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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:
12 October 2023 (12.10.2023)

(54) Title: SYSTEMS AND METHODS FOR TRANSPOSING CARGO NUCLEOTIDE SEQUENCES

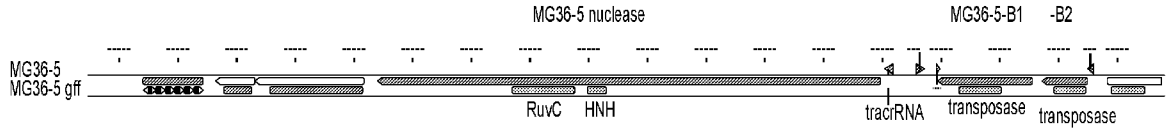

FIG. 4A

(57) Abstract: The present disclosure provides systems and methods for transposing a cargo nucleotide sequence into a target nucleic acid site in a target nucleic acid. These systems and methods may comprise a double-stranded nucleic acid comprising the cargo nucleotide sequence, wherein the cargo nucleotide sequence is configured to interact with a recombinase or transposase complex, an effector complex comprising an effector and at least one engineered guide polynucleotide configured to hybridize to the target nucleic acid, and the recombinase or transposase complex wherein said recombinase or transposase complex is configured to recruit the cargo nucleotide to the target nucleic acid site.

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A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. C12N 15/90; C12N 9/22; C12N 15/11 (2023.01)
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CPC - INV. C12N 15/90; C12N 9/1241; C12N 9/22; C12N 15/102; C12N 15/11

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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)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic database consulted during the international search (name of database and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2020/0190487 A1 (THE BROAD INSTITUTE INC.) 18 June 2020; claim [1]; [0008], [0009], [0022], [0023], [0127], [0129], [0146], [0174], [0177], [0192], [0195], [0241] [0609]	1-5, 96, 99-100
Y	SERGANOV, A ET AL. "Ribosomal protein S15 represses its own translation via adaptation of an rRNA-like fold within its mRNA" pages 1898-1908. The EMBO Journal. Vol. 22, No. 8. 15 April 2003; abstract; DOI: 10.1093/emboj/cdg170	1-5, 96
Y	HAFT, DH ET AL. "A Guild of 45 CRISPR-Associated (Cas) Protein Families and Multiple CRISPR/Cas Subtypes Exist in Prokaryotic Genomes" pages 474-483. PLOS Computational Biology. Vol. 1, No. 6. 11 November 2005; pg. 2, column 1, paragraph 4; pg. 9, column 2, paragraph 1; pg. 11; DOI: 10.1371/journal.pcbi.0010060	99
Y --- A	PEDERSON, C ET AL. "Parabacteroides distasonis strain FDAARGOS_759 chromosome, complete genome" GenBank entry (online). National Center for Biotechnology Information. 04 June 2020; Retrieved from the Internet on 12 June 2023: <URL: https://www.ncbi.nlm.nih.gov/nucleotide/CP054012.1>; pg. 1	100 --- 101
A	WO 2018/229189 A1 (4D PHARMA RESEARCH LIMITED) 20 December 2018; pg. 8, lines 11-12	6-7
A	WO 2016/110512 A1 (DSM IP ASSETS B.V.) 14 July 2016; pg. 8, line 29	6-7
A	WO 2021/209820 A1 (INSTITUT PASTEUR) 21 October 2021; pg. 8, line 29	6-7
A	US 2019/0367949 A1 (LIFEEDIT INC.) 05 December 2019; [0044]	101

☐ 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
"D" document cited by the applicant in the international application
"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
12 June 2023 (12.06.2023)	AUG 21 2023
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
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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.

   b. ☒ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),

      ☒ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US23/63181

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.: 8-22, 30-35, 37-39, 47-58, 68-73, 81-95, 112-124
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:
-***-Please See Supplemental Page-***-

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.:
Groups I+, claims 1-7, 23-29, 40-46, 59-67, 74-80, and 96-111, class 2, type II Cas effector (Cas effector type), SEQ ID NO: 1 (Cas effector sequence), SEQ ID NO: 17 (left-hand recombinase sequence), SEQ ID NO: 19 (right-hand recombinase sequence), SEQ ID NO: 11 (guide polynucleotide sequence), no specified Tn7 component sequence (Tn7 component sequence).

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.

-***-Continued From Box No. III: Observations where unity of invention is lacking-***-

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 must be paid.

Groups I+, claims 1-7, 23-29, 40-46, 59-67, 74-80, and 96-111, class 2, type II Cas effector (Cas effector type), SEQ ID NO: 1 (Cas effector sequence), SEQ ID NO: 17 (left-hand recombinase sequence), SEQ ID NO: 19 (right-hand recombinase sequence), SEQ ID NO: 11 (guide polynucleotide sequence), no specified Tn7 component sequence (Tn7 component sequence), are directed to systems for transporting cargo nucleotide sequences into a target nucleic acid site, and engineered nuclease systems.

The systems of Claims 1-5, 6 (in-part), 7, 96, and 99-101 are believed to encompass the first named invention of Groups I+ and are the claims that will be searched to the extent that they comprise a Cas effector encompassing a class 2, type II Cas effector (first exemplary Cas effector type) comprising SEQ ID NO: 1 (Cas effector sequence), a left-hand recombinase sequence encompassing SEQ ID NO: 17 (first exemplary left-hand recombinase sequence), a right-hand recombinase sequence encompassing SEQ ID NO: 19 (first exemplary right-hand recombinase sequence), and a guide polynucleotide encompassing SEQ ID NO: 12 (first exemplary guide polynucleotide sequence). The first invention does not include a specified Tn7 component sequence. This first named invention of Group I+ has been selected to encompass the first species of each of the genera found in claims 1, 6-7, 23, 28-29, 40, 45-46, 59-66, 74, 79-80, and 96-111 based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines.

Applicant is invited to elect additional Cas effectors types and sequences, left- and right-hand recombinases, and guide polynucleotides, and, if appropriate, the sequence of a Tn7 type transposase component, with specified SEQ ID NO: for each, or with specified substitution(s) at specified site(s) of a SEQ ID NO:, such that the sequence of each elected species is fully specified (i.e. no optional or variable residues or substituents), and where available as an option within at least one searchable claim, to be searched. Additional sequence(s) will be searched upon the payment of additional fees. Applicants must specify the searchable claims that encompass any additionally elected sequence(s). Applicants must further indicate, if applicable, the claims which encompass 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. An exemplary election would be class 2, type V Cas effector (Cas effector type), SEQ ID NO: 22 (Cas effector sequence), SEQ ID NO: 123 (left-hand recombinase sequence), SEQ ID NO: 124 (right-hand recombinase sequence), SEQ ID NO: 90 (guide polynucleotide sequence), and SEQ ID NO: 23 (Tn7 component sequence).

No technical features are shared between the polypeptide and nucleic acid sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features including: a system for transposing a cargo nucleotide sequence into a target nucleic acid site in a target nucleic acid comprising: a Cas effector complex comprising a Cas effector, a small prokaryotic ribosomal protein subunit S15, and an engineered guide polynucleotide configured to hybridize to the target nucleic acid site, a recombinase or transposase complex, in particular a Tn7 type transposase complex, configured to bind the Cas effector complex, and a double-stranded nucleic acid configured to interact with the recombinase or transposase complex and comprising a left-hand recombinase sequence, the cargo nucleotide sequence, and a right-hand recombinase sequence; and an engineered nuclease system comprising an endonuclease comprising a RuvC domain and an HNH domain, wherein the endonuclease is derived from an uncultivated microorganism, and an engineered guide polynucleotide, wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize into a target nucleic acid sequence; these shared technical features are previously disclosed by US 2020/0190487 A1 to THE BROAD INSTITUTE, INC. et al. (hereinafter "Broad") in view of the publication entitled "Ribosomal protein S15 represses its own translation via adaptation of an rRNA-like fold within its mRNA" by Serganov, et al. (hereinafter "Serganov").

Broad discloses a system for transposing a cargo nucleotide sequence into a target nucleic acid site in a target nucleic acid (engineered nucleic acid targeting system for insertion of donor polynucleotides to a target sequence of a target polynucleotide; claim 1) comprising: a Cas effector complex comprising a Cas effector (Cas protein (effector); claim 1), and an engineered guide polynucleotide configured to hybridize to the target nucleic acid site (a guide molecule capable of complexing with the Cas protein and directing sequence specific binding of the guide-Cas protein complex to a target sequence of a target polynucleotide; claim 1), a recombinase or transposase complex, in particular a Tn7 type transposase complex, configured to bind the Cas effector complex (a complex of CRISPR-associated transposase proteins including Tn7 transposases (configured to bind the Cas effector complex); claim 8), and a double-stranded nucleic acid configured to interact with the recombinase or transposase complex and comprising a left-hand recombinase sequence, the cargo nucleotide sequence, and a right-hand recombinase sequence (donor polynucleotide for insertion into the target polynucleotide flanked by a right end sequence element and a left end sequence element (recombinase sequences); claim 13); and an engineered nuclease system comprising an endonuclease comprising a RuvC domain and an HNH domain (the nuclease lobe contains the HNH and RuvC nuclease domains; paragraph [0279]), and an engineered guide polynucleotide (a guide molecule capable of complexing with the Cas protein and directing sequence specific binding of the guide-Cas protein complex to a target sequence of a target polynucleotide; claim 1), wherein the engineered guide RNA is configured to form a complex with the endonuclease and the engineered guide RNA comprises a spacer sequence configured to hybridize into a target nucleic acid sequence (a guide molecule capable of complexing with the Cas protein and directing sequence specific binding (hybridize) of the guide-Cas protein complex to a target sequence of a target polynucleotide; claim 1).

Broad does not disclose a small prokaryotic ribosomal protein subunit S15, and wherein the endonuclease is derived from an uncultivated microorganism.

Serganov discloses a small prokaryotic ribosomal protein subunit S15 (ribosomal protein S15 binds to RNA; abstract). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Broad to incorporate a small prokaryotic ribosomal protein subunit S15, as disclosed by Serganov, in order to increase the capability of the Cas protein to bind RNA by providing an additional RNA binding subunit.

-***-Continued Within the Next Supplemental Box-***-

-***-Continued from previous Supplemental Box-***-

Additionally, it would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the disclosure of Broad to incorporate an endonuclease derived from an uncultivated microorganism, in order to experimentally identify useful endonucleases from previously uncultivated species.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Broad and Serganov references, unity of invention is lacking.