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- (81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.
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(54) **Title:** QUANTITATIVE AMPLICON SEQUENCING FOR MULTIPLEXED COPY NUMBER VARIATION DETECTION AND ALLELE RATIO QUANTITATION

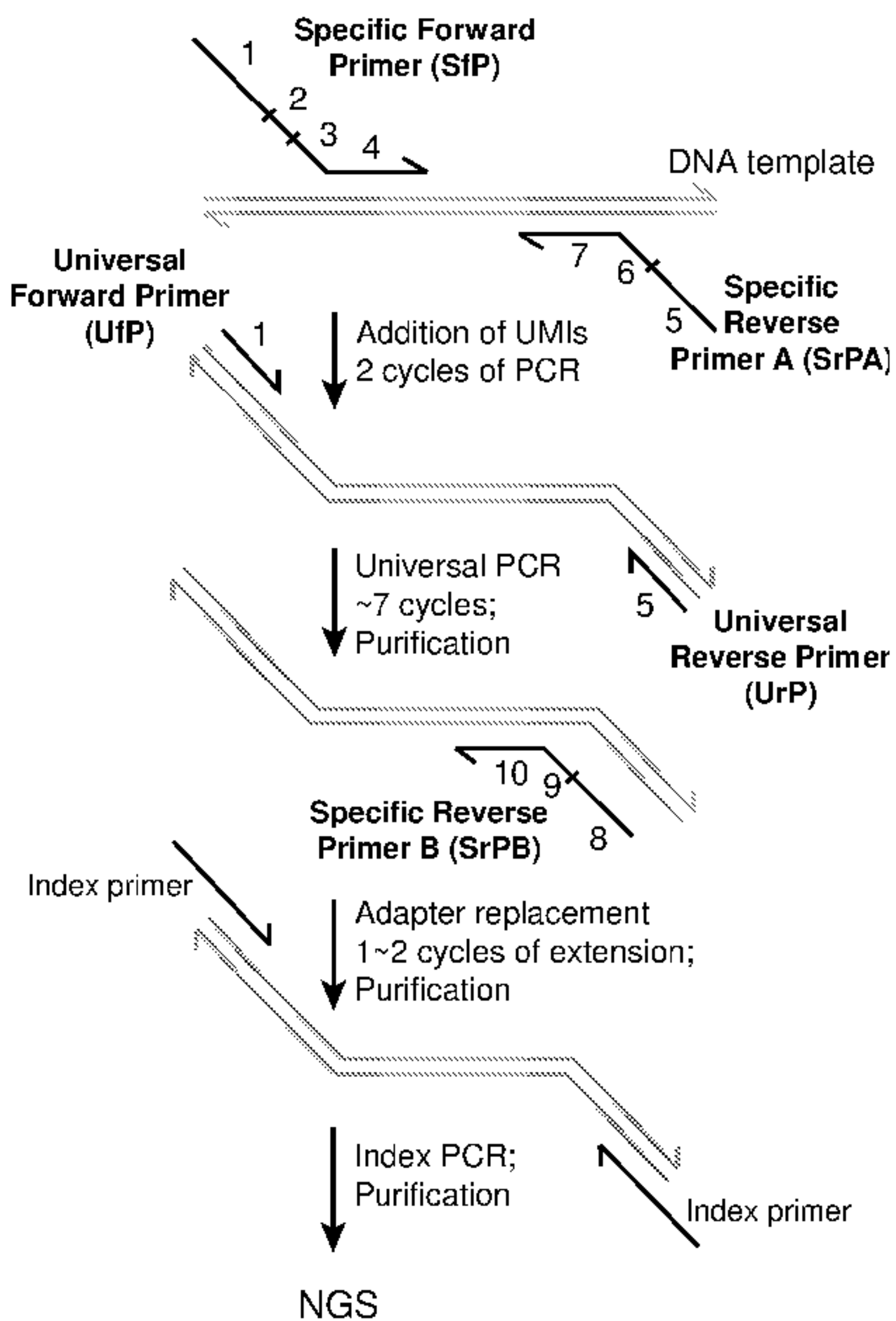


FIG. 1

(57) **Abstract:** Provided herein are methods of quantitative amplicon sequencing, for labeling each strand of targeted genomic loci in a DNA sample with an oligonucleotide barcode sequence by polymerase chain reaction, and amplifying the genomic region(s) for high-throughput sequencing. The methods can be used for the simultaneous detection of copy number variation (CNV) in a set of genes of interest, by quantifying the frequency of extra copies of each gene. In addition, these methods provide for the quantitation of the allele ratio of different genetic identities for targeted genomic loci using multiplexed PCR. In addition, these methods provide for the detection of mutations and quantitation of the variant allele frequency.

Published:

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- *with sequence listing part of description (Rule 5.2(a))*

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/012089

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/6806; C12Q 1/686 (2020.01)

CPC - C12Q 1/6869; C12Q 1/6876; C12Q 2600/156; C12Q 2600/16 (2020.02)

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)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

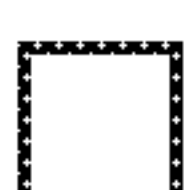
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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

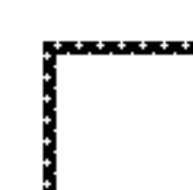
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2018/0023135 A1 (KEYGENE N.V.) 25 January 2018 (25.01.2018) entire document	1-3
Y	US 2007/0196849 A1 (SPIER) 23 August 2007 (23.08.2007) entire document	1-3
A	US 2014/0309128 A1 (BIO-RAD LABORATORIES, INC.) 16 October 2014 (16.10.2014) entire document	1-3
A	WO 2018/162538 A1 (F. HOFFMANN-LA ROCHE AG et al) 13 September 2018 (13.09.2018) entire document	1-3
A	US 2018/0211003 A1 (PACIFIC BIOSCIENCES OF CALIFORNIA, INC.) 26 July 2018 (26.07.2018) entire document	1-3



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

26 February 2020

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2020/012089

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.: 4-57
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:

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.:

- 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.