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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

[Continued on next page]

(54) Title: MOLECULAR BARCODING FOR MULTIPLEX SEQUENCING

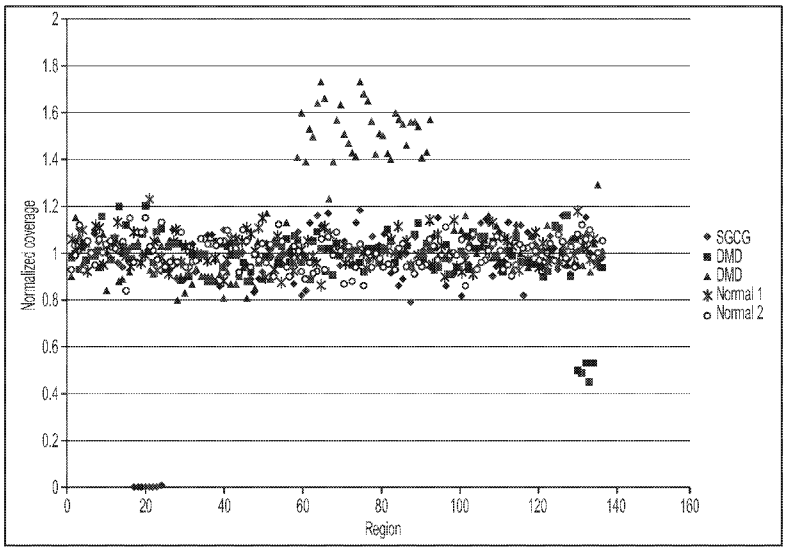

FIG. 2

(57) Abstract: Described herein are methods, compositions and kits for preparing samples for multiplex next generation nucleic acid sequencing. The methods entail the use of in-line barcodes that minimize barcode-confusing chimeras, purification procedures with low cost, and/or a quantitative amplification to generate a desired amount of polynucleotides for sequencing.

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— *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))*

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19 February 2015

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12P 19/34; C40B 40/06; C07H 21/02, 21/04 (2014.01)
CPC - C12Q 1/686; B01J 2219/00722; C12Q 1/689
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: C12Q 1/686; B01J 2219/00722; C12Q 1/689

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC: C12Q 1/686; B01J 2219/00722; C12Q 1/689 (text search)
USPC: 435/91.2, 91.52; 506/16; 536/23.1, 24.33

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
Search terms: index tag or barcode, barcode chimera, first adaptor and second adaptor, double adaptor or indexing, blunt-ended target, ligation, sequencing library, library quantitation, qPCR, hamming code

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	ROHLAND et al. Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. May 2012 Vol 22 No 5 Pages 939-946 and Suppl. pages. Especially pg 940 col 2 para 1, pg 941 fig 2, pg 944 col 2 para 2, Suppl pg 3 para 3, Suppl pg 4 para 2, Suppl pg 5 para 1-2, Suppl pg 24-26 Table S6(a).	1-3, (5,6)/(2,3) ---------- 4, (5,6)/4, 22-26
Y	US 2011/0257031 Al (BODEAU et al.) 20 October 2011 (20.10.2011). Especially para [0055], sheet 7 fig 7.	4, (5,6)/4, 24, (25,26)/24
Y	ROCHE. Parallel Copy Number Variation and Sequence Variation Detection Using Amplicon based Sequencing on the GS Junior System (online) April 2012 [retrieved 1 December 2014]. Available on the internet: <URL:http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0CB4QFjAA&url=http%3A%2F%2F454.com%2Fdownloads%2FGSJuniorCNVDetectionApplicationNote.pdf&ei=bZV_VJ7DBqWQigKSi4DgDA&usg=AFQjCNEgHN-9wqyVIMSVfTRtkDzSHEpYNw&sig2=L_Qj4XAwE5DsDJj_IxkvGg&bvm=bv.80642063,d.cGE>. Especially pg 2 col 1 para 4, pg 3 col 1 para 2, pg 3 fig 3.	22-26
A	BIOO Scientific "NEXTflex DNA Barcodes - 48" [online] 2011 [retrieved 22 September 2014]. Available on the internet:<URL: http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=3&ved=0CDQQFjAC&url=http%3A%2F%2Fwww.zoology.ubc.ca%2F~rieseberg%2FRiesebergResources%2Fwp-content%2Fuploads%2F2012%2F03%2F514104-NEXTflex-DNA-Barcodes-48.pdf&ei=_JwhVIrzC4GnyASc6IK4Bw&usg=AFQjCNHNq8tPTJSD-QuWwdUJvV-ExC0xlw&sig2=OLX8qlZgEuuklEgQf81YZA&bvm=bv.75775273,d.aWw >. p 2, para 2	19

☐ 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
1 December 2014 (01.12.2014)	17 DEC 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	Lee W. Young 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/US 14/41315

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:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/41315

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.: 7-18 and 27-38
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.:
1-6, 22-26

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 14/41315

continued from 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

Group I: Claims 1-6, 22-26, drawn to a method for reducing the incidence of barcode confusing chimerism in a sample for sequencing, comprising incubating each of a plurality of samples with a first adaptor and a second adaptor, wherein:
(i) each sample comprises a plurality of double-stranded target polynucleotides each having two 5 '-phosphorylated blunt ends;
(ii) each first adaptor is partially double-stranded comprising a first partially double-stranded fragment and a double-stranded polynucleotide barcode having a unphosphorylated blunt end, wherein all first adaptors have the same first fragment but a unique barcode, wherein neither strand of the first adaptors is longer than 40 bases, and wherein each barcode is between 6 basepairs (bp) and 8 bp long, has no more than two consecutive nucleotides being the same, and differs from any other barcode by at least 2 bp;
(iii) each second adaptor is partially double-stranded having an unphosphorylated blunt end, wherein all second adaptors have the same sequence in which neither strand is longer than 40 bases, and wherein the incubation is carried out under suitable conditions for the target polynucleotides to ligate to a first adaptor at one end and to a second adaptor at the other end.
The inventions of Group I may further include the steps of
(b) purifying the target polynucleotides ligated with adaptors from free adaptors that are not ligated to target polynucleotides with a size-selection bead or column;
(c) performing nick translation on the target polynucleotides to generate fully double stranded polynucleotides;
(d) purifying the nick translated target polynucleotides with the bead or column;
(e) pooling the samples to obtain a pooled sample;
(f) performing PCR amplification in the pooled sample with a first primer partially complementary to the first fragment and a second primer partially complementary to the second adaptor to obtain amplicons with sequences at both end, due to incorporation of the primers, suitable for sequencing; and
(g) performing quantitative PCR (qPCR) on the amplicons to obtain a sample having a desired amount of polynucleotides for sequencing.
The inventions of Group I may further include the methods of detecting copy number variations in a sample of genomic DNA comprising sequencing steps in claim 2.

Group II+: Claims 19-21, drawn to a kit comprising at least 48 polynucleotide sequences, each of which comprises a different barcode selected from Table 1. [instant application pgs. 15-17, SEQ ID NOs: 11-106].
Group II+ will be searched upon payment of additional fee(s). An exemplary election would be: the first polynucleotide sequence recited in Table 1 (SEQ ID NO: 11). Is believed that claims 19-21 read on this named invention. Applicants must indicate, if applicable, which claims read on this 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 named invention to be searched/examined. Another exemplary election would be SEQ ID NO: 12 (claims 19-21).

The inventions listed as Groups I and II+ 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 a method for preparing a sample for sequencing, comprising samples, first adaptor and a second adaptor, wherein the first adaptor comprises of a double-stranded polynucleotide barcode, not required by Group II+.

Group II+ has the special technical feature of a kit comprising at least 48 polynucleotide sequences, not required by Group I.

Among the inventions listed as Groups II+ is a kit comprising at least 48 polynucleotide sequences, each of which comprises a different barcode. The inventions do not share a special technical feature, because a kit comprising at least 48 polynucleotide sequences is known in the art (see below) and because no significant structural similarities can readily be ascertained among barcode sequences, therefore, each barcode sequence is considered as an individual invention.

Common Technical Feature:

Group II+ is related to Group I in that it is a composition that could be used in the method of Group I and share the common technical feature of polynucleotide barcodes.

However, said common technical feature does not represent a contribution over the prior art, as in anticipated by US 2009/0163366 A1 to NICKERSON et al. (hereinafter "Nickerson").

----------continued on next sheet------

continued from previous sheet

Nickerson teaches polynucleotide barcodes (para [0009-0015]; "The invention utilizes nucleic acid constructs containing at least the following elements i) through v), arranged in the recited order in the 3'-to-5' direction:
[0010] i) a complement of a first universal primer,
[0011] ii) a first target sequence,
[0012] iii) a polynucleotide spacer (optional),
[0013] iv) a complement of a second universal primer, and
[0014] v) a second target sequence.
[0015] In some embodiments, the first target sequence includes a sample-specific barcode sequence which identifies the source of the sample (e.g., position of sample on the plate, plate number, different treatment conditions, disease, tissue, etc.); and the second target sequence includes a gene-specific barcode identifying the gene of interest").

In addition, concerning Group II+, a kit containing at least 48 unique DNA barcodes. This shared technical feature does not represent a contribution over the prior art, and is anticipated the technical publication titled "NEXTflex DNA Barcodes - 48" by BIOO Scientific (hereinafter "Bioo Scientific")[online] 2011 [retrieved 22 September 2014]. Available on the internet:<URL: http://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=3&ved=0CDQQFjAC&url=http%3A%2F%2Fwww.zoology.ubc.ca%2F~rieseberg%2FRiesebergResources%2Fwp-content%2Fuploads%2F2012%2F03%2F514104-NEXTflex-DNA-Barcodes-48.pdf&ei=_JwhVIrzC4GnyASc6IK4Bw&usg=AFQjCNHNq8tPTJSD-QuWwdUJvV-ExC0xlw&sig2=OLX8qIZgEuuklEgQf81YZA&bvm=bv.75775273,d.aWw >.

Bioos Scientific teaches a kit containing 48 unique DNA barcodes (pg 2 para 2; The NEXTflex DNA Barcodes contain 48 barcoded DNA Adapters with enough material for 8 reactions each, for a total of 384 reactions").

As these common technical features were 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 and II+ lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Note concerning Item 4:
Claims 7-18 and 27-38 are multiple dependent claims and are not drafted according to the second and third sequences of PCT Rule 6.4(a).