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(54) Title: NUCLEASE-MEDIATED NUCLEIC ACID MODIFICATION

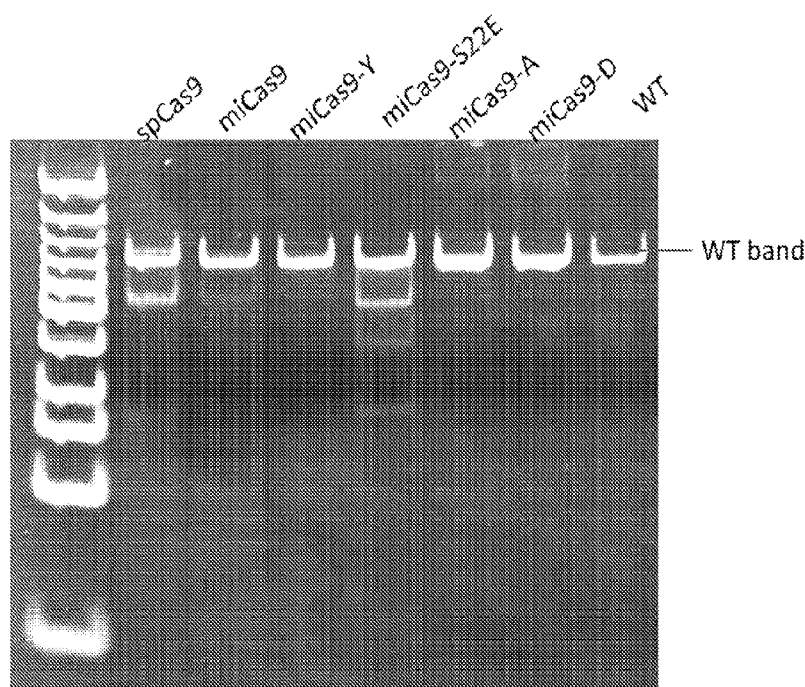


FIG. 1

(57) Abstract: Provided herein is technology relating to molecular biological manipulation of genes and genomes and particularly, but not exclusively, to CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) methods, compositions, systems, and kits for improved genetic editing.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US21/46247

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/63; C12N 9/22; C12N 15/90; C12N 9/96 (2021.01)

CPC - C12N 15/90; A61K 38/465; C12N 15/63; C12N 9/22; C12N 9/96; C12N 15/907

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 data base consulted during the international search (name of data base 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.
X --- Y	WO 2020/018166 A1 (THE REGENTS OF THE UNIVERSITY OF MICHIGAN) 23 January 2020; abstract; page 1, lines 30-35; page 2, lines 1-5; page 3, lines 15-35; page 4, lines 1-25; claims 35-36	1-3, 7-9, 13-27, 31-32, 36-47 --- 4, 10, 28, 33
Y	(NUNEZ, JK et al.) Integrase-mediated spacer acquisition during CRISPR-Cas adaptive immunity. Nature. 12 March 2015, Vol. 519, No. 7542; pages 193-198; abstract; DOI: 10.1038/nature14237	4, 33
Y	(HUAI, C et al.) Structural insights into DNA cleavage activation of CRISPR-Cas9 system. Nature Communications. 9 November 2017, Vol. 8, No. 1, article 1375; pages 1-9; abstract; page 5, second column, third paragraph; page 7, figure 5c; DOI: 10.1038/s41467-017-01496-2	10, 28
A	(STERNBERG, SH et al.) Adaptation in CRISPR-Cas Systems. Molecular Cell. 17 March 2016, Vol. 61, No. 6; pages 797-808; DOI: 10.1016/j.molcel.2016.01.030	1-4, 7-10, 13-28, 31-33, 36-47

☐ 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

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US21/46247

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/US21/46247

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:
-***-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.:
Group I+, Claims 1-47; Cas1 (nuclease domain); S15A (BE27 variant domain substitution)

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.

Group I+, Claims 1-47, Cas1 (nuclease domain), and S15A (BE27 variant domain substitution); are directed toward a GEN-BE27 variant fusion protein comprising a gene editing nuclease domain and a BE27 variant domain and methods, compositions, systems, kits, uses, nucleic acids, vectors, cells, and transgenic animals comprising said variant fusion protein.

The GEN-BE27 variant fusion protein, methods, compositions, systems, kits, uses, nucleic acids, vectors, cells, and transgenic animals will be searched to the extent that they encompass Cas1 (first exemplary nuclease domain) and S15A (first exemplary BE27 variant domain substitution). Applicant is invited to elect additional nuclease domain(s) and/or substitution(s), to be searched. Additional nuclease domain(s) and/or substitution(s) will be searched upon the payment of additional fees. It is believed that claims 1-3, 4 (in-part), 7-10, 13-28, 31-32, 33 (in-part), and 36-47 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass Cas1 (nuclease domain) and S15A (BE27 variant domain substitution). Applicants must specify the claims that encompass any additionally elected nuclease domain(s) and/or substitution(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 Cas1B (nuclease domain).

No technical features are shared between the nuclease domains and substitutions 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 GEN-BE27 variant fusion protein comprising a gene editing nuclease domain and a BE27 variant domain; a composition comprising the GEN-BE27 variant fusion protein; a method of producing a knockin in a target nucleic acid, the method comprising contacting a target nucleic acid with a GEN-BE27 variant fusion protein; a method of producing a knockin in a target nucleic acid, the method comprising contacting a target nucleic acid with a ribonucleoprotein comprising a GEN-BE27 variant fusion protein and a gRNA comprising a sequence complementary to the target nucleic acid; a kit comprising a GEN-BE27 variant fusion protein; a system comprising a GEN-BE27 variant fusion protein; use of a GEN-BE27 variant fusion protein to produce a transgenic cell; use of a GEN-BE27 variant fusion protein to produce a transgenic animal; a nucleic acid encoding a GEN-BE27 variant fusion protein; a vector comprising a nucleic acid encoding a GEN-BE27 variant fusion protein; a cell comprising a GEN-BE27 variant fusion protein; a cell comprising a nucleic acid encoding a GEN-BE27 variant fusion protein; a cell expressing a GEN-BE27 variant fusion protein; a cell expressing a nucleic acid encoding a GEN-BE27 variant fusion protein; a transgenic animal expressing a GEN-BE27 variant fusion protein; a transgenic animal expressing a nucleic acid encoding a GEN-BE27 variant fusion protein; these shared technical features are previously disclosed by WO 2020/018166 A1 (THE REGENTS OF THE UNIVERSITY OF MICHIGAN) (hereinafter 'Michigan').

Michigan discloses a GEN-BE27 variant fusion protein comprising a gene editing nuclease domain and a BE27 variant domain (a GEN-BE27 variant fusion protein comprising a gene editing nuclease domain and a BE27 variant domain; page 27, lines 20-25; page 56, lines 1-3; claim 1); a composition comprising the GEN-BE27 variant fusion protein (a composition comprising the GEN-BE27 variant fusion protein; claim 10); a method of producing a knockin in a target nucleic acid (a method of producing a knockin in a target nucleic acid; claim 20), the method comprising contacting a target nucleic acid with a GEN-BE27 variant fusion protein (the method comprising contacting a target nucleic acid with a GEN-BE27 variant fusion protein; page 27, lines 20-25; page 56, lines 1-3; claim 20); a method of producing a knockin in a target nucleic acid (a method of producing a knockin in a target nucleic acid; claim 21), the method comprising contacting a target nucleic acid with a ribonucleoprotein comprising a GEN-BE27 variant fusion protein and a gRNA comprising a sequence complementary to the target nucleic acid (the method comprising contacting a target nucleic acid with a ribonucleoprotein comprising a GEN-BE27 variant fusion protein and a gRNA comprising a sequence complementary to the target nucleic acid; page 27, lines 20-25; page 56, lines 1-3; claim 21); a kit comprising a GEN-BE27 variant fusion protein (a kit comprising a GEN-BE27 variant fusion protein; page 61, lines 25-31; claim 25); a system comprising a GEN-BE27 variant fusion protein (a system comprising a GEN-BE27 variant fusion protein; page 27, lines 20-25; page 56, lines 1-3; claim 26); use of a GEN-BE27 variant fusion protein to produce a transgenic cell (use of a GEN-BE27 variant fusion protein to produce a transgenic cell; page 27, lines 20-25; page 56, lines 1-3; claim 27); use of a GEN-BE27 variant fusion protein to produce a transgenic animal (use of a GEN-BE27 variant fusion protein to produce a transgenic animal; page 27, lines 20-25; page 56, lines 1-3; claim 28); a nucleic acid encoding a GEN-BE27 variant fusion protein (a nucleic acid encoding a GEN-BE27 variant fusion protein; page 27, lines 20-25; page 56, lines 1-3; claim 29); a vector comprising a nucleic acid encoding a GEN-BE27 variant fusion protein (a vector comprising a nucleic acid encoding a GEN-BE27 variant fusion protein; page 27, lines 20-25; page 56, lines 1-3; claim 30); a cell comprising a GEN-BE27 variant fusion protein (a cell comprising a GEN-BE27 variant fusion protein; page 27, lines 20-25; page 56, lines 1-3; claim 31); a cell comprising a nucleic acid encoding a GEN-BE27 variant fusion protein (a cell comprising a nucleic acid encoding a GEN-BE27 variant fusion protein; page 27, lines 20-25; page 56, lines 1-3; claim 32); a cell expressing a GEN-BE27 variant fusion protein (a cell expressing a GEN-BE27 variant fusion protein; page 27, lines 20-25; page 56, lines 1-3; claim 33); a cell expressing a nucleic acid encoding a GEN-BE27 variant fusion protein (a cell expressing a nucleic acid encoding a GEN-BE27 variant fusion protein; page 27, lines 20-25; page 56, lines 1-3; claim 34); a transgenic animal expressing a GEN-BE27 variant fusion protein (a transgenic animal expressing a GEN-BE27 variant fusion protein; page 27, lines 20-25; page 56, lines 1-3; claim 35); a transgenic animal expressing a nucleic acid encoding a GEN-BE27 variant fusion protein (a transgenic animal expressing a nucleic acid encoding a GEN-BE27 variant fusion protein; page 27, lines 20-25; page 56, lines 1-3; claim 36).

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 the Michigan reference, unity of invention is lacking.