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- Published:

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

— with sequence listing part of description (Rule 5.2(a))

(88) Date of publication of the international search report:
08 May 2025 (08.05.2025)
- (54) Title: METHODS AND KITS CONFIGURED FOR HIGH-THROUGHPUT INTERROGATION OF NUCLEIC ACID-CONTAINING SAMPLES WITH HIGH DYNAMIC RANGE

(57) Abstract: This application describes compositions, methods, and kits for simultaneous, multiplexed detection and quantification of nucleic acid molecules. In particular, compression PCR is disclosed for compressing the dynamic range of highly complex biological samples, and improve the accuracy and efficiency of high-throughput sequencing analysis.
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WO 2024/254462 A3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 24/33027

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C12Q 1/6806 (2024.01)

ADD. C12N 15/11 (2024.01)

CPC - INV. C12Q 1/6869, C12Q 1/6876, C12Q 1/6844

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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 2019/0153508 A1 (SAFEGUARD BIOSYSTEMS HOLDINGS LTD.) 23 May 2019 (23.05.2019) para [0007], para [0012], para [0017], para [0065], para [0071], para [0082], para [0104], para [0178], claim 1, claim 14, claim 17	1-5
Y	EP 2 769 007 B1 (ARMOUR) 07 December 2016 (07.12.2016) para [0009], para [0011]	1-5



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

29 September 2024

Date of mailing of the international search report

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Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 24/33027

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:

a. ☒ forming part of the international application as filed.

b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),

☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 24/33027

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.: 6-52 and 58
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:
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 1-5, directed to a method for sequencing a plurality of target sequences in a nucleic acid mixture sample comprising:
(a) subjecting the mixture to multiplexed asymmetric PCR reactions for multiple target sequences using (i) an excess primer (at 5-fold excess concentration), (ii) a limiting primer each comprising strand-specific binding sequences; and
(b) preparing a high throughput sequencing or hybridization library from the amplification products.

Group II: Claims 53-57, directed to a kit comprising a panel of primer pairs comprising an excess primer and a limiting primer, at a concentration for asymmetric PCR amplification of a desired target amplicon.

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:

----- continued on next 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.:
1-5

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 24/33027

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

Special Technical Features

Group I requires a method for sequencing a plurality of target sequences using asymmetric PCR, not required by group II.

Group II requires a composition comprising a kit having primer pairs for asymmetric PCR, not required by group I.

Common Technical Features

The common technical feature shared by Groups I and II is a panel (multiplexed) primer pairs, each pair comprising an excess primer and a limiting primer for asymmetric PCR amplification of desired target amplicons.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by US 2019/0153508 A1 to Safeguard Biosystems Holdings Ltd., (hereinafter "Safeguard Biosystems"). Safeguard Biosystems teaches primer pairs, each pair comprising an excess primer and a limiting primer for multiplex asymmetric PCR amplification (para [0104] "kits suitable for carrying out the asymmetric PCR methods describe herein. The kits typically include at least an Asymmetric Primer Pair." claim 1 "A method for ... asymmetric polymerase chain reaction (PCR)", claim 14 "The method of claim 1, which is a multiplex PCR reaction ", para [0082]"the initial concentration of the Extended [excess]Primer is preferably in an excess of the concentration of Unextended Primer, for example about a 2-fold to 30-fold molar excess", para [0071]"The target nucleic acid molecules are preferably DNA").

As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

RE: Item 4: Claims 6-52 and 58 have been held unsearchable because they are not drafted in accordance with the second and third sentences of Rule 6.4(a).