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— *with international search report (Art. 21(3))*
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23 July 2020 (23.07.2020)

(54) Title: DIRECTIONAL TARGETED SEQUENCING

(57) Abstract: The present disclosure provides methods and systems for processing nucleic acid molecules. The methods may comprise performing one or more extension or amplification processes to provide libraries for subsequent analysis using nucleic acid sequencing. Logical partitioning and directionality considerations may facilitate efficient and cost-effective amplification of target nucleic acid sequences.

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INTERNATIONAL SEARCH REPORT

International application No
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A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/6853 C12Q1/6806
ADD.

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)
C12Q

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)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	METZLER M ET AL: "Asymmetric multiplex-polymerase chain reaction - a high throughput method for detection and sequencing genomic fusion sites in t(4;11)", BRITISH JOURNAL OF HAEMATOLOGY, WILEY-BLACKWELL PUBLISHING LTD, GB, vol. 124, 23 September 2004 (2004-09-23), pages 47-54, XP008122029, ISSN: 0007-1048 figure 1	1-67, 174-191

X	WO 2017/117440 A1 (BIO-RAD LABORATORIES INC [US]) 6 July 2017 (2017-07-06)	174-191
A	claims 1, 8; example 3	1-67

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[X] Further documents are listed in the continuation of Box C.

[X] 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
7 April 2020	24/06/2020

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2015/039006 A1 (GEN HOSPITAL CORP [US]) 19 March 2015 (2015-03-19)	174-191
A	paragraphs [0012], [0174]; figure 1	1-67

X	WO 2018/165593 A1 (IREPERTOIRE INC [US]) 13 September 2018 (2018-09-13)	174-191
A	figure 1C	1-67

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INTERNATIONAL SEARCH REPORT

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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 additional sheet

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 an 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.:

1-67(completely); 174-191(partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-67(completely); 174-191(partially)

A method of analyzing a nucleic acid sample, comprising:
(a) providing a sample comprising a plurality of nucleic acid molecules including a target nucleic acid sequence;
(b) subjecting said plurality of nucleic acid molecules to a condition sufficient to generate one or more copies of said target nucleic acid sequence, by annealing first and second primers of a plurality of first and second primers and second primers to nucleic acid molecules of said plurality of nucleic acid molecules;
(c) separating said one or more first copies of said target nucleic acid sequence, or complements thereof, from other materials; and
(d) subjecting said one or more first copies of said target nucleic acid sequence, or complements thereof, to a condition sufficient to generate one or more second copies of said target nucleic acid sequence, or complements thereof, wherein generating said one or more second copies, or complements thereof, comprises annealing third primers of a plurality of third primers to said one or more first copies, or complements thereof,wherein said plurality of third primers are configured to anneal to said one or more first copies, downstream of said first primers or second primers, or complements thereof;wherein said plurality of third primers comprise a portion of a first sequencing adapter of a plurality of first sequencing adapters,
Furthermore, optionally,
(e) separating said one or more second copies of said target nucleic acid sequence, or complements thereof, from other materials;
(f) subjecting said one or more second copies of said target nucleic acid sequence, or complements thereof, to conditions sufficient to generate one or more third copies of said target nucleic acid sequence, or complements thereof, wherein generating said one or more third copies, or complements thereof, comprises annealing first and second sequencing adapters of a plurality of sequencing adapters
Yet furthermore, a kit comprising
(a) a plurality of first primers, which plurality of first primers are configured to anneal to nucleic acid molecules comprising a target nucleic acid sequence;(b) a plurality of second primers, which plurality of second primers are configured to anneal to nucleic acid molecules comprising complements of said target nucleic acid sequence; and(c) a plurality of third primers, which plurality of third primers are configured to anneal to nucleic acid molecules adjacent to said target nucleic acid sequence or complements thereof.

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2. claims: 68-135

A method of analyzing a nucleic acid sample, comprising:(a) providing a sample comprising a plurality of nucleic acid molecules, which plurality of nucleic acid molecules comprise a target nucleic acid sequence, optionally, wherein a subset of said plurality of nucleic acid molecules further comprise a directing sequence upstream of said first primers;(b) subjecting said plurality of nucleic acid molecules to a condition sufficient to generate one or more copies of said target nucleic acid sequence, or complements thereof, wherein generating said one or more first copies, or complements thereof, comprises annealing first primers of a plurality of first primers and second primers of a plurality of second primers to nucleic acid molecules of said plurality of nucleic acid molecules;(c) separating said one or more first copies of said target nucleic acid sequence, or complements thereof, from other materials;(d) subjecting said one or more first copies of said target nucleic acid sequence, or complements thereof, to conditions sufficient to generate one or more second copies of said target nucleic acid sequence, or complements thereof, wherein generating said one or more second copies, or complements thereof, comprises annealing first sequencing adapters of a plurality of first sequencing adapters and second sequencing adapters of a plurality of second sequencing adapters to said one or more first copies, or complements thereof,wherein said plurality of first primers comprise (i) a portion of a first sequencing adapter of said plurality of first sequencing adapters, or a complement thereof; or (ii) a filler sequence that does not have substantial sequence identity to a natural sequence.

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3. claims: 136-173(completely); 174-191(partially)

A method of analyzing a nucleic acid sample, comprising:(a) providing a sample comprising a plurality of nucleic acid molecules, which plurality of nucleic acid molecules comprise a target nucleic acid sequence;(b) subjecting said plurality of nucleic acid molecules to a condition sufficient to generate one or more first copies of said target nucleic acid sequence, or complements thereof, which one or more copies, or complements thereof, comprise a first primer sequence or complement thereof at a first end and a second primer sequence or complement thereof at a second end, wherein said first primer sequence comprises a biotin moiety;(c) separating said one or more first copies of said plurality of target nucleic acid sequences, or complements thereof, from other materials;(d) subjecting said one or more first copies of said plurality of target nucleic acid sequences, or complements thereof, to a condition sufficient to generate one or more second copies of said target nucleic acid sequence, or complements thereof, which one or more second copies, or complements thereof, comprise a third primer sequence or complement thereof at said first end and said first primer sequence or complement thereof at said

first end;(e) separating said one or more second copies of said target nucleic acid sequence, or complements thereof, from other materials;(f) subjecting said one or more second copies of said target nucleic acid sequence, or complements thereof, to a condition sufficient to generate one or more third copies of said target nucleic acid sequence, or complements thereof, which one or more third copies, or complements thereof, comprise a first sequencing adapter at a first end and a second sequencing adapter at a second end. Furthermore, a kit comprising:(a) a plurality of first primers, which plurality of first primers are configured to anneal to nucleic acid molecules comprising a target nucleic acid sequence;(b) a plurality of second primers, which plurality of second primers are configured to anneal to nucleic acid molecules comprising complements of said target nucleic acid sequence; and(c) a plurality of third primers, which plurality of third primers are configured to anneal to nucleic acid molecules adjacent to said target nucleic acid sequence or complements thereof.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 3397379 A1	07-11-2018
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