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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, 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, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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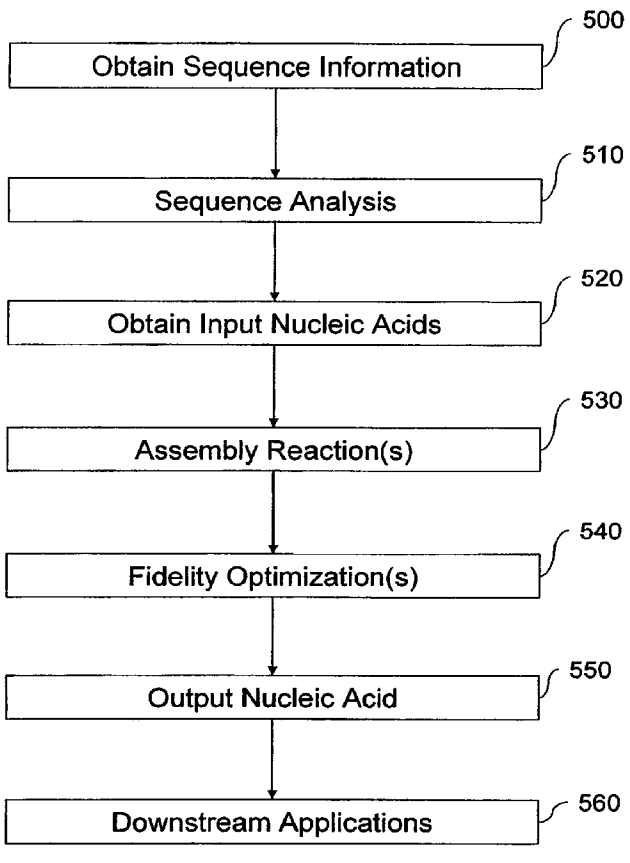
[Continued on next page]

(54) Title: METHODS AND COMPOSITIONS FOR INCREASING THE FIDELITY OF MULTIPLEX NUCLEIC ACID ASSEMBLY



(57) Abstract: Certain aspects of the present invention provide methods for increasing the fidelity of nucleic acid assembly reactions. Fidelity-optimized conditions may be used in certain embodiments. Nucleic acid binding proteins and/or recombinases (e.g., heat stable variants) may be used in some embodiments. A RecA protein (e.g., a heat stable RecA) may be included in some assembly reactions. Aspects of the invention also provide related kits, compositions, devices, and systems to increase the fidelity of multiplex nucleic acid assembly reaction.

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FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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INTERNATIONAL SEARCH REPORT

International application No
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A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68

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)
C12Q

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 practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HENEGARIU O ET AL: "MULTIPLEX PCR: CRITICAL PARAMETERS AND STEP-BY-STEP PROTOCOL" BIOTECHNIQUES, INFORMA LIFE SCIENCES PUBLISHING, WESTBOROUGH, MA, US, vol. 23, no. 3, September 1997 (1997-09), pages 504-511, XP000703350 ISSN: 0736-6205 the whole document	1-4, 6-15, 17-31, 34,39, 49-53

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[X] Further documents are listed in the continuation of Box C.

[X] 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 document 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	Date of mailing of the international search report
9 October 2007	09/01/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Paresce, Donata

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STEMMER W P C ET AL: "Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides" GENE, ELSEVIER, AMSTERDAM, NL, vol. 164, no. 1, 1995, pages 49-53, XP004041916 ISSN: 0378-1119 page 49 - page 51	1,4-10, 32-34, 36, 39-42, 44-49, 51,55-59
X	TIAN J ET AL: "Accurate multiplex gene synthesis from programmable DNA microchips" NATURE, NATURE PUBLISHING GROUP, LONDON, GB, vol. 432, no. 7020, 23 December 2004 (2004-12-23), pages 1050-1054, XP002371017 ISSN: 0028-0836 page 1051 - page 1052	1,4-10, 32-34, 36, 39-42, 44-49, 51,55-59
X	XIONG AI-SHENG ET AL: "A simple, rapid, high-fidelity and cost-effective PCR-based two-step DNA synthesis method for long gene sequences" NUCLEIC ACIDS RESEARCH, vol. 32, no. 12, July 2004 (2004-07), XP002454037 ISSN: 0305-1048 page 2 - page 3	1,5-13, 32-34, 49-54
X	US 2004/241655 A1 (HWANG YUCHI [TW] ET AL) 2 December 2004 (2004-12-02) page 2 - page 4; claims 1-23	1-13, 32-36
X	WO 02/101004 A (SHANGHAI MENDEL DNA CT CO LTD [CN]; HONG GUO FAN [CN]; YANG YONGJIE [C) 19 December 2002 (2002-12-19) page 3 - page 4	1,5-13, 17
X	SHIGEMORI YASUSHI ET AL: "Multiplex PCR: use of heat-stable Thermus thermophilus RecA protein to minimize non-specific PCR products" NUCLEIC ACIDS RESEARCH, vol. 33, no. 14, 2005, XP002454038 ISSN: 0305-1048 page 1 - page 3	1,68
P,X	WO 2006/044956 A (CODON DEVICES INC [US]; AFEYAN NOUBAR [US]; CHURCH GEORGE [US]; JACOBS) 27 April 2006 (2006-04-27) claims 1-304	1-36, 39-61,68

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 1, 9, 55-61 partially

Present claims 1, 9, 55-61 relate to methods which have a given desired property or effect, namely use one or more "fidelity-optimized annealing conditions". However, the description does not provide support and disclosure in the sense of Article 6 and 5 PCT for all such conditions having the said property or effect and there is no common general knowledge of this kind available to the person skilled in the art. This non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search of the claim (PCT Guidelines 9.19 and 9.20).

The search of these claims was consequently restricted to the specifically disclosed conditions having the desired property or effect, such as in claims 2-8, 12-35.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/007988

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.: 1, 9, 55-61 partially
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:
see FURTHER INFORMATION sheet PCT/ISA/210

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:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an 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 reportcovers 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.:

1-36, 39-61, 68

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1; claims 1-36, 39-61, 68

Methods of producing a target nucleic acid from a plurality of starting nucleic acids in an assembly reaction comprising fidelity-optimized reaction conditions.

Invention 2; claims 37 and 38

A composition comprising a plurality of nucleic acid complexes

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Invention 3: claim 62

A method of obtaining a target nucleic acid comprising sending sequence information to a remote site and sending a delivery address to the remote site.

Invention 4; claims 63-65

A system for designing a plurality of nucleic acids to be assembled into a target nucleic acid by means of obtaining the sequence of the target nucleic acid and analyzing said sequence.

Invention 5; claims 66-67

A business method for providing a system for assembling a target nucleic acid and marketing said system.

Invention 6; claims 69-86, 93-94

Methods comprising assembly of nucleic acids in a reaction comprising a recombinase protein.

Invention 7; claim 87

A method of obtaining a target nucleic acid comprising sending sequence information to a remote site and sending a delivery address to the remote site wherein the target nucleic acid is assembled in an assembly reaction comprising a recombinase protein.

Invention 8; claims 88-90

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A system for designing a plurality of nucleic acids to be assembled into a target nucleic acid by means of obtaining the sequence of the target nucleic acid and analyzing said sequence wherein the target nucleic acid is assembled in an assembly reaction comprising a recombinase protein.

Invention 9; claims 91-92

A business method for providing a system for assembling a target nucleic acid and marketing said system wherein the target nucleic acid is assembled in an assembly reaction comprising a recombinase protein.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2007/007988

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2004241655 A1	02-12-2004	CN 1572881 A	02-02-2005
		CN 1854310 A	01-11-2006
		CN 1854311 A	01-11-2006
WO 02101004 A	19-12-2002	CA 2449560 A1	19-12-2002
		EP 1436391 A2	14-07-2004
		JP 2005514003 T	19-05-2005
WO 2006044956 A	27-04-2006	AU 2005295351 A1	27-04-2006
		CA 2584984 A1	27-04-2006
		EP 1812598 A1	01-08-2007
		US 2007269870 A1	22-11-2007