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Declarations under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

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— *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))*

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(54) Title: COMPOSITIONS AND METHODS FOR MULTIPLEXED GENOME EDITING AND SCREENING

(57) Abstract: The present invention includes compositions and methods for multiplexed genome editing and screening *in vivo*. In certain aspects, the invention includes an CCAS library for multiplexed genome-scale mutagenesis.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 18/38242

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/63, C12N 15/82, C12N 15/83 (2018.01)
CPC - C12N 15/63, C12N 15/8203, C12N 15/8216

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.
Y	KIM et al., In vivo high-throughput profiling of CRISPR-Cpf1 activity. Nature Methods, 19 December 2016, Vol. 14, No. 2, pp 153-159. Online methods, p1, col 1, para 2	1-3
A		4
Y	DENEKA et al., Embryonal Fyn-associated Substrate (EFS) and CASS4: the lesser-known CAS protein family members. Gene, 26 June 2015, Vol. 570, No.1, pp: 25-35. p27, col 1, para 3-4	1-3
A		4
A	US 2016/0272965 A1 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY et al.) 22 September 2016 (22.09.2016) para [0126]	4
A	US 2017/0145426 A1 (TSINGHUA UNIVERSITY) 25 May 2017 (25.05.2017) para [0361]	4
A	US 2008/0019998 A1 (WANG et al.) 24 January 2008 (24.01.2008) para [0287]	4

☐ 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	Date of mailing of the international search report
22 September 2018	07 DEC 2018

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
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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.: 32, 62, 63
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:
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.

----Please see continuation in first extra 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 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.:
1-4

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

International application No.
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Continuation of Box No. III. Observations where unity of invention is lacking.

Group I, claims 1-4, directed to a vector comprising a Nuclear Localization Signal (NLS) sequence.

Group II, claims 5-9 and 12-14, directed to a vector comprising a single promoter, two restriction sites, and a posttranscriptional regulatory element sequence.

Group III, claims 10, 11, 15-21 and 35-37, directed to a crRNA array comprising vectors, and a crRNA library comprising a plurality of crRNA arrays, a kit comprising the same.

Group IV, claims 22-31, 33 and 34, directed to a method for simultaneously mutagenizing multiple target sequences in a cell or inducible, sequential mutagenesis in a cell comprising administering to a cell the crRNA library, a method of identifying synergistic drivers of transformation and/or tumorigenesis or identifying and mapping genetic interactions between a plurality of genes in vivo.

Group V, claims 38-61, directed to a gene editing system capable of inducible, sequential mutagenesis in a cell, the system comprising one or more vectors and a Cre recombinase, and a method of inducible, sequential mutagenesis in a cell by administering the vector(s) and the Cre recombinase.

The inventions listed as Groups I-V do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

Group I has the special technical feature of a vector comprising a NLS sequence, that is not required by Groups II-V.

Group II has the special technical feature of a vector comprising a single promoter and a posttranscriptional regulatory element sequence, that is not required by Groups I or III-V.

Group III has the special technical feature of a crRNA array, that is not required by Group I, II or IV-V.

Group IV has the special technical feature of administering to a cell a crRNA library or administering a cell mutagenized by a crRNA library to an animal, that is not required by Groups I, II, III or V.

Group V has the special technical feature of a vector comprising a lox66 sequence and an inverted lox71 sequence, and a Cre recombinase, that is not required by Groups I-IV.

Shared technical features

The inventions of Groups I-II share the common technical feature of a vector comprising a first LTR sequence, an EFS sequence, a Cpf1 sequence, an antibiotic resistance sequence, and a second LTR sequence.

The inventions of Groups II and III further share the common technical feature of a vector comprising a first LTR sequence, a promoter sequence, a direct repeat sequence of Cpfl, an EFS sequence, a posttranscriptional regulatory element sequence, and a second LTR sequence.

The inventions of Groups III and IV further share the common technical feature of a crRNA library, wherein the crRNA library comprises a plurality of vectors comprising a plurality of crRNA arrays, wherein each crRNA array independently comprises a 5' nucleotide sequence that is homologous to a first nucleotide sequence on the vector, a first crRNA sequence, a direct repeat sequence of Cpfl, a second crRNA sequence, a terminator sequence and a 3' sequence that is homologous to a second sequence on the vector, and wherein the first crRNA is complementary to a first target sequence and the second crRNA is complementary to a second target sequence.

The inventions of Groups IV and V further share the common technical feature of a method of inducible, sequential mutagenesis in a cell or in an animal, the method comprising administering to the cell or animal a plurality of said vectors.

However, these shared technical features are previously made obvious over the article entitled 'In vivo high-throughput profiling of CRISPR-Cpf1 activity' by Kim et al., (hereinafter 'Kim') in view of the article entitled 'Embryonal Fyn-associated Substrate (EFS) and CASS4: the lesser-known CAS protein family members' by Deneka et al., (hereinafter 'Deneka').

--continued in next sheet--

INTERNATIONAL SEARCH REPORT

International application No.
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Continuation of Box No. III. Observations where unity of invention is lacking.

--continued from last sheet--

Kim teaches a vector comprising a first long terminal repeat (LTR) sequence, a Cpfl sequence, a Nuclear Localization Signal (NLS) sequence, an antibiotic resistance sequence, and a second LTR sequence (p160, col 1, para 2 - 'To prepare lentiviral vectors, AsCpf1- and LbCpf1-encoding sequences from plasmids (Addgene; #69982, #69988) were cloned into the lentiCas9-Blast plasmid (Addgene; #52962) that has been shown to be capable of producing high-titer virus21; these are referred to as Lenti_AsCpf1-Blast and Lenti_LbCpf1-Blast, respectively'; Note, above constructs contain a first long terminal repeat (LTR) sequence, a Cpf1 sequence, a Nuclear Localization Signal (NLS) sequence, an antibiotic resistance sequence, and a second LTR sequence, as shown in Supplementary Figure 1 and Supplementary Note 1). Kim does not expressly teach the vector comprises an Embryonal Fyn Associated Substrate (EFS) sequence.
Deneka teaches EFS promoter (p4, para 2 - 'This resource proposes several transcriptional regulators for EFS based on consensus binding sites in its promoter region for ATF (Activating transcription factor), NF-kb, NF-kb1, GATA-3, C/EBPa (CCAAT/enhancer-binding protein alpha), glucocorticoid receptors a and b, and p53.'). It would have been obvious to one of ordinary skill in the art that the EFS promoter of Deneka could be used with the lentivirus vector of Kim for gene editing in embryonic cells (e.g., ES cells) or immune cells since Deneka teaches EFS promoter is abundantly expressed in embryonal and immune cells (p3, last para - 'Initial studies profiling EFS mRNA indicated broad expression, with maximal levels in the placenta, the embryonal central nervous system, heart, testes and lungs [27]. EFS expression in the thymus and lymphocytes is functionally important for T cell maturation and prevention of autoimmunity, discussed below').

Kim further teaches the vector comprising a promoter sequence, a direct repeat sequence of Cpf1, a first restriction site, a second restriction site, a posttranscriptional regulatory element sequence, and a first and second LTR sequence (p160, col 1, para 2 - 'To obtain the backbone vector for the plasmid library, we removed a SpCas9 scaffold region from lentiGuide-Puro (Addgene; #52963) that can lead to high-titer virus production21; this vector is hereby referred to as the Lenti_ gRNA-Puro vector (Supplementary Fig. 1a and Supplementary Note 2)'; p154, col 2, Figure 1 legend - 'Figure 1, Paired library preparation and high-throughput evaluation of Cpf1 activity. (a) Schematic representation of oligonucleotides containing pairs of target and guide RNA sequences.'; Note, Addgene #52963 vector backbone comprises a first LTR sequence; elongation factor 1 promoter; a first restriction site, a second restriction site, puromycin resistance gene, posttranscriptional regulatory element of woodchuck hepatitis virus; and a second LTR sequence as shown in Supplementary Figure 1 and Supplementary Notes 1 and 2; Also note that oligonucleotides containing pairs of target and guide RNA sequences in Figure 1 provide the direct repeat sequence of Cpfl in the library construct). Kim does not expressly teach a first crRNA sequence, a second direct repeat sequence of Cpfl, or a second crRNA sequence. Since Kim teaches use of crRNA sequences complementary to multiple different targets (Supplementary Table 4; abstract "target sequence and guide RNA pairs was delivered into human cells using lentiviral vectors"), and further teaches multiplexed editing (p153, col 2, para 1 - 'To evaluate the activity of Cpf1 with various guide RNA sequences at matched and mismatched target sequences in a high-throughput manner, we prepared a lentiviral library of guide RNA target sequence pairs'), it would have been obvious to one of ordinary skill in the art that more than one crRNA sequences could be used in the construct to enable simultaneous editing of multiple targets. Further, it would have been obvious that for each crRNA, a direct repeat of Cpf1 sequence would be required, for targeting of Cpf1.

Kim further teaches a crRNA library, wherein the crRNA library comprises a plurality of said vectors comprising a plurality of crRNA arrays, wherein each crRNA array independently comprises a 5' nucleotide sequence that is homologous to a first nucleotide sequence on the vector, a first crRNA sequence, a direct repeat sequence of Cpfl, a second crRNA sequence, a terminator sequence and a 3' sequence that is homologous to a second sequence on the vector, and wherein the first crRNA is complementary to a first target sequence and the second crRNA is complementary to a second target sequence (abstract "A library of [greater than] 11,000 target sequence and guide RNA pairs was delivered into human cells using lentiviral vectors"; p. 160, col 1, para 1 "To construct a plasmid library, 8,327 for AsCpf1 and 3,634 oligonucleotides for LbCpf1 were synthesized and purchased from CustomArray, Inc. (Bothell, WA). We designed each oligonucleotide to contain the guide-RNA-encod?ing sequence and target sequence in a total length of 122~130 bases (Fig. 1a and Supplementary Note 1)"), and a method of inducible, sequential mutagenesis in a cell or in an animal, the method comprising administering to the cell or animal a plurality of said vectors (p. 154, col 1, para 2 "the multiplicity of infection (MOI) in the cell libraries affects the AsCpf1-induced indel frequencies by using three different cell libraries with different MOIs (0.4, 2, and 10) of guide-RNA-expressing lentivirus "; p. 160, col2, para 3 "Cell library generation. To generate the cell library, HEK293T cells (1.5 ? 106-2.0 ? 106) that were well-attached to a 100-mm dish were transduced with the lentiviral vector"; p. 161, col 1, para 2 "AsCpf1 was delivered into the transduced cells using a lentiviral vector expressing the gene encoding AsCpf1 as described above. 5 d after delivery of the AsCpf1-encoding construct, genomic DNA was isolated from the cells and subjected to deep sequencing").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I-V inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, continuation of number 4 above: claims 32, 62, 63 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).