Appeal decision

Appeal No. 2018-12916

Appellant	Sigma-Aldrich Co. LLC
Patent Attorney	YAMAO, Norihito
Patent Attorney	TOMITA, Kenji
Patent Attorney	INAI, Fumio

The case of appeal against the examiner's decision of refusal of Japanese Patent Application No. 2015-545838, entitled "CRISPR-based genome modification and regulation" (International publication, June 12, 2014, WO2014/089290; and national publication, February 1, 2016, National Publication of International Patent Application No. 2016-502840, number of claims (21)) has resulted in the following appeal decision.

Conclusion

The examiner's decision is revoked.

The Invention of the present application shall be granted a patent.

Reason

No. 1 History of the procedures

The international filing date of the present application (hereinafter, referred to as "Present Application") is December 5, 2013 (priority claimed under the Paris Treaty: December 6, 2012, U.S.A., January 30, 2013, U.S.A., and March 15, 2013, U.S.A.), and the subsequent history of the procedures is as follows:

August 9, 2017:	Notice of reasons for refusal issued
January 22, 2018:	Written statement and written amendment submitted
May 21, 2018:	Examiner's decision of refusal

September 28, 2018: Written demand for trial submitted

November 14, 2018: Written amendment based on the written demand for trial submitted

No. 2 Outline of the examiner's decision

The outline of the examiner's decision of refusal dated May 21, 2018 is as follows:

Since Inventions of Claims 1 to 21 of Present Application could have easily been invented by a person ordinarily skilled in the art of the invention (hereinafter, refer to as "a person skilled in the art") based on inventions disclosed in Cited Documents 1 and 2, they cannot be patented under the provisions in Article 29, (2) of the Patent Act.

List of the Cited Documents, etc.

1: Science Express, 03 Jan 2013, pp. 1-7 and
Supplementary materials (doi: 10.1126/science.1231143)

2: Genome Research, Jul 2012, Vol. 22, pp. 1327-1333

No. 3 The Inventions

1 The Inventions

(1) Regarding Inventions 1 and 11

Inventions of Claims 1 to 21 of Present Application (hereinafter, respectively referred to as "Invention 1" to "Invention 21") are inventions specified with the matters disclosed in Claims 1 to 21 of the scope of claims amended by the amendment of January 22, 2018, and of them, Inventions 1 and 11 are as shown below.

"[Claim 1]

A composition comprising two RNA-guided nickase systems, in which composition each RNA-guided nickase system (i) has a functional nuclease domain in which an RNA-guided endonuclease is modified so that a single strand of the double stranded structure is cleaved, wherein the RNA-guided endonuclease is derived from type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-related (Cas) (CRISPR /Cas) system protein and the type II CRISPR/Cas system is a Cas9 protein, and (ii) comprises a guide RNA comprising a first region that is complementary to the target site in the single strand of the double stranded structure and a second region that interacts with RNA-guided endonuclease, wherein the target sites of two RNA-guided nickase systems exist in the opposing chain of the double stranded structure, each target site is located immediately in front of a protospacer adjacent motif (PAM), and double strand break can be introduced by independently cleaving the opposing chain of the double stranded structure by both of RNA-guided endonucleases of two RNA-guided nickase systems."

"[Claim 11]

A method for modifying a double stranded structure in a eukaryotic cell (excluding methods for carrying out operations, treatments, or diagnoses for human), the method comprising:

a) introducing into the eukaryotic cell two RNA-guided nickase systems or nucleic acids that encode the RNA-guided nickase systems, and arbitrarily a donner polynucleotide, wherein each RNA-guided nickase system:

(i) has a functional nuclease domain in which an RNA-guided endonuclease is modified so that a single strand of the double stranded structure is cleaved, wherein RNA-guided endonuclease is derived from type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-related (Cas) (CRISPR /Cas) system protein and the type II CRISPR/Cas system is a Cas9 protein; and

(ii) is a guide RNA comprising a first region that is complementary to the target site in the single strand of the double stranded structure and a second region that interacts with the RNA-guided endonuclease, wherein the target sites of two RNA-guided nickase systems exist in the opposing chain of the double stranded structure, each target site is located immediately in front of a protospacer adjacent motif (PAM); and

b) culturing the eukaryotic cell so that double strand break can be introduced by independently cleaving the opposing chain of the double stranded structure by both of RNA-guided endonucleases of two RNA-guided nickase systems, and repair of the double strand break by the DNA repair process introduces modification of the double stranded structure."

(2) Overview of Inventions 2 to 10 and 12 to 21

Inventions 2 to 4 are inventions that are merely further restrictions of Invention 1 with further restrictions, Inventions 5 to 8 are inventions relating to multiple nucleic acids, and Inventions 9 and 10 relate to vectors comprising multiple nucleic acids of Inventions 5 to 8. Meanwhile, Inventions 12 to 21 are inventions that are merely further restrictions of Invention 11.

2 Reference date for examination of inventive step of Inventions 1 to 21

As shown in above 1, (1), the matter for specifying both Inventions 1 and 11 is the use of "two" "RNA-guided nickase systems."

As stated in above No. 1, Present Application claims priority based on the

applications of December 6, 2012 in the U.S.A (hereinafter, referred to as "the First Priority Date"), January 30, 2013 (hereinafter, referred to as "the Second Priority Date"), February 5, 2013 (hereinafter, referred to as "the Third Priority Date") and March 15, 2013.

The application documents for the application based on the claim of priority on the Third Priority Date (61/761, 046) mention that "two" "RNA-guided nickase systems" are used, but the application documents of the application based on the claim of priority on the First Priority Date and the Second Priority (61/734, 256 and 61/758, 624) do not have any description concerning the matter, and therefore, it cannot be deemed that, even if common technical knowledge as of the First Priority Date or the Second Priority Date is taken into consideration, the matter was obvious even without explicit description. Then, since it can be deemed that use of "two" "RNA-guided nickase systems" was described for the first time in application documents for the application based on the claim of priority on the Third Priority Date (61/761, 046), the reference date for the examination of inventive step of Inventions 1 and 11 whose matter specifying the invention is the above fact is the Third Priority Date.

In addition, as indicated in above 1 (2), since the matter specifying the invention of Inventions 2 to 10 and 12 to 21 is also same as in Inventions 1 and 11 to use "two" "RNA-guided nickase systems," the reference date for the examination of inventive step of Inventions 2 to 10 and 12 to 21 is also the Third Priority Date.

In the following, if "the Priority Date of the Invention" is mentioned, unless otherwise noted, it means the Third Priority Date, February 5, 2013.

No. 4 Described matters in the Cited Documents

1 Cited Document 1

Cited Document 1, which is a publication distributed before the Priority Date of the Invention and is cited by the examiner's decision, is an academic article titled "Multiple Genomic Engineering Using CRISPR/CAS Systems" and describes the following matters.

(1-1) "Functional elucidation of causal genetic variants and elements requires precise genome editing technologies It has been indicated that a type II CRISPR (...) adaptive immune system derived from prokaryotic organisms promotes RNA-guided site-specific DNA cleavage. We engineered two different type II CRISPR systems and demonstrated that Cas9 nuclease could be induced by a short RNAs so that precise cleavage occurs in the endogenous genomic loci of human and mouse cells. Cas9

could also be converted into a nicking enzyme to facilitate homology-directed repair with minimal mutagenic activity. Lstly, multiple guide sequences can be encoded into a single CRISPR array for enabling simultaneous editing of several sites within the mammalian genome, demonstrating easy programmability and wide applicability of the RNA-guided nuclease technology." (Summary).

(1-2) "The type II CRISPR locus of Streptococcus pyogenes SF370 consists of four genes, including Cas9 nuclease, as well as two non-coding CRISPR RNAs; tracrRNA and precursor crRNA array, and the precursor crRNA array includes nuclease guide sequences (spacers) interspaced by identical direct repeats (DRs) (Figure S1) (...). We designed our initial spacer to target a 30 base pairs (bp) site (protospacer) in the human EMX1 locus that precedes an NGG trinucleotide, the requisite protospacer adjacent motif (PAM) (Figures 1C and S1) (...)" (page 1, left column, paragraph 2 to right column, paragraph 1).

(1-3) "... The wild type SpCas9 is able to mediate site-specific DSBs, which can be repaired through either NHEJ or homology-directed repair (HDR). In order to convert the nuclease into a DNA nickase, we engineered an aspartate-to-alanine substitution (D10A) in the RuvCI domain of SpCas9 (...)(SpCas9n; Figure 4A) , because nicked genomic DNA is typically repaired either seamlessly or through high-fidelity HDR. ... We then tested Cas9-mediated HDR at the same EMX1 locus with a homology repair template to introduce a pair of restriction sites near the protospacer (Fig. 4C). SpCas9 and SpCas9n catalyzed integration of the repair template into EMX1 locus at similar levels (Figure 4D), which we further verified via Sanger sequencing (Figure 4E). These results demonstrate the utility of CRISPR for facilitating targeted genomoic insertions. Given the 14-bp (12 bp from the seed sequence and 2 bp from PAM) target specificity (Fig. 3B) of the wild-type SpCas9, the use of a nickase may reduce off-target mutations." (page 2, left column, paragraph 3).

(1-4) "Finally, the natural architecture of the CRISPR loci with arrayed spacers (Figure S1) suggests the possibility of multiplexed genome engineering. By using a single CRISPR array encoding a pair of EMX1- and PVALB spacers, we detected efficient cleavages at both loci (Figure 4F). We further tested targeted deletion of larger genomic regions through concurrent DSBs by using spacers against two targets within EMX1 spaced by 119 bp and observed a 1.6% deletion efficacy (3 out of 182 amplicons, Fig. 4G), thus demonstrating the CRISPR/Cas system can mediate multiplexed editing

within a single genome." (page 2, left column, paragraph 4).

(1-5) "

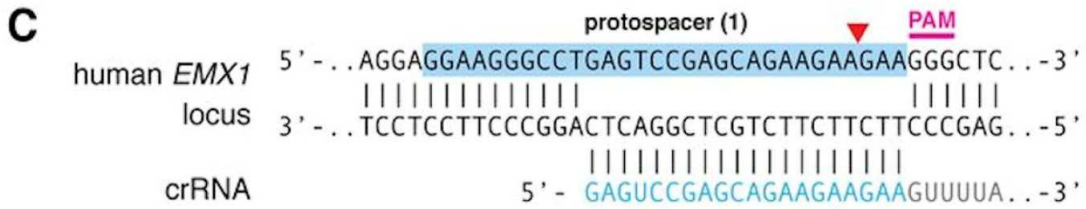

Figure 1: Type II CRISPR locus of Streptococcus pyogenes SF370 can be reconstituted in a mammalian cells to facilitate targeted DSBs of DNA. ... (C) Schematic representation of the base pairing between target locus and EMX1-targeting crRNA. The red arrow indicates putative cleavage site. ..." (Figure 1).

(1-6) "

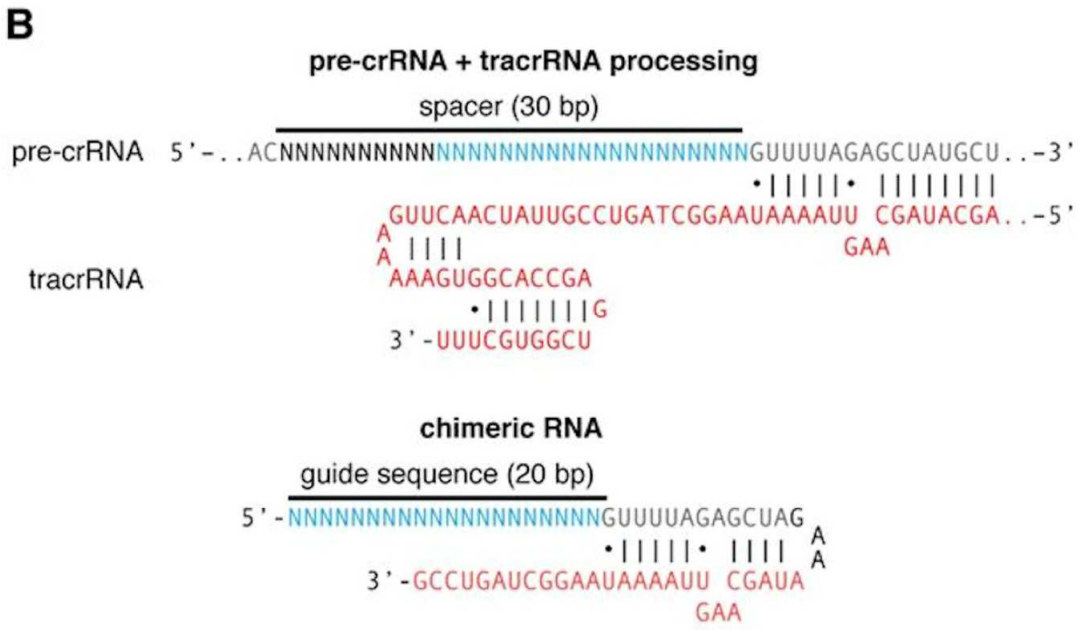

Figure 2: SpCas9 can be reprogrammed to target multiple loci in mammalian cells. ... (B) The schematic view of the pre crRNA:tracrRNA complex(top) showing hybridization between the direct repeat (gray) region of the pre-crRNA and tracrRNA. Schematic view of a chimeric RNA design (...) (bottom). tracrRNA sequence is shown in red, and the 20 bp spacer sequence in blue. ..." (Figure 2).

(1-7) "

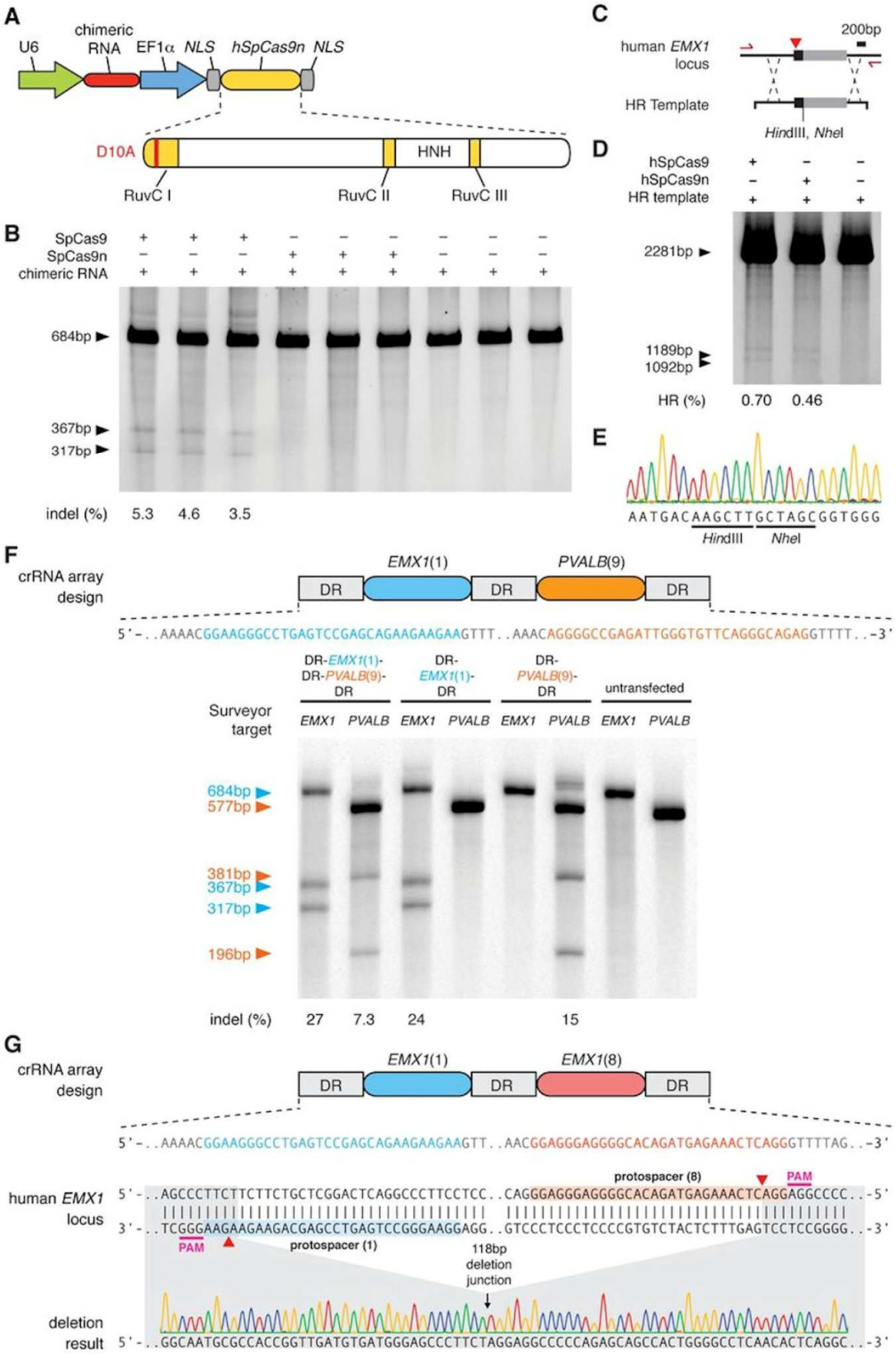
A
chimeric RNA
U6
EF1α
NLS
hSpCas9n
NLS
D10A
HNH
RuvC I
RuvC II
RuvC III
B
SpCas9
SpCas9n
chimeric RNA
684bp
367bp
317bp
indel (%) 5.3 4.6 3.5
C
human EMX1 locus
200bp
HR Template
HindIII, NheI
D
hSpCas9
hSpCas9n
HR template
2281bp
1189bp
1092bp
HR (%) 0.70 0.46
E
AATGACAAGCTTGCTAGCGGTGGG
HindIII
NheI
F
crRNA array design
EMX1(1)
PVALB(9)
DR
5'-..AAAACGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGTTT..AAACAGGGGCCGAGATTGGGTGTTCAGGGCAGAGGTTTT..-3'
DR-EMX1(1)-DR-PVALB(9)-DR
DR-EMX1(1)-DR
DR-PVALB(9)-DR
untransfected
Surveyor target
EMX1 PVALB EMX1 PVALB EMX1 PVALB EMX1 PVALB
684bp
577bp
381bp
367bp
317bp
196bp
indel (%) 27 7.3 24 15
G
crRNA array design
EMX1(1)
EMX1(8)
DR
5'-..AAAACGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGTT..AACGGAGGGAGGGGCACAGATGAGAAACTCAGGGTTTTAG..-3'
human EMX1 locus
protospacer (8)
PAM
5'-..AGCCCTTCTTCTTCTGCTCGGACTCAGGCCCTTCCTCC..CAGGGAGGGAGGGGCACAGATGAGAAACTCAGGAGGCCCC..-5'
3'-..TCGGGAAGAAGAAGACGAGCCTGAGTCCGGGAAGGAGG..GTCCCTCCCTCCCCGTGTCTACTCTTTGAGTCCTCCGGGG..-3'
PAM
protospacer (1)
118bp deletion junction
deletion result
5'-..GGCAATGCGCCACCGGTTGATGTGATGGGAGCCCTTCTAGGAGGCCCCCAGAGCAGCCACTGGGGCCTCAACACTCAGGC..-3'

Figure 4: Applications of Cas9 for homologous recombination and multiplex genome engineering. (A) Mutation of the RuvC I domain converts Cas9 into a nicking enzyme (SpCas9n). (B) Coexpression of EMX1-targeting chimeric RNA with SpCas9n leads to indels, whreas SpCas9 does not (N = 3). (C) Schematic representation of the recombination strategy. A homology repair (HR) template is designed to insert restriction sites into EMX1 locus. Primers used to amplify the modified region are shown as red arrows. (D) Restriction fragment length polymorphism gel analysis. Arrows indicate fragments generated by HindIII digestion. (E) Example of chromatogram showing successful recombination. (F) SpCas9 can facilitate multiplex genome modification by using a crRNA array that contains two spacers targeting EMX1 and PVALB. Schematic view showing the design of crRNA array (top). Both spacers mediate efficient protospacer cleavage (bottom). (G) SpCas9 can be used to achieve precise genomic deletion. Two spacers targeting EMX1 (top) mediated a 118-bp genomic deletion (bottom).)" (Figure 4).

(1-8) "(D) Restriction-fragment-length polymorphism analysis for detecting homologous recombination

HEK293FT and N2A cells are transfected with plasmid DNA, and incubated .... The target genomic region was PCR amplified using primers outside the homology arms of the homologous recombination (HR) template. PCR products were separated on a 1% agarose gel and extracted with MinElute Gel Extraction kit. Purified products were digested with HindIII (...), analyzed ..." (Supplementary materials, page 4, paragraph 2).

2. Cited Document 2

Cited Document 2, which is a publication distributed before the Priority Date of the Invention and is cited by the examiner's decision, is an academic article titled "Precision Genome Engineering with Programmable DNA-Nicking Enzymes" and describes the following matters. Since the original text is written in English language, a translation prepared by the body is shown below.

(2-1) "Zinc-finger nucleases (ZFNs)are powerful tools of genome engineering but are limited by their reliance, on error-prone non-homologous end-joining (NHEJ) repair of DNA double strand breaks (DSBs), which gives rise to randomly generated, unwanted small insertions or deletions (indels) at both on-target and off-target sites. In this article, we present programmable DNA-nicking enzymes (nickases) that produce single-

strand breaks(SSBs) or nicks, instead of DSBs, which are error-free homologous recombination (HR) rather than mutagenic NHEJ. Different from corresponding nucleases, zinc finger nickases allow site-specific genome modifications only at the on-target site, without the induction of unwanted indels. We propose that programmable nickases would be of broad utility in research, medicine. and biotechnology, enabling precision genome engineering in any cell or organism." (Summary).

(2-2) "ZFN and TALENs cleave DNA, producing site-specific DSBs in a genome. DSBs are dangerous signals and, unless properly repaired, in many cases, leads to death of cells and cancers. Cells are equipped with two competing DSB repair systems: HR and NHEJ (...). In the presence of homologous DNA, DSBs can be repaired by HR. The HR machinery uses homologus trast, the two end points of a DSB can be efficiently targeted by NHEJ without the use of homologous DNA. Unlike HR, NHEJ is errorprone, often inducing small insertion deletions(indels) at breakpoint junctions. Repair of nuclease induced site-specific DSBs by HR or NHEJ give rise to targeted genome modifications.

Despite the broad utility of these enzymes in basic research, biotechnology, and medicine, genome engineering with programmable nucleases is limited by the inevitable production of DSBs and reliance on error-prone NHEJ. As a result, programmable nucleases often induce, randomly generated unwanted indels at the on-target site, even in the presence of homologous donor DNA. The reason is that, in cells of higher eukaryotes and living organisms, NHEJ is a dominant pathway of DSB repair over HR (...). To make things worse, these enzymes induce off-target mutations at sites highly homologous to the intendrd target site,where they produce off-target DSBs (...). In addition, the repair of off-target DSBs via NHEJ can give rise to unwanted chromosomal rearrangements (...). Furthermore, nucleases excessively inducing off-target DSBs are toxic to cells, and making it difficult, if not impossible, to isolate gene-edited cells (...).

In principle, it should be possible to overcome these limitations by using an enzyme that either (1)induces DSB only at the intended target site, or (2)does not induce a DSB at the target site but still elicits site-specific mutations. In this article, we demonstrat that a site-specific DNA nicking enzymes (nickases) (this has been achieved by modifying Fok I nuclease domain of ZFN) can induce SSBs in the genome, whose repair via highly accurate HR gives rise to targeted genome modifications. What is important is that SSBs are not repaired by error-prone NHEJ, and, therefore, do not give rise to indels at both on-target and off-target sites. As explained above, SSB-induced

zinc finger (ZF) nickases could serve as highly specific mutagens with no or little off-target effect" (page 1327, left column, paragraph 2 to right column, paragraph 2).

(2-3) "Genome editing with zinc finger nickases

Next, we tested whether these nickases can induce targeted genome modifications at the endogenous site via HR. Human K562 cells were transfected with nickase plasmids and a homologous donor DNA that contained XbaI site not present in the homogenous chromosomal region (Figure 3A). PCR amplicons of this chromosomal region were partially digested by XbaI, and genome editing efficiency by nickase is indicated to be 1% to 3% (Figure 3B). ...

In addition, genome editing frequencies were measured by cloning and sequencing the PCR products. It was found that L_KK/R_EL nuclease induces both randomly generated indels (17 clones/52 clones in total, 33%) and homology-directed incorporation of the XbaI sites (13/52, 25% (Figure 3C; supplemental Figure 1)). In contrast, the L_KK/R_el nickase did not induce indels at all(0/149 clones), but induced HR mediated modification at a frequency of 9% (= 13/149). Although these results are not more efficient than ZFN, they show that ZFN nickase induces true genome modification in mammalian cells and that genome editing with nickase is not accompanied by unwanted indels, demonstrating a crucial advantages of nickases over nucleases." (page 1328, right column, paragraphs 2 to 3).

(2-4) "Two pairs of nickases produce a DSB.

For confirming that nickases induce an SSB at the genomic target site, two nickases were introduced into human cultured cells. Two nickases that generate aSSB on opposite strands may induce a DSB then the two SSBs occur close to each other. This composite DSB could be efficiently repaired by NHEJ. For testing this idea, plasmids that encode two types of nickases: L_KK/R_el and L_el/R _KK, were transfected to K562 cells. ...indels induced by error-prone NHEJ were detected by using mismatch sensitive T7 endonuclease I (T7E1) (Figure 4 ...). PCR amplicons from cells cotransfected with plasmids encoding the two nickases were partially cleaved at the expected position, indicating that the presence of indels at the CCR5 site. Induction of indels at the spacer region was confirmed by analysis of DNA sequencing (Figure 4B). ... In sharp contrast, each nickase alone (L_KK/R_el and L_el/R_KK) did not induced any mutations (assay sensitivity is ~ 1%)" (page 1328, right column, last paragraph to page 1329, left column, paragraph 1).

(2-5) "Nickase-induced SSBs do not give rise to indels

In addition, high-throughput DNA sequencing was carried out, and it was confirmed that ZF nickases did not induce indels via NHEJ at either on-target sites or off-target sites. By analyzing genome DNA isolated from K562 transfected with plasmids encoding ZFN-224 or L_KK/R_el nickase, the frequencies of indels at the CCR5 on-target site and several off-target sites revealed in recent research (...) was measured. As expected, ZFN-224 induced indels at these sites at frequencies up to 20% (Figure 5; supplemental Table 1). In sharp contrast, compared to cells comprising empty vector control, cells that expressed nickase did not indicate any evidence of formation of indel at these sites including CCR5 sites. Apparently, nickase-induced SSBs were faithfully repaired by the endogenous base excision repair (BER) system (...). To wrap up, both T7E1 assay and deep sequence analysis indicate that, different from ZFN, ZFN nickase does not induce NHEJ, which tends to cause errors in repairing a DNA cleavage" (page 1329, left column, paragraph 2 to right column, paragraph 1).

(2-6) "Programmable nickases have many advantages over nucleases. First, off-target SSBs produced by nickases would be efficiently and faithfully sealed by highly accurate base excision repair,leaving no footprints remains at off-target sites. In contrast, off-target DSBs produced by a nuclease are repaired by error-prone NHEJ, which give rise to undesirable indels. In addition, SSBs occur naturally and exist in the genome far oftener than DSBs and are less toxic than DSBs, which often cause death of cells and cancers. Not all but many nucleases are cytotoxic and make isolation of clonal population with edited genes difficult. SSB-prodicing nickases are likely to be less stressful to cells. In addition, nucleases induce unwanted indels at the on-target site even in the presence of homologous donor DNA. Our deep sequencing analysis indicates that nickases do not induce indels at the on-target site. ..." (page 1330, left column, paragraph 2).

(2-7) "In conclusion, we demonstrated SSB-producing programmable nickases could be used for targeted genome modification. Different from corresponding nucleases, ZF nickases allowed efficient genome editing without inducing unwanted indels at the target site. Furthermore, nickase-mediated off-target mutations were not detectable even with high-throughput sequencing, demonstrating an unprecedented precision in genome editing. We propose that programmable nickases are novel tools for precision genome engineering, enabling targeted mutagenesis in any cell or organism" (page 1331,

left column, the last paragraph to right column, paragraph 1).

(2-8) "

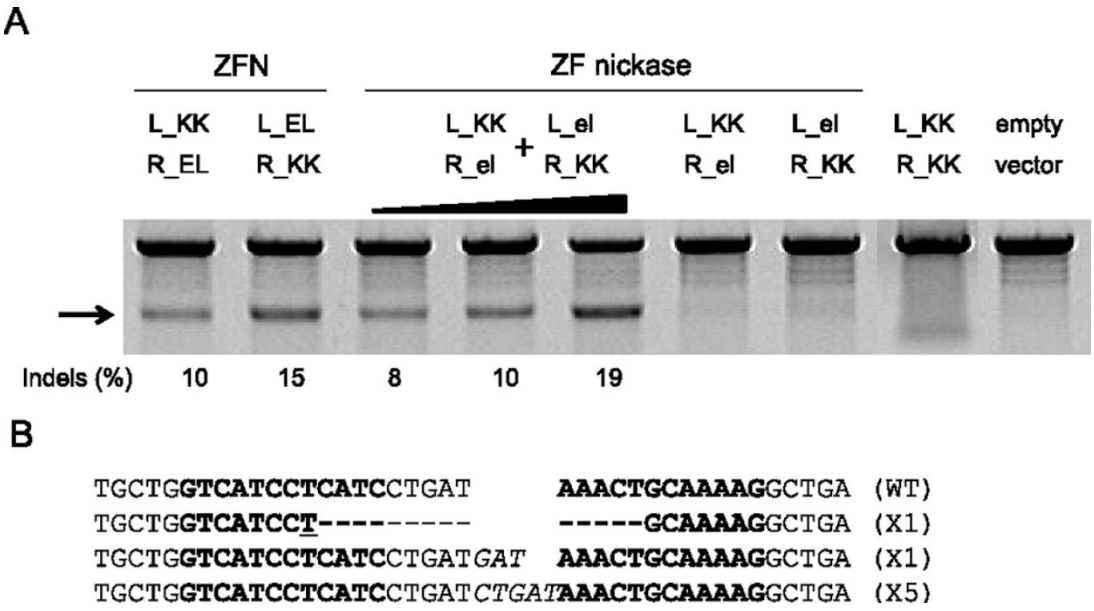

Figure 4 Two pairs of ZF nickases produce a DSB in the genome. (A) Nuclease or nickase-driven indels detected in T7E1 assay. PCR products amplified using genomic DNA from cells transfected with plasmid (4 μg/monomer) that encodes nickases or nuclease were subjected to with T7E1 digestion and analyzed by agarose gel electrophoresis. (Arrow) The expected position of the resulting DNA band. (The black triangle above the pictures of gels) Thw increase of the transfected plasmid (4, 8, and 10 μg/each monomer). (B) DNA sequences of the CCR5 wild-type and mutant clones. The two half-sites are shown in thick letters. Microhomologies are underlined and inserted bases are shown with italic characters. Dashes indicate deleted bases. The number of occurrences is shown in brackets. X1 and X5 are the number of each clone. (WT) means wild type" (Figure 4).

No. 5 Comparison / judgment

1 Regarding Invention 1

(1) Cited Invention 1a

Description (1-1), which is the summary portion of Cited Document 1 that is an academic article concerning genome editing, discloses that Cas9 can be converted to a nicking enzyme facilitate homology-directed repair(hereinafter, referred to as "HDR")

with minimal mutagenic activity.

In addition, in this regard, it is acknowledged that Description (1-3) discloses that nickase SpCas9n was prepared by alanine substitution of asparagine acid (D10A) in the RuvCI domain of SpCas9, and that nickase SpCas9n catalyzed insertion of the repair template to EMX1 locus (Figure 4D) when HDR via Cas9 in the EMX1 locus was tested using a homologous repair template (Figure 4C). Furthermore, it is acknowledged that Description (1-7) discloses in Figure 4A as concrete experimental data thereof that chimeric RNA and nickase SpCas9n were used, and it is further acknowledged that it is disclosed in the legend for Figure 4B that the target of the chimeric RNA is EMX1, and it is still further disclosed in Figure 4C that the HR template has been designed so that the restriction site of HindIII is inserted into the EMX1 locus, and, still furthermore, it is acknowledged that Figure 4D and its legend disclose that, in the presence of nickase SpCas9n and the HR template, the restriction-fragment-length polymorphism analysis which confirmed fragment generated by HindIII digestion indicated that HDR occurred. In addition, Description (1-8) discloses that the above restriction-fragment-length polymorphism analysis was carried out using HEK293FT cells, etc. Furthermore, judging from the fact that Description (1-8) states that HR template means homologous recombination template, it is acknowledged that "homology repair template," "HR template," and "homologous recombination template" have the same meaning in Cited Document 1.

Then, it is acknowledged that Cited Document 1 discloses that HDR was caused to occur in the EMX1 locus of the HEK293FT cell by nickase SpCas9n, chimeric RNA whose target was EMX1 locus, and the homology repair template that had been designed so that the restriction site is inserted into the EMX1 locus.

In addition, it is acknowledged that Description (1-2) discloses that the target site of the EMX1 locus is a site of the EMX1 locus located in front of the NGG that is PAM, and that Description (1-5) discloses as Figure 1C that the target site of the EMX1 locus is located immediately in front of PAM.

Judging from the above, it is acknowledged that Cited Document 1 discloses the following invention (hereinafter, referred to as "Cited Invention 1a").

"A composition comprising (i) a nickase SpCas9n, (ii) a chimeric RNA of which target is the site of the EMX1 locus located immediately in front of PAM, and (iii) a homologous repair template designed so that the restriction site is inserted into the EMX1 locus composition."

(2) Comparison

The "chimeric RNA" of Cited Invention 1a is such that "whose target is the site of the EMX1 locus located immediately in front of PAM," and, as already indicated in above (1), can cause HDR through nickase SpCas9n at the target site of the EMX1 locus in the presence of the homology repair template, and, judging from the lower part of Figure 2B in Description (1-6), its structure includes, in sequence from the end of 5', a domain complementary to the target site (blue), a region that forms a stem-loop structure, and a region derived from tracrRNA. Taking into consideration common technical knowledge concerning RNA that has such structure as of the priority date of the Invention (in short, see descriptions concerning "chimeric RNA" in "Science, Aug 2012, Vol. 337, pp. 816-821, Supplementary Materials" "guide RNA" in "Science, published online 3 Jan 2013, Vol. 339, pp. 823-826," and "sgRNA" in "eLife, 29 Jan 2013, 2:e00471, DOI:10.7554/eLife.00471), it is acknowledged that the "chimeric RNA" of Cited Invention 1a is the "guide RNA comprising a first region that is complementary to the target site in the single strand of the double stranded structure and a second region that interacts with RNA-guided endonuclease" of Invention 1, and corresponds to one of which "target site is located immediately in front of a protospacer adjacent motif (PAM)."

In addition, as already indicated in the above (1), while "nickase SpCas9n" of Cited Invention 1a is prepared by D10 amino acid substitution of the nuclease SpCas9, judging from Description (1-2), since nuclease SpCas9 is derived from type II CRISPR locus, it can be deemed that nickase SpCas9n is derived from type II. Then, it is acknowledged that "nickase SpCas9n" of Cited Invention 1a "has a functional nuclease domain in which an RNA-guided endonuclease is modified so that a single strand of the double stranded structure is cleaved" as in Invention 1, and corresponds to one "wherein RNA-guided endonuclease is derived from type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-related (Cas) (CRISPR /Cas) system protein and the type II CRISPR/Cas system is a Cas9 protein."

Judging from the above, it is acknowledged that a Corresponding Feature and a Different Feature between Invention 1 and Cited Invention 1a are as follows:

[Corresponding Feature]

"A composition comprising an RNA-guided nickase system, in which composition the RNA-guided nickase system (i) has a functional nuclease domain in which an RNA-guided endonuclease is modified so that a single strand of the double stranded structure is cleaved, wherein the RNA-guided endonuclease is derived from

type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-related (Cas) (CRISPR /Cas) system protein and the type II CRISPR/Cas system is a Cas9 protein, and (ii) is a guide RNA comprising a first region that is complementary to the target site in the single strand of the double stranded structure and a second region that interacts with RNA-guided endonuclease, wherein the target sites of the RNA-guided nickase system are located immediately in front of a protospacer adjacent motif (PAM)."

[Different Feature 1a]

While it is specified for Invention 1 that the composition of Invention 1 comprises two RNA-guided nickase systems, that these targets sites exist in the opposing chain of the double stranded structure, and that double strand break can be introduced into these RNA-guided endonucleases by independently cleaving the opposing chain of the double stranded structure, the composition of Cited Invention 1a comprises merely one RNA-guided nickase system and is not such that a double strand break can be introduced.

(3) Judgment

A Other descriptions in Cited Document 1

In addition to matters shown in above (1), Description (1-1) of Cited Document 1 discloses that multiple guide sequences can be encoded to a single CRISPR array in order to enable simultaneous editing of multiple sites in a mammalian genome.

In this regard, it is acknowledged that Description (1-4) discloses that cleavages were detected in both loci using a single CRISPR array that encodes a pair of spacers whose targets were EMX1 and the spacer whose target was PVALB (Figure 4F), and that, by simultaneously generating DSBs using spacers to two targets in EMX1 119 bp away, deletion of the target in larger genome region was observed (Figure 4G).

It is acknowledged that, as concrete experimental data thereof, Description (1-7) discloses in Figure 4F and its legend that nuclease SpCas9 promoted multiple genome modification, by using a single crRNA array comprising two spacers whose targets are EMX1 and PVALB, and that, in Figure 4G and its legend, genome deletion for 118 bp was intermediated using two spacers whose targets were nuclease SpCas9 and EMX1. Then, although it can be deemed that Cited Document 1 concretely discloses that, using two RNA-guided nuclease systems, double strand break (DSB) was simultaneously generated at two sites in different or identical target loci, this is merely a case in which nuclease SpCas9 is used.

On the other hand, Cited Document 1 merely discloses that an RNA-guided nickase system is for introducing a nick (single-strand break) that is repaired by HDR as seamless and with high fidelity (description (1-3)), and use of two of such systems is not disclosed, and, even more, it cannot be deemed that it is disclosed or suggested that DSB is generated by independently cleaving the target site existing in the opposing chain of double stranded structure using two RNA-guided nickase systems.

B Descriptions in Cited Document 2

Judging from the descriptions in the summary portion, Description (2-1), and the introduction portion, Description (2-2), it can be deemed that Cited Document 2 which is an academic article concerning genome editing describes the problem that ZFN and TALEN used for genome editing generate DSBs in target and off-target sites of the genome, and unwanted small insertions and/or deletions (indels) are caused by repair of the DSBs by NHEJ, and, at the same time, discloses that the problem can be solved by generating single-strand break (hereinafter, referred to as "SSB") in the genome using a site-specific DNA nick enzyme (hereinafter, referred to as "ZF nickase") formulated by modifying Fok1 nuclease domain of ZFN. With respect to this ZF nickase and SSB generated by the ZF nickase, to be more specific, the description (2-2) discloses that SSB is not repaired by NHEJ, and, therefore, no indel occurs, at both on-target sites and off-target sites, and, on the other hand, Description (2-3) discloses that target genome modification via HR can be induced without accompaniment of any unwanted indel by inducing SSB with ZF nickase, and, furthermore, Description (2-5) discloses that, different from ZFN, ZF nickase does not induce any NHEJ in repairing DNA cleavages. In addition, as another advantage of this ZF nickase as compared with ZFN, Description (2-6) discloses that off-target SSB generated by ZF nickase can be efficiently and faithfully blocked by very precise base excision repair, and that any nickase that generates SSB causes less stress to cells as compared with nucleases that generate DSB. In addition, Description (2-7), which corresponds to the conclusion portion of Cited Document 2, discloses that ZFN nickase enables efficient genome editing without inducing any unwanted indel at the target site, that off-target mutation through ZF nickase cannot be detected even by high throughput sequence and unprecedented accuracy in genome editing has been demonstrated, and that the programmable ZF nickase is a new tool for precision genomic engineering.

Summing up the above, it can be deemed that Cited Document 2 discloses that, although ZF nickase can generate DNA cleavage at target and off-target sites of the genome the same as ZFN, since SSB is generated, neither repair by NHEJ nor any

unwanted indel due to such repair occurs, different from DSB generated by ZFN, and that, by generating SSB with ZF nickase, target genome modification via HR can be induced, and these can solve the problems that occur when DSB is generated using ZFN or TALEN in genome editing.

Here, judging from Descriptions (2-4) and (2-8), Cited Document 2 describes an experiment in which DSBs are generated by generating one SSB each for one chain and the other chain in proximity to each other by two pairs of ZF nickases. However, it is disclosed at the beginning of Description (2-4) that the purpose of this experiment is to confirm that ZF nickase induces SSB in the genome target site, and, in addition, judging from Descriptions (2-4) and (2-8), it can be deemed that DSBs generated by two pairs of ZF nickases in this experiment are repaired by NHEJ; in other words, judging from Descriptions (2-1) and (2-2), since it is disclosed that DSBs generated here are repaired by the repair pathway of NHEJ that is viewed as problematic in Cited Document 2 because it tends to cause uwanted indels, it can be deemed that this experiment is not for the purpose of generating DSBs with two pairs of ZF nickases, or carrying out genome editing by generating DSBs, but, as described at the beginning of above Description (2-4), for the purpose of confirming that ZF nickases induce SSBs at the genome target site. Then, even if statements in Descriptions (2-4) and (2-8) are taken into consideration, it cannot be deemed that Cited Document 2 describes generation of DSB by two pairs of ZF nickases in genome editing.

As described above, it cannot be deemed that Cited Document 2 describes or suggests generation of DSB using two pairs of ZF nickases.

C Judgment on Different Feature 1a

Cited Invention 1a is an invention related to a composition comprising RNA-guided nickase system for use in genome editing, and judging from the fact that Description (1-1) discloses that nicking enzyme promotes HDR with the minimum mutagenicity as already indicated in above (1), it can be deemed that Cited Invention 1a relates to a composition for promoting HDR with minimal mutagenic activity.

In addition, as already examined in above item i, Cited Document 1 neither discloses nor suggests generating DSB using two pairs of RNA-guided nickase systems by independently cleaving the target sites that exist in the opposing chain of the double stranded structure. As already examined in the above item i, it can be deemed that Cited Document 2 neither discloses nor suggests generating DSBs using ZF nickases in genome editing, and, rather, discloses that, by generating not DSB but SSB using ZF nickase, repair by NHEJ and unwanted indel can be avoided and, at the same time,

genome editing via HR can be carried out by generating SSB.

Then, it can be deemed that a person skilled in the art who reads the description in Cited Document 2 will not be motivated in genome editing to use Cited Invention 1a, which relates to a composition for promoting HDR with minimal mutagenic activity for forming DSB, in which repair by NHEJ and unwanted indel due to repair by NHEJ could occur.

Accordingly, Different Feature 1a between Invention 1 and Cited Invention 1a cannot be deemed to be such that a person skilled in the art could have easily made Invention 1 by combining Cited Invention 1a with technical matters described in Cited Document 2.

(4) Summary

Judging from the above, Invention 1 cannot be deemed to have been easily invented even by a person skilled in the art based on technical matters disclosed in Cited Invention 1a as well as Cited Documents 1 and 2.

2 Regarding Inventions 2 to 10

Since Inventions 2 to 4 are inventions that are merely restrictions of Invention 1 and are provided with the same constitution as that of Invention 1, due to the same reason as in the case of Invention 1, it cannot be deemed that a person skilled in the art could have easily invented Inventions 2 to 4 based on technical matters described in Cited Invention 1a as well as Cited Documents 1 and 2.

Since Inventions 5 to 8 relate to multiple nucleic acids that encode the composition of Invention 1, Inventions 9 and 10 relate to a vector comprising multiple nucleic acids, and all of them have a constitution that corresponds to the constitution of Invention 1, due to the same reason as in the case of Invention 1, it cannot be deemed that a person skilled in the art could have easily invented Inventions 5 to 8, 9 and 10 based on technical matters described in Cited Invention 1a as well as Cited Documents 1 and 2.

3 Regarding Invention 11

(1) Cited Invention 1b

Since Cited Document 1 describes matters indicated in above 1, (1), it is acknowledged that Cited Document 1 discloses the following invention (hereinafter, referred to as "Cited Invention 1b").

"A method a) by introducing into an HEK293FT cell,
(i) a nickase SpCas9n, (ii) a chimeric RNA whose target is the site of EMX1 locus located directly in front of PAM, and (iii) a homologous repair template designed so that the restriction site is inserted into the EMX1 locus,
b) to cause homologous sequence-dependent repair (HDR) in the EMX1 loci of HEK293FT cells."

(2) Comparison

As already examined in above 1, (2), it is acknowledged that the chimeric RNA of Cited Invention 1b is "a guide RNA comprising a first region that is complementary to the target site in the single strand of the double stranded structure and a second region that interacts with RNA-guided endonuclease" of Invention 11, and corresponds to one in which "target site is located immediately in front of a protospacer adjacent motif (PAM)," and "nickase SpCas9n" of Cited Invention 1b "has a functional nuclease domain in which an RNA-guided endonuclease is modified so that a single strand of the double stranded structure is cleaved" of Invention 11, and corresponds to one in which "wherein RNA-guided endonuclease is derived from type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-related (Cas) (CRISPR /Cas) system protein and the type II CRISPR/Cas system is a Cas9 protein."

In addition, "HEK293FT cells" of Cited Invention 1b correspond to "eukaryotic cell" of Invention 11, and, since "to cause homologous sequence-dependent repair (HDR) in the EMX1 loci of HEK293FT cells" of Cited Invention 1b means that, in Cited Invention 1b, homologous sequence-dependent repair (HDR) occurs if a nick is generated by nickase SpCas9n at the target site in the EMX1 locus that was the target site, it corresponds to "for modifying double stranded structures in eukaryotic cells" of Invention 11, and, at the same time, corresponds to "culturing eukaryotic cells so that chain cleavage is introduced by cleaving the chain of the double stranded structure with the RNA-guided endonuclease of RNA-guided nickase system, and repair of chain cleavage by the DNA repair process introduces modification of double stranded structure" of Invention 11.

In addition, it is obvious that the method of Cited Invention 1b is not any method for carrying out operation, treatment, or diagnosis on human.

Judging from the above, the following Corresponding Features and Different Features can be recognized between Invention 11 and Cited Invention 1b.

[Corresponding Feature]

"A method for modifying a double stranded structure in a eukaryotic cell (excluding methods for carrying out operations, treatments or diagnoses for human), the method comprising:
a) introducing into the eukaryotic cell an RNA-guided nickase system or nucleic acid that encodes the RNA-guided nickase systems, and arbitrarily a donner polynucleotide, wherein the RNA-guided nickase system:
(i) has a functional nuclease domain in which an RNA-guided endonuclease is modified so that a single strand of the double stranded structure is cleaved, wherein the RNA-guided endonuclease is derived from type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR-related (Cas) (CRISPR /Cas) system protein and the type II CRISPR/Cas system is a Cas9 protein; and
(ii) is a guide RNA comprising a first region that is complementary to the target site in the single strand of the double stranded structure and a second region that interacts with the RNA-guided endonuclease, wherein the target sites of two RNA-guided nickase systems exist in the opposing chain of the double stranded structure, and each target site is located immediately in front of a protospacer adjacent motif (PAM); and
b) culturing the eukaryotic cell so that double strand break can be introduced by independently cleaving the opposing chain of the double stranded structure by both RNA-guided endonucleases of two RNA-guided nickase systems, and repair of the double strand break by the DNA repair process introduces modification of the double stranded structure."

[Different Feature 1b]

The fact that, while Invention 11 specifies that two RNA-guided nickase systems are introduced, that their target sites exist in the opposing chain of the double stranded structure, and that both of those RNA-guided endonucleases introduce double strand break by independently cleaving the opposing chains of the double stranded structure, Cited Invention 1b merely introduces one RNA-guided nickase system and does not introduce any double strand break.

(3) Judgment

Judging from the fact that it can be deemed that the method of Cited Invention 1b is an invention related to a method for genome editing using an RNA-guided nickase system, and, as already shown in above 1, (1), since Description (1-1) discloses that the nicking enzyme promotes HDR with the minimum mutagenicity, it can be deemed that the method of Cited Invention 1b is a method for promoting HDR with minimal

mutagenic activity in genome editing.

As already examined in above 1, (3), item i, Cited Document 1 neither describes nor suggests generating DSB by independently cleaving the target site existing in the opposing chain to the double stranded structure using two RNA-guided nickase systems.

Meanwhile, as already examined in above 1, (3), item ii, it can be deemed that Cited Document 2 neither describes nor suggests generating DSB using ZF nickase in genome editing, but, rather, by generating not DSB but SSB using ZF nickase, repair by NHEJ and unwanted indel caused thereby can be avoided, and, at the same time, genome editing via HR by generating SSB can be carried out.

Then, it can be deemed that a person skilled in the art who reads the description in Cited Document 2 will not be motivated in genome editing to use the method of Cited Invention 1b that is a method for promoting HDR with the minimum mutagenicity for forming DSB in which repair by NHEJ and unnecessary indel due to the repair by NHEJ could occur.

Accordingly, it cannot be deemed that a person skilled in the art could have easily made Different Feature 1b between Invention 11 and Cited Invention 1b by combining Cited Invention 1b with the technical matters disclosed in Cited Document 2.

(4) Summary

Judging from the above, it cannot be deemed that a person skilled in the art could have easily invented Invention 11 based on Cited Invention 1b as well as technical matters described in Cited Documents 1 and 2.

4. Regarding Inventions 12 to 21

Since Inventions 12 to 21 are inventions that are merely restrictions of Invention 11 and are provided with the same constitution as that of Invention 11, due to the same reason as in the case of Invention 11, it cannot be deemed that a person skilled in the art could have easily invented Inventions 12 to 21 based on technical matters described in Cited Invention 1a as well as Cited Documents 1 and 2.

No. 6 Closing

As described above, a person skilled in the art could not have easily invented Inventions 1 to 21 based on inventions disclosed in Cited Documents 1 and 2.

Therefore, Present Application cannot be refused with the reasons for refusal stated in the examiner's decision.

In addition, beyond that, no reasons for refusal were found.

Therefore, the appeal decision shall be made as described in the conclusion.

October 15, 2019

Chief administrative judge: NAGAI, Keiko
Administrative judge: KOGURE, Michiaki
Administrative judge: TAMURA, Kiyoko