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61/492,174 1 June 2011 (01.06.2011) US(71) Applicant (for all designated States except US): **PRECISION BIOSCIENCES, INC.** [US/US]; 302 East Pettigrew Street, Dibrell Building, Suite A-100, Durham, NC 27701 (US).

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[Continued on next page]

(54) Title: METHODS AND PRODUCTS FOR PRODUCING ENGINEERED MAMMALIAN CELL LINES WITH AMPLIFIED TRANSGENES

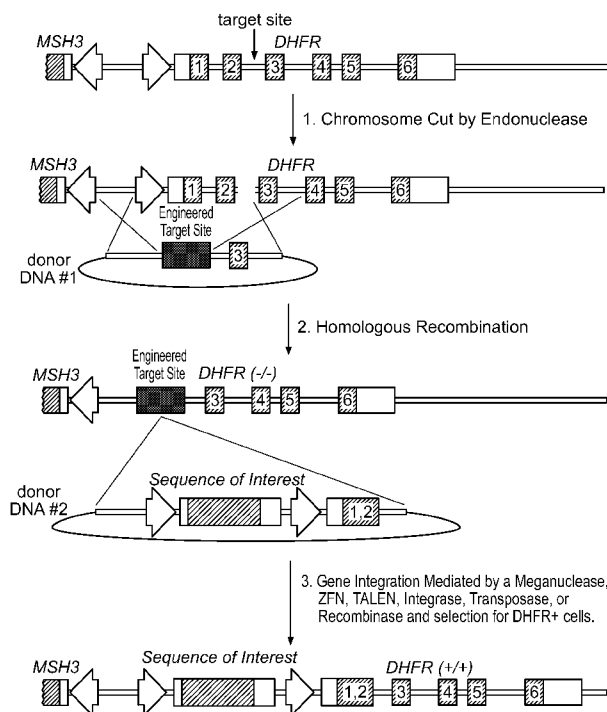


FIG. 4

(57) Abstract: Methods of inserting genes into defined locations in the chromosomal DNA of cultured mammalian cell lines which are subject to gene amplification are disclosed. In particular, sequences of interest (e.g., genes encoding bio-therapeutic proteins) are inserted proximal to selectable genes in amplifiable loci, and the transformed cells are subjected to selection to induce co-amplification of the selectable gene and the sequence of interest. The invention also relates to meganucleases, vectors and engineered cell lines necessary for performing the methods, to cell lines resulting from the application of the methods, and use of the cell lines to produce protein products of interest.



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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:

8 May 2014

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/040599

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12N 15/66 (2012.01)

USPC - 435/455

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(8) - C12N 9/00, 9/14, 9/16, 15/09, 15/63, 15/64, 15/65, 15/66, 15/67, 15/68, 15/69, 15/85, 15/87, 15/90 (2012.01)

USPC - 435/41, 72, 89, 91.1, 91.41, 183, 195, 196, 440, 455, 462, 464

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 practicable, search terms used)

Patbase, Google Patent, Google Scholar, PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2006/0206949 A1 (ARNOULD et al) 14 September 2006 (14.09.2006) entire document	1, 4 ----- 2, 3
X -- Y	WO 2008/096070 A3 (GAUCHER et al) 23 October 2008 (23.10.2008) entire document	9-15 ----- 2, 3
A	US 2003/0224481 A1 (ELLEDEGE et al) 04 December 2003 (04.12.2003) entire document	1-4, 9-15
A	WO 2010/046786 A1 (CABANIOLS et al) 29 April 2010 (29.04.2010) entire document	1-4, 9-15
A	WO 2007/137267 A2 (RUTTER et al) 29 November 2007 (29.11.2007) entire document	1-4, 9-15
A	WO 2010/026443 A1 (SMITH) 11 March 2010 (11.03.2010) entire document	1-4, 9-15

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

* 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

27 November 2012

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/040599

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.: 5-8, 16-19, 23-30, 34-41, 45-50
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 Extra 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 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 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.:
1-4 and 9-15

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

International application No.

PCT/US2012/040599

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I: claims 1-4 and 9-15 are drawn to a recombinant mammalian cell comprising an engineered target site stably integrated within a selectable gene within an amplifiable locus, wherein the engineered target site disrupts the function of the selectable gene and wherein the engineered target site comprises a recognition sequence for a site specific endonuclease.

Group II: claims 20-22, 31-33, 42-44 are drawn to a method for inserting an exogenous sequence into an amplifiable locus of a mammalian cell.

Group III: claims 51 and 52 are drawn to a recombinant meganuclease comprising the amino acid sequence of SEQ ID NO:15.

Group IV: claims 53 and 54 are drawn to a recombinant meganuclease which recognizes and cleaves a recognition of SEQ ID NO:14.

Group V: claims 55 and 56 are drawn to a recombinant meganuclease comprising the amino acid sequence of SEQ ID NO:9.

Group VI: claims 57 and 58 are drawn to a recombinant meganuclease which recognizes and cleaves a recognition of SEQ ID NO:7.

Group VII: claims 59 and 60 are drawn to a recombinant meganuclease comprising the amino acid sequence of SEQ ID NO:10.

Group VIII: claims 61 and 62 are drawn to a recombinant meganuclease which recognizes and cleaves a recognition of SEQ ID NO:8.

Group IX: claims 63 and 64 are drawn to a recombinant meganuclease comprising the amino acid sequence of SEQ ID NO:13.

Group X: claims 65 and 66 are drawn to a recombinant meganuclease which recognizes and cleaves a recognition of SEQ ID NO:12.

Group XI: claims 67 and 68 are drawn to a recombinant meganuclease comprising the amino acid sequence of SEQ ID NO:29.

Group XII: claims 69 and 70 are drawn to a recombinant meganuclease which recognizes and cleaves a recognition of SEQ ID NO:30.

The inventions listed in Groups I through XII do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I, a recombinant mammalian cell is not present in Groups II-XII; the special technical features of Group II, a method for inserting an exogenous sequence into an amplifiable locus of a mammalian cell are not present in Groups I and III-XII; the special technical features of Group III, a recombinant meganuclease comprising the amino acid sequence of SEQ ID NO:15, are not present in Groups I, II, and IV-XII; the special technical features of Group IV, a recombinant meganuclease which recognizes and cleaves a recognition of SEQ ID NO:14, is not present in Groups I-III and V-XII; the special technical features of Group V, a recombinant meganuclease comprising the amino acid sequence of SEQ ID NO:9, are not present in Groups I-IV and VI-XII; the special technical features of Group VI, a recombinant meganuclease which recognizes and cleaves a recognition of SEQ ID NO:7, are not present in Groups I-V and VII-XII; the special technical features of Group VII, a recombinant meganuclease comprising the amino acid sequence of SEQ ID NO:10, is not present in Groups I-VI and VIII-XII; the special technical features of Group VIII, a recombinant meganuclease which recognizes and cleaves a recognition of SEQ ID NO:8, are not present in Groups I-VII and IX-XII; the special technical features of Group IX, a recombinant meganuclease comprising the amino acid sequence of SEQ ID NO:13, are not present in Groups I-VIII and X-XII; the special technical features of Group X, a recombinant meganuclease which recognizes and cleaves a recognition of SEQ ID NO:12, are not present in Groups I-IX, XI, and XII; the special technical features of Group XI, a recombinant meganuclease comprising the amino acid sequence of SEQ ID NO:29, are not present in Groups I-X and XII; the special technical features of Group XII, a recombinant meganuclease which recognizes and cleaves a recognition of SEQ ID NO:30, are not present in Groups I-XI.

Groups I through XII share the technical features of a recombinant mammalian cell comprising an engineered target site stably integrated within a selectable gene within an amplifiable locus, wherein the engineered target site disrupts the function of the selectable gene and wherein the engineered target site comprises a recognition sequence for a site specific endonuclease. However, these shared technical features do not represent a contribution over the prior art. Specifically, US 2006/0206949 A1 to Arnould et al. discloses a recombinant mammalian cell (recombinant vector cell, animal, or plant, abstract; meganuclease-induced homologous recombination in yeast and mammalian cells, Para. [0238]) comprising an engineered target site stably integrated within a selectable gene within an amplifiable locus (URA3 SSA target was a modified ura3 gene (amplifiable locus) with 2 direct repeats of 600 base pairs separated by 4.3 kb, containing a kanamycin resistance cassette (selectable gene) and the HIV2 6335 (endonuclease) cleavage site, Para. [0283]), wherein the engineered target site disrupts the function of the selectable gene and wherein the engineered target site comprises a recognition sequence for a site specific endonuclease (URA3 SSA target was introduced...the strain was unable to grow on a minimal medium lacking uracil but was resistant to G418; when this target was cleaved (by the endonuclease) and recombined properly, the yeast was able to grow on media without uracil and was sensitive to G418 (selectable markers), Para. [0283]; a site of interest comprising at least one recognition and cleavage site of said meganuclease, Para. [0161]).

The inventions listed in Groups I-XII therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.