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(54) Title: SYSTEMS AND METHODS TO DETECT RARE MUTATIONS AND COPY NUMBER VARIATION

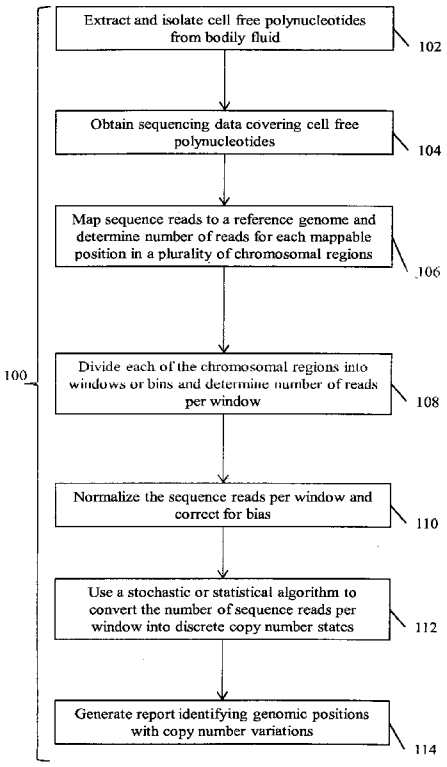

Fig. 1

(57) Abstract: The present disclosure provides a system and method for the detection of rare mutations and copy number variations in cell free polynucleotides. Generally, the systems and methods comprise sample preparation, or the extraction and isolation of cell free polynucleotide sequences from a bodily fluid; subsequent sequencing of cell free polynucleotides by techniques known in the art; and application of bioinformatics tools to detect rare mutations and copy number variations as compared to a reference. The systems and methods also may contain a database or collection of different rare mutations or copy number variation profiles of different diseases, to be used as additional references in aiding detection of rare mutations, copy number variation profiling or general genetic profiling of a disease.

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INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68; C40B 40/08; G01N 33/48 (2014.01)
USPC - 435/6.12; 506/17; 702/20
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): C12Q 1/68; C40B 40/08; G01N 33/48, 33/50 (2014.01)
USPC: 435/6.12, 6.1 1, 6.1, 4; 506/17, 13, 23, 16, 15; 702/20, 19; CPC: C12Q 1/6827, 1/6883; C07H 21/00

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)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C.B, DE-A, DE-T, DE-U, GB-A, FR-A); Proquest Dialog; PubMed/PubMed Central; Google; Google Scholar: extracellular, 'cell free,' 'DNA,' 'nucleic acid,' polynucleotide, sequence, determining, 'Copy Number variation,' 'CNV,' reads, threshold, minimum, reference, control, normalization, mapping, alignment, 'rare variant,' 'Rare mutation'

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 2012/0100548 A1 (RAVA, RP et al.) April 26, 2012; paragraphs [0007], [0022], [0045], [0064]-[0076], [0090], [0101]-[0121], [0155]-[0161], [0206]-[0229], [0276]-[0279]	50-53, 70-72, 116-124, 184 ----- 1-3, 4/1-3, 5/1-3, 6/1-3, 7/1-3, 8/1-3, 9/8/1-3, 10/8/1-3, 11/8/1-3, 12/1-3, 13/12/1-3, 14/1-3, 15/14/1-3, 16/1-3, 17/1-2,
X --- Y	US 8209130 B1 (KENNEDY, C et al.) June 26, 2012; column 3, lines 10-33; column 7, lines 20-25; column 8, lines 49-65; column 9, lines 26-44; column 11, line 63 to column 12, line 62; column 22, lines 48-67; column 23, lines 11-20	78-83, 85-96, 98-101, 103-104, 107-109, 110-115, 143-145, 146/143-145, 175/143-145, 147-148, 159, 160/147-148, 175/147-148, 175/159, 186-188, 194-195, 206 ----- 84, 97, 102, 161, 162, 175/161, 191, 199

☒ 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

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/00048

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 201 1/060240 A1 (AKMAEV, VR et al.) May 19, 201 1; [0008], [0018]-[0021], [0049]-[0055], [0079]	1-3, 4/1-3, 5/1-3, 6/1-3, 7/1-3, 8/1-3, 9/8/1-3, 10/8/1-3, 11/8/1-3, 12/1-3, 13/12/1-3, 14/1-3, 15/14/1-3, 16/1-3, 17/1-2, 18/17/1-3, 19/1-3, 20/19/1-3, 21/19/1-3, 22/1-3, 23/1-3, 24/1-3, 25/19/1-3, 26/19/1-3, 27/19/1-3, 28/19/1-3, 29/1-3, 30/29/1-3, 31/1-3, 32/31/1-3, 33/30/29/1-3, 34/33/29/1-3, 35/1-3, 36/1-3, 37/36/1-3, 38/1-3, 39, 40, 41/1-3, 42/1-3, 43/1-3, 44, 45/1-3, 46, 47/1-3, 48/45/1-3, 49/1-3, 54/1, 54/2, 55/1-3, 56/55/1-3, 57/1-3, 58/1-3, 59/1-3, 60/1-3, 61/1-3, 62/1-3, 63/1-2, 64/1-2, 65/64/1-2, 66/64/1-22, 67/64/1-2, 68/1-3, 69/1-3, 73, 74, 76/1-2, 77, 185
Y	US 2012/0059594 A1 (HATCHWELL, E et al.) March 8, 2012; paragraphs [0103], [0120]	125-133
Y	US 2010/0062494 A1 (CHURCH, GM et al.) March 11, 2010; paragraphs [0021]-[0028], [0062]	19/1-3, 20/19/1-3, 21/19/1-3, 24/1-3, 25/19/1-3, 26/19/1-3, 27/19/1-3, 28/19/1-3, 32/31/1-3, 46, 48/45/1-3
Y	LI, H et al. Mapping Short DNA Sequencing Reads And Calling Variants Using Mapping Quality Scores. Genome Res. November 2008, Vol. 18, No. 11; pages 1851-1858; page 1853, second column, fourth paragraph; page 1856, first column, fourth paragraph.	31/1-3, 32/31/1-3
Y	RAND, KN et al. Headloop Suppression PCR And Its Application To Selective Amplification Of Methylated DNA Sequences. Nucleic Acids Res. 09 August 2005, Vol. 33, No. 14, document e127; page 5, first column, second paragraph.	35/1-3
Y	FORSTER, M et al. From Next-Generation Sequencing Alignments To Accurate Comparison And Validation Of Single-Nucleotide Variants: The Pibase Software. Nucleic Acids Research. 07 January 2013. Vol. 41. No. 1. document e16; page 11, first column, second paragraph; first column, third paragraphs.	62/1-3
Y	WO 2012/106559 A1 (CRAIG, D et al.) August 9, 2012; paragraphs [0136], [0137], [0176] Y 64/1-2, 65/64/1-2, 66/64/1-2, 67/64/1-2	64/1-2, 65/64/1-2, 66/64/1-2, 67/64/1-2
Y	FREEMAN, JD et al. Profiling The T-Cell Receptor Beta-Chain Repertoire By Massively Parallel Sequencing. Genome Research. 18 June 2009, Vol. 19; pages 1817-1824. DOI: 10.1 101/gr.092924.109.	102
A	US 2012/0046877 A1 (HYLAND, F et al.) February 23, 2012; entire document	1-104, 107-133, 143-164, 175, 184-192, 194-200, 206
A	US 2012/0214678 A1 (RAVA, R et al.) August 23, 2012; entire document	1-104, 107-133, 143-164, 175, 184-192, 194-200, 206
A	SCHMITT, MW et al. Detection Of Ultra-Rare Mutations By Next-Generation Sequencing. PNAS, Proceedings of the National Academy of Sciences of the United States of America. 04 September 2012. Vol. 109. No. 36. pages 14508-14513; entire document.	1-104, 107-133, 143-164, 175, 184-192, 194-200, 206
A	TSAI, H et al. Discovery Of Rare Mutations in Populations: TILLING By Sequencing. Plant Physiology. July 201 1. Vol. 156. No. 3; pages 1257-1268; entire document.	1-104, 107-133, 143-164, 175, 184-192, 194-200, 206
P, Y	US 2013/0085681 A1 (DECIU, C et al.) April 4, 2013; entire document	1-104, 107-133, 143-164, 175, 184-192, 194-200, 206

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/00048

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.: 75, 134-142, 176-183
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. Ill 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.:

Group I: **Claims** 1-74, 76-77, 116-133, 184, 185
Group II: **Claims** 78-104, 107-115, 143-164, 175, 186-192, 194-200, 206

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.:

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/US 14/00048

-'"-Continued from Box No. Ill: 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.

Group I: Claims 1-74, 76-77, 116-133, 184 and 185 are directed toward methods comprising: a. sequencing extracellular polynucleotides from a bodily sample from a subject, wherein each of the extracellular polynucleotide generate a plurality of sequencing reads; b. filtering out reads that fail to meet a set threshold; c. mapping the sequence reads obtained from step (a), after reads are filtered out, to a reference sequence, and computer readable medium for performing said methods.

Group II: Claims 78-104, 107-1 15, 143-164, 175, 186-192, 194-200 and 206 are directed toward methods comprising: a. providing at least one set of tagged parent polynucleotides, and for each set of tagged parent polynucleotides; b. amplifying the tagged parent polynucleotides in the set to produce a corresponding set of amplified progeny polynucleotides; c. sequencing a subset (including a proper subset) of the set of amplified progeny polynucleotides, to produce a set of sequencing reads; and d. collapsing the set of sequencing reads to generate a set of consensus sequences, and a computer readable medium for performing said methods

Group III: Claims 105 and 106 are directed toward a method comprising detecting genetic variation in non-uniquely tagged initial starting genetic material with a sensitivity of at least 5%, at least 1%, at least 0.5%, at least 0.1% or at least 0.05%.

Group IV: Claims 165-174 and 193 are directed toward a method of communicating sequence information about at least one individual polynucleotide molecule, comprising: a. providing at least one individual polynucleotide molecule; b. encoding sequence information in the at least one individual polynucleotide molecule to produce a signal; and c. passing at least part of the signal through a channel to produce a received signal, wherein the received signal comprises noise and/or distortion, and a computer readable medium for performing said method.

Group V: Claims 201-205 are directed toward a composition comprising between 100 and 100,000 human haploid genome equivalents of cfDNA polynucleotides, wherein the polynucleotides are tagged.

The inventions listed as Groups I-V 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 include a. sequencing extracellular polynucleotides from a bodily sample from a subject, wherein each of the extracellular polynucleotide generate a plurality of sequencing reads, which are not present in any other Group, the special technical features of Group II including amplifying the tagged parent polynucleotides, which is not present in any other Group, the special technical features of Group III including detecting genetic variation in non-uniquely tagged initial starting genetic material with a sensitivity of at least 5%, at least 1%, at least 0.5%, at least 0.1 % or at least 0.05%, which is not present in any other Group, the special technical features of Group IV including a signal comprising noise and/or distortion, which is not present in any other Group, the special technical features of Group V including a plurality of human haploid genome equivalents, which are not present in any other Group.

Groups I-V share the technical features including polynucleotides and sequence information. Groups I-III share the technical features including detecting genetic variation. Groups I, II and IV share the technical features including a system comprising a computer readable medium comprising non-transitory machine-executable code that, upon execution by a computer processor, implements a method, the method comprising: a. accessing a data file, and performing a method. Groups I and II share the technical features including nucleic acid amplification; generating a plurality of sequence reads; filtering out reads that fail to meet a set threshold; mapping the sequence reads to a reference sequence. Groups I and V share the technical features including sequencing cell free (extracellular) polynucleotides; and genomes. Groups II and V share the technical features including tagged polynucleotides.

However, these shared technical features are previously disclosed by US 2012/0100548 A 1 to Rava, et al. (hereinafter 'Rava'). Rava discloses polynucleotides (nucleic acids (polynucleotides); abstract) and sequence information (sequence information; abstract); detecting genetic variation (determining copy number variation of a nucleic acid sequence (detecting genetic variation); abstract); a system (a computer processing system; paragraph [0221]) comprising a computer readable medium (comprising a computer readable medium; paragraphs [0219], [0220]) comprising non-transitory machine-executable code that, upon execution by a computer processor, implements a method (comprising computer-readable instructions for carrying-out a method for a computer adapted or configured to perform the method (comprising non-transitory machine-executable code that, upon execution by a computer processor, implements a method); paragraphs [0220], [0221]), the method comprising: a. accessing a data file (receiving sequencing data (accessing a data file); paragraph [0219]), and performing a method (performing a method; paragraphs [0220], [0221]); nucleic acid amplification (nucleic acid amplification; paragraph [0020]); generating a plurality of sequence reads (generating a plurality of sequence reads; paragraph [01 14]); mapping the sequence reads to a reference sequence (aligning reads to a reference genome (mapping the sequence reads to a reference sequence); paragraph [01 14]); sequencing cell free (extracellular) polynucleotides (sequencing cell free (extracellular) polynucleotides; paragraph [0021]); genomes (genomes; paragraph [0002]) and tagged polynucleotides (tagged polynucleotides; paragraphs [0008], [0059], [0076]); and the use of a threshold value for a qualifying data set that serves as a limit of diagnosis (use of a threshold value for a qualifying data set that serves as a limit of diagnosis, wherein the threshold value is exceeded in a diagnostic process; paragraph [0073]), wherein the threshold value is exceeded in a diagnostic process (use of a threshold value for a qualifying data set that serves as a limit of diagnosis, wherein the threshold value is exceeded in a diagnostic process; paragraph [0073]). Rava does not disclose filtering out reads that fail to meet a set threshold. However, based on the requirement of a threshold value being exceeded in order to enable the performance of a diagnostic method, as previously disclosed by Rava (use of a threshold value for a qualifying data set that serves as a limit of diagnosis, wherein the threshold value is exceeded in a diagnostic process; paragraph [0073]), it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have implemented filtering-out reads and data sets that failed to meet a predetermined threshold, as determined according to Rava, for effectively removing data from consideration in the diagnosis which may have been of suspect quality.

Since none of the special technical features of the Groups I-V inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Rava reference, unity of invention is lacking.