

EXHIBIT B-1

U.S. Patent No. 9,752,188 to Schmitt et al. (“Schmitt”); Schmitt et al., Detection of ultra-rare mutations by next-generation sequencing, *PNAS*, (Sep. 4, 2012), 109(36):14508-14513 (“Schmitt 2012”); 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 Forsheew et al., “Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA,” *Science Translational Medicine*, 2012, 4(136) (“Forsheew”)

Schmitt, Schmitt 2012, Fan and Forsheew render obvious, at least under Guardant’s apparent infringement theory, the below-described claims of U.S. Patent No. 9,834,822 (“the '822 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 patent is therefore prior art to the '822 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 of the relationship between the Schmitt '188 patent and its Provisionals. Forsheew 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 '822 patent. Schmitt 2012 was published online on August 1, 2012¹, and therefor is prior art to the '822 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, Schmitt 2012, Forsheew 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, Schmitt 2012, Forsheew and/or Fan does not disclose one or more limitations of the claims, it would have been obvious to combine the teachings of these references 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 '822 patent. Because Guardant has yet to identify any limitation of the asserted claims that it contends is not fully disclosed by Schmitt, Schmitt 2012, Forsheew 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.

¹ See <http://www.pnas.org/content/suppl/2012/08/01/1208715109.DCSupplemental>.

Row	'822 Claim Limitation	Prior Art Disclosure – Schmitt '188 Alone or in Combination With Other Prior Art
1A	1. A method comprising: (a) providing a population of cell free DNA (“cfDNA”) molecules obtained from a bodily sample from a subject	<p>It would have been obvious to a POSA as of September 2012 to combine the teachings of Schmitt with 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”) or Forshew et al., “Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA,” 4(136) Sci. Translational Med. 1 (“Forshew”) to use the Duplex Consensus Sequencing (“DCS”) method for sequencing and detecting genetic aberrations in fetal cfDNA (Fan) or tumor cfDNA (Forshew). Besides the disclosure in Schmitt that cfDNA molecules can be obtained from a plasma sample of a subject, both Fan and Forshew 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 fetal aneuploidy. 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. Fan, Abstract, 16270, Table S1.</p> <p>Forshew describes “tagged-amplicon deep sequencing (TAm-Seq)” that involved amplification and sequencing of selected genomic regions corresponding to genes implicated in cancer. Forshew, Abstract; <i>see also id.</i>, 3, Table S1. In addition to applying Tam-Seq to solid tumor samples, Forshew used this approach to directly identify mutations in plasma of cancer patients. <i>Id.</i>, 4, Tables 1-2. Forshew extracted cell-free DNA from between 0.85 and 2.2 ml of plasma. <i>Id.</i>, 10. Table S6 lists the estimated amount of cell-free DNA sequenced from a number of patients, which ranged from 0.9 ng to 19.7 ng. <i>Id.</i>, Table S6.</p> <p>Moreover, a POSA would have Schmitt and Schmitt 2012 to teach a method that could be used to quantify single nucleotide variant tumor markers in cell-free DNA least because those references expressly contemplate applications involving the analysis of cell-free DNA, because they teach methods of error correction known to be critical to identifying rare mutations in cell-free DNA and because they teach methods of detecting mutations at precisely the frequencies that were known to be likely to occur in cell-free DNA.</p>
1B	(b) converting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides, wherein each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a cfDNA molecule of the population of cfDNA molecules, and (ii) an identifier sequence comprising one or more polynucleotide barcodes	<p>A “parent polynucleotide,” as used in the '822 patent, is simply the DNA fragment that is tagged, amplified to generate copies, sequenced, and analyzed, e.g., for the presence of mutations. '822 patent, Figure 9. For the DCS approach, Schmitt teaches ligating “SMI-containing adaptors” comprising a plurality of barcodes to both ends of parental DNA molecules to uniquely identify families or copies of these templates. The SMI adaptor comprises a “sequence (or ‘tag’) of nucleotides that is degenerate or semi-degenerate.” Schmitt, 6:46-51. The SMI adaptor thus comprises a “sequence of nucleotides used as a tag or identifier,” which constitutes a barcode as described in the '822 patent. Schmitt sometimes refers to the SMI sequences as “n-mer” sequences. <i>Id.</i>, 6:46-66; U.S. Provisional Application 61/613,413 (“Schmitt '413 provisional”), [0016].</p> <p>Schmitt describes various embodiments of its approach. In some, Schmitt describes the use of a large number of <i>unique</i> barcode sequences such that there is a high probability that a different barcode sequence is ligated to each parental DNA template. Schmitt, 6:61-63 (using “a sufficiently large number of unique tags to label a set of sheared DNA fragments from a segment of DNA”). But Schmitt also teaches a “hybrid” approach that attaches a small number of <i>non-unique</i> barcodes to parental fragments, where the non-unique barcode sequence <i>in combination</i> with the end-sequences of the parental DNA template provides a unique sequence signature to identify parental DNA templates and copies thereof. <i>Id.</i>, 9:1-13 (explaining that its approach “does not strictly require the use of an SMI tag, as the sheared ends can be used as identifiers to differentiate unique individual molecules from PCR duplicates”), Figure 4. This “hybrid method us[es] a</p>

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		<p>combination of sheared ends <i>and a shorter n-mer tag (such as 1 or 2 or 3 or 4 or more degenerate or semi degenerate bases),</i>” which together “serve as unique molecular identifiers.” <i>Id.</i>, 9:1-13, Figure 4 (emphasis added); Schmitt '413 provisional, [0030].</p> <p>Schmitt’s “hybrid” approach for tagging DNA fragments results in non-uniquely tagged parent polynucleotides. In this embodiment, if the tag sequence is a 4-mer, for example, then the total number of possible unique tag sequences that may be attached on both ends is only 65,536. 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 65,536 possible tag sequences in Schmitt’s “hybrid” approach, probability dictates that each tag sequence will be ligated more than once, which means that each tag sequence is non-unique relative to a fragmented genome or to naturally fragmented cfDNA, which has an average size of about 140 to 170 base pairs. Forshew, 1. Thus, Schmitt’s “hybrid method” discloses the use of non-unique tags.</p> <p>In addition, the description for Figure 3 of Schmitt explains that the tagged DNA fragments comprise both a barcode sequence and a sequence derived from the parent DNA molecule. “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.” Schmitt, 4:4-29; <i>see also id.</i>, Figure 4, 4:30-54 (“When these [4-mer tagged] molecules are amplified with PCR and sequenced, they will yield the following sequence reads”). Accordingly, Schmitt teaches that each of the non-uniquely tagged parent polynucleotides comprises (i) a sequence from a DNA fragment of the population of DNA fragments, and (ii) an identifier sequence comprising one or more polynucleotide barcodes.</p> <p>Schmitt’s “hybrid method” is nearly identical to an embodiment of the '822 patent, which explains that “non-uniquely tagged” polynucleotides can nonetheless be uniquely identifiable:</p> <p style="padding-left: 40px;">[A] plurality of barcodes may be used such that barcodes are not necessarily unique to one another in the plurality. In this example, the barcodes may be ligated to individual molecules such that the combination of the bar code and the sequence it may be ligated to creates a unique sequence that may be individually tracked. As described herein, detecting of non-unique barcodes in combination with sequence data of beginning (start) and end (stop) portions of sequence reads may allow assignment of a unique identity to a particular molecule.</p> <p>'822 patent, 39:13-22 (emphasis added).</p> <p>Indeed, the challenged claims of the '822 patent specifically claim non-uniquely tagged but uniquely identifiable polynucleotides. Claim 1 requires “converting the population of cfDNA molecules into a population of non-uniquely tagged parent polynucleotides.” '822 patent, 62:22-24. Claim 1 also requires that the parent polynucleotides are uniquely identifiable such that amplified copies of the parent polynucleotides can be grouped into families, “whereby each of the families comprises sequence reads amplified from the same tagged parent polynucleotide.” '822 patent, 62:40-42. By teaching a method that uses “shorter n-mer sequences” of up to 4 bases in length, in combination with the sheared ends of</p>

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		the DNA fragment, to “serve as unique molecular identifiers,” Schmitt teaches precisely the approach set forth in Claim 1.
1C	(c) amplifying the population of non-uniquely tagged parent polynucleotides to produce a corresponding population of amplified progeny polynucleotides	Schmitt teaches the limitations set forth in steps (c)-(d) of claim 1 of the '822 patent. The DCS method includes the “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. ” Schmitt, 3:10-20 (emphasis added); <i>see also id.</i> , 21:55-57; Schmitt '413 provisional, [0009], [0063].
1D	(d) sequencing the population of amplified progeny polynucleotides to produce a set of sequence reads	<i>See</i> row 1C.
1E	(e) mapping sequence reads of the set of sequence reads to one or more reference sequences from a human genome	<p>Schmitt discloses mapping sequence reads to a human reference genome. According to Schmitt, “[r]eads were aligned to the human genome with the Burrows Wheeler Aligner (BWA).” Schmitt, 20:39-64; <i>see also id.</i>, 23:10-14 (referring to the well-known “hg19” human reference genome). The POSA would have understood that aligning sequence reads to a human genome constitutes mapping of the sequence reads to a reference sequence from a human genome.</p> <p>Schmitt also explains that “[t]he entire human genome sequence (hg19) was used as reference for the mitochondrial DNA experiment, and reads that mapped to chromosomal DNA were removed.” <i>Id.</i>, 24:33-35. Schmitt further explains that “[r]eads sharing identical tag sequences were then grouped together and collapsed to consensus reads.” <i>Id.</i>, 24:35-37. Schmitt thus teaches mapping sequence reads to one or more reference sequences from a human genome.</p> <p>To the extent that the '822 patent claims are interpreted to require the mapping step to precede the grouping step, Schmitt et al., “Detection of ultra-rare mutations by next-generation sequencing,” Proc. Natl. Acad. Sci. USA 2012, 109(36), 14508-14513 (“Schmitt 2012”) teaches mapping the sequence reads to a reference sequence, followed by grouping. Schmitt 2012, S11 (“Reads were then aligned to the reference genome with the Burrows-Wheeler aligner (BWA) and nonmapping reads were discarded. . . . Reads sharing identical tag sequences were then grouped together and collapsed to consensus reads.”). In one embodiment, Schmitt 2012 teaches that the “entire human genome sequence (hg19) was used as reference” <i>Id.</i>, S11; Schmitt, 24:33-35. Thus, it would have been obvious to a POSA based on Schmitt in combination with Schmitt 2012 to conduct mapping either before or after grouping.</p> <p>Accordingly, Schmitt, either alone or in combination with Schmitt 2012, teaches mapping sequence reads to one or more reference sequences from a human genome.</p>
1F	(f) grouping the sequence reads into families, each of the families comprising sequence reads comprising the same identifier sequence and having the same start and stop positions, whereby each of the families comprises sequence reads amplified from the same tagged parent polynucleotide	<p>Schmitt teaches step (f) of claim 1 of the '822 patent. Schmitt explains that, as part of its DCS method:</p> <p style="padding-left: 40px;">Following tagging with a double-stranded SMI and PCR amplification, 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.</p> <p>Schmitt, 21:55-61; <i>see also id.</i>, 20:39-64; Schmitt '413 provisional, [0063] (“Reads having common (i.e., identical) SMI sequences were grouped together, and were collapsed to generate a consensus read.”). Thus, Schmitt teaches a method in</p>


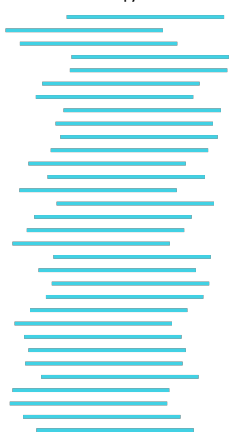

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		<p>which sequence reads arising “from a single DNA molecule” (i.e., the same tagged parent polynucleotide) are grouped into families wherein each family comprises sequence reads with an “identical” SMI tag sequence (i.e., the same identifier sequence).</p> <p>Schmitt further teaches that the DCS method can also be performed by “[c]ombining information regarding the shear points of DNA with the SMI tag sequence.” Schmitt, 17:61-18:2; <i>see also id.</i>, 9:9-14. A POSA would have understood that the “hybrid” method of Schmitt describes grouping into families wherein each family comprises sequence reads having the same identifier sequence and the same sequences from both ends (i.e., start and stop positions) of the sequence derived from the original fragment.</p> <p>Claim 1(f) does not require grouping to be based on either identifier sequence or start and stop positions. Instead, it requires that the reads in each family “compris[e]” the same identifier sequence and “hav[e]” the same start and stop positions, which the reads necessarily will have if they arise from the same parent polynucleotide. Nevertheless, if the claim is interpreted to require grouping based on the same identifier and the same start and stop positions, it would have been obvious to a POSA to do so because (1) Schmitt teaches using a combination of sheared ends of original fragments with the SMI tag sequence to perform the grouping and a POSA would understand the “start and stop positions” to encompass the sequences at the sheared ends, and (2) grouping based on start and stop positions provides an extra level of information in establishing families of amplicons arising from the same tagged parent polynucleotide, beyond just the identifier sequence.</p> <p>Schmitt also explains and shows that each of the grouped families comprises 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. Figures 3(a), 3(b), and 3(c) show three families, where each family contains sequence reads amplified from the same tagged parent polynucleotide. <i>Id.</i>, 4:4-9, Figure 3; Schmitt '413 provisional, [0013], Figure 3.</p>
1G	(g) at each genetic locus 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	<p>Schmitt teaches step (g) of claim 1 of the '822 patent. 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 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.” <i>Id.</i>, 20:53-56. A POSA would have understood that each consensus sequence provides a consensus base call for each family at a position with sufficient coverage and agreement to form a consensus.</p> <p>Schmitt further explains that parts (e) and (f) of Figure 3 (shown below) “show[] consensus sequences from all independently captured, randomly sheared fragments containing a particular genomic site are identified and compared to determine the frequency of genetic variants at this locus within the sampled population.” Schmitt, 4:24-29, Figure 3 (emphasis added). 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, a consensus sequence that contains a particular genetic locus of the reference sequence provides a base call at that particular genetic locus.</p>

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		<p>Figure 3</p> <p>a b c</p> <p>d Error-corrected consensus sequences Non-mutants True mutants</p> <p>e</p> <p>f Mutation counting among consensus sequences</p> <ul style="list-style-type: none"> ● Mutant: 3/10 ○ Wild-type: 7/10 Mutation prevalence: 30% <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. Schmitt, 4:20-29, 23:61-24:1.</p>

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		<p>Schmitt further teaches providing consensus sequences spanning a 758 kb region of the genome – i.e., which can comprise a plurality of genetic loci of interest. Schmitt, 23:61-24:1. 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.” <i>Id.</i>, 22:50-53. Following 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].</p> <p>Additionally, Schmitt teaches consensus sequences for identifying mutations “throughout the mitochondrial genome” – i.e., a plurality of genetic loci. Schmitt, 25:20-30. Referring to Figure 5C, Schmitt explains that “DCS analysis...reveals the true distribution of mitochondrial mutations [throughout the mitochondrial genome].” <i>Id.</i>, 4:55-66, Figure 5C.</p> <p>The POSA would have understood that these examples are representative, and DCS would also be able to identify mutations throughout the genome from cfDNA samples. “The compatibility of DCS with existing sequencing workflows, the potential for greatly reducing the error rate of DNA sequencing, and the multitude of applications for the double-stranded SMI sequences validate DCS as a technique that may play a general role in next generation DNA sequencing.” Schmitt, 18:44-61. 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.</p>
1H	(h) determining a frequency of one or more bases called at the locus from among the families.”	<p>Schmitt teaches step (h) of claim 1 of the '822 patent. Figure 3 in Schmitt, shown below, illustrates comparing consensus sequences to determine the frequency of genetic variants such as mutations, which comprise one or more bases called at the locus from among the families. Schmitt, 4:25-29, Figure 3. Figure 3 identifies the total number of consensus sequences that map to the locus (10), the number of consensus sequences that map to the locus that include a mutation (3), and a frequency of the mutation (“Mutant: 3/10”). Schmitt thus teaches determining a frequency of one or more bases called at the locus from among the families.</p>

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		<p>Figure 3</p> <p>Schmitt, Figure 3.</p> <p>Accordingly, a POSA would have found all the limitations of claim 1 of the '822 patent obvious in view of Schmitt in combination with Schmitt 2012 and either Fan or Forshew.</p>
2	2. The method of claim 1, further comprising detecting, at one or more loci, at least one single nucleotide variant, at least one gene fusion and at least one copy number variant.	<p><i>See Claim 1.</i></p> <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.</p>

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		<p>In further embodiments, the method confirms the presence of a 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.</p> <p>Schmitt, 3:31-40; <i>see also id.</i>, 4:4-29, Figure 3, (“(c) shows true mutations . . . present on both strands of a captured fragment appear in all members of a family pair.”); Schmitt '413 provisional, [0013], Figure 3.</p> <p>While the POSA would have understood “mutation” to encompass each of the claimed genetic aberrations, Schmitt explicitly teaches that DCS can detect “single base substitution” and “CNV.” Schmitt, 29:57-30:10. 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.” <i>Id.</i> (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]; Kivioja et al., “Counting absolute numbers of molecules using unique molecular identifiers,” <i>Nat. Methods</i> 2011, 9(1), 72-74 (“Kivioja”), 9:72-74.</p> <p>Further, the POSA would have understood that the DCS method of Schmitt can detect more than one type of genetic aberration in the same experiment. Sequencing approaches as of September 2012, 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 only need 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.</p>

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		<div style="display: flex; justify-content: space-around;"> <div style="width: 45%;"> <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> </div> <div style="width: 45%;"> <p style="text-align: center;">Insertion</p> <p>Single-Stranded Consensus Sequences α AGCTAGAAAAGCTAGCT β or β TCGATCTTTTGATCGA α</p> <p>Duplex Consensus Sequence TCGATCTTTTGATCGA AGCTAGAAAAGCTAGCT</p> <p>Reference Sequence AGCTAGCTAGCT TCGATCGATCGA</p> </div> </div> <div style="display: flex; justify-content: space-around;"> <div style="width: 45%;"> <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) TCGATCGATCGA AGCTAGCTAGCT</p> </div> <div style="width: 45%;"> <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> </div> </div> <p style="text-align: center;">Copy Number Variants</p> <p>Duplex Consensus Sequences</p> <div style="display: flex; justify-content: space-around;"> <div style="text-align: center;"> <p>normal copy number</p>  </div> <div style="text-align: center;"> <p>increase in copy number</p>  </div> <div style="text-align: center;"> <p>decrease in copy number</p>  </div> </div>

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		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 supra</i> Figures.
3	3. The method of claim 1, wherein converting comprises any of blunt-end ligation, sticky end ligation, molecular inversion probes, PCR, ligation-based PCR, single strand ligation and single strand circularization.	<p><i>See Claim 1.</i></p> <p>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. 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.” <i>Id.</i>, 7:58-62 (emphasis added). Schmitt thus expressly teaches attaching using blunt end ligation.</p> <p>Accordingly, it would have been obvious to a POSA, in view of Schmitt, to attach barcodes to DNA fragments using blunt end ligation.</p>
4	4. The method of claim 1, wherein the one or more reference sequences comprise a sequence from a human genome assembly.	<i>Not asserted by Guardant.</i>
5	5. The method of claim 1, wherein making the base call comprises voting, averaging, maximum a posteriori or maximum likelihood detection, dynamic programming, Bayesian methods, hidden Markov methods or support vector machine methods.	<p><i>See Claim 1.</i></p> <p>As the '822 patent specification acknowledges, “voting, averaging, statistical, maximum a posteriori or maximum likelihood detection, dynamic programming, Bayesian, hidden Markov or support vector machine methods” for generating consensus sequences were “known in the art.” '822 patent, 45:64-46:5. Further, it was common knowledge in the art that methods for generating consensus base calls comprised voting. <i>See, e.g.</i>, U.S. Patent No. 7,406,385 (“Sorenson”), 17:33-45, 18:41-61. Thus, it would have been obvious to a POSA to collapse sequence reads in each family to yield a consensus sequence (i.e., a base call) by the claimed methods.</p>
6	6. The method of claim 1, wherein determining the frequency comprises detecting a rare mutation.	<p><i>See Claim 1.</i></p> <p>The '822 patent explains that the term “rare mutation” encompasses a broad range of mutations and “may comprise a genetic aberration that includes, but is not limited to a single[] base substitution, or small indels, transversions, translocations, inversion, deletions, truncations or gene truncation.” '822 patent, 50:46-49. The '822 patent explains that the frequency of a “rare mutation” varies with the gene and disease state. <i>Id.</i>, 59:31-34. (“Rare mutations are found at an incidence of 5% in two genes, PIK3CA and TP53, respectively, indicating that the subject has an early stage cancer.”).</p> <p>Schmitt teaches determining the frequency of a rare mutation. As discussed above, Figure 3 in Schmitt illustrates comparing consensus sequences to detect rare mutations and to determine the frequency of genetic variants such as mutations. Schmitt, 4:25-29, Figure 3. Schmitt explains that in some embodiments, “the DCS method may be used in methods for high sensitivity detection of rare mutant and variant DNA” <i>Id.</i>, 17:9-11. For example, Schmitt teaches using DCS for the “accurate recovery of mutant sequence [A6293T] . . . down to one mutant molecule per 10,000 wild type molecules.” <i>Id.</i>, 29:57-30:10. The detection of one mutant molecule per 10,000 non-mutants, wherein the mutant</p>

Row	'822 Claim Limitation	Prior Art Disclosure – Schmitt '188 Alone or in Combination With Other Prior Art
		molecule comprises a known single base substitution, is the detection of a rare mutation. A POSA would have understood that Schmitt's method of comparing consensus sequences to determine the frequency of genetic variants includes determining the frequency of rare mutations.
7	7. The method of claim 1, further comprising generating a set of consensus sequences from the sequence reads, wherein determining the frequency of one or more bases comprises detecting a presence of sequence variations in the set of consensus sequences compared with the one or more reference sequences.	<p><i>See Claim 1.</i></p> <p>As discussed above for step (h) of claim 1 and for claim 6, Schmitt discloses determining the frequency of genetic variants such as mutations by identifying the presence of such variations in the set of consensus sequences. Schmitt further teaches aligning the consensus sequences to the human genome and describes hg19 as a reference sequence. Schmitt, 21:10-19, 23:10-14. A POSA would have understood Schmitt to teach aligning the consensus sequences to the human genome as a means to identify mutations (i.e., sequence variations) in the consensus sequences because using a reference sequence is a necessary step in identifying genetic mutations in a DNA sequence, which can only be identified as containing a genetic variant only when the sequence is different from the reference at one or more nucleotide positions. Accordingly, Schmitt teaches determining the frequency of sequence variations in the consensus sequences compared with one or more reference sequences.</p>
8	8. The method of claim 1, wherein d) comprises sequencing a panel of actionable cancer-related genes.	<p><i>See Claim 1.</i></p> <p>Forsheew teaches a tagged-amplicon deep sequencing (“TAm-Seq”) method that can amplify and sequence large genomic regions from cell-free tumor DNA. Forsheew, Abstract. Specifically, Forsheew teaches 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.” <i>Id.</i>, 3. Forsheew teaches using the same primer pairs to amplify plasma DNA, followed by sequencing. <i>Id.</i>, 3, SI1, Table S1. The TP53, PTEN, EGFR, BRAF, KRAS, and PIK3CA genes are “potentially actionable genes known to undergo somatic genomic alterations in cancer.” Wagle et al., “High-throughput Detection of actionable Genomic alterations in clinical tumor samples by targeted, Massively Parallel sequencing,” <i>Cancer Discov.</i> 2012, (2)1:82-93, 84, Supplementary Table 1. The KRAS gene is a “clinically validated and approved [actionable] alteration” for colon cancer and the PIK3CA gene is an “actionable alteration (targeted by drugs [] in clinical development)” for colon and breast cancers. <i>Id.</i>, 87, Table 2. Forsheew thus teaches sequencing a panel of actionable cancer-related genes. Given that Schmitt contemplated the use of deep sequencing methods, such as the DCS method, in cancer screening applications, it would have been obvious to a POSA to apply the DCS method of Schmitt to sequence a panel of actionable cancer-related genes, as taught by Forsheew.</p>
9	9. The method of claim 1, wherein mapping the sequence reads comprises using information about a length of each of the sequence reads.	<p><i>See Claim 1.</i></p> <p>As described above, Schmitt 2012 teaches that after sequencing, “[r]eads were [] aligned to the reference genome with the Burrows-Wheeler aligner (BWA) and nonmapping reads were discarded.” Schmitt 2012, SI1. <i>See also</i> row 1E. Schmitt teaches the same. Schmitt, 20:57-58. A POSA would have understood that aligning (i.e., mapping) reads with the Burrows-Wheeler aligner uses information about a length of each of the sequence reads. Li and Durbin, “Fast and accurate short read alignment with Burrows–Wheeler transform,” <i>Bioinformatics</i> 2009, 25(14), 1754-1760, 1757. Thus, Schmitt and Schmitt 2012 both teach mapping reads using information about a length of each of the sequence reads.</p>
10	11. The method of claim 1, wherein the	<i>See Claim 1.</i>

Row	'822 Claim Limitation	Prior Art Disclosure – Schmitt '188 Alone or in Combination With Other Prior Art
	population of cfDNA molecules is tagged with from 10 to 100,000 different identifiers.	As described above, Schmitt teaches attaching tags of 1-4 nucleotide bases. Schmitt, 9:1-13. In this embodiment, if the tag sequence is a 4-mer, there are up to 256 different sequences possible and the number of different combinations when considering 4-mer tags at both ends of a fragment is 65,536. An n-mer tag yields 4 ⁿ different tag sequences. The number of different combinations for arranging x different tag sequences at both ends of a polynucleotide is x ² . For a group of 256 different tag sequences, there are 256 ² , or 65,536 different combinations. Schmitt thus teaches non-uniquely tagging a population of DNA fragments, wherein the population of DNA fragments is tagged with from 10 to 100,000 different identifiers.
11	12. The method of claim 1, wherein the population of polynucleotides is tagged with n different unique identifiers, wherein n is no more than 100*z, wherein z is a mean of an expected number of duplicate molecules having the same start and stop positions in the sample.	<i>Not asserted by Guardant.</i>
12	13. The method of claim 1, wherein no more than 100 nanograms of polynucleotides from the bodily sample are converted in b).	<i>See Claim 1.</i> Either Fan or Forshev explicitly discloses providing no more than 100 ng 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” is between 1.2 and 8.0 ng of DNA depending on the sample). Forshev extracted about 0.9-19.7 ng of DNA from cell-free plasma for sequencing library preparation. Forshev, Table S6 (listing the estimated amount of cell-free DNA sequenced from patients, which ranged from 0.9 ng to 19.7 ng).
13	18. The method of claim 1, wherein d) comprises sequencing a panel of tumor suppressor genes.	<i>See Claim 1.</i> As discussed above for claim 8, Forshev teaches using the primer pairs to amplify regions of the TP53, PTEN, EGFR, BRAF, KRAS, and PIK3CA genes from plasma DNA, followed by sequencing. Forshev, 3, SI1, Table S1. Forshev characterizes TP53 and PTEN as tumor suppressor genes. <i>Id.</i> , 1 (“These methods, however, interrogate individual or few loci and have limited ability to identify mutations in genes that lack mutation hotspots, such as the TP53 and PTEN tumor suppressor genes (32).”) Forshev thus teaches sequencing a panel of tumor suppressor genes. <i>Id.</i> Given that Schmitt contemplated the use of deep sequencing methods coupled with error correction via the DCS method in cancer screening applications, it would have been obvious to a POSA to apply the DCS method of Schmitt to sequence a panel of tumor suppressor genes, such as that taught by Forshev.
14	19. The method of claim 1, further comprising removing a subset of the sequence reads from further analysis prior to e).	<i>Not asserted by Guardant.</i>
15	20. The method of claim 1, wherein the population of cfDNA molecules is tagged	<i>See Claim 1.</i>

Row	'822 Claim Limitation	Prior Art Disclosure – Schmitt '188 Alone or in Combination With Other Prior Art
	with from 50 to 10,000 different identifiers.	As described above, Schmitt teaches attaching tags of 1-4 nucleotide bases. Schmitt, 9:1-13. In this embodiment, if the tag sequence is a 2-mer, there are up to 16 different sequences possible and the number of different combinations when considering 2-mer tags at both ends of a fragment is 256. Schmitt thus teaches non-uniquely tagging a population of DNA fragments, wherein the population of DNA fragments is tagged with from 50 to 10,000 different identifiers.

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.