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- *with international search report (Art. 21(3))*
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- *with sequence listing part of description (Rule 5.2(a))*

(88) **Date of publication of the international search report**:
26 March 2015

(54) **Title**: METHODS AND SYSTEMS FOR MICROBIOME CHARACTERIZATION, MONITORING AND TREATMENT

(57) **Abstract**: The present disclosure provides methods for profiling a microbiome and therapeutic compositions for treatment. Additionally, the methods, systems, compositions and kits provided herein are directed to assessing or predicting health status in a subject. Some of the embodiments include generating a report.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/47491

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 63/00; C12Q 1/68; C12N 1/20; C40B 40/12 (2014.01)
CPC - A61K 35/74; C12Q 1/6827; C12N 1/20; B01J 2219/00659
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)
CPC: A61K 35/74; C12Q 1/6827; C12N 1/20; B01J 2219/00659

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC: A61K 35/74; C12Q 1/6827; C12N 1/20; B01J 2219/00659 (text search)
USPC: 424/93.4; 435/6.12, 252.4; 506/9; 702/19 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic data bases: PatBase; Google Scholar; Google Patents
16S or 23S ribosomal RNA (16s rRNA or 23S rRNA), full length metagenomic sequencing, 1500 bp 16S rRNA, single read, Pacific Biosciences SMRT*, barcoded sequences,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BOWMAN et al. Analysis of Full-Length Metagenomic 16S Genes by SMRT Sequencing. American Society for Microbiology 2013 General Meeting 19 May 2013 Poster Session pg 116 Poster 390. Available on the internet: <http://www.asm.org/index.php/asm-events/post-meeting-materials [select ASM2013 Final Program, then pg Posters 19 May 2013 pg 116]. Actual poster can be downloaded from: < http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CB4QFjAA&url=http%3A%2F%2Ffiles.pacb.com%2Fpdf%2FAnalysis_of_Full_Length_Metagenomic_16S_Genes_by_SMRT_Sequencing.pdf&ei=jQNUVPyJMom4oQSblYKoDA&usg=AFQjCNFkUKTIHMccYDqyLGmaKpl_56Zxsg&sig2=KtfmYrsWRWGc2XtvSxieeQ&bvm=bv.78677474,d.cGU >. Especially pg 4 of attached PDF.	1
A	US 2013/0121968 A1 (QUAY et al.) 16 May 2013 (16.05.2013). para [0012], [0025].	1
A	EID et al. Real-time DNA sequencing from single polymerase molecules. Science 2 January 2009 Vol 323 No 5910 Pages 133-138. Especially pg 134 col 3 para 4, pg 136 fig 3B.	1

☐ 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 12 January 2015 (12.01.2015)	Date of mailing of the international search report 23 JAN 2015
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: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 14/47491

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, 10, 11, 15, 20-25, 28-29, 31-38
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:
-----go to Extra Sheet for continuation------

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

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.

Continuation of Box III (Lack of Unity of Invention)

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: Claim 1, drawn to a method of classifying a microbe, comprising:
obtaining a nucleic acid sequence of a 16S or 23S ribosomal subunit contained within a single read length of a first microbe; and comparing said nucleic acid sequence of a first microbe to a reference; and identifying the first microbe at the strain level based on the comparing.

Group II: Claims 2-6, drawn to a method of profiling a microbiome in a subject, comprising:
obtaining nucleic acids sequences of a 16S ribosomal subunit from at least one microbe in a biological sample obtained from the subject; analyzing said at least one microbe within said biological sample based upon the nucleic acids sequences obtained; and determining a profile of the microbiome based on said analyzing. Group II may include (claim 3) the nucleic acid sequences of 23S ribosomal subunit.

Group III: Claim 7, drawn to a method of determining metabolic pathways that are indicative of a health status in a subject, comprising: obtaining RNA sequences from a biological sample from a subject, such that the entire transcript is contained within a single read length; analyzing said transcripts by a sequencing method; comparing the sequenced transcripts to a reference; and determining the metabolic pathways that are indicative of a health status.

Group IV: Claim 9, 14, 16-19 and 30, drawn to a method of treating a disease in a subject, comprising:
(a) measuring a microbiome profile in a biological sample obtained from the subject, wherein the microbiome profile comprises at least one microbe;
(b) detecting a presence or absence of the disease in the subject based upon said measuring; and
(c) treating the disease in the subject based upon said detecting
Group IV may involve (claim 14) (c) recommending to the subject at least one microbial-based therapeutic or cosmetic for treatment of said disease based on the detecting.

Group V: Claims 12-13, drawn to a method, comprising:
(a) obtaining data comprising a measurement of a microbiome panel in a biological sample obtained from a subject, wherein said microbiome panel comprises at least two microbes;
(b) generating a microbiome profile of said microbiome panel based upon the measurement data;
(c) comparing said microbiome profile of said microbiome panel to a reference profile; and
(d) determining a likelihood of a disease status in said subject based said comparing

Group VI: Claims 26-27, drawn to a computer readable medium comprising:
(a) computer-executable instructions for analyzing data comprising measurement of a microbiome profile from a biological sample obtained from a subject, wherein the microbiome profile comprises at least one marker selected from at least one microbe; and
(b) computer-executable instructions for determining a presence or absence of at least disease in the subject based upon the analyzing.

The inventions listed as Groups I-VI 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:

Special Technical Features:

Group I has the special technical feature of classifying a microbe to a strain level based on the nucleic acid sequence of a 16S or 23S ribosomal subunit, not required of Groups II-VI.

Groups I and III have the special technical feature of obtaining sequence information comprising the full length of a transcript in a single read length, not required by Groups II, IV-VI.

Group II has the special technical feature of profiling a microbiome in a biological sample by obtaining the nucleic acid sequences of a 16S and 23S ribosomal subunit and analyzing the sequences obtained, not required by Groups I, III-VI.

Group III has the special technical feature of determining metabolic pathways that are indicative of a health status based on obtaining RNA sequences from a biological sample, analyzing the sequences, and comparing the sequences to a reference, not required by Groups I, II, IV-VI.

Group IV has the special technical feature of treating or recommending treatment of a disease based on measuring a microbiome profile, not required by Groups I-III, V-VI.

Group V has the special technical feature of a microbiome panel comprising at least two microbes and determining the likelihood of a disease status of a subject based on a comparison of a microbiome profile of the microbiome panel in comparison to a reference profile, not required by Groups I-IV and VI.

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continued from previous sheet

Group VI has the special technical feature of a computer readable medium comprising computer-executable instructions, not required by Groups I-V.

Group VI has the special technical feature of the microbiome profile comprises at least one marker, where the marker may include a nucleic acid, a protein, or a metabolite [see instant application para [00110-00111]], not required by Groups I-V.

Common Technical Features:

1.Groups I and II share the common technical features of obtaining a 16S or 23S ribosomal subunit nucleic acid sequence.

2.Groups I and III share the common technical feature of obtaining sequence information comprising the full length of a transcript in a single read length.

3.Groups II, IV-VI share the common technical feature of measuring or generating a microbiome profile.

4. Groups III, IV and V share the common technical feature of determining health or disease status.

5. Groups III and V share the common technical feature of comparing to a reference profile.

However said common technical features do not represent a contribution over the prior art and is obvious over US 2013/0121968 A1 (QUAY) [published 16 May 2013], in view of the publication titled "Real-time DNA sequencing from single polymerase molecules" by EID et al. (hereinafter "Eid") [published 2 January 2009 Science Vol 323 No 5910 Pages 133-138]

As to common technical feature 1, Quay teaches obtaining a 16S ribosomal subunit nucleic acid sequence (para [0025]; "Quantifying 16S ribosomal RNA may be conducted using any conventional means including commercially available kits. Methods include [...] next generation cDNA sequencing".

As to common technical feature 3, Quay teaches measuring or generating a microbiome profile (para [0012];" In one embodiment, the method further comprises isolating and quantifying at least a portion of 16S ribosomal RNA of the sample to determine the metagenomic profile of the sample")

As to common technical features 4, Quay teaches determining health or disease status (para [0012]; "Provided herein is a method of diagnosing the presence or absence of a medical condition in a mammal comprising: comparing, using at least a general purpose computer, a multiplex profile of a metatranscriptome profile and a metogenomic profile, from a sample from the mammal, to a multiplex profile of a population of patients diagnosed with said medical condition to determine the presence or absence of the medical condition. In one embodiment, the method further comprises isolating and quantifying at least a portion of 16S ribosomal RNA of the sample to determine the metagenomic profile of the sample. In another embodiment, the method further comprises isolating and quantifying at least a portion of messenger RNA of said sample to determine the metatranscriptome profile").

As to common technical feature 5, Quay teaches comparing to a reference profile (para [0012]).

Quay does not specifically teach common technical feature 2, obtaining sequence information comprising the full length of a transcript in a single read length. However, Eid teaches obtaining a single read length of at least 2520 bp (pg 134 col 3 para 4; A representative read (Fig. 3B) showed the expected continuous signature sequence pattern of alternating periods of A555-dCTP and A647-dGTP pulses"; pg 136 fig 3B; " Fig. 3. Long read length activity of DNA polymerase"; [illustrated read length in fig 3B: >2,520 bases]. It would have been obvious that the full length transcript reads of many RNAs could have been obtained using the sequencing method and instrumentation taught by Eid. An artisan of ordinary skill would have known that the average length of a microbial 16S RNA, for instance, is only 1500 bp.

As the common technical feature was known in the art at the time of the invention, this cannot be considered a common special technical feature that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I-VI lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature

Note on item 4: Claims 8, 10, 11, 15, 20-25, 27-29, 31-38 are multiple dependent claims and are held unsearchable because they are not drafted according to the second and third sentences of PCT Rule 6.4(a).

Note concerning claim 14: Claim element (b) of claim 14 has accidentally omitted the word "disease", and for the purposes of the ISR, interpreted as "(b) detecting a presence or absence of a disease in said subject based upon said measuring"

Note concerning claim 25: Claims 25 and 27 are interpreted to depend from claim 24, not claim 21 as is written, because claim 24 has the appropriate antecedent precedent for "the computer system" indicated in claims 25 and 27.