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Declarations under Rule 4.17:

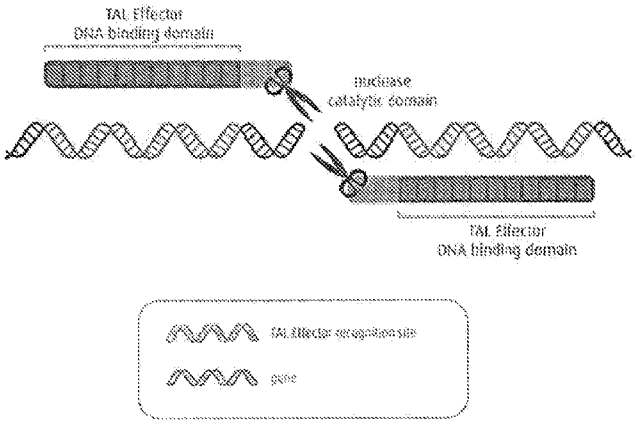
— *as to the identity of the inventor (Rule 4.17(i))*

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

[Continued on next page]

(54) Title: GENOME SURGERY WITH PAIRED, PERMEANT ENDONUCLEASE EXCISION

FIGURE 1



(57) Abstract: The use of P2E2 constructs in genome surgery includes a cell penetration component, a DNA binding component and a restriction endonuclease. The method for performing genome surgery includes: a) providing one or more recombinant of the P2E2 constructs; b) penetrating a cell with the recombinant P2E2 protein construct; c) forming a protein product in the cell by the processes of transcription and translation or by direct introduction of the P2E2 protein construct to the cell; d) attaching the protein product of the P2E2 construct to one or more targeted genomic sequences within the cell; and e) the endonuclease of the P2E2 construct cutting both strands of the genome at target locations.

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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:
17 April 2014

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/049987

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/85 (2014.01)
USPC - 435/91.4
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 1/15, 15/63, 15/64, 15/66, 15/85, 15/86 (2014.01)
USPC - 424/188.1; 435/89, 91.4, 320.1, 462

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 48/00; C12N 15/62, 15/85, 15/102; C07K 14/005 (2013.01)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, Google Patents, Google, 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	WO 2011/146121 A1 (GREGORY et al) 24 November 2011 (24.11.2011) entire document	21, 29 --- 1-18, 20
Y	US 2011/0145940 A1 (VOYTAS et al) 16 June 2011 (16.06.2011) entire document	1-9, 11-18, 20
Y	US 2010/0285464 A1 (CARMI et al) 11 November 2010 (11.11.2010) entire document	10-15
A	US 2006/0100134 A1 (GUO et al) 11 May 2006 (11.05.2006) entire document	1-18, 20, 21, 29
A	LI et al. 'Rapid and highly efficient construction of TALE-based transcriptional regulators and nucleases for genome modification:' Plant Mol Biol. 78(4-5): 407–416. 22 January 2012. entire document	1-18, 20, 21, 29
A	US 2011/0301073 A1 (GREGORY et al) 08 December 2011 (08.12.2011) entire document	1-18, 20, 21, 29

☐ 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	Date of mailing of the international search report
05 February 2014	14 FEB 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/049987

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 Extra Sheets

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.:
1-18, 20, 21, 29, limited to wherein the restriction endonuclease for targeting DNA sequences is selected to be AcsI or ApoI, both enzymes having recognition site R/AATTY.

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.

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 1-34 are drawn to a chemical tool for genome surgery comprising P2E2 constructs of a cell penetration component, a DNA binding component and a restriction endonuclease; and methods comprising the same.

The first invention of Group I+ is restricted to a P2PE2 construct, and a method for performing genome surgery comprising the same, comprising providing one or more recombinant P2E2 constructs comprising a cell penetration component, a DNA binding component and a restriction endonuclease, wherein the restriction endonuclease of the P2E2 recombinant protein cuts the genome at target sites, wherein the restriction endonuclease for targeting DNA sequences is selected to be AcsI or ApoI, both enzymes having recognition site R/AATTY. It is believed that claims 1-18, 20, 21, and 29 read on this first named invention and thus these claims will be searched without fee to the extent that they read on AcsI or ApoI and a recognition site of R/AATTY.

Applicant is invited to elect additional restriction endonucleases for each construct and method to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be a P2PE2 construct, and a method for performing genome surgery comprising the same, for performing genome surgery, comprising providing one or more recombinant P2E2 constructs comprising a cell penetration component, a DNA binding component and a restriction endonuclease, wherein the restriction endonuclease of the P2E2 recombinant protein cuts the genome at target sites, wherein the restriction endonuclease for targeting DNA sequences is selected to be HindIII having recognition site A/AGCTT. Additional restriction endonucleases will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ 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 Groups I+ formulas do not share a significant structural element, requiring the selection of alternatives for the restriction endonuclease, wherein the restriction endonuclease for targeting DNA sequences is selected from the list in claim 22.

The Groups I+ share the technical features of a method for performing genome surgery on an integrated viral genome within a host genome comprising: providing one or more recombinant P2E2 constructs comprising a cell penetration component, a DNA binding component and a endonuclease; penetrating a cell with the recombinant P2E2 protein construct; forming a protein product in the cell by the processes of transcription and translation or by direct introduction of the P2E2 protein construct to the cell; attaching the protein product of the P2E2 construct to respective target sites on two strands of the genome within the cell, the attaching of the two individual recombinant proteins positioning the endonuclease of each recombinant protein over a pair of sequences opposed to each other across a gap between the two strands of the genome within the cell; and the endonuclease of the P2E2 construct cutting both strands of the genome at target locations; further comprising identifying an integrated viral genome within a host genome; identifying a target region of nucleic acid sequences within the integrated viral genome; cutting a strand of the integrated viral genome within the cell; and a chemical tool for genome surgery comprising P2E2 constructs of a cell penetration component, a DNA binding component and a restriction endonuclease. However, these shared technical features do not represent a contribution over the prior art.
Specifically, US 2011/0301073 A1 to Gregory et al. discloses a method for performing genome surgery on an integrated viral genome within a host genome (methods of using novel DNA-binding domains for modulation of gene expression and/or genomic editing, Abstract; TALE-fusions and expression vectors encoding TALE fusions can be administered directly to the patient for modulation of gene expression and for therapeutic or prophylactic applications, for ...viral diseases, e.g., HIV, Para. [0229]; for example, an exogenous nucleic acid can comprise an infecting viral genome, a plasmid or episome introduced into a cell, or a chromosome that is not normally present in the cell, Para. [0115]) comprising: providing one or more recombinant P2E2 constructs comprising a cell penetration component, a DNA binding component and a endonuclease (the invention use DNA-binding proteins comprising one or more TALE-repeat units fused to functional protein domains (collectively "TALE-fusions"), to form ...engineered nucleases ("TALENs"), Para. [0017]; a DNA binding polypeptide comprising at least one TALE repeat unit ...in certain embodiments, one or more amino acids in the TALE repeat domain are altered such that the domain binds to a selected target sequence, Para. [0018]; fused to a cell penetrating peptide, Para. [0384]); penetrating a cell with the recombinant P2E2 protein construct (introduction of exogenous molecules into cells, Para. [0115]); forming a protein product in the cell by the processes of transcription and translation or by direct introduction of the P2E2 protein construct to the cell (expression of a fusion protein in a cell can result from delivery of the fusion protein to the cell or by delivery of a polynucleotide encoding the fusion protein to a cell, wherein the polynucleotide is transcribed, and the transcript is translated, to generate the fusion protein, Para. [0118]); attaching the protein product of the P2E2 construct to respective target sites on two strands of the genome within the cell, the attaching of the two individual recombinant proteins positioning the endonuclease of each recombinant protein over a pair of sequences opposed to each other across a gap between the two strands of the genome within the cell (contacting a target site in the endogenous cellular gene with a fusion TALE protein wherein the TALE comprises an engineered TALE repeat domain such that the TALE has specificity for a desired sequence ... the step of contacting further comprises contacting a second target site in an endogenous cellular gene with a second engineered TALE fusion protein, ...the first and second target sites are adjacent ... the first and second target sites are in the same gene, for example when a pair of TALEN fusion proteins is used to cleave in the same gene. The first and second target sites are separated by any of base pairs ("gap size"), for example, 1 to 20 (or any number therebetween) or even more base pairs, Paras. [0024] and [0025]); and the endonuclease of the P2E2 construct cutting both strands of the genome at target locations (two sets of target sites are contacted by two pairs of TALENs, and are used to create a specific deletion ...at the two sets of targets, Para. [0025]; two DSBs (double strand breaks) are introduced by the targeted nucleases described herein, resulting in the deletion of the DNA in between the DSBs, Para. [0100]); further comprising identifying an integrated viral genome within a host genome and identifying a target region of nucleic acid sequences within the integrated viral genome (another aspect provided by the invention is a method for identifying a suitable nucleic acid target for TALE binding, Para. [0040]; and a chemical tool for genome surgery comprising P2E2 constructs of a cell penetration component, a DNA binding component and a restriction endonuclease (the invention use DNA-binding proteins comprising one or more TALE-repeat units fused to functional protein domains (collectively "TALE-fusions"), to form ...engineered nucleases ("TALENs"), Para. [0017]; a DNA binding polypeptide comprising at least one TALE repeat unit ...in certain embodiments, one or more amino acids in the TALE repeat domain are altered such that the domain binds to a selected target sequence, Para. [0018]; fused to a cell penetrating peptide, Para. [0384]).