



## (51) International Patent Classification:

C12N 15/00 (2006.01) C12N 5/00 (2006.01)  
C07K 14/47 (2006.01) C07K 16/00 (2006.01)

(74) Agents: PASTERNAK, Dahna, S. et al.; Robins & Pasternak LLP, 1731 Embarcadero Road, Suite 230, Palo Alto, California 94303 (US).

## (21) International Application Number:

PCT/US2008/008455

## (22) International Filing Date:

10 July 2008 (10.07.2008)

## (25) Filing Language:

English

## (26) Publication Language:

English

## (30) Priority Data:

60/959,202 12 July 2007 (12.07.2007) US  
60/993,624 13 September 2007 (13.09.2007) US

(71) Applicant (for all designated States except US): SANG-AMO BIOSCIENCES, INC. [US/US]; Point Richmond Tech Center, 501 Canal Blvd., Suite A100, Richmond, California 94804 (US).

## (72) Inventors; and

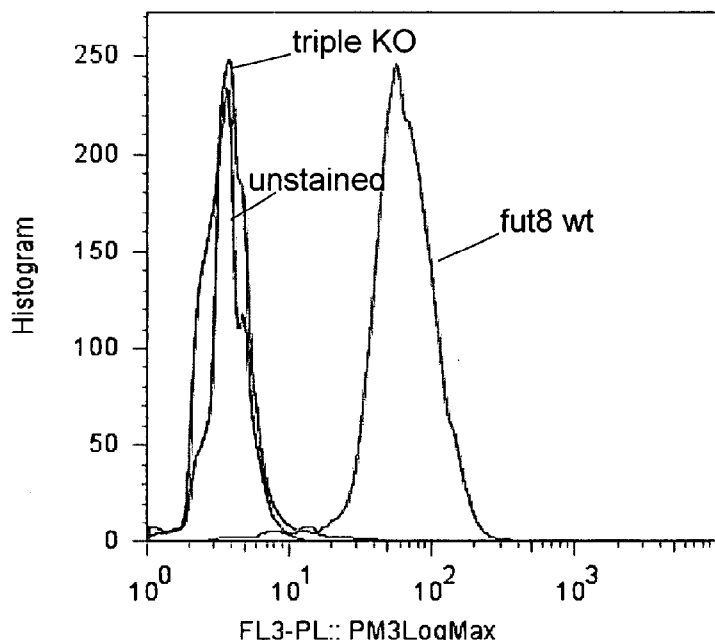
(75) Inventors/Applicants (for US only): COLLINGWOOD, Trevor [NZ/US]; 1111 Cambridge Street, Novato, California 94947 (US). COST, Gregory J. [US/US]; 2310 Ellsworth St., Apt. 3, Berkeley, California 94704 (US).

(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.

(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).

[Continued on next page]

(54) Title: METHODS AND COMPOSITIONS FOR INACTIVATING ALPHA 1,6 FUCOSYLTRANSFERASE (FUT 8) GENE EXPRESSION

**FIG. 9**

(57) Abstract: Disclosed herein are methods and compositions for inactivating a FUT8 gene, using fusion proteins comprising a zinc finger protein and a cleavage domain or cleavage half-domain. Polynucleotides encoding said fusion proteins are also provided, as are cells comprising said polynucleotides and fusion proteins.

**Published:****(88) Date of publication of the international search report:**

27 August 2009

- *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 amendments (Rule 48.2(h))*

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2008/008455

## A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/00 C07K14/47 C12N5/00 C07K16/00

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)

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, BIOSIS, EMBASE, CHEM ABS Data, Sequence Search, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                                                                                         | Relevant to claim No. |
|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| P,X       | <p>IMAI-NISHIYA HARUE ET AL: "Double knockdown of <math>\hat{I}+1,6</math>-fucosyltransferase (FUT8) and GDP-mannose 4,6-dehydratase (GMD) in antibody-producing cells: a new strategy for generating fully non-fucosylated therapeutic antibodies with enhanced ADCC"</p> <p>BMC BIOTECHNOLOGY, BIOMED CENTRAL LTD. LONDON, GB, vol. 7, no. 1, 30 November 2007 (2007-11-30), page 84, XP021035667</p> <p>ISSN: 1472-6750</p> <p>the whole document</p> <p style="text-align: center;">-----<br/>-/--</p> | 1,7-15                |

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

☐ 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

23 January 2009

Date of mailing of the international search report

16/07/2009

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040,  
Fax: (+31-70) 340-3016

Authorized officer

Seranski, Peter

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2008/008455

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                       |
|------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category*                                            | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Relevant to claim No. |
| A                                                    | <p>MORI K ET AL: "Engineering Chinese hamster ovary cells to maximize effector function of produced antibodies using FUT8 siRNA"<br/>BIOTECHNOLOGY AND BIOENGINEERING, WILEY &amp; SONS, HOBOKEN, NJ, US,<br/>vol. 88, no. 7,<br/>30 December 2004 (2004-12-30), pages 901-908, XP002358546<br/>ISSN: 0006-3592</p> <p style="text-align: center;">-----</p>                                                                                                                                                                          | 1,7-15                |
| Y                                                    | <p>BAE KWANG-HEE ET AL: "One-step selection of artificial transcription factors using an in vivo screening system."<br/>MOLECULES AND CELLS 30 JUN 2006,<br/>vol. 21, no. 3, 30 June 2006 (2006-06-30), pages 376-380, XP002511744<br/>ISSN: 1016-8478<br/>the whole document</p> <p style="text-align: center;">-----</p>                                                                                                                                                                                                            | 1,7-15                |
| Y                                                    | <p>MENG XIANGDONG ET AL: "Profiling the DNA-binding specificities of engineered Cys2His2 zinc finger domains using a rapid cell-based method."<br/>NUCLEIC ACIDS RESEARCH 2007,<br/>vol. 35, no. 11, 2007, page e81, XP002511745<br/>ISSN: 1362-4962<br/>the whole document</p> <p style="text-align: center;">-----</p>                                                                                                                                                                                                              | 1,7-15                |
| A                                                    | <p>SATOH MITSUO ET AL: "NON-FUCOSYLATED THERAPEUTIC ANTIBODIES AS NEXT-GENERATION THERAPEUTIC ANTIBODIES"<br/>EXPERT OPINION ON BIOLOGICAL THERAPY, ASHLEY, LONDON, GB,<br/>vol. 6, no. 11,<br/>1 November 2006 (2006-11-01), pages 1161-1173, XP008078583<br/>ISSN: 1471-2598</p> <p style="text-align: center;">-----</p>                                                                                                                                                                                                           | 1,7-15                |
| A                                                    | <p>SHINKAWA T ET AL: "The absence of fucose but not the presence of galactose or bisecting N-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity"<br/>JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BIRMINGHAM,; US,<br/>vol. 278, no. 5,<br/>31 January 2003 (2003-01-31), pages 3466-3473, XP002355427<br/>ISSN: 0021-9258</p> <p style="text-align: center;">-----</p> <p style="text-align: center;">-/--</p> | 1,7-15                |

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2008/008455

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                   | Relevant to claim No. |
|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A         | <p>KANDA YUTAKA ET AL: "Comparison of cell lines for stable production of fucose-negative antibodies with enhanced ADCC"</p> <p>BIOTECHNOLOGY AND BIOENGINEERING, WILEY &amp; SONS, HOBOKEN, NJ, US,</p> <p>vol. 94, no. 4, 1 July 2006 (2006-07-01), pages 680-688, XP002415543</p> <p>ISSN: 0006-3592</p> <p style="text-align: center;">-----</p> | 1,7-15                |

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2008/008455

## 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 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.:

see additional sheet(s)

### 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:

1. claims: 1 (complete), 7-15 (partial)

A zinc finger DNA binding domain as defined in claim 1;  
fusion protein comprising said zinc finger DNA binding  
domain; polynucleotide encoding said protein; isolated cell  
comprising said protein  
---

2. claims: 2 (complete), 7-15 (partial)

A zinc finger DNA binding domain as defined in claim 2;  
fusion protein comprising said zinc finger DNA binding  
domain; polynucleotide encoding said protein; isolated cell  
comprising said protein  
---

3. claims: 3-5 (complete), 7-15 (partial)

A zinc finger DNA binding domain as defined in claim 3;  
fusion protein comprising said zinc finger DNA binding  
domain; polynucleotide encoding said protein; isolated cell  
comprising said protein.  
---

4. claims: 6 (complete), 7-15 (partial)

A zinc finger DNA binding domain as defined in claim 6;  
fusion protein comprising said zinc finger DNA binding  
domain; polynucleotide encoding said protein; isolated cell  
comprising said protein  
---

5. claim: 16

A cell line in which Fut8 is partially or fully inactivated  
---

6. claims: 17-20

Method of inactivating an endogenous cellular Fut8 gene in a  
cell.  
---

7. claims: 21-24

A method of producing a recombinant protein of interest in a  
host cell.  
---