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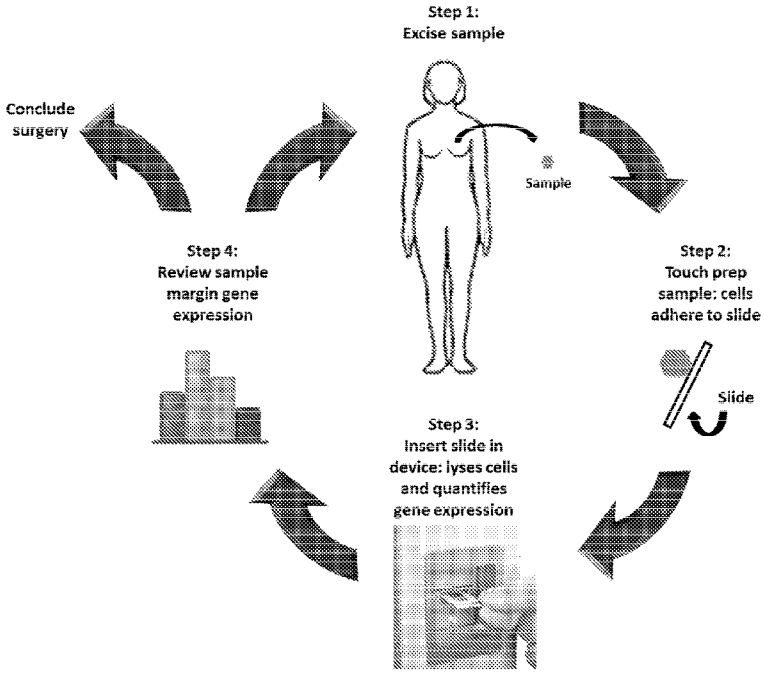
[Continued on next page]

(54) Title: METHODS, COMPOSITIONS, AND DEVICES FOR RAPID ANALYSIS OF BIOLOGICAL MARKERS

FIG. 1A



(57) Abstract: Provided herein are devices and methods for rapid analysis of biological samples. In particular, devices and methods described herein can be applied to rapid nucleic acid analysis of solid tissue samples.

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INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12M 1/00; C12Q 1/68; G06F 19/20; C12P 19/34 (2015.01)
CPC - C12Q 1/6806; C12P 19/34; G06F 19/20
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): C12M 1/00; C12Q 1/68; G06F 19/20; C12P 19/34 (2015.01)
CPC: C12Q 1/6806; C12P 19/34; G06F 19/20

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)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); Google; Google Scholar; PubMed; device, apparatus, unit, system, measure, determine, evaluate, assay, assess, expression, transcript, nucleic, 'mRNA, computer, processor, microprocessor, analzyer, indicate, diagnose, detect, condition, disease, disorder, cancer, neoplasia, malignant, tumor, sample, cell, blood, inlet, input

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2013/010134 A2 (CELULA INC.) January 17, 2013; paragraphs [0009]-[0011], [0057], [0068], [0037], [0055], [0036], [0034], [0071], [0067], [0072], [0046], [0056], [0041]; Claim 8	1-76
Y	US 2007/0213939 A1 (LIEW, CC et al.) September 13, 2007; abstract; paragraphs [0288], [0279], [0282], [0011], [0405], [0013], [0391], [0214], [0020], [0123], [0555], [0057]-[0060], [0113], [0048], [0242], [0129], [0231], [0137], [0135], [0589], [0153], [0313], [0161], [0203], [0440]	1-76
Y	US 2013/0295580 A1 (MCDEVITT, JT et al.) 7 November 2013; paragraphs [0009], [0010]	7, 61/7-76/7
Y	WICK, MR, MD et al. Diagnostic Histochemistry, Chapter 1: Tissue Procurement, Processing, And Staining Techniques. August 2008; pages 1-10; ISBN: 9780521874106; figure 1.3, page 10, paragraph 4; page 4, paragraph 2, page 2, paragraph 2.	12-15, 61/12-76/15
Y	EP 1312682 A1 (TAKARA BIO INC.) 21 May 2003; abstract; paragraphs [0010], [0011], [0012], [0021], [0024], [0029]	27, 39-44, 61/27-76/27, 61/39-76/44
Y	PARK, S et al. Advances In Microfluidic PCR For Point-Of-Care Infectious Disease Diagnostics. Biotechnol. Adv. November 2011; Vol. 29, No. 6; pages 830-839 (Author's manuscript, pages 1-24); page 7, paragraph 1; page 5, paragraph 2; page 8, paragraph 2; abstract. DOI: 10.1016/j.biotechadv.2011.06.017.	19, 20, 61/19-76/20
Y	HORVATH et al. Novel Insights Into Breast Cancer Genetic Variance Through RNA Sequencing. Scientific Reports, July 2013; Vol. 3; 2256; pages 1-13; DOI: 10.1038/srep02256. abstract, page 2, column 2, paragraph 3.	36, 37, 61/36-76/36, 61/37-76/37
Y	US 2010/0256465 A1 (BERNSTEIN, H et al.) 7 October 2010; paragraphs [0004], [0005], [0043]	55, 61/55-76/55

☒ 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
29 October 2015 (29.10.2015)	08 DEC 2015
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/36480

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:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

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 forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/36480

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:

-***-Please See Supplemental Page-***-

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.:

-***-Please See Supplemental Page-***-

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/US15/36480

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GENBANK ACCESSION NM_080282: Homo sapiens ATP-Binding Cassette, Sub-Family A (ABC1) Member 10 mRNA. 26 February 2014; pages 1-7.	37, 61/37-76/37
Y	US 6682889 B1 (WANG, SS et al.) January 27, 2004; column 12, lines 7-24	44, 61/44-76/44
Y	US 2007/0077582 A1 (SLEPNEV, VI) April 5, 2007; paragraphs [0004], [0007]	28, 61/28-76/28
Y	US 2013/0280706 A1 (JUDICE, SA) October 24, 2013; paragraphs [0081], [0054]	29, 30, 61/29-76/29, 61/30-76/30

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US15/36480

-***-Continued from Box No. III: Observations Where Unity of Invention Is Lacking:

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 must be paid.

Groups I+: Claims 1-76 are directed toward a device comprising: a) a sample input unit that receives a cellular specimen comprising a target nucleic acid; b) a nucleic acid analysis unit that measures a target nucleic acid expression level of the target nucleic acid, wherein measuring the target nucleic acid expression level comprises an isothermal amplification of the target nucleic acid; and c) a computational unit that interprets the target nucleic acid expression level as an indication of the presence or absence of a condition affecting the cellular specimen, wherein the sample input unit, nucleic acid analysis unit, and computational unit are integrated within the device.

The device will be searched to the extent that the target nucleic acid encompasses genetic locus ABCA10 (first exemplary target nucleic acid genetic locus). It is believed that Claims 1-35, 36 (in-part), 37 (in-part) and 38-76 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass this target nucleic acid genetic loc(us/i). Applicants must specify the claims that encompass any additionally elected target nucleic acid genetic loc(us/i). Applicants must further indicate, if applicable, the claims which encompass 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. An exemplary election would be: ABCA9 genetic loci

Group II: Claim 77 is directed toward a method of amplifying a target nucleic acid in a sample comprising: a. obtaining a cellular specimen that contains the target nucleic acid, wherein the obtaining comprises a touch prep method; b. contacting the target nucleic acid with an oligonucleotide that hybridizes to the target nucleic acid, a plurality of nucleotides and a polymerase.

Group III: Claim 78 is directed toward a method of amplifying a target nucleic acid in a sample comprising: a. obtaining a cellular specimen that contains the target nucleic acid, wherein the obtaining comprises a brush biopsy; b. contacting the target nucleic acid with an oligonucleotide that hybridizes to the target nucleic acid, a plurality of nucleotides and a polymerase.

Group IV: Claims 79-105 are directed toward a method of amplifying a target nucleic acid, comprising contacting the target nucleic acid with: a) an oligonucleotide designed to hybridize to the target nucleic acid, wherein the oligonucleotide: i. comprises a ribonucleotide; and ii. possesses a 3' terminal modification that prevents polymerase-mediated extension of the oligonucleotide when: either: (1) in the absence of an enzyme activity that removes the 3' terminal modification, and (2) the oligonucleotide is bound to a non-target nucleic acid.

The inventions listed as Groups I+-IV 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 Groups I + include a computational unit, which is not present in any other Group, the special technical features of Group II include a touch prep method, which is not present in any other Group; the special technical features of Group III including a brush biopsy, which is not present in any other Group; the special technical features of Group IV including a 3' terminal modification.

Groups I+-IV share the technical features including amplifying a target nucleic acid in a sample. Groups I+, II and III share the technical features including a cellular specimen. Groups II-IV share the technical features including a method of amplifying a target nucleic acid comprising: a target nucleic acid; contacting the target nucleic acid with an oligonucleotide that hybridizes to the target nucleic acid, and a polymerase. Groups II and III share the technical features including a method of amplifying a target nucleic acid in a sample comprising: a. obtaining a cellular specimen that contains the target nucleic acid, wherein the obtaining comprises a method; b. contacting the target nucleic acid with an oligonucleotide that hybridizes to the target nucleic acid, a plurality of nucleotides and a polymerase. Groups I+ share the technical features including: a device comprising: a) a sample input unit that receives a cellular specimen comprising a target nucleic acid; b) a nucleic acid analysis unit that measures a target nucleic acid expression level of the target nucleic acid, wherein measuring the target nucleic acid expression level comprises an isothermal amplification of the target nucleic acid; and c) a computational unit that interprets the target nucleic acid expression level as an indication of the presence or absence of a condition affecting the cellular specimen, wherein the sample input unit, nucleic acid analysis unit, and computational unit are integrated within the device, wherein the plurality of target nucleic acids correspond to a plurality of genetic loci located in one or more genes; and a method comprising: a) obtaining a cellular specimen containing a target nucleic acid; b) inserting the cellular specimen into a device; c) assessing a presence, absence or risk of a condition or disease in the cellular specimen; and d) directing a user of the device to perform or not perform a procedure based on a result of the assessing.

-***-Continued on Next Supplemental Page-***-

-***-Continued from Previous Supplemental Page:

However, these shared technical features are previously disclosed by US 2007/0213939 A1 to Liew, et al. (hereinafter 'Liew') in view of WO 2013/010134 A2 (CELULA, INC.) (hereinafter 'Celula').

Liew discloses amplifying a target nucleic acid (paragraph [0019]) in a sample (paragraph [0013]); including a cellular specimen (including tissue; paragraph [0030]); a method of amplifying a target nucleic acid (paragraph [0112]) comprising: a target nucleic acid (paragraphs [0019], [0112]); contacting the target nucleic acid with an oligonucleotide that hybridizes to the target nucleic acid (contacting the template nucleic acid with primers (contacting the target nucleic acid with an oligonucleotide that hybridizes to the target nucleic acid); paragraph [0112]), and a polymerase (paragraph [0112]); a method of amplifying a target nucleic acid (paragraphs [0019], [0112]) in a sample (paragraphs [0013], [0030]) comprising: a. obtaining a cellular specimen that contains the target nucleic acid (obtaining a tissue sample (cellular specimen) that contains a target nucleic acid; paragraphs [0013], [0019], [0030]); b. contacting the target nucleic acid with an oligonucleotide that hybridizes to the target nucleic acid (contacting the template (target) nucleic acid with a primer (an oligonucleotide that hybridizes to the target nucleic acid); paragraphs [0019], [0112]), a plurality of nucleotides (paragraph [0112]) and a polymerase (paragraph [0112]); a device (paragraphs [0284], [0288]), wherein measuring the target nucleic acid expression level (paragraphs [0013], [0019]) comprises an isothermal amplification of the target nucleic acid (paragraph [0347]); and c) a computational unit (paragraphs [0282]) that interprets the target nucleic acid expression level (for processing the data for diagnostic purposes (that interprets the target nucleic acid expression level); paragraph [0283]) as an indication of the presence or absence of a condition affecting the cellular specimen (to diagnose a test subject from whom the sample was obtained (as an indication of the presence or absence of a condition affecting the cellular specimen); paragraphs [0030], [0283]), wherein the plurality of target nucleic acids correspond to a plurality of genetic loci located in one or more genes (paragraph [0014]); and a method comprising: a) obtaining a cellular specimen containing a target nucleic acid (paragraphs [0013], [0019], [0030]); assessing a presence, absence or risk of a condition or disease in the cellular specimen (diagnosing a disease or condition in the cellular specimen (assessing a presence, absence or risk of a condition or disease in the cellular specimen); paragraphs [0013], [0030], [0283]); and a sample obtained may be a biopsy sample (wherein a sample obtained may be a biopsy sample; paragraph [0363]); and wherein the results of the diagnosis may be communicated to a healthcare provider or diagnostic facility (wherein the results of the diagnosis may be communicated to a healthcare provider or diagnostic facility; paragraph [0288]). Liew does not disclose wherein the obtaining comprises a method; a) a sample input unit that receives a cellular specimen comprising a target nucleic acid comprising: a nucleic acid analysis unit that measures a target nucleic acid expression level of the target nucleic acid; wherein the sample input unit, nucleic acid analysis unit, and computational unit are integrated within the device; inserting a cellular specimen into the device; and directing a user of the device to perform or not perform a procedure based on a result of the assessing. Celula discloses an analysis system (abstract) comprising a sample input unit (comprising a sample access (input) module (unit); paragraph [0036]; figure 1) that receives a cellular specimen (that receives a cell sample (specimen); paragraph [0036]) comprising a target nucleic acid (paragraph [0029]) comprising: a nucleic acid analysis unit (an imaging module (a nucleic acid analysis unit); paragraph [0069]) that measures a target nucleic acid expression level of the target nucleic acid (that detects fluorescent signals associated with the target nucleic acid (that measures a target nucleic acid expression level of the target nucleic acid); paragraph [0069]); wherein the sample input unit (paragraph [0036]), and nucleic acid analysis unit (analysis unit (nucleic acid analysis unit); paragraph [0036]) are integrated within the device (paragraph [0036]); and inserting a cellular specimen into the device (inserting a slide comprising a specimen (a cellular specimen) into the device; paragraph [0069]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Liew, for incorporating the use of a method of obtaining a sample, the method including the steps necessary to perform a biopsy, for enabling a practitioner to collect a biopsy sample, as previously disclosed by Liew, in an accurate and minimally invasive manner. Furthermore, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Liew, for incorporating directing a user of the device to perform, or not perform, a procedure based on a result of the assessing, such as directing a healthcare provider to perform anti-cancer measures, such as surgery or chemotherapy, on the basis of the diagnosis provided by the system previously disclosed by Liew, for enabling the healthcare provider to provide effective treatment to a subject that has a cancer detected by the system previously disclosed by Liew. Additionally, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Liew, for providing an integrated analysis system, including a sample input, processing and readout analysis unit, as previously disclosed by Celula, further including the computational unit previously disclosed by Liew, integrated into a single device, for obtaining a single system capable of fully processing and analyzing a sample, then obtaining a diagnosis for the sample, and communicating said information to a healthcare professional, as disclosed by Liew, for enabling the healthcare professional to provide necessary and relevant treatment to a patient that provides a sample.

Since none of the special technical features of the Groups I+-IV inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Liew and Celula references, unity of invention is lacking.