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(71) Applicant: NEW YORK UNIVERSITY [US/US]; 70 Washington Square South, New York, New York 10012 (US).

(72) Inventors: LONG, Chengzu; 333 E. 30th Street, #18K, New York, New York 10016 (US). YANG, Qiaoyan; 70 Washington Square South, New York, New York 10012 (US).

(74) Agent: LOPINSKI, John et al.; Hodgson Russ LLP, 140 Pearl Street, Suite 100, Buffalo, New York 14202-4040 (US).

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CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

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(54) Title: ENHANCEMENT OF SAFETY AND PRECISION FOR CRISPR-CAS INDUCED GENE EDITING BY VARIANTS OF DNA POLYMERASE USING CAS-PLUS VARIANTS

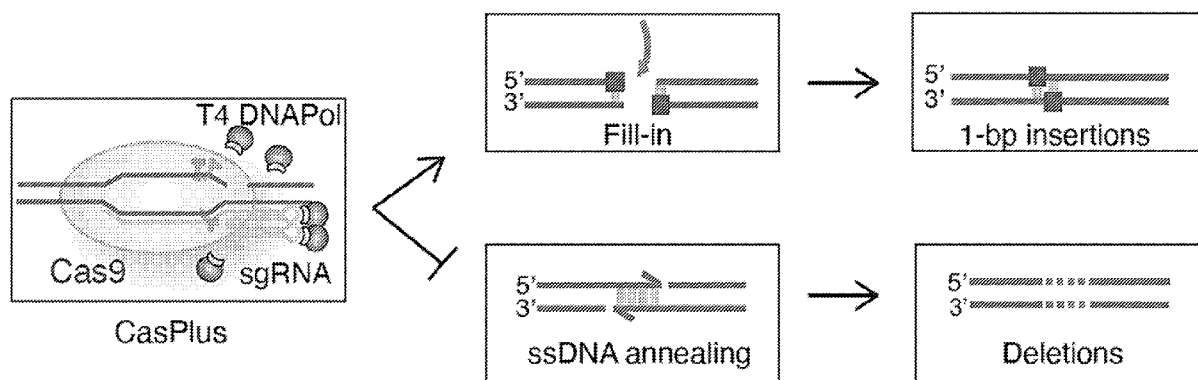


FIG. 1A

(57) Abstract: Provided are compositions and methods that include an engineered DNA polymerase used in combination with a Cas9 protein. The combination exhibits improved on-target chromosomal alterations, increases the proportion of precise 1- to 3-base-pair insertions at target sites, and reduces translocations caused by previously available systems. Wherein a fusion protein comprises a segment of an MS2 bacteriophage coat protein.



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US23/66316

<p>A. CLASSIFICATION OF SUBJECT MATTER</p> <p>IPC - INV. C12N 15/10; C07K 14/195; C12N 9/12; C12N 15/11 (2023.01) ADD.</p> <p>CPC - INV. C12N 15/102; C07K 14/195; C12N 9/1252; C12N 15/11 ADD. C07K 2319/00; C12N 2310/20; C12Q 2521/101</p> <p>According to International Patent Classification (IPC) or to both national classification and IPC</p>																																		
<p>B. FIELDS SEARCHED</p> <p>Minimum documentation searched (classification system followed by classification symbols) See Search History document</p> <p>Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched See Search History document</p> <p>Electronic database consulted during the international search (name of database and, where practicable, search terms used) See Search History document</p>																																		
<p>C. DOCUMENTS CONSIDERED TO BE RELEVANT</p> <table border="1"> <thead> <tr> <th>Category*</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>X</td> <td>US 5,945,312 A (GOODMAN MYRON F) 31 August 1999; Abstract; column 7, lines 31-39; claim 2</td> <td>1-2</td> </tr> <tr> <td>Y</td> <td></td> <td>4-8, 12-15, 19-23</td> </tr> <tr> <td>Y</td> <td>WO 2021/204877 A2 (ASTRAZENECA AB) 14 October 2021; Paragraphs: [0039], [0052]; claims 8 and 16</td> <td>4-8, 12-15, 19-23</td> </tr> <tr> <td>Y</td> <td>CN 106987571 A (SHANGHAI JIAOTONG UNIVERSITY) 28 July 2017; abstract</td> <td>13-15</td> </tr> <tr> <td>Y</td> <td>US 2020/0190487 A1 (THE BROAD INSTITUTE INC.) 18 June 2020; Abstract; paragraphs: [0807], [0839], [0849], [0856], [1103]</td> <td>19-23</td> </tr> </tbody> </table> <p><input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.</p> <table border="0"> <tr> <td>* Special categories of cited documents:</td> <td>"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</td> </tr> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"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</td> </tr> <tr> <td>"D" document cited by the applicant in the international application</td> <td>"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</td> </tr> <tr> <td>"E" earlier application or patent but published on or after the international filing date</td> <td>"&" document member of the same patent family</td> </tr> <tr> <td>"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)</td> <td></td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td></td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	X	US 5,945,312 A (GOODMAN MYRON F) 31 August 1999; Abstract; column 7, lines 31-39; claim 2	1-2	Y		4-8, 12-15, 19-23	Y	WO 2021/204877 A2 (ASTRAZENECA AB) 14 October 2021; Paragraphs: [0039], [0052]; claims 8 and 16	4-8, 12-15, 19-23	Y	CN 106987571 A (SHANGHAI JIAOTONG UNIVERSITY) 28 July 2017; abstract	13-15	Y	US 2020/0190487 A1 (THE BROAD INSTITUTE INC.) 18 June 2020; Abstract; paragraphs: [0807], [0839], [0849], [0856], [1103]	19-23	* Special categories of cited documents:	"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	"A" document defining the general state of the art which is not considered to be of particular relevance	"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	"D" document cited by the applicant in the international application	"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	"E" earlier application or patent but published on or after the international filing date	"&" document member of the same patent family	"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	
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<p>Date of the actual completion of the international search</p> <p>18 August 2023 (18.08.2023)</p>		<p>Date of mailing of the international search report</p> <p>OCT 31 2023</p>																																
<p>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</p>		<p>Authorized officer</p> <p>Shane Thomas</p> <p>Telephone No. PCT Helpdesk: 571-272-4300</p>																																

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US23/66316

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 13ter 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

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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.:
Groups I+, Claims 1-23, and T4 DNA polymerase with D219A mutation (DNA polymerase type), F916P (Cas9 mutation).

- 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

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****-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 1-23, and T4 DNA polymerase with D219A mutation (DNA polymerase type), F916P (Cas9 mutation) are directed towards DNA polymerase proteins and systems and methods associated therewith.

The DNA polymerase proteins, systems, and methods of Claims 1 (in-part), 2, 4-5, 6 (in-part), 7-8, 12, 13 (in-part), 14-15, 19-23 are believed to encompass the first named invention of Groups I+ and are the claims that will be searched without fee to the extent that they encompass T4 DNA polymerase with D219A mutation (first exemplary DNA polymerase type), F916P (first exemplary Cas9 mutation). 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, 13 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 DNA polymerase type(s), Cas9 mutation(s) to be searched. Additional DNA polymerase type(s), Cas9 mutation(s) will be searched upon the payment of additional fees. Applicants must specify the searchable claims that encompass any additionally elected DNA polymerase type(s), Cas9 mutation(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. Exemplary elections would be RB69 DNA polymerase with D222A mutation (DNA polymerase type), F916del (Cas9 mutation).

Groups I+ share the technical features including: a DNA polymerase protein comprising a mutation; a system for editing a DNA substrate, said system comprising the DNA polymerase protein, and a Cas9 nuclease; a method comprising introducing the system into eukaryotic cells, wherein the DNA polymerase protein, the Cas9 nuclease, and an included guide RNA create an indel at a location in DNA that is determined by the sequence of the guide RNA. These shared technical features are previously disclosed by the publication entitled "Targeting DNA polymerase to DNA double-strand breaks reduces DNA deletion size and increases templated insertions generated by CRISPR/Cas9" by Yoo, et al. (hereinafter "Yoo").

Yoo discloses a DNA polymerase protein comprising a mutation (DNA polymerase I with mutations; page 3947, 1st column, 5th paragraph and 2nd column, 5th paragraph); a system for editing a DNA substrate, said system comprising the DNA polymerase protein, and a Cas9 nuclease (fusing pol I to Cas9 (system) increased the percentage of 1-bp deletions and decreased >1-bp deletions targeting the CLCN5 gene (editing a DNA substrate) in HEK293T cells; page 3947, 1st column, 5th paragraph and 2nd column, 5th paragraph; fig. 1c); a method comprising introducing the system into eukaryotic cells, wherein the DNA polymerase protein, the Cas9 nuclease, and an included guide RNA create an indel at a location in DNA that is determined by the sequence of the guide RNA (target plasmid DNA expressing sgRNA/Cas9-Klenow increased the percentage of 1-bp deletions and decreased >1-bp deletions (create an indel) targeting the CLCN5 gene (editing a DNA substrate) in HEK293T cells (introducing the system into eukaryotic cells); page 3945, 2nd column, 3rd paragraph; page 3947, 1st column, 5th paragraph and 2nd column, 5th paragraph; fig. 1c).

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