IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Chuu, et al.
U.S. Patent No.: 8,318,430, claims 1-18
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Title: METHODS OF FETAL ABNORMALITY DETECTION

Second Declaration of Cynthia Casson Morton

I, Cynthia Casson Morton, declare as follows:

Credentials

1) Currently I hold the position of William Lambert Richardson Professor in the Department of Obstetrics, Gynecology and Reproductive Biology at Harvard Medical School. I am also a Professor in the Department of Pathology at Harvard Medical School and the Director of Cytogenetics at Brigham and Women's Hospital. My *curriculum vitae* was submitted previously in this proceeding as Exhibit 1014.

2) I have reviewed and am familiar with the following documents:

- U.S. Patent No. 8,318,430 to Chuu et al. ["the '430 patent", Ex. 1001];
- U.S. Pat No. 7,332,277 to Dhallan ["Dhallan", Ex. 1004];
- US Patent Publication 2008/0090239 to Shoemaker, et al., ["Shoemaker", Ex. 1008];
- Binladen et al., PLoS One. 2007 Feb 14;2(2):e197 ["Binladen", Ex. 1005];
- Decision: Institution of *Inter Partes* Review [Paper 11 in IPR2013-00276];
- Decision: Institution of *Inter Partes* Review [Paper 10 in IPR2013-00277];
- Verinata Health, Inc.'s Patent Owner Response Pursuant to 37 C.F.R. §42.120 [Paper 19 in IPR2013-00276];
- Verinata Health, Inc.'s Patent Owner Response Pursuant to 37 C.F.R. §42.120 [Paper 19 in IPR2013-00277];
- Declaration of Dr. Atul Butte for IPR2013-00276 [Ex. 2003];
- Declaration of Dr. Atul Butte for IPR2013-00277 [Ex. 2003]; and
- Transcript of the Deposition of Dr. Atul Butte [Ex. 1041].

I. SUMMARY OF MY OPINIONS

3) As described in detail below, it is my opinion that a person of ordinary skill in the art, upon reading the Binladen, Dhallan, and Shoemaker references, would have had both the technical knowledge and the motivation to combine the methods disclosed therein. In particular, they would have been motivated to utilize these combined teachings to develop a multiplexed massively parallel sequencing (MPS) method for determining fetal aneuploidy using enriched, non-random polynucleotides from cell-free DNA found in pregnant women's plasma or serum.

4) Although the individual steps recited in claims 1-18 of the '430 patent may not be found in a single reference, a person of ordinary skill in the art in 2009 would immediately understand the benefits of using multiplexing MPS, such as the methods taught in both Binladen and Shoemaker, with the locus-based assays for detection of fetal aneuploidy, such as the assays taught in Dhallan and Shoemaker. The use of cell-free DNA for determination of fetal aneuploidy was also quite well known, and a person of ordinary skill would have understood the benefits of using cell-free DNA generally obtained through a non-invasive procedure. Because employing multiplexed MPS methods using enriched DNA assays to detect fetal

aneuploidy in multiple samples would provide immediate benefits such as increased efficiency, speed, and a decreased cost of sequencing per sample, a person of ordinary skill would naturally have been motivated to combine the subject matter of these references to arrive at the invention as described in claims 1-18 of the '430 patent.

5) Moreover, in my view the technical hurdles described by Verinata's expert, Dr. Atul Butte, would not have prevented a person of ordinary skill in the art from combining the teachings of the combined references or to doubt the expected success of utilizing multiplexing with MPS to determine fetal aneuploidy [*infra* at ¶¶ 44-46]. Specifically, it was known in 2009 that multiplexing could be used with MPS, and in fact it was so commonplace that kits to do so were commercially available [*infra* at ¶¶ 38 and 40, see also Exhibit 1010]. It was also known that MPS worked perfectly well on cell-free DNA extracted from plasma samples of pregnant women, and laboratories such as Dr. Stephen Quake's laboratory at Stanford University and Dr. Dennis Lo's laboratory at the Chinese University of Hong Kong had published articles using MPS to quantitate maternal and fetal cell-free DNA for detection of fetal aneuploidies [see, *e.g.*, Ex. 1033; Ex. 1036; Ex. 1011 and Ex. 1045]. Finally, the use of enriched loci from the cell-free DNA in a pregnant woman's plasma had been used to detect fetal aneuploidies, as

demonstrated by the work of Dhallan and his collaborators [see, *e.g.*, Ex. 1004]. The other technical hurdles that Dr. Butte argues would have precluded combining the exemplary techniques of Dhallan, Binladen and Shoemaker are, in my opinion, irrelevant.

6) Accordingly, based on the state of the art in 2009, the commercially-available systems for massively parallel sequencing ("MPS"), the commercially available kits available for multiplexing on such MPS systems, and various publications in the field of medical genetics and prenatal diagnosis, a person of ordinary skill could have employed multiplexed MPS as the detection method for the fetal aneuploidy assay methods taught in Dhallan and Shoemaker with a reasonable expectation that combining these methods would be successful.

II. A PERSON OF ORDINARY SKILL IN THE RELEVANT FIELD

7) Dr. Butte, in his declaration [Ex. 2003], sets forth his view of a person of ordinary skill in the relevant field:

> "In my opinion, a person of ordinary skill in the relevant field in January 2010 would include someone with a master's or Ph.D degree in molecular biology, genetics, or a related field and, through either education or work

> experience, about 2-3 years of experience with nucleic acid sequencing, sample preparation, and prenatal diagnostics."

8) Importantly, a person of ordinary skill in in the relevant fields would have knowledge about the performance of the MPS systems themselves, as opposed to just experience analyzing the data produced from such sequencing systems. Knowledge of factors that affect the performance of these sequencing systems, as well as methods for adapting or troubleshooting the biochemistry associated with these systems also is, in my view, important, as it informs the person of ordinary skill on the technical challenges that may be imposed by modification of sequencing methods.

9) The conclusions I reached in my previous declaration—and the views expressed in this declaration—are consistent with the expertise and knowledge of a person of ordinary skill in the art.

10) I am acquainted with Dr. Butte from his time as an Instructor in Pediatrics at Children's Hospital in Boston. I know him to be very knowledgeable of bioinformatics, medical informatics and data analysis. However, to my knowledge, Dr. Butte has not worked in or supervised a biochemical or "wet lab", and has never performed or directly supervised the technical aspects of nucleic acid sequencing. It is not only possible, but

probable – and in fact his own deposition testimony corroborates - that he was not aware of the evolution of molecular and biochemistry techniques used in MPS in 2008-2009, including the sample multiplexing employed in the art with MPS (as evidenced by the Binladen and Shoemaker references) and the development of products for use with available MPS systems, such as the Illumina, Inc. multiplexing kit.[1]

11) It is thus my view that a person of ordinary skill (such as a first year postdoctoral student working in a molecular genetics "wet" laboratory in 2009) would have had both the knowledge of the state of the art of MPS and an ability to understand the basic scientific premises described in Binladen and Shoemaker. A person of ordinary skill in the art would have understood the scientific basis of methods for detecting fetal aneuploidy taught in Dhallan and Shoemaker, and would have understood that combining such methods with multiplexing and MPS to process multiple samples simultaneously would require nothing more than routine and highly predictable modifications.

[1] When Dr. Butte was asked if he supervises a wet laboratory in his current position at Stanford, Dr. Butte answered "No." [Ex. 1041 at p. 20:4] Furthermore, Dr. Butte stated that in 2009 neither he nor anyone in his laboratory was a direct customer of Illumina. [Ex. 1041 at p. 22:24 – 23:6]

III. THE COMBINED TEACHINGS OF DHALLAN, BINLADEN AND SHOEMAKER REFERENCES DESCRIBE ALL SUBJECT MATTER IN CLAIMS 1-18 OF THE '430 PATENT

12) As I previously testified in my first declaration, each of Dhallan, Binladen and Shoemaker teach selective amplification of DNA [Ex. 1002 at ¶¶ 39 and 103]. Both Dhallan and Shoemaker teach selective amplification of non-random polynucleotide sequences for prenatal detection of fetal aneuploidy [Ex. 1002 at ¶ 103]. Dhallan teaches use of sequencing of maternal and fetal cell-free DNA to detect selectively-targeted and amplified fetal and maternal loci indicative of individual chromosomes [Ex. 1002 at ¶¶ 46 and 110]. More specifically Dhallan teaches selective amplification of selected loci in cell-free DNA from a maternal plasma or serum sample [Ex. 1002 at ¶¶ 39 and 103].

13) Also in my previous testimony, I described the teachings of Shoemaker and Binladen on the use of nucleic acid indices (also referred to as nucleic acid tags, bar codes or locator elements) in conjunction with multiplexed sequencing systems to identify samples from different sources, whether from different individuals (Binladen) or from samples taken from the same individual at different time points (Shoemaker) [Ex. 1002 at ¶¶ 17-18, 40 and 104; and Exs. 1005 and 1008 generally]. Further, Dhallan and

Shoemaker both teach quantification of maternal and fetal alleles from a maternal sample [Ex. 1002 at ¶¶ 47-48 and 111-112], including alleles from cell-free DNA of a maternal serum or plasma sample. These alleles are from selected loci of interest known to be associated with a specific chromosome, *e.g.*, chromosome 21. So Dhallan and Shoemaker both teach using detection and quantification of alleles associated with individual chromosomes to identify a fetal aneuploidy [*Id.*].

14) Binladen and Shoemaker both teach the use of nucleotide sample indices (irrespective of whether they are referred to as barcodes, unique tags or locator elements) with massively parallel sequencing systems to analyze multiple samples simultaneously [Ex. 1002 at ¶¶ 17-18, 40 and 105; and Exs. 1005 and 1008 generally]. Further, both Dhallan and Shoemaker specifically use 100 different loci on each chromosome in their analysis of the loci from different chromosomes [Ex. 1002 at ¶¶ 42 and 106].

15) Thus, as described in detail in my previous declaration in relation to claims 1-18 [Ex. 1002] and that of Ariosa expert, Prof. Robert Nussbaum [Ex. 1003], each and every limitation of the claims of the ‘430 patent are taught by Dhallan, Binladen and Shoemaker. Specific teachings of each reference are described in more detail below.

IV. THE TEACHINGS OF DHALLAN ALLOW DETECTION OF SELECTED LOCI USING VARIOUS TECHNIQUES

16) Dr. Butte in his declaration testified that, in his opinion, one of ordinary skill in the art in January 2010 would have recognized that the teachings of Binladen or Shoemaker could not reasonably be combined with those of Dhallan because the technologies were incompatible [Ex. 2003 at ¶¶ 215, 218-219]. In Dr. Butte's view, the so-called "Dhallan Process" "critically relies on restriction digestible primers for amplification" to incorporate fluorescent labels into a 5' overhang left after digestion of the nucleic acids [*Id*.]. These fluorescent products are detected by visualizing the products on a gel and "analyzing band intensities for each of the alleles" to "determine the presence or absence of chromosomal abnormalities" [Ex. 2003 at ¶ 220].

17) I agree with Dr. Butte that certain embodiments in Dhallan teach the particular detection methods Dr. Butte characterizes as the "Dhallan Process"; however, Dhallan also teaches (and Dr. Butte ignores) a number of detection methods which do *not* require the use of restriction digestible primers. Dhallan describes multiple embodiments of the invention, including various embodiments that utilize different methods for detection of the nucleic acids. In fact, Dhallan describes a wide variety of methods for sequence determination of the selected loci:

> In some embodiments, determining the sequence includes using a method that is allele specific PCR, mass spectrometry, hybridization, primer extension, fluorescence resonance energy transfer (FRET), sequencing, Sanger dideoxy sequencing, DNA microarray, GeneCHIP arrays, HuSNP arrays, CodeLink Arrays, BeadArray Technology, MassARRAY, MassEXTEND, SNP-IT, TaqMan, InvaderStrand Assay, southern blot, slot blot, dot blot, or MALDI-TOF mass spectrometry.

[Ex. 1004 at col. 6: 26-34]. Moreover, Dhallan also teaches quantitation of tens to hundreds to thousands of loci from multiple chromosomes [Ex. 1004 at col. 7:9-16].

18) Many of the detection mechanisms extant in 2002 and 2003 required fluorescence detection; thus, Dhallan proposes various embodiments to incorporate labels into the amplicons of selected loci including, as Dr. Butte reports, endonuclease digestion [*e.g.*, Ex. 1004 at col. 8: 9-31; col 10:41-52; and col. 10:53 - col. 11:4]. However, Dhallan also teaches fragmentation of DNA and extension to incorporate labels [*e.g.*, Ex. 1004 at col. 11: 61 - col. 12: 17]; *in vitro* transcription of the loci followed by RNase A cleavage and detection using a SpectroCHIP [*e.g.*, Ex. 1004 at col. 12: 40-47]; incorporation of labelling nucleotides during amplification [*e.g.*, Ex. 1004 at col. 13:66 - col. 13:5]; exonuclease treatment and labelling of loci [*e.g.*, Ex.

1004 at col. 6: 36-42]; and the use of a probe using a reporter dye that anneals to the locus of interest [*e.g.*, Ex. 1004 at col. 14: 15-25]. Thus, in my view Dhallan discloses a number of labelling and detection methods, only one of which is drawn to use of restriction endonucleases.

19) Dr. Butte, in my opinion, is taking a significantly more limited view of the teachings of Dhallan than one of ordinary skill in the art would have taken. It is my understanding that all embodiments described in Dhallan are relevant for the analysis of what a person of ordinary skill would have understood as being taught by Dhallan – not just the particular method identified by Dr. Butte.

20) In my opinion, one of ordinary skill in the art in 2009 would view Dhallan as teaching a variety of methods for concurrent determination of sequences of multiple, specific loci of interest on multiple chromosomes for use in prenatal diagnosis.

21) There were, however, a number of technological advances in detection methods between the priority date of Dhallan (circa 2002/2003) and January 2010. Specifically, massively parallel, next generation sequencing was developed and commercialized during this time [Ex. 1002 at ¶15 and Ex. 1003 at ¶19], and by 2008 and 2009 massively parallel

sequencing was already being applied to prenatal detection of fetal aneuploidy [*e.g.*, Ex. 1033; Ex. 1036; Ex. 1011; Ex. 1045].

22) Molecular biology methods and systems are continually optimized, and researchers working in molecular genetics would have been aware of new and improved detection methods to enhance their research activities. In my view, one of ordinary skill in the art would not have continued to utilize the first- or second- generation detection methods disclosed in Dhallan to detect the non-random loci analyzed in Dhallan once the far-superior next generation, massively parallel sequencing techniques that were developed and commercialized in the mid-2000s became available and were widely adopted.

V. THE TEACHINGS OF BINLADEN ARE USEFUL FOR MULTIPLEXED SEQUENCING OF MATERNAL SAMPLES

23) Dr. Butte in his declaration states that the tags of Binladen are unsuitable for fetal aneuploidy detection [Ex. 2003 at ¶¶198-207]. Specifically, Dr. Butte takes issue with the fact that Binladen teaches tagging of individual species of animals rather than different individuals of the same species [*e.g.*, Ex. 2003 at ¶ 60]. Dr. Butte in his declaration states further that Binladen amplified and detected mitochondrial DNA rather than genomic DNA [*e.g.*, Ex. 2003 at ¶ 61], and that Binladen discloses a non-

trivial sequencing error rate using their particular dinucleotide tags [*e.g.*, Ex. 2003 at ¶¶ 62-68]. I disagree with Dr. Butte's assessment of Binladen.

24) First, I believe it is immaterial that Binladen's sample tags are indicative of individuals from different species, as opposed to different individuals of the same species. Sample tags (also known in the art as barcodes, indexes or indices or locator elements) are used commonly to "tag" different sample sources of nucleic acids, where a different tag is used to tag each source. Once nucleic acid sequences from each source are differentially tagged, nucleic acid sequences from each source or sample can be pooled for sequencing then sorted into their original sample source using the tags [*e.g.*, Ex. 1005 at p. 2]. It is my opinion that a scientist of ordinary skill in the art would understand that sample tags are indicative of a template source, whether the source is different individuals of the same species, individuals of different species, or many samples taken, *e.g.*, at different time points from the same individual. The nucleotide tag is used after sequencing to "deconvolute" the pooled sequences to identify the source of each sequence.

25) In addition, Dr. Butte states that because the nucleic acids used in Binladen was mitochondrial DNA rather than genomic DNA from maternal plasma or serum, one of ordinary skill would not view the teachings of

Binladen as applicable to fetal aneuploidy detection [Ex. 2003 at ¶ 60]. Again, I disagree with Dr. Butte's conclusion. My view—which I believe would be shared by others of ordinary skill in the art—is that once a DNA sample is isolated, whether it be genomic DNA or mitochondrial DNA, the steps of selective amplification and isolation and the subsequent sequencing of amplification products are equally applicable.

26) Dr. Butte states that mitochondrial DNA differs from a blood sample with fetal and maternal genomic DNA because mitochondrial DNA consists of a single haploid genome, rather than two diploid genomes (e.g., a mixed sample of fetal and maternal nucleic acids) [*e.g.*, Ex. 2003 at ¶¶ 204 and 206]. However, it is my opinion that one of ordinary skill in the art would view DNA as DNA. First, it should be noted that isolation of cellular nucleic acids typically results in a "mixed sample" of mitochondrial and genomic DNA and RNA. The primers used to select and amplify specific loci are added to the combination of nucleic acids from the cell, and mitochondrial and genomic DNA would both be expected to be amplified as long as appropriate primers are used. Ultimately, the amplified sequences from both the mitochondrial and genomic DNA can then be sequenced. It is my view that one of ordinary skill in the art would not be dissuaded from using the methods of Binladen with a mixed sample of fetal and maternal

nucleic acids, particularly when utilizing single molecule counting methods such as next generation, massively parallel sequencing. In fact, such single molecule counting techniques would be even more effective in complex mixtures of genomes, as single molecule counting techniques are far superior to prior art techniques in identifying low level nucleic acids present in a mixed sample.

27) Finally, Dr. Butte states that the tags of Binladen are unsuitable for fetal aneuploidy detection because the tags tested by Binladen resulted in variations in sequence distribution and a non-trivial error rate [*e.g.*, Ex. 2003 at ¶¶ 199-203]. However, again I disagree with Dr. Butte. Binladen discloses use of different tags and examined the observed and expected sequence distributions of these tags. Binladen found that when using dinucleotide tags, the identity of the dinucleotide tag has an important effect [Ex. 1005 at p. 7]. Binladen also found that tetranucleotide primers resulted in a lower rate of sequence mis-assignment [*Id*.]. However, Binladen proposes "primer design guidelines" for the tag sequences to be used with multiplexed massively parallel sequencing [Ex. 1005 at p. 8]. In particular, Binladen provides guidance as to both the optimal nucleotide content (e.g., a conserved 5' nucleotide) and length (e.g., longer and of identical length) to

enhance performance [Ex. 1005, p. 7].[2] Thus, it is my opinion that it would have been well within the skill of one of ordinary skill in the art, such as a first-year postdoctoral student, to design and optimize sample tags for use with massively parallel sequencing of multiple maternal DNA samples.

28) Further, not only does the Binladen publication provide suggestions that might improve efficiency and accuracy of the tags, but Binladen lays a blueprint for how one of ordinary skill would test sample tags to be used in an experiment. For example, Daines, et al. ("High-Throughput Multiplex Sequencing to Discover Copy Number Variants in Drosophila", Genetics, 182:935-41 (2009) [Ex. 1046]) modified Solexa sequencing primers by adding 3-bp barcodes. The modified sequencing primers were used to prepare libraries such that the 5' ends of the sequencing products indicated the sample source. In Daines, et al., DNA of various microbes were differentially amplified and labeled with barcoded oligonucleotide adapters and the resulting libraries were mixed in equal molar amounts and sequenced simultaneously on one lane of an Illumina Genome Analyzer. Daines, et al., using essentially the same methods to assess the accuracy of

[2] I testified to this in my deposition. When asked if Binladen reports wide variations in results, I replied, "[b]ut he provides the reason why" [Ex. 2005 at 101:10].

tags or barcodes as Binladen, concluded that the reads generated exhibited an extremely low error and cross-contamination rate [*Id*., at p. 938].

29) Thus, it is my opinion that one of ordinary skill in the art as defined by Dr. Butte—or even a scientist with less training—would view the Binladen reference as simply teaching use of different nucleotide "tags" or "barcodes" to indicate different sources of DNA that, once tagged, can be pooled and sequenced. Once sequenced, the sequence of the tags/barcodes are used to deconvolute the sequence information to identify the source of each sequence. Moreover, it is my opinion that one of ordinary skill, upon reading the guidance provided by Binladen on optimal content and length of the tags [Ex. 1005 at pp. 7-8], would be able to easily apply the teachings of Binladen to optimize the tags to decrease the error rate and increase the accuracy of putative sample tags.

VI. SHOEMAKER TEACHES SAMPLE TAGS FOR IDENTIFICATION OF INDIVIDUAL SAMPLES IN MULTIPLEXED SEQUENCING

30) Dr. Butte in his declaration takes the position that the "locator elements" of Shoemaker would not be recognized by one of ordinary skill in the art as sample tags or barcodes that indicate the sample source of a nucleic acid [*e.g.*, Ex. 2003 at ¶¶ 70-73]. Instead, Dr. Butte states the locator elements of Shoemaker identify "bins" which correspond to individual wells [*Id.*]. However, because Shoemaker's individual wells contain single cells, it is my opinion that one of ordinary skill in the relevant fields would understand that in Shoemaker the locator tags that identify an individual cell are actually being used to identify the sample source of the nucleic acids. Dr. Butte in his deposition testimony acknowledged that this is the case [Ex. 1041 at p. 109:22 – 110:6]:

> Q: Does this [particular passage in Shoemaker] describe the use of a unique tag sequence for an individual cell?
> A: My understanding is that the unique tag sequence identifies a well. And if they believe one cell is in one well, it would correspond to one cell.
> Q: But Shoemaker does say that the methods comprise labeling one or more regions of genomic DNA in each cell, correct?

A: It does say, "The methods comprised labeling one or more regions of genomic DNA in each cell," yes.

31) It is my opinion that one of ordinary skill in the art would understand that the locator elements used in Shoemaker are nucleotide tags used to identify a source of nucleic acids used in sequencing, whether that source is different cells (*e.g.*, Shoemaker), samples taken from the same human at different time points (*e.g.*, Shoemaker), samples taken from different individuals (*e.g.*, samples from many different individual species such as those disclosed in Binladen), or samples from individual human patients (*e.g.*, the '430 patent).

VII. BINLADEN AND DHALLAN CAN BE REASONABLY COMBINED

32) Dr. Butte in his declaration states that in his opinion, one of ordinary skill in the art would recognize that the teachings of Binladen cannot reasonably be combined with those of Dhallan, due to Dhallan's reliance on restriction endonuclease digestible primers. Dr. Butte states that the tags taught by Binladen would be cleaved off by the restriction enzyme that Dhallan uses to generate the 5' overhang [*e.g.*, Ex. 2003 at ¶¶ 214-225]. However, as discussed above at ¶ 18, the teachings of Dhallan are not

limited to embodiments requiring use of a restriction endonuclease, and it is my opinion that one of ordinary skill in the art would substitute the robust next generation, massively parallel sequencing techniques developed and commercialized in the mid-2000s for the first- and second-generation detection techniques disclosed in Dhallan. It is also my opinion that in doing so, one of ordinary skill would use basic common sense and **not** utilize the embodiments of Dhallan where restriction sites are added to the primers, or deliberately add barcodes or sample tags to loci only to cleave them off as Dr. Butte suggests. It is my view that one of ordinary skill would recognize that in substituting next generation sequencing techniques for the detection techniques taught in Dhallan, it would be completely nonsensical to use a restriction endonuclease to remove the sample tags.

VIII. SHOEMAKER AND DHALLAN CAN BE REASONABLY COMBINED

33) Dr. Butte has opined that Shoemaker could not be combined with and is not compatible with Dhallan for the same reasons that Binladen cannot reasonably be combined with Dhallan; that is, because "Dhallan critically relies on restriction digestible primers followed by cleaving the primer sequence by enzyme digestion" [Ex. 2003 at ¶¶ 226-230]. However, I disagree with Dr. Butte in this instance as I do with his opinion regarding

combining Binladen and Dhallan [*supra* at ¶ 35]. The teachings of Dhallan are not limited to embodiments requiring use of a restriction endonuclease [*supra* at ¶ 18]. For example, Dhallan also teaches fragmentation of DNA and extension to incorporate labels [*e.g.*, Ex. 1004 at col. 11: 61- col. 12: 17]; *in vitro* transcription of the loci followed by RNase A cleavage and detection using a SpectroCHIP [*e.g.*, Ex. 1004 at col. 12: 40-47]; incorporation of labelling nucleotides during amplification [*e.g.*, Ex. 1004 at col. 13:66- col. 13: 5]; exonuclease treatment and labelling of the loci [Ex. 1004 at col. 6: 36-42]; and using a probe using a reporter dye that anneals to the locus of interest [*e.g.*, Ex. 1004 at col. 14: 15-25].

34) It is my opinion that one of ordinary skill in the art would have been familiar with the next generation, massively parallel sequencing techniques developed and commercialized in the mid-2000s at least by January 2010, and would have been motivated to substitute these next generation sequencing techniques, such as those described in Binladen and Shoemaker, for the first- and second-generation detection techniques disclosed in Dhallan. Essentially, it is my opinion that in January 2010, one of ordinary skill in the art would want to use the most accurate and efficient detection techniques available.

35) The use of improved sequencing techniques would not employ the addition of restriction sites into amplification primers and a person of ordinary skill would not be led away from using next generation, massively parallel sequencing techniques based on the embodiments of Dhallan that utilize restriction enzymes. A person of ordinary skill in the art would understand that it does not make sense to deliberately engineer amplification primers to include barcodes or sample tags to be incorporated into resulting products only to subsequently cleave them off using a restriction enzyme, as Dr. Butte suggests. One of ordinary skill would recognize that cleaving the sample tag would negate its entire purpose, and would render the detection inoperable – and thus would not use the restriction enzyme. Instead, a person of ordinary skill in the art would use one of the other techniques described by Dhallan in conjunction with next generation sequencing.

IX. BINLADEN AND SHOEMAKER CAN BE REASONABLY COMBINED

36) The teachings of Binladen and Shoemaker can also be reasonably combined, as the molecular biology techniques taught in each reference are compatible and could easily have been used together. Although Shoemaker teaches locator tags for identification of different cell sources (e.g., samples taken from the same human at different time points or cells from a mixed

sample), it would be clear to a person of ordinary skill in the art that the locator tags of Shoemaker and the sample tags of Binladen – which are both nucleotide indexes that identify a source of nucleic acids – are equally useful in identifying a particular sample source be it samples from different individuals or multiple samples from a single individual. Conceptually, the use of the locator elements of Shoemaker and the sample tags of Binladen are identical [*supra* at ¶¶ 27-29]. Combining the prenatal assay techniques of Shoemaker, which includes the use of at least 100 selected loci from different chromosomes for detection of fetal aneuploidy, with use of sample tags for samples from different individuals as taught in Binladen would have been a simple modification with a reasonable expectation of success.

X. THERE IS MOTIVATION TO COMBINE DHALLAN WITH BINLADEN AND/OR SHOEMAKER

37) It is my opinion that one of ordinary skill in the art would have been motivated to substitute the next generation, massively parallel sequencing methods of Binladen and Shoemaker for the first- and second-generation detection methods disclosed in Dhallan (*e.g.*, Sanger sequencing using slab gels, capillary electrophoresis, DNA microarrays, mass spectrometry). This is particularly true given that massively parallel sequencing of maternal and

fetal cell-free DNA for detection of fetal aneuploidies in maternal serum and plasma was well known as evidenced at least by the following references:

- Ex. 1033, Chiu, et al., "Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma, PNAS 105(51):20458-63 (2008);
- Ex. 1036, Chiu, et al., "Non-invasive prenatal diagnosis by single molecule counting technologies", Trends in Genetics, 25(7):324-31 (2009);
- Ex. 1011, Fan, et al., "Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood", PNAS, 105(42):16266-71 (2008); and
- Ex. 1045, Lo, et al., US Pat. Pub. No. 2009/0029377, entitled "Diagnosing Fetal Chromosomal Aneuploidy Using Massively Parallel Genomic Sequencing."

38) Moreover, using sample tags to identify different sources of DNA in order to maximize sequencing capacity of the high throughput sequencing systems of the late 2000s was well known in the art as evidenced by Binladen and Shoemaker. Indeed, a commercial kit was available in 2008 through Illumina, Inc. [Ex. 1010]—the company that sells the sequencing system used in the Examples of the '430 patent [Ex. 1001 at col. 18:52-54].

39) Further, it is my opinion that one with ordinary skill in the relevant field—such as a first-year postdoc working in my laboratory in 2009 who was familiar with MPS—would have been motivated to combine the references to greatly enhance detection of the selected loci of Dhallan, maximize sequencing capacity of the sequencing systems available in the mid- to late 2000s, and decrease the overall cost of sequencing. Dhallan teaches a method for selectively amplifying specific loci on specific human chromosomes to detect fetal gene disorders in a maternal cell free sample [Ex. 1004 at col. 7:54-63 and col. 47:38-40]. However, Dhallan teaches detection of the selected loci by the methods that were available at the time of the Dhallan filing (i.e., 2002/2003), including Sanger sequencing using slab gels, capillary electrophoresis, DNA microarrays, mass spectrometry [Ex. 1004 at col. 8:60 – col. 9:7]. High throughput or massively parallel sequencing was not well-known or widely-commercialized until roughly 2005-2006 [Ex. 1002 at ¶15; Ex. 1003 at ¶19]; however, by 2008, several companies offered high throughput sequencing systems [Ex. 1002 at ¶ 14], and prior to 2010, high throughput sequencing had been applied to prenatal diagnostics using maternal serum and plasma samples [Ex. 1033; Ex. 1036; Ex. 1011; Ex. 1045].

40) As for the indexing feature, in 2008 Illumina offered a kit that allowed for tagging or indexing of different samples to maximize sequencing capacity which could be used in conjunction with their commercially-available high throughput sequencing system [Ex. 1010 at p.1]. This four-page Illumina, Inc. product flyer is cited on the front of the '430 patent and in the declaration of Ariosa expert, Prof. Robert Nussbaum. On the first page of Ex. 1010, it states explicitly "[t]o make multiplexed sequencing on the Genome Analyzer *available to any laboratory*, Illumina offers the Mulitplexing Sample Preparation Oligonucleotide Kit and the Mulitplexing Sequencing Primers and PhiX Control Kit" [Ex. 1010 at p. 1 (emphasis added)]. The kit contained the indexed primers, as well as software to deconvolute the sample indexes [see Ex. 1010 at p. 1 and 3].

41) Dr. Butte in his declaration states that there must be some rationale (i.e., some teaching, suggestion, or motivation) for combining cited references, and that a suggestion or motivation may come from sources such as explicit statements in the prior art, or from the knowledge or common sense of one of ordinary skill in the relevant fields. [Ex. 2003 at ¶ 27]; however, Dr. Butte states that there is no rationale or motivation to combine Dhallan with Binladen and/or Shoemaker [Ex. 2003 at ¶¶ 190-197]. Dr. Butte states further that he does not believe that a person of ordinary skill,

reviewing the asserted references in January 2010, would consider the Dhallan, Binladen and Shoemaker references as conveying a path for assembling different components to arrive at the methods of the ‘430 claims. Again, I disagree with Dr. Butte.

42) It is my opinion that one of ordinary skill in January 2010 would not only be aware of the use of next generation, massively parallel sequencing [*e.g.*, Ex. 1033; Ex. 1036; Ex. 1011; Ex. 1045], but would have been aware of the commercially-available indexing kit available through Illumina, Inc. in 2008 [Ex. 1010] that allowed for sequencing of 96 different samples on a single flow cell. Thus, not only was barcoding or sample indexing known in the art as evidenced by both Binladen and Shoemaker, but as early as 2008 Illumina, Inc. offered a sample indexing kit that was compatible with the Genome Analyzer, the same sequencing system used in generating the data reported in the ‘430 patent. [Ex. 1001 at col. 18:52-540].

43) It is my opinion that one of ordinary skill in January 2010 would be motivated to index individual samples and pool them for sequencing to maximize sequencing capacity and to minimize sequencing cost. For example, the Illumina, Inc. product flyer from 2008 [Ex. 1010] states, “[h]arnessing this sequencing power in a multiplexed fashion increases experimental throughput while reducing time and cost.” Similarly, Binladen

states that "in many studies [the] amount of single molecule sequences produced by single GS20 runs is unnecessary and economically unfeasible unless several PCR products [are] processed simultaneously and correctly assigned." [Ex. 1005 at p. 1]. Additionally, I testified to this motivating factor in my deposition [Ex. 2005 at 97:24 – 98:4], and Prof. Nussbaum testified to this in his declaration [Ex. 1003 at ¶ 20].

XI. THERE WOULD HAVE BEEN A REASONABLE EXPECTATION OF SUCCESS TO COMBINE DHALLAN WITH BINLADEN AND/OR SHOEMAKER

44) Dr. Butte does not address directly whether one of ordinary skill in the art would have had a reasonable expectation of success in combining the techniques of Dhallan with those of Binladen or Shoemaker, though Dr. Butte does opine that Binladen cannot be combined with Dhallan [Ex. 2003 at ¶¶ 214-225], nor can Shoemaker be combined with Dhallan [Ex. 2003 at ¶¶ 226-230]. Again, I must disagree with Dr. Butte's conclusions.

45) The Binladen and Shoemaker references demonstrate that use of barcodes or sample tags was well known in the art in the mid-2000s. Given that there was a commercially-available kit from Illumina in 2008 [Ex. 1010] that provided the sample tagged amplification primers as well as access to deconvoluting software, in my opinion one of ordinary skill in the

art would have had a reasonable expectation of success combining the selective amplification of cell-free DNA from maternal serum or plasma taught by Dhallan with the sample tags and next generation sequencing techniques taught by Binladen and/or Shoemaker.

46) In my view, one of ordinary skill in the art would have understood that substituting next generation, massively parallel sequencing techniques for the first- and second-generation detection techniques (e.g., Sanger sequencing using slab gels, capillary electrophoresis, DNA microarrays, mass spectrometry) that were available at the time of the filing of Dhallan would result in a much improved and robust method. That is, in the mid- to late 2000s, a first year postdoc working with sequencing technologies would have had a reasonable expectation of success when substituting the revolutionary next generation sequencing methodologies developed in the mid-2000s for older, less sensitive detection methodologies to enhance the fetal aneuploidy determination methods of Dhallan. In my opinion, this substitution would not only be successful, but would also be more sensitive, rapid and cost effective.

47) I declare that everything said in this statement is based on my knowledge and analysis of the literature. All my statements are true and I am

aware that deliberately false statements are punishable by fine or imprisonment or both, according to section 1001 of chapter 18 of the U.S. Penal Code.

Date: April 3, 2014

Cynthia Casson Morton

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