(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 November 2009 (19.11.2009)

PCT

(10) International Publication Number
WO 2009/138954 A3

(51) International Patent Classification:
C12P 19/34 (2006.01) *C12N 15/10* (2006.01)
C07H 21/00 (2006.01) *C12Q 1/68* (2006.01)
C12M 1/00 (2006.01)

(21) International Application Number:
PCT/IB2009/051958

(22) International Filing Date:
12 May 2009 (12.05.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/071,710 14 May 2008 (14.05.2008) US

(71) Applicant *(for all designated States except US)*: **BRITISH COLUMBIA CANCER AGENCY BRANCH** [CA/CA]; 675 West 10th Avenue, Vancouver, British Columbia V5Z 1L3 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **COOPE, Robin** [CA/CA]; 570 West 7th Avenue, Suite 100, BC Cancer Agency Genome Sciences Centre, Vancouver, British Columbia V5Z 4S6 (CA). **HORSPOOL, Daniel** [CA/CA]; 675 West 10th Avenue, BC Cancer Agency Genome Sciences Centre, Vancouver, British Columbia V5Z 1L3 (CA). **HOLT, Robert A.** [CA/CA]; 675 West 10th Avenue, BC Cancer Agency Genome Sciences Centre, Vancouver, British Columbia V5Z 1L3 (CA).

(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, 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, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

[Continued on next page]

(54) Title: GENE SYNTHESIS BY CONVERGENT ASSEMBLY OF OLIGONUCLEOTIDE SUBSETS

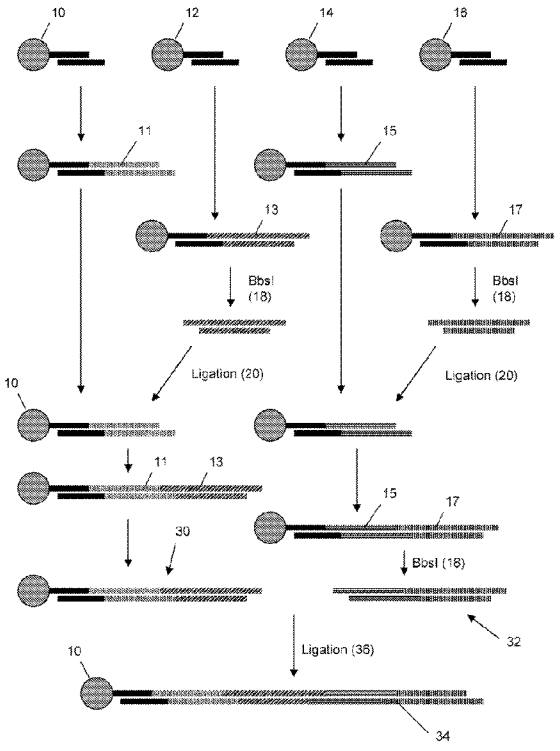


Fig. 1A

(57) Abstract: The invention provides a system and method for synthesizing polynucleotides by solid phase assembly oligonucleotide precursors, in accordance with the method, a polynucleotide is partitioned into an ordered set of subunits, wherein each subunit is assembled in a single reaction from a subset of oligonucleotide precursors that uniquely anneal together to produce the subunit. The subunits are then assembled to form the desired polynucleotide. An important feature of the invention is the selection of subunits that are free of undesired sequence elements, such as palindromes, repetitive sequences, and the like, which would result in more than one subunit product alter ligating a pool of oligonucleotide precursors.

WO 2009/138954 A3

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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**:
7 January 2010

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2009/051958

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C12P 19/34*** (2006.01) , ***C07H 21/00*** (2006.01) , ***C12M 1/00*** (2006.01) , ***C12N 15/10*** (2006.01) , ***C12Q 1/68*** (2006.01)
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: ***C12P 19/34*** (2006.01) , ***C07H 21/00*** (2006.01) , ***C12M 1/00*** (2006.01) , ***C12N 15/10*** (2006.01) , ***C12Q 1/68*** (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Scopus, Canadian Patent Database, Delphion, & PatentScope. Keywords: palindrom*, assembl*, ligat*, gene and synthes*, oligo*, non-palindrom*, solid support

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2003/0228602 A1 (PARKER, H-Y & MULLIGAN, JT) 11 December 2003. * Abstract; Fig. 1; Para. 0059, 0063, 0107 *	1-14, 17-23
Y	CHEN, P-F & PLATSOUCAS, CD. Development of the non-palindromic adaptor polymerase chain reaction (NPA-PCR) for the amplification of alpha- and beta-chain T-cell receptor cDNAs. Scandinavian Journal of Immunology. May 1992, Vol. 35, pages 539-549, ISSN 0300-9475. * Page 546, col. 2 *	1-14, 17-23
Y	HOOVER, DM & LUBKOWSKI, J. DNAWorks: an automated method for designing oligonucleotides for PCR-based gene synthesis. Nucleic Acids Research. 15 May 2002, Vol. 30, e43, ISSN 0305-1048. * Abstract; Page 3, lines 17-21 *	1-14, 17-23

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

[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 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	Date of mailing of the international search report
14 August 2009 (14-08-2009)	18 September 2009 (18-09-2009)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Authorized officer R. Atkins 819-994-4195

INTERNATIONAL SEARCH REPORT	International application No. PCT/IB2009/051958

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant	Relevant to claim No.
Y	US 7323320 B2 (OLEINIKOV, AV) 29 January 2008. * Whole document, especially: col. 9-12 *	3, 5, 7-9, 13, 14, 17-23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2009/051958

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the 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. [ ] Claim Nos. :
because they relate to subject matter not required to be searched by this Authority, namely :

2. [ ] Claim 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. [ ] Claim 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 :

Group 1 - Claims 1 to 14, and 17 to 23 feature an ordered set of palindromeless subunits; and
Group 2 - Claims 15 and 16 feature oligonucleotides capable of annealing to one another to form a polynucleotide having non-ligatable nicks.

There is a lack of unity *a priori* since the methods and systems for synthesizing a polynucleotide of Group 1 feature different subject matter from the method for synthesizing a polynucleotide of Group 2.

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 claim Nos. :

4. [X] 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 claim Nos. : 1-14, 17-23

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
Information on patent family members

International application No.
PCT/IB2009/051958

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
US 2003/0228602 A1	11-12-2003	AU 2003218470 A1	20-10-2003
		CA 2478983 A1	16-10-2003
		CA 2543014 A1	28-04-2005
		EP 1499626 A2	26-01-2005
		EP 1673381 A2	28-06-2006
		JP 2005521419 T	21-07-2005
		JP 2007508825 T	12-04-2007
		US 7482119 B2	27-01-2009
		US 2005106606 A1	19-05-2005
		US 2009137027 A1	28-05-2009
		WO 03085094 A2	16-10-2003
		WO 03085094 A3	25-11-2004
		WO 2005037850 A2	28-04-2005
		WO 2005037850 A3	09-09-2005
US 7323320 B2	29-01-2008	AU 2003270666 A1	30-04-2004
		CA 2498746 A1	25-03-2004
		EP 1546387 A2	29-06-2005
		EP 1546387 A4	12-12-2007
		JP 2005538725 T	22-12-2005
		US 7563600 B2	21-07-2009
		US 2005287585 A1	29-12-2005
		US 2006035218 A1	16-02-2006
		WO 2004024886 A2	25-03-2004
		WO 2004024886 A3	20-01-2005