Patent Right	Date	February 25, 2020	Court	Intellectual Property High Court, First Division
	Case number	2019 (Gyo-Ke) 10011		

- A case in which, with regard to an invention titled "CRISPR-Cas SYSTEMS AND METHODS FOR ALTERING EXPRESSION OF GENE PRODUCTS", it was not acknowledged that the invention of the present application is identical with Cited Invention 1 of an earlier application, because the invention of the present application is characterized in that an efficiency of genome modification increases by focusing on "a length of a tracr sequence" and adopting a configuration that the "tracr sequence is 30 or more nucleotides in length".
- A case in which it was determined that the invention of the present application, which is characterized in that an efficiency of genome modification in eukaryotic cells increases by adopting the configuration that a "tracr sequence is 30 or more nucleotides in length", would not have been easily conceivable to a person ordinarily skilled in the art on the basis of Cited Invention 2 which is at an in vitro level.

Case type: Rescission of Appeal Decision of Refusal

Result: Granted

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

Related rights, etc.: Appeal against Examiner's Decision of Refusal No. 2017-13796, Patent Application No. 2016-128599

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 "CRISPR-Cas SYSTEMS AND METHODS FOR ALTERING EXPRESSION OF GENE PRODUCTS", 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 inventive step on the basis of Cited Invention 2 (Reason 2 for Rescission).

2. The present judgment, in summary, held as follows and rescinded the appeal decision for reasons of each of erroneous determination of Article 29-2 of the Patent Act and erroneous determination of inventive step.

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

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 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 common general technical knowledge as of filing into consideration.

B. .. The invention of the present application is characterized in that an efficiency of genome modification increases by focusing on "a length of a tracr sequence" and adopting the configuration that the "tracr sequence is 30 or more nucleotides in length".

On the other hand, Cited Document 1 merely discloses that a guide RNA comprises three regions of a first region to a third region .., the length of the stem can range from about 6 to about 20 base pairs in length .., in general, the length of 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 .., the combined length of the second and third regions of the guide RNA can range from about 30 to about 120 nucleotides in length ...

C. According to the description of the present application .., that "the portion of the sequence 3' of the loop corresponds to the tracr sequence", it is found that the tracr sequence of the present invention is equivalent to the combination of one side of the stem of the second region with the third region in Cited Invention 1. However, Cited Document 1 does not express a technical idea of defining the length itself of the tracr sequence (the combination of one side of the stem of the second region with the third region).

Further, there is no sufficient evidence to find that there was common general

technical knowledge of a person ordinarily skilled in the art such that the length of tracr sequence is made to be 30 or more nucleotides in length at the time of the priority date of the present application.

D. Therefore, it cannot be deemed that Cited Document 1 discloses that the configuration that "tracr sequence is 30 or more nucleotides in length" was adopted. Further, even by taking the common general technical knowledge into consideration, it cannot be also deemed that the matter disclosed in Cited Document 1 is equivalent to the statement that such configuration was adopted.

(2) Reason 2 for Rescission (Erroneous determination of inventive step on the basis of Cited Invention 2)

A. To a person ordinarily skilled in the art who has read the experimental results in Cited Example 2, .. with regard to a length of a tracr sequence, it can be understood that a tracr sequence having a length of 26 nucleotides is preferable to a tracr sequence which is shorter than 26 nucleotides. However, it is not found in Cited Example 2 that in the case where tracr sequences which are longer than 26 nucleotides are compared, the longer the tracr sequence, the more preferable.

In addition, taking all evidences of the present case into consideration, it is not sufficient to find that at the time of the priority date of the present application, there was common general technical knowledge which shows that the greater the length of a tracr sequence, the more preferable.

B. On the other hand, according to the description of the present application (..), general explanation is made .. with regard to a relationship between a length of a tracr sequence and an efficiency of genome modification. From Fig. 16 and Fig. 17 of the description of the present application, it can be understood that in case where protospacer 1 and protospacer 3 are targeted, a chimeric RNA having a tracr sequence length of 32 is superior to a chimeric RNA having a tracr sequence length of 26 in terms of efficiency of genome modification.

Thus, even if the description of Cited Document 2 and common general technical knowledge as of the priority date of the present application are taken into consideration, with regard to a length of tracr RNA of Cited Invention 2, it cannot be deemed that a person ordinarily skilled in the art was motivated to change from 26, which is concretely disclosed in Cited Document 2, to 30 or more in view of improving efficiency of genome modification.

C. Further, at the time of the priority date of the present application, there were neither technical papers nor any patent literature which reported that a CRISPR/Cas system derived from acquired immunity of bacteria and archaea (..), as disclosed in

the abstract of Cited Document 2, could be applied not to a mixture in a buffer solution (in vitro level) but to eukaryotic cells. It can be evaluated that an effect of improving efficiency of genome modification in eukaryotic cells, which is achieved by adopting the technical means of setting a length of tracr sequence to 30 or more, is beyond the expectation and prediction by a person ordinarily skilled in the art.

D. Therefore, even if the description of Cited Document 2 and common general technical knowledge as of the priority date of the present application are taken into consideration, it cannot be deemed that the matter for defining the present invention, which was listed as Difference 4 .. , would have been easily conceivable to a person ordinarily skilled in the art .. .

Judgment rendered on February 25, 2020
2019 (Gyo-Ke) 10011 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

Defendant: Commissioner of the Japan Patent Office

Main Text

1. The decision rendered by the Japan Patent Office on September 14, 2018 for the case of Appeal against Examiner's Decision of Refusal No. 2017-13796 shall be rescinded.
2. The court costs shall be borne by the Defendant.

Facts and Reasons

No. 1 Claim

The same as the main text, first paragraph.

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 "CRISPR-Cas SYSTEMS AND METHODS FOR ALTERING EXPRESSION OF GENE PRODUCTS" on June 29, 2016 (Patent Application No. 2016-128599; Division of Patent Application No. 2015-547555 (Priority claimed: December 12, 2012, United States of America); Publication Date: September 29, 2016; Exhibit Ko 9).

(2) With regard to this patent application, the Plaintiffs received an Examiner's decision of refusal on May 9, 2017 (Exhibit Ko 13). Accordingly, the Plaintiffs filed

a request for an appeal against the Examiner's decision of refusal on September 15, 2017 (Exhibit Ko 14). The Japan Patent Office examined the above request as the case of Appeal against Examiner's Decision of Refusal No. 2017-13796.

(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 one or more nucleotide sequences encoding one or more CRISPR-Cas system guide RNAs which hybridize with targeted sequences in polynucleotide loci in a eukaryotic cell, wherein the guide RNAs comprise a guide sequence, a tracr sequence, and a tracr mate sequence; and / b) a second regulatory element operably linked to a nucleotide sequence encoding a type II Cas9 protein, wherein the protein further comprises a nuclear localization signal (NLS), / wherein components (a) and (b) are located on the same or different vectors of the system, / wherein the tracr sequence is 30 or more nucleotides in length, / whereby the one or more guide RNAs target the polynucleotide loci in the eukaryotic cell and the Cas9 protein cleaves the polynucleotide locus, whereby a sequence of the polynucleotide locus is modified, and, wherein the Cas9 protein and the one or more guide RNAs do not naturally occur together.

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 and the comparison between the Present Invention and Cited Invention 1.

a. Findings on 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, and, / (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, / wherein a combined length of the second and third regions of the guide RNA can range from about 30 to about 120 nucleotides in length, and 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.

b. Common Feature between the Present Invention and Cited Invention 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 one or more nucleotide sequences encoding one or more CRISPR-Cas system guide RNAs which hybridize with targeted sequences in polynucleotide loci in a eukaryotic cell, wherein the guide RNAs comprise a guide sequence, a tracr sequence, and a tracr mate sequence; and / b) a second regulatory element operably linked to a nucleotide sequence encoding a type II Cas9 protein, wherein the protein further comprises a nuclear localization signal (NLS), / wherein components (a) and (b) are located on different vectors of the system, / whereby the one or more guide RNAs target the polynucleotide loci in the eukaryotic cell and the Cas9 protein cleaves the polynucleotide locus, whereby a sequence of the polynucleotide locus are modified, and, wherein the Cas9 protein and the one or more guide RNAs do not naturally occur together.

c. Prima Facie Difference

In the Present Invention, a "tracr sequence is 30 or more nucleotides in length"; that is, a lower limit value of a length of a tracr sequence is defined. In contrast, Cited Invention 1 does not clearly define a length of a portion corresponding to the "tracr sequence" of the Present Invention. However, so far as a combined length of "the second and third regions" ranges "from about 30 to about 120 nucleotides in length", Cited Invention 1 also encompasses a case which is shorter than 30 nucleotides.

(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) chimera A that is a chimeric RNA, wherein the chimeric RNA contains 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; / b) a type II Cas9 protein, / wherein the tracrRNA is 26 nucleotides in length, / whereby the Cas9 protein cleaves the targeted sequence, and / wherein the Cas9 protein and the chimeric RNA do not naturally occur together.

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) one or more CRISPR-Cas system guide RNAs which hybridize with targeted sequences, wherein the guide RNAs comprise a guide sequence, a tracr

sequence and a tracr mate sequence; and / b) a type II Cas9 protein, / whereby the Cas9 protein cleaves the targeted sequence, and / wherein the Cas9 protein and the one or more guide RNA do not naturally occur together.

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

(Different Feature 1)

In the Present Invention, a guide RNA "hybridizes with a targeted sequence in a polynucleotide locus in a eukaryotic cell" and a type II Cas9 protein "comprises a nuclear localization signal (NLS)", whereby the guide RNA targets "the polynucleotide locus 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)

In the Present Invention, "a sequence of the polynucleotide locus is modified." In contrast, in Cited Invention 2, 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 one or more nucleotide sequences encoding" a CRISPR-Cas system guide RNA; and "a second regulatory element operably linked to a nucleotide sequence encoding" a type II Cas9 protein, "wherein components (a) and (b) 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".

(Different Feature 4)

In the Present Invention, a tracr sequence is "30 or more nucleotides in length". In contrast, in Cited Invention 2, the corresponding tracr RNA is "26 nucleotides in length".

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] Recent advances in genome sequencing techniques and analysis methods have significantly accelerated the ability to catalog and map genetic factors associated with a diverse range of biological functions and diseases. 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. Adding the CRISPR-Cas system to the repertoire of genome sequencing techniques and analysis methods accelerates the ability to catalog and map genetic factors associated with a diverse range of biological functions and diseases.

d. Means for Solving the Problem

[0010] In a preferred embodiment of the present invention, the cell is a eukaryotic cell, in a more preferred embodiment the cell is a mammalian cell, and in a yet more preferred embodiment the mammalian cell is a human cell.

[0012] In one aspect, the invention of the present application provides a vector system comprising one or more vectors. In some embodiments, the CRISPR complex comprises one or more nuclear localization sequences of sufficient strength to drive accumulation of said CRISPR complex in a detectable amount in the nucleus of a eukaryotic cell. Without wishing to be bound by theory, it is believed that a nuclear localization sequence is not necessary for CRISPR complex activity in

eukaryotes, but that including such sequences enhances activity of the system, especially as to targeting nucleic acid molecules in the nucleus. 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. The CRISPR enzyme is codon-optimized for expression in a eukaryotic cell.

[0014] 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.

[0015] Advantageous vectors include lentiviruses and adeno-associated viruses, and types of such vectors can also be selected for targeting particular types of cells.

[0024] Aspects of the present invention comprehend site-specific gene knockout in the endogenous genome: The present invention is advantageous over using site-specific nuclease technologies based on zinc finger and TAL effectors as it does not require elaborate design and may be used to simultaneously knock out multiple genes within the same genome. In a further aspect, the present invention comprises site-specific genome editing. The present invention is advantageous over using natural or artificial site-specific nucleases or recombinases, as it may be able to introduce site-specific double strand breaks to facilitate homologous recombination at the targeted genome loci.

e. Mode for Carrying Out the Invention

[0053] 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.

[0054] 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.

[0063] 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 11B (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.

f. Examples

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

[0145] Figure 2 illustrates the bacterial CRISPR system described in this example. Figure 2A illustrates a schematic showing the CRISPR locus 1 from Streptococcus pyogenes SF370 and a proposed mechanism of CRISPR-mediated DNA cleavage by this system. .. Figure 2B illustrates engineering of S. pyogenes Cas9 (SpCas9) and RNase III (SpRNase III) with a nuclear localization signal (NLS) to enable import into the mammalian nucleus.

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

[0159] 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 16b and 16c (as shown in Attachment 1), 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, are illustrated by histogram in

Figures 17a and 17b (as shown in Attachment 1), corresponding to Figures 16b and 16c, respectively ("N.D." indicates no indels detected).

[0162] 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 16b and 17a). 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 16c and 17b).

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.

(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, and b) a second regulatory element operably linked to a nucleotide sequence encoding the type II Cas9 protein, wherein components (a) and (b) are located on the same or different vectors, wherein the type

II Cas9 protein comprises a nuclear localization signal (NLS), and wherein the tracr sequence is 30 or more nucleotides in length.

Among these, with regard to the constituent feature on a length of the guide sequence (the tracr sequence is 30 or more nucleotides in length), the description of the present application states experimental results using a chimeric RNA (chiRNA; comprising a guide sequence, a tracr mate sequence, and a tracr sequence in a single transcript) and a Cas9 enzyme, wherein the chimeric RNA incorporates different lengths of wild-type tracrRNA sequence as short RNA molecules. In the description of the present application, it is shown that chimeric RNAs containing longer fragments of wild-type tracrRNA (chiRNA(+67) and chiRNA(+85)) mediated DNA cleavage at all three EMX1 targeted sites (Figures 16b and 17a), and chiRNA(+67) and chiRNA(+85) were able to mediate significant cleavage at the two PVALB protospacers (Figures 16c and 17b), and as the length of the tracr sequence increased, efficiency of genome modification increased (Example 4).

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 (as shown in Attachment 2). 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 is a "vector system."

d. 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."

e. Cited Document 1 discloses that each guide RNA comprises three regions, and that a combined length of the second and third regions of the guide RNA can range from about 30 to about 120 nucleotides in length ([0067], [0071]).

Thus, Cited Document 1 discloses that "a combined length of the second and third regions of the guide RNA ranges from about 30 to about 120 nucleotides in length."

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) (CRISPR-Cas) vector system,

H. CRISPR-Cas vector system

C. comprising one or more vectors comprising:

B-a. a first regulatory element operably linked to one or more nucleotide sequences encoding one or more CRISPR-Cas system guide RNAs which hybridize

with targeted sequences in polynucleotide loci in a eukaryotic cell, wherein the guide RNAs comprise a guide sequence, a tracr sequence, and a tracr mate sequence; and

B-b. a second regulatory element operably linked to a nucleotide sequence encoding a type II Cas9 protein, wherein the protein further comprises a nuclear localization signal (NLS);

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

E. wherein the tracr sequence is 30 or more nucleotides in length;

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

G. wherein the Cas9 protein and the one or more guide RNAs do not naturally occur together.

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" of Cited Invention 1 corresponds to "a guide sequence" of the Present Invention.

The second and third regions consisting of "a second internal region that forms a stem loop structure" and "a third 3' region that remains essentially single-stranded" of Cited Invention 1 correspond to a sequence of "loop" in addition to a "tracr RNA sequence" and a "tracr mate sequence" of the Present Invention, in view of "the portion is provided in the lower portion of Figure 11B, 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 [0063] 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" of Cited Invention 1 corresponds to "the guide RNA" "comprising a guide sequence, a tracr sequence, and a tracr mate sequence" 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" of Cited Invention 1 corresponds to "a) a first regulatory element operably linked to one or more nucleotide sequences encoding one or more CRISPR-Cas system guide RNAs which hybridize with targeted sequences in polynucleotide loci in a eukaryotic cell, wherein the guide RNAs comprise a guide sequence, a tracr sequence, and a tracr mate sequence" of the Present Invention.

d. Constituent Feature B-b

"A promoter control sequence" of Cited Invention 1 corresponds to a regulatory element of the Present Invention. Further, Cited Invention 1 comprises at least one nuclear localization signal, and the nuclear localization signal is expressed together with the nucleotide sequence encoding a Cas9 protein.

Therefore, "(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" of Cited Invention 1 corresponds to "b) a second regulatory element operably linked to a nucleotide sequence encoding a type II Cas9 protein, wherein the protein further comprises a nuclear localization signal (NLS)" of the Present Invention.

e. Constituent Feature D

Cited Document 1 discloses not only an embodiment in which the vectors (i) and (ii) are different from each other, but also an embodiment in which the vectors (i) and (ii) are the same ([0005], [0088]). Thus, these embodiments correspond to constituent feature D of the Present Invention.

f. Constituent Feature F

In Cited Invention 1, the above two vectors (i) and (ii) are the constituent features, and a vector system comprising these two 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.

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

g. Constituent Feature G

Cited Invention 1 is a vector system comprising a vector obtained by modifying a Cas9 protein derived from a naturally occurring type II CRISPR/Cas system, including at least one nuclear localization signal, as mentioned in e. above. Further, the guide RNA used in Cited Invention 1 is a chimeric RNA artificially engineered to contain a first region, a second internal region, and a third 3' region.

Therefore, the vector system of Cited Invention 1 corresponds to the CRISPR-Cas vector system "wherein the Cas9 protein and the one or more guide RNAs do not naturally occur together."

h. Constituent Feature H

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

i. Summary

According to the foregoing, the Present Invention and Cited Invention 1 correspond to each other as found by the decision of the present case (No. 2, 3(2)b above), and have the following different feature (the same as No. 2, 3(2)c above).

(Different Feature)

In the Present Invention, a "tracr sequence is 30 or more nucleotides in length"; that is, a lower limit value of the length of the tracr sequence is defined. In contrast, Cited Invention 1 does not clearly define a length of a portion corresponding to the "tracr sequence" of the Present Invention, and a combined length of "the second and

third regions" ranges "from about 30 to about 120 nucleotides in length."

(5) Examination on Different Feature

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.

b. With regard to the relationship between a length of a tracr sequence and efficiency of genome modification, the description of the present application generally explains in [0162] that "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." In particular, as those with a superior increase in the efficiency of genome modification, chimeric RNAs with n of 67 and 85; i.e., the lengths of tracr sequences are 45 and 63, were taken up, and the description of the present application additionally explains that "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 16b and 17a). 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 16c and 17b)."

Further, referring to Figures 16 and 17 of the description of the present application, it can be seen that in the case where protospacer 1 and protospacer 3 were targeted, not only when n was +67, +85, but also when n was +54; i.e., a chimeric RNA in which a tracr sequence was 32 in length, efficiency of modification was greater than that of a chimeric RNA with n of +48; i.e., a chimeric RNA in which a tracr sequence was 26 in length. In addition, it can be understood that the Present Invention sets the length of the tracr sequence to 30 or more nucleotides, and thereby achieves a new effect which is different from that of Cited Invention 1.

Thus, the invention of the present application is characterized in that efficiency of genome modification increases by focusing on "a length of a tracr sequence" and adopting the configuration that "a tracr sequence is 30 or more nucleotides in length".

On the other hand, Cited Document 1 merely discloses that a guide RNA comprises three regions of a first region to a third region ([0067]), a length of a stem can range from about 6 to about 20 base pairs in length ([0069]), in general, a length of 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 ([0070]), and a combined length of the second and third regions of the guide RNA can range from about 30 to about 120 nucleotides in length ([0071]).

c. According to the statement that "the portion of the sequence 3' of the loop corresponds to the tracr sequence" in [0063] of the description of the present application, it is found that the tracr sequence of the Present Invention is equivalent to the combination of one side of the stem of the second region with the third region in Cited Invention 1. However, Cited Document 1 does not express a technical idea of defining the length itself of the tracr sequence (the combination of one side of the stem of the second region with the third region).

Further, there is no sufficient evidence to find that there was common general technical knowledge of a person ordinarily skilled in the art such that a length of a tracr sequence is made to be 30 or more nucleotides in length at the time of the priority date of the present application.

d. Therefore, it cannot be deemed that Cited Document 1 discloses that the

configuration that "a tracr sequence is 30 or more nucleotides in length" was adopted. Further, even by taking common general technical knowledge into consideration, it also cannot be deemed that the matter disclosed in Cited Document 1 is equivalent to the statement that such configuration was adopted.

(6) Assertion by the Defendant

The Defendant asserts that in the case where protospacers 2, 4, and 5 are targeted, no difference can be found between the guide RNA(+48) with a tracr sequence of 26 nucleotides in length and the guide RNA(+54) with a tracr sequence of 32 nucleotides in length (Figures 16 and 17). In addition, the Defendant asserts that it cannot be deemed that the Present Invention, which defines a tracr sequence of 30 or more nucleotides in length, has an effect of increasing efficiency of modification, independent of a targeted sequence, and thus, it cannot be found that the Present Invention achieves a new effect which is different from Cited Invention 1.

However, as mentioned above, the description of the present application states that with regard to different targeted sequences of protospacer 1 and protospacer 3, efficiency of genome modification of a chimeric RNA with a tracr sequence of 32 nucleotides in length is increased as compared to that of a chimeric RNA with a tracr sequence of 26 nucleotides in length. Thus, it cannot be deemed that the Present Invention, which defines a tracr sequence of 30 or more nucleotides in length, has no possibility of increasing efficiency of genome modification in a eukaryotic cell in the cases other than protospacer 1 and protospacer 3.

Therefore, the assertion by the Defendant is unfounded.

(7) Summary

As mentioned above, a prima facie difference between the Present Invention and Cited Invention 1 as found in the decision of the present case; i.e., a "tracr sequence is 30 or more nucleotides in length", is a substantial difference, and it cannot be found that the Present Invention is identical with Cited Invention 1. Therefore, the decision of the present case erred in determining that a patent shall not be granted for the Present Invention under the provision of Article 29-2 of the Patent Act.

Therefore, Reason 1 for Rescission is well founded.

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

(1) Statement of Cited Document 2 (Exhibits Ko 2-1·2)

a. Abstract

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-

associated (Cas) systems provide bacteria and archaea with adaptive immunity against viruses and plasmids by using CRISPR RNAs (crRNAs) to guide the silencing of invading nucleic acids. We show here that in a subset of these systems, the mature crRNA that is base-paired to trans-activating crRNA (tracrRNA) forms a two-RNA structure that directs the CRISPR-associated protein Cas9 to introduce double-stranded breaks in target DNA. The dual-tracrRNA:crRNA, when engineered as a single-strand RNA chimera, also directs sequence-specific Cas9 double-stranded DNA cleavage.

Our study reveals a family of endonucleases that use dual-RNAs for site-specific DNA cleavage and highlights the potential to exploit the system for RNA-programmable genome editing.

b. Main text, page 816, middle column, lines 25 to 35

We show here that in type II systems, Cas9 proteins constitute a family of enzymes that require a base-paired structure formed between the activating tracrRNA and the targeting crRNA to cleave target double-stranded DNA. Site-specific cleavage occurs at locations determined by both base-pairing complementarity between the crRNA and the target protospacer DNA and a short motif [referred to as the protospacer adjacent motif (PAM)] juxtaposed to the complementary region in the target DNA.

c. Figure 3 (As shown in Attachment 3)

Cas9-catalyzed cleavage of target DNA requires an activating domain in tracrRNA and is governed by a seed sequence in the crRNA. (A) Cas9-tracrRNA: crRNA complexes were reconstituted using 42-nucleotide crRNA-sp2 and truncated tracrRNA constructs and were assayed for cleavage activity as in Figure 1B. (omitted) (C) Minimal regions of tracrRNA and crRNA capable of guiding Cas9-mediated DNA cleavage (blue shaded region).

d. Main text, page 820, left column, lines 5 to 18

We designed two versions of a chimeric RNA containing a target recognition sequence at the 5' end followed by a hairpin structure retaining the base-pairing interactions that occur between the tracrRNA and the crRNA. In cleavage assays using plasmid DNA, we observed that the longer chimeric RNA was able to guide Cas9-catalyzed DNA cleavage in a manner similar to that observed for the truncated tracrRNA:crRNA duplex.

e. Figure 5 (As shown in Attachment 3)

Cas9 can be programmed using a single engineered RNA molecule combining tracrRNA and crRNA features.

(A) (Top) In type II CRISPR/Cas systems, Cas9 is guided by a two-RNA structure formed by activating tracrRNA and targeting crRNA to cleave site-specifically targeted double-stranded DNA.

(Bottom) A chimeric RNA generated by fusing the 3' end of crRNA to the 5' end of tracrRNA.

f. Main text (page 820, right column, lines 2 to 9)

Zinc-Finger Nucleases (ZFN) and Transcription-Activator Like Effector Nucleases (TALEN) have attracted considerable interest as artificial enzymes engineered to manipulate genomes. We propose an alternative methodology based on RNA-programmed Cas9 that could offer considerable potential for gene-targeting and genome-editing applications.

g. Plasmid DNA cleavage assay (SUPPLEMENTARY MATERIALS AND METHODS)

Native or restriction digest-linearized plasmid DNA (300 ng (~8 nM)) was incubated for 60 min at 37°C with purified Cas9 protein (50 to 500 nM) and tracrRNA:crRNA complex (50 to 500 nM, 1:1) in a Cas9 plasmid cleavage buffer solution (20 mM HEPES pH 7.5, 150 mM KCL, 0.5 mM DTT, 0.1 mM EDTA) with or without 10 mM $MgCl_2$.

h. Dual-RNA requirements for target DNA binding and cleavage (page 818, left column, line 1 to middle column, line 8)

tracrRNA might be required for target DNA binding and/or to stimulate the nuclease activity of Cas9 downstream of target recognition. Addition of tracrRNA substantially enhanced target DNA binding by Cas9, whereas we observed little specific DNA binding with Cas9 alone or Cas9-crRNA (Figure S9). This indicates that tracrRNA is required for target DNA recognition, possibly by properly orienting the crRNA for interaction with the complementary strand of target DNA. This interaction creates a structure in which the 5'-end 20 nucleotides of the crRNA, which vary in sequence in different crRNAs, are available for target DNA binding. The bulk of the tracrRNA downstream of the crRNA base pairing region is free to form additional RNA structure(s) and/or to interact with Cas9 or the target DNA site. To determine whether the entire length of the tracrRNA is necessary for site specific Cas9-catalyzed DNA cleavage, we tested Cas9-tracrRNA:crRNA complexes reconstituted using full-length mature (42-nucleotide) crRNA and various truncated forms of tracrRNA lacking sequences at their 5' or 3' ends. These complexes were tested for cleavage using a short target double-stranded DNA. A substantially truncated version of the tracrRNA retaining nucleotides 23 to 48 of the native

sequence was capable of supporting robust dual-RNA guided Cas9-catalyzed DNA cleavage (Figure 3, A and C, and Figure S10, A and B).

i. Main text, (page 820, left column, lines 19 to 29)

The shorter chimeric RNA did not work efficiently in this assay, confirming that nucleotides that are 5 to 12 positions beyond the tracrRNA:crRNA base-pairing interaction are important for efficient Cas9 binding and/or target recognition. We obtained similar results in cleavage assays using short double-stranded DNA as a substrate, further indicating that the position of the cleavage site in target DNA is identical to that observed using the dual tracrRNA:crRNA as a guide (Figure 5C and Figure S14, B and C, as shown in Attachment 3).

(2) Summary of the Disclosure of Cited Document 2

a. Chimeric RNA

Cited Document 2 discloses that "We designed two versions of a chimeric RNA containing a target recognition sequence at the 5' end followed by a hairpin structure retaining the base-pairing interactions that occur between the tracrRNA and the crRNA" (the above (1)d), and that in "Plasmid DNA cleavage assay", a target plasmid DNA, Cas9 protein, and RNA were mixed in a buffer solution (the above (1)g), and that such mixing caused a target recognition sequence to hybridize with a targeted sequence, as shown in Figure 5A (the above (1)e).

Further, in chimera A in Figure 5B, it is shown that tracrRNA is 26 nucleotides in length (a portion of 26 letters of G to U which are located to the right of "3'-").

Thus, it can be found that Cited Document 2 discloses "a chimeric RNA containing 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 "the tracrRNA is 26 nucleotides in length".

b. Type II Cas9 Protein

Cited Document 2 discloses that "In type II CRISPR/Cas systems, Cas9 is guided by a two-RNA structure formed by activating tracrRNA and targeting crRNA to cleave site-specifically targeted double-stranded DNA" (the above (1)e), and that "The dual-tracrRNA:crRNA, when engineered as a single-stranded RNA chimera, also directs sequence-specific Cas9 double-stranded DNA cleavage" (the above (1)a). Thus, it can be found that Cited Document 2 discloses that "a type II Cas9 protein" is guided to "cleave a targeted sequence" by a dual-tracrRNA:crRNA or a chimeric RNA.

c. CRISPR-Cas System

The chimeric RNA of Cited Document 2 is one in which the dual (double-

stranded)-tracrRNA:crRNA is artificially engineered as a single-stranded chimera (the above (1)a). Thus, it can be deemed that "clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems" comprising this chimeric RNA as a constituent component is "an engineered, non-naturally occurring" "clustered regularly interspaced short palindromic repeats (CRISPR) - CRISPR-associated (Cas) (CRISPR-Cas) system."

(3) Common Feature and Different Features between the Present Invention and Cited Invention 2

a. According to the above (2), it can be found that Cited Invention 2 disclosed in Cited Document 2 is as found in the decision of the present case (the above No. 1, 3(3)a). Further, it can be found that a common feature and different features between the Present Invention and Cited Invention 2 are as found in the decision of the present case (the above No. 1, 3(3)b, c).

b. Plaintiffs' Assertion that Different Features Had Been Overlooked

(a) The Plaintiffs assert that Different Features 5 and 6 should have been found with regard to the Present Invention and the Cited Invention 2, and that further determinations on Different Features 5 and 6 should have been made.

However, the Present Invention deals with a CRISPR-Cas vector system comprising a) a first regulatory element operably linked to a nucleotide sequence encoding a CRISPR-Cas system polynucleotide sequence, wherein the polynucleotide sequence comprises a guide sequence, tracrRNA, and a tracr mate sequence, and b) a second regulatory element operably linked to a nucleotide sequence encoding a type II Cas9 protein, wherein the vector system comprises a nuclear localization signal. The Present Invention merely defines functions of the vector system as follows: [i] the guide sequence targets a polynucleotide locus in a eukaryotic cell; [ii] the Cas9 protein cleaves the polynucleotide locus; and [iii] a sequence of the polynucleotide locus is modified by the above [i] and [ii]. Since the Present Invention does not specifically define the formation of a complex based on a) and b) (Different Feature 5 as asserted by the Plaintiffs) as well as the environment where cleavage is performed or the object of cleavage (Different Feature 6 as asserted by the Plaintiffs), there is no reason why Cited Invention 2 should be found with these configurations added.

Therefore, the Plaintiffs' assertion that Different Features 5 and 6 should have been found with regard to the Present Invention and the Cited Invention 2 is unfounded.

(b) Even if it is considered that there are Different Features 5 and 6, in determining Different Feature 1, the decision of the present case states that "adding a

nuclear localization signal to a Cas9 protein, which is a conventional method of transferring a protein into a nucleus in order to bring a CRISPR-Cas system to a nuclear genome of a eukaryotic cell, could be done without any particular ingenuity by a person ordinarily skilled in the art", and determined on whether it would be easily conceivable to bring the CRISPR-Cas system to the nucleus of the eukaryotic cell, which is a premise for the formation of a complex.

In addition, in determining Different Feature 1, the decision of the present case states that "it is a natural idea for a person ordinarily skilled in the art to attempt to make the CRISPR-Cas system function for a genome of a eukaryotic cell." Further, in determining Different Feature 3, the decision of the present case states that "in order to attempt to make a desired protein or a nucleic acid function inside a cell, using vectors which express the protein or the nucleic acid is a conventional method. .. It is sufficiently motivated to attempt to make the CRISPR-Cas system of Cited Invention 2 function for the genome in a eukaryotic cell together with a recombinant template. That is, components constituting them; i.e., the Cas9 protein, a chimeric RNA, and a recombinant template, would have been appropriately made to be a vector system by a person ordinarily skilled in the art." Thus, whether the invention would be easily conceivable is substantially determined based on the premise that the cleavage object and the cleavage environment are different between the Present Invention and Cited Invention 2.

(c) Therefore, the Plaintiffs' assertion that Different Features 5 and 6 should have been found, and further determined with regard to the Present Invention and the Cited Invention 2 is unfounded.

(4) Determination on Different Feature 4

A(a) Cited Document 2 shows that in a test using double-stranded Cas9-tracrRNA:crRNA complexes reconstituted by combining full-length mature (42 nucleotides) crRNA and various truncated forms of tracrRNA lacking sequences at their 5' or 3' ends, the tracrRNA retaining nucleotides 23 to 48 of a native sequence (a tracr sequence is 26 nucleotides in length) is effective for Cas9-catalyzed DNA cleavage (the above (1)c, h, Figure 3A).

In addition, Cited Document 2 shows that tracrRNA:crRNA could be engineered as a single-stranded chimera (the above (1)a), and that long chimera A retaining nucleotides 23 to 48 (a tracr sequence is 26 nucleotides in length) guided Cas9-catalyzed DNA cleavage in a manner similar to that observed for the double-stranded tracrRNA:crRNA complex, and on the other hand, short chimera B (a tracr sequence is 18 nucleotides in length) could not guide DNA cleavage (the above (1)d,

e, Figure 5B).

At the time of the priority date of the present application, a person ordinarily skilled in the art who had read the above experimental results of Cited Document 2 understood that a tracr sequence which is shorter than 26 nucleotides in length is inferior in an cleavage effect due to Cas9, and thus, for cleavage of a targeted sequence due to Cas9 protein, it is necessary to include a tracr sequence which is at least 26 nucleotides in length retaining nucleotides 23 to 48 of a native sequence.

However, although it can be understood that with regard to a length of a tracr sequence, a tracr sequence which is longer than 26 nucleotides in length is preferable to a tracr sequence which is shorter than 26 nucleotides in length, it is not found in Cited Document 2 that in the case where tracr sequences which are longer than 26 nucleotides in length are compared, the longer the tracr sequence, the more preferable.

In addition, taking all evidences of the present case into consideration, it is not sufficient to find that at the time of the priority date of the present application, there was common general technical knowledge which shows that the greater a length of a tracr sequence, the more preferable.

(b) On the other hand, with regard to the relationship between a length of a tracr sequence and efficiency of genome modification, the description of the present application generally explains in [0162] that "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." Further, from Figure 16 and Figure 17 of the description of the present application, it can be understood that in the case where protospacer 1 and protospacer 3 are targeted, a chimeric RNA having a tracr sequence which is 32 in length is superior to a chimeric RNA having a tracr sequence which is 26 in length in terms of the efficiency of genome modification.

Thus, even if the disclosure of Cited Document 2 and common general technical knowledge as of the priority date of the present application are taken into consideration, with regard to a length of tracrRNA of Cited Invention 2, it cannot be deemed that a person ordinarily skilled in the art was motivated to change from 26, which is concretely disclosed in Cited Document 2, to 30 or more in view of improving efficiency of genome modification.

(c) Further, at the time of the priority date of the present application, there were neither technical papers nor any patent literature which reported that a CRISPR/Cas system derived from acquired immunity of bacteria and archaea, as disclosed in the abstract of Cited Document 2 (the above (1)a), could be applied not to a mixture in a buffer solution (in vitro level) but to eukaryotic cells. Thus, it can be evaluated that

an effect of improving efficiency of genome modification in eukaryotic cells, which is achieved by adopting the technical means of setting a length of tracr sequence to 30 or more, is beyond the expectation and prediction by a person ordinarily skilled in the art.

(d) Therefore, even if the description of Cited Document 2 and common general technical knowledge as of the priority date of the present application are taken into consideration, it cannot be deemed that the matter for defining the Present Invention which was listed as Different Feature 4; i.e., a "tracr sequence" is made to be "30 or more nucleotides in length", would have been easily conceivable to a person ordinarily skilled in the art.

b. Assertion by the Defendant

The Defendant asserts that from the disclosure of Cited Document 2, it can be understood that tracrRNA containing 26 nucleotides within 23 to 48 nucleotides in length can guide DNA cleavage due to Cas9, even if additional nucleotides are present at the 5' and 3' ends of the tracrRNA. In addition, the Defendant asserts that in Cited Document 2, there is no disclosure which prevents the addition of nucleotides to the 5' or 3' end to make the tracrRNA further longer, and that Figure 3A shows that in addition to the above minimal region, the further longer tracrRNA consisting of the "15-53", "23-89", and "15-89" regions can also be used with crRNA to guide DNA cleavage due to Cas9. Based on these, the Defendant asserts that a person ordinarily skilled in the art would have slightly increased the length of the "tracrRNA" of Cited Invention 2 to be about 30 nucleotides in length, as appropriate.

However, although Figure 3A shows the results of experiments using long tracrRNA in combination with crRNA as a double-strand, it is not disclosed that the particularly long tracrRNA is superior in the cleavage of a targeted sequence. In addition, in order to increase a length of a tracr sequence of the Cited Invention 2 from 26 to 30, it is necessary to increase the length of the tracr sequence by 15% or more. Thus, it cannot be deemed that such increase is the extent to make the tracr sequence to be longer slightly. Further, as mentioned above, at the time of the priority date of the present application, there were no common general technical knowledge which shows that the greater a length of a tracr sequence, the more preferable. Furthermore, at the time of the priority date of the present application, there were neither technical papers nor any patent literature which reported that a CRISPR/Cas system was applied to eukaryotic cells. In view of the foregoing, it should be evaluated that an effect of improving efficiency of genome modification in eukaryotic cells, which is achieved by adopting the technical means of setting a length of a tracr sequence to 30 or more, is beyond the expectation and prediction by a

person ordinarily skilled in the art.

Therefore, the above assertion is not acceptable.

(5) Summary

As mentioned above, it cannot be deemed that Different Feature 4 between the Present Invention and Cited Invention 2 would have been easily conceivable. Thus, the decision of the present case erred in determining that a patent shall not be granted for the Present Invention under the provision of Article 29, paragraph (2) of the Patent Act.

Therefore, Reason 2 for Rescission is well founded.

4. Conclusion

According to the foregoing, Reasons 1 and 2 for Rescission are both well founded.

Therefore, the decision of the present case shall be rescinded, and the judgment is rendered as mentioned in the main text.

Intellectual Property High Court, First Division

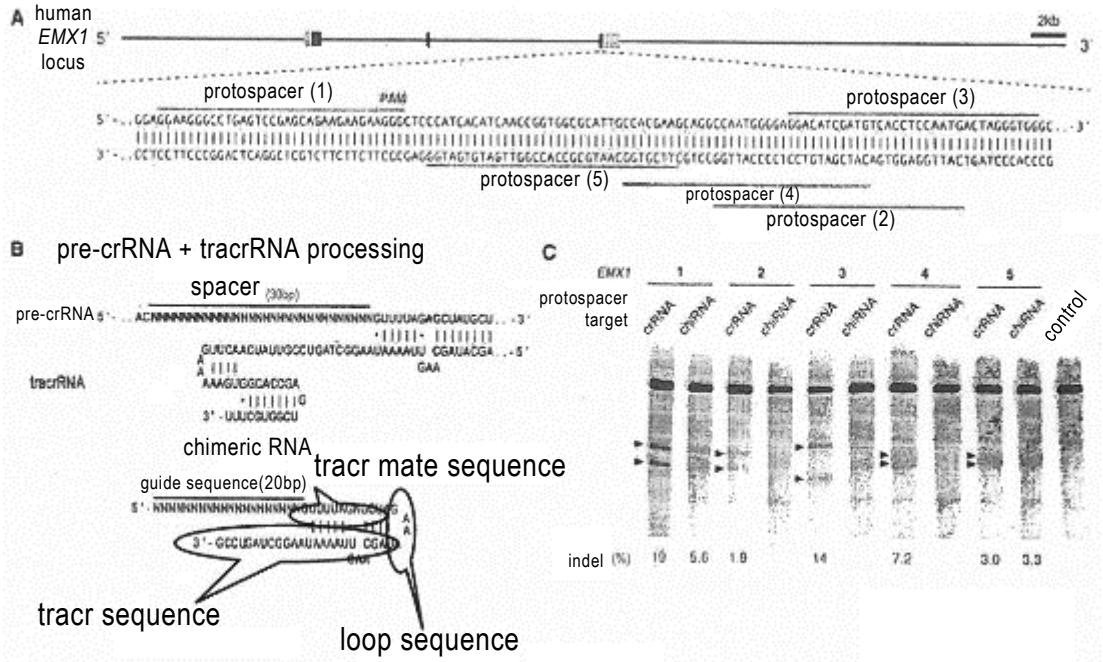
Presiding Judge TAKABE Makiko

Judge KOBAYASHI Yasuhiko

Judge SEKINE Sumiko

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

[Figure 11]



Figures 11A to C

[Figure 16]

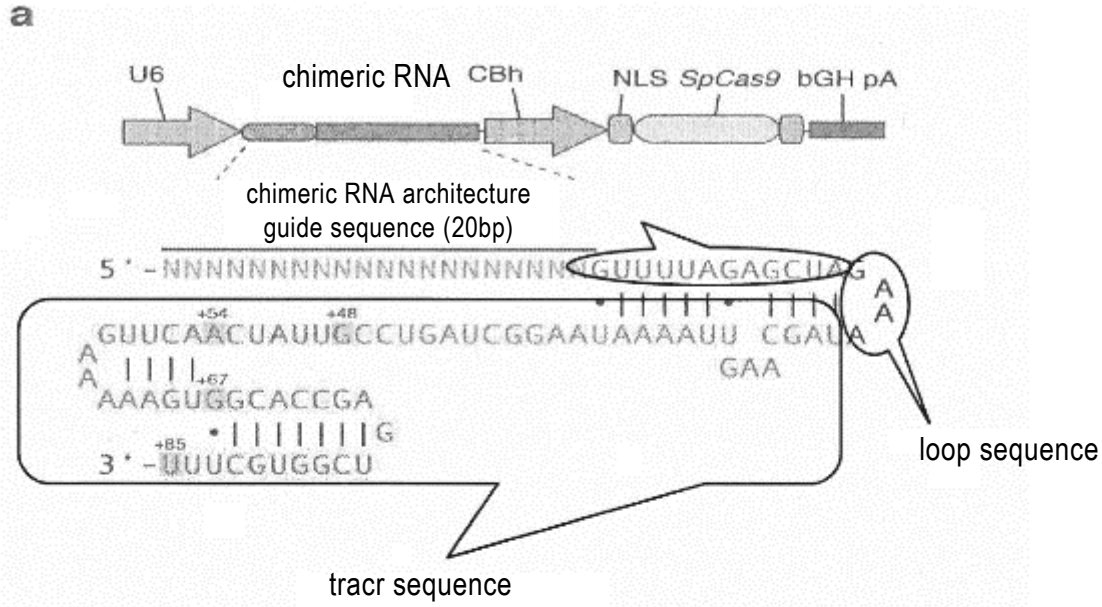


Figure 16A

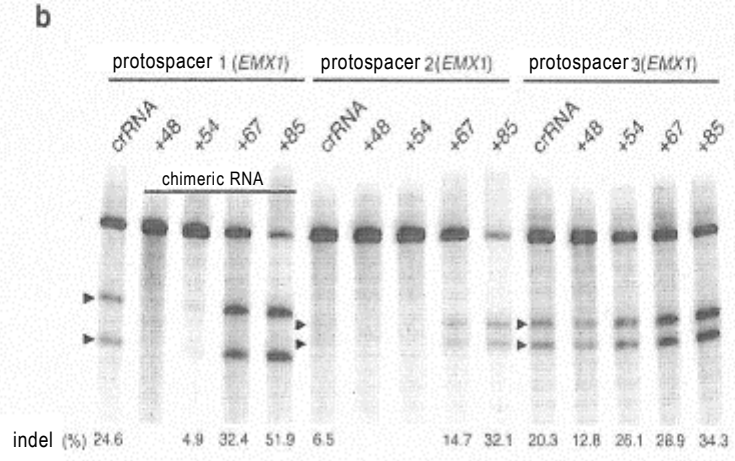


Figure 16B

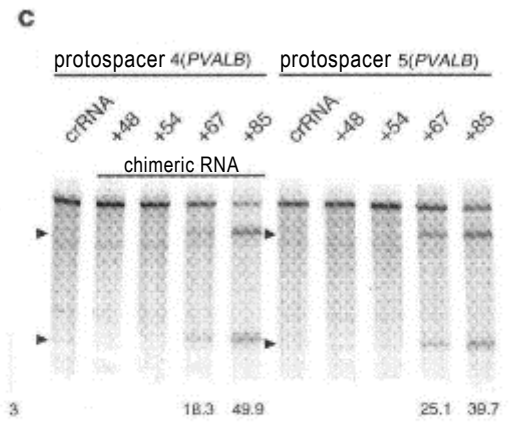
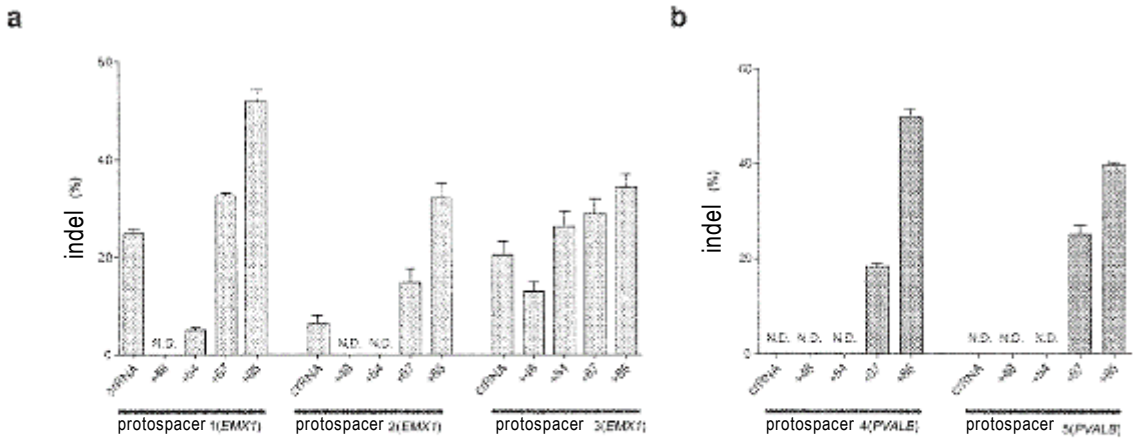


Figure 16C

[Figure 17]



Figures 17A-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]

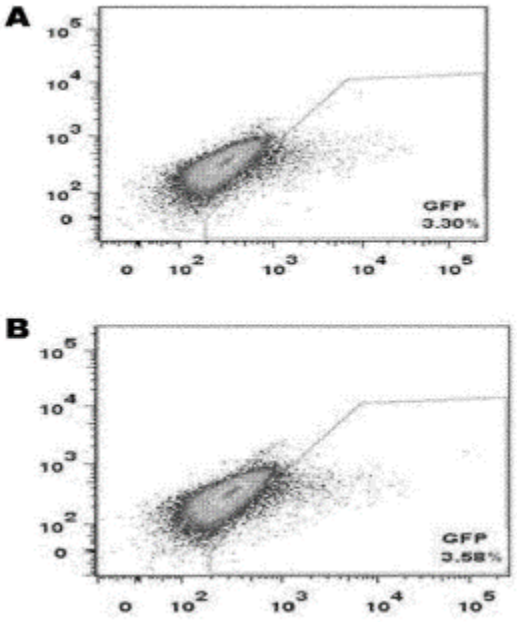


FIG. 4

[Figure 4-2]

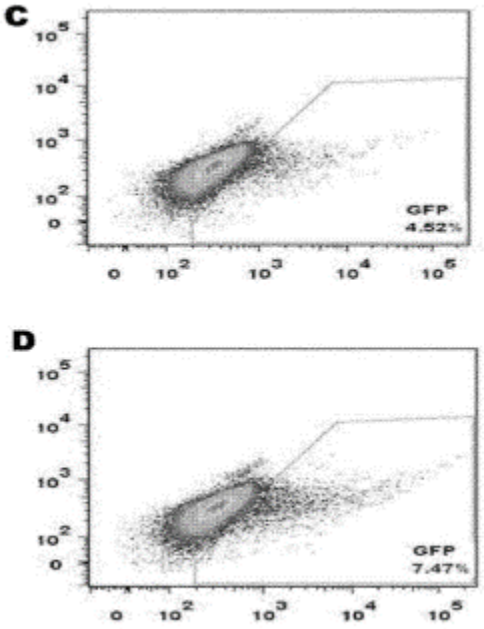


FIG. 4

[Figure 4-3]

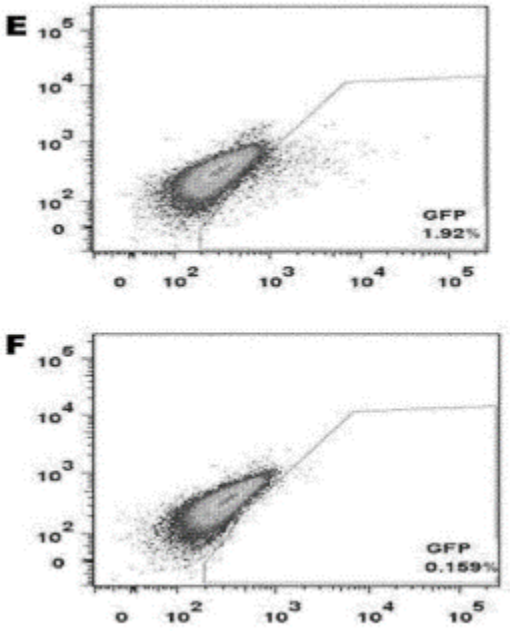


FIG. 4

[Figure 5]

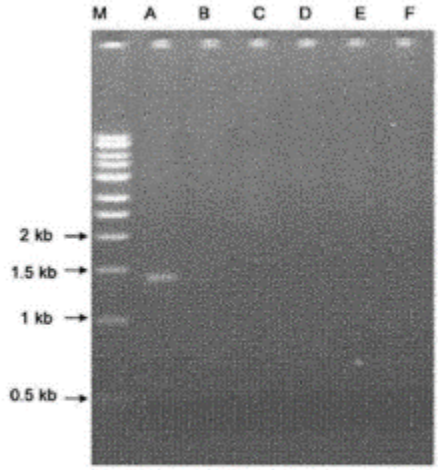
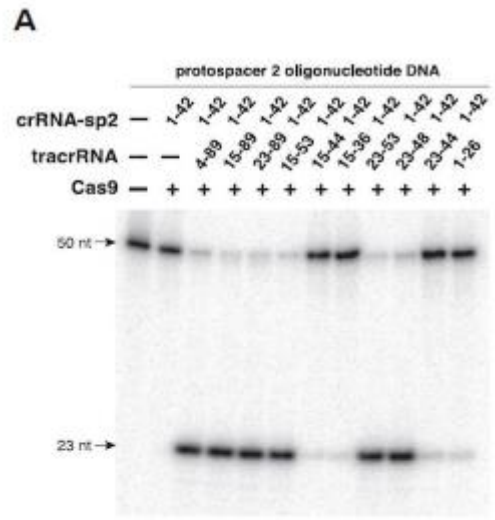
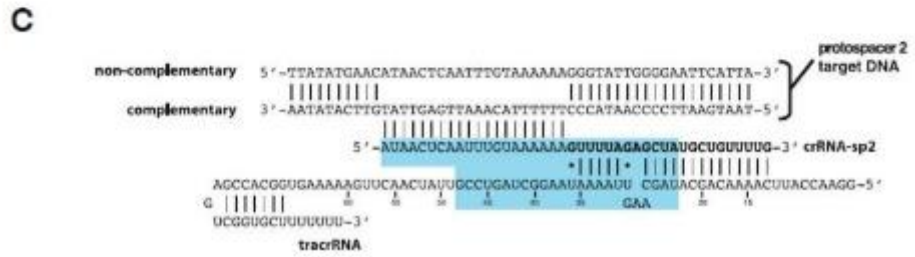


FIG. 5

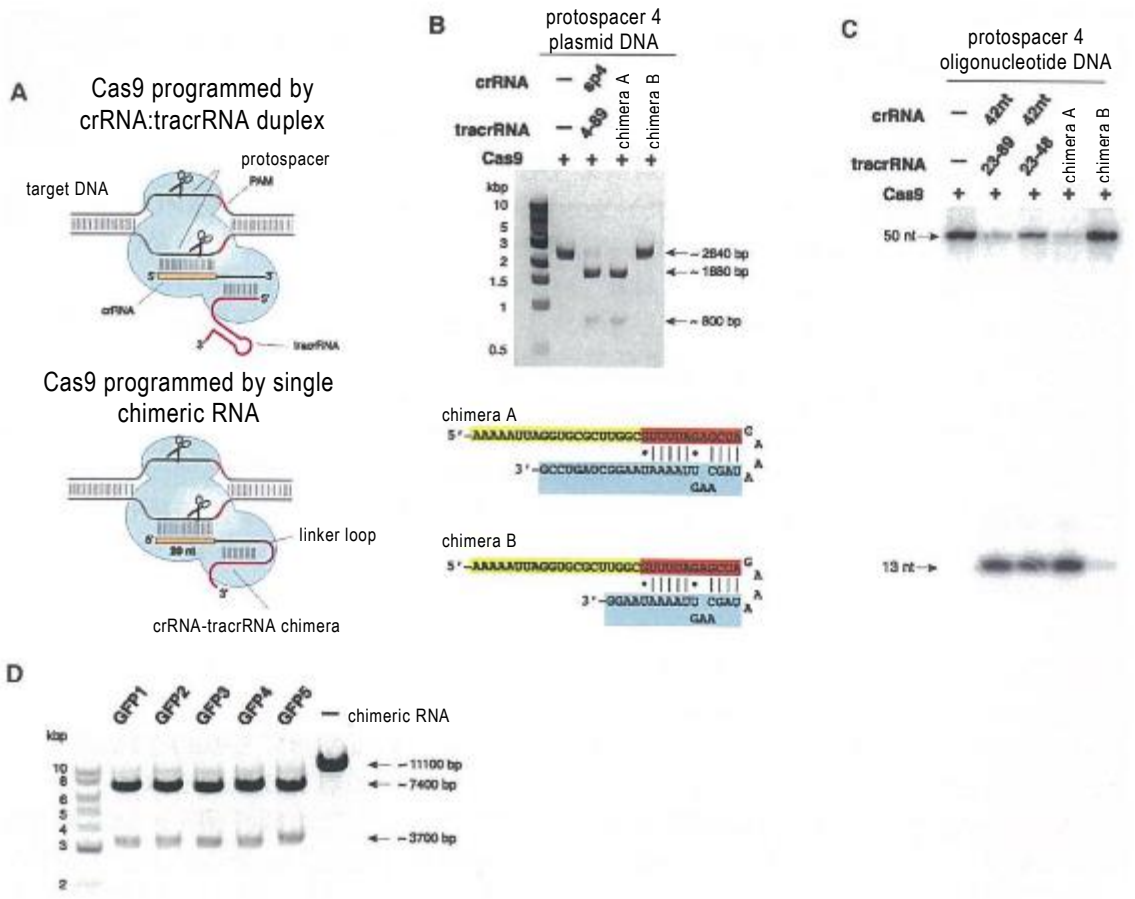
Attachment 3 (List of the Drawings of Cited Invention 2)

[Figure 3]





[Figure 5]


A
Cas9 programmed by crRNA:tracrRNA duplex
target DNA
protospacer
PAM
crRNA
tracrRNA
Cas9 programmed by single chimeric RNA
linker loop
crRNA-tracrRNA chimera
B
protospacer 4 plasmid DNA
crRNA
tracrRNA
Cas9
sp4
4-69
chimera A
chimera B
kbp
10
5
3
2
1.5
1
0.5
~2840 bp
~1880 bp
~800 bp
chimera A
chimera B
C
protospacer 4 oligonucleotide DNA
crRNA
tracrRNA
Cas9
42nt
42nt
23-69
23-48
chimera A
chimera B
50 nt
13 nt
D
GFP1
GFP2
GFP3
GFP4
GFP5
chimeric RNA
kbp
10
8
6
5
4
3
2
~11100 bp
~7400 bp
~3700 bp

[Figure S14]

Figure S14