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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, 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, 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, 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.

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19 February 2015

(54) **Title:** COMPOSITIONS AND METHODS FOR CLOSTRIDIAL TRANSFORMATION

(57) **Abstract:** The invention provides compositions and methods for clostridial bacteria that have been engineered to produce and/or to improve efficiency of production of industrial bioproducts.



INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/043424

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N9/10

ADD.

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)

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, BIOSIS, Sequence Search, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	V BUTKUS ET AL: "Investigation of restriction-modification enzymes from M. varians RFL19 with a new type of specificity toward modification of substrate", NUCLEIC ACIDS RESEARCH, vol. 13, no. 16, 26 August 1985 (1985-08-26), pages 5727-5748, XP055144431, GB ISSN: 0305-1048, DOI: 10.1093/nar/13.16.5727 abstract ----- -/-	1-20, 31-33,36



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 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

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Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/043424

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PURDY D ET AL: "CONJUGATIVE TRANSFER OF CLOSTRIDIAL SHUTTLE VECTORS FROM ESCHERICHIA COLI TO CLOSTRIDIUM DIFFICILE THROUGH CIRCUMVENTION OF THE RESTRICTION BARRIER", MOLECULAR MICROBIOLOGY, WILEY-BLACKWELL PUBLISHING LTD, GB, vol. 46, no. 2, 1 October 2002 (2002-10-01), pages 439-452, XP001159767, ISSN: 0950-382X, DOI: 10.1046/J.1365-2958.2002.03134.X page 448, column 1, paragraph 26-39 -----	46,48-50
A	L.D Mermelstein ET AL: "In vivo methylation in Escherichia coli by the Bacillus subtilis phage .phi.3T I methyltransferase to protect plasmids from restriction upon transformation of Clostridium acetobutylicum ATCC 824", Appl. Environ. Microbiol., 1 January 1993 (1993-01-01), pages 1077-1081, XP055144733, Retrieved from the Internet: URL:http://aem.asm.org/content/59/4/1077.full.pdf abstract -----	1-20, 31-33, 36,46, 48-50
A	HEAP J T ET AL: "A modular system for Clostridium shuttle plasmids", JOURNAL OF MICROBIOLOGICAL METHODS, ELSEVIER, AMSTERDAM, NL, vol. 78, no. 1, 1 July 2009 (2009-07-01), pages 79-85, XP026148475, ISSN: 0167-7012, DOI: 10.1016/J.MIMET.2009.05.004 [retrieved on 2009-05-13] abstract figure 1 table 2 -----	1-20, 31-33, 36,46, 48-50
A	C. LEANG ET AL: "A Genetic System for Clostridium ljungdahlii: a Chassis for Autotrophic Production of Biocommodities and a Model Homoacetogen", APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 79, no. 4, 30 November 2012 (2012-11-30), pages 1102-1109, XP055110483, ISSN: 0099-2240, DOI: 10.1128/AEM.02891-12 abstract ----- -/--	1-20, 31-33, 36,46, 48-50

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/043424

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JENNERT K C B ET AL: "GENE TRANSFER TO CLOSTRIDIUM CELLULOLYTICUM ATCC35319", MICROBIOLOGY, SOCIETY FOR GENERAL MICROBIOLOGY, READING, GB, vol. 146, 1 January 2000 (2000-01-01), pages 3071-3080, XP002515778, ISSN: 1350-0872 abstract -----	1-20, 31-33, 36,46, 48-50
A	HONGJUN DONG ET AL: "Engineering Clostridium Strain to Accept Unmethylated DNA", PLOS ONE, vol. 5, no. 2, 9 February 2010 (2010-02-09), page e9038, XP055144560, DOI: 10.1371/journal.pone.0009038 abstract -----	1-20, 31-33, 36,46, 48-50
A	N. TAKAHASHI ET AL: "A DNA Methyltransferase Can Protect the Genome from Postdisturbance Attack by a Restriction-Modification Gene Complex", JOURNAL OF BACTERIOLOGY, vol. 184, no. 22, 15 November 2002 (2002-11-15), pages 6100-6108, XP055144423, ISSN: 0021-9193, DOI: 10.1128/JB.184.22.6100-6108.2002 abstract -----	1-20, 31-33, 36,46, 48-50

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/043424

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.:
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.:

1-20, 36(completely); 31-33, 46, 48-50(partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-20, 36(completely); 31-33, 46, 48-50(partially)

An isolated polynucleotide having at least 90% sequence identity to SEQ ID NO: 1, wherein the polynucleotide encodes for a polypeptide with methyltransferase activity, and methods using thereof.

2. claims: 22-30, 34, 35(completely); 31-33(partially)

An isolated polynucleotide having at least 90% sequence identity to SEQ ID NO: 4, wherein the polynucleotide encodes a polypeptide with endonuclease activity, and methods using thereof.

3. claim: 21

A method of producing a recombinant Clostridium bacterial transformant, comprising introducing a polynucleotide encoding for a DNA methyltransferase into a Escherichia bacterial host cell.

4. claims: 37(completely); 43-50(partially)

A shuttle plasmid comprising pDW280 (SEQ ID NO: 15), and methods using thereof.

5. claims: 38(completely); 43-50(partially)

A shuttle plasmid comprising pMCS537 (SEQ ID NO: 16), and methods using thereof.

6. claims: 39(completely); 43-50(partially)

A shuttle plasmid comprising pMCS200 (SEQ ID NO: 17), and methods using thereof.

7. claims: 40(completely); 43-50(partially)

A shuttle plasmid comprising pMCS201 (SEQ ID NO: 18), and methods using thereof.

8. claims: 41(completely); 43-50(partially)

A shuttle plasmid comprising pMCS444 (SEQ ID NO: 19), and methods using thereof.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

9. claims: 42(completely); 43-50(partially)

A shuttle plasmid comprising pMCS445 (SEQ ID NO: 20).
