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[Continued on next page]

(54) Title: METHODS OF AMPLIFYING WHOLE GENOME OF A SINGLE CELL

(57) Abstract: Methods and compositions for amplifying the whole genome of a single cell are provided.

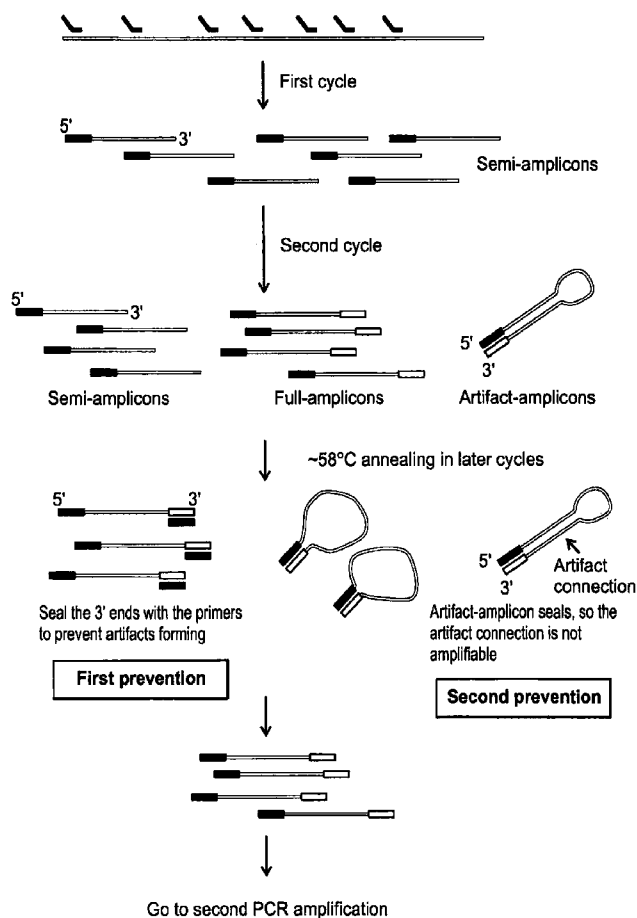


FIG. 1





(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, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/38930

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68 (2012.01)

USPC - 435/6.12; 435/6.11

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)

USPC: 435/6.12; 435/6.11

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

USPC: 435/6.12, 6.11, 6.1

(keyword limited; terms below)

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

PatBase; Google; PubMed

Search terms: whole genome, single cell, single-stranded, amplification, PCR, primer, primer-dimer, degenerate, common, constant, fixed, Phi29, Bst, SEQ ID NO:1, SEQ ID NO:2

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y — A	US 2003/0108870 A1 (JI et al.) 12 June 2003 (12.06.2003) para [0023], [0025]-[0027], [0058], [0060]-[0061], [0064], [0077], [0103], [0180]	1, 3-10, 21-22 — 2
Y — A	US 2003/0100006 A1 (SENAPATHY) 29 May 2003 (29.05.2003) para [0012]-[0013], [0016], [0031], [0081]	1, 3-10, 21-22 — 2
Y — A	US 2004/0209298 A1 (KAMBEROV et al.) 21 October 2004 (21.10.2004) para [0073], [0091]	3-4
A	US 2009/0018031 A1 (TRINKLEIN et al.) 15 January 2009 (15.01.2009) SEQ ID NO:3398	2
A	✓ GOETZ et al. Oncorhynchus mykiss regulator of G-protein signalling 14 mRNA, complete cds. GenBank Accession No. AY606041. 1 May 2006 (01.05.2006) [Retrieved from the internet on 1 November 2012 (01.11.2012)] <url:http://www.ncbi.nlm.nih.gov/nuccore/ay606041>	2
A	✓ PELAK et al. The characterization of twenty sequenced human genomes. PLoS Genet. 9 September 2010 (09.09.2010), Vol. 6, No. 9, Article e1001111, pages 1-10	1-10, 21-22
A	✓ REGIER et al. Increased yield of PCR product from degenerate primers with nondegenerate, nonhomologous 5' tails. Biotechniques. January 2005, Vol. 38, No. 1, pages 34-38	1-10, 21-22
A	✓ FAN et al. Whole-genome molecular haplotyping of single cells. Nat. Biotechnol. 2011, Vol. 29, No. 1, pages 51-57 published online 19 December 2010.	1-10, 21-22



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

1 November 2012 (01.11.2012)

Date of mailing of the international search report

23 NOV 2012

Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/38930

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item I.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 filed or furnished:

a. (means)

☐

on paper

☒

in electronic form

b. (time)

☐

in the international application as filed

☐

together with the international application in electronic form

☒

subsequently to this Authority for the purposes of search

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 in 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 12/38930

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:

This application contains the following inventions or groups of inventions which are not so linked 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 1-10, 21 and 22 directed to a method of amplifying the whole genome of a single cell.

Group II claims 11-20 directed to a method of processing at least one cell or a plurality of cells for DNA amplification.

Group III claims 23-46 directed to a method of amplifying the genome of a single cell or one or more cells.

----- (Continued on 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.:
1-10, 21 and 22

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 12/38930

Continuation of Box III - observations where unity of invention is lacking:

Group IV claims 47-61 directed to a method for linearly amplifying single stranded genomic DNA from one or more cells, a method of amplifying the genome of a one or more cells and a method of analyzing a cancer cell.

The inventions listed as Groups I-IV do not relate to a single inventive concept under 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 feature of each Group is delineated as above. The shared technical feature of all the groups is genomic DNA. Groups I, III, and IV further share the special technical feature of a method of amplifying genomic DNA from one or more cells. Groups I and III share the special technical features of providing genomic DNA from the single cell in single stranded form in a reaction vessel; adding primers having a common sequence, a variable sequence and a fixed sequence to the reaction vessel to produce a reaction mixture; subjecting the reaction mixture to a low temperature at which annealing of the primers to the single stranded genomic DNA takes place; adding at least one of a DNA polymerase having strand displacement activity or 5' to 3' exonuclease activity to the reaction mixture and subjecting the reaction mixture to a temperature at which DNA amplification takes place to produce single stranded DNA. However, this is not an improvement over the prior art of US 2003/0108870 A1 to Ji et al. (hereinafter Ji) that teaches a method of amplifying the whole genome (para [0180]) of a single cell (para [0023] - "This method can be used to amplify trace amounts of DNA, including genomic DNA from...a single cell.") comprising providing genomic DNA from the single cell in a single stranded form in a reaction vessel (para [0023], [0025]-[0026], [0060]); adding primers having a common sequence and a variable sequence to the reaction vessel to produce a reaction mixture (para [0025] - "a first primer with a random sequence of nucleotides at its 3' end and a generic sequence 5' of the random nucleotides"); subjecting the reaction mixture to a low temperature at which annealing of the primers to the single stranded genomic DNA takes place (para [0026], [0060]); adding at least one of a DNA polymerase having strand displacement activity or 5' to 3' exonuclease activity to the reaction mixture and subjecting the reaction mixture to a temperature at which DNA amplification takes place to produce single or double stranded DNA (para [0026], [0060]); subjecting the reaction mixture to a temperature to produce single stranded amplicons (para [0026], [0061] - repeating of denaturation step). Further to Group I Ji teaches repeating steps (c) to (e) to produce amplicons (para [0026], [0061] - repeating of denaturation, annealing, and extension steps); and analyzing the genomic DNA for congenital disorders or known phenotypical consequences including cancer (para [0103] - "Certain SNPs result in disease-causing mutations such as, for example, heritable breast cancer...It is specifically contemplated that DNA amplified by the disclosed methods may be useful in the detection of SNPs or other polymorphisms in an individual."). Ji does not teach that the disclosed primers have a fixed sequence in addition to the common sequence and the variable sequence. US 2003/0100006 A1 to SENAPATHY teaches primers comprising a fixed sequence and a variable ("randomized") sequence (para [0016]), and also teaches that said primers are useful for PCR amplification (para [0013]) of genomic DNA (para [0031]). Senapathy further teaches that methods using the disclosed primers allow the sequencing of a long DNA molecule without fragmenting or subcloning the long DNA molecule (para [0012]), and provides for PCR amplification of longer DNA than was previously possible (para [0081]). It thus would have been obvious to one of skill in the art to include a fixed sequence, as taught by Senapathy, in the primers taught by Ji, in order to enable amplification and analysis of longer sections of the genomic DNA without needing to fragment or subclone the DNA. Group III differs from Group I in that Group III further requires adding at least one of a DNA polymerase having strand displacement activity or 5' to 3' exonuclease activity to the reaction mixture and subjecting the reaction mixture to a temperature at which DNA amplification takes place to produce first round amplicons of the single stranded genomic DNA having a primer sequence at the 3' ends; subjecting the reaction mixture to a low temperature at which annealing of the primers to the single stranded genomic DNA takes place and at which annealing of the primers to first round amplicons takes place; adding at least one of a DNA polymerase having strand displacement activity or 5' to 3' exonuclease activity or a polymerase having a 5' flap endonuclease activity to the reaction mixture and subjecting the reaction mixture to a temperature at which DNA amplification takes place to produce first round amplicons of the single stranded genomic DNA having a primer sequence at the 3' end and second round amplicons of the first round amplicons with the second round amplicons having complementary primer sequences at their 3' and 5' ends; subjecting the reaction mixture to a temperature to anneal free primer to the 3' end of second round amplicons or to anneal the 3' end of a second round amplicon to its 5' end thereby forming a loop thereby making the second round amplicons unavailable for amplification; and (j) repeating steps (f) to (i) to produce amplicons of the genomic DNA. Group II differs from Groups I, III, and IV in that Group II requires separating the genomic DNA into individual portions of genomic DNA which is not required by the other Groups. Group IV differs from Groups I-III in that Group IV requires amplifying the single stranded genomic DNA in the presence of primers and a DNA polymerase having strand displacement activity or 5' to 3' exonuclease activity or 5' flap endonuclease activity to produce a first mixture of the single stranded genomic DNA and first round amplicons of the single stranded genomic DNA having a primer sequence at the 3' end; amplifying the first mixture in the presence of primers and a DNA polymerase having strand displacement activity or 5' to 3' exonuclease activity or 5' flap endonuclease activity to produce a second mixture of the single stranded genomic DNA, first round amplicons of the single stranded genomic DNA having a primer sequence at the 3' end and second round amplicons having complementary primer sequences at their 3' and 5' ends; annealing free primer to the 3' end of the second round amplicons or annealing the 3' end of a second round amplicon to its 5' end thereby forming a loop thereby making the second round amplicons unavailable for amplification; and amplifying the second mixture in the presence of primers and a DNA polymerase having strand displacement activity or 5' to 3' exonuclease activity or 5' flap endonuclease activity to produce amplicons of the genomic DNA which is not required by any of the other Groups.

Therefore the inventions listed as Groups I-IV do not relate to a single general inventive concept under PCT Rule 13.1 because they do not share a same or corresponding special technical feature. The first named Group to be searched without addition fees will be Group I claims 1-10, 21 and 22. In order for all inventions to be examined, the appropriate examination fees must be paid.