

EXHIBIT D-1

U.S. Patent No. 9,752,188 to Schmitt et al. (“Schmitt”); Fan et al., “Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood,” Proc. Natl. Acad. Sci. USA 2008, 105(42), 16266-16271 (“Fan”); and Forshew et al., “Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA,” Science Translational Medicine, 2012, 4(136) (“Forshew”)

Schmitt, Fan and Forshew render obvious, at least under Guardant’s apparent infringement theory, the below-described claims of U.S. Patent No. 9,902,992 (“the '992 patent”) under 35 U.S.C. § 103. The Schmitt '188 patent is a Section 371 national stage application from PCT/US2013/032665, which PCT was filed on Mar. 15, 2013, and which PCT claims the benefit of U.S. Provisional Applications 61/613,413 filed on Mar. 20, 2012, 61/625,623, filed Apr. 17, 2012, and 61/625,319, filed Apr. 17, 2012. Schmitt '188 patent is therefore prior art to the '992 patent. As shown in Appendix A hereto, the cited disclosures of the Schmitt '188 patent are present in at least one of its claimed priority Provisional applications. Foundation Medicine reserves its right to supplement the disclosure relationship between the Schmitt '188 patent and its Provisionals. Forshew was published on May 30, 2012, and is therefore prior art to the '992 patent. Fan was published on October 21, 2008, and is therefore prior art to the '992 patent

Nothing stated in this chart shall be treated as an admission or suggestion that Foundation Medicine agrees with Guardant regarding either the scope of any of the asserted claims or that Foundation Medicine’s accused products meet any limitations of the claims.

The chart below provides representative examples of where each element of each claim is found within Schmitt, Forshew and/or Fan at least under Guardant’s apparent interpretation of the claims as applied in Guardant’s infringement contentions. The cited evidence is merely illustrative, and Foundation Medicine reserves the right to cite alternative or additional evidence.

To the extent that Guardant contends that Schmitt, Forshew and/or Fan does not disclose one or more limitations of the claims, it would have been obvious to combine the teachings of Schmitt with: (1) the knowledge of one of ordinary skill in the art to show all the limitations of the claims; (2) the teachings of any of the prior art references set forth in Foundation Medicine’s other invalidity charts with respect to the one or more limitations; and/or (3) the teachings of any of the prior art references set forth in Foundation Medicine’s omnibus prior art Table for the '992 patent.

Because Guardant has yet to identify any limitation of the asserted claims that it contends is not fully disclosed by Schmitt, Forshew and/or Fan, together or in combination with other prior art cited by Foundation Medicine, Foundation Medicine expressly reserves the right to rebut any such contention, including by identifying additional obviousness combinations, if any such contention is made by Guardant.

Row	'992 Claim Limitation	Prior Art Disclosure – Schmitt '188 Alone or in Combination With Other Prior Art
1A	1. A method for detecting genetic aberrations in cell-free DNA (“cfDNA”) molecules from a subject, comprising:	To the extent that the preamble is limiting, Schmitt teaches a method for detecting genetic aberrations in cfDNA from a subject because: (1) it describes the DCS method for accurately detecting genetic mutations, which provides a solution for discriminating between sequencing errors and true rare mutations in the “deep sequencing” of “heterogeneous” samples of DNA, examples of which are tumor/normal DNA in plasma of cancer individuals or fetal/maternal DNA in plasma of pregnant mothers (Schmitt, 1:28-55; U.S. Provisional Application 61/613,413 (“Schmitt '413 provisional”), [0002]-[0003]), and (2) it discusses and cites papers regarding detecting genetic mutations in fetal DNA by sequencing maternal plasma DNA (Schmitt, 1:28-55; Schmitt '413 provisional, [0002]-[0003]).
1B	a) providing cfDNA molecules obtained from a bodily sample of the subject;	<p>Besides Schmitt’s disclosure that cfDNA molecules can be obtained from a plasma sample of a subject for DCS, Fan et al., “Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood,” Proc. Natl. Acad. Sci. USA 2008, 105(42), 16266-16271 (“Fan”) and Forshew et al., “Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA,” Science Translational Medicine, 2012, 4(136) (“Forshew”) both disclose providing cfDNA molecules obtained from a bodily sample of the subject.</p> <p>Fan sequenced cell-free DNA from the plasma of pregnant women to detect the genetic aberration known as fetal aneuploidy. Fan, Abstract. Fan used cell-free plasma DNA samples where about 1-8 nanograms of DNA fragments were extracted from 1.3-3.2 ml of cell-free plasma for sequencing library preparation. <i>Id.</i>, 16270, Table S1.</p> <p>Forshew describes “tagged-amplicon deep sequencing (TAm-Seq)” to identify mutations in both solid tumor samples as well as in plasma of cancer patients. Forshew, Abstract, 4, Tables 1-2. Forshew extracted cell-free DNA from between 0.85 and 2.2 ml of plasma. <i>Id.</i>, 10.</p>
1C	b) attaching tags comprising barcodes having a plurality of different barcode sequences to the cfDNA molecules to tag at least 20% of the cfDNA molecules, which attaching comprises ligating adaptors comprising the barcodes to both ends of the cfDNA molecules, wherein ligating comprises using more than 10X molar excess of the adaptors as compared to the cfDNA molecules, thereby generating tagged parent polynucleotides;	<p>First, the term “barcode” is used in the '992 patent consistent with its ordinary meaning in the art as of September 2012: a nucleotide or a sequence of nucleotides used as a tag or identifier. '992 patent, 15:50-61; 38:14-19; 39:17-21; 64:2-13. The term “parent polynucleotide” also is used in the '992 patent consistent with its ordinary meaning in the art as of September 2012: the starting DNA fragment that is tagged, amplified to generate copies, sequenced, and analyzed for the presence of mutations. '992 patent, Figure 9; 45:17-26.</p> <p>For the DCS approach, Schmitt teaches ligating barcode tags, or “SMI-containing adaptors” to parental DNA molecules to uniquely identify families or copies of these templates. The SMI adaptor molecules can be “ligated to both ends of a target nucleic acid molecule.” Schmitt, 7:38-41; Schmitt '413 provisional, [0018]. Schmitt teaches that the unique identification can be based on either: (1) the use of a large number of barcode sequences such that there is a high probability that a unique barcode sequence is ligated to each parental DNA template; or (2) a “hybrid” approach that attaches a small number of non-unique barcodes to parental fragments, where the non-unique barcode sequence in combination with the end-sequences of the parental DNA template provides a unique sequence signature to identify parental DNA templates and copies thereof. Schmitt, 9:1-14, Figure 4; Schmitt '413 provisional, [0030]. Thus, in either case, the tags comprise a plurality of different barcode sequences.</p> <p>The relevance of ligation efficiency was well-known to the POSA, which was to improve the sequencing library’s diversity or representation of the genome by optimizing one or more steps of sample preparation. Arneson et al., “Whole-Genome Amplification by Adaptor-Ligation PCR of Randomly Sheared Genomic DNA</p>

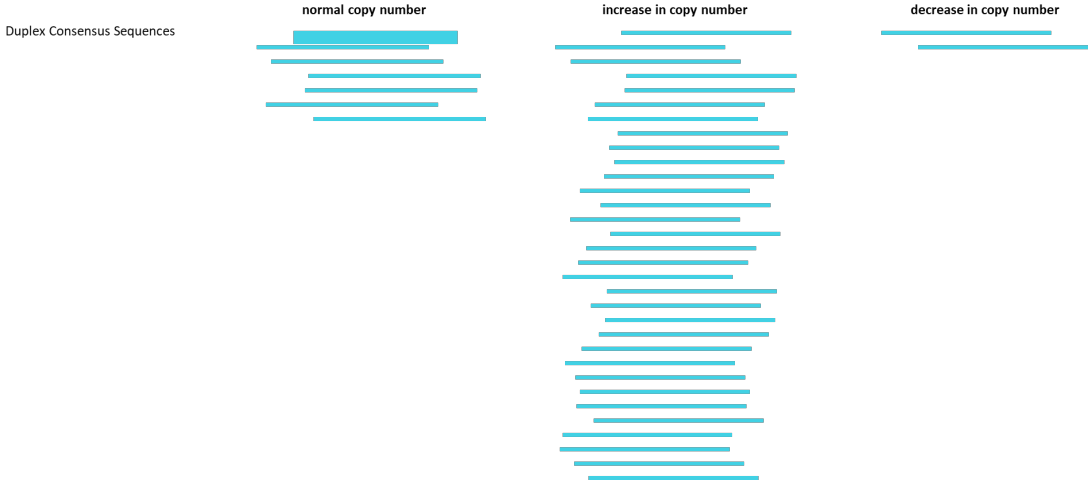
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		<p>(PRSG),” CSH Protocols, 2008, 3(1) (“Arneson”), 1. Schmitt does not explicitly recite tagging “at least 20%” of the cfDNA molecules by ligation, but Schmitt teaches using state of the art ligation techniques, which a POSA would have understood to result in at least a 10-20% yield of tagged DNA fragments. For example, Schmitt disclosed the use of both blunt-end ligation and TA ligation (i.e., ligation of molecules with a thymine (T) or adenine (A) overhang). Schmitt, 7:58-62; 15:5-20; Schmitt '413 provisional, [0021]. As of September 2012, it was known in the art that the blunt-end ligation technique was capable of generating a 10-20% final library yield. <i>See, e.g.</i>, Meyer and Kircher, “Illumina sequencing library preparation for highly multiplexed target capture and sequencing,” Cold Spring Harb. Protoc. 2010, (6), prot5448 (“Meyer and Kircher”), 2, 7. It was also common knowledge in the art that TA ligation was likely to result in a higher yield of tagged fragments. Quail et al., “A large genome center's improvements to the Illumina sequencing system,” Nat. Methods 2008, 5(12), 1005-1010 (“Quail”), 1006; Williford and Betrán, “Gene Fusion,” eLS 2013, 1-8 (“Williford”). This is because the specificity of TA ligation technique made it a superior technique over blunt ligation due to the propensity of blunt-ended template molecules to self-ligate (instead of ligating to adaptors). Quail, 1006; Williford.</p> <p>Schmitt explains that when the DCS method is employed using TA ligation, the “efficiency of adaptor ligation” is “comparable to those seen with standard library preparation methods.” Schmitt, 22:43-56; Schmitt '413 provisional, [0065]. Because standard blunt end ligation methods generated a 10-20% yield, and because TA ligation was more effective than blunt-end ligation, a POSA would have understood the method of Schmitt would achieve a 10-20% or higher yield.</p> <p>Moreover, a POSA would have understood that ligation efficiency could be improved using a variety of techniques known in the art as of September 4, 2012.</p> <p>For example, by September 4, 2012, Quail taught that ligation efficiency can be improved using ultrapure commercial ligases. Quail, 1006-1007. Further, by September 4, 2012, the New England Biolabs (NEB) “Quick Ligation Kit” (NEB Catalog # M2200) was a known kit that improved ligation efficiency, as evidenced by a NEB publication that compared the ligation efficiencies of T4 ligase alone with the Quick Ligation Kit, and with a newer mix called Blunt/TA Master Mix. This NEB publication shows that the percent ligation product yield of two adaptors at 10x molar excess to nanogram quantities of insert using T4 ligase alone was about 30%, which increased to almost 70% with the Quick Ligation Kit. Lohman, Efficient Adaptor Ligation for the Preparation of dsDNA Libraries using the Blunt/TA Ligase Master Mix (“Lohman”), 1 (listing Quick Ligation Kit #M2200), 3, Figure 2.</p> <p>Accordingly, a POSA would have understood Schmitt to teach directly a method wherein at least 10-20% of DNA molecules are tagged, and it also would have been obvious to a POSA that at a ligation yield wherein 20% or more molecules are tagged could be achieved using methods known in the art.</p> <p>Finally, the use of more than 10x molar excess of adaptors as compared to parental DNA templates was both well known in the art and expressly taught by Schmitt. For example, Parkinson 2011 describes two protocols for generating NGS libraries: (1) the standard Illumina library preparation method and (2) a modified “tagmentation” library method. Parkinson et al., “Preparation of high-quality next-generation sequencing</p>

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		<p>libraries from picogram quantities of target DNA,” Genome Res. 2012, 22(1), 125-133 (“Parkinson”), 16. In the standard Illumina library preparation method, DNA was sonicated and 250-350 bp fragments were recovered, end-repaired to generate blunt ends for A-tailing, and then ligated with Illumina adapters at “10x molar excess.” <i>Id.</i> Likewise, Schmitt discloses a standard library preparation protocol that explicitly teaches ligation with 10x molar excess of SMI adaptors relative to DNA fragments. First Schmitt discloses that DNA fragments larger than the optimal range of ~200-500 bp were removed. Schmitt, 19:65-67; Schmitt '413 provisional, [0056]. Then, Schmitt states that “custom adaptors were ligated by combining 750 ng of T-tailed DNA with 250 pmol adaptors in a reaction containing 3000 units T4 DNA ligase.” Schmitt, 20:9-15; Schmitt '413 provisional, [0056]. The conversion of the fragments to moles is: 750 ng of dsDNA with a size of about 500 bp is about 2.4 pmol, and 750 ng of dsDNA with a size of about 200 bp is about 6 pmol. Thus, 250 pmol of adaptors is about 40-103 molar excess as compared to the parental DNA fragments, which satisfies the claim requirement of “using more than 10x molar excess.”</p>
1D	c) amplifying the tagged parent polynucleotides to produce amplified tagged progeny polynucleotides;	<p>Schmitt’s DCS method amplifies tagged parent polynucleotides to produce amplified tagged progeny polynucleotides. For example, Figure 1 illustrates an overview of DCS. SMI adaptors are ligated to sheared double-stranded DNA (i.e., parent polynucleotides) such that “every DNA fragment becomes labeled with two distinct SMI sequences.” Schmitt, Figure 1, 3:44-62; Schmitt '413 provisional, Figure 1, [0011]. These tagged-parent fragments are PCR-amplified “to generate families of PCR duplicates,” which are amplified tagged progeny polynucleotides as required by the '992 claims. Schmitt, Figure 4, 4:30-54; Schmitt '413 provisional, Figure 4, [0014] (regarding the hybrid method tagging parental DNA fragments with 4-mer tags that are amplified and sequenced).</p> <p>Schmitt teaches sequencing of the amplified tagged progeny polynucleotides to produce a plurality of sequence reads from each of the tagged parent polynucleotides, wherein each sequence read comprises a barcode sequence and a sequence derived from the parental DNA molecule. Schmitt, 3:10-20. “FIG. 3 illustrates error correction through Duplex Consensus Sequencing (DCS) analysis according to one embodiment. (a-c) shows sequence reads . . . sharing a unique set of SMI tags are grouped into paired families with members having strand identifiers in either the $\alpha\beta$ or $\beta\alpha$ orientation. Each family pair reflects one double-stranded DNA fragment.” <i>Id.</i>, 4:4-29; Schmitt '413 provisional, [0013]; <i>see also</i> Schmitt, Figure 4, 4:30-54 (“When these molecules [4-mer tagged parental fragments] are amplified . . . and sequenced, they will yield the following sequence reads”).</p>
1E	d) sequencing the amplified tagged progeny polynucleotides to produce a plurality of sequence reads from each of the tagged parent polynucleotides, wherein each sequence read of the plurality of sequence reads comprises a barcode sequence and a sequence derived from a cfDNA molecule of the cfDNA molecules;	See row 1D.
1F	e) mapping sequence reads of the plurality of sequence reads to one or more reference sequences from a human genome;	Schmitt discloses mapping sequence reads to a human reference genome: “Reads were aligned to the human genome with the Burrows Wheeler Aligner (BWA).” Schmitt, 20:39-64; Schmitt '413 provisional, [0060]; <i>see also</i> Schmitt, 23:10-14 (regarding the well-known “hg19” human reference genome); Schmitt '413 provisional, [0066]. The POSA would understand that aligning sequence reads to a human genome constitutes mapping of the sequence reads to a

Row	'992 Claim Limitation	Prior Art Disclosure – Schmitt '188 Alone or in Combination With Other Prior Art
		reference sequence from a human genome.
1G	f) grouping the sequence reads mapped in e) into families based at least on barcode sequences of the sequence reads, each of the families comprising sequence reads comprising the same barcode sequence, . . .	Schmitt teaches grouping sequence reads into families based at least on barcode sequences, where each of the families comprise sequence reads with the same barcode sequence. “FIG. 3 illustrates error correction through Duplex Consensus Sequencing (DCS) analysis according to one embodiment. (a-c) shows sequence reads . . . sharing a unique set of SMI tags are grouped into paired families with members having strand identifiers in either the $\alpha\beta$ or $\beta\alpha$ orientation.” Schmitt, 4:4-29, 20:39-64; Schmitt '413 provisional, [0013], [0060].
1H	whereby each of the families comprises sequence reads amplified from the same tagged parent polynucleotide;	Schmitt explains and shows that each of the grouped families comprise sequence reads amplified from the same tagged parent polynucleotide. In the description for Figure 3, Schmitt states that “[e]ach family pair reflects one double-stranded DNA fragment.” Schmitt, 4:9-10; Schmitt '413 provisional, [0013]. Figure 3(a), 3(b), and 3(c) shows three families, where each family contains sequence reads amplified from the same tagged parent polynucleotide. “FIG. 3 . . . (a)-(c) shows sequence reads . . . sharing a unique set of SMI tags are grouped into paired families with members having strand identifiers in either the $\alpha\beta$ or $\beta\alpha$ direction.” Schmitt, 4:4-9, Figure 3; Schmitt '413 provisional, [0013], Figure 3.
1I	g) at each of a plurality of genetic loci in the one or more reference sequences, collapsing sequence reads in each family to yield a base call for each family at the genetic locus; and	<p>Schmitt teaches that after grouping into families, each family of reads is collapsed to generate a consensus read. Schmitt, 20:50-52 (“Reads having common (i.e., identical) SMI sequences were grouped together, and were collapsed to generate a consensus read.”); Schmitt '413 provisional, [0060]. Schmitt explains that to generate a consensus sequence, “[s]equencing positions were discounted if the consensus group covering that position consisted of fewer than 3 members, or if fewer than 90% of the sequences at that position in the consensus group had the identical sequence.” Schmitt, 20:53-56; Schmitt '413 provisional, [0060]. A POSA would have understood that each consensus sequence provides a consensus base call for each family at those positions with sufficient coverage and agreement to form a consensus.</p> <p>Schmitt further explains that parts (e) and (f) of Figure 3 “show consensus sequences from all independently captured, randomly sheared fragments containing <i>a particular genomic site</i> . . . identified and compared to determine the frequency of <i>genetic variants at this locus</i> within the sampled population.” Schmitt, 4:24-29, Figure 3 (emphasis added); Schmitt '413 provisional, [0013], Figure 3. A POSA would have understood the term “genomic site” to refer to a genetic locus of the reference sequence to which the consensus sequences are mapped and in which a variant may be detected. Thus, the consensus sequence that contains a particular genetic locus of the reference sequence provides a base call at that particular genetic locus.</p> <p>A POSA would have understood “a plurality of genetic loci” to mean more than one location or fixed position in a genome that is of potential interest. In other words, genetic loci is a relative term dependent upon the focus of the investigator. For example, genetic loci of interest could be nucleotide positions that have been identified to have functional mutations for particular genes or the millions of small nucleotide polymorphisms of the human haplotype map.</p> <p>Schmitt further teaches providing consensus sequences spanning a 758 kb region of the genome—which can comprise a plurality of genetic loci of interest. Schmitt, 23:61-24:1; Schmitt '413 provisional, [0068]; <i>see also</i> Albert et al., “Direct selection of human genomic loci by microarray hybridization,” Nat. Methods 2007, 4(11), 903-905 (“Albert”), Abstract. Schmitt explains that “[t]he adaptor-ligated library was PCR amplified and subjected to SureSelect capture, with targeting of an arbitrary 758 kb portion of the genome.” Schmitt, 22:50-53; Schmitt '413 provisional, [0065]. Following</p>

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		sequencing, “the SMI tags were used to group together PCR duplicates that arose from individual single-stranded DNA molecules and to create a consensus sequence from the family of duplicates.” Schmitt, 23:19-22; Schmitt '413 provisional, [0067]. Accordingly, Schmitt teaches collapsing sequence reads in each family to yield a consensus sequence for each family (i.e., a base call) at each genetic locus of a plurality of genetic loci.
1J	h) detecting, at one or more genetic loci, a plurality of genetic aberrations, wherein the plurality of genetic aberrations comprises two or more different members selected from the group of members consisting of a single base substitution, a copy number variation (CNV), an insertion or deletion (indel), and a gene fusion.	<p>Schmitt discloses detecting at least single base substitutions and CNV at genetic loci. The DCS consensus sequence and error-correction process seeks to discriminate “true mutation” from sequencing error. “In further embodiments, the method confirms the presence of true mutation by (i) identifying a mutation present in the paired target nucleic acid strands having one or more nucleotide positions that disagree; (ii) comparing the mutation present in the paired target nucleic acid strands to the error corrected double-stranded consensus sequence; and (iii) confirming the presence of a true mutation when the mutation is present on both of the target nucleic acid strands and appears in all members of a paired target nucleic acid family.” Schmitt, 3:31-40; Schmitt '413 provisional, [0010]; <i>see also</i> Schmitt, Figure 3, 4:4-29 (“(c) shows true mutations . . . present on both strands of a captured fragment appear in all members of a family pair.”); Schmitt '413 provisional, Figure 3, [0013].</p> <p>While the POSA would have understood “mutation” to encompass each of the four claimed genetic aberrations, Schmitt explicitly teaches DCS can detect “single base substitution” and “CNV.” For example, in the experiment to assess sensitivity, single-strand consensus sequencing was conducted to detect single base substitutions. “Consensus Sequencing Accurately Recovers Spiked-in Control Mutations. A series of M13mp2 variants were constructed which contain known single base substitutions.” Schmitt, 29:57-60 (emphasis added). Regarding CNV, Schmitt states that SMI tags allow for “single-molecule counting for accurate determination of DNA or RNA copy number” that is applicable for “accurate detection of altered genomic copy number . . . such as trisomy 21.” <i>Id.</i>, 18:3-21; Schmitt '413 provisional, [0048].</p> <p>Further, the POSA would have understood that Schmitt’s methods can detect more than one type of genetic aberration in the same experiment. Sequencing approaches, including NGS, provide the raw data, i.e., sequence reads, that inherently contains different types of genetic aberrations. The POSA would have understood that different types of mutations could be identified from the same data set. <i>See, e.g.</i>, Meyerson et al., “Advances in understanding cancer genomes through second-generation sequencing,” <i>Nature Review Genetics</i>, Vol. 11 (2010), 685-696 (“Meyerson”), Figure 3; Schweiger et al., “Genome-wide massively parallel sequencing of formaldehyde fixed-paraffin embedded (FFPE) tumor tissues for copy-number- and mutation-analysis,” <i>PLoS One</i> 2009, 4(5), e5548 (“Schweiger”), 3, (detecting copy number and SNPs in same sequencing experiment); McKernan et al., “Sequence and structural variation in a human genome uncovered by short-read, massively parallel ligation sequencing using two-base encoding,” <i>Genome Res.</i> 2009, 19(9), 1527-1541 (“McKernan”), Abstract, (detecting SNPs, indels, and gene fusions in same sequencing experiment). DCS generates consensus sequences from raw sequence reads, and the POSA would need only to compare differences between the consensus sequences and reference sequences to determine the nature of the genetic aberration, i.e., single nucleotide substitutions, indel, gene fusion, etc. <i>See infra</i> Figures. For CNV detection, DCS utilizes the same family consensus sequence data where each family of reads corresponds to a single parental fragment such that the number of parental fragments mapping to a genomic locus can be counted. <i>See infra</i> Figures.</p>

Row	'992 Claim Limitation	Prior Art Disclosure – Schmitt '188 Alone or in Combination With Other Prior Art	
		<p style="text-align: center;">Deletion</p> <p>Single-Stranded Consensus Sequences α AGCTATAGCT β or β TCGATATCGA α</p> <p>Duplex Consensus Sequence TCGATATCGA AGCTATAGCT</p> <p>Reference Sequence AGCTAGCTAGCT TCGATCGATCGA</p>	<p style="text-align: center;">Insertion</p> <p>Single-Stranded Consensus Sequences α AGCTAGAAAACTAGCT β or β TCGATCTTTTGATCGA α</p> <p>Duplex Consensus Sequence TCGATCTTTTGATCGA AGCTAGAAAACTAGCT</p> <p>Reference Sequence AGCTAGCTAGCT TCGATCGATCGA</p>
		<p style="text-align: center;">Gene Fusion</p> <p>Single-Stranded Consensus Sequences α CTAGCTTCGATC β or β GATCGAAGCTAG α</p> <p>Duplex Consensus Sequence GATCGAAGCTAG CTAGCTTCGATC</p> <p>Reference Sequence AGCTAGCTAGCT (gene 1) TCGATCGATCGA (gene 2) AGCTAGCTAGCT</p>	<p style="text-align: center;">Single Nucleotide Variant</p> <p>Single-Stranded Consensus Sequences α AGCTATCTAGCT β or β TCGATAGATCGA α</p> <p>Duplex Consensus Sequence TCGATAGATCGA AGCTATCTAGCT</p> <p>Reference Sequence AGCTAGCTAGCT TCGATCGATCGA</p>

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		<p style="text-align: center;">Copy Number Variants</p>  <p>Duplex Consensus Sequences</p> <p style="text-align: center;">normal copy number increase in copy number decrease in copy number</p>
2	2. The method of claim 1, comprising providing less than 100 nanograms (ng) of the cfDNA molecules.	<p><i>See Claim 1.</i></p> <p>Both Fan and Forshew explicitly disclose providing less than 100 ng (for claim 2) or less than 10 ng (for claim 3) of cfDNA molecules from the plasma of human subjects for sequencing. Fan extracted about 1-8 ng of DNA from cell-free plasma for sequencing library preparation. Fan, 16270, Table S1 (“[a]pproximate amt of input DNA for sequencing library construction” that lists between 1.2 and 8.0 ng of DNA depending on the sample). Forshew extracted about 0.9-19.7 ng of DNA from cell-free plasma for sequencing library preparation. Forshew, Table S6 (listing the estimated amount of cell-free DNA sequenced, ranging from 0.9 ng to 19.7 ng).</p>
3	3. The method of claim 1, comprising providing less than 10 nanograms (ng) of the cfDNA molecules.	<p><i>Not asserted by Guardant.</i></p>
4	4. The method of claim 1, comprising providing between 100 and 100,000 human haploid genome equivalents of the cfDNA molecules, wherein the cfDNA molecules are tagged with between 2 and 1,000,000 unique identifiers.	<p><i>See Claim 1.</i></p> <p>Through its teachings on sequencing nanogram amounts of cfDNA, both Fan and Forshew disclose providing between 100 or 100,000 (for claim 4), or between 1,000 and 50,000 (for claim 5), human haploid genome equivalents of the cfDNA molecules. Forshew teaches the standard scientific formula that 1 haploid genome is about 3.3 pg of DNA. Forshew, S11. Applying this formula to the nanogram amounts of cfDNA disclosed in Fan or Forshew, one can calculate the number of human haploid genome equivalents of cfDNA. Fan reported between 1-8 ng (1,000-8,000 pg) of plasma DNA used for library construction, which corresponds to between about 303 and 2,424 human haploid genome equivalents. Fan, Table S1. This range of 303-2,424 human haploid genome equivalents falls within claim 4’s range of</p>

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		<p>100-100,000. Certain samples used in Fan fall within claim 5's range of 1,000 and 50,000, e.g., the "P1" sample with 8 ng of cfDNA corresponds to about 2,424 human haploid genome equivalents. <i>Id.</i> Forsheew used about 1-20 ng (1,000-20,000 pg) of plasma DNA, which corresponds to about 303-6,060 human haploid genome equivalents. Forsheew, Table S6. This range of 303-6,060 human haploid genome equivalents falls within claim 4's range of 100-100,000. Certain samples used in Forsheew fall within claim 5's range of 1,000 and 50,000, e.g., sample number "2" with 4.2 ng of cfDNA corresponds to about 1,273 human haploid genome equivalents. <i>Id.</i></p> <p>Schmitt discloses tagging parental fragments like cfDNA with between 2 and 1,000,000 unique identifiers. Schmitt's "hybrid" approach teaches a "shorter n-mer tag (such as 1 or 2 or 3 or 4 or more degenerate or semi degenerate bases) in the adaptor may also serve as unique molecular identifiers." Schmitt, 4:30-54, 9:1-13, Figure 4; Schmitt '413 provisional, [0014], [0030]. When the SMI sequence is a n-mer 4 nucleotides in length that is ligated, or "tagged," to parental DNA fragments, there would be 256 unique tag sequences (or 65,536 when considering 4-mer tags at both ends of a fragment), which satisfies claim 4's limitation requiring tagging with between 2 and 1,000,000 unique identifiers. If the tag sequence is a 2-mer, then the number of unique tag sequences is 16 (or 256, considering the number of tags at both ends). Thus, Schmitt's hybrid approach teaches tagging parental DNA fragments with between 2 and 1,000 (and between 2 and 1,000,000) unique identifiers, and Fan or Forsheew teach between 1000 and 50,000, or 100 and 100,000, human haploid genome equivalents of the cfDNA molecules.</p>
5	5. The method of claim 1, comprising providing between 1,000 and 50,000 human haploid genome equivalents of the cfDNA molecules, wherein the cfDNA molecules are tagged with between 2 and 1,000 unique identifiers.	<i>See row 4.</i>
6	6. The method of claim 1, wherein each of the plurality of different barcode sequences is at least 5 nucleotides in length.	<p><i>See Claim 1.</i></p> <p>Unlike claims 4 and 5, claim 6 does not limit the number of unique tag sequences that are attached to the parental fragments. Schmitt discloses 12-mer tags, which are 12 nucleotides in length, ligated to parental DNA fragments. Schmitt, 3:44-62, Figure 1, 6:46-7:5; Schmitt '413 provisional, [0011], Figure 1, [0016].</p>
7	7. The method of claim 1, wherein the attaching comprises non-uniquely tagging the cfDNA molecules with at least 10 and at most 1,000 different barcode sequences.	<p><i>See Claim 1.</i></p> <p>The '992 patent explains that cfDNA molecules or parent polynucleotides are "non-uniquely tagged" whenever the number of different identifiers attached to the cfDNA molecules or polynucleotides is at least 2 and fewer than the number of cfDNA molecules or polynucleotides. '992 patent, 43:15-21.</p> <p>As explained for claims 4 and 5, Schmitt describes a "hybrid" approach where short n-mers of length 1-4 are ligated to parental fragments. Schmitt, 4:30-54, Figure 4; Schmitt '413 provisional, [0014]. Schmitt's "hybrid" approach for tagging DNA fragments results in non-unique tagging. A human genome has about 3 billion base pairs, and Schmitt teaches selection of fragments in the optimal range of 200-500 base pairs, which would result in millions of fragments per genome. Schmitt, 22:43-46; Schmitt '413 provisional, [0065]. Given that there are no more than 256 possible tag sequences in the 2-mer scenario (as explained above), probability dictates each tag sequence will be ligated more than</p>

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		once, which means each tag sequence is non-unique relative to a fragmented genome or to naturally fragmented cfDNA that has an average size of about 140 to 170 base pairs. Forsheiw, 1 (citing publications on ctDNA and cffDNA). Thus, Schmitt discloses non-uniquely tagging with at least 10 and at most 1,000 different barcode sequences, and as explained for claim 1, Fan or Forsheiw discloses cfDNA.
8	8. The method of claim 1, wherein the attaching comprises uniquely tagging the cfDNA molecules.	<i>Not asserted by Guardant.</i>
9	9. The method of claim 1, wherein the attaching comprises performing blunt-end ligation or sticky end ligation.	<i>See Claim 1.</i> Schmitt teaches this limitation. Schmitt explains that the SMI adaptor comprising the SMI sequence (i.e., barcode) is attached to the target nucleic acid sequence by ligation, and adaptors can be attached to both ends of the parental fragment. Schmitt, 3:1-6; 3:44-62, 15:21-38; Schmitt '413 provisional, [0008]. Schmitt further explains: “The SMI ligation adaptor may be any suitable ligation adaptor that is complementary to a ligation adaptor added to a double-stranded target nucleic acid sequence including, but not limited to a T-overhang, an A-overhang, a CG overhang, a blunt end, or any other ligatable sequence.” Schmitt, 7:58-62; 15:5-20; Schmitt '413 provisional, [0020]. Schmitt thus expressly teaches attaching using blunt end or sticky end ligation.
10	10. The method of claim 1, wherein the attaching comprises non-uniquely tagging the cfDNA molecules such that no more than 5% of the tagged parent polynucleotides are uniquely tagged.	<i>Not asserted by Guardant.</i>
11	11. The method of claim 1, wherein at least 50% of the cfDNA molecules are tagged by the attaching.	<i>Not asserted by Guardant.</i>
12	12. The method of claim 1, wherein at least 80% of the cfDNA molecules are tagged by the attaching.	<i>Not asserted by Guardant.</i>
13	13. The method of claim 1, further comprising selectively enriching for polynucleotides mapping to one or more selected reference sequences prior to the sequencing, wherein the selectively enriching comprises (i) subjecting the cfDNA molecules to selective amplification against the one or more selected reference sequences, (ii) subjecting the tagged parent polynucleotides to selective amplification against the one or more selected reference sequences, (iii) subjecting the amplified	<i>See Claim 1.</i> The term “selectively enriching” had a well-understood meaning in the art as of September 2012, and a POSA would have understood the term to refer to isolating particular genomic regions of interest for analysis, such as by sequencing. Mertes et al., “Targeted enrichment of genomic DNA regions for next-generation sequencing,” Brief Funct. Genomics 2011, 10(6), 374-386 (“Mertes”), 374-75. Enrichment techniques were well known and used in the art and include hybrid capture (also known as hybrid selection, target capture, or capture) and PCR amplification. <i>Id.</i> , 375. Example 1 of Schmitt explains that, prior to sequencing, “[t]arget capture was performed with the Agilent SureSelect system.” Schmitt, 20:24-27; 22:50-67; Schmitt '413 provisional, [0058]. The target capture in Example 1 utilized 120 nucleotide “[c]apture baits” that were designed in view of the 758 kb genomic region reference sequence. Schmitt, 20:27-30; Schmitt '413 provisional, [0058]. Schmitt explains “selection and capture may be accomplished by any selection by hybridization method.” Schmitt, 22:60-61; Schmitt '413 provisional, [0065]. Thus, Schmitt discloses at least claim limitation (iv), “selective sequence capture against the one or more selected reference.”

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	progeny polynucleotides to selective sequence capture against the one or more selected reference sequences, or (iv) subjecting the cfDNA molecules to selective sequence capture against the one or more selected reference sequences.	
14	14. The method of claim 13, wherein the selectively enriching comprises enriching for polynucleotides mapping to the following genes: V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), adenomatous polyposis coli (APC), and tumor protein 53 (TP53).	<p>Claim 14 depends from Claim 13, which depends from Claim 1. As discussed above, Claim 1 is obvious over Schmitt and Fan or Forsheiw. Schmitt also teaches the limitations set forth in Claim 13 of the '992 Patent.</p> <p>Claim 13 requires the method to further comprise “selectively enriching for polynucleotides mapping to one or more selected reference sequences prior to the sequencing, wherein the selectively enriching comprises (i) subjecting the cfDNA molecules to selective amplification against the one or more selected reference sequences, (ii) subjecting the tagged parent polynucleotides to selective amplification against the one or more selected reference sequences, (iii) subjecting the amplified progeny polynucleotides to selective sequence capture against the one or more selected reference sequences, or (iv) subjecting the cfDNA molecules to selective sequence capture against the one or more selected reference sequences.” '992 patent, 65:5-17. The term “selectively enriching” had a well-understood meaning in the art as of September 2012, and a POSA would have understood the term to refer to isolating particular genomic regions of interest for analysis, such as by sequencing. Mertes et al., “Targeted enrichment of genomic DNA regions for next-generation sequencing,” <i>Brief Funct. Genomics</i> 2011, 10(6), 374-386 (“Mertes”), 374-75. Enrichment techniques were well known and used in the art and include hybrid capture (sometimes referred to as hybrid selection, target capture, or capture) and PCR amplification. <i>Id.</i>, 375. Example 1 of Schmitt explains that, prior to sequencing, “[t]arget capture was performed with the Agilent SureSelect system.” Schmitt, 20:24-27; 22:50-67; Schmitt '413 provisional, [0058]. The target capture in Example 1 utilized 120 nucleotide “capture baits” that were designed in view of the 758 kb genomic region reference sequence. Schmitt, 20:27-30; Schmitt '413 provisional, [0058]. Schmitt explains “selection and capture may be accomplished by any selection by hybridization method.” Schmitt, 22:60-61; Schmitt '413 provisional, [0065]. Thus, Schmitt discloses at least claim limitation (iv) of Claim 13, “selective sequence capture against the one or more selected reference sequences.”</p> <p>Claim 14 requires selective enrichment to include three specific genes, KRAS, TP53, and APC. Schmitt teaches that DCS can include a selective enrichment step with capture probes “that target the desired double-stranded DNA sequence,” but does not explicitly suggest enrichment of the three genes recited in the claim. Schmitt, 22:50-67; Schmitt '413 provisional, [0065]. Forsheiw describes amplification and sequencing of particular regions of interest from cfDNA of ovarian and breast cancer patients. Forsheiw et al., “Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA,” <i>Science Translational Medicine</i>, 2012, 4(136) (“Forsheiw”), 2. These regions of interest included KRAS and TP53. “We designed a set of 48 primer pairs to amplify 5995 bases of genomic sequence . . . of TP53 and PTEN, and selected regions in EGFR, BRAF, KRAS, and PIK3CA (table S1) by overlapping short amplicons (Fig. 1A).” <i>Id.</i>, 3. Schwarzenbach teaches the analysis of cfDNA for specific gene mutations in all three claimed genes. “Tumour-specific gene mutations. The analysis of cfDNA for specific gene mutations, such as those in KRAS and TP53, is desirable because these genes have a high mutation frequency in many tumour types and contribute to tumour progression. Additionally, clinically relevant mutations in BRAF, epidermal growth factor receptor (EGFR) and adenomatous polyposis coli (APC) have now been studied in cfDNA.” Schwarzenbach et al., “Cell-free nucleic acids as biomarkers in cancer patients,” <i>Nat. Rev. Cancer</i> 2011, 11(6), 426-437 (“Schwarzenbach”), 428. Because Schmitt</p>

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		teaches selective enrichment generally (“target the desired double-stranded DNA sequence”), the POSA would have found it obvious to select at least KRAS, TP53, and APC as genes for selective enrichment in view of Schwarzenbach’s teachings on cfDNA tumor-specific gene mutations.
15	15. The method of claim 1, wherein sequencing comprises massively parallel sequencing.	<i>See Claim 1.</i> Schmitt’s DCS approach can include massively parallel sequencing. Figure 1 is an “overview of Duplex Consensus Sequencing” and the last step in Figure 1 states “[m]assively parallel sequencing.” Schmitt, 3:44-62, Figure 1; Schmitt '413 provisional, [0011], Figure 1.
16	16. The method of claim 1, wherein the amplified tagged progeny polynucleotides are sequenced to produce an average of 5 to 10 sequence reads for each family.	<i>Not asserted by Guardant.</i>
17	17. The method of claim 1, wherein the base call for each family possesses an error rate below 0.0001%.	<i>Not asserted by Guardant.</i>
18	18. The method of claim 1, wherein each base of the tagged parent polynucleotides has at least 99% chance of being represented by at least one sequence read among the sequence reads mapped in e).	<i>Not asserted by Guardant.</i>
19	19. The method of claim 4, wherein grouping the sequence reads mapped in e) is further based on one or more of: sequence information at a beginning of the sequence derived from the cfDNA molecule, sequence information at an end of the sequence derived from the cfDNA molecule, and length of the sequence read.	Schmitt explains that, as part of its DCS method, “a family of molecules is obtained that arose from a single DNA molecule; members of the same PCR ‘family’ are then grouped together by virtue of having a common (i.e., the same) SMI tag sequence.” Schmitt, 21:55-59; Schmitt '413 provisional, [0063]. Schmitt teaches the “hybrid” approach where unique identification is based on “[c]ombining information regarding the shear points of DNA with the SMI tag sequence.” Schmitt, 17:61-18:2; Schmitt '413 provisional, [0047]; see also Schmitt, 9:9-14; Schmitt '413 provisional, [0030]. Schmitt also teaches that SMI tags can be attached to both ends of DNA. EX1011, 7:38-48, EX1012, [0018]. A POSA would have understood these teachings to describe grouping the sequence reads into families based on the barcode sequence (the SMI tag) and information at the beginning <i>and/or end of the sequence derived from the parent polynucleotide (the sheared ends).</i>
20	20. The method of claim 4, wherein grouping the sequence reads mapped in e) is further based on a plurality of: sequence information at a beginning of the sequence derived from the cfDNA molecule, sequence information at an end of the sequence derived from the cfDNA molecule, and length of the sequence read.	Schmitt’s “hybrid” approach groups sequence reads based on families having a common identifier sequence that includes a short n-mer tag sequence and shear point sequences of fragmented DNA. Schmitt, 9:1-19, 26:59-27:8; Schmitt '413 provisional [0030], [0075]. The SMI sequence includes the beginning and end portions of the parent DNA fragment when the hybrid approach attaches the short n-mer tags to both ends. Schmitt, 7:38-48, 9:1-19; Schmitt '413 provisional, [0018], [0030]. Therefore, Schmitt discloses grouping based on the plurality of sequence information at the beginning and the end of parental DNA. Schmitt also teaches grouping can involve the length of the sequence read because DCS matches sense and antisense strand reads. Furthermore, because Schmitt teaches that sequence reads are mapped to a reference genome with the Burrows-Wheeler Aligner (“BWA”), grouping sequence reads based on the length of the sequence read (along with unique identifiers) is implicit or obvious. Schmitt, 20:57-61; Schmitt '413 provisional, [0060]. It was known as of September 2012 that BWA considers read lengths in determining the allowed maximum number of

Row	'992 Claim Limitation	Prior Art Disclosure – Schmitt '188 Alone or in Combination With Other Prior Art
		differences to the reference genome.
21	21. The method of claim 1, wherein at least one single base substitution is detected.	<i>Not asserted by Guardant.</i>
22	22. The method of claim 1, wherein the two or more members comprise a copy number variation (CNV).	<i>See row 19.</i>
23	23. The method of claim 1, wherein at least one indel is detected.	<i>Not asserted by Guardant.</i>
24	24. The method of claim 1, wherein at least one gene fusion is detected.	<i>Not asserted by Guardant.</i>
25	25. The method of claim 1, wherein at least one single base substitution and at least one copy number variation is detected.	<i>Not asserted by Guardant.</i>
26	26. The method of claim 1, further comprising detecting, at one or more genetic loci, one or more genetic aberrations selected from: a transversion, a translocation, an inversion, a deletion, aneuploidy, partial aneuploidy, polyploidy, chromosomal instability, chromosomal structure alterations, chromosome fusions, a gene truncation, a gene amplification, a gene duplication, a chromosomal lesion, a DNA lesion, abnormal changes in nucleic acid chemical modifications, abnormal changes in epigenetic patterns and abnormal changes in nucleic acid methylation.	<i>Not asserted by Guardant.</i>
27	27. The method of claim 21, wherein the single base substitution is detected with a sensitivity of at least 1%.	<i>Not asserted by Guardant.</i>
28	28. The method of claim 21, wherein the single base substitution is detected with a sensitivity of at least 0.1%.	<i>Not asserted by Guardant.</i>
29	29. The method of claim 1, wherein the plurality of genetic aberrations comprises three or more different members selected from the group of members consisting of a single base substitution, a copy number	<p><i>See Claim 1.</i></p> <p>Per claim 1, the POSA would have understood that Schmitt's methods can detect more than one type of genetic aberration in the same experiment. <i>See</i> Korbel et al., "Paired-end mapping reveals extensive structural variation in the human genome," <i>Science</i> 2007, 318(5849), 420-426 ("Korbel"), Abstract; Wheeler et al., "The complete genome of an</p>

Row	'992 Claim Limitation	Prior Art Disclosure – Schmitt '188 Alone or in Combination With Other Prior Art
	variation (CNV), an insertion or deletion (indel), and a gene fusion.	individual by massively parallel DNA sequencing,” Nature 2008, 452(7189), 872-876 (“Wheeler”), 872. DCS generates consensus sequences and the POSA would have needed only to compare differences between the consensus sequences and reference sequences to determine the nature of the genetic difference, i.e., single nucleotide substitutions, indel, gene fusion, etc. See row 1J Figures. For CNV detection, DCS utilizes the same family consensus sequence data where each family of reads corresponds to a single parental fragment such that the number of parental fragments mapping to a genomic locus can be counted. <i>Id.</i> Further, the sequence analysis software programs available as of September 2012 were capable of detecting all of the claimed types of genetic aberrations. <i>Id.</i> ; Ding, R189-R190, Table 1. To the extent it is required, it would have been obvious and routine for the POSA to make any necessary modifications to the open source software to incorporate the approach of DCS whether error correction by DCS occurs prior to the bioinformatic analysis of the sequence reads for mutation detection or error correction by DCS is integrated into the bioinformatic analysis.
30	30. The method of claim 1, wherein the plurality of genetic aberrations comprises a single base substitution, a copy number variation (CNV), an insertion or deletion (indel), and a gene fusion.	<i>See row 29.</i>
31	31. The method of claim 1, wherein the plurality of genetic aberrations comprises a plurality of each of two or more different members selected from the group of members consisting of a single base substitution, a copy number variation (CNV), an insertion or deletion (indel), and a gene fusion.	<i>See row 29.</i>
32	32. The method of claim 1, wherein each of the tagged parent polynucleotides is uniquely tagged.	<i>Not asserted by Guardant.</i>
33	33. The method of claim 1, wherein each of the tagged parent polynucleotides is non-uniquely tagged.	<i>See Claim 1.</i> Schmitt teaches non-uniquely tagging each of the parental DNA molecules. Schmitt’s “hybrid” approach ligates short n-mers of length 1-4 to parental fragments. Schmitt, 4:30-54, Figure 4; Schmitt '413 provisional, [0014]. For example, if the tag sequence is a 2-mer, then the number of unique tag sequences is 16. If one calculates 2-mers attached at both ends, then the number of unique tag sequence combinations is 256. A human genome has about 3 billion base pairs, and Schmitt teaches selection of fragments in the optimal range of 200-500 base pairs, which would result in millions of fragments per genome. Schmitt, 22:43-46; Schmitt '413 provisional, [0065]. Cell-free samples that have a smaller average fragment size as compared to standard genomic DNA library preparations would likely result in a greater number of parental fragments per ng of DNA, and therefore there is even a greater probability that each tag sequence will be ligated to more than one parental fragment. Forshew, 1 (citing publications on ctDNA and cffDNA). Thus, Schmitt’s “hybrid” approach for tagging DNA fragments results in non-unique tagging of each parent fragment.

Appendix A: Provisional Support for Schmitt

Schmitt issued from U.S. Application No. 14/386,800 (the “800 application”), which is a national stage application of PCT/US2013/032665, which was filed on March 15, 2013, and which claims the benefit of U.S. Provisional Application 61/613,413 filed on March 20, 2012 (“413 provisional”). The '800 application validly claims priority to the '413 provisional and names the same inventors as the '413 provisional. As detailed above, the '413 provisional also provides written description support and enablement of at least one claim in Schmitt, as identified in the table below. The teachings that Petitioner relies upon also were carried forward from the '413 provisional to Schmitt.

1. A method of generating an error-corrected sequence read of a double stranded target nucleic acid molecule, comprising	<i>See</i> '413 provisional, [0043] (“... a method of generating an error corrected double-stranded consensus sequence is provided”); <i>see also</i> [0010], [0013], and p. 45, claim 13.
i. a degenerate or semi-degenerate single molecule identifier (SMI) sequence that alone or in combination with the target nucleic acid shear points uniquely labels the double stranded target nucleic acid molecule; and	<i>See</i> '413 provisional, [0016] (“... the SMI adaptor molecule includes a double stranded, complementary “SMI sequence (or "tag") of nucleotides that is degenerate or semi-degenerate”); <i>see also</i> Figure 3 and [0013]; [0047]
ii. a nucleotide sequence that tags each strand of the adaptor-target nucleic acid complex such that each strand of the adaptor-target nucleic acid complex has a distinctly identifiable nucleotide sequence relative to its complementary strand,	<i>See</i> '413 provisional, [0062] (“Every duplicate that arises from a single strand of DNA will have the same SMI, and thus each strand in a DNA duplex pair generates a distinct, yet related population of PCR duplicates after amplification owing to the complementary nature of the SMIs on the two strands of the duplex.”); <i>see also</i> [0012] and Figure 2 [0048]
b) amplifying each strand of the adaptor-target nucleic acid complex to produce a plurality of first strand adaptor-target nucleic acid complex amplicons and a plurality of second strand adaptor-target nucleic acid complex amplicons;	<i>See</i> '413 provisional, [0009] (“... a method of obtaining the sequence of a double-stranded target nucleic acid is provided (also known as Duplex Consensus Sequencing or DCS) is provided. Such a method may include steps of ligating a double-stranded target nucleic acid molecule to at least one SMI adaptor molecule to form a double-stranded SMI-target nucleic acid complex; amplifying the double-stranded SMI-target nucleic acid complex, resulting in a set of amplified SMI-target nucleic acid products; and sequencing the amplified SMI-target nucleic acid products.”); <i>see also</i> [0011]-[0013] and Figures 1-3.
c) sequencing the adaptor-target nucleic acid complex amplicons to produce a plurality of first strand sequence reads and a plurality of second strand sequence reads;	<i>See</i> '413 provisional, [0009] (see excerpt for step b) above); <i>see also</i> [0011]-[0013] and Figures 1-3.
d) comparing at least one sequence read from the plurality of first strand sequence reads with at least one sequence read from the plurality of second strand sequence reads and generating an error corrected sequence read of the double stranded target nucleic acid molecule by discounting nucleotide positions that do not agree.	<i>See</i> '413 provisional, [0029] (“The sequences of the two duplex strands seen in the two sequence reads may then be compared, and sequence information and mutations will be scored only if the sequence at a given position matches in both of the reads.”); <i>see also</i> [0010], [0011]-[0013] and Figures 1-3.