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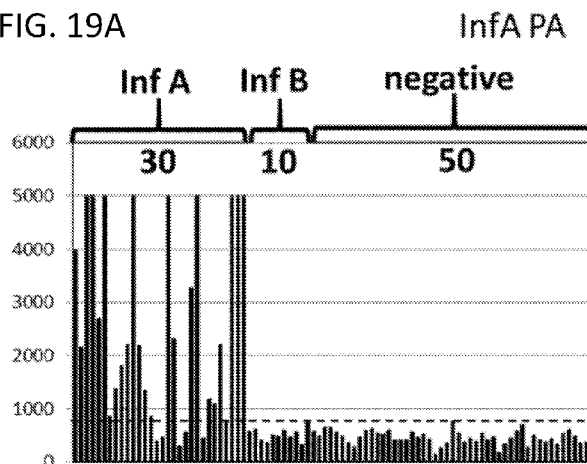
- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(88) Date of publication of the international search report:

19 October 2017

(54) Title: AUTOMATED NESTED RECOMBINASE POLYMERASE AMPLIFICATION

FIG. 19A



(57) Abstract: A flu assay system including a sample module, a microfluidic nucleic acid amplification device, and an analyzer to facilitate fully automated nested recombinase polymerase amplification (RPA) on a sample delivered to the nucleic acid amplification device via the sample module. The assay includes providing a sample to a microfluidic device, and amplifying a target polynucleotide sequence in the sample. Amplifying the target polynucleotide sequence includes performing a first round of amplification on the sample to yield a first amplification product, and performing a second round of amplification on the first amplification product to yield a second amplification product. The second amplification product includes a smaller sequence completely contained within the first amplification product produced during the first round of amplification.



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/20782

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(8) - C12Q 1/68; C12P 19/34; C12N 9/16 (2017.01)
 CPC - C12Q 1/6884, 1/6816, 2521/531, 2600/118

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.
X ----- Y	US 2012/0244534 A1 (Ching et al.) 27 September 2012 (27.09.2012). Especially para [0009], [0011], [0012], [0018], [0039], [0065], [0079], [0088], pg 17 table II, claim 27.	1, 2, 12-22, 24, 26 ----- 3-11, 23, 25, 27
Y	US 2011/0053153 A1 (Piepenburg et al.) 03 March 2011 (03.03.2011). Especially para [0011], [0015], [0018], [0054], sheet 4 fig 4.	3-11
Y	US 2009/0111089 A1 (Lindstrom et al.) 30 April 2009 (30.04.2009). Especially para [0121], [0144], [0148]	23, 25, 27
Y	Hocek et al. Nucleobase modification as redox DNA labelling for electrochemical detection. Chem Soc Rev December 2011 Vol 40 No 12 Pages 5802-5814. Especially Pg 5809 col 2 par 3; pg 5810 scheme 6a, pg 5811 col 2 para 2-3	7-11

☐ 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

14 August 2017

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/20782

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:

-----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.:
Claims 1-27

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 17/20782

-----continuation of 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 paid.

Group I: Claims 1-27, drawn to a method of nucleic acid amplification in a microfluidic device.

Group II: Claims 28-85, drawn to a card or a microfluidic device having two or more reaction chambers configured for nucleic acid amplification.

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 comprising amplifying a target polynucleotide in a first and a second round of amplification, not required by Group II.

Group II has the special technical feature of a card or a device configured to have reaction chambers, detection modules, pumps, inlet and outlet ports, not required by Group I.

Common Technical Feature:

Groups I and II share the special technical feature of (i) carrying out a first nucleic acid amplification on a sample fluid to form a first amplification product; and (ii) carrying out a second nucleic amplification on the first amplification product to form second amplification products, in a microfluidic device.

However, said common technical feature does not represent a contribution over the prior art, and is anticipated by US 2012/0244534 A1 to Ching et al. (hereinafter "Ching").

Ching teaches (i) carrying out a first nucleic acid amplification on a sample fluid to form a first amplification product; and (ii) carrying out a second nucleic amplification on the first amplification product to form second amplification products, in a microfluidic device (para [0009]; "the invention provides a method of detecting the presence or absence of one or more target polynucleotides in a sample having the following steps: (a) amplifying in a fluidly closed reaction system one or more target polynucleotides from a sample using first-stage amplification reagents in a first reaction mixture to form one or more first amplicons, the first-stage amplification reagents including initial primers for each target polynucleotide; (b) isolating a sample of the first reaction mixture in the fluidly closed reaction system; and (c) amplifying in the fluidly closed reaction system the one or more first amplicons in the sample using second-stage amplification reagents in a second reaction mixture to form one or more second amplicons, the second-stage amplification reagents including at least one secondary primer for each of the one or more first amplicons, such that each second primer is nested in such first amplicon relative to an initial primer of such first amplicon"; claim 27: microfluidic device)

As the common technical feature was 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.

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.