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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 December 2003 (24.12.2003)

PCT

(10) International Publication Number
WO 2003/106684 A3

(51) International Patent Classification[7]: C12N 15/67, 15/09, 15/11, 15/79, 15/90, 5/10

(21) International Application Number: PCT/NL2003/000432

(22) International Filing Date: 13 June 2003 (13.06.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
02077350.3 14 June 2002 (14.06.2002) EP

(71) Applicant *(for all designated States except US)*: **CHROMAGENICS B.V.** [NL/NL]; Plantage Muidergracht 12, NL-1018 TV Amsterdam (NL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **OTTE, Arie, Pieter** [NL/NL]; Apkenstraat 37, NL-1447 PN Purmerend (NL). **KRUCKEBERG, Arthur, Leo** [NL/NL]; Oosterpark 50, NL-1092 AP Amsterdam (NL). **SEWALT, Richard, George, Antonius, Bernardus** [NL/NL]; Oude Looierstraat 100-3, NL-1016 VK Amsterdam (NL).

(74) Agent: **PRINS, A.W.**; Nieuwe Parklaan 97, NL-2587 BN Den Haag (NL).

(81) Designated States *(national)*: AE, AG, AL, AM, AT (utility model), AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK (utility model), SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

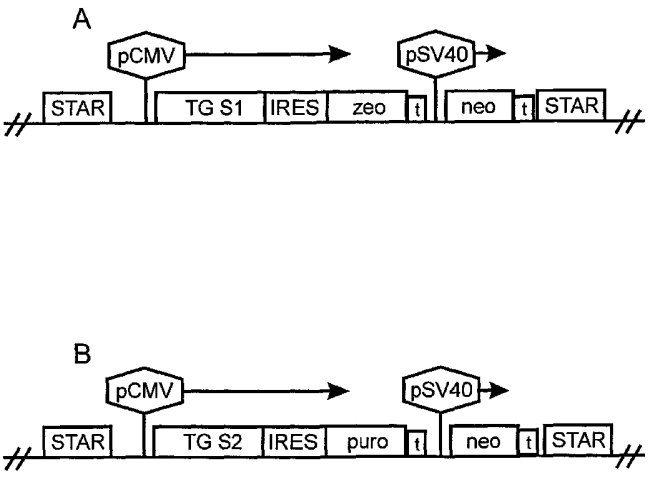
(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

[Continued on next page]

(54) Title: A METHOD FOR SIMULTANEOUS PRODUCTION OF MULTIPLE PROTEINS; VECTORS AND CELLS FOR USE THEREIN

Schematic diagram of the invention



(57) Abstract: The present invention relates to a method for improving expression of two or more proteins in a (host) cell. The method is suited for production of for example recombinant antibodies that can be used in a pharmaceutical preparation or as a diagnostic tool. In one embodiment, the invention provides a method for obtaining a cell which expresses two or more proteins comprising providing said cell with two or more protein expression units encoding said two or more proteins, characterised in that at least two of said protein expression units comprise at least one STAR sequence wherin said STAR sequence protects said transgenes from the effect of gene silencing.

WO 2003/106684 A3

(88) Date of publication of the international search report:
8 April 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

PCT/NL 03/00432

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/67 C12N15/09 C12N15/11 C12N15/79 C12N15/90
C12N5/10

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 7 C12N

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, PAJ, BIOSIS, MEDLINE, EMBASE, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6 149 212 A (BOHLE JR GEORGE WILLIAM ET AL) 21 November 2000 (2000-11-21) column 3, line 26 - line 30 claims 35,36,43-46; figure 3A; example 1A	1-12, 14-28, 30-41
Y	WO 97/27207 A (UNIV NORTH CAROLINA ;SPIKER STEVEN (US); ALLEN GEORGE C (US); HALL) 31 July 1997 (1997-07-31) page 7, line 14 - line 24 page 8, line 34 - page 9, line 1 claims 2,3; figure 3A; example 10	1-12, 14-28, 30-41
Y	WO 00/09749 A (OTTE ARIE PIETER ;STICHTING TECH WETENSCHAPP (NL)) 24 February 2000 (2000-02-24) examples 2,3	1-12, 14-28, 30-41

-/--

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

[X] Patent family members are listed in 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
29 September 2003	09.01.04

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	Tudor, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	HAN K-H ET AL: "MATRIX ATTACHMENT REGIONS (MARS) ENHANCE TRANSFORMATION FREQUENCY AND TRANSGENE EXPRESSION IN POPLAR" TRANSGENIC RESEARCH, LONDON, GB, vol. 6, 1997, pages 415-420, XP002910491 ISSN: 0962-8819 page 416, left-hand column, paragraph 2; figure 1	1-12, 14-28, 30-41
Y	AUTEN J ET AL: "EFFECT OF SCAFFOLD ATTACHMENT REGION ON TRANSGENE EXPRESSION IN RETROVIRUS VECTOR-TRANSDUCED PRIMARY T CELLS AND MACROPHAGES" HUMAN GENE THERAPY, vol. 10, no. 8, 20 May 1999 (1999-05-20), pages 1389-1399, XP000989780 ISSN: 1043-0342 page 1390, right-hand column, line 11 - line 37; figure 1	1-12, 14-28, 30-41
Y	MIELKE C ET AL: "Stabilized, long-term expression of heterodimeric proteins from tricistronic mRNA" GENE, ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, NL, vol. 254, no. 1-2, 22 August 2000 (2000-08-22), pages 1-8, XP004208775 ISSN: 0378-1119 page 2, left-hand column, line 4 - line 25; figure 1	1-12, 14-28, 30-41
A	DATABASE GENESEQ 'Online! EBI, Hinxston, Cambridgeshire, U.K.; 29 October 2001 (2001-10-29), GLUCKSMANN & SILOS-SANTIAGO: "Human seven-transmembrane protein 31945 cDNA sequence." XP002255949 Database accession no. AAH76193 abstract & WO 01/059117 A (SILOS SANTIAGO INMACULADA ;GLUCKSMANN MARIA ALEXANDRA (US); MILLEN) 16 August 2001 (2001-08-16) page 134 - page 137; claim 2	1-12, 14-28, 30-41

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	MIGLIACCIO A R ET AL: "Stable and unstable transgene integration sites in the human genome: extinction of the Green Fluorescent Protein transgene in K562 cells" GENE, ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, NL, vol. 256, no. 1-2, 3 October 2000 (2000-10-03), pages 197-214, XP004238405 ISSN: 0378-1119 page 213, left-hand column, line 41 - right-hand column, line 13	1-12, 14-28, 30-41

INTERNATIONAL SEARCH REPORT

PCT/NL 03/00432

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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 II Observations where unity of invention is lacking (Continuation of item 2 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 all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

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.:

see PCT/ISA/210 annex

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ 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. claim:

Invention 1: Claims 1-12, 14-28, 30-41, all partially; A method for producing two or more proteins in a cell using a protein expression unit comprising at least two gene expression units wherein said at least two expression units comprise at least one STAR3 (SEQ ID NO:3; the first sequence in Table3); a protein expression unit comprising STAR3 (SEQ ID NO:3) for use in said method.

Invention 2: Claims 1-12, 14-28, 30-41, all partially; A method for producing two or more proteins in a cell using a protein expression unit comprising at least two gene expression units wherein said at least two expression units comprise at least one STAR4 (SEQ ID NO:4); a protein expression unit comprising STAR4 (SEQ ID NO:4) for use in said method.

Invention 3: Claims 1-12, 14-28, 30-41, all partially; A method for producing two or more proteins in a cell using a protein expression unit comprising at least two gene expression units wherein said at least two expression units comprise at least one STAR6 (SEQ ID NO:6); a protein expression unit comprising STAR6 (SEQ ID NO:6) for use in said method.

Invention 4: Claims 1-12, 14-28, 30-41, all partially; A method for producing two or more proteins in a cell using a protein expression unit comprising at least two gene expression units wherein said at least two expression units comprise at least one STAR8 (SEQ ID NO:8); a protein expression unit comprising STAR8 (SEQ ID NO:8) for use in said method.

Invention 5: claims 13, 29 (completely) and, partially 1-12, 14-28, 30-41; A method for producing two or more proteins in a cell using a protein expression unit comprising at least two gene expression units wherein said at least two expression units comprise at least one STAR18 (SEQ ID NO:18); a protein expression unit comprising STAR18 (SEQ ID NO:18) for use in said method.

---

2. claim:

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Invention 6: Claims 1-12, 14-28, 30-41, all partially; A method for producing two or more proteins in a cell using a protein expression unit comprising at least two gene expression units wherein said at least two expression units comprise at least STAR1 (SEQ ID NO:1; the first sequence in Figure 6); a protein expression unit comprising STAR1 (SEQ ID NO:1) for use in said method.

Invention 7: as above where the STAR is STAR2 (SEQ ID NO:2)
Invention 8: as above where the STAR is STAR5 (SED ID NO:5)
Invention 9: as above where the STAR is STAR7 (SED ID NO:7)

and so on through figure 6, excluding those sequences previously described in table 3, until....

Invention 119: as above where the STAR is STAR A35 (SEQ ID NO:119)

INTERNATIONAL SEARCH REPORT

PCT/NL 03/00432

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 6149212	A	21-11-2000	NONE		
WO 9727207	A	31-07-1997	US	5773695 A	30-06-1998
			AU	716202 B2	24-02-2000
			AU	2246997 A	20-08-1997
			BR	9707208 A	06-04-1999
			CA	2244204 A1	31-07-1997
			EP	0904276 A1	31-03-1999
			JP	2000504222 T	11-04-2000
			WO	9727207 A1	31-07-1997
WO 0009749	A	24-02-2000	NL	1009862 C2	15-02-2000
			NL	1010670 C1	30-05-2000
			AU	5450599 A	06-03-2000
			EP	1104490 A1	06-06-2001
			JP	2002522094 T	23-07-2002
			WO	0009749 A1	24-02-2000
WO 0159117	A	16-08-2001	AU	3817801 A	20-08-2001
			AU	3819201 A	20-08-2001
			EP	1263950 A2	11-12-2002
			EP	1265997 A1	18-12-2002
			WO	0159117 A2	16-08-2001
			WO	0159118 A1	16-08-2001
			US	6586205 B1	01-07-2003
			US	2003166042 A1	04-09-2003