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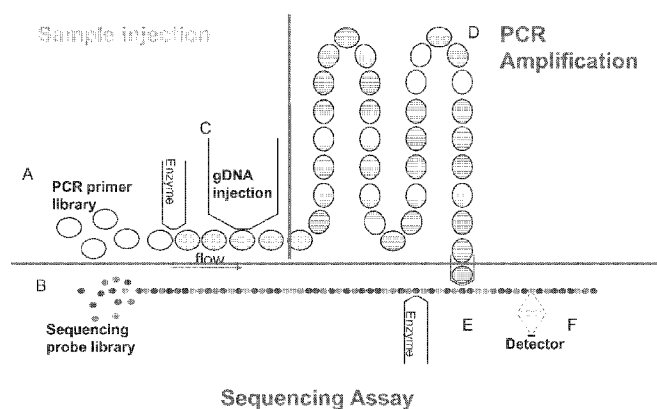
- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
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(88) Date of publication of the international search report:

5 February 2015

(54) Title: SYSTEMS AND METHODS FOR SEQUENCING IN EMULSION BASED MICROFLUIDICS

Figure 1: Microfluidic Workflow



(57) Abstract: Methods, libraries, and kits for nucleotide sequencing are provided.



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US14/40082

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68; C40B 30/10, 40/06 (2014.01)

CPC - C12Q 1/186, 1/6806; G01N 33/50

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)

IPC(8): C12Q 1/68, C40B 30/10, 40/06 (2014.01)

USPC: C12Q 1/186, 1/6806; G01N 33/50

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)

PatSeer; ScienceDirect; PubMed; Google; Google Scholar; sequencing, hybridization, primers, microfluidic, 'Gnubio,' 'sequence by hybridization', droplet, hexamer, 'next generation sequencing'

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y — A	FITZGERALD, J. Advances In RainDance Sequence Enrichment Technology And Applications In Cancer Research. RainDance Technologies presentation. 17 March 2011; pages 1-38. accessed online < http://gqinnovationcenter.com/documents/rendezVous/7-Fitzgerald_RDT.pdf >.	1-13, 21-32, 83-88 — 48-65
Y — A	WO 2012/078710 A1 (RAZ, T et al.) June 14, 2012; abstract; paragraph [0066]	1-13, 83-88 — 48-65
Y — A	WO 2012/135327 A1 (ABATE, A et al.) October 4, 2012; paragraphs [0055]-[0058]; Claim 6	8-11, 21-32, 35-41, 73-88 — 48-65, 74-76
Y	US 2012/0302448 A1 (HUTCHISON, JB et al.) November 29, 2012; paragraphs [0009], [0098]; figures 1-3	7, 13/7, 23-25, 31/23-31/25, 32/31/23-32/31/25
Y — A	ANTHONY, S. Harvard Crack DNA Storage, Crams 700 Terabytes Of Data Into A Single Gram. ExtremeTech Website. 17 August 2012. second paragraph, page 2; fourth paragraph, page 2. accessed online < http://www.extremetech.com/extreme/134672-harvard-cracks-dna-storage-crams-700-terabyte-s-of-data-into-a-single-gram >.	73, 77-82 — 74-76
Y	US 2009/0081675 A1 (COLSTON JR., BW et al.) March 26, 2009; abstract; Claim 1	12, 26, 31/26, 32/31/26,

☒ Further documents are listed in the continuation of Box C.

* 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

15 November 2014 (15.11.2014)

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US14/40082

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.: 14-20, 33, 34, 42-47
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 Supplemental Page-

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.:

Group I: Claims 1-13, 21-32, 35-41, 48-65, 73-88

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/US14/40082

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ARNESON, N et al. Whole-Genome Amplification By Degenerate Oligonucleotide Primed PCR (DOP-PCR). Cold Spring Harbor Protocols. January 2008. Vol. 3, No. 1; pages 1-6; Introduction, page 1; Materials, page 2. DOI: 10.1101/pdb.prot4919.	29, 31/29, 32/31/29
A	MILLER, JR et al. Assembly Algorithms For Next-Generation Sequencing Data. Genomics. 2009. Vol. 95; pages 315-327; fifth paragraph, left column, page 316; sixth paragraph, left column, page 316; DOI:10.1016/j.ygeno.2010.03.001.	48-65
A	US 2012/0208241 A1 (LINK, DR) August 16, 2012; entire document	1-13, 21-32, 35-41, 48-65, 73-88
A	WO 2007/133710 A2 (LINK, DR et al.) November 22, 2007; entire document	1-13, 21-32, 35-41, 48-65, 73-88
A	US 2011/0053798 A1 (HINDSON, BJ et al.) March 3, 2011; entire document	1-13, 21-32, 35-41, 48-65, 73-88

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US14/40082

----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 examined, the appropriate additional examination fees must be paid

Group I: Claims 1-13, 21-32, 35-41, 48-65 and 73-88 are directed toward a method and system for generating and determining a nucleotide sequence; and a library of different primer partitions for use in said method.

Group II: Claims 66-72 are directed toward a method of identifying primer/target nucleic acid partitions as being generated from a same target nucleic acid partition mixture drop.

The inventions listed as Groups I-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: the special technical features of Group I include determining a nucleotide sequence, which is not present in Group II, the special technical features of Group II including identifying extrema in a temporal function.

Groups I-II share the technical features including primer/target nucleic acid partitions comprising mixture drops; receiving a data signal obtained by a detector over a time period, the data signal including signals from a plurality of partitions of a plurality of mixture drops, each mixture drop corresponding to a cohort of partitions and including copies of at least one target nucleic acid; each partition including one or more primers, wherein the data signal includes data about whether at least one primer in a partition hybridizes to a target nucleic acid.

However, these shared technical features are previously disclosed by US 2012/0302448 A1 to Hutchison, et al. (hereinafter 'Hutchison'). Hutchison discloses primer/target nucleic acid partitions (primer/target nucleic acid droplets; paragraph [0007]) comprising mixture drops (paragraph [0011]); receiving a data signal obtained by a detector over a time period (receiving a fluorescence intensity signal over time from a detector; paragraph [0030]), the data signal including signals from a plurality of partitions of a plurality of mixture drops (the data including signals from probes present in a plurality of partitions of a plurality of mixture drops; paragraphs [0021], [0022]), each mixture drop corresponding to a cohort of partitions (each sample mixture droplet corresponding to a plurality of partitions; paragraphs [0020], [0022]) and including copies of at least one target nucleic acid (paragraphs [0021], [0022]); each partition including one or more primers (paragraphs [0021], [0022]), wherein the data signal includes data about whether at least one primer in a partition hybridizes to a target nucleic acid (paragraph [0105]).

Since none of the special technical features of the Groups I-II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Hutchison reference, unity of invention is lacking.