNA (ctDNA) has several applications including, but not limited to, detecting presence of a malignancy, informing a prognosis, assessing treatment efficacy, tracking changes in tumor mutation status, and monitoring for disease recurrence or progression.") (emphasis added). A POSA would have understood "tracking changes in tumor mutation status" to refer to subclonal SNV mutations because it is well established in the art that cancers originate from a monoclonal composition, diverging into genomically distinct subclones. *See, e.g.*, 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 a subsequent evolution leading to selective expansions of genomically distinct subclones with distinct phenotypic properties" and citing numerous articles dating to 1976). Further, a POSA would have understood that to monitor and track cancer effectively requires identifying the various categories of cancer-related mutations, including clonal mutations. *See*

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	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."); Bashashati at 22 (reporting the distribution of different mutations (including clonal mutations) within the tumor and explaining that a "panel of shared, identical mutations, indicative of a single ancestral clonal origin, was evident."); Bai 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."); Bai at 1 (explaining that tumorigenesis "is a multistage process by which monoclonal cancer cells gradually become heterogeneous owing to clonal evolution and genetic/epigenetic instability"). Thus, a POSA would have been motivated to identify and detect clonal mutations (as well as subclonal mutations) to effectively track and monitor cancer, and would have had a reasonable expectation of successfully doing so because Bashashati and Bai teach detection of clonal (and subclonal) mutations in tumor and cfDNA. Further, clonal variations are easier to track because they exist at a higher frequency in plasma, providing further motivation to combine the methods of Patel, Bashashati, Bai, and/or Forshew. *See, e.g.*, Bashashati at 22; Bai at 1–2; Forshew at 1.
4. The method of claim 1, wherein the SNV mutations comprise one or more subclonal SNV mutations.	Patel, alone or the knowledge of a POSA, discloses that the SNV mutations comprise one or more subclonal SNV mutations. *See, e.g.*, Patel at 14 ("Detection of circulating tumor DNA (ctDNA) has several applications including, but not limited to, detecting presence of a malignancy, informing a prognosis, assessing treatment efficacy, tracking changes in tumor mutation status, and monitoring for disease recurrence or progression.") (emphasis added). A POSA would have understood "tracking changes in tumor mutation status" to refer to subclonal SNV mutations because it is well established in the art that cancers originate from a monoclonal composition, diverging into genomically distinct subclones. *See, e.g.*, 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 a subsequent evolution leading to

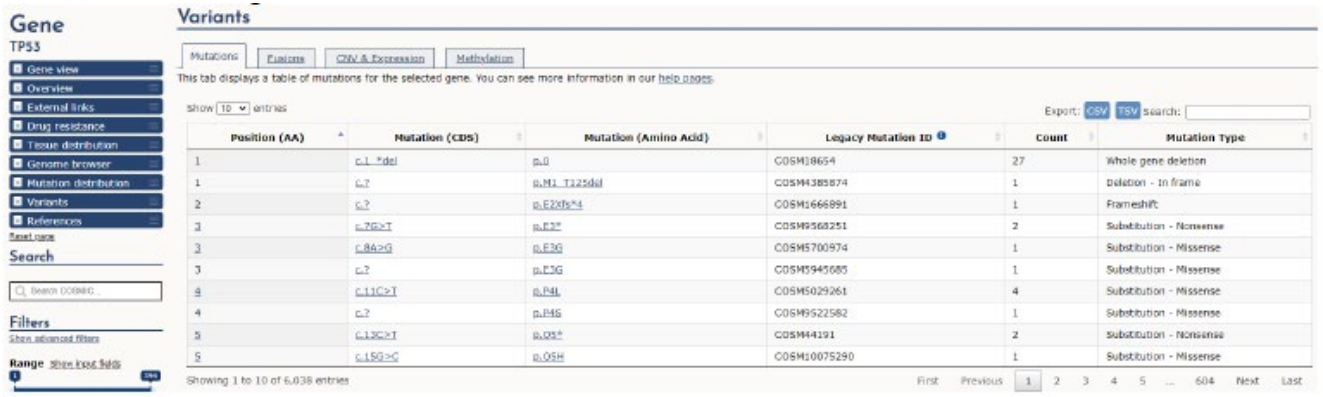
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	selective expansions of genomically distinct subclones with distinct phenotypic properties" and citing numerous articles dating to 1976).
5. The method of claim 1, wherein the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations.	In view of Patel, Bashashati, Bai, and/or the knowledge of a POSA, it would have been obvious that that the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations. *See* claims 3 and 4 above.
6. The method of claim 1, wherein the tumor sample of the subject is a tumor tissue sample.	Patel discloses that the tumor sample of the subject is a tumor tissue sample. *See, e.g.*, 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."). To the extent Natera contends that Patel does not disclose the limitation of tracking mutations in plasma identified from the tumor sample, it would have been obvious to do so in view of Patel, Forshew, and the knowledge of a POSA. *See* Claim 1[a], above.
7. The method of claim 1, wherein the method further comprises determining clonal heterogeneity of the tumor sample.	Patel discloses determining clonal heterogeneity of the tumor sample. *See, e.g.*, claim 3 above; Patel at 14 ("Detection of circulating tumor DNA (ctDNA) has several applications including, but not limited to, . . . tracking changes in tumor mutation status."); Patel at 60–61 ("All clones belonging to a particular sample-specific bin and a particular gene segment bin were compared to the wild-type sequence in the amplicon target region. If a clonal sequence had perfect agreement between its two overlapping reads in the amplicon target region, but deviated from the wild-type sequence, then that clone was identified and counted as a 'consistent variant' . . . If a

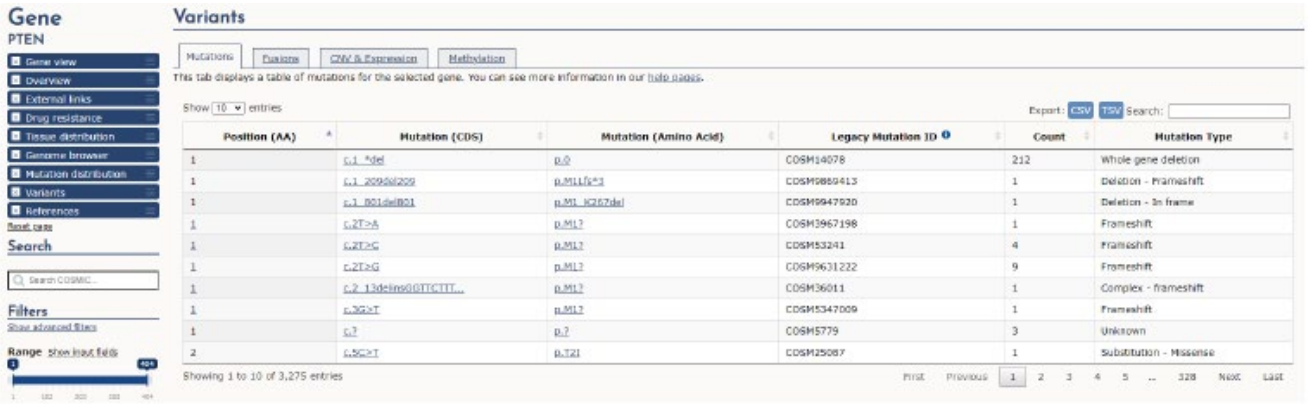
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	clonal sequence had deviations from the amplicon target reference sequence seen in either or both of the forward and reverse reads, but the two reads were not perfectly consistent with each other, then that clone was identified and counted as an 'inconsistent variant'."). To the extent Natera argues that Patel does not disclose determining clonal heterogeneity of the tumor sample, this limitation would have been obvious over Patel in view of Forshew. Forshew 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."); Forshew at 1 (explaining that "mutation profiles in different metastatic clones can differ significantly from each other or from the parent tumor."). A POSA would be motivated to modify the method of Patel with the method of Forshew to determine clonal heterogeneity because it would be useful to gain insight into clonal evolution and compensate for mutational drift. *See, e.g.*, Forshew at 10 ("For example, if a patient has only five copies of a mutant allele per milliliter of plasma (on average), there is a 37% probability that this mutation will not be present in a 0.2-ml sample, and even a perfect assay will fail to detect residual tumor, whereas a method that measures multiple mutations in parallel can have a low likelihood of a false-negative result even if the detection rate for each mutation is less than 50%."). Further, a POSA would reasonably have expected to succeed because it was well known in the art that a method like Patel's could be modified to include a determination of clonal heterogeneity. *See, e.g.*, Forshew at 10; Bashashati at 25.
8. The method of claim 1, wherein the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation.	Patel discloses targeted multiplex amplification of 20 to 50 target loci each encompassing a different tumor-specific SNV mutation. *See, e.g.*, Patel at 40, Example 2 ("In this example, 40 different genomic target regions were analyzed."); Patel at 65, Example 3 ("In this example, the same 40 genomic target regions were analyzed as in Example 2.").

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9. The method of claim 1, wherein the targeted multiplex amplification amplifies 50 to 100 target loci each encompassing a different tumor-specific SNV mutation.	In view of Patel, Forshew, and/or the knowledge of a POSA, it would have been obvious to a person of ordinary skill in the art to amplify higher numbers of target loci. It is well established in the art that amplifying more loci can "increase [] the robustness for tumor monitoring by compensating for effects of sampling noise or mutational drift." *See* Forshew at 10. In addition, the related '035 patent admits a "general belief in the art is 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. Further, under Natera's apparently construction of the claim term "target loci," the analyzed genes disclosed by Patel—TP53, PTEN, EGFR, BRAF, KRAS, and PIK3CA—include thousands of variants. For example, a POSA would understand that the Catalog of Somatic Mutations in Cancer ("COSMIC") database identifies hundreds to thousands of variants within each of the genes disclosed in Patel. For example, COSMIC identifies 6,038 different variants of the TP53 gene.  Gene TP53 Gene view Overview External links Drug resistance Tissue distribution Genome browser Mutation distribution Variants References Search Filters Range 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 Export: CSV TSV Search: Position (AA) | Mutation (CDS) | Mutation (Amino Acid) | Legacy Mutation ID | Count | Mutation Type 1 | c.1_*del | p.0 | COSM18654 | 27 | Whole gene deletion 1 | c.? | p.M1_T125del | COSM4388874 | 1 | Deletion - In frame 2 | c.? | p.E2Xfs*4 | COSM1666891 | 1 | Frameshift 3 | c.7G>T | p.E3* | COSM9568251 | 2 | Substitution - Nonsense 3 | c.8A>G | p.E3G | COSM5700974 | 1 | Substitution - Missense 3 | c.? | p.E3G | COSM5945685 | 1 | Substitution - Missense 4 | c.11C>T | p.P4L | COSM5029261 | 4 | Substitution - Missense 4 | c.? | p.P4S | COSM9522582 | 1 | Substitution - Missense 5 | c.13C>T | p.Q5* | COSM44191 | 2 | Substitution - Nonsense 5 | c.15G>C | p.Q5H | COSM10075290 | 1 | Substitution - Missense Showing 1 to 10 of 6,038 entries First Previous 1 2 3 4 5 ... 604 Next Last https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53

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	As another example, COSMIC identifies 3,275 different variants in the PTEN gene.  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 To the extent Patel does not specifically teach targeted multiplex amplification of 50 to 100 target loci, it would have been obvious to a POSA to do so in view of the knowledge of a POSA or in view of references such as Page. For example, Page teaches 207 amplicons amplified in a single reaction mixture. *See, e.g.*, Page at 2, 3. A POSA would be motivated to apply the method disclosed by Patel to amplify at least 100 target loci at the time the '454 patent was filed because this would have increased efficiency and obtained more amplicons in a single operation. In addition, there is reasonable expectation of success for a POSA to combine Patel with references such as Page because

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	Patel discloses methods that "utilize next-generation, high-throughput DNA sequencing technologies to identify and quantify nucleic acid variants." Patel at 17.
10. The method of claim 1, wherein the method further comprises designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample.	Patel discloses designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample. *See, e.g.*, Patel at 8 ("Generally, sequence information from the ends of the region of interest or beyond needs to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified."); Patel at 29 ("Purified plasma DNA was then subjected to 2 rounds of amplification by PCR (in triplicate) using primers designed to amplify short DNA segments that included condons 12 and 13 of *KRAS*, codon 858 of *EGRF*, and codon 600 of *BRAF*."); Patel at 35 ("By designing PCR primers that flank very short regions (<50 bp) surrounding these mutation hotspots, adequate amplification of highly fragmented plasma DNA could be ensured and greater sequence depth could be achieved.").
11. The method of claim 1, wherein the method further comprises performing barcoding PCR prior to the sequencing.	Patel discloses performing barcoding PCR prior to the sequencing. *See, e.g.*, Patel at 3 ("Use of primers synthesized from modular segments allowed early barcoding of targeted template DNA during the first round of PCR, enabling subsequent steps to be performed in a combined reaction volume."); Patel at 30 ("The nested primers were labeled with sample-specific barcode sequences to allow multiplexed sequencing of DNA from many samples."); Patel ("In order to build flexibility and scalability into the design of the deep sequencing scheme, barcoded oligonucleotides and gene-specific PCR primers were combined in a modular fashion, as illustrated in Fig. 9 . . . The sequences of the template and barcode-containing oligonucleotides are listed in Table 2."); Patel at 31–32 (Table 2); *Patel* at 33 ("Barcoded PCR products from all samples were mixed to produce 3 separate pools" prior to ultra-deep sequencing); Patel at 35 ("Modular attachment of DNA barcode tags to the 5'-ends of the PCR primers allowed sequencing of up to 256 DNA samples in batch (Fig. 4A and Fig. 9); Patel at 46 ("As described previously, each set of gene-specific primers had a unique, sample-specific DNA sequence (a barcode) near the 5'-end of the primers that were incorporated in a modular fashion."); Patel at 57 ("An index read was also performed, and the length

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	of the index read was increased from the standard 7cycles up to 13 cycles so that our longer custom barcodes and MLT sequences could be appropriately read."); 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."); Patel at 65 ("The reverse primers used in the first round of PCR were the modular barcoded mixtures of gene-specific primers as described above . . ."); Patel at 66 ("Forty different gene regions were targeted, and therefore a combination of 40 different biotinylated forward primers and 40 different modular barcoded gene-specific reverse primers were used in the Round 1 PCR for each sample."); Patel at 67 ("Since the PCR products now had sample-specific barcodes attached, the products of all reactions could be pooled together into a single tube.").
12. The method of claim 1, wherein the method further comprises detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA.	Patel discloses detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. *See, e.g.,* Patel at 14 ("Detection of circulating tumor DNA (ctDNA) has several applications including, but not limited to, detecting presence of a malignancy, informing a prognosis, assessing treatment efficacy, tracking changes in tumor mutation status, and monitoring for disease recurrence or progression."); Patel at 37 (disclosing "Monitoring ctDNA levels in cancer patients"); Patel at 37–39, Table 4 (showing clinical characteristics and mutation findings in various patients); Patel at 40 ("In two cases, the ctDNA level decreased upon treatment with radiation and/or systemic therapy. Aggressive progression of metastatic disease in a different patient was accompanied by a substantial rise in ctDNA. In another two cases, ctDNA levels increased shortly after initiating treatment, perhaps because more tumor DNA was released into the bloodstream as cancer cells were being killed.").
13. The method of claim 1, wherein the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer.	Patel discloses that the cancer is lung cancer. *See, e.g.,* Patel at 28 ("[P]lasma samples were obtained from 30 patients with stage I-IV non-small cell lung cancer.").

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14[pre]. A method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:	Patel 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. *See* claim 1[pre] above.
14[a]. performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;	In view of Patel, Forshew, and/or the knowledge of a POSA, it would have been obvious to perform whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations. *See* claim 1[a] above.
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	Patel 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. *See* claim 1[b] above.

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the target loci are amplified together in the same reaction volume; and	
14[c]. sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads,	The Court construed the term "sequencing the amplicons" to have its plain and ordinary meaning. Doc. 280 at 10. The Court construed the term "sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads" to require that the detecting and sequencing are part of the same step. *Id.* at 11. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Under Natera's apparent interpretation of the Court's adopted construction, Patel 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. *See* claim 1[c] above.
14[d]. wherein the method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus.	Patel discloses a method capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus. *See, e.g.,* Patel at 36, Fig. 6 ("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.").
15. The method of claim 14, wherein the sequencing has a depth of	Patel discloses that the sequencing has a depth of read of at least 50,000 per target locus.

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read of at least 50,000 per target locus.	*See* claim 1[d] above.
16. The method of claim 14, wherein the cell-free DNA comprises circulating tumor DNA.	Patel discloses that the cell-free DNA comprises circulating tumor DNA. *See* claim 2 above.
17. The method of claim 14, wherein the SNV mutations comprise one or more clonal SNV mutations.	In view of Patel, Bashashati, Bai, and/or the knowledge of a POSA, it would have been obvious to a person of ordinary skill in the art that the SNV mutations comprise one or more clonal SNV mutations. *See* claim 3 above.
18. The method of claim 14, wherein the SNV mutations comprise one or more subclonal SNV mutations.	In view of Patel, Bashashati, Bai, and/or the knowledge of a POSA, it would have been obvious to a person of ordinary skill in the art that the SNV mutations comprise one or more subclonal SNV mutations. *See* claim 4 above.
19. The method of claim 14, wherein the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations.	In view of Patel, Bashashati, Bai, and/or the knowledge of a POSA, it would have been obvious to a person of ordinary skill in the art that the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations. *See* claims 3 and 4 above.
20. The method of claim 14, wherein the tumor sample of the	Patel discloses that the tumor sample of the subject is a tumor tissue sample. *See* claim 6 above.

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Claim	**Prior Art**
subject is a tumor tissue sample.	
21. The method of claim 14, wherein the method further comprises determining clonal heterogeneity of the tumor sample.	In view of Forshew, Patel discloses and renders obvious to a person of ordinary skill in the art determining clonal heterogeneity of the tumor sample. *See* claim 7 above.
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.	Patel discloses targeted multiplex amplification of 20 to 50 target loci each encompassing a different tumor-specific SNV mutation. *See* claim 8 above.
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.	In view of Patel, Forshew, and/or the knowledge of a POSA, it would have been obvious to a person of ordinary skill in the art to amplify higher numbers of target loci. *See* claim 9 above.
24. The method of claim 14, wherein the	Patel discloses designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample.

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Claim	**Prior Art**
method further comprises designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample.	*See* claim 10 above.
25. The method of claim 14, wherein the method further comprises performing barcoding PCR prior to the sequencing.	Patel discloses performing barcoding PCR prior to the sequencing. *See* claim 11 above.
26. The method of claim 14, wherein the method further comprises detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA.	Patel discloses detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. *See* claim 12 above.
27. The method of claim 14, wherein the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer.	Patel discloses that the cancer is lung cancer. *See* claim 13 above.
28. The method of claim 14, wherein the	In view of Patel, it would be obvious to a person of ordinary skill in the art to increase sensitivity by increasing sequencing depth and fidelity.

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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.	*See* claims 9 and 14[d] above.

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

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 appendix and supplementary spreadsheet "Bashashati")

Reference: Bai, H. et al., *Detection and Clinical Significance of Intratumoral EGFR Mutational Heterogeneity in Chinese Patients with Advanced Non-Small Cell Lung Cancer*, PLOS ONE Vol. 8 Iss. 2 (Feb. 2013) (Bai)

Reference: Forshew, T. et al., *Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA* 4(136):36RA68 (May 2012) (Forshew)

Reference: Page, K. et al., *Influence of Plasma Processing on Recovery and Analysis of Circulating Nucleic Acids*, PLOS ONE, Vol.8, Iss. 10 (Oct. 18, 2013) (Page)

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)

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

Motivation to Combine

To the extent Natera asserts that Bashashati does not disclose any limitation of Asserted Claims 1-28, it would have been obvious to combine Bashashti with any of the other prior art mentioned herein. A POSA would have considered the disclosures of the aforementioned prior art because the prior art references all concern the same subject matter, namely amplification and/or sequencing of DNA for the purpose of identifying mutations therein.

A POSA would have been motivated to combine the methods of Bashashati, Forshew, and Page to amplify at least 100 target loci at the time the '454 patent was filed because this would have increased efficiency and obtained more amplicons in a single operation. In addition, a POSA would reasonably have expected to succeed because Forshew discloses that more loci would "increase [] the robustness for tumor monitoring by compensating for effects of sampling noise or mutational drift." Forshew at 10.

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A POSA would have understood that to monitor and track cancer effectively requires identifying the various categories of cancer-related mutations, including clonal mutations. Thus, a POSA would have been motivated to identify and detect clonal mutations (as well as subclonal mutations) to effectively track and monitor cancer, and would have had a reasonable expectation of successfully doing so because Bashashati and Bai teach detection of clonal (and subclonal) mutations in tumor and cfDNA.

A POSA would have been motivated to track and detect subclonal mutations because they are an integral part of cancer tumors and knowledge about them would inform diagnosis, monitoring, and treatment. A POSA would have had a reasonable expectation of successfully doing so because Bashashati identified and tracked subclonal mutations. Further, a POSA would have understood "tracking changes in tumor mutation status" to refer to subclonal SNV mutations because it is well established in the art that cancers originate from a monoclonal composition, diverging into genomically distinct subclones.

A POSA would have been motivated to combine the methods of Bashashati, Forshew, and/or Bai because determining the clonal heterogeneity of a tumor would have provided a more complete picture of the genetic makeup of the cancer, which would have aided in the task of monitoring and tracking the cancer and allowed for improved treatment. A POSA would have had a reasonable expectation of success because determining clonal heterogeneity and the benefits of doing so for improved monitoring and treatment were well known in the art.

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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, Bashashati 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. *See, e.g.,* Bashashati at 22, 25; Bashashati supplementary app'x at 7. For example, Bashashati discloses that "[n]ext-generation sequencing technology has enabled the study and quantification of clonal evolution and intratumoural diversity in cancer, including inference from distributions of digital allelic representation of mutations in single samples, along with serial and regional comparisons of mutation profiles." Bashashati at 22. Bashashati also discloses that "sequencing of cell-free circulating tumour DNA (ctDNA) extracted from plasma has been demonstrated to be an effective non-invasive tool for monitoring tumour burden." Bashashati at 22.

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Claim	**Prior Art**
	As another example, Bashashati discloses using plasma samples from a subset of patients "to identify the presence of clonally dominant and subclonal mutations through deep quenching of ctDNA." Bashashati at 22; *see also id.* ("A total of 1349 somatic mutations in all samples were confirmed with deep amplicon resequencing (median 5079-fold coverage; see supplementary material, Table S2)."); Bashashati at 25 ("Mutational events in the ancestral clone are detectable in cell-free circulating tumour DNA from plasma."); *id.* ("Plasma samples collected pre-operatively and pre-anaesthesia were available for four cases from which ctDNA was extracted and subjected to targeted deep-sequencing of validated somatic mutations."). As another example, Bashashati teaches a method in which "[c]irculating tumour DNA was extracted from 1-2 ml of plasma" and then subjected to deep amplicon sequencing to validate candidate SNVs identified from initial sequencing of primary ovarian cancer samples. Bashashati suppmentary app'x at 7.
1[a]. performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;	Bashashati 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. *See, e.g.,* 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.

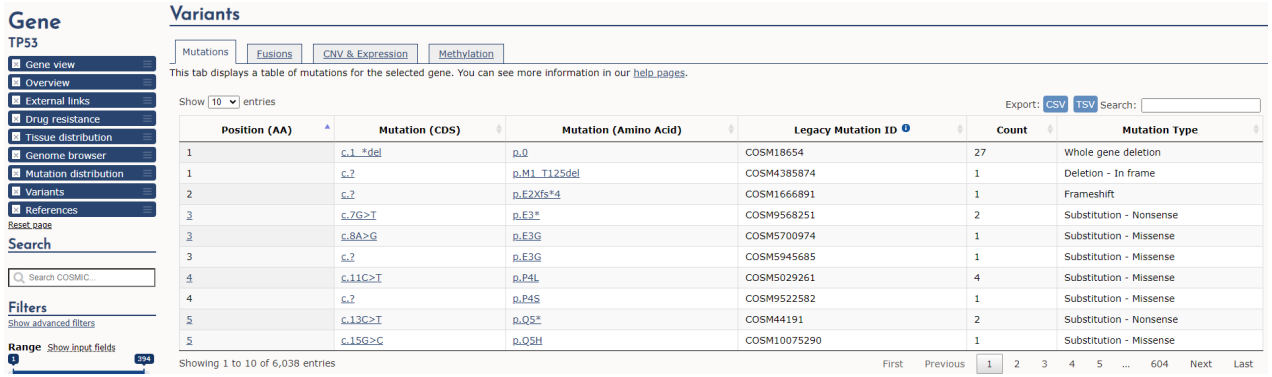
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Claim	**Prior Art**
	As another example, Bashashati discloses a method in which "the exomes of 25 samples (19 tumour, six normal; Table 1) were sequenced to a median exonic coverage of 70.7-fold across different samples (see supplementary material, Table S1 and Figure S1)." Bashashati at 22. As another example, Bashashati discloses a method in which "multiple tissue samples were obtained from primary ovarian tumor and metastatic sites where adequate tumor volume permitted," teaching that "[g]enomic DNA was extracted from frozen samples" and that "[e]xome capture was achieved through solution hybrid selection with the Human All Exon kit SureSelect Target Enrichment System (Agilent) version 1 for Illumina Genome Analyzer paired-end sequencing."). Bashashati supplementary app'x at 5–6. A POSA would understand that this method discloses whole exome sequencing because Bashashati is using the "Human **All** Exon kit." As another example, Bashashati teaches that "[c]andidate SNVs and indels were subjected to deep sequencing using Illumina and Ion Torrent platforms" in order to validate mutations. Bashashati supplementary app'x at 6.
1[b]. performing targeted multiplex amplification to amplify 10 to 500 target loci each 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	Bashashati 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. *See, e.g.,* Bashashati supplementary spreadsheet at cols. T, U; Bashashati supplementary app'x at 6, 7. For example, in Case1a, Bashashati discloses 61 tumor-specific SNVs amplified in plasma. *See* Bashashati supplementary spreadsheet at cols. T, U. As another example, Bashashati teaches amplification of target loci from cell-free DNA isolated from a plasma sample. First, Bashashati states that "[c]irculating tumour DNA was extracted from 1-2 ml of plasma." Bashashati supplementary app'x at 7. Then, Bashashati teaches amplifying circulating tumor DNAQ by the method described in the Supplementary Appendix in the section titled "Deep amplicon sequencing using the Illumina MiSeq platform for cell-free DNA in the

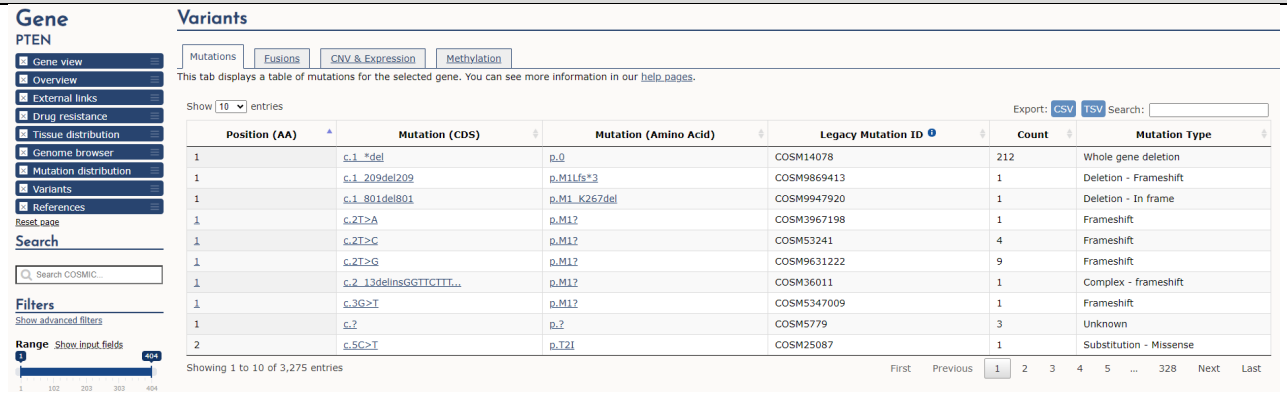
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Claim	**Prior Art**
together in the same reaction volume; and	plasma," which refers back to the section titled "Deep amplicon sequencing using the Illumina GAII platform." *Id.* These sections teach the use of targeted PCR to amplify target loci encompassing different SNVs. Bashashati supplementary app'x at 6 ("Primer Paris were designed to place the variant position within 75bps of either end of the amplicon and to be between 50-300bp in length."). Bashashati also discloses obtaining amplicons having a length of 50-150 bases. *See, e.g.,* Bashashati supplementary app'x at 6. For example, Bashashati discloses a method in which "[p]rimer pairs were designed to place the variant position within 75bps of either end of the amplicon and to be between 50-300bp in length." Bashashati supplementary app'x at 6. A POSA would thus understand that the primer pairs could be designed to place the variant position with 75bps of both ends of the amplicon, leading to an amplicon no longer than 149 base pairs. In view of Bashashati, it would have been obvious to a POSA to amplify the target loci together in the same reaction volume. *See, e.g.,* Bashashati supplementary app'x at 6, 7. For example, Bashashati teaches a method in which "[v]alidations of candidate single nucleotide variants (SNVs) identified from the initial sequencing of the primary ovarian cancer samples were achieved using a deep amplicon sequencing approach." Bashashati supplementary app'x at 7. In this method, candidate SNVs "were fed into the LIFE Technology AmpliSeq custom designed pipeline, after conversion to hg19 coordinates, and three multiplex primers pools were generated." *Id.* Even though the targeted amplification of SNVs from ctDNA was conducted using single primer pairs per reaction, Bashashati thus teaches a POSA that SNVs can be amplified in multiplex reactions. A POSA would have been motivated to do so for the benefit of requiring fewer separate reactions, and would have had a reasonable expectation of successfully doing so because multiplex amplification was well known in the art. *See, e.g.,* Forshew at 3, 6 (Table 2), 9; Forshew supplementary materials at 2, 2 (Fig. 1B), 9 (Table S1), 17–18 (Table S6). In addition, any of the

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Claim	**Prior Art**
	Multiplex PCR References provide motivations and a reasonable expectation of success to perform targeted multiplex amplification to amplify 10 to 500 target loci s recited in this claim limitation. Furthermore, under Natera's apparent interpretation of the Court's 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 Forshew. 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**
	

https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=PTEN

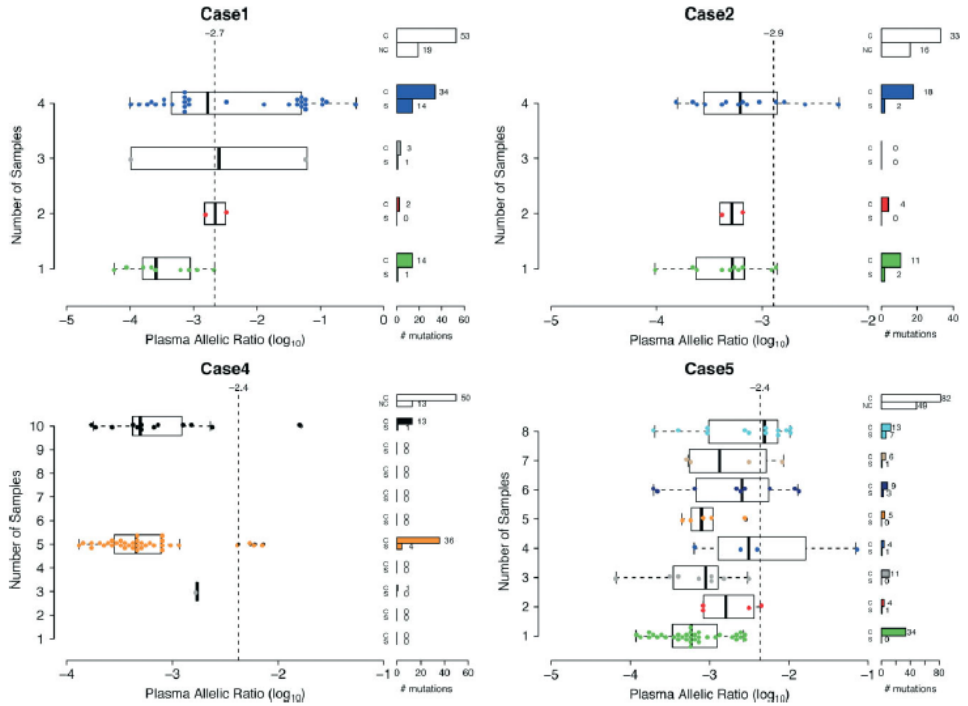
See also
https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=EGFR
https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=BRAF
https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=KRAS
https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=PIK3CA

Additionally, to the extent Natera contends that Bashashati does not teach targeted multiplex amplification of 50 to 100 target loci, it would have been obvious to a POSA to do so in view of the knowledge of a POSA or in view of references such as Page. For example, Page teaches 207 amplicons amplified in a single reaction mixture. See, e.g., Page at 2, 3. A POSA would be motivated to apply the method disclosed by Bashashati to amplify at least 100 target loci at the time the '454 patent was filed because this would have increased efficiency and obtained more amplicons in a single operation. In addition, there is reasonable expectation of success for a POSA to combine Bashashati with references such as Page and Forshew because Forshew discloses that more loci would "increase [] the robustness for tumor monitoring by compensating for effects of sampling noise or mutational drift." Forshew at 10.

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Claim	**Prior Art**
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,	The Court construed the term "sequencing the amplicons" to have its plain and ordinary meaning. Doc. 280 at 10. The Court construed the term "sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads" to require that the detecting and sequencing are part of the same step. *Id.* at 11. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Under Natera's apparent interpretation of the Court's adopted construction, Bashashati discloses sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads. *See, e.g.,* Bashashati supplementary app'x at 6–7; Bashashati at 25, 26 (Fig. 2); Bashashati supplementary spreadsheet at col. T. For example, Bashashati discloses a method in which "[s]omatic mutation predictions were subjected to rigorous validation by targeted deep amplicon sequencing in the tumour and normal DNA templates" and in which cell-free DNA in the plasma was subjected to deep amplicon sequencing using the Illumina MiSeq platform. Bashashati supplementary app'x at 6–7. As another example, Bashashati explains that "[p]lasma samples collected pre-operatively and pre-anaesthesia were available for four cases from which ctDNA was extracted and subjected to targeted deep-sequencing of validated somatic mutations (see supplementary material, Appendix)." Bashashati at 25. Bashashati then "assayed 72 mutations in case 1, 49 in case 2, 63 in case 4 and 131 in case 5" and found that, "[a]cross the four cases, 215/312 (69%) mutations contained sequencing coverage and 38/215 (18%) were detectable with mutation allele frequency above statistical background noise." Bashashati at 25; *see also* Bashashati supplementary spreadsheet at col. T (anything with RefCount.plasma). Bashashati presents these findings in Figure 2:

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	 **Figure 2.** Deep sequencing results of cell-free circulating tumour DNA from the plasma in cases 1, 2, 4 and 5. The distribution of plasma variant allelic ratio (log10 scale) is separated by the number of tumour samples in which the mutation was originally discovered. Cases 4 and 5 both also include the fallopian tube when counting the number of tumour samples. The number of mutations based on positions with coverage (c), no coverage (nc) and those with coverage that were significantly (s) detected, based on the binomial exact test (adjusted $p < 0.05$), are shown. The minimum variant allelic ratio detectable using the exact test for each patient is denoted by the vertical dashed line. The Court's Claim Construction Order states that "NeoGenomics is correct that the sequencing must be done on the amplicons obtained from the targeted multiplex amplification, not on some other set of amplicons obtained from some other source or process; the term "the" must mean something. Doc. 280 at 8. To the extent the Court's construction is interpreted to require sequencing the amplicons from the multiplex PCR step rather than sequencing only a subset of the amplicons from the

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	multiplex PCR step, this would have been obvious in view of Bashashati alone or in combination with other references such as Forshew. For example, Bashashati teaches constructing sequencing libraries "using the SPRIworks Fragment Library System I with no on-board size selection." Bashashati at 7. For example, Forshew teaches that the multiplex PCR step was designed to avoid unwanted side products. *See* Forshew 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."); Forshew 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.
1[d]. wherein the sequencing has a depth of read of at least 50,000 per target locus.	Bashashati discloses a sequencing depth of read of at least 50,000 per target locus. *See, e.g.,* Bashashati supplementary spreadsheet at cols. U, T (showing depth often in excess of 50,000, with a maximum depth of 969,361 and an average depth of 62,692).
2. The method of claim 1, wherein the cell-free DNA comprises circulating tumor DNA.	Bashashati discloses that cell-free DNA comprises circulating tumor DNA. *See, e.g.,* Bashashati at 22 ("[S]equencing of cell-free circulating tumour DNA (ctDNA) extracted from plasma has been demonstrated to be an effective non-invasive tool for monitoring tumour burden."); Bashashati at 25 ("Mutational events in the ancestral clone are detectable in cell-free circulating tumour DNA from plasma."); Bashashati at 26, Figure 2 (showing "[d]eep sequencing results of cell-free circulating tumour DNA from the plasma.").
3. The method of claim 1, wherein the SNV mutations comprise one or more clonal SNV mutations.	In view of Bashashati, Bai, and/or the knowledge of a POSA, it would have been obvious that the SNV mutations comprise one or more clonal SNV mutations. *See, e.g.,* Bashashati at Abstract, 22, 24, 25, 28, 32, Fig. 1, Supplementary Appx at 10.

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Claim	**Prior Art**
	See 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."); *see also id.* ("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.") Bashashanti also discloses, "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." Bashashati at 22. Further, to the extent Natera contends that Bashashati does not disclose this limitation, a POSA would have understood that to monitor and track cancer effectively requires identifying the various categories of cancer-related mutations, including clonal mutations. *See* 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."); Bashashati at 22 (reporting the distribution of different mutations (including clonal mutations) within the tumor and explaining that a "panel of shared, identical mutations, indicative of a single ancestral clonal origin, was evident."); Bai 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."); Bai at 1 (explaining that tumorigenesis "is a multistage process by which monoclonal cancer cells gradually become heterogeneous owing to clonal evolution and genetic/epigenetic instability") Thus, a POSA would have been motivated to identify and detect clonal mutations (as well as subclonal mutations) to effectively track and monitor cancer, and would have had a reasonable

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	expectation of successfully doing so because Bashashati and Bai teach detection of clonal (and subclonal) mutations in tumor and cfDNA.
4. The method of claim 1, wherein the SNV mutations comprise one or more subclonal SNV mutations.	In view of Bashashati, Bai, and/or the knowledge of a POSA, it would have been obvious that the SNV mutations comprise one or more subclonal SNV mutations. *See* claim 3, above. *See also* Bashashati at 22, 25, 26, 27-32, Figs. 1-5; Supplementary App'x at 10. *See, e.g.,* Bashashati at 22 ("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 25 ("Mutational events in the ancestral clone are detectable in cell-free circulating tumour DNA from plasma."); Bashashati at 26 ("Observations of subclonal NF1 deletions reinforced evidence from the mutation data that important driver mutations may be acquired late in the evolutionary history of the tumour."); *see also, e.g.*, Bashashati at 25 ("Mutational event in the ancestral clone are detectable in cell-free circulating tumour DNA from plasma"); Bashashati at 25 ("The intratumoural variation in genomic architectures of HGSCs"); Bashashati at 27 ("The origins of fallopian tube lesions and reconstruction of HGSC evolutionary histories"); Bashashati at 29 ("Intra-sample clonal diversity revealed by cellular frequency analysis of mutational profiles"); Bashashati at 30 ("Diverse genomic landscapes associate with heterogeneous transcriptional profiles"); Bashashati at 31 ("Genomic aberrations impacting transcriptional networks"). Additionally, as described above, a POSA would have been motivated to track and detect subclonal mutations because they are an integral part of cancer tumors and knowledge about them would inform diagnosis, monitoring, and treatment. A POSA would have had a reasonable expectation of successfully doing so because Bashashati identified and tracked subclonal mutations. *See, e.g.*, Bashashati at 22 ("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 25 ("Mutational events in the ancestral clone are detectable in cell-free circulating tumour DNA from plasma."); Bashashati at 26 ("Observations of

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	subclonal NF1 deletions reinforced evidence from the mutation data that important driver mutations may be acquired late in the evolutionary history of the tumour."). Further, Patel discloses that "[d]etection of circulating tumor DNA (ctDNA) has several applications including, but not limited to, detecting presence of a malignancy, informing a prognosis, assessing treatment efficacy, tracking changes in tumor mutation status, and monitoring for disease recurrence or progression."). Patel at 14 (emphasis added). A POSA would have understood "tracking changes in tumor mutation status" to refer to subclonal SNV mutations because it is well established in the art that cancers originate from a monoclonal composition, diverging into genomically distinct subclones. *See, e.g.*, 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 a 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.	In view of Bashashati, Bai, and/or the knowledge of a POSA, it would have been obvious that the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations. *See* claims 3 and 4 above.
6. The method of claim 1, wherein the tumor sample of the subject is a tumor tissue sample.	Bashashati discloses that the tumor sample of the subject is a tumor tissue sample. *See, e.g.,* Bashashati at 22, Table 1 ("Tissue was obtained from a total of 31 tumour sites (range 3–10 samples/patient, including two fallopian tube lesions) with matched normal DAN for each patient (Table 1)."); Bashashati at 1 ("The 19 tumour samples were used as index discovery samples to identify sets of mutations for interrogation by deep amplicon sequencing . . .").

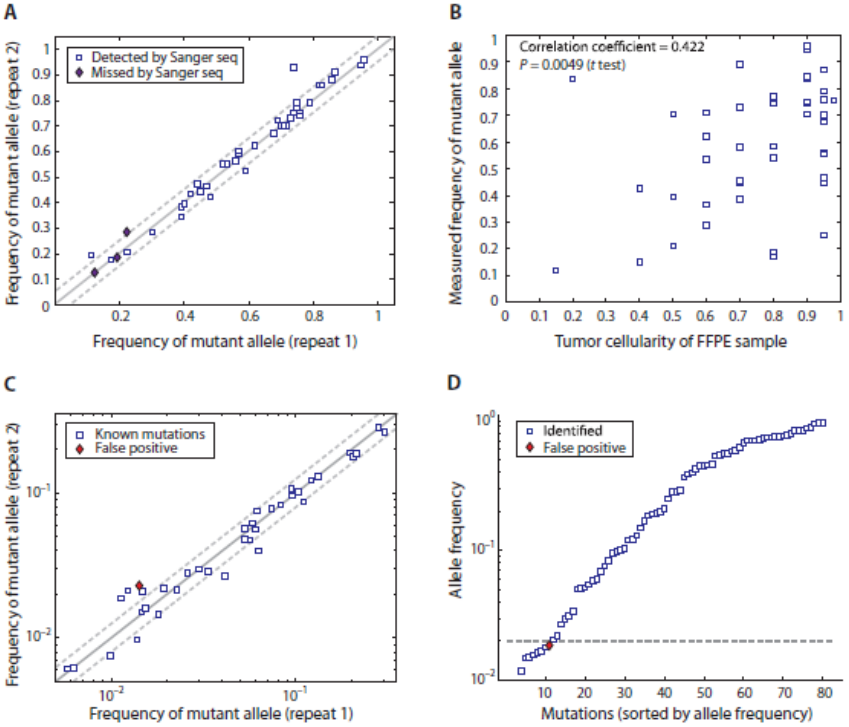
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Claim	**Prior Art**
7. The method of claim 1, wherein the method further comprises determining clonal heterogeneity of the tumor sample.	In view of Bashashati, Forshew, and/or the knowledge of a POSA, it would have been obvious to determine clonal heterogeneity of the tumor sample. *See, e.g.,* Bashashati at 28 (analyzing a fallopian tube lesion and determining that it "was composed of heterogeneous subclones" with further analysis revealing "two coexisting populations" in the lesion.); Bashashati at 25 (tracking these different mutations in cfDNA using personalized NGS assays, thus monitoring clonal heterogeneity, and explaining that "Cases 1 and 5 were enriched for the presence of mutations in the plasma that were also present in all intrapatient tumour samples, and thus part of the ancestral clone (Fisher exact test $p < 0.05$; Figure 2)"); *See, e.g.,* Bashashati at 22 ("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 25 ("Mutational events in the ancestral clone are detectable in cell-free circulating tumour DNA from plasma."); Bashashati at 26 ("Observations of subclonal NF1 deletions reinforced evidence from the mutation data that important driver mutations may be acquired late in the evolutionary history of the tumour."); *see also, e.g.*, Bashashati at 25 ("Mutational event in the ancestral clone are detectable in cell-free circulating tumour DNA from plasma"); Bashashati at 25 ("The intratumoural variation in genomic architectures of HGSCs"); Bashashati at 27 ("The origins of fallopian tube lesions and reconstruction of HGSC evolutionary histories"); Bashashati at 29 ("Intra-sample clonal diversity revealed by cellular frequency analysis of mutational profiles"); Bashashati at 30 ("Diverse genomic landscapes associate with heterogeneous transcriptional profiles"); Bashashati at 31 ("Genomic aberrations impacting transcriptional networks"). To the extent Bashashati is deemed not to disclose determining clonal heterogeneity of the tumor sample, a POSA would have been motivated to combine the teachings of Bashashati with those of Forshew and Bai. This is because determining the clonal heterogeneity of a tumor would have provided a more complete picture of the genetic makeup of the cancer, which would have aided in the task of monitoring and tracking the cancer and allowed for improved treatment. A POSA would have had a reasonable expectation of success because determining clonal heterogeneity and the benefits of doing so for improved monitoring and treatment were well known in the art.

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Claim	**Prior Art**
	It was well known in the art before 2015 that tumor tissue is genetically heterogeneous. *See, e.g.,* Forshew at 1, 10; Bashashati at Abstract, 1, 27–28, Bai at 2–3. Forshew teaches that "mutation profiles in different metastatic clones can different significantly from each other or the partent primary tumor" and that "[e]volutionary changes within the cancer can alter the mutation spectrum of the disease and its responsiveness to therapies," such that there was a need to identify the range of mutations present in a cancer patient to improve monitoring and treatment. Forshew at 1, 10. Forshew also teaches determining allele frequency in the ovarian cancer formalin-fixed, paraffin-embedded (FFPE) tissue samples, see Forshew at 4, Figure 2: Further evidence of motivation and reasonable expectation of success to determine clonal heterogeneity of the tumor sample is found in NEOGEN00275297 ("we are thinking of how this might be used to monitor the tumour heterogeneity and clonal selection during treatment").

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	 **Fig. 2.** Identification of mutations in ovarian cancer FFPE samples by TAm-Seq. (**A**) Concordance between duplicate measurements of AFs of mutations identified in fragmented DNA extracted from FFPE samples. The mutation frequency in each library was calculated as the fraction of reads with the mutant (nonreference) base. Solid line indicates equality. Dotted lines indicate a difference in AF of 0.05. (**B**) Correlation of AF with FFPE tumor cellularity. The measured mutant AF (average of both repeats) correlated significantly with the cellularity, estimated from histology (table S3). (**C**) Concordance between duplicate measurements of AFs of mutations identified in a mixture of DNA extracted from different FFPE samples. (**D**) Summary of mutations called in FFPE using TAm-Seq, sorted by increasing AF. Dotted line indicates AF of 2%. Further, Bai discloses that "[m]utant frequencies varied widely throughout individual tumors, ranging between 5%-100% by DHPLC and 1%-100% by ARMS," and that a percentage of "samples were identified to carry intratumoral EGFR mutational heterogeneity by DHPLIC and ARMS." Bai

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	at 3; see also Bai at 4, Figure 1. And Bashashati teaches determining clonal heterogeneity of tumors in its disclosure of characterizing and monitoring tumor cells in cancer patients. *See, e.g.,* Bashashati at 28 ("Results from PIK3CA mutation sequencing suggested the [fallopian tube] lesion in case 4 was composed of heterogeneous subclones" with further analysis revealing "two cooexisting populations of nuclei in the [fallopian tube] lesion"); Bashashati at 27–28 (determining a fallopian tube lesion harbored mutations not present in all samples and determining phylogenetic relationships, and determining likely origin on particular tumor as metastatic implant). Bashashati also shows that a POSA would have appreciated the benefit of monitoring clonal heterogeneity of the tumor sample for improved treatment. *See* Bashashati at Abstract ("[W]e observed mixed cell populations in the early fallopian tube lesion . . . Our results revealed that [high-grade serous ovarian cancers] 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 and investigation of drug-resistance mechanisms.").
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.	Bashashati discloses performing targeted multiplex amplification to amplify 20 to 50 target loci each encompassing a different tumor-specific SNV mutation. *See, e.g.,* Bashashati supplementary spreadsheet at cols. T, U (teaching amplification of 61 tumor-specific SNVs in Case 1a); Bashashati at 25 ("Plasma samples collected pre-operatively and pre-anaesthesia were available for four cases from which ctDNA was extracted and subjected to targeted deep-sequencing of validated somatic mutations (see supplementary material, Appendix). We assayed 72 mutations in case 1, 49 in case 2, 63 in case 4 and 131 in case 5."); Bashashati supplementary app'x at 6 ("Candidate SNVs and indels were subjected to deep sequencing using Illumina and Ion Torrent platforms."). *See also* Claim 1[b] above.

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Claim	**Prior Art**
9. The method of claim 1, wherein the targeted multiplex amplification amplifies 50 to 100 target loci each encompassing a different tumor-specific SNV mutation.	Bashashati discloses, alone or in combination with the knowledge of a POSA, performing targeted multiplex amplification to amplify 50 to 100 target loci each encompassing a different tumor-specific SNV mutation. See, e.g., Bashashati at 25 ("Plasma samples collected pre-operatively and pre-anaesthesia were available for four cases from which ctDNA was extracted and subjected to targeted deep-sequencing of validated somatic mutations (see supplementary material, Appendix). We assayed 72 mutations in case 1, 49 in case 2, 63 in case 4 and 131 in case 5."); Bashashati supplementary app'x at 6 ("Candidate SNVs and indels were subjected to deep sequencing using Illumina and Ion Torrent platforms."). *See also* Claim 1[b] above.
10. The method of claim 1, wherein the method further comprises designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample.	Bashashati discloses, alone or in combination with the knowledge of a POSA, designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample. *See, e.g.,* Bashashati supplementary app'x at 6 ("Primer pairs were designed to place the variant position within 75bps of either end of the amplicon and to be between 50-300bp in length."); Bashashati supplementary app'x at 7 ("The primers were designed to generate 100bp amplicons to accommodate the sequencing of DNA from formalin-fixed paraffin-embedded (FFPE) tissues."); Bashashati supplementary app'x at 7 ("DNA primers were synthesized in 96-well plates at a 25nmol scale with standard desalting (IDT Coralville, IA USA).").
11. The method of claim 1, wherein the method further comprises performing barcoding PCR prior to the sequencing.	Bashashati discloses performing barcording PCR prior to the sequencing. *See, e.g.,* Bashashati supplementary app'x at 7 ("Adapter-ligated products were purified on Qiaquick spin columns (Qiagen, Valencia, CA, USA) and PCR-amplified using Phusion DNA polymerase (NEB, Ipswich, MA, USA) in 10 cycles using PE primer 1.0 (Illumina) and a custom multiplexing PCR Primer [5 CAAGCAGAAGACGGCATACGAGAT NNNNNNCGGTCTCGGCATTCCTGC TGAACCGCTCTTCCGATCT-3] where NNNNNN was replaced with unique fault tolerant

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	hexamer barcodes for each template . . . Between the paired 75bp reads a third 7 base pair read was performed using the following custom sequencing primer [5-GATCGGAAGAGCGGTTCAGCAGGAATGCCGAGACCG] to sequence the hexamer barcode.").
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.	Bashashati discloses detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. *See, e.g.,* Bashashati at 22 ("[T]he divergence of clonal cell populations confers a heterogeneous genomic tapestry with implications for clinical endpoints, including the acquisition of metastatic potential."); Bashashati at 27 ("The FT lesion in case 5 harbored seven mutations that were not present in all samples, indicating that the clones comprising case 5h were unlikely to have been ancestral to the tumours . . . We suggest that case 5h is therefore more likely to be a metastatic implant in the FT rather than a precursor or early lesion."); Bashashati at 22 ("In case 6, we compared a primary sample to a recurrence obtained 42 months later, following multiple chemotherapeutic regimens (total 21 cycles) (see supplementary material, Figure S7).").
13. The method of claim 1, wherein the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer.	Bashashati discloses or renders obvious wherein the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer. For example, the References cited by Bashashati disclose the implementation of related techniques for colorectal cancer and breast cancer. Bashashati at 33; Bashashati Supplementary Appendix at 12. To the extent that Bashashati does not disclose this limitation, it would have been obvious to a POSA to apply the techniques and methods of Bashashati to any of colorectal cancer, lung cancer, bladder cancer, or breast cancer based on the teachings of Bashashati and the knowledge of a POSA. For example, Bashashati teaches that clonal diversity affect the progression and treatment of "ovarian carcinoma and other primary cancers." Colorectal cancer, lung cancer, bladder cancers, and breast cancer are also examples of primary cancers. Accordingly, a POSA would be motivated to study these other cancers for the same reasons that Bashashati studied ovarian cancer. And a POSA would have a reasonable expectation of success analyzing other cancers because a POSA would understand

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	that the techniques Bashashati used to analyze ovarian cancer would be equally applicable to other primary cancers.
14[pre]. A method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:	To the extent the preamble is construed to be limiting, Bashashati 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. *See* claim 1[pre] above.
14[a]. performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;	Bashashati 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. *See* claim 1[a] above.
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	Bashashati 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. *See* claim 1[b] above.

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therefrom to obtain amplicons, wherein the target loci are amplified together in the same reaction volume; and	
14[c]. sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads,	The Court construed the term "sequencing the amplicons" to have its plain and ordinary meaning. Doc. 280 at 10. The Court construed the term "sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads" to require that the detecting and sequencing are part of the same step. *Id.* at 11. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Under Natera's apparent interpretation of the Court's adopted construction, Bashashati discloses sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads. *See* claim 1[c] above.
14[d]. wherein the method is capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus.	Bashashati discloses a method capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus. *See, e.g.,* Bashashati supplementary spreadsheet at cols. U and T (AltCount.Plasma and RefCount.plasma will give VAF, which is less than 0.015% in some cases).
15. The method of claim 14, wherein the sequencing has	Bashashati discloses the sequencing has a depth of read of at least 50,000 per target locus.

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a depth of read of at least 50,000 per target locus.	*See* claim 1[d] above.
16. The method of claim 14, wherein the cell-free DNA comprises circulating tumor DNA.	Bashashati discloses that cell-free DNA comprises circulating tumor DNA. *See* claim 2 above.
17. The method of claim 14, wherein the SNV mutations comprise one or more clonal SNV mutations.	In view of Bashashati, Bai, and/or the knowledge of a POSA, it would have been obvious to a POSA that the SNV mutations comprise one or more clonal SNV mutations. *See* claim 3 above.
18. The method of claim 14, wherein the SNV mutations comprise one or more subclonal SNV mutations.	In view of Bashashati, Bai, and/or the knowledge of a POSA, it would have been obvious to a POSA that the SNV mutations comprise one or more subclonal SNV mutations. *See* claim 4 above.
19. The method of claim 14, wherein the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations.	In view of Bashashati, it would have been obvious to a POSA that the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations. *See* claim 5 above.
20. The method of claim 14, wherein the tumor sample of the subject is a tumor tissue sample.	Bashashati discloses that the tumor sample of the subject is a tumor tissue sample. *See* claim 6 above.

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Claim	**Prior Art**
21. The method of claim 14, wherein the method further comprises determining clonal heterogeneity of the tumor sample.	In view of Bashashati, Forshew, and/or the knowledge of a POSA, it would have been obvious to a POSA that the method further comprises determining clonal heterogeneity of the tumor sample. *See* claim 7 above.
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.	Bashashati discloses, alone or in combination with the knowledge of a POSA, performing targeted multiplex amplification to amplify 20 to 50 target loci each encompassing a different tumor-specific SNV mutation. *See* claim 8 above.
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.	Bashashati discloses, alone or in combination with a POSA, performing targeted multiplex amplification to amplify 50 to 100 target loci each encompassing a different tumor-specific SNV mutation. *See* claim 9 above.
24. The method of claim 14, wherein the method further comprises designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations	Bashashati discloses designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample. *See* claim 10 above.

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identified in the tumor sample.	
25. The method of claim 14, wherein the method further comprises performing barcoding PCR prior to the sequencing.	Bashashati discloses performing barcording PCR prior to the sequencing. *See* claim 11 above.
26. The method of claim 14, wherein the method further comprises detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA.	Bashashati discloses detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. *See* claim 12 above.
27. The method of claim 14, wherein the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer.	Bashashati discloses or renders obvious wherein the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer. *See* claim 13 above.
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.	Bashashati discloses a method capable of detecting an SNV mutation that is present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus. *See* claim 14[d] above.

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EXHIBIT 2-F – FINAL 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):36RA68 (May 2012) (Forshew)

Reference: Narayan, A. et al., *Ultrasensitive Measurement of Hotspot Mutations in Tumor DNA in Blood Using Error-Suppressed Multiplexed Deep Sequencing*, Cancer Res; 72(14) (July 15, 2012) (Narayan)

Reference: United States Patent Application Publication No. 2010/0261189 (published Oct. 14, 2010) (Bentley)

Reference: Bai, H. et al., *Detection and Clinical Significance of Intratumoral EGFR Mutational Heterogeneity in Chinese Patients with Advanced Non-Small Cell Lung Cancer*, PLOS ONE Vol. 8 Iss. 2 (Feb. 2013) (Bai)

Reference: Page, K. et al., *Influence of Plasma Processing on Recovery and Analysis of Circulating Nucleic Acids*, PLOS ONE, Vol.8, Iss. 10 (Oct. 18, 2013) (Page)

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

Reference: Bashashati, A. et al., *Distinct evolutionary trajectories of primarily high-grade serous ovarian cancers revealed through spatial mutational profiling*, J Pathol 2013; 231 : 21–34 (June 7, 2013) (including its supplementary appendix and supplementary spreadsheet "Bashashati")

This chart hereby incorporates by reference all prior art references contained within the "General Knowledge of a POSA" section of NeoGenomics' Invalidity Contentions cover pleading.

Motivation to Combine

To the extent Natera asserts that Forshew does not disclose any limitation of Asserted Claims 1-28, it would have been obvious to combine Forshew with any of the other prior art mentioned herein. A POSA would have considered the disclosures of the aforementioned prior art because the prior art references all concern the same subject matter, namely amplification and/or sequencing of DNA for the purpose of identifying mutations therein.

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A POSA would have been motivated to combine Forshew with Patel, Bentley and/or Narayan because Forshew provides an express invitation to perform its methods with higher read depth to achieve higher sensitivity and detect SNVs at lower levels of frequency. *See* Forshew at 10 ("Our approach may be sufficient for analyzing plasma from patients with certain advanced cancers, but further improvement may be necessary before this method can be more widely used in the clinic. Higher read depth or fidelity, additional replicates, or improved algorithms could allow for enhanced mutation detection without change to protocols."). Further, a POSA would reasonably expect to succeed because Patel, Bentley and Narayan disclose methods of achieving higher sensitivity through increased depth of read, thus demonstrating that Forshew's TAm-Seq method can detect SNV mutations present in less than or equal to 0.015% of cfDNA by using sequencing depths greater than 50,000.

A POSA would also have been motivated to combine Forshew with Bai, Patel, Bashashati, and/or the knowledge of a POSA because a POSA have understood that to monitor and track cancer effectively requires identifying the various categories of cancer-related mutations, including clonal and subclonal mutations. A POSA would have had a reasonable expectation of successfully doing so because Forshew, Bai, Patel, and Bashashati each teach detection of clonal and subclonal mutations in tumor and cfDNA.

A POSA would also have been motivated to combine Forshew with Page. For example, Page teaches 207 amplicons amplified in a single reaction mixture. See, e.g., Page at 2, 3. A POSA would be motivated to apply the method disclosed by Page to amplify at least 100 target loci at the time the '454 patent was filed because this would have increased efficiency and obtained more amplicons in a single operation. In addition, there is reasonable expectation of success for a POSA to combine Forshew with references such as Page because Forshew discloses that more loci would "increase [] the robustness for tumor monitoring by compensating for effects of sampling noise or mutational drift." Forshew at 10.

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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)	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. *See, e.g.,* 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 7 ("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

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mutations in the plasma sample, the method comprising:	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.e.*, SNVs). Additionally, to the extent the preamble is construed to be limiting, Narayan 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. *See, e.g.,* Narayan at Abstract, 3492, 3493. For example, Narayan discloses a method that "uses next-generation sequencing to quantify the small fraction of DNA molecules that contain tumor-specific mutations within a background of normal DNA in plasma." Narayan at 3492; *see also id.* ("The release of cell-free DNA into the bloodstream from dying tumor cells has been well documented in patients with various types of cancer. There has been growing interest in trying to use such circulating tumor DNA (ctDNA) as a noninvasive biomarker to detect the presence of malignancy, follow treatment response, gauge prognosis, or monitor for recurrence. Because unique somatic mutations can be used as telltale marks to distinguish tumor-derived DNA is plasma, a new class of highly specific DNA-based cancer biomarkers can be envisioned with clinical applications that may complement those of conventional serum protein markers."). *See also* Narayan at 3492 ("Amplification of both mutated and wild-type sequences is carried out by unbiased PCR in a single tube, ensuring highly accurate and reproducible quantitation. The scheme is designed to have the flexibility to simultaneously analyze mutations in several genes from multiple patient samples, making it practically feasible to screen plasma samples for mutant ctDNA without previous knowledge of the tumor's mutation profile."); Narayan at 3493 ("30 patients with stage I-IV non-small cell lung cancer (NSCLC) were enrolled in this study between July 2009 and July 2010 . . . A total of 117 samples were obtained."); *id.* ("Blood was collected in EDTA-containing tubes (Becton Dickinson) and was centrifuged at 1,000 x *g* for 10 minutes within 3 hours of collection. Plasma was transferred to cryovials . . . DNA was extracted from 0.2 mL of each

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	plasma sample using the QIAamp DNA Blood Mini Kit (Qiagen), according to the manufacturer's protocol. Amplification of mutation hotspot regions was carried out in triplicate for each sample using 2 successive rounds of PCR, with primers designed to flank codons 12 and 13 of *KRAS*, codon 858 of *EGRF*, and codon 600 of *BRAF*.").
1[a]. performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;	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. *See, e.g.,* 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.e.*, SNVs)); Forshew at 3 (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 TP53 by Sanger sequencing"). Because TP53 is a tumor suppressor gene associated with cancer, a POSA would have understood that Sanger sequencing to identify mutations in TP53 constitutes identification of tumor-specific SNV mutations. Forshew 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)." Forshew at 3. Forshew identifies 40 single-base substitutions (i.e., SNVs) in the ovarian tumor sample. Forshew 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. Furthermore, Narayan teaches performing sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations.

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	For example, Narayan discloses obtaining "[f]ormalin-fixed, paraffin-embedded tumor specimens [from] 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." Narayan at 3493. And Narayan discloses that "[g]enomic DNA was isolated from [these samples] using the QuickExtract FFPE DNA Extraction Kit (Epicentre). 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 carried out on gel-purified amplicons, and mutations were identified from chromatograms using Mutation Surveyor software (Softgenetics)." Narayan at 3493–94. Further, Narayan teaches that knowing the mutations present in the cancer DNA can increase the sensitivity of its approach. For example, Narayan explains that "[h]ad we selectively enrolled patients with known tumor mutations, we would have likely seen more cases with measurable ctDNA" and that "[b]y including a larger panel of cancer-specific mutations in future studies, we expect to be able to measure mutant ctDNA in a higher percentage of patients." Narayan at 3497. Accordingly, Narayan teaches and recognizes the benefits that whole exome sequencing or whole genome sequencing on a tumor sample of the subject can have on sensitivity.
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	Forshew 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. *See, e.g.,* Forshew 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"); Forshew at 3 ("We therefore applied a two-step amplification process: a limited-cycle preamplification step where all primer sets were used together to capture

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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	the starting molecules present in the template, followed by individual amplification to purify and select for intended targets. (Fig. 1B) (Supplementary Methods)."); Forshew supplemental materials at 2 ("Preamplification methods 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."); Forshew at 2, Fig. 1B (depicting the targeted multiplex 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; *see also* 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. *See* 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. *See* 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; *see also id.* at 17-18 (Table S6); Forshew at 6 (Table 2). Additionally, Bentley discloses performing targeted multiplex amplification to amplify 10 to 500 target loci each encompassing a different mutation. *See, e.g.,* Bentley at [0090], [0110], Table 4, Table 5.

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	For example, Bentley discloses more than 100 target specific primers for HLA variants for use in its targeted multiplex amplification methods. *See* Table 4; Table 5. As another example, Bentley describes "highly multiplexed amplicon sequencing" in which "the HLA genotypes for at least eight loci (HLA-A, B, C, DRB1, DQA1, DQB1, DPA1, DPB1), as well as for DRB 3, 4, and 5 can be obtained from the data generated by sequencing." Bentley at [0090]. Bentley further explains that the "HLA amplicons for a subject may be obtained in one or more multiplex reactions that comprise primer pairs to amplify individual amplicons." Bentley at [0110].
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,	The Court construed the term "sequencing the amplicons" to have its plain and ordinary meaning. Doc. 280 at 10. The Court construed the term "sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads" to require that the detecting and sequencing are part of the same step. *Id.* at 11. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Under Natera's apparent interpretation of the Court's adopted construction, 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. *See, e.g.,* 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."). Forshew further discloses analyzing the sequence data to detect tumor-specific SNV mutations by using a bioinformatics pipeline. *See* Forshew supplemental materials at 4 ("De-multiplexing and alignment," "Mutation identification," and "Mutation detection"); *see also* Forshew at 5, Table 1 (identifying tumor-specific SNV

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	mutations in plasma samples from seven ovarian cancer patients); Forshew supplemental materials at 19, Table S7 ("[S]ubsequent data analysis was performed using the CASAVA 1.8 analysis pipeline (Illumina) to identify somatic mutations."). The Court's Claim Construction Order states that "NeoGenomics is correct that the sequencing must be done on the amplicons obtained from the targeted multiplex amplification, not on some other set of amplicons obtained from some other source or process; the term "the" must mean something. Doc. 280 at 8. To the extent to the Court's construction is interpreted to require sequencing amplicons from the multiplex PCR step rather than sequencing only a subset of the amplicons from the multiplex PCR step, such a modification of Forshew would have been obvious to a person of ordinary skill in the art. Forshew teaches that the multiplex PCR step was designed to avoid unwanted side products. *See* Forshew 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."); Forshew 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, Forshew 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 amplification using a single amplification reaction step can be used to attach sequencing platform-specific barcodes prior to sequencing. *See, e.g.,* Hoffman et al., *DNA bar coding and pyrosequencing to identify rare HIV drug resistance mutations*, Nucleic Acids Research, 2007, Vol. 35, No. 13, at 3 (teaching that "[g]enomic DNA samples of interest are amplified using primers that include 5'

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	extension providing binding sites for the 454A and B primers" that are required for sequencing); *id.* at 4, Figure 2 (showing the single amplification step, reproduced below).

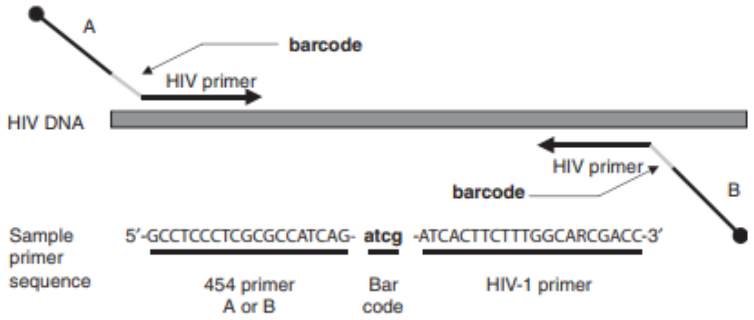


Figure 2. The DNA bar coding system. Primers are shown diagrammatically, illustrating the placement of the DNA bar code between the 454 primers (**A** and **B**) and the HIV-complementary sequences. An example of a primer sequence is shown at the bottom. DNA 5′ ends in the primers are shown as the black balls.

Additionally, Narayan 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.

See, e.g., Narayan at Abstract, 3493, 3495.

For example, Narayan discloses a method of sequencing the results of multiplex PCR amplification to identify SVN mutations present in the cfDNA. *See* Narayan at 3493 ("All samples were loaded onto a single lane of an Illumina HiSeq2000 flow cell and were subjected to paired-end sequencing (75 base pairs per read)."); Narayan at 3493 ("To determine the relative abundance of tumor-specific mutations, we carried out massively parallel sequencing of PCR amplicons derived from plasma DNA fragments containing known mutation hotspots."); Narayan at 3495 ("Importantly, the design of short PCR amplicons enabled us to devise a sequencing strategy that could distinguish mutant from wild-type DNA molecules with very high confidence.").

Bentley also discloses sequencing amplicons to obtain sequence reads, and detecting one or more HLA variants.

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	See, e.g., Bentley at [0009], [0056], [0090], [0092],]0114], Claim 1 For example, Bentley discloses a method that comprises "amplifying a plurality of first amplicons from a double stranded nucleic acid sample, wherein the first amplicons are amplified with a plurality of pairs of nucleic acid primers . . .; amplifying the first amplicons to produce a plurality of populations of second amplicons . . .; sequencing the plurality of populations of second amplicons to generate a nucleic acid sequence composition for each of the plurality of second amplicons; and detecting variation in the sequence composition from one or more of the second amplicons for one or more of the HLA loci." Bentley at [0009]. As another example, Bentley discloses "using sets of specific nucleic acid primers to amplify a selected target region or regions from a sample comprising the target nucleic acid. Further, the sample may include a population of nucleic acid molecules that are known or suspected to contain sequence variants comprising sequence composition associated with a research or diagnostic utility where the primers may be employed to amplify and provide insight into the distribution of sequence variants in the sample." Bentley at [0056] (also explaining that amplicons "are sequenced and the collection of sequences are used to determine an allelic frequency of one or more variants present"). As another example, Bentley discloses that the "highly multiplexed amplicon sequencing of the described embodiments employ sample-specific internal sequence tags . . . in the primers that allow pooling of samples yet maintain the ability to assign sequences to a specific individual. In the described embodiments, the HLA genotypes for at least eight loci . . . , as well as for DRB 3, 4, and 5 can be obtained from the data generated by sequencing." Bentley at [0090]. As another example, Bentley discloses that "the variants present in exon 2 and exon 3 are determined by sequencing the products of first amplicons generated by PCR from an individual." Bentley at [0092]. As another example, Bentley discloses that "following PCR amplification, the beads that have the single-stranded second HLA amplicon template are isolated, e.g., via a moiety such as a biotin that is present on an amplification primer during the emulsion PCR, and the template is sequenced using DNA sequencing technology described elsewhere in this specification." Bentley at [0113]. Further, Bentley discloses that "[o]nce the sequencing data is obtained for the sequence of the individual DNA molecules, the unambiguous

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	HLA sequence can be determined by comparing these sequence files to an HLA sequence database for the known HLA alleles." Bentley at [0114].
1[d]. wherein the sequencing has a depth of read of at least 50,000 per target locus.	In view of Forshew in combination with Bentley, Narayan, and/or the knowledge of a POSA, it would have been obvious to perform sequencing with a depth of read of at least 50,000 per target locus. 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, *e.g.*, because higher read depth could allow for enhanced detection of lower levels of mutation without change to protocols. *See* Forshew at 10. 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 order of magnitude lower frequencies." Forshew at 10; *see also* 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." For example, Forshew teaches: "Sequencing adaptors were separately attached at either end and the products mixed together, such that single-end sequencing generated separate sets of forward and reverse reads. We 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. This produced an average readdepth of 3250 for each of 96 barcoded samples for 48 amplicons read in two possible orientations." Forshew at 3. The read depth for a single replicate of a given locus would therefore be 6,500 per replicate, or 13,000 across the two replicates used for TAm-Seq. *See* Forshew at 3, 9, 7, FIG. 4D legend ("TAm-Seq results are reported as the mean frequency of duplicate analyses"), 4, FIG. 2 legend ("measured mutant AF (average of both repeats)"), Forshew Supplementary Information at 4, 17, 18.

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	Accordingly, Forshew teaches both the motivation and reasonable expectation of success to increase the read depth to at least 50,000 per target locus. Further evidence of motivation and reasonable expectation of success to increase the read depth to at least 50,000 per target locus is found in NEOGEN00275278–NEOGEN00275334 and NEOGEN00275270–NEOGEN0027527. *See, e.g.*, NEOGEN00275326 (describing tables plotting the "number of reads for each barcode according to which block of 48 barcodes it belongs to") and NEOGEN00275329 (providing read counts of greater than 50,000 for some loci (e.g., in columns labeled FLD0200, FLD0217, FLD0233, FLD0234, FLD0235, FLD0236, FLD0237, FLD0238, and FLD0239). A POSA would have been motivated to increase sequencing read depth to obtain greater sensitivity and would have had a reasonable expectation of success in doing so because such an approach was well known in the art as shown by, for example, the teachings of Bentley and/or Narayan. *See, e.g.,* Bentley at ¶ 85 (explaining that "sequencing many nucleic acid templates in parallel provides the sensitivity for the presently described invention" and teaching that the higher the number of reads, the lower the limit for the minimum frequency of detectable SNVs); *see also* Bentley at 9, Table 2 (teaching what a POSA would have understood about the relationship between "Number of Reads" and the "Minimum frequency of SNP in population detectable" with both 95% and 99% confidence) (reproduced below); Narayan at 3494 (disclosing "a median depth of 108,467 read pairs per mutation site per sample after filtering and demultiplexing a total of 86,359,980 raw sequences generated on a single lane of an Illumina HiSeq 2000 flow cell" and explaining that "[b]y designing PCR primers that flank very short regions (<50 bp) surrounding these mutation hotspots, we could ensure adequate amplification of highly fragmented plasma DNA and achieve greater sequence depth").

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<table>
<tr><th colspan="2">U.S. Patent No. 11,530,454</th></tr>
<tr><th>Claim</th><th>Prior Art</th></tr>
<tr><td></td><td>TABLE 2
<table><tr><th>SNP Classes</th><th>Number of Reads</th><th>Minimum frequency of SNP in population detectable with 95% confidence</th><th>Minimum frequency of SNP in population detectable with 99% confidence</th></tr><tr><td>1</td><td>200000</td><td>0.002%</td><td>0.003%</td></tr><tr><td>2</td><td>100000</td><td>0.005%</td><td>0.007%</td></tr><tr><td>5</td><td>40000</td><td>0.014%</td><td>0.018%</td></tr><tr><td>10</td><td>20000</td><td>0.028%</td><td>0.037%</td></tr><tr><td>50</td><td>4000</td><td>0.14%</td><td>0.18%</td></tr><tr><td>100</td><td>2000</td><td>0.28%</td><td>0.37%</td></tr><tr><td>200</td><td>1000</td><td>0.55%</td><td>0.74%</td></tr><tr><td>500</td><td>400</td><td>1.39%</td><td>1.85%</td></tr><tr><td>1000</td><td>200</td><td>2.76%</td><td>3.64%</td></tr></table>
Additionally, Patel discloses the sequencing has a depth of read of at least 50,000 per target locus.

See, e.g., Patel at 72 ("The total number of raw clonal paired-end sequences was 20,511,389. After application of the various filters described above, the remaining sequences numbered 11,184,975. The 40 different gene target regions were fairly evenly represented among the filtered sequences. The median sequence count for the 40 gene-specific regions (all barcodes) was 166,867.").</td></tr>
<tr><td>2. The method of claim 1, wherein the cell-free DNA comprises circulating tumor DNA.</td><td>Forshew discloses that the cell-free DNA comprises circulating tumor DNA.

See, e.g., Forshew at Abstract ("Plasma of cancer patients contains cell-free tumor DNA that carries information on tumor mutations and tumor burden."); see also, e.g., Forshew at 4 ("For all seven patients, TP53 tumor mutations were identified in the circulating DNA at frequencies of 4% to 44% (Table 1).").

Narayan also discloses the cell-free DNA comprises circulating tumor DNA.</td></tr>
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	See, e.g., Narayan at 3492 ("The release of cell-free DNA into the bloodstream from dying tumor cells has been well documented in patients with various types of cancer. There has been growing interest in trying to use such circulating tumor DNA (ctDNA) as a noninvasive biomarker to detect the presence of malignancy, follow treatment response, gauge prognosis, or monitor for recurrence."); Narayan at Abstract ("Detection of cell-free tumor DNA in the blood has offered promise as a cancer biomarker . . . Here we present an approach that uses next-generation sequencing to quantify the small fraction of DNA molecules that contain tumor-specific mutations within a background of normal DNA in plasma.").
3. The method of claim 1, wherein the SNV mutations comprise one or more clonal SNV mutations.	Forshew discloses that the SNV mutations comprise one or more clonal SNV mutations. It is well established in the art that cancer cells are assumed to originate from monoclonal compositions. *See, e.g.,* Bai et al., *Detection and Clinical Significance of Intratumoral EGFR Mutations Heterogeneity in Chinese Patients with Advanced Non-Small Cell Lung Cancer*, 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 et al., *Distinct evolutionary trajectories of primary high-grade serous ovarian cancers revealed through spatial mutational profiling*, J Pathol 2013; 231, 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. Forshew 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." *See, e.g.*, Forshew at 1. 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.

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	Forshew 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 draft." Forshew at 10. Forshew 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)." Forshew at 1. 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. Further evidence of motivation and reasonable expectation of success to detect clonal SNV mutations is found in NEOGEN00275297 ("I think by comparing two genes in the way you suggested we should indeed be able to follow tumour evolution"). *See also id.* ("we are thinking of how this might be used to monitor the tumour heterogeneity and clonal selection during treatment"). Further, in view of Bai, Forshew, Patel, Bashashati, and/or the knowledge of a POSA, it would have been obvious that the SNV mutations comprise one or more clonal SNV mutations. A POSA would have understood that to monitor and track cancer effectively requires identifying the various categories of cancer-related mutations, including clonal mutations. See 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."); Bashashati at 22 (reporting the distribution of different mutations (including clonal mutations) within the tumor and explaining that a "panel of shared, identical mutations, indicative of a single ancestral clonal origin, was evident."); Bai 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."); Bai at 1 (explaining that tumorigenesis "is a multistage process by which monoclonal cancer cells gradually become heterogeneous owing to clonal evolution and genetic/epigenetic instability")

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	Thus, a POSA would have been motivated to identify and detect clonal mutations (as well as subclonal mutations) to effectively track and monitor cancer, and would have had a reasonable expectation of successfully doing so because Forshew, Bashashati, and Bai teach detection of clonal (and subclonal) mutations in tumor and cfDNA.
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. *See, e.g.,* 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 subloncal DNA. Forshew at 1. This challenge necessarily implies the existence of both clonal and subclonal DNA within a tumor. *See, e.g.,* Forshew at 7, Fig. 4A (disclosing a subclonal SNV mutation by identifying a particular *EGFR* mutation present in only two regions from the omental mass, unlike the *TP53* 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. *See, e.g.,* 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 a subsequent evolution leading to selective expansions of genomically distinct subclones with distinct phenotypic properties" and citing numerous articles dating to 1976). Further evidence of motivation and reasonable expectation of success to detect subclonal SNV mutations is found in NEOGEN00275297 ("I think by comparing two genes in the way you suggested we should indeed be able to follow tumour evolution"). *See also id.* ("we are thinking of how this might be used to monitor the tumour heterogeneity and clonal selection during treatment").

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	Additionally, in view of Forshew, Bai, Patel, Bashashati, and/or the knowledge of a POSA, it would have been obvious that the SNV mutations comprise one or more subclonal SNV mutations. *See* claim 3, above. *See also* Bashashati at 22, 25, 26. As described above, *see* claim 3, a POSA would have been motivated to track and detect subclonal mutations because they are an integral part of cancer tumors and knowledge about them would inform diagnosis, monitoring, and treatment. A POSA would have had a reasonable expectation of successfully doing so because Bashashati identified and tracked subclonal mutations. *See, e.g.,* Bashashati at 22 ("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 25 ("Mutational events in the ancestral clone are detectable in cell-free circulating tumour DNA from plasma."); Bashashati at 26 ("Observations of subclonal NF1 deletions reinforced evidence from the mutation data that important driver mutations may be acquired late in the evolutionary history of the tumour."). Further, Patel discloses that "[d]etection of circulating tumor DNA (ctDNA) has several applications including, but not limited to, detecting presence of a malignancy, informing a prognosis, assessing treatment efficacy, *tracking changes in tumor mutation status*, and monitoring for disease recurrence or progression."). Patel at 14 (emphasis added). A POSA would have understood "tracking changes in tumor mutation status" to refer to subclonal SNV mutations because it is well established in the art that cancers originate from a monoclonal composition, diverging into genomically distinct subclones. *See, e.g.*, 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 a 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	Forshew discloses that the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations. *See* claims 3 and 4 above. *See, e.g.,* Forshew at 7, Fig. 4A, 9, 10.

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clonal SNV mutations and one or more subclonal SNV mutations.	Further evidence of motivation and reasonable expectation of success to detect one or more clonal SNV mutations and one or more subclonal SNV mutations is found in NEOGEN00275297 ("I think by comparing two genes in the way you suggested we should indeed be able to follow tumour evolution"). *See also id.* ("we are thinking of how this might be used to monitor the tumour heterogeneity and clonal selection during treatment"). Furthermore, In view of Bai, Patel, Bashashati, and the knowledge of a POSA, it would have been obvious that the SNV mutations comprise one or more subclonal SNV mutations and one or more subclonal SNV mutations. See claims 3 and 4 above.
6. The method of claim 1, 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. *See, e.g.,* Forshew at 9 ("Whole-genome sequencing of tumor material was used to identify tumor mutations in a patient with breast cancer undergoing two phases of chemotherapy."); Forshew 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."). Narayan also discloses the tumor sample of the subject is a tumor tissue sample. *See, e.g.,* Narayan at 3493 ("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."). Further, Bai discloses a method wherein the tumor sample of the subject is a tumor tissue sample. *See, e.g.*, Bai at 2 ("All specimens had been evaluated for EGFR mutation by DHPLC routinely and were sorted into 40 wild-type and 45 mutant-type samples. From each Formalin-Fixed Paraffin-Embedded (FFPE) block, one 15-µm-thick section was stained with hematoxylin and eosin (H&E). To ensure the samples analyzed had more than 90% tumor contents, a protocol is routinely performed in our group . . .").

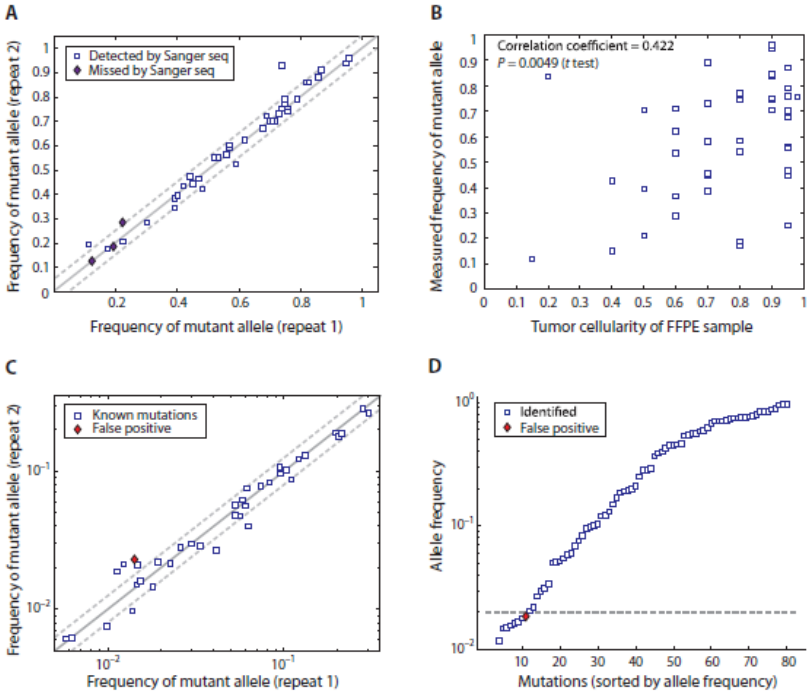
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7. The method of claim 1, wherein the method further comprises determining clonal heterogeneity of the tumor sample.	Forshew discloses that the method further comprises determining clonal heterogeneity of the tumor sample. *See, e.g.,* Forshew at 10 ("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."); Forshew at 1 (explaining that "mutation profiles in different metastatic clones can differ significantly from each other or from the parent tumor."); Forshew at 7, Fig. 4A (determining the heterogeneity of tumor samples from an ovarian cancer patient at relapse and disclosing that the *TP53* mutation could be in the left and right ovaries and omental mass at different frequencies). Further evidence of motivation and reasonable expectation of success to determine clonal heterogeneity of the tumor sample is found in NEOGEN00275297 ("we are thinking of how this might be used to monitor the tumour heterogeneity and clonal selection during treatment"). To the extent Forshew is deemed not to disclose determining clonal heterogeneity of the tumor sample, a POSA would have been motivated to combine the teachings of Forshew with those of Bashashati and Bai. This is because determining the clonal heterogeneity of a tumor would have provided a more complete picture of the genetic makeup of the cancer, which would have aided in the task of monitoring and tracking the cancer and allowed for improved treatment. A POSA would have had a reasonable expectation of success because determining clonal heterogeneity and the benefits of doing so for improved monitoring and treatment were well known in the art. *See, e.g.*, Bashashati at 28 (analyzing a fallopian tube lesion and determining that it "was composed of heterogeneous subclones" with further analysis revealing "two coexisting populations" in the lesion.); Bashashati at 25 (tracking these different mutations in cfDNA using personalized NGS assays, thus monitoring clonal heterogeneity, and explaining that "Cases 1 and 5 were enriched for the presence of mutations in the plasma that were also present in all intrapatient tumour samples, and thus part of the ancestral clone (Fisher exact test $p < 0.05$; Figure 2)"). To the extent Forshew is deemed not to disclose determining clonal heterogeneity of the tumor sample, a POSA would have been motivated to combine the teachings of Forshew with those of Bashashati and Bai. This is because determining the clonal heterogeneity of a tumor would have provided a more complete picture of the

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	genetic makeup of the cancer, which would have aided in the task of monitoring and tracking the cancer and allowed for improved treatment. A POSA would have had a reasonable expectation of success because determining clonal heterogeneity and the benefits of doing so for improved monitoring and treatment were well known in the art. It was well known in the art before 2015 that tumor tissue is genetically heterogeneous. *See, e.g.*, Forshew at 1, 10; Bashashati at Abstract, 1, 27–28, Bai at 2–3. Forshew teaches that "mutation profiles in different metastatic clones can different significantly from each other or the partent primary tumor" and that "[e]volutionary changes within the cancer can alter the mutation spectrum of the disease and its responsiveness to therapies," such that there was a need to identify the range of mutations present in a cancer patient to improve monitoring and treatment. Forshew at 1, 10. Forshew also teaches determining allele frequency in the ovarian cancer formalin-fixed, paraffin-embedded (FFPE) tissue samples. *See, e.g.,* Forshew at 4, Figure 2:

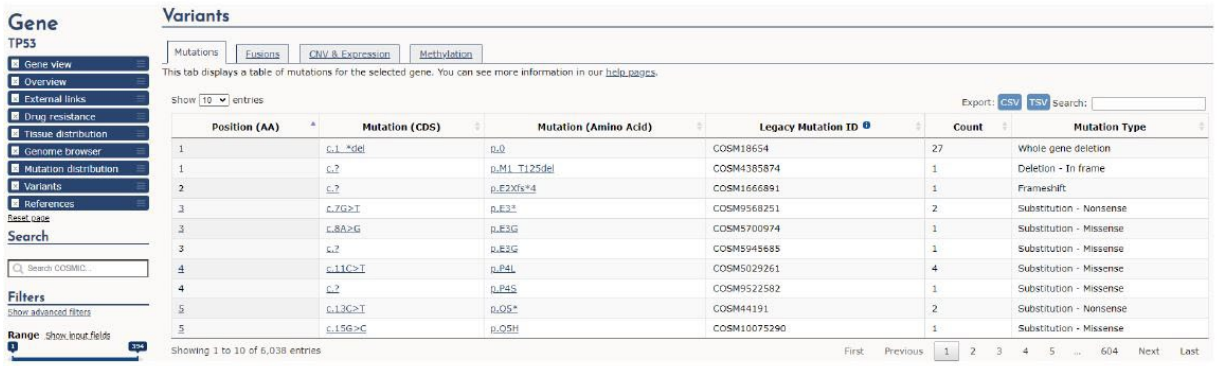
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	 **Fig. 2.** Identification of mutations in ovarian cancer FFPE samples by TAm-Seq. (**A**) Concordance between duplicate measurements of AFs of mutations identified in fragmented DNA extracted from FFPE samples. The mutation frequency in each library was calculated as the fraction of reads with the mutant (nonreference) base. Solid line indicates equality. Dotted lines indicate a difference in AF of 0.05. (**B**) Correlation of AF with FFPE tumor cellularity. The measured mutant AF (average of both repeats) correlated significantly with the cellularity, estimated from histology (table S3). (**C**) Concordance between duplicate measurements of AFs of mutations identified in a mixture of DNA extracted from different FFPE samples. (**D**) Summary of mutations called in FFPE using TAm-Seq, sorted by increasing AF. Dotted line indicates AF of 2%. As another example, Forshew teaches detecting an *EGFR* mutation in plasma, which "was not found in the ovarian mass removed by interval debulking surgery 15 months before the blood sample was collected." Forshew at 4. Forshew further discloses that "[the] EGFR mutation was detected in the two omental samples

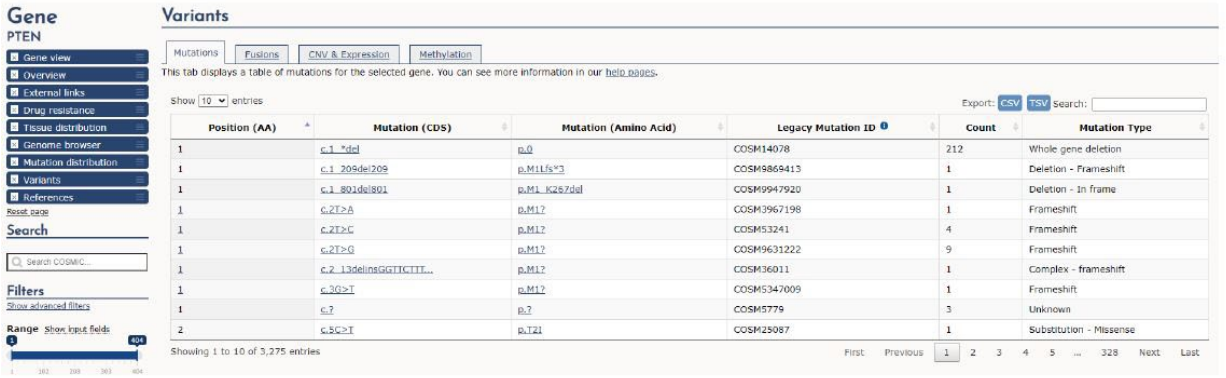
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	above the 0.99 confidence margin (fig. S3) at AF of 0.7%, but was not detected in the six ovarian samples (below the 0.8 confidence margin)." *Id.* Further, Bai discloses that "[m]utant frequencies varied widely throughout individual tumors, ranging between 5%-100% by DHPLC and 1%-100% by ARMS," and that a percentage of "samples were identified to carry intratumoral EGFR mutational heterogeneity by DHPLIC and ARMS." Bai at 3; see also Bai at 4, Figure 1., And Bashashati teaches determining clonal heterogeneity of tumors in its disclosure of characterizing and monitoring tumor cells in cancer patients. *See, e.g.*, Bashashati at 28 ("Results from PIK3CA mutation sequencing suggested the [fallopian tube] lesion in case 4 was composed of heterogeneous subclones" with further analysis revealing "two cooexisting populations of nuclei in the [fallopian tube] lesion"); Bashashati at 27–28 (determining a fallopian tube lesion harbored mutations not present in all samples and determining phylogenetic relationships, and determining likely origin on particular tumor as metastatic implant). Bashashati also shows that a POSA would have appreciated the benefit of monitoring clonal heterogeneity of the tumor sample for improved treatment. *See* Bashashati at Abstract ("[W]e observed mixed cell populations in the early fallopian tube lesion . . . Our results revealed that [high-grade serous ovarian cancers] 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 and investigation of drug-resistance mechanisms.").
8. The method of claim 1, wherein the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different	Forshew discloses that the targeted multiplex amplification amplifies 20 to 50 target loci each encompassing a different tumor-specific SNV mutation. *See, e.g.,* Forshew at 2 (performing targeted multiplex amplification to amplify 20 and 48 target loci); Forshew 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 *TP53* and *PTEN*, as well as selected regions in *EGFR*, *BRAF*, *KRAS*, and *PIK3CA* by overlapping short amplicons); Forshew at 6 (Table 2); Forshew supplemental materials at 17-18 (Table S6); Forshew at 10 (disclosing that more loci would "increase [] the robustness for tumor monitoring by compensating for effects of sampling noise or mutational drift").

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tumor-specific SNV mutation.	
9. The method of claim 1, 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. *See, e.g.,* Forshew at 2, Fig. 1A (using TAm-Seq with 48 sequence-specific primer pairs to amplify 5995 bases of genomic sequence covering coding regions of *TP53* and *PTEN*, and selected regions in *ECFR*, *BRAF*, *KRAS*, and *PIK3CA* by overlapping short amplicons); Forshew supplemental materials at 9–10, Table S1; Forshew at 6 (Table 2); Forshew supplemental materials at 17-18 (Table S6); Forshew 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 Clam 8, Forshew renders obvious amplifying higher numbers of target loci. Furthermore, under Natera's apparent interpretation of the Court's 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 Forshew. For example, COSMIC identifies 6,038 different variants in the TP53 gene.  Gene TP53 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 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 Export: CSV TSV Search: Position (AA) | Mutation (CDS) | Mutation (Amino Acid) | Legacy Mutation ID | Count | Mutation Type 1 c.1_*del p.0 COSM18654 27 Whole gene deletion 1 c.? p.M1_T125del COSM4385874 1 Deletion - In frame 2 c.? p.E2Xfs*4 COSM1666891 1 Frameshift 3 c.7G>T p.E3* COSM9568251 2 Substitution - Nonsense 3 c.8A>G p.E3G COSM5700974 1 Substitution - Missense 3 c.? p.E3G COSM5945685 1 Substitution - Missense 4 c.11C>T p.P4L COSM5029261 4 Substitution - Missense 4 c.? p.P4S COSM9522582 1 Substitution - Missense 5 c.13C>T p.Q5* COSM44191 2 Substitution - Nonsense 5 c.15G>C p.Q5H COSM10075290 1 Substitution - Missense Showing 1 to 10 of 6,038 entries First Previous 1 2 3 4 5 ... 604 Next Last

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	https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=TP53 As another example, COSMIC identifies 3,275 different variants in the PTEN gene.  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 Additionally, to the extent Natera contends that Forshew does not teach targeted multiplex amplification of 50 to 100 target loci, it would have been obvious to a POSA to do so in view of the knowledge of a POSA or in view of references such as Page. For example, Page teaches 207 amplicons amplified in a single reaction mixture. *See, e.g.*, Page at 2, 3. A POSA would be motivated to apply the method disclosed by Forshew to amplify at least 100 target loci at the time the '454 patent was filed because this would have increased efficiency and obtained more amplicons in a single operation. In addition, there is reasonable expectation of success for a POSA to combine Forshew with references such as Page because Forshew discloses that more loci would

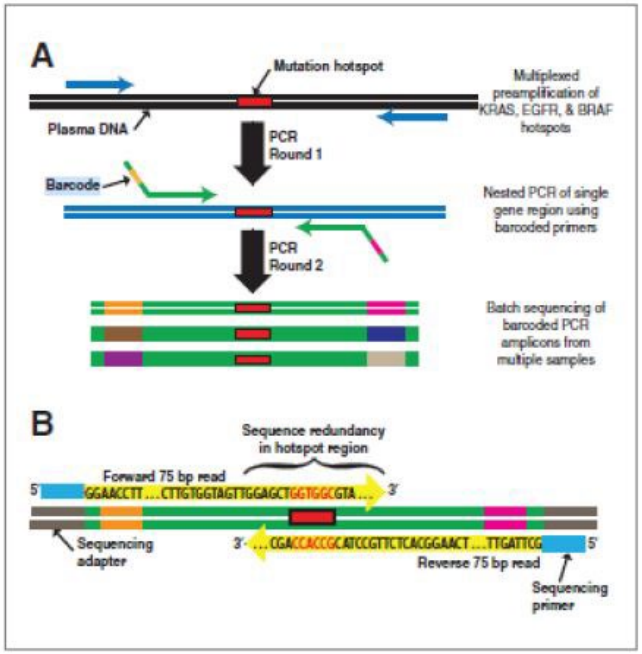
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	"increase [] the robustness for tumor monitoring by compensating for effects of sampling noise or mutational drift." Forshew at 10. In addition, the related '035 patent admits a "general belief in the art is that multiplexing PCR for sequencing is 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.
10. The method of claim 1, wherein the method further comprises designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations identified in the tumor sample.	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. *See, e.g.,* Forshew supplemental materials at 2 ("Target-specific primers were designed with universal primer sequences (termed CS1 and CS2) appended at the 5'-end (table S1)."); Forshew at 2, Fig. 1 ("Primers were designed to amplify regions of interest in overlapping short amplicons."); Forshew supplemental materials at 2 (Primer design for Tam-Seq); Forshew at 2 (Figure 1B showing the "initial preamplification step was performed for 15 cycles using a poos of target-specific primer pairs."). Additionally, Narayan discloses the method further comprises designing PCR primers or hybrid capture probes targeting the plurality of SNV mutations. *See, e.g.,* Narayan at 3493 ("Amplification of mutation hotspot regions was carried out in triplicate for each sample using 2 successive rounds of PCR, with primers designed to flank codons 12 and 13 or *KRAS*, codon 858 of *EGFR*, and codon 600 of *BRAF*."); Narayan at 3493 ("By designing PCR primers that flank very short regions (<50 bp) surrounding these mutation hotspots, we could ensure adequate amplification of highly fragmented plasma DNA and achieved greater sequence depth."); Narayan at 3495 ("Importantly, the design of short PCR amplicons enabled us to devise a sequencing strategy that could distinguish mutant form wild-type DNA molecules with very high confidence.").
11. The method of claim 1, wherein the method further	Forshew discloses that the method further comprises performing barcoding PCR prior to the sequencing.

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comprises performing barcoding PCR prior to the sequencing.	*See, e.g.,* Forshew at 2, Fig. 1 ("[S]ample-specific barcodes were attached to the harvested amplicons in a further PCR."); Forshew 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."); Forshew supplemental materials at 5, Figure S1; Forshew supplemental materials at 11, Table S2 ("Platform-specific adaptors and barcodes are attached through PCR following the single-plex amplification step."). Narayan also discloses the method further comprises performing barcoding PCR prior to the sequencing. *See, e.g.,* Narayan at Abstract ("In addition, the attachment of modular barcode tags to the DNA fragments to be sequenced facilitated the simultaneous analysis of more than 100 patient samples."); Narayan at 3493 ("The barcode sequences were 6 nucleotides in length and were designed to differ from all other barcodes in the set at a minimum of 2 positions so that a single sequencing error would not lead to misclassification of samples."); Narayan at 3494 ("Modular attachment of DNA barcode tags to the 5'-ends of the PCR primers allowed sequencing of up to 256 DNA samples in batch (Fig. 1A and Supplementary Fig. S1)."

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	 Figure 1. Schematic of the error-suppressed multiplexed deep sequencing approach. A, cell-free DNA purified from plasma undergoes 2 rounds of amplification by PCR. The first round amplifies mutation hotspot regions of several genes from a given sample in a single tube. The second round separately amplifies each hotspot region using nested primers incorporating unique combinations of barcodes to label distinct samples. The barcoded PCR products are then pooled and subjected to deep sequencing. Millions of sequences are sorted and counted to determine the ratio of mutant to wild-type molecules derived from each sample. The total number of plasma DNA fragments is measured by real-time PCR and can be used to calculate the absolute concentration of mutant ctDNA. B, sequence redundancy in mutation hotspot regions is produced by partial overlap of paired-end reads from the forward and reverse strands of each clone. This yields highly accurate base calls, permitting detection and quantitation of rare mutations with greater sensitivity.

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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.	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. *See, e.g.,* Forshew 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."); Forshew 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 *EGFR* (exon 21), at AF of 6% (patient 27, Table 1)."); Forshew 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%."). Narayan additionally discloses a method further comprising detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. *See, e.g.,* Narayan at 3492 ("There has been growing interest in trying to use such circulating tumor DNA (ctDNA) as a noninvasive biomarker to detect the presence of malignancy, follow treatment response, gauge prognosis, or monitor for recurrence. Because unique somatic mutations can be used as telltale marks to distinguish tumor-derived DNA in plasma, a new class of highly specific DNA-based cancer biomarkers can be envisioned with clinical applications that may complement those of conventional serum protein markers."); Narayan at 3497 ("Aggressive progression of metastatic disease in a different patient was accompanied by a substantial rise in ctDNA.").
13. The method of claim 1, wherein the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer.	Forshew discloses the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer. *See, e.g.,* 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.") Narayan also discloses that the cancer is lung cancer.

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	See, e.g., Narayan at 3493 (". . . 30 patients with stage I–IV non-small cell lung cancer (NSCLC) were enrolled in this study . . .").
14[pre]. A method for preparing a plasma sample of a subject having cancer or suspected of having cancer useful for detecting one or more single nucleotide variant (SNV) mutations in the plasma sample, the method comprising:	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, the method comprising. Narayan 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. *See* claim 1[pre] above.
14[a]. performing whole exome sequencing or whole genome sequencing on a tumor sample of the subject to identify a plurality of tumor-specific SNV mutations;	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. *See* claim 1[a] above.
14[b]. performing targeted multiplex amplification to amplify 10 to	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

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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	subject or DNA derived therefrom to obtain amplicons, wherein the target loci are amplified together in the same reaction volume. *See* claim 1[b] above.
14[c]. sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads,	The Court construed the term "sequencing the amplicons" to have its plain and ordinary meaning. Doc. 280 at 10. The Court construed the term "sequencing the amplicons to obtain sequence reads, and detecting one or more of the tumor-specific SNV mutations present in the cell-free DNA from the sequence reads" to require that the detecting and sequencing are part of the same step. *Id.* at 11. In addition, Natera has failed to supplement its response to NeoGenomics' Interrogatory No. 14 to properly address NeoGenomics' Initial Invalidity Contentions served on February 14, 2024 and the Court's Claim Construction Order issued on May 15, 2024 (Doc. 280). NeoGenomics reserves the right to supplement its contentions to the extent Natera supplements its response to Interrogatory No. 14 or there are any modifications to the Court's claim construction ruling. Under Natera's apparent interpretation of the Court's adopted construction, 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. *See* claim 1[c] above.

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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 Forshew in combination with Bentley, Narayan, and/or 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. *See* claim 1[d] above. *See, e.g.*, Forshew at 3 ("Background frequencies of nonreference reads were ~0.1% (median, 0.03%; mean, 0.2%; in keeping with Q30 quality threshold applied)"); Forshew 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); Forshew at 9 ("Enhanced detection down to 1 mutant allele in 10,000 or more wild-type alleles can be obtained."). As to the degree of sensitivity, Forshew specifically teaches the importance of increasing sensitivity and how to improve sensitivity of the TAm-Seq assay, including by increasing sequencing depth and fidelity. Forshew at 10. Further evidence of motivation and reasonable expectation of success is found in NEOGEN00275278–NEOGEN00275334 and NEOGEN00275270–NEOGEN0027527. As an example, NEOGEN00275278 describes experiments performed by Forshew around March 2011 demonstrating successful detection of TP53 mutations in cell-free DNA. For example, NEOGEN00275278 states: "Last week we successfully identified the same mutation by sequencing cell free DNA from the blood directly. To do this we extracted the DNA from blood plasma at two different treatment time points. We then pre amplified the DNA using primers to a number of genes including TP53. We then put this preamplified DNA on a Fluidigm Access Array before sequencing with an Illumina sequencer. We were able to sequence all of TP53 and PTEN and a number of other regions. Through this we identified the same mutation. In the first time point we found that 17% of this DNA was mutated (336/2014 reads). At the second time point this had dropped to 1% (21/2614 reads)." NEOGEN00275326 and NEOGEN00275329 provide further evidence of motivation and reasonable expectation of success for increased read depths. *See, e.g.*, NEOGEN00275326 (describing tables plotting the "number of reads for each barcode according to which block of 48 barcodes it belongs to") and NEOGEN00275329 (providing read counts of greater than 50,000 for some loci (e.g., in columns labeled FLD0200, FLD0233,

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	FLD0234, FLD0235, FLD0236, FLD0237, FLD0238, and FLD0239). As Forshew specifically teaches, "[h]igher read depth or fidelity, additional replicates, or improved algorithms could allow for enhanced mutation detection without change to protocols." Forshew at 10. *See also* Forshew at 3 ("The minimal nonreference AFs that could be detected depend on the read depth and background rates of nonreference reads… for example, a minimum of 10 reads implies that sequencing depth of 5000 would be required to detect mutations at AF as low as 0.2%."). A POSA would have been motivated to combine Forshew with Bentley and Narayan to attain the level of sensitivity to detect an SNV mutation present in less than or equal to 0.015% of the cell-free DNA comprising the SNV locus because Bentley and Narayan would have provided useful information on the sensitivity that could be achieved with Forshew's TAm-Seq protocol. *See* Bentley at 9, Table 2 (teaching that a read depth of 40,000 would successfully detect SNPs between a frequency of 0.014% and 0.018% and that a sequencing depth of 100,000 would successfully detect SNPs between a frequency of 0.005% and 0.007%) (reproduced below);

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<table>
<tr><th colspan="2">U.S. Patent No. 11,530,454</th></tr>
<tr><th>Claim</th><th>Prior Art</th></tr>
<tr><td></td><td>TABLE 2
<table><tr><th>SNP Classes</th><th>Number of Reads</th><th>Minimum frequency of SNP in population detectable with 95% confidence</th><th>Minimum frequency of SNP in population detectable with 99% confidence</th></tr><tr><td>1</td><td>200000</td><td>0.002%</td><td>0.003%</td></tr><tr><td>2</td><td>100000</td><td>0.005%</td><td>0.007%</td></tr><tr><td>5</td><td>40000</td><td>0.014%</td><td>0.018%</td></tr><tr><td>10</td><td>20000</td><td>0.028%</td><td>0.037%</td></tr><tr><td>50</td><td>4000</td><td>0.14%</td><td>0.18%</td></tr><tr><td>100</td><td>2000</td><td>0.28%</td><td>0.37%</td></tr><tr><td>200</td><td>1000</td><td>0.55%</td><td>0.74%</td></tr><tr><td>500</td><td>400</td><td>1.39%</td><td>1.85%</td></tr><tr><td>1000</td><td>200</td><td>2.76%</td><td>3.64%</td></tr></table></td></tr>
<tr><td>15. The method of claim 14, wherein the sequencing has a depth of read of at least 50,000 per target locus.</td><td>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.

See claim 1[d] above.</td></tr>
<tr><td>16. The method of claim 14, wherein the cell-free DNA comprises circulating tumor DNA.</td><td>Forshew discloses that the cell-free DNA comprises circulating tumor DNA.

Narayan also discloses that the cell-free DNA comprises circulating tumor DNA.

See claim 2 above.</td></tr>
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Claim	**Prior Art**
17. The method of claim 14, wherein the SNV mutations comprise one or more clonal SNV mutations.	In view of Forshew, in combination with Bai, Patel, Bashashati, and/or the knowledge of a POSA, it would have been obvious that the SNV mutations comprise one or more clonal SNV mutations. *See* claim 3 above.
18. The method of claim 14, wherein the SNV mutations comprise one or more subclonal SNV mutations.	In view of Forshew, in combination with Bai, Patel, Bashashati, and/or the knowledge of a POSA, it would have been obvious that the SNV mutations comprise one or more subclonal SNV mutations. *See* claim 4 above.
19. The method of claim 14, wherein the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations.	In view of Forshew, in combination with Bai, Patel, Bashashati, and/or the knowledge of a POSA, it would have been obvious to a POSA that the SNV mutations comprise one or more clonal SNV mutations and one or more subclonal SNV mutations. *See* claim 5 above.
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. Narayan also discloses that the tumor sample of the subject is a tumor tissue sample. Additionally, Bai discloses the tumor sample of the subject is a tumor tissue sample. *See* claim 6 above.

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Claim	**Prior Art**
21. The method of claim 14, wherein the method further comprises determining clonal heterogeneity of the tumor sample.	In view of Forshew, in combination with Bai, Patel, Bashashati, and/or the knowledge of a POSA, it would have been obvious to a POSA to determine clonal heterogeneity of the tumor sample. *See* claim 7 above.
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. *See* claim 8 above.
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. *See* claim 9 above.

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Claim	**Prior Art**
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. *See* claim 10 above.
25. The method of claim 14, wherein the method further comprises performing barcoding PCR prior to the sequencing.	Forshew discloses a method further comprises performing barcoding PCR prior to the sequencing. Narayan also discloses a method that further comprises performing barcoding PCR prior to the sequencing. *See* claim 11 above.
26. The method of claim 14, wherein the method further comprises detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA.	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. Narayan also discloses a method that further comprises detecting recurrence and/or metastases of the cancer from the SNV mutations detected in the cell-free DNA. *See* claim 12 above.
27. The method of claim 14, wherein the	Forshew discloses that the cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer.

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Claim	**Prior Art**
cancer is colorectal cancer, lung cancer, bladder cancer, or breast cancer.	Narayan also discloses that the cancer is lung cancer. *See* claim 13 above.
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 combined with Bentley, Narayan, and/or 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. *See* claims 1[d] and 14[d] above.

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