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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 October 2013 (24.10.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/159035 A3

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

(21) International Application Number:
PCT/US2013/037439

(22) International Filing Date:
19 April 2013 (19.04.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

61/635,723	19 April 2012 (19.04.2012)	US
61/700,873	13 September 2012 (13.09.2012)	US
61/798,421	15 March 2013 (15.03.2013)	US

(71) Applicant: **MEDICAL COLLEGE OF WISCONSIN, INC.** [US/US]; Office Of Technology Development, 8701 Watertown Plank Road, Milwaukee, WI 53226 (US).

(72) Inventors: **MITCHELL, Aoy, Tomita**; 13835 Stonefield Court, Elm Grove, WI 53122 (US). **MITCHELL, Michael**; 13835 Stonefield Court, Elm Grove, WI 53122 (US).

(74) Agent: **VATLAND, Janice, A.**; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210-2206 (US).

(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, BN, 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, PA, 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, 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))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

[Continued on next page]

(54) Title: HIGHLY SENSITIVE SURVEILLANCE USING DETECTION OF CELL FREE DNA

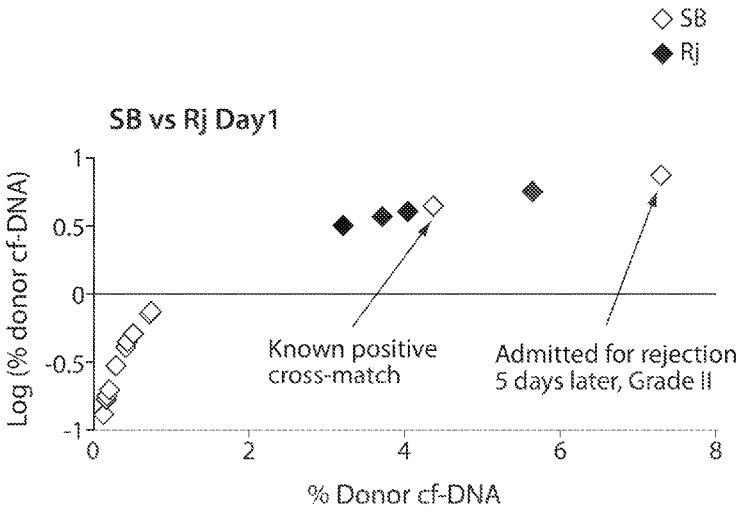

Fig. 2

(57) Abstract: Provided herein are methods and computer-readable storage media related to cell-free DNA and uses thereof to determine risk of a condition in a subject. In one embodiment, the method comprises analyzing nucleic acids from cell-free DNA extracted from a biological sample obtained from the subject to identify a plurality of loci, the nucleic acids comprising first nucleic acids of the subject and second nucleic acids not native to the subject; determining an allele of each of the plurality of loci; selecting at least one informative locus from the plurality of loci based on the determining of the allele; calculating an estimated allele frequency of a first allele at the at least one informative locus using a statistical distribution; determining an amount of cell-free DNA not native to the subject in the cell-free DNA based on the estimated allele frequency; and determining a risk in the subject.

WO 2013/159035 A3

WO 2013/159035 A3

(88) Date of publication of the international search report:
3 April 2014

Previous Correction:
see Notice of 27 December 2013

(15) Information about Correction:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/037439

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2014.01)
USPC - 435/6.1
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)
IPC(8) - C12Q 1/68; G01N 33/00; G06F 19/18 (2014.01)
USPC - 73/865.8; 435/6.1, 375, 395

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - C12Q 1/6883, 2600/112, 2600/118, 2600/156 (2013.01)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Patbase, Google Patents, Google, Pubmed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2009/0087847 A1 (LO et al) 02 April 2009 (02.04.2009) entire document	1, 9-11 --- 2-7, 48-52, 54, 55, 82-84,104-106
Y	MEHRA et al. 'Gene Expression Profiles and B-Type Natriuretic Peptide Elevation in Heart Transplantation: More Than a Hemodynamic Marker'. Circulation Vol. 114 [Suppl. I]. Pages I-21-I-26. 2006. entire document	2-7
Y	ROEDDER et al. 'Biomarkers in solid organ transplantation: establishing personalized transplantation medicine'. Genome Medicine Vol. 3:37, Pages 1-12. 08 June 2011. entire document	5
Y	SINGH et al. 'Aspergillus Infections in Transplant Recipients'. Clinical Microbiology Review Vol. 18. No. 1. Pages 44-49. January 2005. entire document	6, 7
Y	US 2010/0326218 A1 (BOECKH et al) 30 December 2010 (30.12.2010) entire document	48-52, 54, 55, 82-84, 104-106
A	SNYDER et al. 'Universal noninvasive detection of solid organ transplant rejection'. PNAS Vol. 108. No. 15. Pages 6229-6234. 12 April 2011. entire document	1-7, 9-11, 48-52, 54, 55, 82-84, 104-106
P,Y	WO 2012/122374 A2 (BRISCOE et al) 13 September 2012 (13.09.2012) entire document	1-7, 9-11, 48-52, 54, 55, 82-84, 104-106

☐ Further documents are listed in the continuation of Box C. ☐

* 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
25 September 2013	04 FEB 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/037439

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.: 8, 12-47, 53, 56-81, 85-103, 107-117, 122-133, 138-141, 147-150, 157
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 Extra 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 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.:
1-7, 9-11, 48-52, 54, 55, 82-84, 104-106

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/US2013/037439

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I: claims 1-7, 9-11, 48-52, 54, 55, 82-84, and 104-106 are drawn to a method of assessing risk and treating a subject, the method comprising analyzing cell-free DNA from a biological sample obtained from a subject, determining the amount of cell-free DNA not native to a subject and then administering therapy to the subject.

Group II: claims 118-121 are drawn to a method of evaluating a subject, comprising: calculating a value for a Predictive Model of Formula 1, Formula 2 or Formula 3.

Group III: claims 134-137, 142-146, and 151-156 are drawn to a computer-readable storage medium.

The inventions listed in Groups I-III do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I, a method of assessing risk and treating a subject, the method comprising analyzing cell-free DNA from a biological sample obtained from a subject, determining the amount of cell-free DNA not native to a subject and then administering therapy to the subject are not present in Groups II and III; the special technical features of Group II are drawn to a method of evaluating a subject, comprising: calculating a value for a Predictive Model of Formula 1, Formula 2 or Formula 3, are not present in Groups I and III; and the special technical features of Group III, a computer-readable storage medium are not present in Groups I and II.

Additionally, Groups I-III share the technical features of a method comprising determining an allele of each of a plurality of loci; selecting at least one informative locus from the plurality of loci based on the determining of the allele; calculating an estimated allele frequency of a first allele at the at least one informative locus using a statistical distribution; determining an amount of cell-free DNA not native to a subject in the cell-free DNA based on the estimated allele frequency; and determining a risk in the subject based on the determined amount of the cell-free DNA not native to the subject in the cell-free DNA; quantifying an amount of cell-free DNA extracted from a biological sample obtained from a recipient of a transplant; determining a risk of a systemic disease in the recipient of a transplant based on the determined amount of the cell-free DNA; and calculating a value for a Predictive Model of Formula 1, Formula 2 or Formula 3. However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2009/0087847 A1 to Lo et al. discloses a method comprising: determining an allele of each of a plurality of loci (wells would be expected to contain both loci, Para. [0152]; wells containing just the overrepresented allele with respect to wells containing just the reference allele, Para. [0159]; determining allelic imbalance, Para. [0162]); selecting at least one informative locus from the plurality of loci based on the determining of the allele (determining the fractional concentration of fetal DNA would be through the quantification of polymorphic [allelic] differences between the pregnant women and the fetus...targeting polymorphic [allelic] sites at which the pregnant woman is homozygous and the fetus is heterozygous, The amount of specific allele can be compared with the amount of the common allele, Para. [0278]; The proportion of the overrepresented allele among all the informative wells (Pr) was determined, Para. [0221]; "informative loci" refers to a locus where the genotype of the subject is homozygous for the major allele, while the genotype of the nucleic acid not native to the subject is homozygous or heterozygous for the minor allele, as defined by the instant invention); calculating an estimated allele frequency of a first allele at the at least one informative locus using a statistical distribution (abundance [frequency] of the A and the G alleles in the DNA sample, Para. [0076] 20 wells are positive for the A allele, 24 wells are positive for the G allele, and 28 wells are positive for both alleles. The A allele would be regarded as the reference allele because less wells are positive for this allele...mr [reference allele per well] can be calculated using the Poisson [staticitical] distribution, Para. [0299]; the number of positive wells for the two alleles would be 1: 1 and, thus, the expected proportion of informative wells, Para. [0300]); determining an amount of cell-free DNA not native to a subject in the cell-free DNA based on the estimated allele frequency (the sequencing technique on plasma cell-free DNA may be used to detect the chromosomal aberrations in the plasma DNA for the detection of a specific cancer, Para. [0280]; a second set of quantitative data indicating a second amount of a background [non-native] nucleic acid sequence different from the clinically relevant nucleic acid sequence...determining a parameter from the two data sets, Para. [0016]; the abundance [frequency] of the A and the G alleles in the DNA sample, Para. [0076]); and determining a risk in the subject based on the determined amount of the cell-free DNA not native to the subject in the cell-free DNA and quantifying an amount of cell-free DNA extracted from a biological sample obtained from a recipient of a transplant; and determining a risk in the subject based on the determined amount of the cell-free DNA not native to the subject in the cell-free DNA (a second set of quantitative data indicating a second amount of a background nucleic acid sequence [non-native] different from the clinically relevant nucleic acid sequence...the reference nucleic acid sequence is...the background nucleic acid sequence...comparing the parameter to the first cutoff value...and based on the comparison, determining a classification of whether a nucleic acid sequence imbalance [risk] exists, Para. [0016]; the sequencing technique on plasma cell-free DNA may be used to detect the chromosomal aberrations in the plasma DNA for the detection of a specific cancer, Para. [0280]; this invention...relates to the diagnostic testing of genotypes and diseases...detection of cancer and...monitoring transplantation...in a sample, Para. [0003]).

Further, WO 2011/015944 A1 to Gornik et al. discloses calculating a value for a Predictive Model of Formula 1 (Results show that cell-free DNA measured in both plasma and serum of patients with acute pancreatitis on the first day after hospital admission is significantly higher in patients who developed severe pancreatitis than in those with mild disease, Pg. 26, Lns. 15-17; Total hospital length of stay (LOS) and hospital mortality were used as outcome measures, Pg. 19, Lns. 26-28).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Lo et al. to include calculating a value for a Predictive Model of Formula 1 as taught by Gornik et al. in order to determine the risk or likelihood of developing a disease or condition.

The inventions listed in Groups I-III therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.