REQUEST FOR *EX PARTE* REEXAMINATION TRANSMITTAL FORM

Mail Stop *Ex Parte* Reexam
Commissioner for Patents
Alexandria, VA 22313-1450

Attorney Docket No.: 465157US110RX

Date: January 8, 2016

1. ■ This is a request for *ex parte* reexamination pursuant to 37 CFR 1.510 of patent number 8,318,430 issued November 27, 2012. The request is made by:

   ☐ patent owner. ■ third party requester.

2. ■ The name and address of the person requesting reexamination is:

   Ariosa Diagnostics, Inc.
   5945 Optical Court
   San Jose, CA 95138

3. ■ Credit card payment is being made online (if electronically filed), or is attached hereto (if paper filed), in the amount of $12,000.00 to cover the reexamination fee, 37 CFR 1.20(c)(1);

4. ■ Any refund should be made by ■ Credit to Deposit Account No. 15-0030
   37 CFR 1.26(c). If payment is made by credit card, refund must be to credit card account.

5. ■ A copy of the patent to be reexamined having a double column format on one side of a separate paper is enclosed. 37 CFR 1.510(b)(4)

6. ☐ CD-ROM or CD-R in duplicate, Computer Program (Appendix) or large table
   ☐ Landscape Table on CD

7. ☐ Nucleotide and/or Amino Acid Sequence Submission
   If applicable, items a. – c. are required.

   a. ☐ Computer readable Form (CRF)
   b. Specification Sequence Listing on:
      i. ☐ CD-ROM (2 copies) or CD-R (2 copies); **or**
      ii. ☐ paper
   c. ☐ Statements verifying identity of above copies

8. ☐ A copy of any disclaimer, certificate of correction or reexamination certificate issued in the patent is included.

9. ■ Reexamination of claim(s) 1-30 is requested.

10. ■ A copy of every patent or printed publication relied upon is submitted herewith including a listing thereof on Form PTO/SB/08, PTO-1449, or equivalent.

11. ☐ An English language translation of all necessary and pertinent non-English language patents and/or printed publications is included.

12. ■ The attached detailed request includes at least the following items:

a. A statement identifying each substantial new question of patentability based on prior patents and printed publications. 37 CFR 1.510(b)(1)

b. An identification of every claim for which reexamination is requested, and a detailed explanation of the pertinency and manner of applying the cited art to every claim for which reexamination is requested. 37 CFR 1.510(b)(2)

13. ☐ A proposed amendment is included (only where the patent owner is the requester). 37 CFR 1.510(e)

14. ■ a. It is certified that a copy of this request (if filed by other than the patent owner) has been served in its entirely on the patent owner as provided in 37 CFR 1.33(c).
The name and address of the party served and the date of service are:

WILSON SONSINI GOODRICH & ROSATI – VERINATA

650 Page Mill Road

Palo Alto, CA 94304

Date of Service: January 8, 2016 ; or

☐ b. A duplicate copy is enclosed since service on patent owner was not possible.

15. Correspondence Address: Direct all communication about the reexamination to:

■ The address associated with Customer Number: 22850

16. ■ The patent is currently the subject of the following concurrent proceeding(s):

☐ a. Copending reissue Application No. _____.

☐ b. Copending reexamination Control No. _____.

☐ c. Copending Interference No. _____.

■ d. Copending litigation styled:

Verinata Health, Inc. et al. v. Ariosa Diagnostics, Inc. et al., Case No. 3:12-cv-05501-SI (N.D. Cal), currently stayed

/Greg H. Gardella/ — Authorized Signature

January 8, 2016 — Date

Greg H. Gardella — Typed/Printed Name

46,045 — Registration No.

☐ For Patent Owner Requester

■ For Third Party Requester

10/08

Form PTO 1449 (Modified)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTY DOCKET NO. 465157US110RX	CONTROL NO. Not yet assigned
LIST OF REFERENCES CITED BY APPLICANT		APPLICANT Yue-Jen CHUU, et al.	
		FILING DATE Herewith	GROUP Not yet assigned

U.S. PATENT DOCUMENTS

EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUB CLASS	FILING DATE IF APPROPRIATE
	A	8,318,430	11/27/12	Chuu et al.			
	B	7,332,277	02/19/08	Dhallan			
	C	2006/0121452	06/08/06	Dhallan			
	D	2008/0090239	04/17/08	Shoemaker et al.			

OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, etc.)

	E	Prosecution history of U.S. Patent No. 8,318,430 (U.S. Patent Application No. 13/368,035)
	F	The Use of Coded PCR Primers Enables High-Throughput Sequencing of Multiple Homolog Amplification Products by 454 Parallel Sequencing, Jonas Binladen et al., PLoS ONE. 2007; 2(2): e197 (“Binladen”)
	G	*Identification of Genetic Variants Using Barcoded Multiplexed Sequencing, Nat. Methods*, Craig et al., Nat. Methods, 5(10):887-93 (2008) (“Craig”)
	H	Illumina Brochure “Multiplexed Sequencing with the Illumina Genome Analyzer System”, 2008 (“Illumina Brochure”)
	I	*A pyrosequencing-tailored nucleotide barcode design unveils opportunities for large-scale sample multiplexing*, Parameswaran, et al., Nucleic Acids Research, 35(19):e130 (2007) (“Parameswaran”)
	J	*Error-correcting barcoded primers allow hundreds of samples to be pyrosequenced in multiplex*, Hamady, et al., Nat. Methods, 5(3):235-37 (2008) (“Hamady”)
	K	Decision Denying Institution of Inter Partes Review: IPR2015-00873 – USP 7,879,828 (Apotex v. Wyeth)
	L	File History for *Inter Partes* Review of claims 1-18 of the ‘430 patent (IPR2013-00276)
	M	File History for *Inter Partes* Review of claims 19-30 of the ‘430 patent (IPR2013-00277)
	N	Supplementary Material referred to on page 8 of Craig
	O	Declaration of Dr. Steven Rosenberg
	P	Appeal No. 2015-1073: *Schott Gemtron Corp. v. SSW Holding Company Inc.* -- Corrected Brief for Intervenor – Director of the United States Patent and Trademark Office
	Q	Printout of http://www.nature.com/nmeth/journal/v5/n10/full/nmeth.1251.html
	R	Printout of http://www.nature.com/nmeth/journal/v5/n3/full/nmeth.1184.html
	S	Printout of http://nar.oxfordjournals.org/content/35/19/e130.full
	T	Federal Circuit Mandate Entering the Judgment and Order in Case No. 15-1215
	U	Federal Circuit Judgment and Order Vacating the Final Written Decisions in IPR2013-00276 and IPR2013-00277
	V	Claim Chart for the First Ground of Rejection: Dhallan II v. Craig and Illumina Brochure
	W	Claim Chart for the Second Ground of Rejection: Dhallan II v. Parameswaran and Hamady
	X	Claim Chart for the Third Ground of Rejection: Dhallan I v. Binladen

		☐ Additional References sheet(s) attached	
Examiner		Date considered	

*Examiner: Initial if reference is considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

U.S. Patent No. 8,318,430	§	Attorney Docket No.: 465157US
	§	
Issued: November 27, 2012	§	
	§	
Filed: Feb. 7, 2012	§	
	§	
For: METHODS OF FETAL ABNORMALITY DETECTION	§	
	§	
	§	

REQUEST FOR *EX PARTE* REEXAMINATION OF U.S. PATENT NO. 8,318,430

Mail Stop *Ex Parte* Reexam
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

Pursuant to the provisions of 35 U.S.C. § 302 and 37 C.F.R. § 1.510 et seq., the undersigned, on behalf of Ariosa Diagnostics, Inc., requests *ex parte* patent reexamination of claims 1-30 of U.S. Patent No. 8,318,430 ("the '430 patent," Exhibit A).

The '430 patent is assigned on its face to Verinata Health, Inc. ("Verinata" or "Patent Owner"). Formerly known as Artemis Health, Inc., Verinata Health, Inc., is a wholly owned subsidiary of Illumina, Inc.

Co-Pending Litigation

The '430 patent is the subject of a litigation captioned *Verinata Health, Inc. et al. v. Ariosa Diagnostics, Inc. et al.*, Case No. 3:12-cv-05501-SI (N.D. Cal), currently stayed.

Requester notes that USPTO policy dictates that patent reexaminations involved in concurrent litigation are to be accorded a special status. "Any cases involved in litigation, whether they are reexamination proceedings or reissue applications, will have priority over all

other cases." MPEP § 2261. As such, it is respectfully requested that the USPTO accord this proceeding special status such that it may advance to a timely conclusion.

Ex Parte Patent Reexamination Filing Requirements

Pursuant to 37 C.F.R. § 1.510(b)(1), statements pointing out at least one substantial new question of patentability based on material, non-cumulative prior art patents for claims 1-30 of the '430 patent are provided in Section VI. of this Request. Although some of these prior art references were previously cited in the record during the original prosecution of the '430 patent, these references have not been considered in the new light demonstrated by the proposed substantial new questions of patentability.

Pursuant to 37 C.F.R. § 1.510(b)(2), reexamination of claims 1-30 of the '430 patent is requested, and a detailed explanation of the pertinency and manner of applying the cited prior art to claims 1-30 is provided in Section VII. of this Request.

Pursuant to 37 C.F.R. § 1.510(b)(3), copies of every patent relied upon or referred to in the statement pointing out each substantial new question of patentability or in the detailed explanation of the pertinency and manner of applying the cited prior art are provided as Exhibits A-X of this Request.

Pursuant to 37 C.F.R. § 1.510(b)(4), a copy of the '430 patent is provided as Exhibit A of this Request.

Pursuant to 37 C.F.R. § 1.510(b)(5), the attached Certificate of Service indicates that a copy of this Request, in its entirety, has been served on Patent Owner at the following address of record for Patent Owner, in accordance with 37 C.F.R. § 1.33(c):

WSGR/VERINATA
650 Page Mill Road
Palo Alto, CA 94304

Also submitted herewith is the fee set forth in 37 C.F.R. § 1.20(c)(1).

Pursuant to 37 C.F.R. § 1.510(b)(6), Requester hereby certifies that neither the statutory estoppel provisions of 35 U.S.C. § 315(e)(1) nor 35 U.S.C. § 325(e)(1) prohibit Requester from filing this *ex parte* patent reexamination request for two reasons, both of which are explained more detail in Section II., below. **First**, the Final Written Decision declined to institute trial based on the ground presented herein at Section VII.C on the basis that that ground was redundant with another ground on which trial was instituted. Ex. L at 20; Ex. M at 20-21. Accordingly, the estoppel provisions of 35 U.S.C. § 315(e)(1) cannot apply to that ground. *Apotex v. Wyeth*, IPR2015-00873, Paper 8, p. 9 ("[B]ecause the Board denied institution of [a ground] as redundant, and Petitioner could not have raised [the ground] again once institution was denied as to that ground. Estoppel under 35 U.S.C. § 315(e)(1), therefore, does not bar Petitioner from maintaining a proceeding before the Office on [that ground]"). **Second**, estoppel does not apply to the remaining grounds presented herein because on December 23, 2015 the Federal Circuit issued the formal mandate entering the judgment of the Federal Circuit which vacates the Final Written Decision of the Patent Trial and Appeal Board (PTAB) in the matter of *Ariosa Diagnostics, Inc. v. Verinata Health, Inc.*, IPR2013-00276 and IPR2013-00277. *See* Exs. T and U. When a judgment is vacated, the effect is to "nullify the judgment entirely and place the parties in the position of no trial having taken place at all." *United States v. Williams*, 904 F.2d 7, 8 (7th Cir. 1990); *United States v. Ayres*, 76 U.S. 608, 610 (1869) ("[I]t is quite clear, that [an] order granting the new trial has the effect of vacating the former judgment, and to render it null and void, and the parties are left in the same situation as if no trial had ever taken place in the cause."); *see also United States v. Lawson*, 736 F.2d 835 (2d Cir.1984) ("It has long been established … that when a judgment has been reversed and the case remanded for a new trial, the

effect is to nullify the judgment entirely and place the parties in the position of no trial having taken place"). In a similar context the PTAB held that the one–year bar of 37 C.F.R. § 315(a) does not apply where a district court complaint was dismissed without prejudice because "[t]he Federal Circuit has consistently interpreted the effect of such dismissals as leaving the parties as though the action had never been brought." *Macauto v. BOS GmbH & KG*, IPR2012-00004, Paper 18, p. 15 (PTAB Jan 24, 2013). The same reasoning applies to the estoppel provision of 35 U.S.C. §315(e)(1) – the vacatur of the final written decisions places the parties in the position as if no final written decisions had been rendered. *Ariosa Diagnostics* is thus not estopped from filing the present request under 37 C.F.R. § 315(e)(1) or 37 C.F.R. § 325(e)(1).

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TABLE OF EXHIBITS

Exhibit R	Printout of http://www.nature.com/nmeth/journal/v5/n3/full/nmeth.1184.html
Exhibit S	Printout of http://nar.oxfordjournals.org/content/35/19/e130.full
Exhibit T	Federal Circuit Mandate Entering the Judgment and Order in Case No. 15-1215
Exhibit U	Federal Circuit Judgment and Order Vacating the Final Written Decisions in IPR2013-00276 and IPR2013-00277
Exhibit V	Claim Chart for the First Ground: Dhallan II in combination with Craig and the Illumina Brochure
Exhibit W	Claim Chart for the Second Ground: Dhallan II in combination with Parameswaran and Hamady
Exhibit X	Claim Chart for the Third Ground: Dhallan I in combination with Binladen

I. INTRODUCTION

This request for reexamination and the proposed grounds of rejection raised herein are supported by a declaration from Dr. Steven Rosenberg, a pioneer in the diagnostics field that developed two multivariate diagnostic tests which are in clinical use today. *See* Ex. O. Dr. Rosenberg's declaration summarizes and reflects his knowledge, technical expertise, and understanding of the scope and content of the prior art applied in this request for reexamination. *Id.* at ¶¶ 2-3, 35-44, MPEP § 2258. The state of the art at the time of the filing of the '430 patent is presented by Dr. Rosenberg in this request for reexamination.

The '430 patent is generally directed to a method for detecting fetal aneuploidies in multiple samples of pregnant women by counting, in a maternal blood sample, the number of DNA fragments from a chromosome suspected of being aneuploid and the number of fragments from a reference chromosome or control region from a chromosome that is not aneuploid. Ex. A at Abstract, 1:23-67, 2:4-11, 6:20-27 and 13:59-64; Ex. O at ¶¶ 14, 45. The two numbers are compared to determine whether there is an abnormal level of DNA associated with the chromosome suspected of being aneuploid. *Id.*; *Id.* at ¶¶ 14, 32, 34, and 45. This method is performed in a multiplexed fashion for a plurality of maternal blood samples using indexing (i.e., tagging or labelling) techniques to distinguish results from different samples. *Id.* at 22:9-29; *Id.* at ¶¶ 14, 31, 45 and 48.

The USPTO has considered the subject matter claimed in the '430 patent allowable because the indexing methods taught in cited secondary references were believed to be incompatible with the sequencing techniques taught in the primary references. *See* Sections IV.C and IV.D, *infra*.

Neither the *ex parte* examiner nor the PTAB has addressed the merits of any of the grounds presented in this request, each of which provides a combination of art in which the

indexing or multiplexing technique is fully compatible with the sequencing method. Parameswaran, P., et al. "A Pyrosequencing-Tailored Nucleotide Barcode Design Unveils Opportunities for Large-Scale Sample Multiplexing." *Nucleic Acids Research*, 35(19):e130 (2007) ("Parameswaran") (Ex. H) and Hamady, M., et al. "Error-Correcting Barcoded Primers Allow Hundreds of Samples to be Pyrosequenced in Multiplex." *Nat. Methods*, 5(3):235-37 (2008) ("Hamady") (Ex. I) were not before the PTAB during the *inter partes* reviews. U.S. Patent Pub. App. No. 2006/0121452 to Dhallan II ("Dhallan II") was likewise not before the PTAB, although a related Dhallan reference ("Dhallan I") (U.S. Patent No. 7,332,277, Ex. C) was considered. *See*, e.g., Ex. L, Paper 11 at 14-20; Ex. M, Paper 11 at 14-21. However, the PTAB expressly declined to consider whether the claimed subject matter was rendered obvious by Dhallan I taken in view of documentation describing commercially available prior art massively parallel sequencing systems. *See* Ex. L, Paper 43 at 19, Ex. M, Paper 43 at 19. Accordingly, even as to Dhallan I the PTAB did not consider whether that reference rendered the claimed subject matter obvious when considered in combination with then-conventional and commercially available massively parallel sequencing systems.

The substantial new questions of patentability presented in this request are straightforward. Dhallan II, filed in 2004, teaches all limitations recited in the claims except the detection method which employs i) indexing or tagging samples from different patients so they can be processed simultaneously (sometimes called multiplexing), and ii) using massively parallel sequencing to sequence the indexed samples. Ex. O at ¶ 35.

Neither of these latter techniques was commercially available at the time of the filing of the Dhallan II reference. However, both were present in massively parallel sequencing systems which were commercially available and widely used by scientists by January 23, 2010, the

earliest claimed priority date of the '430 patent. *See* Exs. F and G. For instance, the Illumina Genome Analyzer was available in 2008, as was a Multiplexing Kit which was marketed in conjunction with the Illumina Genome Analyzer. The combined use of the Illumina Genome Analyzer and the Multiplexing Kit were advertised as providing fast, high throughput analysis of multiple samples with high quality data, improved productivity and reduced time and cost. Ex. G at 1. Dr. Rosenberg explains in his declaration that any first year post-doctoral student in a molecular biology laboratory would have considered it routine (and quite advantageous) to perform the techniques utilized in the aneuploidy detection method taught by Dhallan II in 2004 using the later-developed Illumina Genome Analyzer and Multiplexing Kit as a replacement for the labor-intensive detection technique actually used in the examples of Dhallan II. Ex. O ¶¶ 62-64; Ex. F (Craig, describing the Illumina Genome Analyzer). The Dhallan II reference expressly envisions that various sequencing methods could be used with the disclosed aneuploidy detection assay, stating "[a]ny method that provides information on the sequence of a nucleic acid can be used," and lists over 20 different detection methods that could be used *in addition* to the detection technique actually used in the examples. Ex. E at ¶ [0228]. Thus, the claims of the '430 patent are rendered obvious by Dhallan II in view of Craig (teaching how to perform multiplexed processing on the Illumina Genome Analyzer) and further in view of the Illumina Multiplexing Kit Brochure. Ex. O at ¶¶ 45-52 and 67-197.

The second substantial new question of patentability and proposed rejection is similar. Dhallan II could be alternatively combined with a different massively parallel sequencing platform sold by 454 Life Sciences, later acquired by Roche before the earliest claimed priority date. The Roche/454 massively parallel sequencing platform also could have been advantageously used to perform the method disclosed by Dhallan II, as it too is a more efficient

and cost effective detection method than the detection method taught in Dhallan II. Parameswaran teaches the use of the Roche/454 massively parallel sequencing platform for multiplexed detection of multiple samples (Ex. H), and references such as Hamady teach the efficiency of multiplexing using indexes with the Roche/454 massively parallel sequencing platform (Ex. I). Ex. O at ¶¶ 40-41. Thus the claims of the '430 patent also are rendered obvious by Dhallan II in view of Parameswaran (describing large-scale multiplexing using the Roche/454 massively parallel sequencing platform) and further in view of Hamady (describing use of error correcting indexes for multiplexing on the Roche/454 platform). *Id.* at ¶¶ 53-55, 198-326.

The third substantial new question of patentability is Dhallan I and Binladen. This ground was presented in the IPR petitions but the Board chose to institute trial instead on a related but different ground involving a different primary reference – the combination of Shoemaker, Dhallan I and Binladen. Ex. L, Paper 11 at 20; Ex. M, Paper 11 at 20. The combination of Dhallan I and B presented herein is fundamentally different that the combination of Shoemaker, Dhallan I and Binladen addressed during the IPR. The combination of Dhallan I and Binladen relies on the sequencing techniques of Dhallan I being replaced by those described in Binladen (*i.e.*, multiplexed massively parallel sequencing). In contrast, the ground considered in the IPR involved Shoemaker's sequencing techniques being modified only to include the use of extracellular DNA (from Dhallan I) and multiplexing samples from multiple patients (from Binladen). Ex. L, Paper 1 at 38; Ex. M, Paper 1 at 38. Because the combination of Dhallan I and Binladen also present the technical teaching believed to be missing from the prior art (a multiplexing method which is compatible with the sequencing method), this combination also

presents a substantial new question of patentability and renders the claimed subject matter obvious. Ex. O at ¶¶ 56-61 and 327-466.

II. THE ESTOPPEL PROVISIONS OF 35 USC §§ 315, 325 DO NOT BAR THIS REQUEST

Neither 35 U.S.C. § 315(e)(1) nor 35 U.S.C. § 325(e)(1) prohibit Requester from filing this *ex parte* patent reexamination request. Only the former is relevant here, as the latter applies to Covered Business Method Review proceedings, which have not occurred relative to the '430 patent.

Even if the Final Written Decision had not been vacated, the estoppel provision of 35 U.S.C. § 315(e)(1) cannot apply to grounds presented but not included in the trial proceedings. The Board held in *Apotex Inc. v. Wyeth LLC*, IPR2015-00873, Paper 8 at 8-9 (Sept. 16, 2015) (Ex. K) that

> An inter partes review does not begin until the Office decides to institute review; prior to that point, our Rules refer to a 'preliminary proceeding' that begins with the filing of a petition and ends with a decision whether to institute trial. . . . [G]rounds raised during the preliminary proceeding, but not made part of the instituted trial, are not raised 'during' an inter partes review and cannot be the basis for estoppel under 35 U.S.C. § 315(e)(1). (internal citation omitted).

In *Apotex*, the Board concluded that the 35 U.S.C. § 315(e)(1) provision did not apply "because the Board denied institution of [a ground] as redundant, and Petitioner could not have raised [the ground] again once institution was denied as to that ground. Estoppel under 35 U.S.C. § 315(e)(1), therefore, does not bar Petitioner from maintaining a proceeding before the Office on [that ground]". Ex. K at 9. The Director of the United States Patent and Trademark Office likewise has taken the position that § 315(e)(1) estoppel does not apply to grounds on which trial

was not instituted. In the solicitor's brief in *Schott Gemtron Corp. v. SSW Holding Co.*, Appeal No. 2015-1073, the Office argued that

> Contrary to Schott's argument, estoppel does **not** prevent Schott from asserting the subset of proposed grounds that were not part of the IPR proceeding in this case. Under the AIA, estoppel applies for "any ground that the petitioner raised or reasonably could have **raised during that inter partes review**."

Ex. P at 38 (emphasis in original, citations omitted).

Here, the Board exercised its discretion and declined to institute on the combination of Dhallan I and Binladen (presented herein at Section VII.C) on the basis that it was redundant with another ground involving a different primary reference:

> 3. Obviousness of claims 1-18 over the Combination of Dhallan and Binladen
> Given our determination that there is a reasonable likelihood that Ariosa would prevail on the ground that claims 1-18 are unpatentable as obvious over Shoemaker, Dhallan, and Binladen, we exercise our discretion to deny as redundant Ariosa's asserted ground that claims 1-18 are rendered obvious over Dhallan and Binladen.

Ex. L at 20; Ex. M at 20-21. Accordingly, under the rule set forth in *Apotex* and the solicitor's brief in *Schott,* no estoppel can apply to the combination of Dhallan I and Binladen because trial was not instituted on the ground. Ex. K at 38.

Turning to the remaining grounds presented herein, estoppel does not apply because on December 23, 2015 the Federal Circuit issued the formal mandate entering the judgment of the Federal Circuit, which vacates the final written decisions of the Patent Trial and Appeal Board (PTAB) in the matter of Ariosa Diagnostics, Inc. v. Verinata Health, Inc., IPR2013-00276 and IPR2013-00277. See Exs. T and U. Requester is unaware of any decision specifically addressing the impact of a *vacatur* of a final written decision on the estoppel provisions of

§ 315(e), so it is a case of first impression. Moreover, the legislative history does not appear to address this issue.

That being said, in a closely related context the Board has held that the one-year bar provision of 37 C.F.R. § 315(a) does not apply where a district court complaint was dismissed without prejudice because "[t]he Federal Circuit has consistently interpreted the effect of such dismissals as leaving the parties as though the action had never been brought." *Macauto v. BOS GmbH & KG*, IPR2012-00004, Paper 18, p. 15 (PTAB Jan 24, 2013).

Vacatur of the final written decision has the same effect and should produce the same result, *i.e.*, the parties should be left is the same position as though the decision had not been rendered. The Supreme Court has held that "vacating the former judgment [] render[s] [the judgment] null and void, and the parties are left in the same situation as if no trial had ever taken place in the cause." *United States v. Ayres*, 76 U.S. 608, 610 (1869). The Seventh Circuit similarly has held that when a judgment is vacated, the effect is to "nullify the judgment entirely and place the parties in the position of no trial having taken place at all." *United States v. Williams*, 904 F.2d 7, 8 (7th Cir. 1990); see also *United States v. Lawson*, 736 F.2d 835 (2d Cir.1984) ("It has long been established [] that when a judgment has been reversed and the case remanded for a new trial, the effect is to nullify the judgment entirely and place the parties in the position of no trial having taken place").

Because the controlling precedent dictates that vacatur of a decision or judgment puts the parties in the same position as though the decision or judgment had not been rendered, the reasoning adopted by the Board in *Macauto* applies with equal force here. The vacatur of the final written decisions places the parties in the position as if no final written decisions had been rendered. Ariosa Diagnostics is thus not estopped under 37 C.F.R. § 315(e)(1).

III. CITATION OF PRIOR ART PATENTS AND PRINTED PUBLICATIONS RELIED UPON IN REQUEST FOR REEXAMINATION

Reexamination of claims 1-30 of the '430 patent is requested in view of the following prior art patents and printed publications:

U.S. Patent No. 7,332,277 to Dhallan ("Dhallan I") is attached hereto as Exhibit C. Dhallan I was filed on September 11, 2003 and issued on Feb. 19, 2008, and is available as prior art under 35 U.S.C. § 102(b).

Binladen, J., et al. "The Use of Coded PCR Primers Enables High-Throughput Sequencing of Multiple Homolog Amplification Products by 454 Parallel Sequencing." *PLoS ONE*. 2(2):e197 (2007) ("Binladen") is attached hereto as Exhibit D. Binladen was published in February 2007 and is available as prior art under 35 U.S.C. § 102(b).

U.S. Patent Pub. App. No. 2006/0121452 to Dhallan ("Dhallan II") is attached hereto as Exhibit E. Dhallan II was filed on March 1, 2004 and published on June 8, 2006, and is available as prior art under 35 U.S.C. § 102(b).

Craig, David W., et al. "Identification of Genetic Variants Using Barcoded Multiplexed Sequencing."*Nat. Methods*, 5(10):887-93 (2008) ("Craig") is attached hereto as Exhibit F. Craig was published online on September 14, 2008, and is available as prior art under 35 U.S.C. § 102(b). The online publication date is shown at http://www.nature.com/nmeth/journal/v5/n10/full/nmeth.1251.html, attached as Ex. Q.

Illumina Brochure "Multiplexed Sequencing with the Illumina Genome Analyzer System," 2008 ("Illumina Brochure") is attached hereto as Exhibit G. The Illumina Brochure was publically available in 2008 and is available as prior art under 35 U.S.C. § 102(b). The Illumina Brochure is ascribed a publication date of December 2, 2008 on the face of the '430 patent. Ex. A at 1, Other Publications. Consistent with this representation, the Illumina

Brochure bears an indication that it was copyrighted in 2008 and is "current as of 2 December 2008." Ex. G at 4, footer.

Parameswaran, P., et al. "A Pyrosequencing-Tailored Nucleotide Barcode Design Unveils Opportunities for Large-Scale Sample Multiplexing." *Nucleic Acids Research*, 35(19):e130 (2007) ("Parameswaran") is attached hereto as Exhibit H. Parameswaran was published online on October 11, 2007 and is available as prior art under 35 U.S.C. § 102(b). *See* Ex. S (copy of http://nar.oxfordjournals.org/content/35/19/e130.full).

Hamady, M., et al. "Error-Correcting Barcoded Primers Allow Hundreds of Samples to be Pyrosequenced in Multiplex." *Nat. Methods*, 5(3):235-37 (2008) ("Hamady") is attached hereto as Exhibit I. Hamady was published online on February 10, 2008 and is available as prior art under 35 U.S.C. § 102(b). The online publication date is shown at http://www.nature.com/nmeth/journal/v5/n3/full/nmeth.1184.html, attached as Ex. R.

IV. OVERVIEW OF THE '430 PATENT

A. Background of the '430 Patent and Level of Skill in the Art[1]

By 2008 it was well established that plasma and serum from the blood of pregnant women contained cell-free DNA from both the mother and fetus in concentrations that allow prenatal testing. Ex. A at 1:20-37; Ex. O at ¶ 25. Numerous methods for detecting fetal DNA in a plasma or serum sample from a pregnant woman for identifying genetic traits and abnormalities in the fetus were known. *Id.*; Ex. O at ¶¶ 25-27. Methods for determining whether a fetus had an abnormal chromosome number (aneuploidy) by comparing the amounts of DNA fragments originating from an aneuploid chromosome and a normal chromosome had been widely published. Ex. O at ¶ 27.

[1] *See Ex. O (Rosenberg Decl.)* ¶¶ *28-55* for full Background discussion, including evidentiary support.

Massively parallel sequencing methods were in routine use by 2010. These methods permitted the rapid sequencing of nucleic acids, including those constituting the entire human genome or portions of it. Ex. A at 10:7-27; Ex. O at ¶¶ 19-23.

Multiplexed massively parallel sequencing of a pooled sample (e.g., efficiently sequencing a mixed or pooled sample containing the DNA fragments from several different individuals after indexing the DNA fragments from each individual with a tag or barcode unique to a sample) were also well known, commercially available, and in widespread use as of 2010. Ex. O at ¶¶ 21-23.

B. Summary of the '430 Patent

The '430 patent is generally directed to a method for detecting fetal aneuploidies in multiple samples of pregnant women by counting, in a maternal blood sample, the number of DNA fragments from a chromosome suspected of being aneuploid and the number of fragments from a reference chromosome or control region from a chromosome that is not aneuploid. Ex. A at Abstract, 1:23-67, 2:4-11, 6:20-27 and 13:59-64; Ex. O at ¶¶ 14, 45. The two numbers are compared to determine whether there is an abnormal level of DNA associated with the chromosome suspected of being aneuploid. *Id.*; *Id.* at ¶¶ 14, 32, 34, and 45. This method is performed in a multiplexed fashion for a plurality of maternal blood samples using indexing (i.e., tagging or labelling) techniques to distinguish results from different samples. *Id.* at 22:9-29; *Id.* at ¶¶ 14, 31, 45 and 48.

Specifically, the '430 patent claims a six step process for detecting fetal aneuploidy in samples from pregnant women: obtaining blood samples from multiple pregnant women that contain cell-free DNA, isolating cell-free DNA samples from the plurality of blood samples, enriching at least 100 non-random polynucleotides from a first chromosome tested for being

aneuploid and at least 100 different non-random polynucleotide sequences non-random polynucleotides from a reference chromosome in each cell-free DNA sample by amplification to create a library that contains representative PCR products having the sequence of the non-random polynucleotides, detecting the non-random polynucleotides from the first chromosome tested for being aneuploid and the reference chromosome of each library for each sample, enumerating (*i.e.*, counting) the non-random polynucleotides from the first chromosome or chromosome region tested for being aneuploid and the reference chromosome or chromosome control region of each library from each sample, and determining the presence or absence of a fetal aneuploidy for each sample comparing the enumerated non-random polynucleotides from the first chromosome or chromosome region tested for being aneuploid and the reference chromosome or chromosome control region from each sample library. Ex. A at Abstract, 1:23-67, 2:4-11, 6:20-27 and 13:59-64; Ex. O at ¶ 45.

C. Prosecution History of the '430 Patent

The '035 application was filed on February 7, 2012. The original claims were rejected under 35 U.S.C. 102(b) over Stoughton, et al., U.S. Patent Pub. No. 2008/0070792 on March 13, 2012. Ex. B at 252-254. In response, Applicants made various amendments to the claims after which the Examiner issued a new obviousness rejection based on U.S. Patent Pub. No. 2007/0202525 to Quake and the Illumina Brochure. Ex. B at 72-76.

In rebuttal to the new rejection Applicants argued that the digital PCR (dPCR) technique taught in Quake was not compatible with indexing techniques described in the Multiplexing Sequencing Kit. In its August 31, 2012 response, Applicants stated that

> it is important to note that [all teachings] of **selective enrichment of nucleic acid sequences in the '525 Application are in conjunction only with the use of the dPCR embodiment**. The massively parallel sequencing embodiment is described without

> any such selective enrichment of sequences prior to sequencing. (emphasis added)
>
> . . .
>
> [C]laims 1 and 18 specify the indexing and pooling of the enriched sequences prior to conducting massively parallel sequencing. After sequencing, all the different sequences from multiple patients need to be deconstructed and identified to their chromosomes. . . . It is important to note that the **step of indexing and pooling is inconsistent with the digital PCR embodiment of the 525 Application**. In the dPCR embodiment ***single DNA*** molecules are analyzed in discrete reaction samples. **A pooling step before performing dPCR step would defeat the purpose of the dPCR which tries to dilute out the sample to have a single DNA molecule per reaction site**. Ex. B at 60-61. (emphasis added)

The Examiner agreed with Verinata's argument that digital PCR was not compatible with indexing techniques described in the Illumina Brochure. Hence, the next action was a notice of allowance. Ex. B at 27-34. The statement of reasons for allowance indicated that "2007/0202525 does not teach or suggest 'b) selectively enriching a plurality of non-random polynucleotide sequences of each fetal and maternal cell-free genomic DNA sample' in conjunction with the use of massively parallel sequencing." *Id.* at 33.

Accordingly, the **Examiner appears to have allowed the claims because the Quake reference's dPCR technique was believed to be incompatible with indexing techniques taught in the applied prior art**.

D. The *Inter Partes* Review Proceedings

Ariosa filed two *inter partes* review petitions, one against claims 1-18 and one against claims 19-30. Ex. L, Paper 1; Ex. M, Paper 1. The petitions presented the same prior art and substantially identical argumentation. *Id.*

In the *inter partes* review petitions, Ariosa presented the Shoemaker reference which taught that massively parallel sequencing techniques were useful to detect fetal aneuploidies

using a maternal blood sample. Ex. L, Paper 1 at 40-55; Ex. M, Paper 1 at 38-53. The petitions also explained that the use of maternal blood samples for detecting fetal aneuploidies was known, as shown by both Shoemaker and a different Dhallan reference, U.S. Pat No. 7,332,277 ("Dhallan I"). The petitions further demonstrated that indexing techniques were conventional at the time of filing, as described in Shoemaker and as exemplified by the Binladen reference. *Id.* at 11, 43 and 46; *Id.* at 11, 44 and 47.

The Board agreed with these premises. Ex. L, Paper 11; Ex. M, Paper 11. The Board instituted trial on the ground that the claims were rendered obvious Shoemaker in view of Dhallan I and Binladen. *Id.* at 20; *Id.* at 20. The Board deemed an alternate ground, Dhallan I in view of Binladen, as redundant as declined to institute trial on that ground. *Id.*; *Id.* at 20-21.

During the *inter partes* reviews Verinata did not dispute that the references taught the use of massively parallel sequencing for aneuploidy detection. Ex. L at Paper 20 and Paper 34; Ex. M at Paper 19 and Paper 23 (discussing Dhallan I, of which Dhallan II is a continuation-in-part). Nor did Verinata dispute that use of maternal blood samples and the use indexing or tagging were well known. *Id.* at Paper 10 and Paper 20; *Id.* at Paper 10 and Paper 20. Rather, Verinata presented essentially the same argument it presented during *ex parte* prosecution. Verinata again argued that the specific detection method used in the primary reference was incompatible with the specific indexing technique disclosed in the secondary reference. *Id.* at Paper 20; *Id.* at Paper 20. More particularly, Verinata argued that the detection method taught in the experimental section of Dhallan I was incompatible with the specific indexing technique used in Binladen. *Id.* at 52; *Id.* at 52.

In reply Ariosa argued that the Shoemaker taught the use of massively parallel sequencing with indexes for aneuploidy detection, and Dhallan I taught multiple embodiments

for detecting DNA that could be used with Shoemaker's aneuploidy detection methods. Ex. L, Paper 26 at 8-9; Ex. M, Paper 26 at 8-9. Ariosa further argued, as set forth in the Declaration of Dr. Cynthia Morton, that the level of skill in the art included the ability to order an off-the-shelf indexing kit designed for use with a massively parallel sequencing system, and as a consequence a skilled artisan would have had no trouble implementing indexing as suggested by Binladen or Shoemaker with the aneuploidy detection methods of Dhallan I or Shoemaker. *Id.* at Paper 26 at 9 and Ex. 1042, ¶¶ 5-6, 21 and 22; *Id.* at Paper 26 at 9 and Ex. 1042, ¶¶ 5-6, 21 and 22.

The Board, in its Final Decision, refused to consider these reply arguments on the basis that they were deemed to have been belatedly presented. The Final Written Decision dismissed both arguments because they were not presented in the first instance in the petition:

> Dr. Morton, in her Second Declaration, contends that [Dhallan I] also teaches a number of amplification and/or detection methods which do not require the use of restriction digestible primers" (Ex. 1042 ¶¶ 17, 18), but those portions of [Dhallan I] were not identified or discussed in the Petition or the accompanying Declarations. . . . [As to the second rebuttal argument] [t]his testimony, in effect, replaces the tagging and sequencing techniques of [Dhallan I] and Binladen with the Illumina indexing kit and sequencing platform, but neither [Ariosa] nor Dr. Morton explains why Exhibit 1010 could not have been presented as part of the asserted ground of unpatentability in the first instance with the Petition. Therefore we accord this aspect of Dr. Morton's testimony no weight.

Ex. L, Paper 43 at 17-19; Ex. M, Paper 43 at 17-19. The Board thus declined to consider i) the fact that Dhallan I taught multiple embodiments for detecting DNA that could be used with its aneuploidy detection methods, and ii) the fact that one skilled in the art could have performed Dhallan I's aneuploidy detection techniques with commercially available a multiplexed massively parallel sequencing system, because neither premise was considered to have been fully presented until the reply brief.

The Final Written Decision commented on the compatibility of Binladen's multiplexing technique with the exemplified sequencing method of Dhallan but did not make any findings as to whether Dhallan's sequencing method could be replaced with multiplexed massively parallel sequencing, as is proposed in the grounds presented herein. At pages 12-15 of the Final Written Decisions the Board discusses Patent Owner's contentions that Binladen's tags could not be incorporated in the methods described in Dhallan because they would be "incompatible with the restriction digestible primers critical to the process of Dhallan['s] [examples]." Ex. L, Paper 43; Ex. M, Paper 43. However, the Board expressly refused to consider the broader question of whether the Dhallan's sequencing technique could simply be replaced with the commercially available multiplexed massively parallel sequencing techniques. *Id.* at 16-19; *Id.* at 16-19. Accordingly, to the extent the PTAB addressed the merits of the proffered combination, the PTAB found the asserted combination to be patentable because Dhallan's sequencing method was believed to be incompatible with the indexing techniques taught in Binladen.

Thus the PTAB appears to have "allowed" the claims for substantially the same reason as the *ex parte* examiner, *i.e.*, the base reference's sequencing technique was believed to be incompatible with the indexing techniques taught in the secondary reference.

E. The Appeal to the Federal Circuit

The Court of Appeals for the Federal Circuit vacated and remanded the Final Written Decision. The Federal Circuit held that

> [t]he Board's language on its face supports Ariosa's interpretation of what the Board meant—that the Board was declining to consider Exhibit 1010, even as evidence of the background understanding of skilled artisans as of January 2010, simply because the brochure had not been identified at the petition stage as one of the pieces of prior art defining a combination for obviousness. If that is what the Board meant, the Board erred. Art can legitimately serve to

> document the knowledge that skilled artisans would bring to bear in reading the prior art identified as producing obviousness. (Citing *Randall Mfg. v. Rea*, 733 F.3d 1355, 1362-63 (Fed. Cir. 2013), which held that the Board erred by "narrowly focusing on the four prior-art references cited by the Examiner and ignoring the additional record evidence [] cited to demonstrate the knowledge and perspective of one of ordinary skill in the art.").

Ex. U at 11-12. The formal mandate entering the judgment issued on December 23, 2015. *See* Ex. T.

The *inter partes* reviews are currently pending before the PTAB. The PTAB has not yet issued any order concerning the conduct of the proceedings on remand.

V. CLAIM CONSTRUCTION

Claim terms are generally given their ordinary and customary meaning as would be understood by one of ordinary skill in the art. *Phillips v. AWH Corp.*, 415 F.3d 1303, 1312-13 (Fed. Cir. 2005) (*en banc*). In an *ex parte* reexamination, a claim in an unexpired patent is "given the broadest reasonable interpretation consistent with the specification." MPEP § 2258(I)(G).

A. "selectively enriching a plurality of non-random polynucleotide sequences" (Claims 1 and 19)

The term "selectively enriching" is not explicitly defined by the specification. The term "selective enrichment" of nucleic acids from a sample encompasses, under the broadest reasonable interpretation, increasing the concentration of a selected subset of nucleic acids relative to the remainder of the nucleic acids in the sample.

This phrase is used, for example, in the specification as follows:

> In another embodiment, said selectively enriching comprises performing PCR. In another embodiment, said selectively enriching comprises linear amplification. Ex. A at 2:40-43.

In this passage, the phrase "selectively enriching" embodies a method that selectively increases the relative concentration of a selected DNA region by amplification methods. The experimental section of the specification describes only selective amplification by PCR for enrichment to create multiple copies of a sequence such as a "hot spot." Ex. A at 14:59 - 22:41. Besides amplification, the '430 patent mentions no other methods that could be used for selective enrichment.

In its institution decision the Board interpreted "selectively enriching a plurality of nonrandom polynucleotide sequences" to mean "increasing the copy number of selected non-random polynucleotide sequences." Ex. L, Paper 11 at 7-8; Ex. M, Paper 11 at 7-8. The Board reasoned that

> [b]oth the claim language and the Specification refer to "non-random polynucleotide sequences." Claim 1 requires enriching a "plurality of nonrandom polynucleotide sequences" including "at least 100 different nonrandom polynucleotide sequences" selected from a first chromosome tested for being aneuploid and from a reference chromosome. In addition, the Specification states that, although the prior art teaches methods of "randomly enriching" nucleic acids, there is a need for enriching "nonrandom fetal and maternal polynucleotide sequences" to facilitate aneuploidy detection. Ex. 1001 [Ex. A], 1:33-37; see also Prelim. Resp. 9-10 (citing various portions of Specification). The Specification further discloses methods for selecting the non-random polynucleotide sequences to enrich. For example, "[t]he selection of polynucleotide sequences to enrich can be based on knowledge of regions of chromosomes that have a role in aneuploidy." Ex. 1001 [Ex. A], 5:61-63 (emphasis added). Thus, we agree that the required "selective enrichment" is of "non-random polynucleotide sequences." Furthermore, the Specification provides that "selectively enriching" includes "performing PCR," i.e., polymerase chain reaction, or "linear amplification," methods which increase the copy number of the selected nucleic acids, Ex. 1001 [Ex. A], 2:40-43; Ex. 1002 ¶¶ 9, 10. Accordingly, we interpret "selectively enriching a plurality of nonrandom polynucleotide sequences" to mean "increasing the copy number of selected non-random polynucleotide sequences."

Ex. L, Paper 11, at 7-8; Ex. M, Paper 11 at 7-8. The Board did not consider it necessary to interpret this term in the Final Written Decision. *Id.* at Paper 43; *Id.* at Paper 43. *See also* Ex. O at ¶ 29.

B. "at least 100 different non-random polynucleotide sequences selected from a first chromosome tested for being aneuploid and at least 100 different non-random polynucleotide sequences selected from a reference chromosome" (Claim 1)

At institution of the *inter partes* reviews the Board interpreted the claim phrase "at least 100 different nonrandom polynucleotide sequences selected from a first chromosome tested for being aneuploid and at least 100 different non-random polynucleotide sequences selected from a reference chromosome" as "100 different sequences selected from each of a reference chromosome and a reference chromosome." The relevant portion of the Board's analysis is as follows:

> The claim language makes it clear that the "at least 100 different nonrandom polynucleotide sequences" that are selected from a first chromosome and a reference chromosome are 100 different sequences from each of a first chromosome and a reference chromosome. The Specification explains that a plurality of sequences that are "representative of a plurality of [. . .] regions of a chromosome" are enriched. Ex. 1001 [Ex. A], 3:20-22 (emphasis added), 3:6-7. Furthermore, the number of regions of a chromosome, i.e., the claimed nonrandom polynucleotide sequences, which can be enriched in a sample, can be at least 1 to 1000. *Id.* at 8:7-11, 8:56-61. Accordingly, we interpret the claim phrase "at least 100 different nonrandom polynucleotide sequences selected from a first chromosome tested for being aneuploid and at least 100 different non-random polynucleotide sequences selected from a reference chromosome" as "100 different sequences selected from each of a reference chromosome and a reference chromosome.

Ex. L, Paper 11, at 8-9; Ex. M, Paper 11 at 8-9. The Board did not consider it necessary to interpret this term in the Final Written Decision. *Id.* at Paper 43; *Id.* at Paper 43. *See also* Ex. O at ¶ 30.

C. "at least one chromosome region tested for being aneuploidy" (Claim 19)

Claim 19 contains the same recitation discussed immediately above except that it uses the term "at least one chromosome region tested for being aneuploidy" instead of "a first chromosome tested for being aneuploidy." The Board accorded both claim terms similar meaning, and found that the teachings of Dhallan I met this language of both claims 1 and 19. Ex. L, Paper 11, at 15-20; Ex. M, Paper 11 at 15-20.

D. "sequence reads corresponding to enriched and indexed fetal and maternal non-random polynucleotide sequences" (Claims 1 and 19)

The '430 specification describes "sequence reads" as information obtained by sequencing selectively enriched products from a sample, as exemplified below:

> In one aspect, a method for determining the presence or absence of fetal aneuploidy is provided comprising a) selectively enriching non-random polynucleotide sequences of genomic DNA from a cell-free DNA sample; b) sequencing said enriched polynucleotide sequences; c) enumerating sequence reads from said sequencing step; and d) determining the presence or absence of fetal aneuploidy based on said enumerating. Ex. A at 1:40-48.

As the only sequence reads taught in the '430 are the product of sequencing selectively enriched polynucleotides, the term "sequence reads" is interpreted to include the order of bases determined for enriched polynucleotides. Ex. O at ¶ 31. Importantly, the use of "sequence" in this particular term refers to information rather than a physical molecule. *Id*.

In the institution decision the Board agreed, holding that that the claimed "sequence reads" correspond to the "occurrence(s) of a selected non-random polynucleotide sequence."

> The claim language and Specification make clear that "sequence reads" result from DNA sequencing. Claim 1 requires "performing massively parallel sequencing" of the pooled libraries of enriched and indexed fetal and maternal non-random polynucleotide sequences "to produce sequence reads" corresponding to the enriched sequences and "enumerating sequence reads." See steps (d) and (e) of claim 1. As explained in step (f), the presence or

> absence of a fetal aneuploidy is determined using the "enumerated sequence reads" corresponding to the first chromosome tested for being aneuploidy and the reference chromosome. Likewise, the Specification explains that the claimed method comprises sequencing the enriched non-random polynucleotide sequences and enumerating the sequence reads from the sequencing step to determine the presence or absence of fetal aneuploidy. Ex. 1001, 1: 40-48. Figure 24 illustrates sequence reads mapped on chromosome 21, i.e., SEQ ID NOS: 99-132, and shows the occurrence(s) of selected sequences. *Id.* at 5:38-40.

Ex. L, Paper 11, at 9-10; Ex. M, Paper 11 at 9-10. For these reasons the Board interpreted "sequence reads" to correspond to the "occurrence(s) of a selected non-random polynucleotide sequence." Here again, the Board did not consider it necessary to interpret this term in the Final Written Decision. *Id.* at Paper 43; *Id.* at Paper 43.

The term "non-random polynucleotide sequence" is interpreted to include a nucleic acid molecule that has been selectively enriched, such as one that is amplified by PCR using primers that amplify a specific DNA region in a genome. Ex. O at ¶ 31. The use of "sequence" in this term refers to a physical molecule, as it describes a molecule resulting from a selective enrichment procedure, e.g., a physical copy of a non-random DNA region. *Id.*

E. "reference chromosome" (Claim 1) or "chromosome control region" (Claim 19)

The specification uses the terms "reference chromosome" and "chromosome control region" interchangeably. In the specification, the terms "reference chromosome" and "chromosome control region" are used to describe chromosomes that can serve as comparators to a chromosome being tested for aneuploidy. Ex. A at 2:10-11, 13:6-8, 13:62-63, 19:18-19, 20:58-89, Figs. 8B, 16 and 17. However, the Board noted that the only distinction the claim language makes between the "first chromosome" and the "reference chromosome" or "chromosome control region" is that they are different. The Board interpreted "reference chromosome" and

"chromosome control region" as "a chromosome that is different from the claimed first chromosome tested" and "a chromosome region that is different from the claimed one chromosome region tested", respectively. Ex. L, Paper 11 at 10-11; Ex. M, Paper 11 at 10-12. *See also* Ex. O at ¶ 32.

VI. STATEMENT POINTING OUT EACH SUBSTANTIAL NEW QUESTION OF PATENTABILITY FOR THE CHALLENGED CLAIMS

A. Subject Matter, Which if Taught by Prior Art Patents or Printed Publications, Raises a Significant New Question of Patentability for the Challenged Claims

During both *ex parte* prosecution and the *inter partes* reviews the Office found the claimed subject matter was patentable over certain prior art for essentially the same reason: **the primary references were deemed incompatible with the specific indexing (or multiplexing) methods taught in the secondary references**. *See* Sections IV.C and IV.D, above. The *ex parte* examiner relied upon a reference which taught the use of digital PCR to do so but ultimately agreed with Verinata that digital PCR was not compatible with indexing techniques described in the Illumina Brochure of Ex. G. *See* Ex. B at 32-33 and 58-63. In the *inter partes* reviews the Board observed that "Dr. Morton acknowledged on cross examination that Binladen's indexing (i.e., tagging) scheme could not be used with Dhallan II's restriction-digestible amplification primers." Ex. L, Paper 43 at 17; Ex. M, Paper 43 at 17. The Board expressly refused to consider Ariosa's arguments to the effect that i) other techniques in Dhallan II were readily combinable with Binladen's indexing technique, and ii) one skilled in the art could have readily achieved the claimed subject matter by performing the Dhallan II techniques on the commercially available Illumina Genome Analyzer with the Multiplexing Kit. *Id.* at 17-19, *Id.* at 17-19. The Board felt that these arguments were not presented in sufficient detail until Ariosa's reply and thus were belated and not entitled to consideration. *Id.*

Accordingly, the **claims were allowed or confirmed by the examiner and the Board because, in each proceeding, the primary reference was deemed incompatible with the specific indexing/multiplexing techniques disclosed in the secondary reference**.

As explained below and in the declaration of Dr. Rosenberg, the combination of Dhallan II with Craig and the Illumina Brochure demonstrates that the Dhallan II aneuploidy detection technique (disclosed in 2004) could be performed readily with one of the multiplexed massively parallel sequencing platforms that were made commercially available several years later, in 2008. Ex. O at ¶¶ 45-52 and 67-197. Consistent with this premise, during the IPRs Verinata did not contest that indexing/multiplexing was well-known as of the filing date. Ex. L at Paper 10 and Paper 20; Ex. M at Paper 10 and Paper 20. Verinata likewise did not contest that performing the Dhallan II method on the Illumina platform was well within the abilities of a person of ordinary skill, *e.g.,* a first-year post-doc in a molecular laboratory familiar with DNA detection methods. *Id.* Accordingly, the combination of Dhallan II with the Illumina platform described in Craig and the Illumina Brochure (i.e., fully *replacing* the detection technique of Dhallan II, including Dhallan II's use of restriction digestion, with the multiplexed detection of the Illumina Genome Analyzer and Multiplexed Kit) addresses the perceived deficiency of the previously asserted combinations (i.e., that the technique of the primary reference was incompatable with the indexing technique taught in the secondary reference).

The combination of Dhallan II, Parameswaran and Hamady similarly provides a primary reference (Dhallan II) which is plainly and readily combinable with the indexing technique taught in the secondary references (Parameswaran and Hamady), thereby providing the teaching that was believed to be missing from the previously applied prior art. Dr. Rosenberg explains that the Dhallan II method could have been readily performed on the Roche/454 platform

(instead of the Illumina platform) and that doing so would have had the same benefits of increased throughput decreased per-sample cost. Again, one skilled in the art would fully replace the detection technique of Dhallan II (including Dhallan II's use of restriction digestion) with multiplexed detection using the Roche/454 massively parallel sequencing system as taught in Parameswaran and Hamady. Ex. O at ¶¶ 47, 66. The combination of Dhallan II, Parameswaran and Hamady thus also independently addresses the perceived deficiency of the previously considered prior art and presents a substantial new question of patentability.

B. The Combination of Dhallan II, Craig and the Illumina Brochure Presents a Substantial New Question of Patentability for Claims 1-30 of the '430 Patent

U.S. Patent Pub. App. No. 2006/0121452 to Dhallan II ("Dhallan II") is attached hereto as Exhibit E. Dhallan II was filed on March 1, 2004 and issued on June 8, 2006, and is available as prior art under 35 U.S.C. § 102(b). As explained in Sections VII.A. and VII.B., below, and in the declaration of Dr. Rosenberg, Dhallan II teaches all limitations recited in the claims except a detection method which employs i) indexing or tagging samples from different patients so they can be processed simultaneously (i.e., multiplexing), and ii) using massively parallel sequencing to sequence the indexed samples. Ex. O at ¶ 35.

In the three years following Dhallan II's filing date, Illumina introduced a massively parallel sequencing platform and a "multiplexing kit" which tagged different patient samples with index labels and processed all of the samples simultaneously. Ex. O at ¶¶ 22, 39. One prior art reference which discusses that the Illumina Genome Analyzer (a massively parallel sequencing system) can be used for multiplexed processing is Craig. *See* Ex. F. Craig was published online on September 14, 2008, and is available as prior art under 35 U.S.C. § 102(b).[2]

[2] The online publication date is shown at http://www.nature.com/nmeth/journal/v5/n10/full/nmeth.1251.html, attached as Ex. Q.

Several months after Craig was published Illumina started offering a multiplexing kit for use with the Illumina Genome Analyzer, as shown in a prior art brochure entitled "Multiplexed Sequencing with the Illumina Genome Analyzer System," 2008 ("Illumina Brochure"). *See* Ex. G. The Illumina Brochure was publically available in 2008 and is available as prior art under 35 U.S.C. § 102(b).[3]

Craig's abstract explains that he and his co-authors "developed a generalized framework for multiplexed resequencing of targeted regions of the human genome on the Illumina Genome Analyzer using degenerate indexed DNA sequence barcodes ligated to fragmented DNA prior to sequencing. Using this [barcoding] method, the DNA of multiple HapMap individuals was simultaneously sequenced at several . . . regions." Ex. F at Abstract. The body of the Craig paper further describes how the authors used the Illumina Genome analyzer for the multiplexed or simultaneous processing of samples from different patients, each indexed with a unique bar-code label.

> In this manuscript we report an experimental and analytical approach for simultaneous sequencing of multiple individuals using DNA indexes on the Illumina Genome Analyzer (GA) . . . Simultaneous resequencing of large numbers of individuals for a targeted region is possible by bar-coding or indexing the reads from each individual with a short identifying oligonucleotide. Ex. F at 2.

About three months after the publication of Craig, Illumina commercially launched its multiplexing kits for use with the Illumina Genome Analyzer. The Illumina Brochure announces that "[t]o make multiplexed sequencing on the Genome Analyzer available to any laboratory, Illumina offers the Multiplexing Sample Preparation Oligonucleotide Kit and the Multiplexing Sequencing Primers and PhiX Control Kit." Ex. G at 1. The Illumina Brochure explains that

[3] The Illumina Brochure is ascribed a publication date of December 2, 2008 on the face of the '430 patent. *See* Ex. A at 1.

multiplexed sequencing is faster and cheaper than traditional massively parallel sequencing and produces accurate and reliable data:

> HIGHLIGHTS OF ILLUMINA MULTIPLEXED SEQUENCING
> • Fast, High-Throughput Strategy: Automated sequencing of 96 samples per flow cell
> • Cost-Effective Method: Multisample pooling improves productivity by reducing time and reagent use
> • High-Quality Data: Accurate maintenance of read length for unknown sequences
> • Simplified Analysis: Automated sample association with index using Pipeline Analysis software

Ex. G at 1, callout box. Illumina's multiplexing kit was able to "introduc[e] index sequences onto DNA fragments enables sequencing of 96 different samples on a single flow cell [which] greatly increases experimental scalability, while maintaining extremely low error rates and conserving read length." *Id.* at 2. More particularly, the kit provided 12 index oligos for pooling up to 12 samples per lane, or 96 samples per flow cell. *Id*.

Craig and the Illumina Brochure thus suggest that it was notoriously well known as of the earliest claimed priority date of the '430 patent that sequencing could be advantageously performed with a commercially available massively parallel sequencing platform and a commercially available multiplexing kit specifically designed for use with this platform. Dr. Rosenberg explains in his declaration that this was indeed the case and any person of ordinary skill in this art would have had no difficulty whatsoever performing the Dhallan II sequencing techniques on the Illumina platform with the Illumina multiplexing kit, and in fact, the use of this combined system would be easier, faster and more cost effective than the actual detection methods utilized by Dhallan II in the experiments of the examples. Ex. O at ¶ 52. Thus, the

Dhallan II methods could have been even more effectively and efficiently performed on the Illumina platform with the Illumina multiplexing kit in a multiplexed manner. *Id.* at ¶¶ 45-52.

Therefore, the combination of Dhallan II, Craig and Illlumina unquestionably discloses a technical teaching that was believed to be missing from the prior art, namely, a **method for detecting fetal aneuploidy by sequencing maternal and fetal extracellular DNA in a manner that was compatible with prior art multiplexed massively parallel sequencing techniques**. Not only would a reasonable Examiner consider the combination of Dhallan II, Craig and Illlumina important in deciding whether claims 1-30 are patentable, but would have rejected such claims of the '430 patent in view of this combination of references. *See* MPEP § 2242. Thus, the combination of Dhallan II, Craig and Illlumina presents a substantial new question of patentability for claims 1-30 of the '430 patent.

C. The Combination of Dhallan II, Parameswaran and Hamady Presents a Substantial New Question of Patentability for Claims 1-30 of the '430 Patent

A skilled artisan would also have known that Dhallan II's method for detecting fetal aneuploidy by sequencing maternal and fetal extracellular DNA could have been performed in a multiplexed manner on the Roche/454 massively parallel sequencing platform before the earliest claimed priority date of the '430 patents. The use of multiplexing with the Roche/454 platform is described in Parameswaran (Ex. H) and Hamady (Ex. I). Parameswaran was published online on October 11, 2007 and is available as prior art under 35 U.S.C. § 102(b).[4] Hamady was published online on February 10, 2008 and is available as prior art under 35 U.S.C. § 102(b).[5]

[4] The online publication date is shown at http://nar.oxfordjournals.org/content/35/19/e130.full, attached as Ex. S.
[5] The online publication date is shown at http://www.nature.com/nmeth/journal/v5/n3/full/nmeth.1184.html, attached as Ex. R.

Both Parameswaran and Hamady teach the use of the Roche/454 platform and barcode labelling to simultaneously process (i.e., multiplex) samples from numerous different patients. Parameswaran discloses that

> [i]n two pilot series of barcoded sequencing using the GS20 Sequencer (454/Roche), we found that over 99.8% of obtained sequences could be assigned to 25 independent, uniquely barcoded libraries based on the presence of either a perfect forward or a perfect reverse barcode. The false-discovery rate, as measured by the percentage of sequences with unexpected perfect pairings of unmatched forward and reverse barcodes, was estimated to be <0.005%. Ex. H at Abstract.

Hamady similarly discloses that

> [w]e have constructed error-correcting DNA barcodes that allow one run of a massively parallel pyrosequencer to process up to 1544 samples simultaneously. We have used these barcodes to process 16S ribosomal DNA sequences representing 286 microbial communities, correct 92% of sample assignment errors, and nearly double the known 16S rRNA sequences. In principle, our approach has myriad applications. . . . To test these barcodes, we determined the bacterial composition of 286 environmental samples by PCR amplifying, sequencing, and analyzing 681,688 16S rRNA gene sequences from a single sequencing run of the Genome Sequencer FLX (454 Life Sciences, Branford, CT.). Ex. I at Abstract, 2.

Dr. Rosenberg explains that any first year post-doctoral student in a molecular biology lab would have recognized that Dhallan II's aneuploidy detection techniques, which were disclosed in a 2004 patent filing, could have been even more effectively and efficiently performed on the newer Roche/454 platform in a multiplexed manner. Ex. O at ¶¶ 53-55. There would have been no difficulties in doing so and any optimization would have been well within the level of ordinary skill. *Id.* Accordingly, the Roche/454 system (with multiplexing) could have been predictably used to improve the Dhallan II method in the same manner it was used by Parameswaran and Hamady to improve upon the preexisting detection techniques taught in Dhallan II. *Id.*; *KSR Int'l Co. v. Teleflex Inc.,* 550 U.S. 398, 417 (2007) ("[I]f a technique has

been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond that person's skill.").

Therefore, the combination of Dhallan II, Parameswaran and Hamady provides a technical teaching which was believed to be missing from the applied prior art. In particular, **this combination provides a method for detecting fetal aneuploidy by sequencing maternal and fetal cell-free DNA which was fully and readily compatible with prior art multiplexed massively parallel sequencing techniques**. This teaching is not cumulative to the technological teachings discussed on the record; rather, both the Board and the *ex parte* examiner found that the grounds of unpatentability could not be sustained because of a perceived incompatibility of the base references' aneuploidy detection techniques and the secondary references' indexing techniques techniques. Ex. B at 32-33 and 58-63; Ex. L, Paper 43 at 16-19; Ex. M, Paper 43 at 16-19. Accordingly, the combination of Dhallan II, Parameswaran and Hamady, as discussed and applied with particularity below, presents a new, non-cumulative technical teaching that demonstrates the unpatentability of claims 1-30 of the '430 patent.

D. The Combination Dhallan I and Binladen Presents a Substantial New Question of Patentability for Claims 1-30 of the '430 Patent

As discussed above, Dhallan I and Binladen are prior art against the '430 patent under 35 U.S.C. § 102(b). The combination of Dhallan I and Binladen was previously set forth as a ground of unpatentability for the claims of the '430 patent in IPR2014-00276 and IPR2014-00277. The Board exercised its discretion to deny consideration of the combination of Dhallan I and Binladen on the basis that it was redundant to another ground upon which trial was instituted. Ex. L, Paper 11 at 20; Ex. M, Paper 11 at 20-21.

However, the ground upon which trial was instituted was based on a different primary reference and involved a **fundamentally different combined system**. Trial was instituted on the ground that the claims were rendered obvious by U.S. Patent Pub. App. No. 2008/0090239 to Shoemaker, et al. ("Shoemaker") taken in view of Dhallan I and Binladen. The petition argued that

> a skilled artisan would [] have readily understood that Shoemaker's methods for determining the presence of fetal abnormalities b of cell-free DNA described in Dhallan and the multiplexed detection techniques taught in Binladen.

Ex. L, Paper 1 at 38; Ex. M, Paper 1 at 38. This ground of obviousness relied upon the sequencing techniques of Shoemaker and relied upon Dhallan I only for the use of extracellular DNA. *Id.* Binladen was relied upon only for its teaching concerning the multiplexing processing of multiple patients' samples. *Id.*

The **combination of Dhallan I and Binladen presented herein is fundamentally different that the combination of Shoemaker, Dhallan I and Binladen addressed during the IPR**. The combination of Dhallan I and Binladen presented herein relies on the sequencing techniques of Dhallan I being replaced by those described in Binladen (*i.e.*, multiplexed massively parallel sequencing). Ex. O at ¶¶ 56-61; *see also* Section VII.C, below. In contrast, the ground considered in the IPR involved Shoemaker's sequencing techniques being modified only to include the use of extracellular DNA (from Dhallan I) and multiplexing samples from multiple patients (from Binladen). Ex. L, Paper 1 at 38; Ex. M, Paper 1 at 38. Moreover, the combination of Dhallan I and Binladen provides a technical teaching which was believed to be missing from the applied prior art.

Dr. Rosenberg explains that anyone skilled in the art as of the earliest claimed priority date in 2010 would have understood that the sequencing techniques described in 2003 in

Dhallan I could have been advantageously replaced with the much faster and efficient multiplexed massively parallel sequencing techniques described in Binladen. Ex. O at ¶¶ 56-61. Accordingly, **this combination provides a method for detecting fetal aneuploidy by sequencing maternal and fetal cell-free DNA which was fully and readily compatible with prior art multiplexed massively parallel sequencing techniques**.

This teaching is not cumulative to the technological teachings discussed on the record because the Board and the *ex parte* examiner found that the grounds of unpatentability under consideration could not be sustained because of a perceived incompatibility of the base references' aneuploidy detection technique and the secondary references' multiplexed massively parallel sequencing techniques. Ex. B at 32-33 and 58-63; Ex. L, Paper 43 at 16-19; Ex. M, Paper 43 at 16-19. The combination of Dhallan I and Binladen, as discussed and applied with particularity below, addressed this perceived deficiency and presents a new, non-cumulative technical teaching that demonstrates the unpatentability of claims 1-30 of the '430 patent.

The PTAB's Final Written Decision commented on the compatibility of Binladen's multiplexing technique with the exemplified sequencing method of Dhallan but did not make any findings as to whether Dhallan's sequencing method could be replaced with multiplexed massively parallel sequencing, as is proposed in the grounds presented herein. At pages 12-15 of the Final Written Decision the Board discusses Patent Owner's contentions that Binladen's tags could not be incorporated in the methods described in Dhallan because they would be "incompatible with the restriction digestible primers critical to the process of Dhallan['s] [examples]." Ex. L, Paper 43; Ex. M, Paper 43. However, the Board expressly refused to consider the broader question of whether the Dhallan's sequencing technique could simply be

replaced with the commercially available multiplexed massively parallel sequencing techniques. *Id.* at16-19; *Id.* at 16-19.

VII. DETAILED EXPLANATION OF THE PERTINENCE AND MANNER OF APPLYING THE PRIOR ART REFERENCES TO EVERY CLAIM FOR WHICH REEXAMINATION IS REQUESTED

In accordance with 37 C.F.R. § 1.510(b)(2), Requester provides the following detailed explanation of the pertinency and manner of applying the prior art to claims 1-30 of the '430 patent, for which reexamination is requested.

A. Dhallan II in View of Craig and the Illumina Brochure Renders Obvious Claims 1-30 of the '430 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

As discussed above, Dhallan II, Craig and the Illumina Brochure are prior art against the '430 patent under 35 U.S.C. § 102(b). As shown by the detailed claim-by-claim and limitation-by-limitation analyses below, Dhallan II in view of Craig and the Illumina Brochure discloses each of the limitations set forth in claims 1-30 of the '430 patent. Therefore, claims 1-30 are unpatentable under 35 U.S.C. § 103(a) as being obvious over Dhallan II in view of Craig and the Illumina Brochure.

Regarding Independent Claim 1

As explained by Dr. Rosenberg, Dhallan II was filed in 2004 and teaches all limitations recited in the claims except the detection method which employs i) indexing or tagging samples from different patients so they can be processed simultaneously (i.e., multiplexing), and ii) using massively parallel sequencing to sequence the indexed samples. Ex. O at ¶ 35. Significantly, Verinata did not contest this premise during the *inter partes* reviews. Ex. L at Paper 10 and Paper 20; Ex. M at Paper 10 and Paper 20 (discussing Dhallan I, of which Dhallan II is a continuation-in-part). Both of the missing limitations were present in massively parallel

sequencing systems which became commercially after Dhallan II was filed but before the earliest claimed priority date. Ex. O at ¶ 23. For instance, the Illumina Genome Analyzer was available in 2008 with a Multiplexing Kit which was advertised as being faster and more cost-effective while maintaining data quality. *See* Exs. F and G. Dr. Rosenberg explains that any first year post-doctoral student in a molecular biology laboratory familiar with DNA detection methods would have considered it routine (and quite advantageous) to perform the method taught by Dhallan II in 2004 with the later-developed Illumina Genome Analyzer and Multiplexing Kit. Ex. O at ¶¶ 62-66. The claims of the '430 patent are thus rendered obvious by Dhallan II in view of Craig (describing how the Illumina Genome Analyzer can be used for multiplexed processing) and further in view of the Illumina Multiplexing Kit Brochure (which Illumina released several months after Craig).

More particularly, Dr. Rosenberg explains that it would have been obvious to combine Dhallan II, Craig and the Illumina Brochure in the following manner. Ex. O at ¶¶ 45-52. Dhallan II discloses a fetal aneuploidy determination method which has the following steps: 1) obtaining blood samples from multiple pregnant women that contain cell-free DNA; 2) isolating cell-free DNA samples from the plurality of blood samples; 3) enriching at least 100 nonrandom polynucleotides (i.e., loci of interest) from a first chromosome tested for being aneuploid and at least 100 different non-random polynucleotide sequences non-random polynucleotides from a reference chromosome in each cell-free DNA sample by amplification to create a library (*i.e.*, a preparation) that contains representative PCR products having the sequence of the non-random polynucleotides; 4) detecting the non-random polynucleotides from the first chromosome tested for being aneuploid and the reference chromosome of each library for each sample; 5) enumerating the non-random polynucleotides from the first chromosome or chromosome region

tested for being aneuploid and the reference chromosome or chromosome control region of each library from each sample; and 6) determining the presence or absence of a fetal aneuploidy for each sample by comparing the enumerated non-random polynucleotides from the first chromosome or chromosome region tested for being aneuploid and the reference chromosome or chromosome control region from each sample library. *Id.* at ¶ 45. Claim 1 of the '430 patent follows the same steps except it employs a different detection technique for step (4). *Id.* at ¶ 47. As described in detail below, the detection technique of step (4) of the '430 patent was well known at the time of the filing date of the '430 patent, including the use of multiplexing on the Illumina Genome Analyzer as disclosed in Craig (Ex. F) and the Illumina Brochure (Ex. G). *Id*. at ¶ 49. Both of these references teach detection of amplified polynucleotides from individual samples using indexing and massively parallel sequencing. It would have been well within the ordinary skill of the art to substitute the detection technique Dhallan II with the detection technique of Craig and the Illumina Brochure, and a person of ordinary skill would have had a reasonable expectation of success in doing so. *Id*. at ¶¶ 49-52.

> **Claim 1 [preamble]: A method for determining a presence or absence of a fetal aneuploidy in a fetus for each of a plurality of maternal blood samples obtained from a plurality of different pregnant women, said maternal blood samples comprising fetal and maternal cell-free genomic DNA, said method comprising:**

Dhallan II discloses a method for determining a presence or absence of a fetal aneuploidy in a fetus by taking blood samples from pregnant women which contain cellular and non-cellular DNA. Dhallan II provides

> a method useful for detection of genetic disorders. The method comprises determining the sequence of alleles of a locus of interest, and quantitating a ratio for the alleles at the locus of interest, wherein the ratio indicates the **presence or absence of a chromosomal abnormality**. The present invention also provides a non-invasive method for the detection of **chromosomal abnormalities *in a fetus***. The invention is especially useful as a non-invasive method for determining the sequence of fetal DNA.

> The invention further provides methods of isolation of free DNA from a sample. Ex. E at Abstract.
>
> The present invention is directed to a method for the detection of genetic disorders including chromosomal abnormalities and mutations. The present invention provides a rapid, non-invasive method for determining the sequence of DNA from a fetus. The method is especially useful for detection of chromosomal abnormalities in a fetus including translocations, transversions, monosomies, trisomies, and other **aneuplodies [sic]**, deletions, additions, amplifications, translocations and rearrangements. *Id.* at ¶ [0003].
>
> In one embodiment, the sample containing the nucleic acid is obtained from a pregnant female. In a preferred embodiment, the sample is obtained from a pregnant human female. In a preferred embodiment, the **sample is blood obtained from a pregnant** female. *Id.* at ¶ [0076].
>
> The present invention provides a method for detecting genetic disorders, including but not limited to mutations, insertions, deletions, and chromosomal abnormalities, and is especially useful for the detection of **genetic disorders of a fetus**. The method is especially useful for detection of a translocation, addition, amplification, transversion, inversion, **aneuploidy**, polyploidy, monosomy, trisomy, trisomy 21, trisomy 13, trisomy 14, trisomy 15, trisomy 16, trisomy 18, trisomy 22, triploidy, tetraploidy, and sex chromosome abnormalities including but not limited to XO, XXY, XYY, and XXX. *Id.* at ¶ [0133].
>
> The blood sample is centrifuged to separate the plasma from the maternal cells. The plasma and maternal cell fractions are transferred to separate tubes and re-centrifuged. The **plasma fraction contains cell-free fetal DNA and maternal DNA**. *Id.* at ¶ [0197].

See also Ex. O at ¶¶ 68-74, citing also Ex. E at ¶¶ [0030], [0032], [0132], [0182], [0190], [0196] and [0201].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Claim 1[a]: (a) obtaining a fetal and maternal cell-free genomic DNA sample from each of the plurality of maternal blood samples;

This claim limitation is largely cumulative to the preamble and is likewise disclosed by Dhallan II:

> In one embodiment, the template DNA is **fetal DNA**. Fetal DNA can be obtained from sources including but not limited to **maternal blood**, maternal serum, maternal plasma, fetal cells, umbilical cord blood, chorionic villi, amniotic fluid, urine, saliva, cells or tissues. Ex. E at ¶ [0190].
>
> In another embodiment, the template DNA contains both maternal DNA and fetal DNA. In a preferred embodiment, **template DNA is obtained from blood of a pregnant female**. *Id.* at ¶ [0196].
>
> The blood sample is centrifuged to separate the plasma from the maternal cells. The plasma and maternal cell fractions are transferred to separate tubes and re-centrifuged. **The plasma fraction contains cell-free fetal DNA and maternal DNA**. *Id.* at ¶ [0197].
>
> In another embodiment, the template **DNA is obtained from the plasma or serum of the blood of the pregnant female**. The percentage of fetal DNA in maternal plasma is between 0.39-11.9% (*Pertl, and Bianchi, Obstetrics and Gynecology* 98: 483-490 (2001)). *Id.* at ¶ [0201].
>
> In accordance with an IRB approved study, blood samples were collected from pregnant women after informed consent had been granted. **Blood samples were received from 27 different clinical sites operating in 16 different states located throughout the U.S.** Blood samples were collected from both women carrying male and female fetuses, however, here, we report results obtained from woman carrying male fetuses, as the Y chromosome is the accepted marker when quantitating percentages of fetal DNA. *Id.* at ¶ [1210].

See also Ex. E at ¶¶ [0030] and [0042]. *See also* Ex. O at ¶¶ 75-76.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Claim 1[b]: selectively enriching a plurality of non-random polynucleotide sequences of each fetal and maternal cell-free genomic DNA sample of (a) to

> **generate a library derived from each fetal and maternal cell-free genomic DNA sample of enriched and indexed fetal and maternal non-random polynucleotide sequences, wherein each library of enriched and indexed fetal and maternal non-random polynucleotide sequences includes an indexing nucleotide sequence which identifies a maternal blood sample of the plurality of maternal blood samples, wherein said plurality of non-random polynucleotide sequences comprises at least 100 different non-random polynucleotide sequences selected from a first chromosome tested for being aneuploid and at least 100 different non-random polynucleotide sequences selected from a reference chromosome, wherein the first chromosome tested for being aneuploid and the reference chromosome are different, and wherein each of said plurality of non-random polynucleotide sequences is from 10 to 1000 nucleotide bases in length**,

Dr. Rosenberg explains Dhallan II teaches every aspect of this claim element except for the limitations underlined above, which require that the samples be tagged or indexed, as was conventionally done in off-the-shelf multiplexed assays at the time of filing of the '430 patent. Ex. O at ¶¶ 22-23, 45-52, 66. That Dhallan II teaches these elements is confirmed by Verinata's failure to contest the same during the *inter partes* reviews. Ex. L at Paper 10 and Paper 20, Ex. M at Paper 10 and Paper 20. Dhallan II teaches that

> [t]he method can be used for **determining sequences of multiple loci of interest concurrently**. The template DNA can comprise multiple loci from a single chromosome. The template DNA can comprise multiple loci from different chromosomes. The loci of interest on template DNA can be amplified in one reaction. Alternatively, each of the loci of interest on template DNA can be amplified in a separate reaction. Ex. E at ¶ [0060].
>
> The original template DNA was amplified using 12 base primers that annealed to various regions on chromosome 13**. One hundred different primer sets were used to amplify regions throughout chromosome 13**. For each of the nine SNPs, a primer that annealed approximately 130 bases from the locus of interest and 130 bases downstream of the locus of interest were used. This amplification reaction, which contained a total of 100 different primer sets, was used to amplify the regions containing the loci of interest. *Id.* at ¶ [0127].
>
> By a "locus of interest" is intended a selected region of nucleic acid that is within a larger region of nucleic acid. A locus of interest can include but is not limited to **1-100**, 1-50, 1-20, or 1-10

nucleotides, preferably 1-6, 1-5, 1-4, 1-3, 1-2, or 1 nucleotide(s). *Id.* at ¶ [0177].

The template DNA [maternal and fetal DNA] can be amplified using any suitable method known in the art including but not limited to PCR (polymerase chain reaction), 3SR (self-sustained sequence reaction), LCR (ligase chain reaction), RACE-PCR (rapid amplification of cDNA ends), PLCR (a combination of polymerase chain reaction and ligase chain reaction), Q-beta phage amplification (Shah et al., J. Medical Micro. 33: 1435-41 (1995)), SDA (strand displacement amplification), SOE-PCR (splice overlap extension PCR), and the like. *Id.* at ¶ [0283].

The multiple primer sets will amplify the loci of interest, such that a minimal amount of template DNA is not limiting for the number of loci that can be detected. For example, if template DNA is isolated from a single cell or the **template DNA is obtained from a pregnant female, which comprises both maternal template DNA and fetal template DNA, low concentrations of each primer set can be used in a first amplification reaction to amplify the loci of interest**. *Id.* at ¶ [0288].

For example, **100 SNPs can be analyzed on chromosome 1**. Of these 100 SNPs, assume 50 are heterozygous. The ratio of the alleles at heterozygous SNPs on chromosome 1 can be summed, and should give a ratio of approximately 50:50. **Likewise, of 100 SNPs analyzed on chromosome 21, assume 50 are heterozygous**. The ratio of alleles at heterozygous SNPs on chromosome 21 is summed. With a normal number of chromosomes, the ratio should be approximately 50:50, and thus there should be no difference between the ratio obtained from chromosome 1 and 21. *Id.* at ¶ [0379].

For example**, if 100 maternal loci of interest on chromosome 21 and 100 maternal loci of interest on chromosome 1 are analyzed**, one would predict approximately 50 loci of interest on each chromosome to be homozygous and 50 to be heterozygous. The 50 homozygous loci of interest, or the 50 heterozygous loci of interest or the 50 homozygous and 50 heterozygous loci of interest, or any combination of the homozygous and heterozygous loci of interest on each chromosome can be analyzed using the template DNA from the sample from the pregnant female. *Id.* at ¶ [0384].

In one embodiment, one of the chromosomes used in the comparison can be chromosome 13, 15, 16; 18, 21, 22, X or Y. **In a preferred embodiment, the ratios on chromosomes 13, 18,**

> **and 21 are compared**. *Id.* at ¶ [0395].

See Ex. O at ¶¶ 45-52, and Ex. E at ¶¶ [0059], [0064], [0110], [0114], [0127], [0385], [0402], [0414], [0427] and [1023]. Dr. Rosenberg explains in this declaration that because the enriched and indexed products are created by a selective amplification process, the products from the loci of interest would be **non-random**. Ex. O at ¶ 78.

Dhallan II does not teach the claim recitation that the libraries include "indexed" fetal and maternal non-random polynucleotide sequences or the method includes "an indexing nucleotide sequence which identifies a maternal blood sample of the plurality of maternal blood samples." As Dr. Rosenberg explains, these limitations would be met if the 2004 Dhallan II method were simply performed on the later-developed and much more effective and cost-effective multiplexed massively parallel sequencing systems. Ex. O at ¶¶ 45-52. One such system was sold by Illumina in 2008. *See* Exs. F and G.

Craig, Ex. F, describes that the Illumina Genome Analyzer is useful for multiplexed massively parallel sequencing. Craig discloses the creation of indexed libraries for different patients using the massively parallel sequencing method of the Illumina Genome Analyzer:

> **We developed a generalized framework for multiplexed resequencing of targeted regions of the human genome on the Illumina Genome Analyzer using degenerate indexed DNA sequence barcodes ligated to fragmented DNA prior to sequencing**. Using this [barcoding] method, the DNA of multiple HapMap individuals was simultaneously sequenced at several ENCODE (ENCyclopedia of DNA Elements) regions. Ex. F at Abstract.
>
> Simultaneous **resequencing of large numbers of individuals for a targeted region is possible by bar-coding** or indexing the reads from each individual with a short identifying oligonucleotide . . . In this manuscript we report an experimental and analytical approach for **simultaneous sequencing of multiple individuals using DNA indexes** on the Illumina Genome Analyzer (GA) . . . .We amplified multiple 5kb regions . . . by long-range PCR, for 46 individuals

> genotyped by the ENCODE projects . . . Following ligation, samples from all individuals were pooled into a single sample **(referred to as an indexed library)**, purified, enriched by PCR, and sequenced on the Illumina GA on a single lane of an 8 lane flow-cell. *Id.* at 2.
>
> In this report, we developed per-individual indexing of pooled **PCR amplicons** to carry out targeted sequencing. . . . **Two primary amplicon libraries** (Library A and B, specific targeted regions listed in supplementary table 2) **were constructed** from individually amplified 5 kb regions using long-range PCR. *Id.* at 6.

Several months after the publication of Craig, Illumina itself introduced multiplexing kits which essentially performed the same multiplexing process described in Craig. The Illumina Brochure, Ex. G, announces the availability of Illumina's Multiplexing Sample Preparation Oligonucleotide Kit and the Multiplexing Sequencing Primers and PhiX Control Kit. Ex. G at 1. The Brochure also describes how the kits tag or index libraries with unique identifiers so that hundreds of patients' samples can be processed simultaneously:

> Harnessing this sequencing power in a **multiplex** fashion **increases experimental throughput while reducing time and cost. . . . To make multiplexed sequencing on the Genome Analyzer available to any laboratory**, Illumina offers the Multiplexing Sample Preparation Oligonucleotide Kit and the Multiplexing Sequencing Primers and PhiX Control Kit. . . . In the multiplexed sequencing method, **DNA libraries are "tagged" with a unique identifier, or index,** during sample preparation. Multiple samples are then **pooled** into a single lane on a flow cell and sequenced together in one Genome Analyzer run. An automated three-read sequencing strategy (Figure 1) identifies each uniquely tagged sample for individual downstream analysis. Using this approach, sample identification is highly accurate. Ex. G at 1.
>
> Index sequences are added to adapter-modified DNA fragments during the PCR enrichment step. *Id.* at 2.
>
> Using Illumina's Pipeline Analysis software, **each index is associated with a particular read-pair, identifying samples for analysis**. *Id.* at 3.

Performing the Dhallan II method on the Illumina Genome Analyzer (described in Craig) with the optional Multiplexing Kit (described in the Illumina Brochure) would have involved nothing more than the application of routine skill. Indeed, Dr. Rosenberg explains that any first-year post-doctoral student working in a molecular biology laboratory would have been able to carry out the Dhallan II method on the Illumina system as of the filing date in 2010. Ex. O at ¶¶ 62-64. Any index optimization which may have been required for efficiency in detection of fetal aneuploidy was well within the skill of such a person. *Id.* at ¶¶ 51, 84.

The fact that a skilled artisan would have been able to carry out the Dhallan II aneuploidy method on the Illumina system is evidenced further by U.S. Patent Pub. App. No. 2008/0090239 to Shoemaker, et al. ("Shoemaker") (Ex. J). Shoemaker, filed in 2007, describes a method for aneuploidy detection performed on the Illumina Genome Analyzer in a multiplexed fashion. Ex. J at ¶ [0157] (describing use of the Illumina Genome Analyzer), *see also* ¶¶ [0108], [0114], [0119], [0122], [0127], [0138], [0140], [0157] and [0159] (describing the use of the technique to detect aneuploidy in maternal and fetal DNA). Shoemaker exemplifies that which is apparent from Dhallan II, Craig and the Illumina Brochure – that any skilled artisan would have been motivated to and would have had no difficulty in achieving aneuploidy detection on a commercially available multiplexed massively parallel sequencing platform. Ex. J at Example 4 (¶¶ [0225] - [0236]).

Accordingly, the Illumina Genome Analyzer and multiplexing kits could have been predictably used to improve the Dhallan II method in the same manner it was used to improve techniques for targeting genomic sub-regions or studying small genomes – the specific applications noted in the Illumina Brochure. Ex. G at 1-2. Because doing so would achieve the claimed subject matter, it is obvious and unpatentable. *KSR,* 550 U.S. at 417 ("[I]f a technique

has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond that person's skill.").

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Claim 1[c]: pooling the libraries generated in (b) to produce a pool of enriched and <u>indexed</u> fetal and maternal non-random polynucleotide sequences;

Dr. Rosenberg explains that Dhallan II teaches all aspects of this claim except for the limitation of indexing the libraries, a fact which Verinata did not contest during the *inter partes* review proceedings. Ex. L at Paper 10 and Paper 20; Ex. M at Paper 10 and Paper 20 (discussing Dhallan I, of which Dhallan II is a continuation-in-part). In particular, Dhallan II teaches that

> Alternatively, to avoid competition for nucleotides and to minimize primer dimers and difficulties with annealing temperatures for primers, each locus of interest or small groups of loci of interest can be amplified in separate reaction tubes or wells, **and the products later pooled if desired**. Ex. E at ¶ [0279].

As discussed above in connection with claim 1(b), the Illumina Genome Analyzer and multiplexing kits permitted the sequencing of multiple patient samples simultaneously by tagging or indexing them so that they can be pooled and processed simultaneously. Craig discloses that

> Following ligation, **samples from all individuals were pooled into a single sample (referred to as an indexed library)**, purified, enriched by PCR, and sequenced on the Illumina GA on a single lane of an 8 lane flow-cell. Ex. F at 2.

The supplementary material referred to on page 8 of Craig further provides that:

> A unique indexed-adapter sequence was ligated to each HapMap individuals' adenylated amplicon pool. . . . **Ligated amplicons were then pooled for all individuals** to be sequenced in the same flow lane. Ex. N at 8.

See also Ex. N at Supplementary Figure 1. The Illumina Brochure similarly explains that

> [i]n the multiplexed sequencing method, **DNA libraries are "tagged" with a unique identifier, or index**, during sample preparation. **Multiple samples are then pooled** into a single lane on a flow cell and sequenced together in one Genome Analyzer run. An automated three-read sequencing strategy (Figure 1) identifies each uniquely tagged sample for individual downstream analysis. Ex. G at 1.

As also discussed above and in the declaration of Dr. Rosenberg, any skilled artisan would have readily understood that the Dhallan II aneuploidy detection method, which dates from 2004, could have been more performed with greater throughput and reduced cost on the Illumina Genome Analyzer with the multiplexing kits. Ex. O at ¶¶ 45-52. Because the Illumina system had been used to improve other techniques in the same way and a person of ordinary skill in the art would have recognized that it could be readily applied to the Dhallan II method, the technique is obvious. *KSR,* 550 U.S. at 417.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

> **Claim 1[d]: performing massively parallel sequencing of the pool of enriched and indexed fetal and maternal non-random polynucleotide sequences of (c) to produce sequence reads corresponding to enriched and indexed fetal and maternal non-random polynucleotide sequences of each of the at least 100 different non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and sequence reads corresponding to enriched and indexed fetal and maternal non-random polynucleotide sequences of each of the at least 100 different non-random polynucleotide sequences selected from the reference chromosome;**

As Dr. Rosenberg explains in his declaration, Dhallan II teaches all aspects of claim 1(d) except the underlined limitations, namely, that the sequencing be massively parallel and that the libraries be tagged or indexed. Ex. O at ¶ 35. Again, Verinata did not contest this premise during the *inter partes* review proceedings. Ex. L at Paper 10 and Paper 20; Ex. M at Paper 10 and Paper 20 (discussing Dhallan I, of which Dhallan II is a continuation-in-part).

Dhallan II teaches performing sequencing to produce sequence reads corresponding to the sequences from the chromosome suspected of being aneuploidy and the sequences from the reference chromosome. Ex. O at ¶¶ 31, 35, 37, 45 and 96. More particularly, Dhallan II teaches that the loci of interest may be sequenced using "[a]ny method that provides information on the sequence of a nucleic acid," and the sequence counts may be compared between the suspect chromosome and a reference chromosome:

> Any method that **provides information on the sequence** of a nucleic acid can be used including but not limited to allele specific PCR, PCR, gel electrophoresis, ELISA, mass spectrometry, MALDI-TOF mass spectrometry hybridization, primer extension, fluorescence detection, fluorescence resonance energy transfer (FRET), fluorescence polarization, **DNA sequencing**, Sanger dideoxy sequencing, DNA sequencing gels, . . . Ex. E at ¶ [0228].
>
> In another embodiment, **determining the sequence** of alleles of a locus of interest comprises a method including but not limited to allele specific PCR, gel electrophoresis, ELISA, mass spectrometry, hybridization, primer extension, fluorescence polarization, fluorescence detection, fluorescence resonance energy transfer (FRET), **sequencing**, DNA microarray, SNP-IT, GeneChips, HuSNP, BeadArray, TaqMan assay, Invader assay, MassExtend, MassCleave.TM. (hMC) method, southern blot, slot blot, dot blot, and MALDI-TOF mass spectrometry. *Id.* at ¶ [0046].
>
> In one embodiment, the **determination of the sequence** of the locus of interest comprises detecting the incorporated nucleotide. In one embodiment, the detection is by a method selected from the group consisting of gel electrophoresis, capillary electrophoresis, microchannel electrophoresis, polyacrylamide gel electrophoresis, fluorescence detection, fluorescence polarization, **DNA sequencing**, Sanger dideoxy **sequencing** . . . *Id.* at ¶ [0058].
>
> For example**, if 100 maternal loci of interest on chromosome 21 and 100 maternal loci of interest on chromosome 1 are analyzed**, one would predict approximately 50 loci of interest on each chromosome to be homozygous and 50 to be heterozygous. The 50 homozygous loci of interest, or the 50 heterozygous loci of interest or the 50 homozygous and 50 heterozygous loci of interest, or any combination of the homozygous and heterozygous loci of

> interest on each chromosome can be analyzed using the template DNA from the sample from the pregnant female. *Id.* at ¶ [0384].
>
> In one embodiment, one of the chromosomes used in the **comparison can be chromosome** 13, 15, 16; 18, 21, 22, X or Y. **In a preferred embodiment, the ratios on chromosomes 13, 18, and 21 are compared**. *Id.* at ¶ [0395].

While Dhallan II does not teach that the sequencing is massively parallel or the sequences are indexed, those aspects are taught by Craig and the Illumina Brochure. After Dhallan II filed his application in 2004, Illumina introduced its Genome Analyzer which helped massively parallel sequencing achieve widespread adoption by the earliest claimed priority date of the '430 patent in 2010. Ex. O at ¶ 20. As discussed above, Craig explains that the Illumina Genome Analyzer is useful for multiplexed massively parallel sequencing. The most relevant portions of Craig are reproduced below for convenience:

> **We developed a generalized framework for multiplexed resequencing of targeted regions of the human genome on the Illumina Genome Analyzer using degenerate indexed DNA sequence barcodes ligated to fragmented DNA prior to sequencing**. Using this [barcoding] method, the DNA of multiple HapMap individuals was simultaneously sequenced at several ENCODE (ENCyclopedia of DNA Elements) regions. Ex. F at Abstract.
>
> Simultaneous **resequencing of large numbers of individuals for a targeted region is possible by bar-coding** or indexing the reads from each individual with a short identifying oligonucleotide. . . .In this manuscript we report an experimental and analytical approach for **simultaneous sequencing of multiple individuals using DNA indexes** on the Illumina Genome Analyzer (GA) . . . .We amplified multiple 5kb regions … by long-range PCR, for 46 individuals genotyped by the ENCODE projects. . . . Following ligation, samples from all individuals were pooled into a single sample **(referred to as an indexed library)**, purified, enriched by PCR, and sequenced on the Illumina GA on a single lane of an 8 lane flow-cell. *Id.* at 2.
>
> In this report, we developed per-individual indexing of pooled **PCR amplicons** to carry out targeted sequencing. . . . **Two**

> **primary amplicon libraries** (Library A and B, specific targeted regions listed in supplementary table 2) **were constructed** from individually amplified 5 kb regions using long-range PCR. *Id.* at 6.

Shortly after the publication of Craig, Illumina announced the commercial availability of its Multiplexing Sample Preparation Oligonucleotide Kit and the Multiplexing Sequencing Primers and PhiX Control Kit. Ex. G at 1. As noted above, the Illumina Brochure explains how the kits are used to tag or index libraries with unique identifiers so that hundreds of patients' samples can be processed simultaneously:

> Harnessing this sequencing power in a **multiplex** fashion **increases experimental throughput while reducing time and cost. . . . To make multiplexed sequencing on the Genome Analyzer available to any laboratory**, Illumina offers the Multiplexing Sample Preparation Oligonucleotide Kit and the Multiplexing Sequencing Primers and PhiX Control Kit. . . . In the multiplexed sequencing method, **DNA libraries are "tagged" with a unique identifier, or index,** during sample preparation. Multiple samples are then **pooled** into a single lane on a flow cell and sequenced together in one Genome Analyzer run. An automated three-read sequencing strategy (Figure 1) identifies each uniquely tagged sample for individual downstream analysis. Using this approach, sample identification is highly accurate. Ex. G at 1.
>
> Index sequences are added to adapter-modified DNA fragments during the PCR enrichment step. *Id.* at 2.
>
> Using Illumina's Pipeline Analysis software, **each index is associated with a particular read-pair, identifying samples for analysis**. *Id.* at 3.

The Rosenberg declaration explains that performing the Dhallan II method on the Illumina Genome Analyzer with the optional Multiplexing Kit (or using the multiplexing method taught in Craig) would have involved nothing more than the application of routine skill. Ex. O at ¶¶ 49-50.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

> **Claim 1[e]: based on the indexing nucleotide sequence, for each of the plurality of maternal blood samples, <u>enumerating</u> sequence reads corresponding to enriched and indexed fetal and maternal non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and sequence reads corresponding to enriched and indexed fetal and maternal non-random polynucleotide sequences selected from the reference chromosome; and.**

This claim element recites the step of enumerating the sequence reads from the chromosome being tested and the reference chromosome. Dhallan II teaches this limitation.

> In another embodiment, **the ratio of alleles at a heterozygous locus of interest on a chromosome is compared to the ratio of alleles at a heterozygous locus of interest on a different chromosome**. There is no limitation as to the chromosomes that can be compared. The ratio for the alleles at a heterozygous locus of interest on any chromosome can be compared to the ratio for the alleles at a heterozygous locus of interest on any other chromosome. In a preferred embodiment, the ratio of alleles at multiple heterozygous loci of interest on a chromosome are summed and compared to the ratio of alleles at multiple heterozygous loci of interest on a different chromosome. Ex. E at ¶ [0061].
>
> The **ratio for the alleles at a heterozygous locus of interest on any chromosome can be compared to the ratio for the alleles at a heterozygous locus of interest on any other chromosome**. *Id.* at ¶ [0047].
>
> In another embodiment, **the ratio of alleles at a heterozygous locus of interest on a chromosome is compared to the ratio of alleles at a heterozygous locus of interest on two, three, four or more than four chromosomes**. In another embodiment, the ratio of alleles at multiple loci of interest on a chromosome is compared to the ratio of alleles at multiple loci of interest on two, three, four, or more than four chromosomes. *Id.* at ¶ [0062].

See also Ex. E at ¶ [0063]; Ex. O at ¶¶ 35, 46, 99. Verinata did not contest during the *inter partes* review proceedings that Dhallan II taught this limitation. Ex. L at Paper 10 and Paper 20;

Ex. M at Paper 10 and Paper 20 (discussing Dhallan I, of which Dhallan II is a continuation-in-part).

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Claim 1[f]: for each of the plurality of maternal blood samples, determining the presence or absence of a fetal aneuploidy comprising using a number of enumerated sequence reads corresponding to the first chromosome and a number of enumerated sequence reads corresponding to the reference chromosome of (e).

Dhallan II discloses the step of determining whether the sample contains a fetal aneuploidy by using the enumerated or tallied sequence reads. Ex. O at ¶¶ 35, 46, 89, 99. Here again, Verinata did not contest that Dhallan II discloses this element during the *inter partes* review proceedings. Ex. L at Paper 10 and Paper 20; Ex. M at Paper 10 and Paper 20 (discussing Dhallan I, of which Dhallan II is a continuation-in-part). Dhallan II teaches that aneuploidy is detected by comparing the ratio for the alleles on the chromosome tested for being aneuploid to the ratio of alleles on the reference chromosome:

> The **ratio for the alleles at a heterozygous locus of interest on any chromosome can be compared to the ratio for the alleles at a heterozygous locus of interest on any other chromosome**. Ex. E at ¶ [0047].
>
> For example, 100 SNPs can be analyzed on chromosome 1. Of these 100 SNPs, assume 50 are heterozygous. The ratio of the alleles at heterozygous SNPs on chromosome 1 can be summed, and should give a ratio of approximately 50:50. Likewise, of 100 SNPs analyzed on chromosome 21, assume 50 are heterozygous. The ratio of alleles at heterozygous SNPs on chromosome 21 is summed. With a normal number of chromosomes, the ratio should be approximately 50:50, and thus there should be no difference between the ratio obtained from chromosome 1 and 21. However, if there is an additional copy of chromosome 21, an additional allele will be provided, and the ratio should be approximately 66:33. **Thus, the ratio for nucleotides at heterozygous SNPs can be used to detect the presence or absence of chromosomal abnormalities**. Any chromosomal abnormality can be detected including **aneuploidy**, polyploidy, inversion, a trisomy, a

> monosomy, duplication, deletion, deletion of a part of a chromosome, addition, addition of a part of chromosome, insertion, a fragment of a chromosome, a region of a chromosome, chromosomal rearrangement, and translocation. The method is especially useful for the detection of trisomy 13, trisomy 18, trisomy 21, XXY, and XYY. *Id.* at ¶ [0379].
>
> In one embodiment, one of the chromosomes used in the comparison can be chromosome 13, 15, 16; 18, 21, 22, X or Y. In a preferred embodiment, **the ratios on chromosomes 13, 18, and 21 are compared**. *Id.* at ¶ [0395].
>
> A single locus of interest can be analyzed or multiple loci of interest. The intensity of the maternal allele at multiple loci of interest can be quantitated. **An average can be calculated for a chromosome and compared to the average obtained for a different chromosome**. For example, the average intensity of the maternal allele and the fetal allele inherited from the mother at chromosome 1 can be compared to the average intensity of the maternal allele and the fetal allele inherited from the mother at chromosomes 13, 18, or 21. In a preferred embodiment, chromosomes 13, 15, 18, 21, 22, X and Y, when applicable, are compared. *Id.* at ¶ [0414].
>
> There is no difference in the amount of fetal DNA from one chromosome to another. For instance, the percentage of fetal DNA in any given individual from chromosome 1 is the same as the percentage of fetal DNA from chromosome 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, X and Y. **Thus, the allele ratio calculated for SNPs on one chromosome can be compared to the allele ratio for the SNPs on another chromosome**. For example, the allele ratio for the SNPs on chromosome 1 should be equal to the allele ratio for the SNPs on chromosomes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, X, and Y. However**, if the fetus has a chromosomal abnormality, including but not limited to a trisomy or monosomy, the ratio for the chromosome that is present in an abnormal copy number will differ from the ratio for the other chromosomes**. *Id.* at ¶¶ [1022] – [1023].

See also Ex. O at ¶¶ 35, 46, 99; Ex. E at Abstract, and ¶¶ [0047], [0395], [0378] and [0414].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 1 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 2

Claim 2 of the '430 patent recites "wherein for each of the plurality of maternal blood samples determining the presence or absence of a fetal aneuploidy comprises comparing the number of enumerated sequence reads corresponding to the first chromosome tested for being aneuploid with the number of enumerated sequence reads corresponding to the reference chromosome." As discussed above in connection with claim 1(f), Dhallan II teaches that aneuploidy is detected by comparing the ratio for the alleles on the chromosome tested for being aneuploid to the ratio of alleles on the reference chromosome. That discussion is incorporated herein by reference. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 2. Ex. O at ¶¶ 105-106.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 2 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 3

Claim 3 of the '430 patent recites "wherein said plurality of non-random polynucleotide sequences comprises at least 300 different non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and at least 300 different non-random polynucleotide sequences selected from the reference chromosome." Dhallan II teaches the sequencing of up to tens of thousands of locations (loci) of interest, each of which contains non-random polynucleotide sequences:

> By a "locus of interest" is intended a selected region of nucleic acid that is within a larger region of nucleic acid. A locus of interest can include but is not limited to 1-100, 1-50, 1-20, or 1-10 nucleotides, preferably 1-6, 1-5, 1-4, 1-3, 1-2, or 1 nucleotide(s). Ex. E at ¶ [0177].
>
> In one embodiment, the sequence of alleles of one to tens to hundreds to thousands of loci of interest on a single chromosome on template DNA is determined. In a preferred embodiment, **the sequence of alleles of one to tens to hundreds to thousands of loci of interest on multiple chromosomes is determined**. *Id.* at ¶ [0059].
>
> Alternatively, 2, 3, 4, 5, 6, 7, 8, 9, 10-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-100, 100-250, 250-500, **500-1,000, 1,000-2,000, 2,000-3,000, 3,000-5,000, 5,000-10,000, 10,000-50,000 or more than 50,000 loci of interest can be analyzed at the same time** when a global genetic screening is desired. Such a global genetic screening might be desired when using the method of the invention to provide a genetic fingerprint to identify an individual or for SNP genotyping. *Id.* at ¶ [0226].

See also Ex. E at ¶¶ [0385] and [0406]. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 3. Ex. O at ¶¶ 107-108.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 3 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 4

Claim 4 of the ‘430 patent recites “wherein said plurality of non-random polynucleotide sequences comprises at least 500 different non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and at least 500 different non-random polynucleotide sequences selected from the reference chromosome.” This differs from claim 3 only in that it recites 500 sequences instead of 300. As explained above in connection with claim

3, Dhallan II teaches the sequencing of up to tens of thousands of locations (loci) of interest, each of which contains non-random polynucleotide sequences. Ex. E at ¶¶ [0059], [0226], [0385] and [0406]. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 4. Ex. O at ¶¶ 109-110.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 4 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 5

Claim 5 of the '430 patent recites "wherein each of said plurality of non-random polynucleotide sequences is from 10 to 500 nucleotide bases in length." Dhallan II teaches that the each locus of interest includes up to 100 nucleotides:

> By a "locus of interest" is intended a selected region of nucleic acid that is within a larger region of nucleic acid. A locus of interest can include but is not limited to 1-100, 1-50, 1-20, or 1-10 nucleotides, preferably 1-6, 1-5, 1-4, 1-3, 1-2, or 1 nucleotide(s). Ex. E at ¶ [0177].

Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 5. Ex. O at ¶¶ 111-112.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 5 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 6

Claim 6 of the '430 patent recites "wherein each of said plurality of non-random polynucleotide sequences is from 50 to 150 nucleotide bases in length." Dhallan II teaches that the each locus of interest includes up to 100 nucleotide bases:

> By a "locus of interest" is intended a selected region of nucleic acid that is within a larger region of nucleic acid. A locus of interest can include but is not limited to 1-100, 1-50, 1-20, or 1-10 nucleotides, preferably 1-6, 1-5, 1-4, 1-3, 1-2, or 1 nucleotide(s). Ex. E at ¶ [0177].

Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 6. Ex. O at ¶¶ 113-114.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 6 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 7

Claim 7 of the '430 patent recites "wherein said first chromosome tested for being aneuploid is selected from the group consisting of chromosome 13, chromosome 18, chromosome 21, chromosome X, and chromosome Y." Dhallan II teaches that its methods are used to detect trisomy 13, 18, 21, XXY and XYY:

> Any chromosomal abnormality can be detected including **aneuploidy**, polyploidy, inversion, a trisomy, a monosomy, duplication, deletion, deletion of a part of a chromosome, addition, addition of a part of chromosome, insertion, a fragment of a chromosome, a region of a chromosome, chromosomal rearrangement, and translocation. The method is especially useful for the detection of **trisomy 13, trisomy 18, trisomy 21, XXY, and XYY**. Ex. E at ¶ [0379].
>
> In one embodiment, one of the chromosomes used in the

> comparison can be chromosome 13, 15, 16; 18, 21, 22, X or Y. In a preferred embodiment, the ratios on chromosomes 13, 18, and 21 are compared. *Id.* at ¶ [0395].
>
> A single locus of interest can be analyzed or multiple loci of interest. The intensity of the maternal allele at multiple loci of interest can be quantitated. An average can be calculated for a chromosome and compared to the average obtained for a different chromosome. For example, the average intensity of the maternal allele and the fetal allele inherited from the mother at chromosome 1 can be compared to the average intensity of the maternal allele and the fetal allele inherited from the mother at chromosomes 13, 18, or 21. In a preferred embodiment, chromosomes **13, 15, 18, 21, 22, X and Y**, when applicable, are compared. *Id.* at ¶ [0414].

See also Ex. E at ¶¶ [0114], [0133] and [0384]. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 7. Ex. O at ¶¶ 115-116.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 7 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 8

Claim 8 of the ‘430 patent recites “wherein said fetal aneuploidy comprises fetal aneuploidy of a chromosome selected from the group consisting of chromosome 13, chromosome 18, chromosome 21, chromosome X, and chromosome Y.” This limitation is met by the same disclosure discussed above in connection with claim 7. *See also* Ex. O at ¶¶ 117-118.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 8 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 9

Claim 9 of the '430 patent recites "wherein said fetal aneuploidy is selected from the group consisting of trisomy 21, trisomy 18, trisomy 13, and monosomy X." This limitation is met by the same disclosure discussed above in connection with claim 7. *See also* Ex. O at ¶¶ 119-120.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 9 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 10

Claim 10 of the '430 patent recites "wherein said reference chromosome is selected from the group consisting of chromosome 1, chromosome 2, chromosome 3, chromosome 13, chromosome 18, and chromosome 21." Dhallan II teaches that the comparator chromosomes may be chromosomes 13, 15, 18 and 21:

> In embodiments, the ratio for alleles at heterozygous loci of interest on a chromosome are summed and compared to the ratio for alleles at heterozygous loci of interest on a different chromosome, where a difference in ratios indicates the presence of a chromosomal abnormality. In some of these embodiments, the chromosomes that are compared are human chromosomes such as chromosome 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, X, or Y. Ex. E at ¶ [0047].

> The ratio of alleles at heterozygous loci of interest on a chromosome can be compared to the ratio for alleles at heterozygous loci of interest on a different chromosome. For example, the ratio for multiple loci of interest on chromosome 1 (the ratio at SNP 1, SNP 2, SNP 3, SNP 4, etc.) can be compared to the ratio for multiple loci of interest on chromosome 21 (the ratio at SNP A, SNP B, SNP C, SNP D, etc.). **Any chromosome can be compared to any other chromosome. There is no limit to the number of chromosomes that can be compared.** *Id.* at ¶ [0402].

> A single locus of interest can be analyzed or multiple loci of interest. The intensity of the maternal allele at multiple loci of interest can be quantitated. An average can be calculated for a chromosome and compared to the average obtained for a different chromosome. For example, the average intensity of the maternal allele and the fetal allele inherited from the mother at chromosome 1 can be compared to the average intensity of the maternal allele and the fetal allele inherited from the mother at chromosomes 13, 18, or 21. In a preferred embodiment, chromosomes **13, 15, 18, 21, 22, X and Y**, when applicable, are compared. *Id.* at ¶ [0414].

See also Ex. E at ¶¶ [0061], [0062], [0063] and [0394]. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 10. Ex. O at ¶¶ 121-122.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 10 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 11

Claim 11 of the '430 patent recites "wherein said fetal aneuploidy comprises monosomy, trisomy, tetrasomy, or pentasomy of the first chromosome." Dhallan II teaches that the genetic disorders detected include monosomy and trisomy:

> The present invention is directed to a method for the detection of genetic disorders including chromosomal abnormalities and mutations. The present invention provides a rapid, non-invasive method for determining the sequence of DNA from a fetus. The method is especially useful for detection of chromosomal abnormalities in a fetus including translocations, transversions**, monosomies, trisomies, and other aneuplodies**, deletions, additions, amplifications, translocations and rearrangements. Ex. E at ¶ [0003].
>
> The invention is directed to a method for detection of genetic disorders including mutations and chromosomal abnormalities. In a

> preferred embodiment, the present invention is used to detect mutations, and chromosomal abnormalities including but not limited to translocation, transversion, **monosomy, trisomy, and other aneuploidies**, deletion, addition, amplification, fragment, translocation, and rearrangement. *Id.* at ¶ [0032].
>
> The present invention provides a method for detecting genetic disorders, including but not limited to mutations, insertions, deletions, and chromosomal abnormalities, and is especially useful for the detection of genetic disorders of a fetus. The method is especially useful for detection of a translocation, addition, amplification, transversion, inversion, **aneuploidy, polyploidy, monosomy, trisomy**, trisomy 21, trisomy 13, trisomy 14, trisomy 15, trisomy 16, trisomy 18, trisomy 22, triploidy, tetraploidy, and sex chromosome abnormalities including but not limited to XO, XXY, XYY, and XXX. The method also provides a non-invasive technique for determining the sequence of fetal DNA and identifying mutations within the fetal DNA. *Id.* at ¶ [0133].

See also Ex. E at ¶¶ [0009] and [0182]. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 11. Ex. O at ¶¶ 123-124.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 11 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 12

Claim 12 of the '430 patent recites "wherein said selectively enriching of (b) comprises performing polymerase chain reaction (PCR) amplification." Dhallan II teaches amplification by PCR:

> In one embodiment, the amplification can comprise **polymerase chain reaction (PCR)**. Ex. E at ¶ [0048].
>
> "Amplified" DNA is DNA that has been "copied" once or multiple times, e.g. by **polymerase chain reaction**. *Id.* at ¶ [0212].
>
> The template DNA can be **amplified using any suitable method**

> **known in the art including but not limited to PCR (polymerase chain reaction)**, 3SR (self-sustained sequence reaction), LCR (ligase chain reaction), RACE-PCR (rapid amplification of cDNA ends), PLCR (a combination of polymerase chain reaction and ligase chain reaction), Q-beta phage amplification (Shah et al., J. Medical Micro. 33: 1435-41 (1995)), SDA (strand displacement amplification), SOE-PCR (splice overlap extension PCR), and the like. *Id.* at ¶ [0283].

See also Ex. E at ¶¶ [0447] and [0473]. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 12. Ex. O at ¶¶ 125-127.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 12 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 13

Claim 13 of the '430 patent recites "wherein for each fetal and maternal cell-free genomic DNA sample PCR amplification comprises **hybridizing at least two oligonucleotides** to each of the at least 100 different non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and each of the at least 100 different non-random polynucleotide sequences selected from the reference chromosome." Dhallan II discloses the use of primer pairs, which in use are hybridized to the loci of interest:

> In one embodiment, one **primer pair** is used for each locus of interest. However, multiple primer pairs can be used for each locus of interest. Ex. E at ¶ [0236].
>
> A "primer pair" is intended a pair of forward and reverse primers. Both primers of a **primer pair** anneal in a manner that allows extension of the primers, such that the extension results in amplifying the template DNA in the region of the locus of interest. *Id.* at ¶ [0231].
>
> FIG. 1A. A Schematic diagram depicting a double stranded DNA

> molecule. **A pair of primers**, depicted as bent arrows, flank the locus of interest, depicted as a triangle symbol at base N14. *Id.* at ¶ [0082].

See also Ex. E at ¶¶ [0092], [0127], [0287], [0447] and [0473]. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 13. Ex. O at ¶¶ 128-130.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 13 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 14

Claim 14 of the '430 patent recites "wherein said oligonucleotides do not hybridize to non-random polynucleotide sequences comprising one or more polymorphisms." Dhallan II is drawn to selective amplification and analysis of loci in chromosomes, so the Dhallan II sequences of interest are non-random. A skilled artisan would assume that if in some embodiments the loci of interest are suspected of containing a single nucleotide polymorphism, then in some embodiments at least one of the loci of interest does not contain a single nucleotide polymorphism. Ex. O at ¶ 133. Accordingly, some of the loci of interest that are analyzed in the Dhallan II aneuploidy detection methods would *not* contain polymorphisms, *i.e.*, are homozygous between the fetus and the mother. The primers used for analysis of the homozygous SNPs would thus not hybridize to non-random polynucleotide sequences comprising one or more polymorphisms, as they would hybridize to loci that were the same between the mother and fetus:

> For example, 100 SNPs can be analyzed on chromosome 1. **Of these 100 SNPs, assume 50 are heterozygous**. The ratio of the

> alleles at heterozygous SNPs on chromosome 1 can be summed, and should give a ratio of approximately 50:50. Likewise, of 100 SNPs analyzed on chromosome 21, assume 50 are heterozygous. The ratio of alleles at heterozygous SNPs on chromosome 21 is summed. With a normal number of chromosomes, the ratio should be approximately 50:50, and thus there should be no difference between the ratio obtained from chromosome 1 and 21. Ex. E at ¶ [0379].
>
> For example, if 100 maternal loci of interest on chromosome 21 and 100 maternal loci of interest on chromosome 1 are analyzed, one would predict approximately **50 loci of interest on each chromosome to be homozygous and 50 to be heterozygous**. The 50 homozygous loci of interest, or the 50 heterozygous loci of interest or the 50 homozygous and 50 heterozygous loci of interest, or any combination of the homozygous and heterozygous loci of interest on each chromosome can be analyzed using the template DNA from the sample from the pregnant female. *Id.* at ¶ [0384].

Further, Dhallan II discloses that in some embodiments the primers anneal upstream and downstream of the loci of interest:

> The sequence of the 3' end of the primers is such that the primers anneal at a desired distance upstream and downstream of the locus of interest. *Id.* at ¶ [0083].
>
> The sequence of the SNP-IT primer, which is designed to anneal immediately upstream of the SNP site, is the best sequence available from between the upper and the lower strands. *Id.* at ¶ [1497].

Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 14. Ex. O at ¶¶ 131-133.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 14 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 15

Claim 15 of the '430 patent recites "wherein each of said oligonucleotides has a substantially similar melting temperature." Dhallan II discloses that the primers may have the same melting temperature:

> Primers can be prepared by a variety of methods including but not limited to cloning of appropriate sequences and direct chemical synthesis using methods well known in the art (Narang et al., Methods Enzymol. 68:90 (1979); Brown et al., Methods Enzymol. 68:109 (1979)). Primers can also be obtained from commercial sources such as Operon Technologies, Amersham Pharmacia Biotech, Sigma, and Life Technologies. The primers can have an **identical melting temperature**. The lengths of the primers can be extended or shortened at the 5' end or the 3' end to produce primers with desired melting temperatures. Ex. E at ¶ [0232].

Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 15. Ex. O at ¶¶ 134-135.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 15 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 16

Claim 16 of the '430 patent recites "wherein said massively parallel sequencing generates at least 30 nucleotide bases per sequence read." Craig teaches that the sequences are generally 32 or 42 bases in length:

> Typically 3-10 million short-read (32 or 42 base) sequences were generated for each lane of an 8-lane flow cell. Ex. F at 3.

Dr. Rosenberg confirms that one of skill in the art would understand that Craig teaches the recitation of claim 16. Ex. O at ¶¶ 136-137.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 16 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 17

Claim 17 of the '430 patent recites "wherein said fetal aneuploidy comprises partial monosomy or partial trisomy." Partial monosomy means that part of a chromosome is missing and partial trisomy means that there is an extra copy of part of a chromosome. Ex. O at ¶¶ 138-139. Dhallan II teaches methods for detecting partial monosomy and partial trisomy.

> The term "chromosomal abnormality" refers to a deviation between the structure of the subject chromosome and a normal homologous chromosome. The term "normal" refers to the predominate karyotype or banding pattern found in healthy individuals of a particular species. A chromosomal abnormality can be numerical or structural, and includes but is not limited to aneuploidy, polyploidy, inversion, a trisomy, a monosomy, duplication, deletion, **deletion of a part of a chromosome, addition, addition of a part of chromosome**, insertion, a fragment of a chromosome, a region of a chromosome, chromosomal rearrangement, and translocation. Ex. E at ¶ [0182].

See also Ex. E at Abstract, and ¶¶ [0003], [0032] and [0133]. Dr. Rosenberg confirms that the disclosure of Dhallan II thus teaches the recitation of claim 17. Ex. O at ¶¶ 138-139.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 17 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claim 18

Claim 18 of the '430 patent recites "wherein said plurality of non-random polynucleotide sequences comprises no more than 1000 different non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and no more than 1000 different non-random polynucleotide sequences selected from the reference chromosome." Dhallan II teaches analyzing 1000 or less loci of interest:

> Alternatively, 2**, 3, 4, 5, 6, 7, 8, 9, 10-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-100, 100-250, 250-500, 500-1,000**, 1,000-2,000, 2,000-3,000, 3,000-5,000, 5,000-10,000, 10,000-50,000 or more than 50,000 loci of interest can be analyzed at the same time when a global genetic screening is desired. Such a global genetic screening might be desired when using the method of the invention to provide a genetic fingerprint to identify an individual or for SNP genotyping. Ex. E at ¶ [0226].
>
> Any number of loci of interest can be analyzed on the template DNA from the sample from the pregnant female. For example, 1, 1-5, 5-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, **100-150, 150-200, 200-250, 250-300, 300-500, 500-1000**, 1000-2000, 2000-3000, 3000-4000 or more than 4000 homozygous maternal loci of interest can be analyzed in the template DNA from the sample from the pregnant female. In a preferred embodiment, multiple loci of interest on multiple chromosomes are analyzed. *Id.* at ¶ [0385].
>
> Any number of loci of interest can be analyzed including but not limited to 1, 1-5, 5-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 1**00-150, 150-200, 200-250, 250-300, 300-500, 500-1000**, 1000-2000, 2000-3000, 3000-4000, 4000-8000, 8000-16000, 16000-32000 or greater than 32000 loci of interest. *Id.* at ¶ [0406].

See also Ex. E at ¶ [0059]. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 18. Ex. O at ¶¶ 140-141.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 18 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Independent Claim 19

Independent claim 19 is identical to independent claim 1 except that claim 19 i) uses the term "chromosome control region" instead of "reference chromosome" and ii) uses the term "at least one chromosome region tested for being aneuploidy" instead of "a first chromosome tested for being aneuploidy." As noted above in Sections V.C and V.E, the Board interpreted "chromosome control region" as "a chromosome region that is different from the claimed one chromosome region tested." Dr. Rosenberg likewise interprets that claims 1 and 19 have similar scope under the broadest reasonable interpretation, noting the '430 specification uses the terms "reference chromosome" and "chromosome control region" interchangeably. Ex. O at ¶ 32.

Moreover, Dhallan II teaches the use of loci on particular chromosomes, which are *de facto* chromosome regions. Thus, the use of loci on a reference chromosome in Dhallan II is essentially the use of particular chromosome regions on that chromosome for determination of fetal aneuploidy. Ex. O at ¶ 160.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claim 19 is shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

Regarding Dependent Claims 20-30

Dependent claims 20-30 correspond to dependent claims 2-3, 5, 7-10, 11-13 and 18, respectively. The discussion set forth above in connection with claims 2-3, 5, 7-10, 11-13 and 18

applies with equal force to claims 20-30. Dr. Rosenberg concurs in that assessment. Ex. O at ¶¶ 125-197.

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit V.

Thus, claims 20-30 are shown to be rendered obvious by the combination of Dhallan II, Craig and the Illumina Brochure.

B. Dhallan II in View of Parameswaran and Hamady Renders Obvious Claims 1-30 of the '430 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

The primary difference between this ground and the preceding ground is that Parameswaran and Hamady describe a different commercially available multiplexed massively parallel sequencing system that was available at the time of filing of the '430 patent, the Roche/454 platform. Dr. Rosenberg explains that a skilled artisan would have had no difficulties using multiplexing on the Roche/454 platform, which was launched two years following the earliest priority date of Dhallan II for detection of the indexed fetal and maternal loci for use in Dhallan II's aneuploidy detection methods. Ex. O at ¶ 59. Any artisan of ordinary skill would have understood that doing so would have been faster, cheaper and more effective. *Id.* at ¶ 60.

Parameswaran and Hamady teach the specific methodology for using multiplexing in conjunction with the Roche/454 platform. A person of ordinary skill in the art could easily adapt the specific techniques taught in Parameswaran and Hamady for using the multiplexed detection on the Roche/454 platform to detect the fetal and maternal loci used in Dhallan II's aneuploidy detection methods. Ex. O at ¶¶ 59-60 and 64-66. As discussed above, Dhallan II, Parameswaran and Hamady are prior art against the '430 patent under 35 U.S.C. § 102(b). As shown by the detailed claim-by-claim and limitation-by-limitation analyses below, Dhallan II in view of Parameswaran and Hamady discloses each of the limitations set forth in claims 1-30 of the '430

patent. Therefore, claims 1-30 are unpatentable under 35 U.S.C. § 103(a) as being obvious over Dhallan II in view of Parameswaran and Hamady.

Regarding Independent Claim 1

As explained by Dr. Rosenberg, Dhallan II was filed in 2004 and teaches all limitations recited in the claims except the detection method which employs i) indexing or tagging samples from different patients so they can be processed simultaneously (sometimes called multiplexing), and ii) using massively parallel sequencing to sequence the indexed samples. Ex. O at ¶ 35. Significantly, Verinata did not contest this premise during the *inter partes* reviews. Ex. L at Paper 10 and Paper 20; Ex. M at Paper 10 and Paper 20 (discussing Dhallan I, of which Dhallan II is a continuation-in-part). Both of the missing limitations were present in massively parallel sequencing systems, which were commercially available by January 2010, the earliest claimed priority date. *See* Exs. H and I. For instance, the Roche/454 platform was launched in 2005, and by 2008 it was routinely being used to perform multiplexed sequencing (in which multiple patients' samples are tagged with indexes and processed simultaneously). Dr. Rosenberg explains that any first year post-doctoral student in a molecular biology laboratory would have considered it routine (and quite advantageous) to perform the method taught by Dhallan II in 2004 with the later-developed Roche/454 massively parallel sequencing platform, as described in Parameswaran and Hamady. Ex. O at ¶¶ 20, 53-55, 62-66, and 198. The claims of the '430 patent are thus rendered obvious by Dhallan II in view of Parameswaran and Hamady.

More particularly, Dr. Rosenberg explains that it would have been obvious to combine Dhallan II, Parameswaran and Hamady in the following manner. Ex. O at ¶¶ 53-55. Dhallan II discloses a fetal aneuploidy determination method which has the following steps: 1) obtaining blood samples from multiple pregnant women that contain cell-free DNA; 2) isolating cell-free

DNA samples from the plurality of blood samples; 3) enriching at least 100 nonrandom polynucleotides (i.e., loci of interest) from a first chromosome tested for being aneuploid and at least 100 different non-random polynucleotide sequences non-random polynucleotides from a reference chromosome in each cell-free DNA sample by amplification to create a library (*i.e.*, a preparation) that contains representative PCR products having the sequence of the non-random polynucleotides; 4) detecting the non-random polynucleotides from the first chromosome tested for being aneuploid and the reference chromosome of each library for each sample; 5) enumerating the non-random polynucleotides from the first chromosome or chromosome region tested for being aneuploid and the reference chromosome or chromosome control region of each library from each sample; and 6) determining the presence or absence of a fetal aneuploidy for each sample by comparing the enumerated non-random polynucleotides from the first chromosome or chromosome region tested for being aneuploid and the reference chromosome or chromosome control region from each sample library. *Id.* at ¶ 45. Claim 1 of the '430 patent follows the same steps except it employs a different detection technique for step (4). *Id.* at ¶ 47. As described in detail below, the detection technique of step (4) of the '430 patent was well known at the time of the filing date of the '430 patent, including the use of multiplexing on the Roche/454 platform as disclosed in Parameswaran and Hamady. *Id*. at ¶¶ 49, 53-55. Both of these references teach detection of amplified polynucleotides from individual samples using indexing and massively parallel sequencing. It would have been well within the ordinary skill of the art to substitute the detection technique Dhallan II with the detection technique of Parameswaran and Hamady, and a person of ordinary skill would have had a reasonable expectation of success in doing so. *Id*. at ¶¶ 53-55.

> **Claim 1 [preamble]: A method for determining a presence or absence of a fetal aneuploidy in a fetus for each of a plurality of maternal blood samples obtained**

from a plurality of different pregnant women, said maternal blood samples comprising fetal and maternal cell-free genomic DNA, said method comprising:

Dhallan II discloses a method for determining a presence or absence of a fetal aneuploidy in a fetus by taking blood samples from pregnant women which contain cell-free maternal and fetal DNA. Dhallan II provides

> a method useful for detection of genetic disorders. The method comprises determining the sequence of alleles of a locus of interest, and quantitating a ratio for the alleles at the locus of interest, wherein the ratio indicates the **presence or absence of a chromosomal abnormality**. The present invention also provides a non-invasive method for the detection of **chromosomal abnormalities *in a fetus***. The invention is especially useful as a non-invasive method for determining the sequence of fetal DNA. The invention further provides methods of isolation of free DNA from a sample. Ex. E at Abstract.

> The present invention is directed to a method for the detection of genetic disorders including chromosomal abnormalities and mutations. The present invention provides a rapid, non-invasive method for determining the sequence of DNA from a fetus. The method is especially useful for detection of chromosomal abnormalities in a fetus including translocations, transversions, monosomies, trisomies, and other **aneuplodies [sic]**, deletions, additions, amplifications, translocations and rearrangements. *Id.* at ¶ [0003].

> In one embodiment, the sample containing the nucleic acid is obtained from a pregnant female. In a preferred embodiment, the sample is obtained from a pregnant human female. In a preferred embodiment, the **sample is blood obtained from a pregnant** female. *Id.* at ¶ [0076].

> The present invention provides a method for detecting genetic disorders, including but not limited to mutations, insertions, deletions, and chromosomal abnormalities, and is especially useful for the detection of **genetic disorders of a fetus**. The method is especially useful for detection of a translocation, addition, amplification, transversion, inversion, **aneuploidy**, polyploidy, monosomy, trisomy, trisomy 21, trisomy 13, trisomy 14, trisomy 15, trisomy 16, trisomy 18, trisomy 22, triploidy, tetraploidy, and sex chromosome abnormalities including but not limited to XO, XXY, XYY, and XXX. *Id.* at ¶ [0133].

> The blood sample is centrifuged to separate the plasma from the maternal cells. The plasma and maternal cell fractions are transferred to separate tubes and re-centrifuged. The **plasma fraction contains cell-free fetal DNA and maternal DNA**. *Id.* at ¶ [0197].

See also Ex. O at ¶¶ 199-205, citing also Ex. E at ¶¶ [0030], [0032], [0132], [0182], [0190], [0196] and [0201].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Claim 1[a]: (a) obtaining a fetal and maternal cell-free genomic DNA sample from each of the plurality of maternal blood samples;

This claim limitation is largely cumulative to the preamble and is likewise disclosed by Dhallan II:

> In one embodiment, the template DNA is **fetal DNA**. Fetal DNA can be obtained from sources including but not limited to **maternal blood**, maternal serum, maternal plasma, fetal cells, umbilical cord blood, chorionic villi, amniotic fluid, urine, saliva, cells or tissues. Ex. E at ¶ [0190].
>
> In another embodiment, the template DNA contains both maternal DNA and fetal DNA. In a preferred embodiment, **template DNA is obtained from blood of a pregnant female**. *Id.* at ¶ [0196].
>
> The blood sample is centrifuged to separate the plasma from the maternal cells. The plasma and maternal cell fractions are transferred to separate tubes and re-centrifuged. **The plasma fraction contains cell-free fetal DNA and maternal DNA**. *Id.* at ¶ [0197].
>
> In another embodiment, the template **DNA is obtained from the plasma or serum of the blood of the pregnant female**. The percentage of fetal DNA in maternal plasma is between 0.39-11.9% (Pertl, and Bianchi, Obstetrics and Gynecology 98: 483-490 (2001). *Id.* at ¶ [0201].
>
> In accordance with an IRB approved study, blood samples were collected from pregnant women after informed consent had been granted. **Blood samples were received from 27 different clinical sites operating in 16 different states located throughout the**

> **U.S.** Blood samples were collected from both women carrying male and female fetuses, however, here, we report results obtained from woman carrying male fetuses, as the Y chromosome is the accepted marker when quantitating percentages of fetal DNA. *Id.* at ¶ [1210].

See also Ex. E at ¶¶ [0030] and [0042]. *See* also Ex. O at ¶¶ 206-207.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

> **Claim 1[b]: selectively enriching a plurality of non-random polynucleotide sequences of each fetal and maternal cell-free genomic DNA sample of (a) to generate a library derived from each fetal and maternal cell-free genomic DNA sample of enriched and indexed fetal and maternal non-random polynucleotide sequences, wherein each library of enriched and <u>indexed</u> fetal and maternal non-random polynucleotide sequences includes an <u>indexing nucleotide sequence which identifies a maternal blood sample of the plurality of maternal blood samples</u>, wherein said plurality of non-random polynucleotide sequences comprises at least 100 different non-random polynucleotide sequences selected from a first chromosome tested for being aneuploid and at least 100 different non-random polynucleotide sequences selected from a reference chromosome, wherein the first chromosome tested for being aneuploid and the reference chromosome are different, and wherein each of said plurality of non-random polynucleotide sequences is from 10 to 1000 nucleotide bases in length**,

Dr. Rosenberg explains that Dhallan II teaches every aspect of this claim element except for the limitations underlined above, which require that the samples be tagged or indexed, as was conventionally done in off-the-shelf multiplexed assays at the time of filing. Ex. O at ¶¶ 64 and 208-220. The fact that Dhallan II teaches these elements is confirmed by Verinata's failure to contest the same during the *inter partes* reviews. Ex. L at Paper 10 and Paper 20, Ex. M at Paper 10 and Paper 20. Dhallan II teaches that

> [t]he method can be used for **determining sequences of multiple loci of interest concurrently**. The template DNA can comprise multiple loci from a single chromosome. The template DNA can comprise multiple loci from different chromosomes. The loci of interest on template DNA can be amplified in one reaction. Alternatively, each of the loci of interest on template DNA can be amplified in a separate reaction. Ex. E at ¶ [0060].

The original template DNA was amplified using 12 base primers that annealed to various regions on chromosome 13**. One hundred different primer sets were used to amplify regions throughout chromosome 13**. For each of the nine SNPs, a primer that annealed approximately 130 bases from the locus of interest and 130 bases downstream of the locus of interest were used. This amplification reaction, which contained a total of 100 different primer sets, was used to amplify the regions containing the loci of interest. *Id.* at ¶ [0127].

By a "locus of interest" is intended a selected region of nucleic acid that is within a larger region of nucleic acid. A locus of interest can include but is not limited to **1-100**, 1-50, 1-20, or 1-10 nucleotides, preferably 1-6, 1-5, 1-4, 1-3, 1-2, or 1 nucleotide(s). *Id.* at ¶ [0177].

The template DNA [maternal and fetal DNA] can be amplified using any suitable method known in the art including but not limited to PCR (polymerase chain reaction), 3SR (self-sustained sequence reaction), LCR (ligase chain reaction), RACE-PCR (rapid amplification of cDNA ends), PLCR (a combination of polymerase chain reaction and ligase chain reaction), Q-beta phage amplification (Shah et al., J. Medical Micro. 33: 1435-41 (1995)), SDA (strand displacement amplification), SOE-PCR (splice overlap extension PCR), and the like. *Id.* at ¶ [0283].

The multiple primer sets will amplify the loci of interest, such that a minimal amount of template DNA is not limiting for the number of loci that can be detected. For example, if template DNA is isolated from a single cell or the **template DNA is obtained from a pregnant female, which comprises both maternal template DNA and fetal template DNA, low concentrations of each primer set can be used in a first amplification reaction to amplify the loci of interest**. *Id.* at ¶ [0288].

For example, **100 SNPs can be analyzed on chromosome 1**. Of these 100 SNPs, assume 50 are heterozygous. The ratio of the alleles at heterozygous SNPs on chromosome 1 can be summed, and should give a ratio of approximately 50:50. **Likewise, of 100 SNPs analyzed on chromosome 21, assume 50 are heterozygous**. The ratio of alleles at heterozygous SNPs on chromosome 21 is summed. With a normal number of chromosomes, the ratio should be approximately 50:50, and thus there should be no difference between the ratio obtained from chromosome 1 and 21. *Id.* at ¶ [0379].

> For example**, if 100 maternal loci of interest on chromosome 21 and 100 maternal loci of interest on chromosome 1 are analyzed**, one would predict approximately 50 loci of interest on each chromosome to be homozygous and 50 to be heterozygous. The 50 homozygous loci of interest, or the 50 heterozygous loci of interest or the 50 homozygous and 50 heterozygous loci of interest, or any combination of the homozygous and heterozygous loci of interest on each chromosome can be analyzed using the template DNA from the sample from the pregnant female. *Id.* at ¶ [0384].
>
> In one embodiment, one of the chromosomes used in the comparison can be chromosome 13, 15, 16; 18, 21, 22, X or Y. **In a preferred embodiment, the ratios on chromosomes 13, 18, and 21 are compared**. *Id.* at ¶ [0395].

See Ex. O at ¶¶ 208-220, citing also Ex. E at ¶¶ [0059], [0064], [0110], [0114], [0127], [0385], [0402], [0414], [0427] and [1023]. Dr. Rosenberg explains in this declaration that because the enriched and indexed products are created by a selective amplification process, the products would be non-random polynucleotides. Ex. O at ¶ 78.

Dhallan II does not explicitly teach the claim recitation that the libraries include "indexed" fetal and maternal non-random polynucleotide sequences or the method includes "an indexing nucleotide sequence which identifies a maternal blood sample of the plurality of maternal blood samples." As Dr. Rosenberg explains, these limitations would be met if the 2004 Dhallan II method were simply performed on the later-developed and much more effective and cost-effective multiplexed massively parallel sequencing systems. Ex. O at ¶¶ 53-55 and 63-66. One such system was sold by Roche, which acquired 454 Life Sciences. *See* Exs. H and I.

Parameswaran describes that the Roche/454 platform is useful for multiplexed massively parallel sequencing. Parameswaran discloses the creation of indexed libraries for different patients using the massively parallel sequencing method of the Roche/454 platform:

> To overcome these limitations, we have devised a novel **barcoding approach** to allow for **pooling** and sequencing of DNA from **independent samples**, and to facilitate subsequent segregation of

sequencing capacity. . . . In two pilot series of **barcoded sequencing** using the GS20 Sequencer (454/Roche) we found that over 99.8% of obtained sequences could be assigned to 25 independent, uniquely barcoded libraries based on the presence of either a perfect forward or a perfect reverse barcode. Ex. H at Abstract.

To overcome these limitations, we describe a high-information-content barcoding approach in which **each sample is associated with two uniquely designed, 10-nucleotide barcodes**. . . . Using the GS20 platform (454/Roche), these barcodes allowed us to **simultaneously pyrosequence small RNA libraries** from 25 diverse samples for each pilot run, and unambiguously assign 99.8% of obtained barcoded sequences by bioinformatically probing for an error-free barcode at either end of the sequence of interest. . . . To pursue this analysis, small RNA fractions from each cell or tissue sample were **independently isolated** (using the miRvana small RNA isolation protocol (Ambion)), and processed to produce **50 individually barcoded cDNA libraries** that could be sequenced using the **Roche/454 platform**. *Id.* at 2.

We have devised a barcoding approach to substantially enhance the scope and capacity of multiplexed high-throughput pyrosequencing. *Id.* at 4.

Despite the extended length of the barcoded primers, complex pools of cDNA containing 19-27 nt small RNA inserts were efficiently amplified to yield PCR products that were 115-123 nt long. *Id.* at 5.

DNA from two independent sets of **25 barcoded libraries** (prepared with 19-27 nts RNAs from different in vitro and in vivo virus-infected systems) **were pooled for sequencing** runs, and the data was used to evaluate the efficacy of the barcoding technology. *Id.* at 7.

The barcoding approach requires being able to establish a relationship between ratios of material from various libraries, and the ratios of obtained sequences that correspond to those libraries . . . We have described a **pyrosequencing-tailored barcoding approach** that allows for the unambiguous assignment of nucleic acid sequences from a **mixture of libraries from up to 48 different samples**. *Id.* at 8.

Several months after the publication of Parameswaran, Hamady described another use of the Roche/454 platform for multiplexed massively parallel sequencing. Hamady describes how the kits tag or index libraries with unique identifiers so that hundreds of patients' samples can be processed simultaneously:

> We have constructed **error-correcting DNA barcodes** that allow one run of a **massively parallel** pyrosequencer to **process up to 1544 samples simultaneously**. Ex. I at Abstract.
>
> Use of pyrosequencing … has been limited by the expense of each individual run, and by the difficulty of splitting a single plate across multiple runs. One way around this problem is to use a **barcoding approach**, in which a unique tag is added to each primer before PCR amplification. Because each sample is amplified with a known tagged primer, sequencing can be performed on an equimolar mixture of PCR-amplified DNA from each sample, and **sequences can be assigned** to samples based on the **unique barcode**. *Id.* at 235.
>
> We used 286 of the 1544 candidate codewords to synthesize **barcoded PCR primers** to use in PCR reactions amplifying a region (27F-338R) of the 16S rRNA gene that was previously determined to be the optimal region of the 16S rRNA to use for phylogenetic analysis from pyrosequencing reads. *Id.* at 236.
>
> **For each of 286 samples, the four replicate PCR reactions were combined**, purified with AMpure magnetic purification beads (Agencourt), quantified with the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen) and a fluorospectrometer (Nanodrop ND3300), and combined to create a **master DNA pool** with a final concentration of 21.5 ng/µl, which was sent for pyrosequencing with primer A at **454 Life Sciences** (Branford, CT) as described. *Id.* at 237.

Performing the Dhallan II method on the Roche/454 platform in a multiplexed manner, as described in Parameswaran and Hamady, would have involved nothing more than the application of routine skill. Indeed, Dr. Rosenberg explains that any first-year post-doctoral student working in a molecular biology laboratory would have been able to carry out the Dhallan II method on the Roche/454 system in a multiplexed manner, as described by Parameswaran and Hamady, as of

the filing date in 2010. Ex. O at ¶¶ 53-55 and 63-66 and 198. Any index optimization which may have been required for efficiency in detection of fetal aneuploidy was well within the skill of such a person. *Id.* at ¶¶ 55 and 63-66.

The fact that a skilled artisan would have been able to carry out the Dhallan II aneuploidy method on the Roche/454 system in a multiplexed manner, as described by Parameswaran and Hamady, is evidenced further by U.S. Patent Pub. App. No. 2008/0090239 to Shoemaker, et al. ("Shoemaker") (Ex. J). Shoemaker, filed in 2007, describes a method for aneuploidy detection performed on another commercially available massively parallel sequencing system (the Illumina Genome Analyzer) in a multiplexed fashion. Ex. J at ¶ [0157] (describing use of the Illumina Genome Analyzer), *see also* ¶¶ [0108], [0114], [0119], [0122], [0127], [0138], [0140], [0157] and [0159] (describing the use of the technique to detect aneuploidy in maternal and fetal DNA).

Accordingly, the Roche/454 system and the Parameswaran/Hamady multiplexing techniques could have been predictably used to improve the Dhallan II method in the same manner they were used to improved techniques for "the discovery, identification and quantitation of small RNAs," "the detection of rare variations in cancers" and "assessments of microbial community diversity" – the specific applications noted by Parameswaran and Hamady. Ex. H at 1, Ex. I at 1. Because doing so would achieve the claimed subject matter, it is obvious and unpatentable. *KSR*, 550 U.S. at 417 ("[I]f a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond that person's skill.").

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Claim 1[c]: pooling the libraries generated in (b) to produce a pool of enriched and <u>indexed</u> fetal and maternal non-random polynucleotide sequences;

Dr. Rosenberg explains that Dhallan II teaches all aspects of this claim except for the limitation of indexing the libraries, a fact which Verinata did not contest during the *inter partes* review proceedings. Ex. L at Paper 10 and Paper 20; Ex. M at Paper 10 and Paper 20 (discussing Dhallan I, of which Dhallan II is a continuation-in-part). In particular, Dhallan II teaches that

> Alternatively, to avoid competition for nucleotides and to minimize primer dimers and difficulties with annealing temperatures for primers, each locus of interest or small groups of loci of interest can be amplified in separate reaction tubes or wells, **and the products later pooled if desired**. Ex. E at ¶ [0279].

As discussed above in connection with claim 1(b), Parameswaran describes that the Roche/454 platform is useful for multiplexed massively parallel sequencing, in which samples are indexed or tagged for simultaneous processing. Parameswaran discloses:

> To overcome these limitations, we describe a high-information-content barcoding approach in which **each sample is associated with two uniquely designed, 10-nucleotide barcodes**. . . . Using the GS20 platform (454/Roche), these barcodes allowed us to **simultaneously pyrosequence small RNA libraries** from 25 diverse samples for each pilot run, and unambiguously assign 99.8% of obtained barcoded sequences by bioinformatically probing for an error-free barcode at either end of the sequence of interest. . . . To pursue this analysis, small RNA fractions from each cell or tissue sample were **independently isolated** (using the miRvana small RNA isolation protocol (Ambion)), and processed to produce **50 individually barcoded cDNA libraries** that could be sequenced using the **Roche/454 platform**. Ex. H at 2.
>
> DNA from two independent sets of **25 barcoded libraries** (prepared with 19-27 nts RNAs from different in vitro and in vivo virus-infected systems) **were pooled for sequencing** runs, and the data was used to evaluate the efficacy of the barcoding technology. *Id.* at 7.
>
> We have described a **pyrosequencing-tailored barcoding approach** that allows for the unambiguous assignment of nucleic acid sequences from a **mixture of libraries from up to 48 different samples**. *Id.* at 8.

Hamady similarly describes how the kits tag or index libraries with unique identifiers so that hundreds of patients' samples can be processed simultaneously:

> We have constructed **error-correcting DNA barcodes** that allow one run of a **massively parallel** pyrosequencer to **process up to 1544 samples simultaneously**. Ex. I at Abstract.
>
> **For each of 286 samples, the four replicate PCR reactions were combined**, purified with AMpure magnetic purification beads (Agencourt), quantified with the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen) and a fluorospectrometer (Nanodrop ND3300), and combined to create a **master DNA pool** with a final concentration of 21.5 ng/µl, which was sent for pyrosequencing with primer A at **454 Life Sciences** (Branford, CT) as described. *Id.* at 237.

As also discussed above and in the declaration of Dr. Rosenberg, any skilled artisan would have readily understood that the Dhallan II aneuploidy detection method, which dates from before 2004, could have been performed with greater throughput and reduced cost on the Roche/454 system in a multiplexed manner, as described by Parameswaran and Hamady. Ex. O at ¶¶ 20, 53-55, 62-66, and 198. Because the Roche/454 system had been used to improve other techniques in the same way, and a person of ordinary skill in the art would have recognized that it could be readily applied to the Dhallan II method, the technique is obvious. *KSR,* 550 U.S. at 417.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

> **Claim 1[d]: performing <u>massively parallel sequencing</u> of the pool of enriched and <u>indexed</u> fetal and maternal non-random polynucleotide sequences of (c) to produce sequence reads corresponding to enriched and <u>indexed</u> fetal and maternal non-random polynucleotide sequences of each of the at least 100 different non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and sequence reads corresponding to enriched and <u>indexed</u> fetal and maternal non-random polynucleotide sequences of each of the at least 100 different non-random polynucleotide sequences selected from the reference chromosome;**

As Dr. Rosenberg explains in his declaration, Dhallan II teaches all aspects of claim 1(d) except the underlined limitations above, namely, that the sequencing be massively parallel and that the libraries be tagged or indexed. Ex. O at ¶ 35. Again, Verinata did not contest during the *inter partes* reviews that Dhallan II taught these limitations. Ex. L at Paper 10 and Paper 20; Ex. M at Paper 10 and Paper 20 (discussing Dhallan I, of which Dhallan II is a continuation-in-part). Dhallan II teaches performing sequencing to produce sequence reads corresponding to the sequences from the chromosome suspected of being aneuploidy and the sequences from the reference chromosome. Ex. O at ¶¶ 35 and 45. More particularly, Dhallan II teaches that the loci of interest may be sequenced using "[a]ny method that provides information on the sequence of a nucleic acid," and the sequence counts may be compared between the suspect chromosome and a reference chromosome:

> Any method that **provides information on the sequence** of a nucleic acid can be used including but not limited to allele specific PCR, PCR, gel electrophoresis, ELISA, mass spectrometry, MALDI-TOF mass spectrometry hybridization, primer extension, fluorescence detection, fluorescence resonance energy transfer (FRET), fluorescence polarization, **DNA sequencing**, Sanger dideoxy sequencing, DNA sequencing gels, . . . Ex. E at ¶ [0228].
>
> In another embodiment, **determining the sequence** of alleles of a locus of interest comprises a method including but not limited to allele specific PCR, gel electrophoresis, ELISA, mass spectrometry, hybridization, primer extension, fluorescence polarization, fluorescence detection, fluorescence resonance energy transfer (FRET), **sequencing**, DNA microarray, SNP-IT, GeneChips, HuSNP, BeadArray, TaqMan assay, Invader assay, MassExtend, MassCleave.TM. (hMC) method, southern blot, slot blot, dot blot, and MALDI-TOF mass spectrometry. *Id.* at ¶ [0046].
>
> In one embodiment, the **determination of the sequence** of the locus of interest comprises detecting the incorporated nucleotide. In one embodiment, the detection is by a method selected from the group consisting of gel electrophoresis, capillary electrophoresis, microchannel electrophoresis, polyacrylamide gel electrophoresis,

> fluorescence detection, fluorescence polarization, **DNA sequencing**, Sanger dideoxy **sequencing** . . . *Id.* at ¶ [0058].
>
> For example**, if 100 maternal loci of interest on chromosome 21 and 100 maternal loci of interest on chromosome 1 are analyzed**, one would predict approximately 50 loci of interest on Exhinieach chromosome to be homozygous and 50 to be heterozygous. The 50 homozygous loci of interest, or the 50 heterozygous loci of interest or the 50 homozygous and 50 heterozygous loci of interest, or any combination of the homozygous and heterozygous loci of interest on each chromosome can be analyzed using the template DNA from the sample from the pregnant female. *Id.* at ¶ [0384].
>
> In one embodiment, one of the chromosomes used in the **comparison can be chromosome** 13, 15, 16; 18, 21, 22, X or Y. **In a preferred embodiment, the ratios on chromosomes 13, 18, and 21 are compared**. *Id.* at ¶ [0395].

While Dhallan II does not teach that the sequencing is massively parallel or the sequences are indexed, those aspects are taught by Parameswaran and Hamady. After Dhallan II filed his application in 2004, Roche/454 introduced its GS20 system in 2010, which helped massively parallel sequencing achieve widespread adoption by the earliest claimed priority date of the '430 patent. Ex. O at ¶ 20. As discussed above in connection with claim elements 1(b) and 1(c), Parameswaran and Hamady explain that the Roche/454 system is useful for multiplexed massively parallel sequencing.

The Rosenberg declaration explains that performing the Dhallan II aneuploidy detection method using the Roche/454 system in a multiplexed manner for detection of the analyzed loci, as described by Parameswaran and Hamady, would have involved nothing more than the application of routine skill. Ex. O at ¶¶ 53-55 and 62-66.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Claim 1[e]: based on the indexing nucleotide sequence, for each of the plurality of maternal blood samples, enumerating sequence reads corresponding to enriched and indexed fetal and maternal non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and sequence reads corresponding to enriched and indexed fetal and maternal non-random polynucleotide sequences selected from the reference chromosome; and.

This claim element recites the step of enumerating the sequence reads from the chromosome being tested and the reference chromosome. Dhallan II teaches this limitation:

> In another embodiment, **the ratio of alleles at a heterozygous locus of interest on a chromosome is compared to the ratio of alleles at a heterozygous locus of interest on a different chromosome**. There is no limitation as to the chromosomes that can be compared. The ratio for the alleles at a heterozygous locus of interest on any chromosome can be compared to the ratio for the alleles at a heterozygous locus of interest on any other chromosome. In a preferred embodiment, the ratio of alleles at multiple heterozygous loci of interest on a chromosome are summed and compared to the ratio of alleles at multiple heterozygous loci of interest on a different chromosome. Ex. E at ¶ [0061].
>
> The **ratio for the alleles at a heterozygous locus of interest on any chromosome can be compared to the ratio for the alleles at a heterozygous locus of interest on any other chromosome**. *Id.* at ¶ [0047].
>
> In another embodiment, **the ratio of alleles at a heterozygous locus of interest on a chromosome is compared to the ratio of alleles at a heterozygous locus of interest on two, three, four or more than four chromosomes**. In another embodiment, the ratio of alleles at multiple loci of interest on a chromosome is compared to the ratio of alleles at multiple loci of interest on two, three, four, or more than four chromosomes. *Id.* at ¶ [0062].

See also Ex. E at ¶ [0063]; Ex. O at ¶¶ 226-228. Verinata did not contest during the *inter partes* reviews that Dhallan II taught these limitations. Ex. L at Paper 10 and Paper 20; Ex. M at Paper 10 and Paper 20 (discussing Dhallan I, of which Dhallan II is a continuation-in-part).

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Claim 1[f]: for each of the plurality of maternal blood samples, determining the presence or absence of a fetal aneuploidy comprising using a number of enumerated sequence reads corresponding to the first chromosome and a number of enumerated sequence reads corresponding to the reference chromosome of (e).

Dhallan II discloses the step of determining whether the sample contains a fetal aneuploidy by using the enumerated or tallied sequence reads. Ex. O at ¶¶ 229-231. Here again, did not contest during the *inter partes* reviews that Dhallan II taught these limitations. Ex. L at Paper 10 and Paper 20; Ex. M at Paper 10 and Paper 20 (discussing Dhallan I, of which Dhallan II is a continuation-in-part). Dhallan II teaches that aneuploidy is detected by comparing the ratio for the alleles on the chromosome tested for being aneuploid to the ratio of alleles on the reference chromosome:

> The **ratio for the alleles at a heterozygous locus of interest on any chromosome can be compared to the ratio for the alleles at a heterozygous locus of interest on any other chromosome**. Ex. E at ¶ [0047].
>
> For example, 100 SNPs can be analyzed on chromosome 1. Of these 100 SNPs, assume 50 are heterozygous. The ratio of the alleles at heterozygous SNPs on chromosome 1 can be summed, and should give a ratio of approximately 50:50. Likewise, of 100 SNPs analyzed on chromosome 21, assume 50 are heterozygous. The ratio of alleles at heterozygous SNPs on chromosome 21 is summed. With a normal number of chromosomes, the ratio should be approximately 50:50, and thus there should be no difference between the ratio obtained from chromosome 1 and 21. However, if there is an additional copy of chromosome 21, an additional allele will be provided, and the ratio should be approximately 66:33. **Thus, the ratio for nucleotides at heterozygous SNPs can be used to detect the presence or absence of chromosomal abnormalities**. Any chromosomal abnormality can be detected including **aneuploidy**, polyploidy, inversion, a trisomy, a monosomy, duplication, deletion, deletion of a part of a chromosome, addition, addition of a part of chromosome, insertion, a fragment of a chromosome, a region of a chromosome, chromosomal rearrangement, and translocation. The method is especially useful for the detection of trisomy 13, trisomy 18, trisomy 21, XXY, and XYY. *Id.* at ¶ [0379].
>
> In one embodiment, one of the chromosomes used in the

> comparison can be chromosome 13, 15, 16; 18, 21, 22, X or Y. In a preferred embodiment, **the ratios on chromosomes 13, 18, and 21 are compared**. *Id.* at ¶ [0395].
>
> A single locus of interest can be analyzed or multiple loci of interest. The intensity of the maternal allele at multiple loci of interest can be quantitated. **An average can be calculated for a chromosome and compared to the average obtained for a different chromosome**. For example, the average intensity of the maternal allele and the fetal allele inherited from the mother at chromosome 1 can be compared to the average intensity of the maternal allele and the fetal allele inherited from the mother at chromosomes 13, 18, or 21. In a preferred embodiment, chromosomes 13, 15, 18, 21, 22, X and Y, when applicable, are compared. *Id.* at ¶ [0414].
>
> There is no difference in the amount of fetal DNA from one chromosome to another. For instance, the percentage of fetal DNA in any given individual from chromosome 1 is the same as the percentage of fetal DNA from chromosome 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, X and Y. **Thus, the allele ratio calculated for SNPs on one chromosome can be compared to the allele ratio for the SNPs on another chromosome**. For example, the allele ratio for the SNPs on chromosome 1 should be equal to the allele ratio for the SNPs on chromosomes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, X, and Y. However**, if the fetus has a chromosomal abnormality, including but not limited to a trisomy or monosomy, the ratio for the chromosome that is present in an abnormal copy number will differ from the ratio for the other chromosomes**. *Id.* at ¶¶ [1022] – [1023].

See also Id., citing also Ex. E at Abstract, and ¶¶ [0047], [0395], [0378] and [0414].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 1 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 2

Claim 2 of the '430 patent recites "wherein for each of the plurality of maternal blood samples determining the presence or absence of a fetal aneuploidy comprises comparing the number of enumerated sequence reads corresponding to the first chromosome tested for being aneuploid with the number of enumerated sequence reads corresponding to the reference chromosome." As discussed above in connection with claim 1(f), Dhallan II teaches that aneuploidy is detected by comparing the ratio for the alleles on the chromosome tested for being aneuploid to the ratio of alleles on the reference chromosome. That discussion is incorporated herein by reference. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 2. Ex. O at ¶¶ 233-234.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 2 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 3

Claim 3 of the '430 patent recites "wherein said plurality of non-random polynucleotide sequences comprises at least 300 different non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and at least 300 different non-random polynucleotide sequences selected from the reference chromosome." Dhallan II teaches the sequencing of up to tens of thousands of locations (loci) of interest, each of which contains non-random polynucleotide sequences:

> By a "locus of interest" is intended a selected region of nucleic acid that is within a larger region of nucleic acid. A locus of interest can include but is not limited to 1-100, 1-50, 1-20, or 1-10 nucleotides, preferably 1-6, 1-5, 1-4, 1-3, 1-2, or 1 nucleotide(s).

> Ex. E at ¶ [0177].
>
> In one embodiment, the sequence of alleles of one to tens to hundreds to thousands of loci of interest on a single chromosome on template DNA is determined. In a preferred embodiment, **the sequence of alleles of one to tens to hundreds to thousands of loci of interest on multiple chromosomes is determined**. *Id.* at ¶ [0059].
>
> Alternatively, 2, 3, 4, 5, 6, 7, 8, 9, 10-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-100, 100-250, 250-500, **500-1,000, 1,000-2,000, 2,000-3,000, 3,000-5,000, 5,000-10,000, 10,000-50,000 or more than 50,000 loci of interest can be analyzed at the same time** when a global genetic screening is desired. Such a global genetic screening might be desired when using the method of the invention to provide a genetic fingerprint to identify an individual or for SNP genotyping. *Id.* at ¶ [0226].

See also Ex. E at ¶¶ [0385] and [0406]. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 3. Ex. O at ¶¶ 235-236.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 3 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 4

Claim 4 of the ‘430 patent recites “wherein said plurality of non-random polynucleotide sequences comprises at least 500 different non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and at least 500 different non-random polynucleotide sequences selected from the reference chromosome.” This differs from claim 3 only in that it recites 500 sequences instead of 300. As explained above in connection with claim 3, Dhallan II teaches the sequencing of up to tens of thousands of locations (loci) of interest, each of which contains non-random polynucleotide sequences. Ex. E at ¶¶ [0059], [0226],

[0385] and [0406]. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 4. Ex. O at ¶¶ 237-238.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 4 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 5

Claim 5 of the '430 patent recites "wherein each of said plurality of non-random polynucleotide sequences is from 10 to 500 nucleotide bases in length." Dhallan II teaches that the each locus of interest includes up to 100 nucleotides:

> By a "locus of interest" is intended a selected region of nucleic acid that is within a larger region of nucleic acid. A locus of interest can include but is not limited to 1-100, 1-50, 1-20, or 1-10 nucleotides, preferably 1-6, 1-5, 1-4, 1-3, 1-2, or 1 nucleotide(s). Ex. E at ¶ [0177].

Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 5. Ex. O at ¶¶ 239-240.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 5 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 6

Claim 6 of the '430 patent recites "wherein each of said plurality of non-random polynucleotide sequences is from 50 to 150 nucleotide bases in length." Dhallan II teaches that the each locus of interest includes up to 100 nucleotide bases:

> By a "locus of interest" is intended a selected region of nucleic acid that is within a larger region of nucleic acid. A locus of interest can include but is not limited to 1-100, 1-50, 1-20, or 1-10 nucleotides, preferably 1-6, 1-5, 1-4, 1-3, 1-2, or 1 nucleotide(s). Ex. E at ¶ [0177].

Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 6. Ex. O at ¶¶ 241-242.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 6 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 7

Claim 7 of the '430 patent recites "wherein said first chromosome tested for being aneuploid is selected from the group consisting of chromosome 13, chromosome 18, chromosome 21, chromosome X, and chromosome Y." Dhallan II teaches that its method is used to detect trisomy 13, 18, 21, XXY and XYY:

> Any chromosomal abnormality can be detected including **aneuploidy**, polyploidy, inversion, a trisomy, a monosomy, duplication, deletion, deletion of a part of a chromosome, addition, addition of a part of chromosome, insertion, a fragment of a chromosome, a region of a chromosome, chromosomal rearrangement, and translocation. The method is especially useful for the detection of **trisomy 13, trisomy 18, trisomy 21, XXY, and XYY**. Ex. E at ¶ [0379].
>
> In one embodiment, one of the chromosomes used in the comparison can be chromosome 13, 15, 16; 18, 21, 22, X or Y. In a preferred embodiment, the ratios on chromosomes 13, 18, and 21 are compared. *Id.* at ¶ [0395].
>
> A single locus of interest can be analyzed or multiple loci of interest. The intensity of the maternal allele at multiple loci of interest can be quantitated. An average can be calculated for a chromosome and compared to the average obtained for a different

> chromosome. For example, the average intensity of the maternal allele and the fetal allele inherited from the mother at chromosome 1 can be compared to the average intensity of the maternal allele and the fetal allele inherited from the mother at chromosomes 13, 18, or 21. In a preferred embodiment, chromosomes **13, 15, 18, 21, 22, X and Y**, when applicable, are compared. *Id.* at ¶ [0414].

See also Ex. E at ¶¶ [0114], [0133] and [0384]. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 7. Ex. O at ¶¶ 243-244.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 7 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 8

Claim 8 of the '430 patent recites "wherein said fetal aneuploidy comprises fetal aneuploidy of a chromosome selected from the group consisting of chromosome 13, chromosome 18, chromosome 21, chromosome X, and chromosome Y." This limitation is met by the same disclosure discussed above in connection with claim 7. *See also* Ex. O at ¶¶ 245-246.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 8 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 9

Claim 9 of the '430 patent recites "wherein said fetal aneuploidy is selected from the group consisting of trisomy 21, trisomy 18, trisomy 13, and monosomy X." This limitation is

met by the same disclosure discussed above in connection with claim 7. *See also* Ex. O at ¶¶ 247-248.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 9 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 10

Claim 10 of the '430 patent recites "wherein said reference chromosome is selected from the group consisting of chromosome 1, chromosome 2, chromosome 3, chromosome 13, chromosome 18, and chromosome 21." Dhallan II teaches that the comparator chromosomes may be chromosomes 13, 15, 18 and 21:

> In embodiments, the ratio for alleles at heterozygous loci of interest on a chromosome are summed and compared to the ratio for alleles at heterozygous loci of interest on a different chromosome, where a difference in ratios indicates the presence of a chromosomal abnormality. In some of these embodiments, the chromosomes that are compared are human chromosomes such as chromosome 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, X, or Y. Ex. E at ¶ [0047].
>
> The ratio of alleles at heterozygous loci of interest on a chromosome can be compared to the ratio for alleles at heterozygous loci of interest on a different chromosome. For example, the ratio for multiple loci of interest on chromosome 1 (the ratio at SNP 1, SNP 2, SNP 3, SNP 4, etc.) can be compared to the ratio for multiple loci of interest on chromosome 21 (the ratio at SNP A, SNP B, SNP C, SNP D, etc.). **Any chromosome can be compared to any other chromosome. There is no limit to the number of chromosomes that can be compared.** *Id.* at ¶ [0402].
>
> A single locus of interest can be analyzed or multiple loci of interest. The intensity of the maternal allele at multiple loci of interest can be quantitated. An average can be calculated for a chromosome and compared to the average obtained for a different chromosome. For example, the average intensity of the maternal

> allele and the fetal allele inherited from the mother at chromosome 1 can be compared to the average intensity of the maternal allele and the fetal allele inherited from the mother at chromosomes 13, 18, or 21. In a preferred embodiment, chromosomes **13, 15, 18, 21, 22, X and Y**, when applicable, are compared. *Id.* at ¶ [0414].

See also Ex. E at ¶¶ [0061], [0062], [0063] and [0394]. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 10. Ex. O at ¶¶ 249-250.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 10 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 11

Claim 11 of the '430 patent recites "wherein said fetal aneuploidy comprises monosomy, trisomy, tetrasomy, or pentasomy of the first chromosome." Dhallan II teaches that the genetic disorders detected include monosomy and trisomy:

> The present invention is directed to a method for the detection of genetic disorders including chromosomal abnormalities and mutations. The present invention provides a rapid, non-invasive method for determining the sequence of DNA from a fetus. The method is especially useful for detection of chromosomal abnormalities in a fetus including translocations, transversions**, monosomies, trisomies, and other aneuplodies**, deletions, additions, amplifications, translocations and rearrangements. Ex. E at ¶ [0003].

> The invention is directed to a method for detection of genetic disorders including mutations and chromosomal abnormalities. In a preferred embodiment, the present invention is used to detect mutations, and chromosomal abnormalities including but not limited to translocation, transversion, **monosomy, trisomy, and other aneuploidies**, deletion, addition, amplification, fragment, translocation, and rearrangement. *Id.* at ¶ [0032].

> The present invention provides a method for detecting genetic disorders, including but not limited to mutations, insertions, deletions, and chromosomal abnormalities, and is especially useful for the detection of genetic disorders of a fetus. The method is especially useful for detection of a translocation, addition, amplification, transversion, inversion, **aneuploidy, polyploidy, monosomy, trisomy**, trisomy 21, trisomy 13, trisomy 14, trisomy 15, trisomy 16, trisomy 18, trisomy 22, triploidy, tetraploidy, and sex chromosome abnormalities including but not limited to XO, XXY, XYY, and XXX. The method also provides a non-invasive technique for determining the sequence of fetal DNA and identifying mutations within the fetal DNA. *Id.* at ¶ [0133].

See also Ex. E at ¶¶ [0009] and [0182]. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 11. Ex. O at ¶¶ 251-252.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 11 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 12

Claim 12 of the '430 patent recites "wherein said selectively enriching of (b) comprises performing polymerase chain reaction (PCR) amplification." Dhallan II teaches amplification by PCR:

> In one embodiment, the amplification can comprise **polymerase chain reaction (PCR)**. Ex. E at ¶ [0048].
>
> "Amplified" DNA is DNA that has been "copied" once or multiple times, e.g. by **polymerase chain reaction**. *Id.* at ¶ [0212].
>
> The template DNA can be **amplified using any suitable method known in the art including but not limited to PCR (polymerase chain reaction)**, 3SR (self-sustained sequence reaction), LCR (ligase chain reaction), RACE-PCR (rapid amplification of cDNA ends), PLCR (a combination of polymerase chain reaction and ligase chain reaction), Q-beta phage amplification (Shah et al., J. Medical Micro. 33: 1435-41 (1995)), SDA (strand displacement

> amplification), SOE-PCR (splice overlap extension PCR), and the like. *Id.* at ¶ [0283].

See also Ex. E at ¶¶ [0447] and [0473]. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 12. Ex. O at ¶¶ 253-255.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 12 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 13

Claim 13 of the '430 patent recites "wherein for each fetal and maternal cell-free genomic DNA sample PCR amplification comprises **hybridizing at least two oligonucleotides** to each of the at least 100 different non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and each of the at least 100 different non-random polynucleotide sequences selected from the reference chromosome." Dhallan II discloses the use of primer pairs, which in use are hybridized to the loci of interest:

> In one embodiment, one **primer pair** is used for each locus of interest. However, multiple primer pairs can be used for each locus of interest. Ex. E at ¶ [0236].
>
> A "primer pair" is intended a pair of forward and reverse primers. Both primers of a **primer pair** anneal in a manner that allows extension of the primers, such that the extension results in amplifying the template DNA in the region of the locus of interest. *Id.* at ¶ [0231].
>
> FIG. 1A. A Schematic diagram depicting a double stranded DNA molecule. **A pair of primers**, depicted as bent arrows, flank the locus of interest, depicted as a triangle symbol at base N14. *Id.* at ¶ [0082].

See also Ex. E at ¶¶ [0092], [0127], [0287], [0447] and [0473]. Dr. Rosenberg confirms that one

of skill in the art would understand that Dhallan II teaches the recitation of claim 13. Ex. O at ¶¶ 256-258.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 13 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 14

Claim 14 of the '430 patent recites "wherein said oligonucleotides do not hybridize to non-random polynucleotide sequences comprising one or more polymorphisms." Dhallan II is drawn to selective amplification and analysis of loci in chromosomes, so the Dhallan II sequences of interest are non-random. A skilled artisan would assume that if in some embodiments the loci of interest are suspected of containing a single nucleotide polymorphism, then in some embodiments at least one of the loci of interest does not contain a single nucleotide polymorphism. Ex. O at ¶ 261. Accordingly, some of the loci of interest that are analyzed in the Dhallan II aneuploidy detection methods would *not* contain polymorphisms, *i.e.*, are homozygous between the fetus and the mother. The primers used for analysis of the homozygous SNPs would thus not hybridize to non-random polynucleotide sequences comprising one or more polymorphisms, as they would hybridize to loci that were the same between the mother and fetus:

> For example, 100 SNPs can be analyzed on chromosome 1. **Of these 100 SNPs, assume 50 are heterozygous**. The ratio of the alleles at heterozygous SNPs on chromosome 1 can be summed, and should give a ratio of approximately 50:50. Likewise, of 100 SNPs analyzed on chromosome 21, assume 50 are heterozygous. The ratio of alleles at heterozygous SNPs on chromosome 21 is summed. With a normal number of chromosomes, the ratio should be approximately 50:50, and thus there should be no difference

> between the ratio obtained from chromosome 1 and 21. Ex. E at ¶ [0379].
>
> For example, if 100 maternal loci of interest on chromosome 21 and 100 maternal loci of interest on chromosome 1 are analyzed, one would predict approximately **50 loci of interest on each chromosome to be homozygous and 50 to be heterozygous**. The 50 homozygous loci of interest, or the 50 heterozygous loci of interest or the 50 homozygous and 50 heterozygous loci of interest, or any combination of the homozygous and heterozygous loci of interest on each chromosome can be analyzed using the template DNA from the sample from the pregnant female. *Id.* at ¶ [0384].
>
> Further, Dhallan II discloses that in some embodiments the primers anneal upstream and downstream of the loci of interest. The sequence of the 3' end of the primers is such that the primers anneal at a desired distance upstream and downstream of the locus of interest. *Id.* at ¶ [0083].
>
> The sequence of the SNP-IT primer, which is designed to anneal immediately upstream of the SNP site, is the best sequence available from between the upper and the lower strands. *Id.* at ¶ [1497].

Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 14. Ex. O at ¶¶ 259-261.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 14 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 15

Claim 15 of the '430 patent recites "wherein each of said oligonucleotides has a substantially similar melting temperature." Dhallan II discloses that the primers may have the same melting temperature:

> Primers can be prepared by a variety of methods including but not limited to cloning of appropriate sequences and direct chemical synthesis using methods well known in the art (Narang et al.,

> Methods Enzymol. 68:90 (1979); Brown et al., Methods Enzymol. 68:109 (1979)). Primers can also be obtained from commercial sources such as Operon Technologies, Amersham Pharmacia Biotech, Sigma, and Life Technologies. The primers can have an **identical melting temperature**. The lengths of the primers can be extended or shortened at the 5' end or the 3' end to produce primers with desired melting temperatures. Ex. E at ¶ [0232].

Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 15. Ex. O at ¶¶ 262-263.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 15 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 16

Claim 16 of the '430 patent recites "wherein said massively parallel sequencing generates at least 30 nucleotide bases per sequence read." Hamady teaches this limitation:

> After removal of low-quality sequences and trimming of primer sequences, 437,544 sequences remained, **each representing between ~240–280 bases of 16S rRNA sequence**. Ex. I at 3.

Dr. Rosenberg confirms that one of skill in the art would understand that Hamady teaches the recitation of claim 16. Ex. O at ¶¶ 264-265.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 16 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 17

Claim 17 of the '430 patent recites "wherein said fetal aneuploidy comprises partial monosomy or partial trisomy." Partial monosomy means that part of a chromosome is missing and partial trisomy means that there is an extra copy of part of a chromosome. Ex. O at ¶ 267. Dhallan II teaches methods for detecting partial monosomy and partial trisomy.

> The term "chromosomal abnormality" refers to a deviation between the structure of the subject chromosome and a normal homologous chromosome. The term "normal" refers to the predominate karyotype or banding pattern found in healthy individuals of a particular species. A chromosomal abnormality can be numerical or structural, and includes but is not limited to aneuploidy, polyploidy, inversion, a trisomy, a monosomy, duplication, deletion, **deletion of a part of a chromosome, addition, addition of a part of chromosome**, insertion, a fragment of a chromosome, a region of a chromosome, chromosomal rearrangement, and translocation. Ex. E at ¶ [0182].

See also Ex. E at Abstract, and ¶¶ [0003], [0032] and [0133]. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 17. Ex. O at ¶¶ 266-267.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 17 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claim 18

Claim 18 of the '430 patent recites "wherein said plurality of non-random polynucleotide sequences comprises no more than 1000 different non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and no more than 1000 different non-

random polynucleotide sequences selected from the reference chromosome." Dhallan II teaches analyzing 1000 or less loci of interest:

> Alternatively, 2**, 3, 4, 5, 6, 7, 8, 9, 10-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-100, 100-250, 250-500, 500-1,000**, 1,000-2,000, 2,000-3,000, 3,000-5,000, 5,000-10,000, 10,000-50,000 or more than 50,000 loci of interest can be analyzed at the same time when a global genetic screening is desired. Such a global genetic screening might be desired when using the method of the invention to provide a genetic fingerprint to identify an individual or for SNP genotyping. Ex. E at ¶ [0226].
>
> Any number of loci of interest can be analyzed on the template DNA from the sample from the pregnant female. For example, 1, 1-5, 5-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, **100-150, 150-200, 200-250, 250-300, 300-500, 500-1000**, 1000-2000, 2000-3000, 3000-4000 or more than 4000 homozygous maternal loci of interest can be analyzed in the template DNA from the sample from the pregnant female. In a preferred embodiment, multiple loci of interest on multiple chromosomes are analyzed. *Id.* at ¶ [0385].
>
> Any number of loci of interest can be analyzed including but not limited to 1, 1-5, 5-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 1**00-150, 150-200, 200-250, 250-300, 300-500, 500-1000**, 1000-2000, 2000-3000, 3000-4000, 4000-8000, 8000-16000, 16000-32000 or greater than 32000 loci of interest. *Id.* at ¶ [0406].

See also Ex. E at ¶ [0059]. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan II teaches the recitation of claim 18. Ex. O at ¶¶ 268-269.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 18 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Independent Claim 19

Independent claim 19 is identical to independent claim 1 except that claim 19 i) uses the

term "chromosome control region" instead of "reference chromosome" and ii) uses the term "at least one chromosome region tested for being aneuploidy" instead of "a first chromosome tested for being aneuploidy." As noted above in Sections V.C and V.E, the Board interpreted "chromosome control region" as meaning "a chromosome region that is different from the claimed one chromosome region tested." Dr. Rosenberg likewise interprets that claims 1 and 19 have similar scope under the broadest reasonable interpretation, noting the '430 specification uses the terms "reference chromosome" and "chromosome control region" interchangeably. Ex. O at ¶ 32.

Moreover, Dhallan II teaches the use of loci on particular chromosomes, which are *de facto* chromosome regions. Thus, the use of loci on a reference chromosome in Dhallan II is essentially the use of particular chromosome regions on that chromosome for determination of fetal aneuploidy. Ex. O at ¶ 160.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claim 19 is shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

Regarding Dependent Claims 20-30

Dependent claims 20-30 correspond to dependent claims 2-3, 5, 7-10, 11-13 and 18, respectively. The discussion set forth above in connection with claims 2-3, 5, 7-10, 11-13 and 18 applies with equal force to claims 20-30. Dr. Rosenberg concurs in that assessment. Ex. O at ¶¶ 304-326.

Additional correspondence between these claims and the cited references is shown in the claim chart submitted herewith as Exhibit W.

Thus, claims 20-30 are shown to be rendered obvious by the combination of Dhallan II, Parameswaran and Hamady.

C. Dhallan I in Combination with Binladen Renders Obvious Claims 1-30 of the '430 Patent Under (pre-AIA) 35 U.S.C. § 103(a)

As discussed above, Dhallan I and Binladen are prior art against the '430 patent under 35 U.S.C. § 102(b). As shown by the detailed claim-by-claim and limitation-by-limitation analyses below, Dhallan I in combination with Binladen discloses each of the limitations set forth in claims 1-30 of the '430 patent. Therefore, claims 1-30 are unpatentable under 35 U.S.C. § 103(a) as being obvious over Dhallan I in combination with Binladen.

The PTAB's Final Written Decision commented on the compatibility of Binladen's multiplexing technique with the exemplified sequencing method of Dhallan but did not make any findings as to whether Dhallan's sequencing method could be replaced with multiplexed massively parallel sequencing, as is proposed in the grounds presented herein. At pages 12-15 of the Final Written Decision the Board discusses Patent Owner's contentions that Binladen's tags could not be incorporated in the methods described in Dhallan because they would be "incompatible with the restriction digestible primers critical to the process of Dhallan['s] [examples]." Ex. L, Paper 43; Ex. M, Paper 43. However, the Board expressly refused to consider the question of whether the Dhallan's sequencing technique could simply be replaced with the commercially available multiplexed massively parallel sequencing techniques as broadly described in Binladen. *Id.* at 16-19; *Id.* at 16-19.

Regarding Independent Claim 1

Dhallan II is a continuation-in-part of Dhallan I. Dr. Rosenberg explains that, like Dhallan II, Dhallan I teaches all limitations recited in the claims except the detection method

which employs i) indexing or tagging samples from different patients so they can be processed simultaneously (i.e., multiplexing), and ii) using massively parallel sequencing to sequence the indexed samples. Ex. O at ¶ 35. Significantly, Verinata did not contest this premise during the *inter partes* reviews. Ex. L at Paper 10 and Paper 20; Ex. M at Paper 10 and Paper 20. As discussed above, both of the missing limitations were present in massively parallel sequencing systems which were commercially available by January 2010, the earliest claimed priority date. *See* Ex. C.

Dr. Rosenberg explains that at the time of filing any person skilled in DNA detection methods would have considered it routine and quite advantageous to perform the method taught by Dhallan I in 2003 with the multiplexed massively parallel sequencing technique discussed in Binladen in 2007. Ex. O at ¶¶ 56-60, 63-66 and 327. Binladen describes a technique for multiplexing on the Roche/454 massively parallel sequencing system, which was commercially available at the time Binladen was published in 2007. Ex. D at *Background*. In the ensuing years multiplexed massively parallel processing become commonplace, as evidenced by the fact that in late 2008 another commercially available massively parallel sequencing system was available with an off-the-shelf kit for multiplexing. *See* Exs F and G. This fact supports Dr. Rosenberg's opinion that any artisan having ordinary skill would have expected at the time of filing to be successful in performing the aneuploidy detection technique of Dhallan I using multiplexed massively parallel sequencing described in Binladen. Ex. O at ¶¶ 60, 64-66 and 327. The claims of the '430 patent are thus rendered obvious by Dhallan I in view of Binladen. *Id.* at ¶¶ 327-466.

More particularly, Dr. Rosenberg explains that it would have been obvious to combine Dhallan I and Binladen in the following manner. Ex. O at ¶¶56-61. Dhallan II discloses a fetal

aneuploidy determination method which has the following steps: 1) obtaining blood samples from multiple pregnant women that contain cell-free DNA; 2) isolating cell-free DNA samples from the plurality of blood samples; 3) enriching at least 100 nonrandom polynucleotides (i.e., loci of interest) from a first chromosome tested for being aneuploid and at least 100 different non-random polynucleotide sequences non-random polynucleotides from a reference chromosome in each cell-free DNA sample by amplification to create a library (*i.e.*, a preparation) that contains representative PCR products having the sequence of the non-random polynucleotides; 4) detecting the non-random polynucleotides from the first chromosome tested for being aneuploid and the reference chromosome of each library for each sample; 5) enumerating the non-random polynucleotides from the first chromosome or chromosome region tested for being aneuploid and the reference chromosome or chromosome control region of each library from each sample; and 6) determining the presence or absence of a fetal aneuploidy for each sample by comparing the enumerated non-random polynucleotides from the first chromosome or chromosome region tested for being aneuploid and the reference chromosome or chromosome control region from each sample library. *Id.* at ¶ 45. Claim 1 of the '430 patent follows the same steps except it employs a different detection technique for step (4). *Id.* at ¶ 47. As described in detail below, the detection technique of step (4) of the '430 patent was well known at the time of the filing date of the '430 patent, including the use of multiplexing on the Roche/454 platform as disclosed in Binladen. *Id*. at ¶ 57. Binladen teaches detection of amplified polynucleotides from individual samples using indexing and massively parallel sequencing. It would have been well within the ordinary skill of the art to substitute the detection technique Dhallan II with the detection technique of Parameswaran and Hamady, and a person of ordinary skill would have had a reasonable expectation of success in doing so. *Id.* at ¶¶ 58-62.

Claim 1 [preamble]: A method for determining a presence or absence of a fetal aneuploidy in a fetus for each of a plurality of maternal blood samples obtained from a plurality of different pregnant women, said maternal blood samples comprising fetal and maternal cell-free genomic DNA, said method comprising:

Dhallan I discloses a method for determining a presence or absence of a fetal aneuploidy in a fetus by taking blood samples from pregnant women which contain by cellular and non-cellular DNA. Dhallan I provides:

> In embodiments the sample is from a pregnant female. In an embodiment, the sample is obtained from a pregnant human female. In an embodiment, the sample is blood obtained from a pregnant female and, e.g., the nucleic acid is isolated from plasma obtained from blood of a pregnant female . . . Ex. C at 16:32-37.
>
> The ratio of the alleles at the loci of interest can be used to determine the presence or absence of a chromosomal abnormality. *Id.* at 68:56-60.
>
> The ratio of the alleles at the loci of interest can be used to determine the presence or absence of a chromosomal abnormality and detect a genetic disorder in the fetus. In a preferred embodiment, the maternal allele at a locus of interest is used to determine the presence or absence of a chromosomal abnormality in the fetus. *Id.* at 70:24-30.

See also Ex. O at ¶¶ 328-335, citing also Ex. E at ¶¶ [0030], [0032], [0132], [0182], [0190], [0196] and [0201].

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Claim 1[a]: (a) obtaining a fetal and maternal cell-free genomic DNA sample from each of the plurality of maternal blood samples;

This claim limitation is largely cumulative to the preamble and is likewise disclosed by Dhallan I:

> In one embodiment, the template DNA is **fetal DNA**. Fetal DNA can be obtained from sources including but not limited to **maternal blood**, maternal serum, maternal plasma, fetal cells,

> umbilical cord blood, chorionic villi, amniotic fluid, urine, saliva, cells or tissues. Ex. C at 30:28-32.
>
> In another embodiment, the template DNA contains both maternal DNA and fetal DNA. In a preferred embodiment, **template DNA is obtained from blood of a pregnant female.** *Id.* at 31:32-34.
>
> The blood sample is centrifuged to separate the plasma from the maternal cells. The plasma and maternal cell fractions are transferred to separate tubes and re-centrifuged. **The plasma fraction contains cell-free fetal DNA and maternal DNA**. *Id.* at 31:44-48.
>
> In accordance with an IRB approved study, blood samples were collected from pregnant women after informed consent had been granted. **Blood samples were received from 27 different clinical sites operating in 16 different states located throughout the U.S.** Blood samples were collected from both women carrying male and female fetuses, however, here, we report results obtained from woman carrying male fetuses, as the Y chromosome is the accepted marker when quantitating percentages of fetal DNA. *Id.* at 219:55-63.

See also Ex. E at ¶¶ [0030] and [0042]. *See also* Ex. O at ¶¶ 336-338, also citing Ex. C at 5:39-41 and 32:22-25.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

> **Claim 1[b]: selectively enriching a plurality of non-random polynucleotide sequences of each fetal and maternal cell-free genomic DNA sample of (a) to generate a library derived from each fetal and maternal cell-free genomic DNA sample of enriched and indexed fetal and maternal non-random polynucleotide sequences, wherein each library of enriched and <u>indexed</u> fetal and maternal non-random polynucleotide sequences includes an <u>indexing nucleotide sequence which identifies a maternal blood sample of the plurality of maternal blood samples</u>, wherein said plurality of non-random polynucleotide sequences comprises at least 100 different non-random polynucleotide sequences selected from a first chromosome tested for being aneuploid and at least 100 different non-random polynucleotide sequences selected from a reference chromosome, wherein the first chromosome tested for being aneuploid and the reference chromosome are different, and wherein each of said plurality of non-random polynucleotide sequences is from 10 to 1000 nucleotide bases in length**,

Dr. Rosenberg explains Dhallan I teaches every aspect of this claim element except for the limitations underlined above, which require that the samples be tagged or indexed, as was conventionally done in off-the-shelf multiplexed assays at the time of filing of the '430 patent. Ex. O at ¶¶ 42-43, 56-57 and 64. That Dhallan I teaches these elements is confirmed by Verinata's failure to contest the same during the *inter partes* reviews. Ex. L at Paper 10 and Paper 20; Ex. M at Paper 10 and Paper 20. Dhallan I teaches that

> [t]he method can be used for **determining sequences of multiple loci of interest concurrently**. The template DNA can comprise multiple loci from a single chromosome. The template DNA can comprise multiple loci from different chromosomes. The loci of interest on template DNA can be amplified in one reaction. Alternatively, each of the loci of interest on template DNA can be amplified in a separate reaction. Ex. C at 7:19-24.
>
> The original template DNA was amplified using 12 base primers that annealed to various regions on chromosome 13. One hundred different primer sets were used to amplify regions throughout chromosome 13. For each of the nine SNPs, a primer that annealed approximately 130 bases from the locus of interest and 130 bases downstream of the locus of interest were used. *Id.* at 25:1-7.
>
> By a "locus of interest" is intended a selected region of nucleic acid that is within a larger region of nucleic acid. A locus of interest can include but is not limited to **1-100**, 1-50, 1-20, or 1-10 nucleotides, preferably 1-6, 1-5, 1-4, 1-3, 1-2, or 1 nucleotide(s). *Id.* at 29:6-10.
>
> **The template DNA [maternal and fetal DNA] can be amplified using any suitable method** known in the art including but not limited to PCR (polymerase chain reaction), 3SR (self-sustained sequence reaction), LCR (ligase chain reaction), RACE-PCR (rapid amplification of cDNA ends), PLCR (a combination of polymerase chain reaction and ligase chain reaction), Q-beta phage amplification (Shah et al., J. Medical Micro. 33: 1435-41 (1995)), SDA (strand displacement amplification), SOE-PCR (splice overlap extension PCR), and the like. *Id.* at 47:38-46.
>
> The multiple primer sets will amplify the loci of interest, such that a minimal amount of template DNA is not limiting for the number of loci that can be detected. For example, if template DNA is

> isolated from a single cell or the **template DNA is obtained from a pregnant female, which comprises both maternal template DNA and fetal template DNA, low concentrations of each primer set can be used in a first amplification reaction to amplify the loci of interest**. *Id.* at 48:6 - 49:4.
>
> For example, **100 SNPs can be analyzed on chromosome 1**. Of these 100 SNPs, assume 50 are heterozygous. The ratio of the alleles at heterozygous SNPs on chromosome 1 can be summed, and should give a ratio of approximately 50:50. **Likewise, of 100 SNPs analyzed on chromosome 21, assume 50 are heterozygous**. The ratio of alleles at heterozygous SNPs on chromosome 21 is summed. With a normal number of chromosomes, the ratio should be approximately 50:50, and thus there should be no difference between the ratio obtained from chromosome 1 and 21. *Id.* at 65:59 - 66:1.
>
> For example**, if 100 maternal loci of interest on chromosome 21 and 100 maternal loci of interest on chromosome 1 are analyzed**, one would predict approximately 50 loci of interest on each chromosome to be homozygous and 50 to be heterozygous. The 50 homozygous loci of interest, or the 50 heterozygous loci of interest or the 50 homozygous and 50 heterozygous loci of interest, or any combination of the homozygous and heterozygous loci of interest on each chromosome can be analyzed using the template DNA from the sample from the pregnant female. *Id.* at 66:52-61.
>
> In one embodiment, one of the chromosomes used in the comparison can be chromosome 13, 15, 16; 18, 21, 22, X or Y. **In a preferred embodiment, the ratios on chromosomes 13, 18, and 21 are compared**. *Id.* at 68:31-34.

See Ex. O at ¶¶ 339-354, citing also Ex. C at 7:54-63, 25:1-10, 32:22-25, 47:38-40, 48:64 - 49:4 and 219:57-63. Dr. Rosenberg explains in this declaration that because the enriched and indexed products are created by a selective amplification process, the products would be **non-random** polynucleotides. Ex. O at ¶ 78.

Dhallan I does not teach the claim recitation that the libraries include "indexed" fetal and maternal non-random polynucleotide sequences or the method includes "an indexing nucleotide sequence which identifies a maternal blood sample of the plurality of maternal blood samples."

As Dr. Rosenberg explains, these limitations would be met if the 2003 Dhallan I method were simply performed on the later-developed and much more effective and cost-effective multiplexed massively parallel sequencing systems. Ex. O at ¶¶ 53-55 and 63-66. One such system and method for implementing it is disclosed in Binladen, which utilizes the Roche/454 massively parallel sequencing system to achieve multiplexed detection. Ex. D at 1, col. 1.

Binladen describes that the Roche/454 massively parallel sequencing system is useful for multiplexed massively parallel sequencing. Binladen discloses the creation of indexed libraries for different individuals, amplification of the nucleic acids in the library, and analysis using the massively parallel sequencing method of the Roche/454 massively parallel sequencing system:

> a method where initial PCR primers are 5'-tagged with short nucleotide sequences (tags) in such a way that a unique tagged primer combination can be applied to each specific DNA template source. As sequences generated by the GS20 commence at the very first position of the source DNA fragment, the tags are observed in the generated sequences. Therefore sequences can rapidly be sorted into their original template source using the tags (Figure 1). Currently, the method provides a means for the simultaneous sequencing, generation of single molecule sequences, and assignment In this paper we have overcome this problem, presenting of short (~120 bp) from homologous PCR products obtained from multiple individuals. Ex. D at 2.
>
> For purposes such as comparative genomics, mitochondrial sequencing, and population genetics, it is of interest to combine the selectivity of primer-based PCR, with the sequencing power of the GS20 platform. *Id.* at 1.

Binladen also describes the potential use and advantages of the multiplexed detection on the:

> In conclusion, we believe that this new approach **combining 5'-tagged PCR with GS20 sequencing will be of importance to a broad range of research areas** where **large-scale comparisons of homologous DNA sequences from multiple sources are needed** such as is the case in comparative genomics, population genetics, and phylogenetics. Ex. D at 8.

> [A] likely use of the technique [is] the PCR amplification and sequencing of specific genetic regions from multiple individuals of a single species. *Id.* at 3.

As discussed above in Section VII.B, other publications such as Parameswaran and Hamady had likewise utilized the Roche/454 massively parallel sequencing system prior to 2010 to perform multiplexed detection of samples from multiple individuals. The disclosure of Binladen exemplifies this technique and even provides teachings for how to optimize the index tags to increase efficiency of the system, *e.g.,* use of tetranucleotide tags instead of dinucleotide tags. Ex. D at 3, 7 and 8. Performing the assay methods of Dhallan I to detect fetal aneuploidy in multiple samples from pregnant women using multiplexing on the Roche/454 massively parallel sequencing system would require no more than the application of routine skill available to one of ordinary skill in the art. Ex. O at ¶¶ 57-60 and 63-66. Indeed, Dr. Rosenberg explains that the detection system actually utilized in Dhallan I would be completely substituted by the multiplexed detection system, and would be well within the ordinary skill in the art. *Id.* at ¶¶ 58-59. Any index optimization which may have been required for efficiency in detection of fetal aneuploidy was well within the skill of any first-year post-doctoral student working in a molecular biology laboratory. *Id.* at ¶¶ 53-55 and 62-66.

Accordingly, the methods disclosed in Binladen and multiplexing kits could have been predictably used to improve the Dhallan I method in the same manner it was used to improved techniques for targeting genomic regions in individuals from different species. Ex. O at ¶¶ 58-59 and 63-66. Because doing so would achieve the claimed subject matter, it is obvious and unpatentable. *KSR,* 550 U.S. at 417 ("[I]f a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the

same way, using the technique is obvious unless its actual application is beyond that person's skill.").

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Claim 1[c]: pooling the libraries generated in (b) to produce a pool of enriched and <u>indexed</u> fetal and maternal non-random polynucleotide sequences;

Dr. Rosenberg explains that Dhallan I teaches all aspects of this claim except for the limitation of indexing the libraries, a fact which Verinata did not contest during the *inter partes* review proceedings. Ex. L at Paper 10 and Paper 20; Ex. M at Paper 10 and Paper 20. In particular, Dhallan I teaches that

> Alternatively, to avoid competition for nucleotides and to minimize primer dimers and difficulties with annealing temperatures for primers, each locus of interest or small groups of loci of interest can be amplified in separate reaction tubes or wells, **and the products later pooled if desired**. Ex. C at 46:50-55.

As discussed above in connection with claim 1(b), the Roche/454 massively parallel sequencing system permitted the sequencing of multiple patient samples simultaneously by tagging or indexing them so that they can be pooled and processed simultaneously. Binladen discloses that

> The pooled PCR products were subsequently analysed on the GS20 platform using the complete sample preparation and analytical process, as recommended by the manufacturer (Roche). Ex. D at 5, s*ee also* Figure 1.

As also discussed above and in the declaration of Dr. Rosenberg, any skilled artisan would have readily understood that the Dhallan I aneuploidy detection method, which dates from 2004, could have been performed with greater throughput and reduced cost on the Roche/454 massively parallel sequencing system, as described in Binladen. Ex. O at ¶¶ 56-60, 63-66 and 327. Because the Roche/454 massively parallel sequencing system had been used for

multiplexed genomic analysis in the same way in multiple references available prior to the 2010 filing date of the '430 patent (Binladen, Parameswaran and Hamady) and a person of ordinary skill in the art would have recognized that it could be readily applied to the Dhallan I method, the technique is obvious. *KSR,* 550 U.S. at 417.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

> **Claim 1[d]: performing massively parallel sequencing of the pool of enriched and indexed fetal and maternal non-random polynucleotide sequences of (c) to produce sequence reads corresponding to enriched and indexed fetal and maternal non-random polynucleotide sequences of each of the at least 100 different non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and sequence reads corresponding to enriched and indexed fetal and maternal non-random polynucleotide sequences of each of the at least 100 different non-random polynucleotide sequences selected from the reference chromosome;**

As Dr. Rosenberg explains in his declaration, Dhallan I teaches all aspects of claim 1(d) except the underlined limitations, namely, that the sequencing used is massively parallel sequencing and that the libraries be tagged or indexed. Ex. O at ¶¶ 42-43 and 56-57. Again, Verinata did not contest this premise in the *inter partes* review proceedings. Ex. L at Paper 10 and Paper 20; Ex. M at Paper 10 and Paper 20.

Dhallan I teaches performing sequencing to produce sequence reads corresponding to the sequences from the chromosome suspected of being aneuploid and the sequences from the reference chromosome. Ex. O at ¶¶ 357-359. More particularly, as discussed above in connection with elements 1(a) and 1(b), Dhallan I teaches that the loci of interest may be sequenced using "[a]ny method that provides information on the sequence of a nucleic acid," and the sequence counts may be compared between the suspect chromosome and a reference chromosome:

> 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. C at 6:26-34.
>
> One hundred different primer sets were used to amplify regions throughout chromosome 13. For each of the nine SNPs, a primer that annealed approximately 130 bases from the locus of interest and 130 bases downstream of the locus of interest were used. This amplification reaction, which contained a total of 100 different primer sets, was used to amplify the regions containing the loci of interest. *Id.* at 25:3-7.
>
> In embodiments, alleles of multiple loci of interest are sequenced and their relative amounts quantitated and expressed as a ratio. In one embodiment, the sequence of alleles of one to tens to hundreds to thousands of loci of interest on a single chromosome on template DNA is determined. In another embodiment, the sequence of alleles of one to tens to hundreds to thousands of loci of interest on multiple chromosomes is determined. *Id.* at 7:9-16.
>
> Any method that provides information on the sequence of a nucleic acid can be used including but not limited to… DNA sequencing, Sanger dideoxy sequencing, DNA sequencing gels, capillary electrophoresis on an automated DNA sequencing machine . . . *Id.* at 36:6-14.

While Dhallan I does not teach that the sequencing is massively parallel or the sequences are indexed, those aspects are taught by Binladen. After Dhallan I filed his application in 2003, the Roche/454 massively parallel sequencing system, the GS20, became commercially available. Ex. O at ¶ 59. As discussed above, Binladen (Ex. D) explains that the Roche/454 massively parallel sequencing system is useful for multiplexed massively parallel sequencing for a variety of applications. The most relevant portions of Binladen are reproduced below for convenience:

> We demonstrate that this new approach enables the assignment of virtually all the generated DNA sequences to the correct source once sequencing anomalies are accounted for (miss-assignment rate <0.4%). Therefore, the method enables accurate sequencing

> and assignment of homologous DNA sequences from multiple sources in single high-throughput GS20 run. Ex. D at *Background*.
>
> The pooled PCR products were subsequently analysed on the GS20 platform using the complete sample preparation and analytical process, as recommended by the manufacturer (Roche). *Id.* at 5.

See also Ex. D at Figure 1. As discussed above, the Roche/454 massively parallel sequencing system had been used by multiple groups to detect genomic regions in multiple individuals in a multiplexed fashion. *See* Ex. O at ¶ 24. The Rosenberg declaration explains that performing the Dhallan I method on the Illumina Roche/454 massively parallel sequencing system would have involved nothing more than the application of routine skill. *Id.* at ¶¶ 56-60, 63-66 and 327.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Claim 1[e]: based on the indexing nucleotide sequence, for each of the plurality of maternal blood samples, <u>enumerating</u> sequence reads corresponding to enriched and indexed fetal and maternal non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and sequence reads corresponding to enriched and indexed fetal and maternal non-random polynucleotide sequences selected from the reference chromosome; and.

This claim element recites the step of enumerating the sequence reads from the chromosome being tested and the reference chromosome. Dhallan I teaches this limitation:

> In another aspect, the invention provides a method for detecting a chromosomal abnormality by (a) determining the sequence of alleles of a locus of interest from template DNA, and (b) quantitating the relative amount of the alleles at a heterozygous locus of interest that was identified from the locus of interest of (a), **wherein said relative amount is expressed as a ratio, and wherein said ratio indicates the presence or absence of a chromosomal abnormality**. Ex. C at 16:62 - 17:2.
>
> The **ratio for the alleles at a heterozygous locus of interest on any chromosome can be compared to the ratio for the alleles at a heterozygous locus of interest on any other chromosome**. *Id.* at 74:66 - 74:2.

> The present invention provides a method to quantitate a ratio for the alleles at a heterozygous locus of interest. The loci of interest include but are not limited to single nucleotide polymorphisms, mutations. *Id.* at 66:14-17.

Ex. O at ¶¶ 360-362. Verinata did not contest during the *inter partes* review proceedings that Dhallan I taught this limitation. Ex. L at Paper 10 and Paper 20; Ex. M at Paper 10 and Paper 20.

Binladen teaches that the sequence reads from each of the individual sources that had been pooled for sequencing are enumerated for further analysis:

> We demonstrate that this new approach enables the assignment of virtually all the generated DNA sequences to the correct source once sequencing anomalies are accounted for (miss-assignment rate <0.4%). **Therefore, the method enables accurate sequencing and assignment of homologous DNA sequences from multiple sources in single high-throughput GS20 run**. Ex. D at *Background*.
>
> Subsequently, the **identity of the remaining sequences were globally aligned to the thirteen reference sequences** (Sanger-sequencing generated) using direct and reverse complementation. *Id.* at 5.

See also Ex. O at ¶ 365, explaining that the combination of Dhallan I and Binladen teaches enumerating sequences reads for a first chromosome tested for being aneuploid and a reference chromosome.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

> **Claim 1[f]: for each of the plurality of maternal blood samples, determining the presence or absence of a fetal aneuploidy comprising using a number of enumerated sequence reads corresponding to the first chromosome and a number of enumerated sequence reads corresponding to the reference chromosome of (e).**

Dhallan I discloses the step of determining whether the sample contains a fetal aneuploidy by using the enumerated or tallied sequence reads. Ex. O at ¶¶ 363-365. Here again,

Verinata did not contest that Dhallan I discloses this element during the *inter partes* review proceedings. Ex. L at Paper 10 and Paper 20; Ex. M at Paper 10 and Paper 20. Dhallan I teaches that aneuploidy is detected by comparing the ratio for the alleles on the chromosome tested for being aneuploid to the ratio of alleles on the reference chromosome:

> The method comprises determining the sequence of alleles of a locus of interest, and quantitating a ratio for the alleles at the locus of interest, wherein the ratio indicates the presence or absence of a chromosomal abnormality. Ex. C at Abstract.
>
> The ratio for the alleles at a heterozygous locus of interest on any chromosome can be compared to the ratio for the alleles at a heterozygous locus of interest on any other chromosome. *Id.* at 7:34-37.
>
> For example, 100 SNPs can be analyzed on chromosome 1. Of these 100 SNPs, assume 50 are heterozygous. The ratio of the alleles at heterozygous SNPs on chromosome 1 can be summed, and should give a ratio of approximately 50:50. Likewise, of 100 SNPs analyzed on chromosome 21, assume 50 are heterozygous. The ratio of alleles at heterozygous SNPs on chromosome 21 is summed. With a normal number of chromosomes, the ratio should be approximately 50:50, and thus there should be no difference between the ratio obtained from chromosome 1 and 21. However, if there is an additional copy of chromosome 21, an additional allele will be provided, and the ratio should be approximately 66:33. **Thus, the ratio for nucleotides at heterozygous SNPs can be used to detect the presence or absence of chromosomal abnormalities**. Any chromosomal abnormality can be detected including **aneuploidy**, polyploidy, inversion, a trisomy, a monosomy, duplication, deletion, deletion of a part of a chromosome, addition, addition of a part of chromosome, insertion, a fragment of a chromosome, a region of a chromosome, chromosomal rearrangement, and translocation. The method is especially useful for the detection of trisomy 13, trisomy 18, trisomy 21, XXY, and XYY. *Id.* at 65:59 - 66:13.
>
> In one embodiment, one of the chromosomes used in the comparison can be chromosome 13, 15, 16; 18, 21, 22, X or Y. In a preferred embodiment, **the ratios on chromosomes 13, 18, and 21 are compared**. *Id.* at 68:31-34.

Comparing the ratio for the alleles on the chromosome tested for being aneuploid to the ratio of alleles on the reference chromosome constitutes determining the presence or absence of a fetal aneuploidy comprising using a number of enumerated sequence reads corresponding to the first chromosome and a number of enumerated sequence reads corresponding to the reference chromosome. *See also* Ex. O at ¶¶ 367-368.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 1 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 2

Claim 2 of the '430 patent recites "wherein for each of the plurality of maternal blood samples determining the presence or absence of a fetal aneuploidy comprises comparing the number of enumerated sequence reads corresponding to the first chromosome tested for being aneuploid with the number of enumerated sequence reads corresponding to the reference chromosome." As discussed above in connection with claim 1(f), Dhallan I teaches that aneuploidy is detected by comparing the ratio for the alleles on the chromosome tested for being aneuploid to the ratio of alleles on the reference chromosome. That discussion is incorporated herein by reference. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan I teaches the recitation of claim 2. Ex. O at ¶¶ 367-368.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 2 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 3

Claim 3 of the '430 patent recites "wherein said plurality of non-random polynucleotide sequences comprises at least 300 different non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and at least 300 different non-random polynucleotide sequences selected from the reference chromosome." Dhallan I teaches the sequencing of up to tens of thousands of locations (loci) of interest, each of which contains non-random polynucleotide sequences:

> By a "locus of interest" is intended a selected region of nucleic acid that is within a larger region of nucleic acid. A locus of interest can include but is not limited to 1-100, 1-50, 1-20, or 1-10 nucleotides, preferably 1-6, 1-5, 1-4, 1-3, 1-2, or 1 nucleotide(s). Ex. C at 29:6-10.
>
> In embodiments, alleles of multiple loci of interest are sequenced and their relative amounts quantitated and expressed as a ratio. In one embodiment, the sequence of alleles of one to tens to hundreds to thousands of loci of interest on a single chromosome on template DNA is determined. In another embodiment, the sequence of alleles of one to tens to hundreds to thousands of loci of interest on multiple chromosomes is determined. *Id.* at 7:11-16.
>
> Alternatively, 2, 3, 4, 5, 6, 7, 8, 9, 10-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-100, 100-250, 250-500, 500-1,000, 1,000-2,000, 2,000-3,000, 3,000-5,000, 5,000-10,000, 10,000-50,000 or more than 50,000 loci of interest can be analyzed at the same time when a global genetic screening is desired. *Id.* at 35:48-53.

Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan I teaches the recitation of claim 3. Ex. O at ¶¶ 369-370.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 3 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 4

Claim 4 of the '430 patent recites "wherein said plurality of non-random polynucleotide sequences comprises at least 500 different non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and at least 500 different non-random polynucleotide sequences selected from the reference chromosome." This differs from claim 3 only in that it recites 500 sequences instead of 300. As explained above in connection with claim 3, Dhallan I teaches the sequencing of up to tens of thousands of locations (loci) of interest, each of which contains non-random polynucleotide sequences. Ex. C at 29:6-10, 7:11-16 and 35:48-53. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan I teaches the recitation of claim 4. Ex. O at ¶¶ 371-372.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 4 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 5

Claim 5 of the '430 patent recites "wherein each of said plurality of non-random polynucleotide sequences is from 10 to 500 nucleotide bases in length." Dhallan I teaches that the each locus of interest includes up to 100 nucleotides:

> By a "locus of interest" is intended a selected region of nucleic acid that is within a larger region of nucleic acid. A locus of interest can include but is not limited to 1-100, 1-50, 1-20, or 1-10 nucleotides, preferably 1-6, 1-5, 1-4, 1-3, 1-2, or 1 nucleotide(s). Ex. C at 29:6-10.

Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan I teaches the recitation of claim 5. Ex. O at ¶¶ 373-375.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 5 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 6

Claim 6 of the '430 patent recites "wherein each of said plurality of non-random polynucleotide sequences is from 50 to 150 nucleotide bases in length." Dhallan I teaches that the each locus of interest includes up to 100 nucleotide bases:

> By a "locus of interest" is intended a selected region of nucleic acid that is within a larger region of nucleic acid. A locus of interest can include but is not limited to 1-100, 1-50, 1-20, or 1-10 nucleotides, preferably 1-6, 1-5, 1-4, 1-3, 1-2, or 1 nucleotide(s). Ex. C at 29:6-10.

Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan I teaches the recitation of claim 6. Ex. O at ¶¶ 376-377.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 6 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 7

Claim 7 of the '430 patent recites "wherein said first chromosome tested for being aneuploid is selected from the group consisting of chromosome 13, chromosome 18, chromosome 21, chromosome X, and chromosome Y." Dhallan I teaches that its methods are used to detect trisomy 13, 18, 21, XXY and XYY:

> Any chromosomal abnormality can be detected including **aneuploidy**, polyploidy, inversion, a trisomy, a monosomy,

> duplication, deletion, deletion of a part of a chromosome, addition, addition of a part of chromosome, insertion, a fragment of a chromosome, a region of a chromosome, chromosomal rearrangement, and translocation. The method is especially useful for the detection of **trisomy 13, trisomy 18, trisomy 21, XXY, and XYY**. Ex. C at 66:6-13.
>
> In one embodiment, one of the chromosomes used in the comparison can be chromosome 13, 15, 16; 18, 21, 22, X or Y. In a preferred embodiment, the ratios on chromosomes 13, 18, and 21 are compared. *Id.* at 68:31-34.
>
> A single locus of interest can be analyzed or multiple loci of interest. The intensity of the maternal allele at multiple loci of interest can be quantitated. An average can be calculated for a chromosome and compared to the average obtained for a different chromosome. For example, the average intensity of the maternal allele and the fetal allele inherited from the mother at chromosome 1 can be compared to the average intensity of the maternal allele and the fetal allele inherited from the mother at chromosomes 13, 18, or 21. In a preferred embodiment, chromosomes **13, 15, 18, 21, 22, X and Y**, when applicable, are compared. *Id.* at 71:51-61.

Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan I teaches the recitation of claim 7. Ex. O at ¶¶ 378-379.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 7 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 8

Claim 8 of the ‘430 patent recites “wherein said fetal aneuploidy comprises fetal aneuploidy of a chromosome selected from the group consisting of chromosome 13, chromosome 18, chromosome 21, chromosome X, and chromosome Y.” This limitation is met by the same disclosure discussed above in connection with claim 7. *See also* Ex. O at ¶¶ 380-381.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 8 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 9

Claim 9 of the '430 patent recites "wherein said fetal aneuploidy is selected from the group consisting of trisomy 21, trisomy 18, trisomy 13, and monosomy X." This limitation is met by the same disclosure discussed above in connection with claim 7. *See also* Ex. O at ¶¶ 382-383.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 9 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 10

Claim 10 of the '430 patent recites "wherein said reference chromosome is selected from the group consisting of chromosome 1, chromosome 2, chromosome 3, chromosome 13, chromosome 18, and chromosome 21." Dhallan I teaches that the comparator chromosomes may be chromosomes 13, 15, 18 and 21:

> In embodiments, the ratio for alleles at heterozygous loci of interest on a chromosome are summed and compared to the ratio for alleles at heterozygous loci of interest on a different chromosome, where a difference in ratios indicates the presence of a chromosomal abnormality. In some of these embodiments, the chromosomes that are compared are human chromosomes such as chromosome 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, X, or Y. Ex. C at 7:44-52.
>
> The ratio of alleles at heterozygous loci of interest on a

> chromosome can be compared to the ratio for alleles at heterozygous loci of interest on a different chromosome. For example, the ratio for multiple loci of interest on chromosome 1 (the ratio at SNP 1, SNP 2, SNP 3, SNP 4, etc.) can be compared to the ratio for multiple loci of interest on chromosome 21 (the ratio at SNP A, SNP B, SNP C, SNP D, etc.). **Any chromosome can be compared to any other chromosome. There is no limit to the number of chromosomes that can be compared.** *Id.* at 69:51-60.
>
> A single locus of interest can be analyzed or multiple loci of interest. The intensity of the maternal allele at multiple loci of interest can be quantitated. An average can be calculated for a chromosome and compared to the average obtained for a different chromosome. For example, the average intensity of the maternal allele and the fetal allele inherited from the mother at chromosome 1 can be compared to the average intensity of the maternal allele and the fetal allele inherited from the mother at chromosomes 13, 18, or 21. In a preferred embodiment, chromosomes **13, 15, 18, 21, 22, X and Y**, when applicable, are compared. *Id.* at 71:51-61.

Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan I teaches the recitation of claim 10. Ex. O at ¶¶ 384-385.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 10 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 11

Claim 11 of the '430 patent recites "wherein said fetal aneuploidy comprises monosomy, trisomy, tetrasomy, or pentasomy of the first chromosome." Dhallan I teaches that the genetic disorders detected include monosomy and trisomy:

> The invention is directed to a method for detection of genetic disorders including mutations and chromosomal abnormalities. In a preferred embodiment, the present invention is used to detect mutations, and chromosomal abnormalities including but not limited to translocation, transversion, **monosomy, trisomy, and other aneuploidies**, deletion, addition, amplification, fragment,

> translocation, and rearrangement. Ex. C at 5:63-6:3.
>
> The term "chromosomal abnormality" refers to a deviation between the structure of the subject chromosome and a normal homologous chromosome. The term "normal" refers to the predominate karyotype or banding pattern found in healthy individuals of a particular species. A chromosomal abnormality can be numerical or structural, and includes but is not limited to aneuploidy, polyploidy, inversion, **a trisomy, a monosomy, duplication, deletion,** deletion of a part of a chromosome, addition, addition of a part of chromosome, insertion, a fragment of a chromosome, a region of a chromosome, chromosomal rearrangement, and translocation. *Id.* at 29:38-49.
>
> The present invention provides a method for detecting genetic disorders, including but not limited to mutations, insertions, deletions, and chromosomal abnormalities, and is especially useful for the detection of genetic disorders of a fetus. The method is especially useful for detection of a translocation, addition, amplification, transversion, inversion, **aneuploidy, polyploidy, monosomy, trisomy**, trisomy 21, trisomy 13, trisomy 14, trisomy 15, trisomy 16, trisomy 18, trisomy 22, triploidy, tetraploidy, and sex chromosome abnormalities including but not limited to XO, XXY, XYY, and XXX. The method also provides a non-invasive technique for determining the sequence of fetal DNA and identifying mutations within the fetal DNA. *Id.* at 25:63 - 26:8.

See also Field of the invention. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan I teaches the recitation of claim 11. Ex. O at ¶¶ 386-387.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 11 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 12

Claim 12 of the ‘430 patent recites “wherein said selectively enriching of (b) comprises performing polymerase chain reaction (PCR) amplification.” Dhallan I teaches amplification by PCR:

> "Amplified" DNA is DNA that has been "copied" once or multiple times, e.g. by **polymerase chain reaction**. Ex. C at 33:65-66.
>
> The template DNA can be **amplified using any suitable method known in the art including but not limited to PCR (polymerase chain reaction)**, 3SR (self-sustained sequence reaction), LCR (ligase chain reaction), RACE-PCR (rapid amplification of cDNA ends), PLCR (a combination of polymerase chain reaction and ligase chain reaction), Q-beta phage amplification (Shah et al., J. Medical Micro. 33: 1435-41 (1995)), SDA (strand displacement amplification), SOE-PCR (splice overlap extension PCR), and the like. *Id.* at 47:38-46.
>
> In some embodiments, the method of amplification is PCR. *Id.* at 9:67 – 10:1.
>
> In some embodiments, the method of amplification maybe, for example, polymerase chain reaction, self-sustained sequence reaction, ligase chain reaction, rapid amplification of cDNA ends, polymerase chain reaction and ligase chain reaction, Q-beta phage amplification, strand displacement amplification, or splice overlap extension polymerase chain reaction. In some embodiments, the method of amplification is by PCR. *Id.* at 11:12-19.

See also Ex. C at 33:65-66 and 47:38-46. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan I teaches the recitation of claim 12. Ex. O at ¶¶ 388-390.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 12 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 13

Claim 13 of the '430 patent recites "wherein for each fetal and maternal cell-free genomic DNA sample PCR amplification comprises **hybridizing at least two oligonucleotides** to each of the at least 100 different non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and each of the at least 100 different non-random

polynucleotide sequences selected from the reference chromosome." Dhallan I discloses the use of primer pairs, which in use are hybridized to the loci of interest:

> In one embodiment, one **primer pair** is used for each locus of interest. However, multiple primer pairs can be used for each locus of interest. Ex. C at 37:47-49.
>
> A "primer pair" is intended a pair of forward and reverse primers. Both primers of a **primer pair** anneal in a manner that allows extension of the primers, such that the extension results in amplifying the template DNA in the region of the locus of interest. *Id.* at 36:41-45.
>
> The original template DNA was amplified using 12 base primers that annealed to various regions on chromosome 13. One hundred different primer sets were used to amplify regions throughout chromosome 13. For each of the nine SNPs**, a primer that annealed approximately 130 bases from the locus of interest and 130 bases downstream of the locus of interest were used**. *Id.* at 25:1-7.

See also Ex. C at Fig. 1A, 25:8-15 and 179:39 – 180:39. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan I teaches the recitation of claim 13. Ex. O at ¶¶ 391-393.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 13 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 14

Claim 14 of the '430 patent recites "wherein said oligonucleotides do not hybridize to non-random polynucleotide sequences comprising one or more polymorphisms." Dhallan I is drawn to selective amplification and analysis of loci in chromosomes, so the Dhallan I sequences of interest are non-random. A skilled artisan would assume that if in some embodiments the loci

of interest are suspected of containing a single nucleotide polymorphism, it is estimated that 50% of the loci of interest will not contain a single nucleotide polymorphism. In particular, Dhallan I teaches:

> For example, 100 SNPs can be analyzed on chromosome 1. **Of these 100 SNPs, assume 50 are heterozygous**. The ratio of the alleles at heterozygous SNPs on chromosome 1 can be summed, and should give a ratio of approximately 50:50. Likewise, of 100 SNPs analyzed on chromosome 21, assume 50 are heterozygous. The ratio of alleles at heterozygous SNPs on chromosome 21 is summed. With a normal number of chromosomes, the ratio should be approximately 50:50, and thus there should be no difference between the ratio obtained from chromosome 1 and 21. Ex. C at 65:59 - 66:1.
>
> For example, if 100 maternal loci of interest on chromosome 21 and 100 maternal loci of interest on chromosome 1 are analyzed, one would predict approximately **50 loci of interest on each chromosome to be homozygous and 50 to be heterozygous**. The 50 homozygous loci of interest, or the 50 heterozygous loci of interest or the 50 homozygous and 50 heterozygous loci of interest, or any combination of the homozygous and heterozygous loci of interest on each chromosome can be analyzed using the template DNA from the sample from the pregnant female. *Id.* at 66:52-61.

Accordingly, some of the loci of interest that are analyzed in the Dhallan I aneuploidy detection methods would *not* contain polymorphisms, *i.e.*, are homozygous between the fetus and the mother. *See also* Ex. O at ¶ 395. The primers used for analysis of the homozygous SNPs would thus not hybridize to non-random polynucleotide sequences comprising one or more polymorphisms. *Id*. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan I teaches the recitation of claim 14. *Id.* at ¶¶ 394-395.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 14 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 15

Claim 15 of the '430 patent recites "wherein each of said oligonucleotides has a substantially similar melting temperature." Dhallan I discloses that the primers may have the same melting temperature:

> The primers can have an identical melting temperature. The lengths of the primers can be extended or shortened at the 5' end or the 3' end to produce primers with desired melting temperatures. Ex. C at 36:53-56.

Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan I teaches the recitation of claim 15. Ex. O at ¶¶ 396-397.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 15 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 16

Claim 16 of the '430 patent recites "wherein said massively parallel sequencing generates at least 30 nucleotide bases per sequence read." Binladen teaches that the sequences can be up to 120 bases in length:

> However, under the current status of the sequencing technology, GS20 sequencing reads are limited to approximately 120 bases, thus in this experiment the full sequence (133-141 bp including primer, species dependent) was not returned and our analyses were limited to simple discriminating using the primer at the sequence end of the product. Ex. D at 3.

Dr. Rosenberg confirms that one of skill in the art would understand that Binladen teaches the recitation of claim 16. Ex. O at ¶¶ 398-399.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 16 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 17

Claim 17 of the '430 patent recites "wherein said fetal aneuploidy comprises partial monosomy or partial trisomy." Partial monosomy means that part of a chromosome is missing and partial trisomy means that there is an extra copy of part of a chromosome. Ex. O at ¶ 401. Dhallan I teaches methods for detecting partial monosomy and partial trisomy:

> The term "chromosomal abnormality" refers to a deviation between the structure of the subject chromosome and a normal homologous chromosome. The term "normal" refers to the predominate karyotype or banding pattern found in healthy individuals of a particular species. A chromosomal abnormality can be numerical or structural, and includes but is not limited to aneuploidy, polyploidy, inversion, a trisomy, a monosomy, duplication, deletion, **deletion of a part of a chromosome, addition, addition of a part of chromosome,** insertion, a fragment of a chromosome, a region of a chromosome, chromosomal rearrangement, and translocation. Ex. C at 29:38-49.

See also Ex. C at Abstract. Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan I teaches the recitation of claim 17. Ex. O at ¶¶ 400-401.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 17 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claim 18

Claim 18 of the '430 patent recites "wherein said plurality of non-random polynucleotide sequences comprises no more than 1000 different non-random polynucleotide sequences selected from the first chromosome tested for being aneuploid and no more than 1000 different non-random polynucleotide sequences selected from the reference chromosome." Dhallan I teaches analyzing 1000 or less loci of interest:

> Alternatively, **2, 3, 4, 5, 6, 7, 8, 9, 10-20, 20-25, 25-30, 30-35, 35-40, 40-45, 45-50, 50-100, 100-250, 250-500, 500-1,000**, 1,000-2,000, 2,000-3,000, 3,000-5,000, 5,000-10,000, 10,000-50,000 or more than 50,000 loci of interest can be analyzed at the same time when a global genetic screening is desired. Such a global genetic screening might be desired when using the method of the invention to provide a genetic fingerprint to identify an individual or for SNP genotyping. Ex. C at 35:48-56.
>
> Any number of loci of interest can be analyzed on the template DNA from the sample from the pregnant female. For example, 1, 1-5, 5-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, **100-150, 150-200, 200-250, 250-300, 300-500, 500-1000**, 1000-2000, 2000-3000, 3000-4000 or more than 4000 homozygous maternal loci of interest can be analyzed in the template DNA from the sample from the pregnant female. In a preferred embodiment, multiple loci of interest on multiple chromosomes are analyzed. *Id.* at 67:1-10.
>
> Any number of loci of interest can be analyzed including but not limited to 1, 1-5, 5-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 1**00-150, 150-200, 200-250, 250-300, 300-500, 500-1000**, 1000-2000, 2000-3000, 3000-4000, 4000-8000, 8000-16000, 16000-32000 or greater than 32000 loci of interest. *Id.* at 70:37-44.

Dr. Rosenberg confirms that one of skill in the art would understand that Dhallan I teaches the recitation of claim 18. Ex. O at ¶¶ 402-403.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 18 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Independent Claim 19

Independent claim 19 is identical to independent claim 1 except that claim 19 i) uses the term "chromosome control region" instead of "reference chromosome" and ii) uses the term "at least one chromosome region tested for being aneuploidy" instead of "a first chromosome tested for being aneuploidy." As noted above in Sections V.C and V.E, the Board interpreted "chromosome control region" as "a chromosome region that is different from the claimed one chromosome region tested." Dr. Rosenberg likewise interprets that claims 1 and 19 have similar scope under the broadest reasonable interpretation, noting the '430 specification uses the terms "reference chromosome" and "chromosome control region" interchangeably. Ex. O at ¶ 32.

Moreover, Dhallan I teaches the use of loci on particular chromosomes, which are *de facto* chromosome regions. Thus, the use of loci on a reference chromosome in Dhallan I is essentially the use of particular chromosome regions on that chromosome for determination of fetal aneuploidy. Ex. O at ¶ 160.

Additional correspondence between this claim element and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claim 19 is shown to be rendered obvious by the combination of Dhallan I and Binladen.

Regarding Dependent Claims 20-30

Dependent claims 20-30 correspond to dependent claims 2-3, 5, 7-10, 11-13 and 18, respectively. The discussion set forth above in connection with claims 2-3, 5, 7-10, 11-13 and 18

applies with equal force to claims 20-30. Dr. Rosenberg concurs in that assessment. Ex. O at ¶¶ 442-466.

Additional correspondence between these claim and the cited references is shown in the claim chart submitted herewith as Exhibit X.

Thus, claims 20-30 are shown to be rendered obvious by the combination of Dhallan I and Binladen.

VIII. CONCLUSION

Based on the above, the accompanying references show that there exists at least one substantial new question of patentability as to claims 1-30 of the '430 patent. For the reasons set forth in this Request, it is respectfully requested that the *ex parte* reexamination of the '430 patent be ordered.

Respectfully submitted,

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE REEXAMINATION OF U.S.
PATENT NO. 8,318,430:
INVENTOR: Yue-Jen CHUU, et al.

GROUP: Not yet assigned

CONTROL NO: Not yet assigned

EXAMINER: Not yet assigned

FILED: Herewith

FOR: METHODS OF FETAL ABNORMALITY DETECTION

CERTIFICATE OF SERVICE

MAIL STOP *EX PARTE* REEXAM
COMMISSIONER FOR PATENTS
Alexandria, VA 22313-1450

Commissioner:

Pursuant to Requester's duty enunciated in 37 C.F.R. §1.550(f), Requester's undersigned Representative certifies that the Patent Owner has been duly served with a copy of the Request for *Ex Parte* Reexamination Transmittal Form; *Ex Parte* Request for Reexamination of U.S. Patent No. 8,318,430 with Exhibits A-X, PTO Form 1449 and a copy of this Certificate of Service. The Patent Owner has been served in the manner provided by 37 C.F.R. §1.248(a)(4). Specifically, the Patent Owner was served by first-class U.S. mail on January 8, 2016, addressed to:

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