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(54) Title: COMPOSITIONS FOR SURFACE AMPLIFICATION AND USES THEREOF

(57) Abstract: The present disclosure provides compositions, methods, and systems for enrichment and analysis of template nucleic acid molecules, e.g., of a biological sample. Further provided herein are methods of using a plurality of unique bead species each comprising a unique set of identical primer molecules.

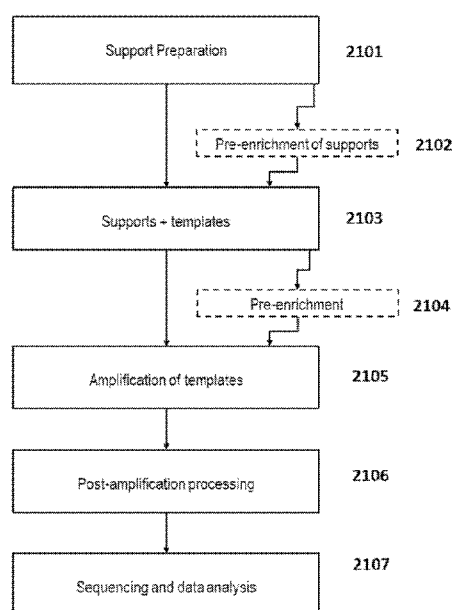


FIG. 21



(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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- *with international search report (Art. 21(3))*
- *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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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/46951

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 9/22, C12N 9/00, C12N 9/14, C12N 9/16, C12Q 1/6806, C12Q 1/6848 (2021.01)

CPC - C12Q 2521/325, C12Y 301/11001, C12Q 1/6869, C12Q 1/6874

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

See Search History document

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 2019/0352707 A1 (ULTIMA GENOMICS, INC.) 21 November 2019 (21.11.2019) Abstract; Claim 1; Claim 53; para [0043-0044]; para [0046]; para [0052]; para [0065]	1-12
Y	US 2018/0363042 A1 (ARCTICZYMES AS et al.) 20 December 2018 (20.12.2018) Abstract; Claim 1; para [0016]	1-12

☐ 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

"D" document cited by the applicant in the international application

"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

25 December 2021

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/46951

Box No. 1 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:
- a. ☐ forming part of the international application as filed:
☐ in the form of an Annex C/ST.25 text file.
☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☒ furnished subsequent to the international filing date for the purposes of international search only:
☒ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
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 forming part of 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 21/46951

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.: 13-33, 37-62, 66-74, 79-86
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:

----Please see continuation in first extra 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 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.:
1-12

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.

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Continuation of Box No. III. 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 searched, the appropriate additional search fees must be paid.

Group I, claims 1-12, directed to a method for processing supports by selective degrading of surface primer molecules that are not bound to template nucleic acid molecules.

Group II, claims 34-36, 87-94, directed to a method for amplifying a first and second template nucleic acids using distinct sets of supports, primers and adaptors, and identifying the sequence of the amplicons.

Group III, claims 63-65, 147-161, directed to a composition, comprising: a mixture comprising a plurality of supports and primers immobilized thereto.

Group IV, claims 75-78, 95-146, directed to a method for processing supports and pre-enriching a mixture of supports.

The inventions listed as Groups I-IV do not relate to a single special technical feature 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 processing supports by selective degrading of surface primer molecules that are not bound to template nucleic acid molecules, that is not required by Groups II-IV.

Group II has the special technical feature of a method for amplifying in solution a first and second template nucleic acids using distinct sets of supports, primers and adaptors, and identifying the sequence of the amplicons, that is not required by Groups I, III and IV.

Group III has the special technical feature of a composition, comprising: a mixture comprising a plurality of supports, that is not required by Groups I, II and IV.

Group IV has the special technical feature of a method for processing supports and pre-enriching a mixture of supports wherein template nucleic acid molecules comprise a cleavable moiety and a first reactive moiety that is configured for capture by a second reactive moiety, and isolating said first support from said mixture of supports onto a substrate surface, that is not required by Groups I-III.

Common technical features:

Groups I-IV share the common technical feature of:
a method for processing supports, comprising:

(a) providing a plurality of supports, wherein said plurality of supports comprises a plurality of surface primer molecules coupled thereto, wherein a plurality of template nucleic acid molecules are immobilized to a support of said plurality of supports via a first set of surface primer molecules of said plurality of surface primer molecules, wherein a second set of surface primer molecules of said plurality of surface primer molecules is not coupled to said plurality of template nucleic acid molecules.

However, this shared technical feature does not represent a contribution over prior art, because this shared technical feature is made obvious by US 2019/0352707 A1 to Ultima Genomics, Inc. (hereinafter 'Ultima').

-----please see continuation on next sheet -----

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/46951

Continuation of Box No. III. Observations where unity of invention is lacking.

-----continued from previous sheet -----

Ultima teaches a method for processing supports, comprising:

(a) providing a plurality of supports, wherein said plurality of supports comprises a plurality of surface primer molecules coupled thereto, wherein a plurality of template nucleic acid molecules are immobilized to a support of said plurality of supports via a first set of surface primer molecules of said plurality of surface primer molecules, wherein a second set of surface primer molecules of said plurality of surface primer molecules is not coupled to said plurality of template nucleic acid molecules (Claim 1 - 'A method for determining a nucleic acid sequence of a target nucleic acid molecule, comprising: (a) providing a plurality nucleic acid molecules immobilized to a support, wherein each of said plurality of nucleic acid molecules exhibits sequence homology to said target nucleic acid molecule, and wherein said support is operatively coupled to a detector; (b) directing a plurality of nucleotides or nucleotide analogs to said support, which plurality of nucleotides or nucleotide analogs comprises at least a first subset of nucleotides or nucleotide analogs and a second subset of nucleotides or nucleotide analogs, wherein (i) each of said first subset of nucleotides or nucleotide analogs comprises a detectable moiety and a terminating subunit, and (ii) none of said second subset of nucleotides or nucleotide analogs comprises said detectable moiety and said terminating subunit'; para [0043] - 'Non-limiting examples of nucleic acid amplification methods include reverse transcription, primer extension, polymerase chain reaction (PCR)'; para [0044] - 'In some cases, such a signal may be indicative of incorporation of one or more nucleotides or nucleotide analogs during a primer extension reaction. In some cases, a detectable moiety is coupled to a nucleotide or nucleotide analog, which nucleotide or nucleotide analog may be used in a primer extension reaction.'; Claim 53 - 'The method of claim 1, wherein said support is a bead,'). Ultima fails to expressly teach a plurality of supports. However, since Ultima teaches the support is a bead, and further teaches a plurality of independently addressable locations (Claim 46 - 'The method of claim 1, wherein said support has a plurality of independently addressable locations, wherein said plurality nucleic acid molecules is immobilized to said support at a given independently addressable location of said plurality of independently addressable location.'), it would have been obvious to one of ordinary skill in the art that the method of Ultima could be modified to instead use a plurality of beads for multiplexing since multiplexing assays with beads were well known in the art.

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I-IV inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, continuation of Item 4 above: claims 13-33, 37-62, 66-74, 79-86 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).