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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 October 2014 (02.10.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/159652 A3

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

(21) International Application Number:
PCT/US2014/024589

(22) International Filing Date:
12 March 2014 (12.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/784,429 14 March 2013 (14.03.2013) US

(71) Applicants: **MAYO FOUNDATION FOR MEDICAL EDUCATION AND RESEARCH** [US/US]; 200 First Street S.W., Rochester, Minnesota 55905 (US). **EXACT SCIENCES CORPORATION** [US/US]; 441 Charmany Drive, Madison, WI 53719 (US).

(72) Inventors: **AHLQUIST, David A.**; 1116 Kennett Lane SW, Rochester, Minnesota 55902 (US). **KISIEL, John B.**; 3418 Wright Road, SW, Rochester, Minnesota 55902 (US). **TAYLOR, William R.**; 218 N. Garden Street, Lake City, Minnesota 55041 (US). **YAB, Tracy C.**; 6371 30th Avenue NW, Rochester, Minnesota 55901 (US). **MAHONEY, Douglas W.**; 735 Riverwood Court, Elgin, Minnesota 55932 (US). **LIDGARD, Graham, P.**; 8921 Timber Wolf Lane, Madison, WI 53717 (US). **ALLAWI, Hatim, T.**; 901 Winding Way, Middleton, WI 53562 (US).

(74) Agent: **GOETZ, Robert A.**; Casimir Jones SC, 2275 Deming Way, Suite 310, Middleton, Wisconsin 53562 (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, IR, IS, JP, KE, KG, 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, SA, 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.

[Continued on next page]

(54) Title: DETECTING NEOPLASM

(57) Abstract: Provided herein is technology relating to detecting neoplasia and particularly, but not exclusively, to methods, compositions, and related uses for detecting premalignant and malignant neoplasms such as pancreatic and colorectal cancer. Accordingly, provided herein is technology for pancreatic cancer screening markers and other gastrointestinal cancer screening markers that provide a high signalto- noise ratio and a low background level when detected from samples taken from a subject (e.g., stool sample). As described herein, the technology provides a number of methylated DNA markers and subsets thereof (e.g., sets of 2, 3, 4, 5, 6, 7, 8, 9, 10, 1 1, 12 or more markers) with high discrimination for Gl neoplasms overall and/or at individual tumor sites.

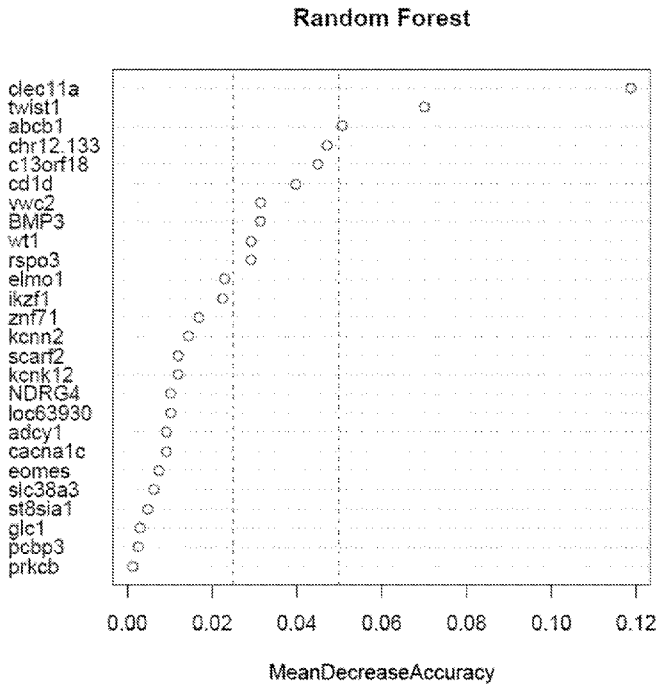

FIG. 1

WO 2014/159652 A3

(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, KM, 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))*

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

(88) Date of publication of the international search report:
27 November 2014

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/024589

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2014.01)
USPC - 435/6.11
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; C40B 40/06; G01N 33/574 (2014.01)
USPC - 435/6.11 ; 506/16

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - C12Q 1/6809, 1/6883, 1/6886, 2600/154 (2014.06)

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

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 2012/0164110 A1 (FEINBERG et al) 28 June 2012 (28.06.2012) entire document	26, 27, 35, 37, 39-42 --- 34, 36, 38
X -- Y	US 2006/0253259 A1 (FERNANDEZ) 09 November 2006 (09.11.2006) entire document	46-50, 52 --- 51
X -- Y	US 2013/0022974 A1 (CHINNAIYAN et al) 24 January 2013 (24.01.2013) entire document	45, 53-57 --- 1-7, 11-16, 20, 22-24, 28-31,43,44,51,58-61,63
Y	US 2011/0318738 A1 (JONES et al) 29 December 2011 (29.11.2011) entire document	1-7, 11-16, 20, 22-24, 28-31, 34, 36, 43, 44, 58-61, 63
Y	US 2008/0213870 A1 (CAO et al) 04 September 2008 (04.09.2008) entire document	61
Y	US 2013/0012410 (ZOU et al) 10 January 2013 (10.01.2013) entire document	38

☐ 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
04 September 2014	29 SEP 2014

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	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/US2014/024589

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs:1 and 2 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/024589

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 first Extra Sheet(s).

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, 11-16, 20, 22-31, 34-61, and 63 to the extent that they read on marker ABCB1 (DMR 11), corresponding to 87229775-87229856 of chromosome 7, and SEQ ID NOs:1 and 2.

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/US2014/024589

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 1-63 are drawn to methods, kits, and systems for screening a neoplasm in a sample obtained from a subject, and compositions comprising oligonucleotide/nucleic acid reagents for the same.

The first invention of Group I+ is restricted to methods, kits, and systems for screening a neoplasm in a sample obtained from a subject, comprising assaying a methylation state of a marker in a sample obtained from the subject, wherein the marker comprises a base in a differentially methylated region (DMR) selected to be marker ABCB1 (DMR 11, wherein DMR 11 is the first specifically named DMR to appear in the claims), corresponding to 87229775-87229856 of chromosome 7; further restricted to oligonucleotides and/or nucleic acids, and compositions and kits comprising the same, said oligonucleotides and/or nucleic acids selected to be SEQ ID NOs: 1 and 2 for the amplification of marker ABCB1 (DMR 11), corresponding to 87229775-87229856 of chromosome 7. It is believed that claims 1-7, 11-16, 20, 22-31, 34-61, and 63 read on this first named invention and thus these claims will be searched without fee to the extent that they read on marker ABCB1 (DMR 11), corresponding to 87229775-87229856 of chromosome 7, and SEQ ID NOs:1 and 2.

Applicant is invited to elect additional markers comprising DMRs and/or oligonucleotides with specified SEQ ID NO for each method, kit, system, or composition to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be methods, kits, and systems for screening a neoplasm in a sample obtained from a subject, comprising assaying a methylation state of a marker in a sample obtained from the subject, wherein the marker comprises a base in a differentially methylated region (DMR) selected to be marker ADCY1 (DMR 14), corresponding to 45613877-45614564 (forward) and 45613878-45614572 (reverse) of chromosome 7; further restricted to oligonucleotides and/or nucleic acids, and compositions and kits comprising the same, said oligonucleotides and/or nucleic acids selected to be SEQ ID NOs: 7 and 8 for the amplification of marker ADCY1 (DMR 14), corresponding to 45613877-45614564 (forward) and 45613878-45614572 (reverse) of chromosome 7. Additional markers and/or oligonucleotides will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ 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 Groups I+ formulas do not share a significant structural element, requiring the selection of alternatives for the marker "differentially methylated region (DMR) selected from a group consisting of DMR 1-107 from Table 1, DMR 1-449 from Table 10, and a DMR corresponding to Chr16 58497395-58497458" and oligonucleotide "oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 1-202".

The Groups I+ share the technical features of a method of screening for a neoplasm in a sample obtained from a subject, the method comprising: 1) assaying a methylation state of a marker in a sample obtained from a subject, and 2) identifying the subject as having a neoplasm when the methylation state of the marker is different than a methylation state of the marker assayed in a subject that does not have a neoplasm, wherein the marker comprises a base in a differentially methylated region (DMR); an oligonucleotide comprising a sequence complementary to a chromosomal region having a base in a DMR; an oligonucleotide sensitive to methylation state of a DMR; a kit and composition comprising a bisulfite reagent, and a control nucleic acid comprising a DMR and having a methylation state associated with a subject who does not have a cancer; a composition comprising a nucleic acid comprising a DMR and a methylation-sensitive restriction enzyme; a composition comprising a nucleic acid comprising a DMR and a polymerase; a method for screening for a neoplasm in a sample obtained from a subject, the method comprising: a) determining a methylation state of a marker in the sample comprising a base in a DMR, b) comparing the methylation state of the marker from the subject sample to a methylation state of the marker from a normal control sample from a subject who does not have a cancer, c) determining a confidence interval and/or a p value of the difference in the methylation state of the subject sample and the normal control sample; a method for screening for a neoplasm in a sample obtained from a subject, the method comprising reacting a nucleic acid comprising a DMR with a bisulfite reagent to produce a bisulfite-reacted nucleic acid, sequencing the bisulfite reacted nucleic acid to provide a nucleotide sequence of the bisulfite-reacted nucleic acid, comparing the nucleotide sequence of the bisulfite-reacted nucleic acid with a nucleotide sequence of a nucleic acid comprising the DMR from a subject who does not have a cancer to identify differences in the two sequences, and identifying the subject as having a neoplasm when a difference is present; a system for screening for a neoplasm in a sample obtained from a subject, the system comprising an analysis component configured to determine the methylation state of a sample, a software component configured to compare the methylation state of the sample with a control sample or a reference sample methylation state recorded in a database, and an alert component configured to determine a single value based on a combination of methylation states and alert a user of a cancer-associated methylation state. However, these shared technical features do not represent a contribution over the prior art.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/024589

Specifically, US 2013/0022974 A1 to Chinnaiyan et al. discloses a method of screening for a neoplasm in a sample obtained from a subject (methods.of screening for the presence of prostate cancer in a subject, comprising contacting a biological sample from a subject with a reagent for detecting the methylation status of one or more genes, Para. [0008]), the method comprising: 1) assaying a methylation state of a marker in a sample obtained from a subject (a biological sample from a subject with a reagent for detecting the methylation status of one or more genes ...detecting the methylation status of said genes using an in vitro assay, Para. [0008]), and 2) identifying the subject as having a neoplasm when the methylation state of the marker is different than a methylation state of the marker assayed in a subject that does not have a neoplasm, wherein the marker comprises a base in a differentially methylated region (DMR) (wherein a higher degree of methylation of said genes in said sample relative to the level of methylation in normal prostate cells in indicative of prostate cancer in said subject, Para. [0008]; 18 regions based on M-NGS data were selected and their methylation status was validated using a standard bisulfite sequencing technique ...this included fifteen differentially methylated regions (DMRs), Para. [0122]); a method for screening for a neoplasm in a sample obtained from a subject (methods of screening for the presence of prostate cancer in a subject, comprising contacting a biological sample from a subject with a reagent for detecting the methylation status of one or more genes, Para. [0008]), the method comprising: a) determining a methylation state of a marker in the sample comprising a base in a DMR (a biological sample from a subject with a reagent for detecting the methylation status of one or more genes ...detecting the methylation status of said genes using an in vitro assay, Para. [0008]; 18 regions based on M-NGS data were selected and their methylation status was validated using a standard bisulfite sequencing technique ...this included fifteen differentially methylated regions (DMRs), Para. [0122]), b) comparing the methylation state of the marker from the subject sample to a methylation state of the marker from a normal control sample from a subject who does not have a cancer (wherein a higher degree of methylation of said genes in said sample relative to the level of methylation in normal prostate cells in indicative of prostate cancer in said subject, Para. [0008]), c) determining a confidence interval and/or a p value of the difference in the methylation state of the subject sample and the normal control sample (the built-in meta-analysis tool rank orders the genelist by the p-value, which is determined by Student's t-test for comparisons made within each available dataset, for example Cancer vs. Normal, Para. [0088]); a method for screening for a neoplasm in a sample obtained from a subject (methods of screening for the presence of prostate cancer in a subject, comprising contacting a biological sample from a subject with a reagent for detecting the methylation status of one or more genes, Para. [0008]), the method comprising reacting a nucleic acid comprising a DMR with a bisulfite reagent to produce a bisulfite-reacted nucleic acid (bisulfite conversion was performed, Para. [0136]), sequencing the bisulfite reacted nucleic acid to provide a nucleotide sequence of the bisulfite-reacted nucleic acid (universally methylated control DNA and unmethylated control fetal DNA were validated by bisulfite sequencing, Para. [0025]), comparing the nucleotide sequence of the bisulfite-reacted nucleic acid with a nucleotide sequence of a nucleic acid comprising the DMR from a subject who does not have a cancer to identify differences in the two sequences, and identifying the subject as having a neoplasm when a difference is present (specific genomic regions of interest in WFDC2 gene promoter were PCR amplified and the DNA methylation status of CG residues was characterized ...the WFDC2 promoter region showed a high frequency of methylation compared to normal counterparts; four different PCR products that monitored 26 CG position in WFDC2 promoter were characterized by EpiTYPER analysis. Samples are represented in rows and CG position monitored is represented in columns. Unmethylated state and increasing degree of methylation at each position per sample is represented as a heatmap, Paras. [0136] and [0137]; wherein a higher degree of methylation of said genes in said sample relative to the level of methylation in normal prostate cells in indicative of prostate cancer in said subject, Para. [0008]); a composition comprising a nucleic acid comprising a DMR (purified DNA was used as template for PCR reactions with primers and synthesized according to bisulfite converted DNA sequences for the regions of interest ...the PCR product was gel purified and cloned into pCR4 TOPO TA sequencing vector, Para. [0100]; this included fifteen DMRs, Para. [0122]) and a methylation-sensitive restriction enzyme (methylation-sensitive
restriction enzymes, Para. [0113]).

Further, US 2006/0253259 A1 to Fernandez discloses a system for screening for a neoplasm in a sample obtained from a subject (systems-biology software, which provides diagnostic or therapeutic guidance, Abstract; detect presence and/or sequence and/or structure of any DNA molecules including profiling for changes in methylation, Para. [0051]), the system comprising an analysis component configured to determine the methylation state of a sample (detect presence and/or sequence and/or structure of any DNA molecules including profiling for changes in methylation, Para. [0051]; methylation abnormalities in the promoter CpG islands of p16, HOX A9, MAGE A1 and MAGE B2 can be detected in sputum of lung cancer patients with DNA sensor 201, Para. [0054]), a software component configured to compare the methylation state of the sample with a control sample or a reference sample methylation state recorded in a database (software to detect selected discriminatory peaks separate cancer from non-cancer groups, Para. [0066]; control parameter, Para. [0096]), and an alert component configured to determine a single value based on a combination of methylation states and alert a user of a cancer-associated methylation state (detect presence and/or sequence and/or structure of any DNA molecules including profiling for changes in methylation, Para. [0051]; methylation abnormalities in the promoter CpG islands of p16, HOX A9, MAGE A1 and MAGE B2 can be detected in sputum of lung cancer patients with DNA sensor 201, Para. [0054]; systems-biology platform 104 may alert medical professionals when host patient is determined via sense or simulation approach to detect genomic mutation, Para. [0158]).

Further, US 2012/0164110 A1 to Feinberg et al. discloses an oligonucleotide comprising a sequence complementary to a chromosomal region having a base in a DMR (the probe, primer, or primer pair, can be capable of selectively hybridizing to the DMR either with or without prior bisulfite treatment of the DMR, Para. [0110]; nucleic acid hybridization reactions ...will vary, depending on the ...degree of complementarity, Para. [0102]; a primer pair specific for methylated residues within a DMR, Para. [0097]); an oligonucleotide sensitive to methylation state of a DMR (a primer pair specific for methylated residues within a DMR. In these embodiments, selective hybridization or binding of at least one of the primers is dependent on the methylation state of the target DNA sequence, Para. [0097]); a kit (the present invention provides a kit for determining a methylation status of one or more DMRs of the invention, Para. [0110]) comprising a bisulfite reagent (the kit can provide reagents for bisulfite pyrosequencing, Para. [0111]), and a control nucleic acid comprising a DMR and having a methylation state associated with a subject who does not have a cancer (differentially methylated regions (DMRs) between B-iPSC and F-iPSC as a negative control, Para. [0044]); a composition comprising a nucleic acid comprising a DMR (a primer pair specific for methylated residues within a DMR. In these embodiments, selective hybridization or binding of at least one of the primers is dependent on the methylation state of the target DNA sequence, Para. [0097]) and a polymerase (reagents for bisulfite pyrosequencing, Para. [0111]; a nucleic acid amplification, polymerase chain reaction (PCR), methylation specific PCR, Para. [0093]; an enzyme such as T7 polymerase, Para. [0108]).

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