Patent Right	Date	February 25, 2020	Court	Intellectual Property High Court, First Division
	Case number	2019 (Gyo-Ke) 10010		

- In Article 29-2 of the Patent Act, the "invention" stated in the description, etc. of the earlier application means an invention which is understood from a matter stated in the description, etc. of the earlier application and from a matter equivalent to that stated in the description, etc. of the earlier application, in which the "matter equivalent to that stated" means a matter which can be derived from the stated matter by taking common general technical knowledge as of filing into consideration.
- A case in which, with regard to a patent application concerning an invention titled "ENGINEERING OF SYSTEMS, METHODS AND OPTIMIZED GUIDE COMPOSITIONS FOR SEQUENCE MANIPULATION", there is a statement in the description, etc. of the earlier application to the extent that a person ordinarily skilled in the art can understand that the invention of the earlier application is shown in the description, etc. of the earlier application and a person ordinarily skilled in the art is enabled to work the invention shown in the earlier application, and therefore, it is determined that the invention of the present application is identical with the invention of the earlier application and is not allowed to be granted a patent.

Case type: Rescission of Appeal Decision of Refusal

Result: Dismissed

References: Article 29, paragraph (1), item (iii), and Article 29-2 of the Patent Act

Related rights, etc.: Appeal against Examiner's Decision of Refusal No. 2017-13795, Patent Application No. 2016-117740

Summary of the Judgment

1. The present case is a lawsuit for rescission of an appeal decision of refusal in which the examiner made a decision of refusal with regard to the Plaintiffs' patent application concerning an invention titled "ENGINEERING OF SYSTEMS, METHODS AND OPTIMIZED GUIDE COMPOSITIONS FOR SEQUENCE MANIPULATION", against which Plaintiffs filed an appeal, and then the decision of the JPO dismissing the request for an appeal against the examiner's decision of refusal was made, and thus Plaintiffs sought the rescission of the appeal decision of refusal.

Reasons for rescission are [i] Erroneous determination of Article 29-2 of the Patent Act on the basis of Cited Invention 1 (Reason 1 for Rescission) and [ii] Erroneous determination of an inventive step on the basis of Cited Invention 2 (Reason 2 for Rescission).

2. The present judgment, in summary, held with regard to Reason 1 for Rescission

as follows and dismissed the Plaintiff's claim.

(1) It is found that the invention of the present application is identical with Cited Invention 1.

It should be deemed that Cited Document 1 discloses not only formally but also substantially that "a guide RNA directs a type II Cas 9 protein to a targeted site in chromosomal sequence in eukaryotic cells where the type II Cas 9 protein introduces a double-stranded break of chromosomal DNA in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified." It can be understood that the vector system of Cited Invention 1 is also disclosed as a vector system which includes the above function.

(2) A. An object of Article 29-2 of the Patent Act .. is as follows. An invention stated in the description, etc. of an earlier application, even if the invention is not stated in the Scope of Claims, is laid open to the public in a publication, etc. of the earlier application. Thus, even if a later application is filed before the publication, etc. of the earlier application, when the invention of the later application is identical with the invention of the earlier application, no new technology is laid open to the public in the publication, etc. of the later application. Therefore, granting a patent to such invention is improper from the viewpoint of the patent system, which intends to protect an invention as a reward for laying a new invention open to the public.

The "invention" stated in the description, etc. of the earlier application in this Article is construed as an invention which is understood from a matter stated in the description, etc. of the earlier application and from a matter equivalent to that stated in the description, etc. of the earlier application. The "matter equivalent to that stated" means a matter which can be derived from the stated matter by taking common general technical knowledge as of filing into consideration.

Thus, even if there is no particular statement in the description, etc. of the earlier application, the invention of the earlier application can be found by taking common general technical knowledge into consideration in order to understand the invention of the earlier application. On the other hand, in case where an invention lacks concreteness or where technical content of an invention is insufficiently disclosed even taking common general technical knowledge of a person ordinarily skilled in the art into consideration, such invention does not fall under the "invention" mentioned above, and does not have an effect of excluding the later application stipulated in this Article. In addition, it should be deemed that the degree of disclosure of technical content required here should be sufficient if the technical content is disclosed to the extent that a person ordinarily skilled in the art can understand that the invention of

the earlier application is shown in the description, etc. of the earlier application and the person ordinarily skilled in the art is enabled to work the invention shown in the earlier application.

B. In the present case, with regard to the above, Examples 1 to 3 in Cited Invention 1 disclose in detail a method for producing each vector of (i) to (iii) in Cited Invention 1, and Example 4 clearly discloses a concrete test method for confirming whether a donor sequence (GFP gene) is incorporated in or near the targeted sequence.

In addition, .. from the experimental results of Example 4, it can be understood that a combination of RNA-guided endonuclease comprising a nuclear localization signal, guide RNA, and donor polynucleotides is incorporated into eukaryotic cells and that double-stranded break and repair occur in the targeted site. The experimental results of Example 5 are not to be interpreted as a bar to the above understanding.

Further, a vector system comprising the above vectors (i) to (iii) has the technical means necessary for appropriate transcription, translation, nuclear translocation, etc. in eukaryotic cells, and the technical means necessary for appropriate modification of the targeted sequence in eukaryotic cells. Thus, it can be understood that even if the vector system is used, such vector system has functions of cleavage of targeted sequences and modification of targeted sequences in eukaryotic cells .. .

From the above, it can be deemed that there is a disclosure in Cited Document 1 to the extent that a person ordinarily skilled in the art can understand that the invention of the earlier application is shown in the description, etc. of the earlier application and a person ordinarily skilled in the art is enabled to work the invention shown in the earlier application. Therefore, it is proper to find that in Cited Document 1, a technology was laid open to the public to the extent that the technology was sufficient to exclude the later application, in which the technology includes a portion of the function such that "a guide RNA directs a type II Cas 9 protein to a targeted site in chromosomal sequence in eukaryotic cells where the type II Cas 9 protein introduces a double-stranded break of chromosomal DNA in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified."

Judgment rendered on February 25, 2020
2019 (Gyo-Ke) 10010 A case of seeking rescission of the JPO decision
Date of conclusion of oral argument: December 18, 2019

Judgment

Plaintiff: The Broad Institute Inc.

Plaintiff: Massachusetts Institute of Technology

Plaintiffs: President and Fellows of Harvard College

Defendant: Commissioner of the Japan Patent Office

Main Text

1. The Plaintiffs' claim shall be dismissed.

2. The court costs shall be borne by the Plaintiffs.

3. An additional period of 30 days shall be set for the Plaintiffs to file a final appeal against this judgment and a petition for acceptance of the final appeal.

Facts and Reasons

No. 1 Claim

The decision rendered by the Japan Patent Office on September 14, 2018 for the case of Appeal against Examiner's Decision of Refusal No. 2017-13795 shall be rescinded.

No. 2 Outline of the Case

1. History of Procedures in the Japan Patent Office

(1) The Plaintiffs filed a patent application with regard to an invention titled "ENGINEERING OF SYSTEMS, METHODS, AND OPTIMIZED GUIDE COMPOSITIONS FOR SEQUENCE MANIPULATION" on June 14, 2016 (Patent Application No. 2016-117740, Division of Patent Application No. 2015-547573 (Priority claimed: December 12, 2012, United States of America), Publication Date:

September 15, 2016, Exhibit Ko 9).

(2) With regard to this patent application, the Plaintiffs received an Examiner's decision of refusal on April 28, 2017 (Exhibit Ko 14). Accordingly, the Plaintiffs filed a request for an appeal against the Examiner's decision of refusal on September 15, 2017 (Exhibit Ko 15). The Japan Patent Office examined the above request as the case of Appeal against Examiner's Decision of Refusal No. 2017-13795.

(3) On September 14, 2018, the Japan Patent Office rendered a decision that the request for the appeal shall be dismissed, as stated in the written decision (a copy) as shown in Attachment (hereinafter referred to as "the decision of the present case"). The certified copy of the decision of this case was served on the Plaintiffs on October 1, 2018.

(4) The Plaintiffs instituted an action for seeking a rescission of the decision of the present case on January 29, 2019.

2. Statement of the Scope of Claims

The statement of Claim 1 in the Scope of Claims to which the decision is directed is as follows (Exhibit Ko 12; hereinafter, the invention stated in the above Claim 1 is referred to as "the Present Invention"; the description according to this application (Exhibit Ko 9) and the drawings attached thereto are collectively referred to as "the description of the present application"). Note that "/" in the text below indicates a line break in the original text (the same shall apply hereinafter).

[Claim 1]

An engineered, non-naturally occurring clustered regularly interspaced short palindromic repeats (CRISPR) - CRISPR-associated (Cas) (CRISPR-Cas) vector system, / comprising one or more vectors comprising: / a) a first regulatory element operably linked to a nucleotide sequence encoding a CRISPR-Cas system polynucleotide sequence comprising a guide sequence, tracrRNA, and a tracr mate sequence, wherein the guide sequence hybridizes with one or more targeted sequences in polynucleotide loci in a eukaryotic cell, / b) a second regulatory element operably linked to a nucleotide sequence encoding a type II Cas9 protein, and / c) a recombination template, / wherein components (a), (b), and (c) are located on the same or different vectors of the system, wherein the system further comprises one or more nuclear localization signals (there can be more than one) (NLS (there can be more than one)) to be expressed together with the nucleotide sequence encoding the Cas9 protein, / whereby the guide sequence targets the one or more polynucleotide loci in the eukaryotic cell and the Cas9 protein cleaves the one or more polynucleotide loci, whereby the sequence of the one or more polynucleotide loci is modified.

3. Summary of Reasons of the Decision of the Present Case

(1) Reasons of the decision of the present case are as stated in the written decision (a copy) in the Attachment. In summary, [i] the Present Invention is identical with the invention as disclosed in Cited Document 1 of the earlier application mentioned below (hereinafter referred to as "Cited Invention 1"), and thus falls under Article 29-2 of the Patent Act, and [ii] the Present Invention could have easily been made by a person ordinarily skilled in the art on the basis of the invention as disclosed in Cited Document 2 mentioned below (hereinafter referred to as "Cited Invention 2") and the well-known art before the priority date of the present application (December 12, 2012), and thus falls under Article 29, paragraph (2) of the Patent Act. For the foregoing reasons, a patent shall not be granted for the Present Invention.

a. Cited Document 1: PCT/US2013/073307 (International Publication No. WO2014/089290, Filing date: December 5, 2013 (Priority claimed: December 6, 2012), Publication date: June 12, 2014, Exhibit Ko 1-1)

b. Cited Document 2: "A Programmable Dual-RNA - Guided DNA Endonuclease in Adaptive Bacterial Immunity" (Science, Aug 2012, Vol. 337, pp. 816-821, Exhibit Ko 2-1) and "Supplementary Materials" (Exhibit Ko 2-2) (Online publication: June 28, 2012).

(2) The decision of the present case found as follows, with regard to Cited Invention 1.

A vector system, comprising / (i) a vector comprising a promoter control sequence operably linked to a nucleic acid encoding at least one type II Cas9 protein comprising at least one nuclear localization signal, / (ii) a vector comprising a promoter control sequence operably linked to DNA encoding at least one guide RNA comprising a first region at the 5' end that is complementary to a targeted site in a chromosomal sequence in a eukaryotic cell, a second internal region that forms a stem loop structure, and a third 3' region that remains essentially single-stranded; / and (iii) a vector comprising at least one donor polynucleotide, / wherein the guide RNA directs a type II Cas9 protein to a targeted site in the chromosomal sequence in a eukaryotic cell, where the type II Cas9 protein introduces a chromosomal DNA double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified.

(3) The decision of the present case found as follows, with regard to Cited Invention 2 and the comparison between the Present Invention and Cited Invention 2.

a. Findings on Cited Invention 2

An engineered, non-naturally occurring clustered regularly interspaced short palindromic repeats (CRISPR) - CRISPR-associated (Cas) (CRISPR-Cas) system, / comprising: / a) a chimeric RNA comprising a target recognition sequence at the 5' end followed by a hairpin structure retaining base-pairing interactions that occur between tracrRNA and crRNA, wherein the target recognition sequence hybridizes with a targeted sequence in a buffer solution; and / b) a type II Cas9 protein, / whereby the Cas9 protein cleaves the targeted sequence.

b. Common Feature between the Present Invention and Cited Invention 2

An engineered, non-naturally occurring clustered regularly interspaced short palindromic repeats (CRISPR) - CRISPR-associated (Cas) (CRISPR-Cas) system, / comprising: / a) a CRISPR-Cas system polynucleotide comprising a guide sequence, tracrRNA, and a tracr mate sequence, wherein the guide sequence hybridizes with a targeted sequence; and / b) a type II Cas9 protein, / whereby the Cas9 protein cleaves the targeted sequence.

c. Different Features between the Present Invention and Cited Invention 2

(Different Feature 1)

In the Present Invention, a guide sequence "hybridizes with one or more targeted sequences in polynucleotide loci in a eukaryotic cell" and the system "further comprises one or more nuclear localization signals (there can be more than one) (NLS (there can be more than one)) to be expressed together with the nucleotide sequence encoding the Cas9 protein", whereby the guide sequence targets "the one or more polynucleotide loci in the eukaryotic cell" and the Cas9 protein cleaves this. In contrast, in Cited Invention 2, a targeted sequence is present in a buffer solution. Further, a Cas9 protein does not comprise a nuclear localization signal.

(Different Feature 2)

The Present Invention comprises a "recombination template", wherein "sequence of the one or more polynucleotide loci is modified." In contrast, in Cited Invention 2 does not comprise a recombination template, a targeted sequence is only cleaved.

(Different Feature 3)

The Present Invention is a CRISPR-Cas "vector" system, comprising: "one or more vectors" comprising: "a first regulatory element operably linked to a nucleotide sequence encoding" the CRISPR-Cas system polynucleotide sequence; and "a second regulatory element operably linked to a nucleotide sequence encoding" a type II Cas9 protein; "wherein components (a), (b), and (c) are located on the same or different vectors of the system". In contrast, Cited Invention 2 is a CRISPR-Cas system using

a "chimeric RNA" and a "Cas9 protein".

4. Reasons for Rescission

(1) Erroneous Determinations of Article 29-2 of the Patent Act Based on Cited Invention 1 (Reason 1 for Rescission)

(2) Erroneous Determinations of Inventive Step Based on Cited Invention 2 (Reason 2 for Rescission)

(omitted)

No. 4 Judgment of This Court

1. Present Invention

(1) Statement of the Description of the Present Application

The description of the present application is stated as follows (Exhibit Ko 9).

a. Technical Field

[0003] The present invention generally relates to systems, methods, and compositions used for the control of gene expression involving sequence targeting, such as genome perturbation or gene-editing, that may use vector systems related to Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and components thereof.

b. Background Art

[0005] Precise genome targeting technologies are needed to enable systematic reverse engineering of causal genetic variations as well as to advance synthetic biology, biotechnological, and medical applications. Although genome-editing techniques such as designer zinc fingers, transcription activator-like effectors (TALE), or homing meganucleases are available , there remains a need for new genome engineering technologies that are affordable, easy to set up, scalable, and amenable to targeting multiple positions within the eukaryotic genome.

c. Problem to be Solved by the Invention

[0007] In the CRISPR/Cas or the CRISPR-Cas system, a single Cas enzyme can be programmed by a short RNA molecule to recognize a specific DNA target; in other words, the Cas enzyme can be recruited to a specific DNA target using said short RNA molecule.

d. Means for Solving the Problem

[0008] In some embodiments, the CRISPR enzyme is a type II CRISPR system enzyme. In some embodiments, the CRISPR enzyme is a Cas9 enzyme. In some embodiments, the Cas9 enzyme is S. pneumoniae, S. pyogenes, or S. thermophilus

Cas9, and may include mutated Cas9 derived from these organisms.

In some embodiments, the CRISPR enzyme is codon-optimized for expression in a eukaryotic cell.

In some embodiments, the guide sequence is at least 15, 16, 17, 18, 19, 20, 25 nucleotides in length, or between 10 to 30, or between 15 to 25, or between 15 to 20 nucleotides in length.

In general, and throughout this description, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked.

One type of vector is a "plasmid," which refers to a circular double-stranded DNA loop into which additional DNA segments can be inserted, such as by standard molecular cloning techniques. Another type of vector is a viral vector, wherein virally-derived DNA or RNA sequences are present in the vector for packaging into a virus (..).

Common expression vectors of utility in recombinant DNA techniques are often in the form of plasmids.

[0010] The term "regulatory element" is intended to include promoters, enhancers, internal ribosomal entry sites (IRES), and other expression control elements (e.g. transcription termination signals, such as polyadenylation signals and poly-U sequences).

In some embodiments, a vector comprises one or more pol III promoters (..), one or more pol II promoters (..), one or more pol I promoters (..), or combinations thereof. Examples of pol III promoters include, but are not limited to, U6 and H1 promoters. Examples of pol II promoters include, but are not limited to, the retroviral Rous sarcoma virus (RSV) LTR promoter (..), the cytomegalovirus (CMV) promoter. Also encompassed by the term "regulatory element" are enhancer elements, such as WPRE; CMV enhancers; the R-U5' segment in LTR of HTLV-1; and SV40 enhancer. A vector can be introduced into host cells to thereby produce transcripts, proteins, or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein.

[0012] In one aspect, the present invention provides a vector comprising a regulatory element operably linked to an enzyme-coding sequence encoding a CRISPR enzyme comprising one or more nuclear localization sequences.

[0023] In aspects of the present invention, the modification comprises an engineered secondary structure. For example, the modification can comprise a reduction in a region of hybridization between a tracr mate sequence and a tracr sequence. For example, the modification also may comprise fusing a tracr mate

sequence and a tracr sequence through an artificial loop. The modification may comprise a tracr sequence having a length between 40 and 120 bp.

In certain embodiments, a length of tracrRNA includes at least nucleotides 1 to 67 and in some embodiments at least nucleotides 1 to 85 of a wild type tracrRNA.

The modification may comprise sequence optimization.

Sequence optimization may be combined with the reduction in a region of hybridization between a tracr mate sequence and a tracr sequence; for example, a reduction in a length of a tracr sequence.

[0025] In an aspect, the present invention provides a CRISPR-Cas system or a CRISPR enzyme system wherein the modification comprises adding a polyT terminator sequence.

[0035] In some aspects of the present invention, a length of tracrRNA required in a construct of the present invention, e.g., a chimeric construct, need not necessarily be fixed, and in some aspects of the present invention it can be between 40 and 120 bp, and in some aspects of the present invention up to a full length of the tracr, e.g., in some aspects of the present invention, until 3' end of tracr as punctuated by a transcription termination signal in a bacterial genome. In certain embodiments, a length of tracrRNA includes at least nucleotides 1 to 67 and in some embodiments at least nucleotides 1 to 85 of a wild type tracrRNA. In some embodiments, at least nucleotides corresponding to nucleotides 1 to 67 or 1 to 85 of wild type S. pyogenes Cas9 tracrRNA may be used.

As to the presence of polyT terminator sequences in tracr + tracr mate transcript, e.g., a polyT terminator (TTTTT or more T), in some aspects of the present invention, it is advantageous that such be added to an end of the transcript, whether it is in two RNA (tracr and tracr mate) or single guide RNA form. Concerning loops and hairpins in tracr and tracr mate transcripts, in some aspects of the present invention, it is advantageous that a minimum of two hairpins be present in the chimeric guide RNA. A first hairpin can be the hairpin formed by complementation between the tracr and tracr mate (direct repeat) sequence. A second hairpin can be at the 3' end of the tracrRNA sequence, and this can provide a secondary structure for interaction with Cas9. Additional hairpins may be added to the 3' of the guide RNA, e.g., in some aspects of the present invention, to increase stability of the guide RNA.

e. Mode for Carrying Out the Invention

[0065] In general, "CRISPR system" refers collectively to transcripts and other elements involved in the expression of or directing the activity of CRISPR-associated ("Cas") genes, including sequences encoding a Cas gene, a tracr (trans-activating

CRISPR) sequence (e.g. tracrRNA or an active partial tracrRNA), a tracr mate sequence (encompassing a "direct repeat" and a tracrRNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a "spacer" in the context of an endogenous CRISPR system), or other sequences and transcripts from a CRISPR locus.

In some embodiments, one or more elements of a CRISPR system are derived from a particular organism comprising an endogenous CRISPR system, such as Streptococcus pyogenes. In general, a CRISPR system is characterized by elements that promote the formation of a CRISPR complex at the site of a targeted sequence (also referred to as a protospacer in the context of an endogenous CRISPR system). In the context of formation of a CRISPR complex, "targeted sequence" refers to a sequence to which a guide sequence is designed to have complementarity, where hybridization between a targeted sequence and a guide sequence promotes the formation of a CRISPR complex.

A sequence or template that may be used for recombination into the targeted locus comprising the targeted sequences is referred to as an "editing template" or "editing polynucleotide" or "editing sequence". In aspects of the present invention, an exogenous template polynucleotide may be referred to as an editing template. In an aspect of the present invention, the recombination is homologous recombination.

[0066] Without wishing to be bound by theory, the tracr sequence, which may comprise or consist of all or a portion of a wild-type tracr sequence (e.g. about or more than about 20, 26, 32, 45, 48, 54, 63, 67, 85, or more nucleotides of a wild-type tracr sequence), may also form part of a CRISPR complex, such as by hybridization along at least a portion of the tracr sequence to all or a portion of a tracr mate sequence that is operably linked to the guide sequence.

[0071] In some embodiments, a vector encodes a CRISPR enzyme comprising one or more nuclear localization sequences (NLS), such as about or more than about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more NLSs.

Non-limiting examples of NLSs include the NLS of the SV40 virus large T-antigen, having the amino acid sequence PKKKRKV.

[0072] In general, the one or more NLSs are of sufficient strength to drive accumulation of the CRISPR enzyme in a detectable amount in the nucleus of a eukaryotic cell.

[0076] In general, a tracr mate sequence includes any sequence that has sufficient complementarity with a tracr sequence to promote one or more of: (1) excision of a guide sequence flanked by tracr mate sequences in a cell containing the

corresponding tracr sequence; and (2) formation of a CRISPR complex (the CRISPR complex comprises the tracr mate sequence hybridized to the tracr sequence) at a targeted sequence.

In some embodiments, the tracr sequence is about or more than about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, or more nucleotides in length. In some embodiments, the tracr sequence and tracr mate sequence are contained within a single transcript, such that hybridization between the two produces a transcript having a secondary structure, such as a hairpin. Preferred loop forming sequences for use in hairpin structures are four nucleotides in length, and most preferably have the sequence GAAA. However, longer or shorter loop sequences may be used, as may be alternative sequences. The sequences preferably include a nucleotide triplet (for example, AAA), and an additional nucleotide (for example C or G). Examples of loop forming sequences include CAAA and AAAG. In an embodiment of the present invention, the transcript or transcribed polynucleotide sequence has at least two hairpins. In preferred embodiments, the transcript has two, three, four, or five hairpins. In a further embodiment of the present invention, the transcript has at most five hairpins. In some embodiments, the single transcript further includes a transcription termination sequence; preferably this is a polyT sequence, such as six T nucleotides. An example illustration of such a hairpin structure is provided in the lower portion of Figure 8B (as shown in Attachment 1), where the portion of the sequence 5' of the final "N" and upstream of the loop corresponds to the tracr mate sequence, and the portion of the sequence 3' of the loop corresponds to the tracr sequence. (In the original text, "Fig. 13B" is shown. However, from the context of the preceding and following text, this is recognized as Fig. 8B.)

f. Examples

(a) Example 1: CRISPR Complex Activity in the Nucleus of a Eukaryotic Cell

[0146] To improve expression of CRISPR components in mammalian cells, two genes of Cas9 (SpCas9) and RNase III (SpRNase III) from the SF370 locus 1 of Streptococcus pyogenes (S. pyogenes) were codon-optimized. To facilitate nuclear localization, a nuclear localization signal (NLS) was included at the amino (N)- or carboxyl (C)-termini of both SpCas9 and SpRNase III (Figure 2B).

A version of SpCas9 with an NLS attached to both N- and C-termini (2×NLS-SpCas9) was also generated.

Whereas the C-terminal NLS was sufficient to target SpRNase III to the nucleus, attachment of a single copy of these particular NLSs to either the N- or C-

terminus of SpCas9 was unable to achieve adequate nuclear localization in this system. In this example, the C-terminal NLS was that of nucleoplasmin (KRPAATKKAGQAKKKK), and the C-terminal NLS was that of the SV40 large T-antigen (PKKKRKV). Of the versions of SpCas9 tested, only 2×NLS-SpCas9 exhibited nuclear localization (Figure 2B).

(b) Example 4: Evaluation of Multiple Chimeric crRNA-tracrRNA Hybrids

[0165] This example states results obtained for chimeric RNAs (chiRNAs; comprising a guide sequence, a tracr mate sequence, and a tracr sequence in a single transcript) having tracr sequences that incorporate different lengths of wild-type tracrRNA sequence.

Cas9 is driven by the CBh promoter and the chimeric RNA is driven by a U6 promoter.

The guide and tracr sequences are separated by the tracr mate sequence GUUUUAGAGCUA followed by the loop sequence GAAA. Results of SURVEYOR assays for Cas9-mediated indels at the human EMX1 and PVALB loci are illustrated in Figures 18b and 18c, respectively.

ChiRNAs are indicated by their "+n" designation, and crRNA refers to a hybrid RNA where guide and tracr sequences are expressed as separate transcripts.

Quantification of these results, performed in triplicate, is illustrated by histograms in Figures 11a and 11b, corresponding to Figures 10b and 10c (as shown in Attachment 1), respectively ("N.D." indicates no indels detected).

[0171] Initially, three sites within the EMX1 locus in human HEK293FT cells were targeted. Efficiency of genome modification of each chiRNA was assessed using the SURVEYOR nuclease assay, which detects mutations resulting from DNA double-strand breaks (DSB) and their subsequent repair by the non-homologous end joining (NHEJ) DNA damage repair pathway. Constructs designated chiRNA(+n) indicate that up to the +n nucleotide of wild-type tracrRNA is included in the chimeric RNA construct, with values of 48, 54, 67, and 85 used for n. Chimeric RNAs containing longer fragments of wild-type tracrRNA (chiRNA(+67) and chiRNA(+85)) mediated DNA cleavage at all three EMX1 targeted sites, with chiRNA(+85) in particular demonstrating significantly higher levels of DNA cleavage than the corresponding crRNA/tracrRNA hybrids that expressed guide and tracr sequences in separate transcripts (Figures 10b and 10a). Two sites in the PVALB locus that yielded no detectable cleavage using the hybrid system (guide sequence and tracr sequence expressed as separate transcripts) were also targeted using chiRNAs. chiRNA(+67) and chiRNA(+85) were able to mediate significant cleavage at the two

PVALB protospacers (Figures 10c and 10b).

[0172] For all five targets in the EMX1 and PVALB loci, a consistent increase in efficiency of genome modification with increasing tracr sequence length was observed. Without wishing to be bound by any theory, the secondary structure formed by the 3' end of the tracrRNA may play a role in enhancing the rate of CRISPR complex formation. An illustration of predicted secondary structures for each of the chimeric RNAs used in this example is provided in Figure 21. Because chiRNAs with longer tracr sequences were able to cleave targets that were not cleaved by native CRISPR crRNA/tracrRNA hybrids, it is possible that chimeric RNA may be loaded onto Cas9 more efficiently than its native hybrid counterpart.

[0173] Figures 11 (as shown in Attachment 1) and 21 illustrate exemplary bicistronic expression vectors for expression of chimeric RNA including up to the +85 nucleotides of wild-type tracrRNA sequence, and SpCas9 with nuclear localization sequences. SpCas9 is expressed from a CBh promoter and terminated with the bGH polyA signal (bGHpA). The expanded sequence illustrated immediately below the schematic corresponds to the region surrounding the guide sequence insertion site, and includes, from 5' to 3', 3'-portion of the U6 promoter (first shaded region), BbsI cleavage sites (arrows), partial direct repeat (tracr mate sequence GTTTTAGAGCTA, underlined), loop sequence GAAA, and +85 tracr sequence (underlined sequence following loop sequence). An exemplary guide sequence insert is illustrated below the guide sequence insertion site, with nucleotides of the guide sequence for a selected target represented by "N".

(2) Features of the Present Invention

The Present Invention relates to genome-editing techniques using a CRISPR-Cas vector.

In the Present Invention, a Cas enzyme (a type II Cas9 protein) is allowed to recognize a specific DNA target (a polynucleotide locus) in a eukaryotic cell by a short RNA molecule (a CRISPR-Cas system polynucleotide sequence comprising a guide sequence, a tracrRNA sequence, and a tracr mate sequence), and the Cas enzyme cleaves the DNA target, whereby the DNA target is modified.

The Present Invention has the following constituent features; that is, a) a first regulatory element operably linked to a nucleotide sequence encoding the CRISPR-Cas system polynucleotide sequence, b) a second regulatory element operably linked to a nucleotide sequence encoding the type II Cas9 protein, and c) a recombination template are located on one or more of the same or different vectors of the system, and comprise a nuclear localization signal to be expressed together with the

nucleotide sequence encoding the Cas9 protein.

2. Reason 1 for Rescission (Erroneous Determinations of Article 29-2 of the Patent Act Based on of Cited Invention 1)

(1) Statement of Cited Document 1 (Exhibit Ko 1-1. The paragraph number is that of National Publication of International Patent Application No. 2016-502840 which is a national publication (Exhibit Ko 1-2). Paragraph [0151] is from the description on which the priority claim is based with regard to Cited Document 1 (Exhibit Ko 105)).

a. Technical Field

[0001] The present disclosure relates to targeted genome modification. In particular, the disclosure relates to RNA-guided endonucleases or fusion proteins comprising CRISPR/Cas-like protein and methods of using said proteins to modify or regulate targeted chromosomal sequences.

b. Background Art

[0002] Targeted genome modification is a powerful tool for genetic manipulation of eukaryotic cells, embryos, and animals. Current methods rely on the use of engineered nuclease enzymes, such as, for example, zinc finger nucleases (ZFN) or transcription activator-like effector nucleases (TALEN). Each new genomic target, however, requires the design of a new ZFN or TALEN comprising a novel sequence-specific DNA-binding module. Thus, preparation of these custom designed nucleases tends to be costly and time-consuming. Moreover, the specificities of ZFN and TALEN are such that they can mediate off-target (..) cleavages.

[0003] Thus, there is a need for a targeted genome modification technology that does not require the design of a new nuclease for each new targeted genomic location .. , there is a need for a technology with increased specificity with few or no off-target effects.

c. Summary of the Invention

[0004] Among the various aspects of the present disclosure is the provision of an isolated RNA-guided endonuclease, wherein the endonuclease comprises at least one nuclear localization signal, at least one nuclease domain, and at least one domain that interacts with a guide RNA to target the endonuclease to a specific nucleotide sequence for cleavage.

In other embodiments, a nucleic acid sequence encoding the RNA-guided endonuclease can be operably linked to a promoter control sequence, and optionally, can be part of a vector. In other embodiments, a vector comprising a sequence

encoding the RNA-guided endonuclease, which can be operably linked to a promoter control sequence, can also comprise a sequence encoding a guide RNA, which can be operably linked to a promoter control sequence.

[Claim 13] A method for modifying a chromosomal sequence in a eukaryotic cell or embryo, / the method comprising: / a) introducing into the eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide; and / b) culturing the eukaryotic cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified.

[Claim 14] The method of claim 13, wherein the RNA-guided endonuclease is derived from a Cas9 protein.

[Claim 15] The method of claim 13 or claim 14, wherein the nucleic acid encoding the RNA-guided endonuclease is mRNA.

[Claim 16] The method of claim 13 or claim 14, wherein the nucleic acid encoding the RNA-guided endonuclease is DNA.

[Claim 17] The method of claim 16, wherein the DNA is part of a vector that further comprises a sequence encoding the guide RNA.

[0005] Another aspect of the present invention encompasses a method for modifying a chromosomal sequence in a eukaryotic cell or embryo. The method comprises introducing into a eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease as defined herein, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide comprising a donor sequence. The method further comprises culturing the cell or embryo such that each guide RNA directs a RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified. In one embodiment, the RNA-guided endonuclease can be derived from a Cas9 protein. In another embodiment, the nucleic acid encoding the RNA-guided endonuclease introduced into the cell or embryo can be mRNA. In a

further embodiment, the nucleic acid encoding the RNA-guided endonuclease introduced into the cell or embryo can be DNA. In a further embodiment, the DNA encoding the RNA-guided endonuclease can be part of a vector that further comprises a sequence encoding the guide RNA.

d. Mode for Carrying Out the Invention

[0014] In one embodiment, the RNA-guided endonuclease is derived from a type II CRISPR/Cas system. In a specific embodiment, the RNA-guided endonuclease is derived from a Cas9 protein.

[0022] In optional embodiments, the RNA-guided endonuclease may be part of a protein-RNA complex comprising a guide RNA. The guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific targeted site (a specific protospacer sequence at the 5' end of the guide RNA base pairs).

[0060] In embodiments in which the optional donor polynucleotide is present, the donor sequence in the donor polynucleotide can be exchanged with or integrated into the chromosomal sequence during repair of the double-stranded breaks. ,For example, in embodiments in which the donor sequence is flanked by upstream and downstream sequences having substantial sequence identity with upstream and downstream sequences, respectively, of the targeted sites in the chromosomal sequence, the donor sequence can be exchanged with or integrated into the chromosomal sequence at the targeted site during repair mediated by homology-directed repair process.

(a) RNA-Guided Endonuclease

[0063] The method comprises introducing into a cell or embryo at least one RNA-guided endonuclease comprising at least one nuclear localization signal, or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal.

[0064] In some embodiments, the RNA-guided endonuclease can be introduced into the cell or embryo as an isolated protein. In other embodiments, the RNA-guided endonuclease can be introduced into the cell or embryo as an mRNA molecule. In still other embodiments, the RNA-guided endonuclease can be introduced into the cell or embryo as a DNA molecule. The DNA sequence can be linear, or the DNA sequence can be part of a vector.

(b) Guide RNA

[0066] The method also comprises introducing into a cell or embryo at least one guide RNA or DNA encoding at least one guide RNA. A guide RNA interacts with the RNA-guided endonuclease to direct the endonuclease to a specific targeted

site, at which site the 5' end of the guide RNA base pairs with a specific protospacer sequence in the chromosomal sequence.

[0067] Each guide RNA comprises three regions: a first region at the 5' end that is complementary to the targeted site in the chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3' region that remains essentially single-stranded. .. The first region of each guide RNA is different such that each guide RNA guides a fusion protein to a specific targeted site. .. The second and third regions of each guide RNA can be the same in all guide RNAs.

[0068] The first region of the guide RNA is complementary to a sequence (i.e., a protospacer sequence) at the targeted site in the chromosomal sequence such that the first region of the guide RNA can base pair with the targeted site. In an exemplary embodiment, the first region of the guide RNA is about 19, 20, or 21 nucleotides in length.

[0069] The guide RNA also comprises a second region that forms a secondary structure. In some embodiments, the secondary structure comprises a stem (or hairpin) and a loop. For example, the loop can range from about 3 to about 10 nucleotides in length, and the stem can range from about 6 to about 20 base pairs in length. Thus, the overall length of the second region can range from about 16 to about 60 nucleotides in length. In an exemplary embodiment, the loop is about 4 nucleotides in length and the stem comprises about 12 base pairs.

[0070] The guide RNA also comprises a third region at the 3' end that remains essentially single-stranded. Thus, the third region has no complementarity to any chromosomal sequence in the cell of interest and has no complementarity to the rest of the guide RNA. The length of the third region can vary. In general, the third region is about 4 or more nucleotides in length. For example, the length of the third region ranges from about 5 to about 60 nucleotides in length.

[0071] The combined length of the second and third regions of the guide RNA can range from about 30 to about 120 nucleotides in length. In one aspect, the combined length of the second and third regions of the guide RNA ranges from about 70 to about 100 nucleotides in length.

[0072] In some embodiments, the guide RNA comprises a single molecule comprising all three regions. In other embodiments, the guide RNA can comprise two separate molecules. The first RNA molecule can comprise the first region of the guide RNA and one half of the "stem" of the second region of the guide RNA. The second RNA molecule can comprise the other half of the "stem" of the second region of the guide RNA and the third region of the guide RNA. Thus, in this embodiment,

the first and second RNA molecules each contain a sequence of nucleotides that are complementary to one another. For example, in one embodiment, the first and second RNA molecules each comprise a sequence (about 6 to about 20 nucleotides) that base pairs to the other sequence to form a functional guide RNA.

[0073] The guide RNA can be introduced into the cell or embryo as an RNA molecule.

[0074] In other embodiments, the guide RNA can be introduced into the cell or embryo as a DNA molecule. In such cases, the DNA encoding the guide RNA can be operably linked to a promoter control sequence for expression of the guide RNA in the cell or embryo of interest. In exemplary embodiments, the RNA encoding a sequence is linked to a mouse or human U6 promoter.

[0075] In some embodiments, the DNA sequence encoding the guide RNA can be part of a vector. Suitable vectors include plasmid vectors and viral vectors.

[0076] In embodiments in which both the RNA-guided endonuclease and the guide RNA are introduced into the cell as DNA molecules, each can be part of a separate molecule or both can be part of the same molecule.

(c) Targeted Site

[0077] An RNA-guided endonuclease in conjunction with a guide RNA is directed to a targeted site in the chromosomal sequence, wherein the RNA-guided endonuclease introduces a double-stranded break in the chromosomal sequence.

(d) Optional Donor Polynucleotide

[0085] Donor polynucleotides comprising the upstream and downstream sequences with sequence similarity to the targeted chromosomal sequence can be linear or circular. In embodiments in which the donor polynucleotide is circular, it can be part of a vector. For example, the vector can be a plasmid vector.

[0088] Typically, the donor polynucleotide will be DNA. The DNA may be single-stranded or double-stranded and/or linear or circular. The donor polynucleotide may be a DNA plasmid, a bacterial artificial chromosome (BAC), a yeast artificial chromosome (YAC), a viral vector, a linear piece of DNA, a PCR fragment, a naked nucleic acid, or a nucleic acid complexed with a delivery vehicle such as a liposome or poloxamer. In certain embodiments, the donor polynucleotide comprising the donor sequence can be part of a plasmid vector. In any of these situations, the donor polynucleotide comprising the donor sequence can further comprise at least one additional sequence.

(e) Example 1: Modification of Cas9 Gene for Mammalian Expression

[0138] A Cas9 gene from Streptococcus pyogenes strain MGAS15252

(Accession number YP_005388840.1) was optimized with Homo sapiens codon preference to enhance its translation in mammalian cells. The Cas9 gene was also modified by adding a nuclear localization signal PKKKRKV (SEQ ID NO:1) at the C terminus for targeting the protein into the nuclei of mammalian cells.

[0140] The modified Cas9 DNA sequence was placed under the control of a cytomegalovirus (CMV) promoter for constituent expression in mammalian cells. The modified Cas9 DNA sequence was also placed under the control T7 promoter for in vitro mRNA synthesis with T7 RNA polymerase.

(f) Example 2: Targeting Cas9

[0141] The adeno-associated virus integration site 1 (AAVS1) locus was used as a target for Cas9-mediated human genome modification. The human AAVS1 locus is located in intron 1 (4427 bp) of protein phosphatase 1, regulatory subunit 12C (PPP1R12C).

[0143] Cas9 guide RNAs were designed for targeting the human AAVS1 locus. A 42 nucleotide RNA (referred to herein as a "crRNA" sequence) comprising (5' to 3') a target recognition sequence (i.e., a sequence complementary to the non-coding strand of the targeted sequence) and a protospacer sequence; a 85 nucleotide RNA (referred to herein as a "tracrRNA" sequence) comprising 5' sequence with complementarity to the 3' sequence of the crRNA and additional hairpin sequence; and a chimeric RNA comprising nucleotides 1-32 of the crRNA, a GAAA loop, and nucleotides 19-45 of the tracrRNA were prepared. The chimeric RNA coding sequence was also placed under the control of human U6 promoter for in vivo transcription in human cells.

[0144] [Table 8] (As shown in Attachment 2)

(g) Example 3: Preparation of Donor Polynucleotide to Monitor Genome Modification

[0145] Targeted integration of a GFP protein into the N terminus of PPP1R12C was used to monitor Cas9-mediated genome modification. To mediate integration by homologous recombination, a donor polynucleotide was prepared. The AAVS1-GFP DNA donor contained a 5' (1185 bp) AAVS1 locus homologous arm, an RNA splicing receptor, a turbo GFP coding sequence, a 3' transcription terminator, and a 3' (1217 bp) AAVS1 locus homologous arm.

[0147] Targeted gene integration will result in a fusion protein between the first 107 amino acids of the PPP1R12C and the turbo GFP. The expected fusion protein contains the first 107 amino acid residues of PPP1R12C (highlighted in grey) from RNA splicing between the first exon of PPP1R12C and the engineered splice

receptor (..).

(h) Example 4: Cas9-Mediated Targeted Integration

[0149] Transfection was performed on human K562 cells. The K562 cell line was obtained from American Type Culture Collection (ATCC) and grown in Iscove's Modified Dulbecco's Medium, supplemented with 10% FBS and 2 mM L-glutamine. Cultures were split one day before transfection (at approximately 0.5 million cells per mL). Cells were transfected with Nucleofector Solution V (Lonza) on a Nucleofector (Lonza) with the T-016 program. Transfection treatments are detailed in Table 7.

[0150] [Table 7] (As shown in Attachment 2)

[0151] Fluorescence-activated cell sorting (FACS) was performed 4 days after transfection. FACS data are presented in Figure 4. The percent GFP detected in each of the four experimental treatments (A to D) was greater than in the control treatments (E, F) (Exhibit Ko 105).

(i) Example 5: PCR Confirmation of Targeted Integration

[0152] Genomic DNA was extracted from transfected cells with GenElute Mammalian Genomic DNA Miniprep Kit (Sigma) 12 days after transfection. Genomic DNA was then PCR amplified with a forward primer located outside the 5' homologous arm of the AAVS1-GFP plasmid donor and a reverse primer located at the 5' region of the GFP. The forward primer was 5'-CCACTCTGTGCTGACCACTCT-3' (SEQ ID NO:18) and the reverse primer was 5'-GCGGCACTCGATCTCCA-3' (SEQ ID NO:19). The expected fragment size from the junction PCR was 1388 bp. The amplification was carried out with JumpStart Taq ReadyMix (Sigma), using the following cycling conditions: 98°C for 2 minutes for initial denaturation; 35 cycles of 98°C for 15 seconds, 62°C for 30 seconds, and 72°C for 1 minute and 30 seconds; and a final extension at 72°C for 5 minutes. PCR products were resolved on 1% agarose gel.

[0153] Cells transfected with 10 μg of Cas9 mRNA transcribed with an Anti-Reverse Cap Analog (ARCA), 0.3 nmol of pre-annealed crRNA-tracrRNA duplex, and 10 μg of AAVS1-GFP plasmid DNA displayed a PCR product of the expected size (see lane A of Figure 5).

(2) Summary of the Disclosure of Cited Document 1 and Cited Invention 1 Found by the Decision of the Present Case

a. The main purpose of Cited Document 1 is to provide an RNA-guided endonuclease directed by a guide RNA as genome-editing techniques. According to this, Cited Document 1 discloses a technology that does not require a design of a new

nuclease for a targeted genomic location, like the conventional genome editing techniques such as ZFN and TALEN, and that also has increased specificity ([0001] to [0004]).

b. Concretely, Cited Document 1 discloses that (1) an RNA-guided endonuclease (Cas9 protein) comprising a nuclear localization signal is introduced into a eukaryotic cell, (2) a guide RNA directs an RNA-guided endonuclease to a targeted site, and (3) in a eukaryotic cell, an RNA-guided endonuclease introduces a double-stranded break in a targeted site and the chromosomal sequence is modified by a donor DNA ([Claim 13], [Claim 14], [0005]).

Among these, as (1) a method of introducing an RNA-guided endonuclease into a eukaryotic cell, Cited Document 1 discloses [i] a method of introducing a protein, [ii] a method of introducing mRNA encoding a protein, and [iii] a method wherein DNA encoding a protein is made to be part of a vector to introduce the part of the vector ([Claim 15] to [Claim 17], [0005], [0064]).

In addition, as (2) a method of introducing a guide RNA into a eukaryotic cell, Cited Document 1 discloses [i] a method of introducing a single molecule (single-stranded chimeric RNA), [ii] a method of introducing two separate molecules (double-stranded crRNA-tracrRNA) ([0072], Example 2, Example 4), and [iii] a method of introducing DNA encoding the RNA in the above [i] or [ii] (single-stranded or double-stranded) wherein the DNA is made to be part of a vector to introduce the part of the vector ([0074], [0075]).

Further, with regard to (3) a donor DNA, Cited Document 1 discloses that the donor DNA is made to be part of a vector to introduce into a eukaryotic cell ([0085], [0088]).

Methods of preparing the above (1) to (3) are disclosed in Examples 1 to 3.

c. In Examples 4 and 5, with regard to Treatment A ((1)[ii], (2)[ii]), Treatments B and C ((1)[i], (2)[i]) and Treatment D ((1)[iii], (2)[iii]) in which the introducing methods were changed from (1) and (2), Fluorescence-Activated Cell Sorting (FACS) (Example 4) and PCR test (Example 5) were used to confirm whether or not a chromosomal sequence is modified in a eukaryotic cell, as compared to control treatment groups (E, F). In this regard, among Treatments A to D, none of Treatments A to C is a vector system. Only Treatment D is a vector system.

d. The decision of the present case found Cited Invention 1 from Cited Document 1, as mentioned in No. 2, 3(2) above.

(3) Findings on Cited Invention 1

a. Cited Document 1 discloses an invention related to targeted genome-editing

([0001]). With regard to the invention, Cited Document 1 discloses the configuration comprising introducing into a eukaryotic cell or embryo at least one RNA-guided endonuclease comprising at least one nuclear localization signal, or a nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal ([Claim 13], [0005]), among which the RNA-guided endonuclease is derived from a Cas9 protein ([Claim 14], [0005]), wherein the nucleic acid encoding the RNA-guided endonuclease is DNA, and the DNA is part of a vector which further comprises a sequence encoding a guide RNA ([Claim 16], [Claim 17], [0075]). Cited Document 1 also discloses the configuration that the nucleic acid sequence encoding the RNA-guided endonuclease can be operably linked to a promoter control sequence, and is part of a vector ([0004]).

Thus, Cited Document 1 discloses "a vector comprising a promoter control sequence operably linked to a nucleic acid encoding at least one type II Cas9 protein comprising at least one nuclear localization signal."

b. With regard to the invention, Cited Document 1 discloses the configuration comprising introducing into a eukaryotic cell or embryo at least one guide RNA or DNA encoding at least one guide RNA ([Claim 13], [0005]), among which the guide RNA comprises three regions; i.e., a first region at the 5' end that is complementary to a targeted site in a chromosomal sequence, a second internal region that forms a stem loop structure, and a third 3' region that remains essentially single-stranded ([0067]), wherein the guide RNA is operably linked to a promoter control sequence for expression of the guide RNA in the cell or embryo of interest ([0074]), and the DNA sequence encoding the guide RNA is part of a vector ([0075]).

Thus, Cited Document 1 discloses "a vector comprising a promoter control sequence operably linked to DNA encoding at least one guide RNA comprising a first region at the 5' end that is complementary to a targeted site in a chromosomal sequence in a eukaryotic cell, a second internal region that forms a stem loop structure, and a third 3' region that remains essentially single-stranded."

c. Cited Document 1 discloses that the invention comprises introducing at least one donor polynucleotide into a eukaryotic cell or embryo ([Claim 13], [0005]), and discloses the configuration that in an embodiment in which the donor polynucleotide is circular, it can be part of a vector ([0085]).

Thus, Cited Document 1 discloses "a vector comprising at least one donor polynucleotide".

d. Cited Document 1 discloses that the invention is a "vector system."

e. Cited Document 1 discloses that the invention has a function of culturing a

eukaryotic cell or embryo such that each guide RNA directs a RNA-guided endonuclease to a targeted site in a chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified ([Claim 13], [0005]).

Thus, Cited Document 1 discloses that "the guide RNA directs a type II Cas9 protein to a targeted site in a chromosomal sequence in a eukaryotic cell where the type II Cas9 protein introduces a chromosomal DNA double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified."

f. According to the foregoing, Cited Document 1 discloses the above configurations a to e. Therefore, it can be found that Cited Document 1 discloses the invention as found in the decision of the present case (Cited Invention 1).

(4) Comparison between the Present Invention and Cited Invention 1

a. The Present Invention is as the above No. 2, 2 and can be divided into the constituent features as follows:

A. An engineered, non-naturally occurring clustered regularly interspaced short palindromic repeats (CRISPR) - CRISPR-associated (Cas)

G. (CRISPR-Cas) vector system;

C. comprising one or more vectors comprising;

B-a. a first regulatory element operably linked to a nucleotide sequence encoding a CRISPR-Cas system polynucleotide sequence comprising a guide sequence, tracrRNA, and a tracr mate sequence, wherein the guide sequence hybridizes with one or more targeted sequences in polynucleotide loci in a eukaryotic cell;

B-b. a second regulatory element operably linked to a nucleotide sequence encoding a type II Cas9 protein; and

B-c. a recombination template;

D. wherein components a), b), and c) are located on the same or different vectors of the system;

E. the system further comprises one or more nuclear localization signals (there can be more than one) (NLS (there can be more than one)) to be expressed together with the nucleotide sequence encoding the Cas9 protein;

F. whereby the guide sequence targets the one or more polynucleotide loci in the eukaryotic cell and the Cas9 protein cleaves the one or more polynucleotide loci, whereby sequence of the one or more polynucleotide loci is modified.

b. Constituent Feature A(C)

(a) The Present Invention is an engineered, non-naturally occurring clustered regularly interspaced short palindromic repeats (CRISPR) - CRISPR-associated (Cas) (CRISPR-Cas) system.

On the other hand, Cited Invention 1 uses an engineered, non-naturally occurring Cas9 protein which is derived from a naturally occurring type II CRISPR/Cas system and is modified to include a nuclear localization signal ([0014], [0063]). Thus, this portion of Cited Invention 1 corresponds to a portion where the vector system is "clustered regularly interspaced short palindromic repeats (CRISPR) - CRISPR-associated (Cas) (CRISPR-Cas)" among constituent feature A of the Present Invention.

(b) Among constituent feature A of the Present Invention, the portion of "vector system" (the same applies to constituent feature C) corresponds to the constituent feature "vector system" of Cited Invention 1.

c. Constituent Feature B-a

"A first region at the 5' end that is complementary to a targeted site in a chromosomal sequence in a eukaryotic cell" in Cited Invention 1 corresponds to "the guide sequence" which "hybridizes with one or more targeted sequences in polynucleotide loci in a eukaryotic cell" in the Present Invention.

The second and third regions which combine "a second internal region that forms a stem loop structure" and "a third 3' region that remains essentially single-stranded" in Cited Invention 1 correspond to a sequence of "loop" in addition to "tracrRNA" and a "tracr mate sequence" in the Present Invention, in view of the statement "the portion is provided in the lower portion of Figure 13B (Judgment Note: this is a typographical error of ‘Figure 8B’), where the portion of the sequence 5' of the final "N" and upstream of the loop corresponds to the tracr mate sequence, and the portion of the sequence 3' of the loop corresponds to the tracr sequence" in [0076] of the description of the present application.

Thus, "at least one guide RNA comprising a first region at the 5' end that is complementary to a targeted site in a chromosomal sequence in a eukaryotic cell, a second internal region that forms a stem loop structure, and a third 3' region that remains essentially single-stranded" in Cited Invention 1 corresponds to "a CRISPR-Cas system polynucleotide sequence comprising a guide sequence, tracrRNA, and a tracr mate sequence", wherein "the guide sequence hybridizes with one or more targeted sequences in polynucleotide loci in a eukaryotic cell" in constituent feature B-a of the Present Invention.

Therefore, "(ii) a vector comprising a promoter control sequence operably linked to DNA encoding at least one guide RNA comprising a first region at the 5' end that is complementary to a targeted site in a chromosomal sequence in a eukaryotic cell, a second internal region that forms a stem loop structure, and a third 3' region that remains essentially single-stranded" in Cited Document 1 corresponds to "one ... vectors comprising" "a) a first regulatory element operably linked to a nucleotide sequence encoding a CRISPR-Cas system polynucleotide sequence comprising a guide sequence, tracrRNA, and a tracr mate sequence, wherein the guide sequence hybridizes with one or more targeted sequences in polynucleotide loci in a eukaryotic cell" in the Present Invention.

d. Constituent Feature B-b

The second regulatory element in the Present Invention comprises one encoding a CRISPR enzyme comprising a nuclear localization sequence (NLS) ([0071]), and thus is a regulatory element operably linked to an enzyme-coding sequence encoding a CRISPR enzyme comprising a nuclear localization sequence ([0012]).

On the other hand, "(i) a vector comprising a promoter control sequence operably linked to a nucleic acid encoding at least one type II Cas9 protein comprising at least one nuclear localization signal" in Cited Invention 1 corresponds to "one ... vectors comprising" "b) a second regulatory element operably linked to a nucleotide sequence encoding a type II Cas9 protein, and further comprising "one or more nuclear localization signals (there can be more than one) (NLS (there can be more than one)) to be expressed together with the nucleotide sequence encoding the Cas9 protein" in the Present Invention.

e. Constituent Feature B-c

"(iii) at least one donor polynucleotide" in Cited Invention 1 corresponds to "a sequence or a template which can be used for recombination into the locus to be targeted, including a targeted sequence" in the Present Invention (an explanation to such matters is given in [0065] of the description of the present application), and thus, corresponds to "a recombination template." Therefore, "a vector comprising at least one donor polynucleotide" in Cited Document 1 corresponds to "one .. vectors comprising" "c) a recombination template" in the Present Invention.

f. Constituent Feature D

Cited Document 1 discloses not only an embodiment in which the vectors (i) to (iii) are different from each other, but also an embodiment in which the vectors (i) to (iii) are the same ([0005], [0088]). Thus, these embodiments correspond to

constituent feature D of the Present Invention.

g. Constituent Feature E

As mentioned in the above d, Cited Document 1 comprises at least one nuclear localization signal to be expressed together with the nucleotide sequence encoding a Cas9 protein, and thus, this corresponds to constituent feature E of the Present Invention.

h. Constituent Feature F

In Cited Invention 1, the above three vectors (i) to (iii) are the constituent features, and a vector system comprising these three vectors is provided with a function in which "the guide RNA directs a type II Cas9 protein to a targeted site in a chromosomal sequence in a eukaryotic cell where the type II Cas9 protein introduces a chromosomal DNA double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified." Thus, "a targeted site in a chromosomal sequence in a eukaryotic cell", "break", and "modify" of Cited Invention 1 correspond to "the one or more polynucleotide loci in a eukaryotic cell", "cleave", and "modify" of the Present Invention, respectively.

Thus, the above function of Cited Invention 1 is the same as the function of the Present invention in which "a guide sequence targets the one or more polynucleotide loci in the eukaryotic cell and the Cas9 protein cleaves the one or more polynucleotide loci, whereby sequence of the one or more polynucleotide loci is modified", and therefore, corresponds to constituent feature F.

i. Constituent Feature G

It is clear that Cited Invention 1 is a CRISPR-Cas vector system, which corresponds to constituent feature G of the Present Invention as well.

j. According to the foregoing, it can be found that the Present Invention is identical with Cited Invention 1.

(5) Assertions by the Plaintiffs

a. The Plaintiffs assert that Cited Document 1 is not supported by experimental data that a targeted site sequence was modified and does not provide a rational basis for assuming that the CRISPR-Cas9 system could be applied to eukaryotic applications. Accordingly, the Plaintiffs assert that [i] it cannot be deemed that the Present Invention is substantially identical with Cited Invention 1, because Cited Invention 1 does not comprise a function of the Present Invention; i.e. "a guide RNA directs a type II Cas9 protein to a targeted site in a chromosomal sequence in a eukaryotic cell where the type II Cas9 protein introduces a chromosomal DNA

double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified", and that [ii] it cannot be deemed that Cited Document 1 has an effect of excluding a later application stipulated in Article 29-2 of the Patent Act, because a system disclosed in Cited Document 1 cannot solve the problem of the Present Invention.

b. Assertion of the above a[i]

(a) Example 4 and Example 5

Cited Document 1 is a Patent Document in which the main purpose is to provide new genome-editing techniques; concretely, to provide an RNA-guided endonuclease directed by a guide RNA. In this regard, Cited Document 1 in Treatment D of Example 4 discloses a method of introducing DNA encoding a protein as part of a vector, as a method of introducing into a eukaryotic cell an RNA-guided endonuclease (a Cas9 protein) comprising a nuclear localization signal, and discloses using U6-chimeric RNA plasmid DNA as a method of introducing a guide RNA into a eukaryotic cell.

Fluorescence-activated cell sorting (FACS) was performed in Example 4, and a PCR experiment was performed in Example 5.

(b) Fluorescence-Activated Cell Sorting (FACS)

Introducing a donor sequence into a targeted sequence resulted in a fusion protein of the first 107 amino acids of PPP1R12C and a turbo GFP (Example 3), and when the fusion protein (expressed GPF protein) was irradiated with laser light, it emitted fluorescence at a specific wavelength (Exhibit Ko 103, page 5, lines 2 to 5). By measuring the fluorescence of the GFP protein, it is possible to presume that the GFP protein is expressed in the cell and that a donor sequence (such as a GFP gene) is incorporated into the targeted sequence.

Cited Document 1 discloses in Example 4 that in Treatment D (Treatments E and F are the control treatment groups), fluorescence-activated cell sorting (FACS) was performed using the Cas9, the guide RNA, and the donor polynucleotides prepared in Examples 1 to 3.

According to Figures 4-1 to 3 (as shown in Attachment 2), which show the results of the experiments in Example 4, a numerical value of Treatment D was 7.47%. This value is considerably higher than values of the control treatment groups; i.e., Treatment E (1.92%) by using only "AAVS1-GFP plasmid DNA" (donor DNA) and Treatment F (0.159%) by using no reagents. According to this, it can be found that a donor sequence (such as a GFP gene) was incorporated into a targeted sequence.

(c) Results of PCR experiment

In PCR experiment, the presence or absence of a PCR product obtained in the experiment can be detected by bands on a gel, whereby it is possible to presume that a donor sequence (GFP gene) is incorporated into or near a targeted sequence (Exhibit Ko 103, page 18, line 10 to page 20, line 16).

Example 5 discloses that the PCR experiment was performed using the same samples as in Example 4. According to Figure 5 (as shown in Attachment 2), which shows the results of the experiments in Example 5, a band of 1388 bp (base length), which was expected for Treatment D, was not detected.

However, in Treatment D in Example 5, it cannot be denied that a band on the predetermined gel could not be detected, as a result of insufficient efficiency of genome modification due to differences in guide RNAs, targeted sequences, etc., similarly to the chimeric RNA with n of +48 (a tracr sequence is 26 nucleotides in length) and the chimeric RNA with n of +54 (a tracr sequence is 32 nucleotides in length) of the description of the present application. Therefore, it is not denied that the vector system of the Cited Invention 1 has a function of incorporating a donor sequence (such as a GFP gene) into a targeted sequence, notwithstanding the results of Treatment D in Example 5.

(d) According to the foregoing, it can be understood from the disclosure of Cited Document 1 that an RNA-guided endonuclease directed by a guide RNA has a function of modifying a chromosomal sequence at a targeted site in a eukaryotic cell.

(e) As vector (i), Cited Document 1 uses a vector which was obtained by optimizing a Cas9 gene obtained from a Streptococcus pyogenes strain for enhancing translation in mammalian cells and adding the nuclear localization signal PKKKRKV (SEQ ID NO: 1) to the C-terminus, followed by placing it under the control of a promoter (Example 1).

In addition, as vector (ii), Cited Document 1 uses a vector which was obtained by placing a chimeric RNA comprising nucleotides 1 to 32 of the crRNA, a GAAA loop, and nucleotides 19 to 45 of the tracrRNA under the control of human U6 promoter (Example 2).

Further, as vector (iii), Cited Document 1 uses "AAVS1-GFP plasmid DNA" by which a target gene is integrated into a targeted sequence by homologous recombination (Example 3).

Furthermore, by showing experimental examples, Cited Document 1 discloses in Example 4 that the vector system comprising the vectors disclosed in Examples 1 to 3 has a function of cleaving a targeted sequence in a eukaryotic cell and modifying the targeted sequence.

Thus, each vector (i) to (iii) in Cited Invention 1 has a technical means necessary for appropriate transcription, translation, and nuclear translocation, etc. in a eukaryotic cell, and a technical means necessary for appropriate modification of a targeted sequence in a eukaryotic cell. Therefore, it can be understood that it is also disclosed that such vectors have functions of cleaving a targeted sequence in a eukaryotic cell and modifying the targeted sequence.

(f) Summary

According to the foregoing, it should be deemed that Cited Document 1 discloses not only formally but also substantially that "a guide RNA directs a type II Cas9 protein to a targeted site in a chromosomal sequence in a eukaryotic cell where the type II Cas9 protein introduces a chromosomal DNA double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified." It can be understood that the vector system of Cited Invention 1 is disclosed as a system which also includes the above functions.

c. Assertion of the above a[ii]

(a) Article 29-2 of the Patent Act provides that if an invention claimed in a patent application is identical with an invention or a device stated in the description or the drawings (hereinafter referred to as "the description, etc. of an earlier application") originally attached to a request of another application for a patent or of another application for a utility model registration which was filed before the date of the patent application and for which the Patent Gazette or the Utility Model Gazette was published after filing the patent application, a patent shall not be granted for the invention.

An object of this Article is as follows. An invention stated in the description, etc. of the earlier application, even if the invention is not stated in the Scope of Claims, is laid open to the public in a publication, etc. of the earlier application. Thus, even if a later application is filed before the publication, etc. of the earlier application, when the invention of the later application is identical with the invention of the earlier application, no new technology is laid open to the public in the publication, etc. of the later application. Therefore, granting a patent to such invention is inappropriate from the viewpoint of the patent system which intends to protect an invention as a reward for laying a new invention open to the public.

The "invention" stated in the description, etc. of the earlier application in this Article is construed as an invention which is understood from a matter stated in the description, etc. of the earlier application and from a matter equivalent to that stated

in the description, etc. of the earlier application. The "matter equivalent to that stated" means a matter which can be derived from the stated matter by taking into consideration common general technical knowledge as of the time of filing.

Thus, even if there is no particular statement of the description, etc. of the earlier application, the invention of the earlier application can be found by taking common general technical knowledge into consideration in order to understand the invention of the earlier application. On the other hand, in the case where an invention lacks concreteness or technical content of an invention is insufficiently disclosed even while taking into consideration common general technical knowledge of a person ordinarily skilled in the art, such invention does not fall under the "invention" mentioned above, and does not have an effect of excluding the later application stipulated in this Article. In addition, it should be deemed that the degree of disclosure of technical content required here should be sufficient if the technical content is disclosed to the extent that a person ordinarily skilled in the art can understand that the invention of the earlier application is shown in the description, etc. of the earlier application and the person ordinarily skilled in the art is enabled to work the disclosed invention of the earlier application.

(b) In the present case, with regard to the above, Examples 1 to 3 in Cited Invention 1 disclose in detail a method for producing each vector (i) to (iii) in Cited Invention 1, and Example 4 clearly discloses a concrete test method for confirming whether a donor sequence (GFP gene) is incorporated in or near a targeted sequence.

In addition, as mentioned above, from the experimental results of Example 4, it can be understood that a combination of the RNA-guided endonuclease comprising the nuclear localization signal, the guide RNA, and the donor polynucleotide is incorporated into the eukaryotic cell and that the double-stranded break and repair occur in the targeted site. The experimental results of Example 5 are not to be interpreted as a bar to the above understanding.

Further, a vector system comprising the above vectors (i) to (iii) has a technical means necessary for appropriate transcription, translation, and nuclear translocation, etc. in a eukaryotic cell, and a technical means necessary for appropriate modification of a targeted sequence in a eukaryotic cell. Thus, it can be understood that even if the vector system is used, such vector system has functions of cleavage of a targeted sequence and modification of the targeted sequence in a eukaryotic cell, as mentioned above.

From the above, it can be deemed that there is a disclosure in Cited Document 1 to the extent that a person ordinarily skilled in the art can understand that the

invention of the earlier application is disclosed in the description, etc. of the earlier application, and the person ordinarily skilled in the art is enabled to work the shown invention of the earlier application. Therefore, it is proper to find that in Cited Document 1, a technology was laid open to the public to the extent that the technology was sufficient to exclude the later application, in which the technology includes a portion of the function such that "a guide RNA directs a type II Cas9 protein to a targeted site in a chromosomal sequence in a eukaryotic cell where the type II Cas9 protein introduces a chromosomal DNA double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified."

d. Other Assertions by the Plaintiffs

(a) The Plaintiffs assert as follows. According to the description on which the priority claim is based with regard to Cited Document 1 (Exhibit Ko 105), there are no statements "integration of the donor sequence and expression of the fusion protein were confirmed" in Example 4. In addition, it cannot be deemed that the FACS alone performed in Example 4 shows that the fluorescence detected is not due to other factors than incorporation into the targeted site. Further, it should be understood that incorporation into the targeted site does not occur unless approximately 10 times or more fluorescence is detected as compared to the control. The Plaintiffs submit a written opinion by a researcher along these lines (Exhibit Ko 103). However, it cannot be deemed that the technical content of the experimental results stated in Cited Document 1 is insufficiently disclosed.

The Plaintiffs assert that the results of the PCR experiment in Example 5 show that a PCR product was not confirmed in Treatment D, indicating that the gene of the donor sequence was not incorporated into the targeted site. However, in Treatment D in Example 5, as mentioned in the above b(c), there is a possibility that the band on the predetermined gel was not detected, as a result of insufficient efficiency of genome modification due to differences in guide RNAs and targeted sequences. Therefore, from the result of Treatment D in Example 5, it should be construed that it is not denied that the vector system of the Cited Invention 1 has a function of incorporating a donor sequence (such as a GFP gene) into a targeted sequence.

(b) The Plaintiffs assert that a person ordinarily skilled in the art would have reviewed the conflicting results obtained by the FACS experiment and the PCR experiment in Cited Document 1 and concluded that a question of whether the CRISPR-Cas9 system functions in a eukaryotic cell is unresolved. Alternatively, the Plaintiffs assert that it was not also possible to deduce that the experiments in Cited

Document1 were successful on the basis of the fact that other scientists could achieve success in a eukaryotic cell using other techniques or constructs.

However, the results of the experiment in Example 4 are sufficient to understand that the RNA-guided endonuclease comprising the nuclear localization signal, the guide RNA, and the donor polynucleotide were introduced into the eukaryotic cell and that the double-stranded break and repair occurred in the targeted site. In addition, the results of the experiment in Example 5 are not to be construed as a bar to the above understanding.

Further, a vector system comprising vectors (i) to (iii) in Cited Invention 1 has a technical means necessary for appropriate transcription, translation, and nuclear translocation, etc. in a eukaryotic cell, and a technical means necessary for appropriate modification of a targeted sequence in a eukaryotic cell, as mentioned in the above b(e). Thus, it can be understood that the vector system of Cited Invention 1 has a function of cleaving a targeted sequence in a eukaryotic cell and modifying the targeted sequence. That is, in Cited Document 1, there is sufficient disclosure to understand that Cited Invention 1 can be worked.

Therefore, it cannot be deemed that the issue of whether a CRISPR-Cas9 system functions in a eukaryotic cell is unresolved in Cited Invention 1.

(c) The Plaintiffs assert that the Present Invention discloses guidance for adapting a CRISPR-Cas9 system to eukaryotic genomic DNA in a eukaryotic cell environment, and emphasize a difference in the function from the CRISPR-Cas9 system of Cited Document 1. However, as determined from the statement of Claim 1, the Present Invention does not make a selection such as adding multiple NLSs to Cas9, making tracrRNAs longer, and inserting, e.g., poly-T as a terminator at the 3' end of a chimeric RNA. Rather, the Present Invention and Cited Invention 1 use a vector system containing common vectors in the configuration of the invention.

The Plaintiffs' assertion emphasizing the difference between the two inventions is unfounded.

(6) Summary

As mentioned above, the Present Invention is identical with the Cited Invention 1, and thus is not allowed to be granted a patent under the provision of Article 29-2 of the Patent Act.

Therefore, Reason 1 for Rescission is unfounded.

3. Conclusion

According to the foregoing, the decision of the present case did not err in concluding that the request for the appeal of the present case shall be dismissed. The

Plaintiffs' claim for seeking a rescission of the decision is unfounded and thus shall be dismissed. Therefore, the judgment is rendered as mentioned in the main text.

Intellectual Property High Court, First Division

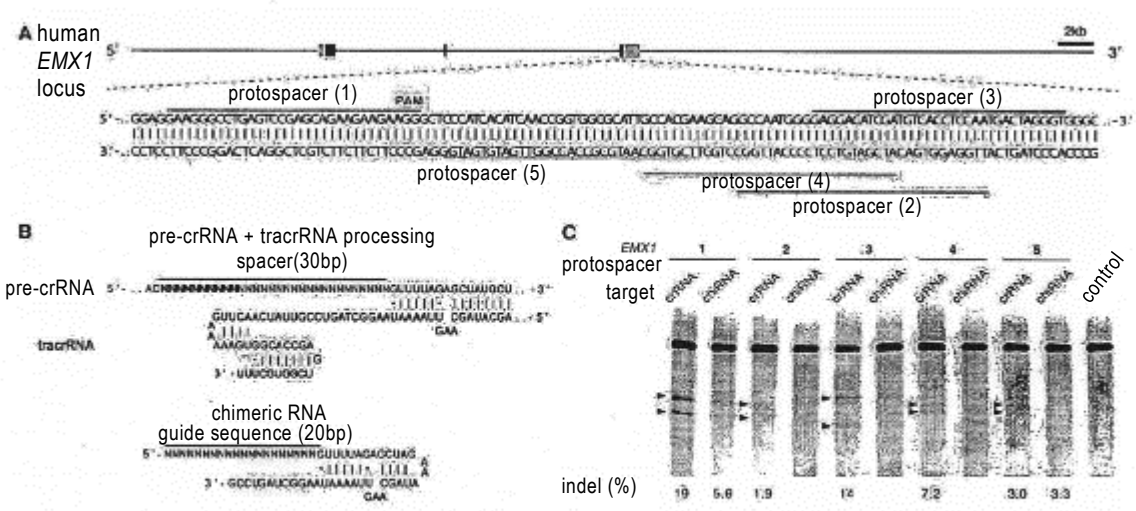
Presiding Judge TAKABE Makiko

Judge KOBAYASHI Yasuhiko

Judge SEKINE Sumiko

Attachment 1 (List of the Drawings of the Present Invention)

[Figure 8]



Figures 8A-C

[Figure 10]

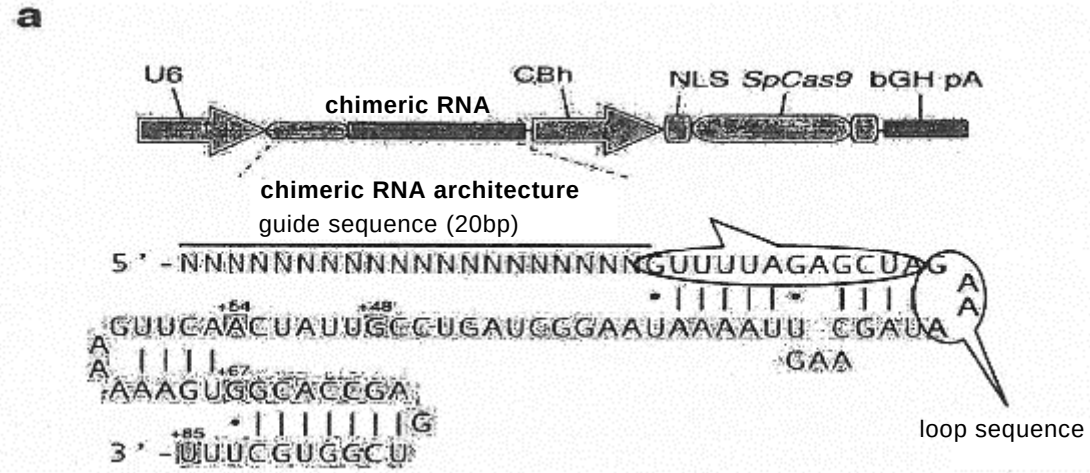


Figure 10A

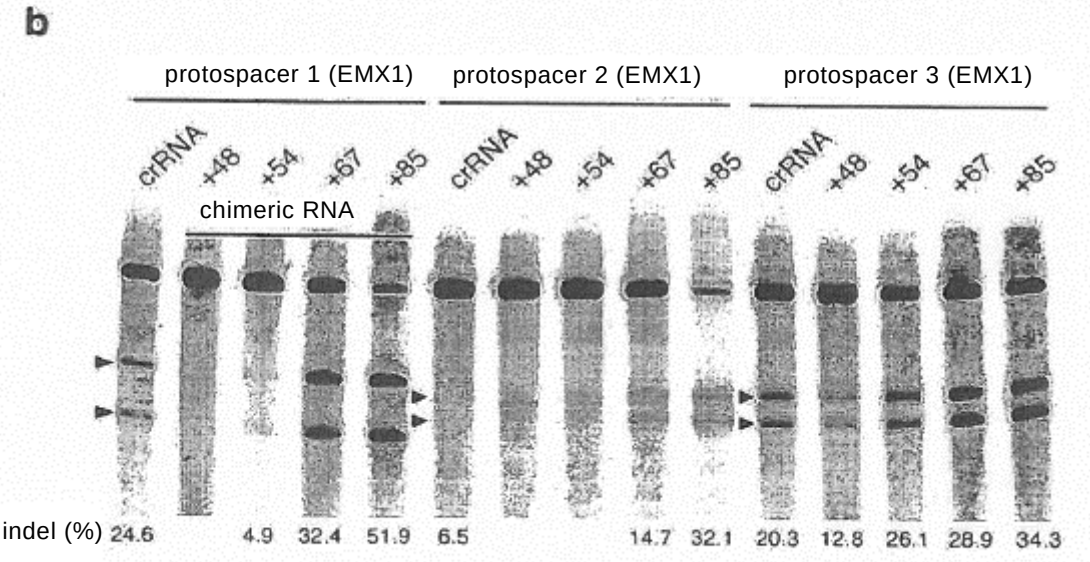


Figure 10B

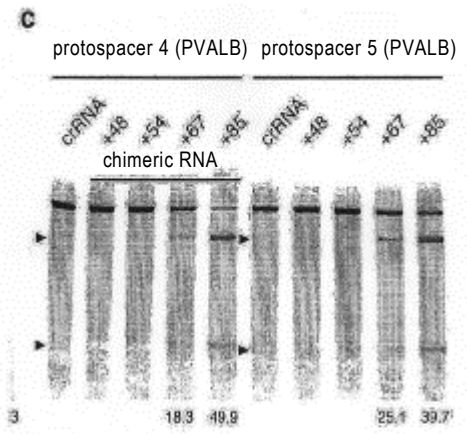
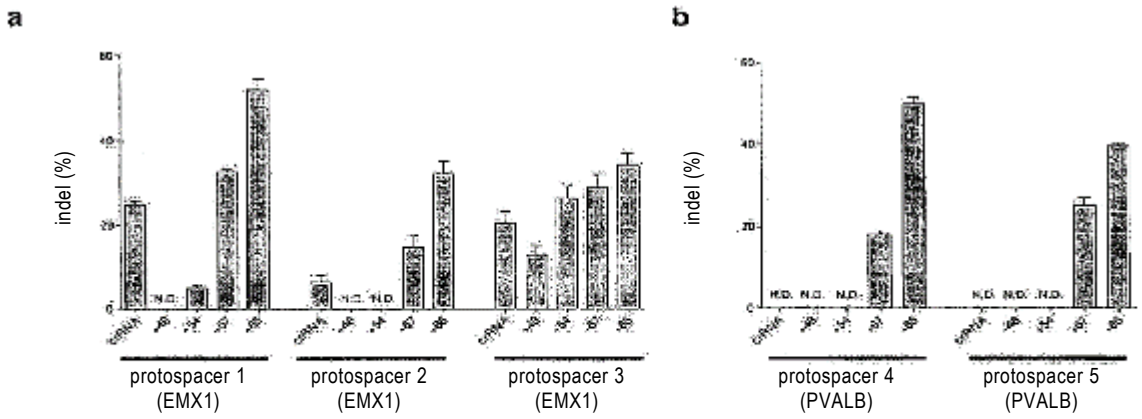


Figure 10C

[Figure 11]



Figures 11A-B

Attachment 2 (List of the Drawings, etc. of Cited Invention 1)

[Table 7]

Table 7 Transfection Treatments

Treatment	Modified Cas9	Guide RNA	Donor sequence
A	Cas9 mRNA transcribed with an Anti-Reverse Cap Analog (10 μg)	Pre-annealed crRNA-tracrRNA duplex (0.3 nmol)	AAVS1-GFP plasmid DNA (10 μg)
B	Cas9 mRNA transcribed with an Anti-Reverse Cap Analog (10 μg)	Chimeric RNA (0.3 nmol)	AAVS1-GFP plasmid DNA (10 μg)
C	Cas9 mRNA capped via post-transcription capping reaction (10 μg)	Chimeric RNA (0.3 nmol)	AAVS1-GFP plasmid DNA (10 μg)
D	Cas9 plasmid DNA (10 μg)	U6-chimeric RNA plasmid DNA (5 μg)	AAVS1-GFP plasmid DNA (10 μg)
E	None	None	AAVS1-GFP plasmid DNA (10 μg)
F	None	None	None

[Table 8]

Table 4. Guide RNAs

RNA	5'-3' Sequence	SEQ ID NO:
AAVS1—crRNA	ACCCCACAGUGGGGCCACUAGUUU UAGAGCUAUGCUGUUUUG	12
tracrRNA	GGAACCAUUCAAAACAGCAUAGCA AGUUAAAAUAAGGCUAGUCCGUUA UCAACUUGAAAAAGUGGCACCGAG UCGGUGCUUUUUUU	13
Chimeric RNA	ACCCCACAGUGGGGCCACUAGUUU UAGAGCUAGAAAUAGCAAGUUAAA AUAAGGCUAGUCCG	14

[Figure 4]

[Figure 4-1]

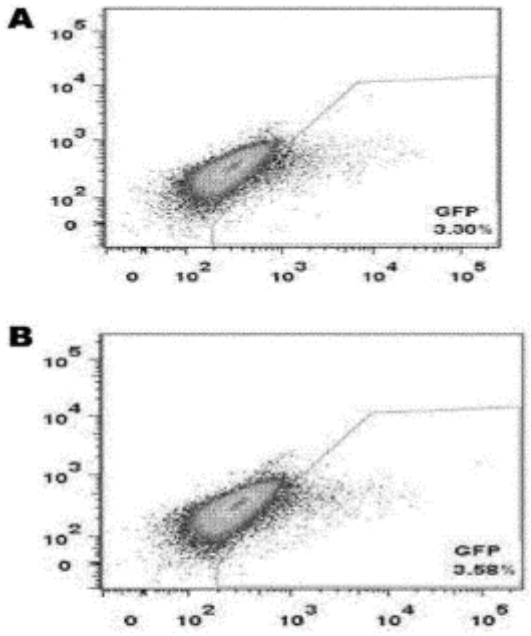


FIG. 4

[Figure 4-2]

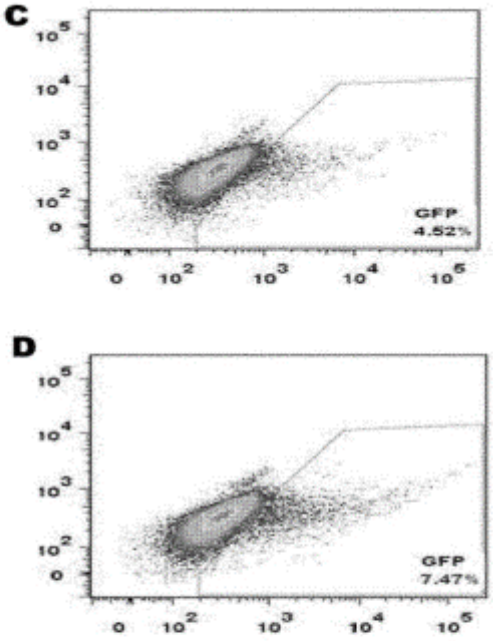


FIG. 4

[Figure 4-3]

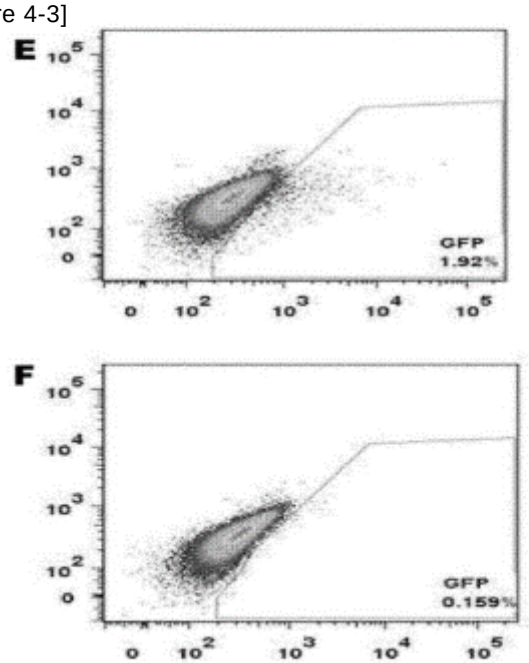


FIG. 4

[Figure 5]

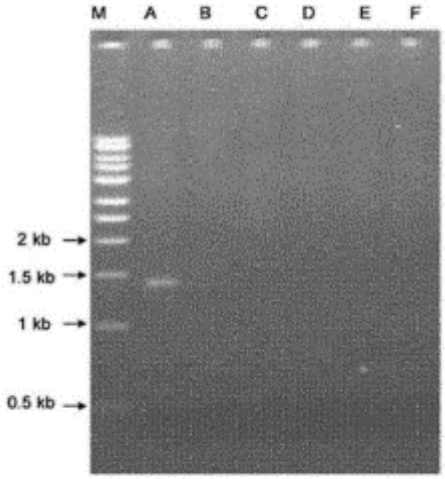


FIG. 5