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- (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, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,

(54) Title: COMPOSITIONS AND METHODS FOR MODIFYING GENOMES USING CPF1 OR CSM1

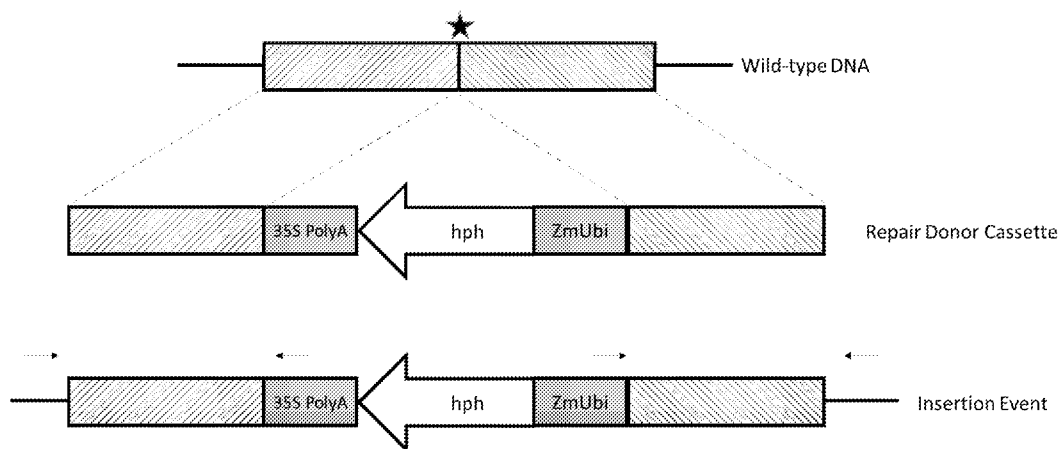


FIG. 1

(57) Abstract: Compositions and methods for modifying genomic DNA sequences are provided. The methods reduce double-strand breaks (DSBs) at pre-determined target sites in a genomic DNA sequence, resulting in mutation, insertion, and/or deletion of DNA sequences at the target site(s) in a genome. Compositions comprise DNA constructs comprising nucleotide sequences that encode a Cpf1 or Csm1 protein operably linked to a promoter that is operable in the cells of interest. The DNA constructs can be used to direct the modification of genomic DNA at pre-determined genomic loci. Methods to use these DNA constructs to modify genomic DNA sequences are described herein. Additionally, compositions and methods for modulating the expression of genes are provided. Compositions comprise DNA constructs comprising a promoter that is operable in the cells of interest operably linked to nucleotide sequences that encode a mutated Cpf1 or Csm1 protein with an abolished ability to reduce DSBs, optionally linked to a domain that regulates transcriptional activity. The methods can be used to up- or down-regulate the expression of genes at predetermined genomic loci.



PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, 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, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, 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, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

**Declarations under Rule 4.17:**

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

**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))*
- *with sequence listing part of description (Rule 5.2(a))*

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

02 November 2017 (02.11.2017)

# INTERNATIONAL SEARCH REPORT

International application No PCT/IB2017/050845
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<b>A. CLASSIFICATION OF SUBJECT MATTER</b> INV. C12N9/22      C12N15/10      C12N15/82      C12N15/62 ADD.		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b>		
Minimum documentation searched (classification system followed by classification symbols) 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 practicable, search terms used) EPO-Internal, WPI Data, BIOSIS, EMBL, FSTA, Sequence Search		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ROBERT D. FAGERLUND ET AL: "The Cpf1 CRISPR-Cas protein expands genome-editing tools", GENOME BIOLOGY, vol. 523, no. 1, 17 December 2015 (2015-12-17), page 481, XP055271439, GB ISSN: 1465-6906, DOI: 10.1186/s13059-015-0824-9 page 2; figure 1 ----- -/--	1-25
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> 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	"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	
"E" earlier application or patent but published on or after the international filing date	"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	
"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)	"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	
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family	
"P" document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search	Date of mailing of the international search report	
7 September 2017	22/09/2017	
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  Obel, Nicolai	

INTERNATIONAL SEARCH REPORT

International application No  
PCT/IB2017/050845

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BERND ZETSCHKE ET AL: "Cpf1 Is a Single RNA-Guided Endonuclease of a Class 2 CRISPR-Cas System", CELL, vol. 163, no. 3, 1 October 2015 (2015-10-01), pages 759-771, XP055267511, US ISSN: 0092-8674, DOI: 10.1016/j.cell.2015.09.038	2,8, 11-13,15
Y	page 768, column 1, line 9 - column 2, line 5 abstract; figure 1	1-10,12, 14,16-25
A	----- SERGEY SHMAKOV ET AL: "Discovery and Functional Characterization of Diverse Class 2 CRISPR-Cas Systems", MOLECULAR CELL., vol. 60, no. 3, 1 November 2015 (2015-11-01), pages 385-397, XP055267512, US ISSN: 1097-2765, DOI: 10.1016/j.molcel.2015.10.008	1-25
A	----- NICOLE RUSK: "Genomics: New kid on the CRISPR block", NATURE METHODS, vol. 12, no. 12, 1 December 2015 (2015-12-01), pages 1117-1117, XP055271447, ISSN: 1548-7091, DOI: 10.1038/nmeth.3680 the whole document	1-25
X	----- US 2014/356956 A1 (CHURCH GEORGE M [US] ET AL) 4 December 2014 (2014-12-04) claim 1 paragraph [0121] paragraph [0082]	21,23
A	----- DATABASE UniProt [Online]  20 January 2016 (2016-01-20), "RecName: Full=CRISPR-associated endonuclease Cpf1 {ECO:0000303 PubMed:26422227}; EC=3.1.-.-; AltName: Full=FnCpf1 {ECO:0000303 PubMed:26422227}"; XP002770633, retrieved from EBI accession no. UNIPROT:A0Q7Q2 Database accession no. A0Q7Q2 sequence	1-25
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INTERNATIONAL SEARCH REPORT

International application No  
PCT/IB2017/050845

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2015/071474 A2 (CRISPR THERAPEUTICS AG [CH]) 21 May 2015 (2015-05-21)  claims 45, 52-55  -----	1,3-7,9, 10,14, 16-20, 22,24,25
X,P	EP 3 009 511 A2 (BROAD INST INC [US]; MASSACHUSETTS INST TECHNOLOGY [US]; HARVARD COLLE) 20 April 2016 (2016-04-20) claims 1,9,19  -----	1-25
X	DATABASE UniProt [Online]  5 October 2010 (2010-10-05), "SubName: Full=Uncharacterized protein {ECO:0000313 EMBL:EF170750.1}";", XP002773449, retrieved from EBI accession no. UNIPROT:D8E0G1 Database accession no. D8E0G1	11, 13-15,24
Y	the whole document	1-10,12, 16-25
Y	----- THE NORTH AMERICAN CONSORTIUM FOR RUMEN BACTERIA ET AL: "Comparative Genome Analysis of: Insights into Their Environmental Niche", MICROBIAL ECOLOGY, SPRINGER-VERLAG, NE, vol. 60, no. 4, 29 June 2010 (2010-06-29), pages 721-729, XP019858682, ISSN: 1432-184X, DOI: 10.1007/S00248-010-9692-8 abstract	1-10,12, 16-25
X	----- DATABASE UniProt [Online]  26 November 2014 (2014-11-26), "SubName: Full=Uncharacterized protein {ECO:0000313 EMBL:KF067988.1}";", XP002773450, retrieved from EBI accession no. UNIPROT:A0A091FC75 Database accession no. A0A091FC75	11, 13-15,24
Y	sequence  -----  -/--	1-10,12, 16-25

**INTERNATIONAL SEARCH REPORT**

International application No PCT/IB2017/050845
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>TAE-YANG JUNG ET AL: "Crystal Structure of the Csm1 Subunit of the Csm Complex and Its Single-Stranded DNA-Specific Nuclease Activity",                      STRUCTURE,                      vol. 23, no. 4, 1 April 2015 (2015-04-01),                      pages 782-790, XP055403999,                      AMSTERDAM, NL                      ISSN: 0969-2126, DOI:                      10.1016/j.str.2015.01.021                      abstract</p> <p align="center">-----</p>	<p>1-10,12, 16-25</p>

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2017/050845

## Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
  - a.  forming part of the international application as filed:
    - in the form of an Annex C/ST.25 text file.
    - on paper or in the form of an image file.
  - b.  furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
  - c.  furnished subsequent to the international filing date for the purposes of international search only:
    - in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
    - on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2.  In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/IB2017/050845

## 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 all searchable 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 report covers only those claims for which fees were paid, specifically claims Nos.:  
  
1-25(partially)
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.:

### 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

Information on patent family members

International application No PCT/IB2017/050845
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Patent document cited in search report	A1	Publication date	Patent family member(s)	Publication date
US 2014356956	A1	04-12-2014	US 2014356956 A1	04-12-2014
			US 2014356959 A1	04-12-2014
			US 2016237456 A1	18-08-2016
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WO 2015071474	A2	21-05-2015	AU 2014350051 A1	07-07-2016
			CA 2930877 A1	21-05-2015
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			US 2016298096 A1	13-10-2016
			WO 2015071474 A2	21-05-2015
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EP 3009511	A2	20-04-2016	EP 3009511 A2	20-04-2016
			HK 1220726 A1	12-05-2017
			TW 201716572 A	16-05-2017
			US 2016208243 A1	21-07-2016
			WO 2016205711 A1	22-12-2016
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**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-25(partially)

Directed to a polypeptide with SEQ ID NO 3.

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2-83. claims: 1-25(partially)

Directed to each further polypeptide identified claims 1 and 2.

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