



(51) International Patent Classification:

A01K 67/027 (2006.01) C12N 15/90 (2006.01)

(21) International Application Number:

PCT/GB2009/000790

(22) International Filing Date:

25 March 2009 (25.03.2009)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

0805487.6	26 March 2008 (26.03.2008)	GB
0903120.4	24 February 2009 (24.02.2009)	GB

(71) Applicant (for all designated States except US): **ITI SCOTLAND LIMITED** [GB/GB]; 180 St. Vincent Street, Glasgow, Strathclyde, Scotland G2 5SG (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **SEIBLER, Jost** [DE/DE]; Artemis Pharmaceuticals GmbH, Neurather Ring 1, 51063 Köln (DE). **SCHEER, Nico** [DE/DE]; Artemis Pharmaceuticals GmbH, Neurather Ring 1, 51063 Köln (DE).

(74) Agent: **GOODFELLOW, Hugh Robin**; CARPMAELS & RANSFORD, 43-45 BLOOMSBURY SQUARE, LONDON, WC1A 2RA (GB).

(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, 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:

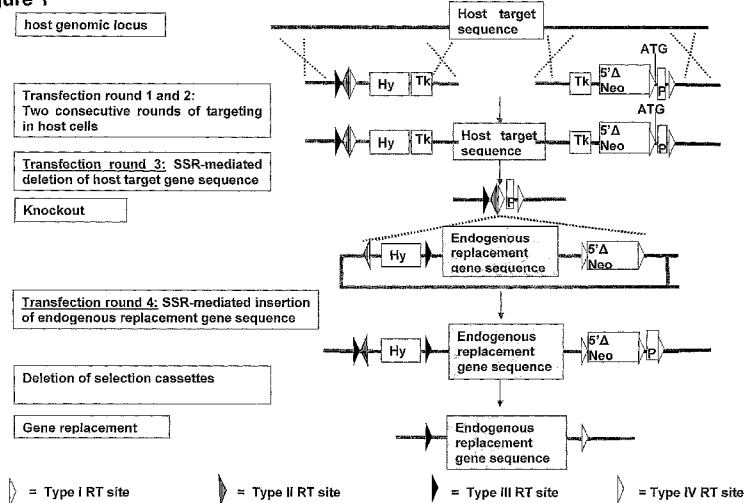
— with international search report (Art. 21(3))
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

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

26 November 2009

(54) Title: EFFICIENT INSERTION OF DNA INTO EMBRYONIC STEM CELLS

Figure 1



(57) Abstract: The present invention relates, in general, to a method for introducing a heterologous replacement gene sequence into a host embryonic stem cell to replace an endogenous host gene target sequence. In particular, the invention relates to a method for inserting large pieces of DNA into embryonic stem cells with improved efficiency, by first deleting the endogenous host gene target sequence, and subsequently utilising two proximally positioned site-specific recombinase target (RT) sites to insert a heterologous replacement gene sequence into the host chromosome.

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2009/000790

A. CLASSIFICATION OF SUBJECT MATTER
INV. A01K67/027 C12N15/90

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)
A01K 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, INSPEC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WALLACE HELEN A C ET AL: "Manipulating the mouse genome to engineer precise functional syntenic replacements with human sequence" CELL, CELL PRESS, CAMBRIDGE, MA, US, vol. 128, no. 1, 1 January 2007 (2007-01-01), pages 197-209, XP002481713 ISSN: 0092-8674 abstract page 198 - page 201 figures 2,4 ----- -/--	1-34

☒ 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.
- *G* document member of the same patent family

Date of the actual completion of the international search

11 September 2009

Date of mailing of the international search report

22/09/2009

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

Surdej, Patrick

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2009/000790

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WIRTH ET AL: "Road to precision: recombinase-based targeting technologies for genome engineering" CURRENT OPINION IN BIOTECHNOLOGY, LONDON, GB, vol. 18, no. 5, 1 October 2007 (2007-10-01), pages 411-419, XP022350911 ISSN: 0958-1669 page 414 - page 416 figure 4	1-34
X	----- SORRELL ET AL: "Targeted modification of mammalian genomes" BIOTECHNOLOGY ADVANCES, ELSEVIER PUBLISHING, BARKING, GB, vol. 23, no. 7-8, 1 November 2005 (2005-11-01), pages 431-469, XP005076498 ISSN: 0734-9750 page 456 - page 459 figures 14-16 -----	1-34