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

(19) World Intellectual Property Organization
International Bureau

PCT

(43) International Publication Date
4 September 2008 (04.09.2008)

(10) International Publication Number
WO 2008/104184 A3

(51) **International Patent Classification:**
C12N 15/10 (2006.01) ***C07K 16/00*** (2006.01)

(21) **International Application Number:**
PCT/DK2008/050048

(22) International Filing Date:
27 February 2008 (27.02.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PA 2007 00316 1 March 2007 (01.03.2007) DK
60/904,772 5 March 2007 (05.03.2007) US

(71) **Applicant** *(for all designated States except US):* **SYMPHOGEN A/S** [DK/DK]; Elektrovej building 375, DK-2800 Kgs. Lyngby (DK).

(72) Inventors; and
(75) Inventors/Applicants *(for US only):* **MEUER, Per-Johan** [SE/DK]; Asminderoedgade 15, 3 tv, DK-2200 Koebenhavn N (DK). **NIELSEN, Lars, Soegaard** [DK/DK]; Nivaapark 58, DK-2990 Nivaa (DK).

(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, SV, SY, TJ, TM, TN, TR,** TT, **TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW**

(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, 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*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
16 October 2008

(54) Title: METHOD FOR CLONING COGNATE ANTIBODIES

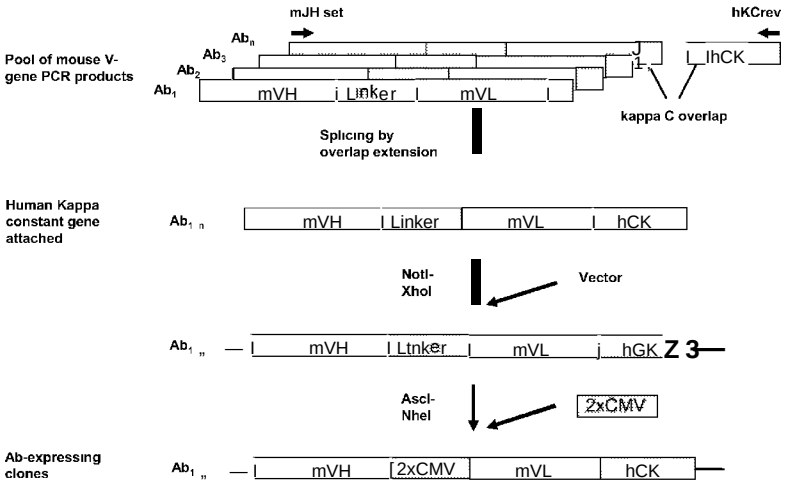

Fig. 2

(57) Abstract: The invention relates to a procedure for linking cognate pairs of V_H and V_L encoding sequences from a population of cells enriched in particular surface antigen markers. The linking procedure involves a multiplex molecular amplification procedure capable of linking nucleotide sequences of interest in connection with the amplification, in particular polymerase chain reaction (multiplex PCR). The method is particularly advantageous for the generation of cognate pair libraries as well as combinatorial libraries of variable region encoding sequences from immunoglobulins. The invention also relates to methods for generation of chimeric human/non-human antibodies and expression libraries generated by such methods.

WO 2008/104184 A3

INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2008/050048

A. CLASSIHCATION OF SUBJECT MATTER
INV. C12N15/10 C07K16/00

According Io International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C07K

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 , WPI Data, CAB Data, Sequence Search, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/042774 A (SYMPHOGEN AS [DK]; OLEKSIEWICZ MARTIN B [DK]; NIELSEN LARS S [DK]; AND) 12 May 2005 (2005-05-12) cited in the application page 57, line 13 - line 17	1-10, 16-32, 34-41, 59-68
Y	page 89, line 17 - page 91, line 18 claims 21-58	43-58, 60-68

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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 bul 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

1P• 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

"Y1 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 docu¬ments, 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
22 August 2008	01/09/2008

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	Hornig, Horst

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document with indication where appropriate, of the relevant passages	Relevant to claim No
X	BABCOOK J S ET AL: "A NOVEL STRATEGY FOR GENERATING MONOCLONAL ANTIBODIES FROM SINGLE, ISOLATED LYMPHOCYTES PRODUCING ANTIBODIES OF DEFINED SPECIFICITIES" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC, US, vol. 93, no. 15, 23 July 1996 (1996-07-23), pages 7843-7848, XP000608647 ISSN: 0027-8424	59
Y	the whole document	43-58, 60-68
X	ORLANDI R ET AL: "CLONING IMMUNOGLOBULIN VARIABLE DOMAINS FOR EXPRESSION BY THE POLYMERASE CHAIN REACTION" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC, US, vol. 86, no. 10, 1 May 1989 (1989-05-01), pages 3833-3837, XP000026475 ISSN: 0027-8424	59
Y	the whole document	43-58, 60-68
X	WO 2007/003041 A (SCHRADER JOHN [CA]) 11 January 2007 (2007-01-11) claim 34	59
X	WO 99/29888 A (SCRIPPS RESEARCH INST [US]) 17 June 1999 (1999-06-17) claims 1-15	59
A	LIU A Y ET AL: "Expression of mouse: :human immunoglobulin heavy-chain cDNA in lymphoid cells." GENE 1987, vol. 54, no. 1, 1987, pages 33-40, XP002454700 ISSN: 0378-1119 the whole document	1-42
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document with indication, where appropriate of the relevant passages	Relevant to claim No
A	US 2006/228350 A1 (WU HERREN [US] ET AL) 12 October 2006 (2006-10-12) the whole document	43-68
A	FR 2 724 393 A (INST NAT SANTE RECH MED [FR]) 15 March 1996 (1996-03-15) the whole document	43-68
A	US 2004/067532 A1 (ZHU LI [US] ET AL) 8 April 2004 (2004-04-08) the whole document	43-68
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A	US 2001/027249 A1 (LE JUNMING [US] ET AL) 4 October 2001 (2001-10-04) the whole document	43-68
A	US 2003/175837 A1 (LE JUNMING [US] ET AL LE JUNMING [US] ET AL) 18 September 2003 (2003-09-18) the whole document	43-68

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK2008/050048

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-

see additi onal sheet .

1. ☐ As all required additonal 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.:

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 POT/ISA/ 210

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

1. claim.: 1-42, (59-68) partially

A method for producing a library of cognate pairs comprising linked variable region encoding sequences, said method comprising: a) providing a lymphocyte-comprising cell fraction from the donor; b) obtaining a population of isolated single cells, comprising distributing cells from said cell fraction individually into a plurality of vessels, wherein at least a subpopulation of the cells express CD43 and CD138 antigen; and c) amplifying and effecting linkage of the variable region encoding sequences contained in said population of isolated single cells by amplifying, in a multiplex molecular amplification procedure, nucleotide sequences of interest using a template derived from an isolated single cell, or a population of isogenic cells; and effecting linkage of the nucleotide sequences of interest amplified, a library of vectors encoding chimeric antibodies each antibody member consisting of a non-human immunoglobulin variable region encoding sequences, and human immunoglobulin heavy and light chain constant regions or fragments thereof, wherein said vectors are obtained by said method above.

1.1. claims: 1-42, (59-68) partially

Idem as subject 1, but limited to: wherein at least a subpopulation of the cells express B220 and MHCII antigen;

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2. claims: 43-58, (59-68) partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A method for generating a vector encoding a chimeric antibody with human constant regions and non-human variable regions, said method comprsing: a) providing a lymphocyte-comprising cell fraction froma non-human animal; b) obtaining a population of isolated single cells, comprising distributing cells from said cell fraction individually into a plurality of vessels; c) amplifying and effecting linkage of the variable region encoding nucleic acids contained in said population of isolated single cells by amplifying, in a multiplex molecular amplification procedure, said nucleic acids using a template derived from an isolated single cell, or a population of isogenic cells; and effecting linkage of the amplified nucleic acids encoding variable regions of heavy and light chains; d) effecting linkage of the amplified variable regions to human constant regions; and e) inserting the obtained nucleic acid into a vector; a library of vectors encoding chimeric antibodies each antibody member consisting of a non-human immunoglobulin variable region encoding sequences, and human immunoglobulin heavy and light chain constant regions or fragments thereof, wherein said vectors are obtained by said method above.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/DK2008/050048

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