(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 August 2012 (02.08.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/103031 A3

(51) **International Patent Classification**:
C12Q 1/68 (2006.01)

(21) **International Application Number**:
PCT/US2012/022261

(22) **International Filing Date**:
23 January 2012 (23.01.2012)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:

61/436,135	25 January 2011 (25.01.2011)	US
61/436,132	25 January 2011 (25.01.2011)	US
13/316,154	9 December 2011 (09.12.2011)	US
13/338,963	28 December 2011 (28.12.2011)	US

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) **Date of publication of the international search report**:
24 January 2013

WO 2012/103031 A3

(54) **Title**: DETECTION OF GENETIC ABNORMALITIES

(57) **Abstract**: The present invention provides assay systems and related methods for determining genetic abnormalities in mixed samples comprising cell free DNA from both normal and putative genetically atypical cells. Exemplary mixed samples for analysis using the assay systems of the invention include samples comprising both maternal and fetal cell free DNA and samples that contain DNA from normal cells and circulating cancerous cells.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/022261

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FAN H C ET AL: "Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC; US, vol. 105, no. 42, 21 October 2008 (2008-10-21), pages 16266-16271, XP002613056, ISSN: 0027-8424, DOI: 10.1073/PNAS.0808319105 [retrieved on 2008-10-06] whole document ----- -/--	1-12

[X] Further documents are listed in the continuation of Box C.

[ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 May 2012	22/08/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Leber, Thomas

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/022261

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-12

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/022261

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHIU ROSSA W K ET AL: "Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC; US, vol. 105, no. 51, 23 December 2008 (2008-12-23), pages 20458-20463, XP002620454, ISSN: 0027-8424, DOI: 10.1073/PNAS.0810641105 [retrieved on 2008-12-10] the whole document	1-12

X	R. W. K. CHIU ET AL: "Supporting Information for XP02620454 (Title: Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma)", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 105, no. 51, 10 December 2008 (2008-12-10), pages 1-17, XP055024153, ISSN: 0027-8424, DOI: 10.1073/pnas.0810641105 the whole document	1-12

A	R. W. K. CHIU ET AL: "Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study", BMJ, vol. 342, no. jan11 1, 11 January 2011 (2011-01-11), pages 1-9, XP055024134, ISSN: 0959-8138, DOI: 10.1136/bmj.c7401 the whole document	1-12

T	ANDREW B. SPARKS ET AL: "Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18", AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY, vol. 206, no. 4, 1 April 2012 (2012-04-01) , pages 319.E1-319.E9, XP055024123, ISSN: 0002-9378, DOI: 10.1016/j.ajog.2012.01.030 the whole document	

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/022261

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	ANDREW B. SPARKS ET AL: "Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy", PRENATAL DIAGNOSIS, vol. 32, no. 1, 6 January 2012 (2012-01-06), pages 3-9, XP055024132, ISSN: 0197-3851, DOI: 10.1002/pd.2922 the whole document -----	1-12

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-12

An assay system for detection of the presence or absence of a fetal chromosomal abnormality based on the relative frequencies of two or more non-polymorphic nucleic acid regions of a first genomic region and two or more non-polymorphic nucleic acid regions of a second genomic region whereby the sample is a mixed sample comprising cell free DNA.

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2. claims: 13-25

An assay system for detection of the presence or absence of a fetal aneuploidy based on the relative frequencies of two or more non-polymorphic nucleic acid regions of a first chromosome and two or more non-polymorphic nucleic acid regions of a second chromosome whereby the sample is a mixed sample comprising cell free DNA.

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3. claims: 26-38

An assay system for detection of the presence or absence of a fetal aneuploidy based on the relative frequencies of two or more nucleic acid regions of a first chromosome and two or more nucleic acid regions of a second chromosome whereby the sample is a maternal sample comprising cell free DNA.

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4. claims: 39-48

An assay system for detection of the presence or absence of a fetal aneuploidy based on the relative frequencies of two or more nucleic acid regions of a chromosome of interest and two or more nucleic acid regions of a reference chromosome whereby the sample is a maternal sample comprising maternal and fetal cell free DNA.

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5. claims: 49-60

An assay system for determining the likelihood of the presence or absence of a fetal aneuploidy based on the relative frequencies of two or more selected polymorphic nucleic acid regions of a first chromosome and two or more selected polymorphic nucleic acid regions of a second chromosome whereby the sample is a maternal sample comprising maternal and fetal cell free DNA.

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6. claims: 61-71

An assay system for determining the likelihood of the presence or absence of a fetal aneuploidy based on the relative frequencies of two or more selected polymorphic nucleic acid regions of an autosomal chromosome and quantifying the relative frequency of the alleles detected whereby the sample is a maternal sample comprising maternal and fetal cell free DNA.
