JUDGMENT

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In regard to the patent invalidation case for the invention “RNA-directed target DNA modification and method and composition for RNA-directed modulation of transcription” of Patent No. 6692856 between the foregoing parties, the judgment is as follows.

Conclusion

The claim is not established.

Trial costs shall be borne by the Petitioner.

Grounds

1 - Procedural history

The application related to Patent No. 6692856 of the petition in the present invalidation trial (henceforth, "the Patent") is Patent Application 2018-97369, a new application filed on May 21, 2018, based on part of Patent Application 2015-514015, which had March 15, 2013 as an international filing date (Paris Treaty priority claim May 25, 2012 in the US, October 19, 2012 in the US, January 28, 2013 in the US, and February 15, 2013 in the US) and registered on April 17, 2020. Other main points in the procedural history are as follows.

2020/04/25	Trial Petition and Petitioner's Exhibit No. 1 to 8 submitted (Petitioner)
2020/09/15	Trial Answer and Respondents' Exhibit No. 1 to 123 submitted (Respondents)
2020/12/13	Notice of Issues for Trial (to Respondents and Petitioner)
2021/03/07	Oral Hearing Brief and Petitioner's Exhibit No. 9 to 22-4 (Petitioner)
2021/03/09	Oral Hearing Brief and Respondents' Exhibit No. 124 to 143 (Respondents)
2021/03/23	Oral Hearing
2021/03/29	Petition (Respondents) submitted
2021/03/31	Petition (Petitioner) submitted
2021/05/09	Petition (Petitioner) and Petitioner's Exhibit No. 23 to 25 submitted
2021/05/10	Petition (Respondents) and Respondents' Exhibit No. 144 to 162 submitted

II. The Invention

The invention as in claims 1 to 111 of the Patent (henceforth, when accompanied by a claim number, "Invention 1" and so forth; jointly, referred to as "the Invention") is as specified by the matter set down in claims 1 to 111 of the Patent. Claims 1 to 111 are as follows.

CLAIMS

1. A method of modifying a target DNA, the method comprising contacting a target DNA in a cell with a complex comprising:

(a) a Cas9 polypeptide and

(b) a DNA-targeting RNA comprising:

(i) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a sequence in the target DNA, and

(ii) a protein-binding segment that interacts with said Cas9 polypeptide, wherein the protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA),
wherein said dsRNA comprises complementary nucleotides of a tracrRNA and a CRISPR RNA (crRNA),

wherein the cell is a plant cell, an animal cell, or a single-cell eukaryotic organism,

wherein the cell is not a human cell in vivo, not a human germ cell, and not a human embryonic cell; and

wherein said modifying is cleavage of the target DNA.

2. The method of claim 1, wherein the cell is an animal cell and the animal cell is a mammalian cell.

3. The method of claim 1 or claim 2, wherein said two complementary stretches of nucleotides hybridize to form 8 to 15 base pairs .

4. The method of claim 1 or claim 2, wherein said two complementary stretches of nucleotides hybridize to form 15 to 18 base pairs.

5. The method of any one of claims 1 to 4, wherein the percent complementarity between the nucleotides that hybridize to form the dsRNA of the protein-binding segment is greater than 70%.

6. The method of any one of claims 1 to 5, wherein the target DNA is chromosomal DNA.

7. The method of any one of claims 1 to 6, wherein the DNA-targeting RNA comprises one or more of: a modified nucleobase, a modified backbone or non-natural internucleoside linkage, a modified sugar moiety, a Locked Nucleic Acid, and a Peptide Nucleic Acid.

8. The method of any one of claims 1 to 6, wherein the DNA-targeting RNA comprises a non-natural internucleoside linkage that comprises one or more of: a phosphorothioate, a phosphoramidate, a non-phosphodiester, a heteroatom, a chiral phosphorothioate, a phosphorodithioate, a phosphotriester, an aminoalkylphosphotriester, a 3'-alkylene phosphonates,

a 5'-alkylene phosphonate, a chiral phosphonate, a phosphinate, a 3'-amino phosphoramidate, an aminoalkylphosphoramidate, a phosphorodiamidate, a thionophosphoramidate, a thionoalkylphosphonate, a thionoalkylphosphotriester, a selenophosphate, and a boranophosphate.

9. The method of any one of claims 1 to 6, wherein the DNA-targeting RNA comprises one or more of: (i) a non-natural internucleoside linkage selected from a phosphorothioate, an inverted polarity linkage, and an abasic nucleoside linkage; (ii) a locked nucleic acid (LNA); and (iii) a modified sugar moiety selected from 2'-O-methoxyethyl, 2'-O-methyl, and 2'-fluoro.

10. The method of any one of claims 1 to 6, wherein the DNA-targeting RNA comprises one or more modified sugar moieties selected from: 2'-O-(2-methoxyethyl), 2'-dimethylaminooxyethoxy, 2'-dimethylaminoethoxyethoxy, 2'-O-methyl, and 2′ -fluoro.

11. The method of any one of claims 1 to 6, wherein the DNA-targeting RNA comprises a nucleobase comprising one or more of: a 5-methylcytosine; a 5-hydroxymethyl cytosine; a xanthine; a hypoxanthine; a 2-aminoadenine; a 6-methyl derivative of adenine; a 6-methyl derivative of guanine; a 2-propyl derivative of adenine; a 2-propyl derivative of guanine; a 2-thiouracil; a 2-thiothymine; a 2-thiocytosine; a 5-propynyl uracil; a 5-propynyl cytosine; a 6-azo uracil; a 6-azo cytosine; a 6-azo thymine; a pseudouracil; a 4-thiouracil; an 8-haloadenin; an 8-aminoadenin; an 8-thioladenin; an 8-thioalkyladenin; an 8-hydroxyladenin; an 8-haloguanin; an 8-aminoguanin; an 8-thiolguanin; an 8-thioalkylguanin; an 8-hydroxylguanin; a 5-halouracil; a 5-bromouracil; a 5-trifluoromethyluracil; a 5-halocytosine; a 5-bromocytosine; a 5-trifluoromethylcytosine; a 5-substituted uracil; a 5-substituted cytosine; a 7-methylguanine; a 7-

methyladenine; a 2-F-adenine; a 2-amino-adenine; an 8-azaguanine; an 8-azaadenine; a 7-deazaguanine; a 7-deazaadenine; a 3-deazaguanine; a 3-deazaadenine; a tricyclic pyrimidine; a phenoxazine cytidine; a phenothiazine cytidine; a substituted phenoxazine cytidine; a carbazole cytidine; a pyridoindole cytidine; a 7-deazaguanosine; a 2-aminopyridine; a 2-pyridone; a 5-substituted pyrimidine; a 6-azapyrimidine; an N-2, N-6 or O-6 substituted purine; a 2-aminopropyladenine; a 5-propynyluracil; and a 5-propynylcytosine.

12. The method of any one of claims 1 to 11, wherein the DNA-targeting RNA is conjugated to a moiety selected from: a polyamine; a polyamide; a polyethylene glycol; a polyether; a cholesterol moiety; a cholic acid; a thioether; a thiocholesterol; an aliphatic chain; a phospholipid; an adamantane acetic acid; a palmityl moiety; an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety; a biotin; a phenazine; a folate; a phenanthridine; an anthraquinone; an acridine; a fluorescein; a rhodamine; a dye; a coumarin; a moiety that improves uptake, enhances resistance to degradation, and/or strengthens sequence-specific hybridization; and a moiety that improves uptake, distribution, metabolism or excretion.

13. The method of any one of claims 1 to 11, wherein the DNA-targeting RNA comprises two RNA molecules, each of which comprises one of the two complementary stretches of nucleotides that hybridize to form the dsRNA, wherein said two RNA molecules are chemically linked to one or more moieties or conjugates.

14. The method of any one of claims 1 to 12, wherein the DNA-targeting RNA comprises two RNA molecules, each of which comprises one of the two complementary stretches of nucleotides that hybridize to form the dsRNA, wherein said two RNA molecules are not covalently linking by intervening nucleotides.

15. The method of any one of claims 1 to 14, wherein the contacting comprises introducing into the cell (a) said Cas9 polypeptide, or a polynucleotide encoding said Cas9 polypeptide, and (b) said DNA-targeting RNA, or one or more DNA polynucleotides encoding said DNA-targeting RNA.

16. The method of claim 15, wherein one or more of:
the polynucleotide encoding said Cas9 polypeptide, and

the one or more DNA polynucleotides encoding said DNA-targeting RNA is a recombinant expression vector.

17. The method of claim 16, wherein the recombinant expression vector is a viral vector.

18. The method of claim 17, wherein the viral vector is selected from the group consisting of retroviral, lentiviral, adenoviral, adeno-associated, and herpes simplex virus vectors.

19. The method of claim 16, wherein the recombinant expression vector is selected from the group consisting of plasmids, cosmids, minicircles, phage, and viral vectors.

20. The method of any one of claims 15 to 19, wherein the method further comprises introducing into the cell a donor polynucleotide.

21. The method according to any one of claims 1 to 20, wherein a protein transduction domain is covalently linked to the amino terminus of the Cas9 polypeptide, wherein said protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of the cell.

22. The method according to any one of claims 1 to 20 wherein a protein transduction domain is covalently linked to the carboxyl terminus of the Cas9 polypeptide, wherein said protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of the cell.

23. The method according to any one of claims 1 to 22, wherein the Cas9 polypeptide comprises one or more mutations in a RuvC domain and/or a HNH domain.

24. The method according to any one of claims 1 to 23, wherein the Cas9 polypeptide has reduced nuclease activity compared to a corresponding wild-type Cas9 protein.

25. The method according to any one of claims 1 to 24, wherein the target DNA is edited via a non-homologous end joining (NHEJ) repair mechanism.

26. The method according to any one of claims 1 to 24, wherein the target DNA is edited via

a homology-directed repair (HDR) mechanism.

27. The method according to any one of claims 1 to 24, wherein the target DNA molecule is edited via insertion of a sequence of a donor polynucleotide into a cleaved strand of the target DNA molecule.

28. A composition comprising:
(a) a Cas9 polypeptide, or a polynucleotide encoding said Cas9 polypeptide, and
(b) a DNA-targeting RNA, or one or more DNA polynucleotides encoding said DNA-targeting RNA, wherein said DNA-targeting RNA comprises:
(i) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a sequence in a target DNA, wherein the target DNA is present in a plant cell, an animal cell, or a single-cell eukaryotic organism, and
(ii) a protein-binding segment that interacts with said Cas9 polypeptide,
wherein the protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA),
wherein said dsRNA comprises complementary nucleotides of a tracrRNA and a CRISPR RNA (crRNA),
wherein the composition is for guiding said Cas9 polypeptide to a target DNA by contacting the target DNA in a eukaryotic cell with a complex comprising said Cas9 polypeptide and said DNA-targeting RNA.

29. A composition comprising:
(a) a Cas9 polypeptide, or a polynucleotide encoding said Cas9 polypeptide, and
(b) a DNA-targeting RNA, or one or more DNA polynucleotides encoding said DNA-targeting RNA, wherein said DNA-targeting RNA comprises:
(i) a DNA-targeting segment comprising a nucleotide sequence having 100% complementarity to an 18-25 nucleotide long target sequence in a target DNA, wherein the target DNA is present in a plant cell, an animal cell, or a single-cell eukaryotic organism, and
(ii) a protein-binding segment that interacts with said Cas9 polypeptide,
wherein the protein-binding segment comprises two complementary stretches of nucleotides that

hybridize to form a double stranded RNA (dsRNA),
wherein said dsRNA comprises complementary nucleotides of a tracrRNA and a CRISPR RNA (crRNA).

30. A composition comprising:
(a) a Cas9 polypeptide, or a polynucleotide encoding said Cas9 polypeptide, and
(b) a DNA-targeting RNA, or one or more DNA polynucleotides encoding said DNA-targeting RNA, wherein said DNA-targeting RNA comprises:
(i) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a sequence in a target DNA, wherein the target DNA is present in a plant cell, an animal cell, or a single-cell eukaryotic organism, and
(ii) a protein-binding segment that interacts with said Cas9 polypeptide,
wherein the protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA),
wherein said dsRNA comprises complementary nucleotides of a tracrRNA and a CRISPR RNA (crRNA),
wherein the composition is characterized by at least one of the following:
(A) said two complementary stretches of nucleotides hybridize to form 8 to 15 base pairs or hybridize to form 15 to 18 base pairs;
(B) a protein transduction domain is covalently linked to the amino terminus of the Cas9 polypeptide, wherein said protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of a cell;
(C) a protein transduction domain is covalently linked to the carboxyl terminus of the Cas9 polypeptide, wherein said protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of a cell; and
(D) one or more of:
the polynucleotide encoding said Cas9 polypeptide, and
the one or more DNA polynucleotides encoding said DNA-targeting RNA,
is a viral expression vector.

31. The composition of any one of claims 28 to 30, wherein said two complementary stretches of nucleotides hybridize to form 8 to 15 base pairs or hybridize to form 15 to 18 base pairs.

32. The composition of any one of claims 28 to 31, wherein the percent complementarity between the nucleotides that hybridize to form the dsRNA of the protein-binding segment is greater than 70%.

33. The composition of any one of claims 28 to 32, wherein the target DNA is chromosomal DNA.

34. The composition of any one of claims 28 to 33, wherein a protein transduction domain is covalently linked to the amino terminus of the Cas9 polypeptide, wherein said protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of a cell.

35. The composition of any one of claims 28 to 33, wherein a protein transduction domain is covalently linked to the carboxyl terminus of the Cas9 polypeptide, wherein said protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of a cell.

36. The composition of any one of claims 28 to 35, wherein the Cas9 polypeptide and the DNA-targeting RNA are in a eukaryotic cell which is not a human cell in vivo.

37. The composition of any one of claims 28 to 36, wherein the DNA-targeting RNA comprises one or more of: a modified nucleobase, a modified backbone or non-natural internucleoside linkage, a modified sugar moiety, a Locked Nucleic Acid, and a Peptide Nucleic Acid.

38. The composition of any one of claims 28 to 36, wherein the DNA-targeting RNA comprises a non-natural internucleoside linkage that comprises one or more of: a phosphorothioate, a phosphoramidate, a non-phosphodiester, a heteroatom, a chiral phosphorothioate, a phosphorodithioate, a phosphotriester, an aminoalkylphosphotriester, a 3'-alkylene phosphonates, a 5'-alkylene phosphonate, a chiral phosphonate, a phosphinate, a 3'-amino phosphoramidate, an aminoalkylphosphoramidate, a phosphorodiamidate, a thionophosphoramidate, a thionoalkylphosphonate, a thionoalkylphosphotriester, a selenophosphate, and a boranophosphate

39. The composition of any one of claims 28 to 36, wherein the DNA-targeting RNA

comprises one or more of: (i) a non-natural internucleoside linkage selected from a phosphorothioate, an inverted polarity linkage, and an abasic nucleoside linkage; (ii) a locked nucleic acid (LNA); and (iii) a modified sugar moiety selected from 2'-O-methoxyethyl, 2'-O-methyl, and 2'-fluoro.

40. The composition of any one of claims 28 to 36, wherein the DNA-targeting RNA comprises one or more modified sugar moieties selected from: 2'-O-(2-methoxyethyl), 2'-dimethylaminooxyethoxy, 2'-dimethylaminoethoxyethoxy, 2'-O-methyl, and 2′ -fluoro.

41. The composition of any one of claims 28 to 36, wherein the DNA-targeting RNA comprises a nucleobase comprising one or more of: a 5-methylcytosine; a 5-hydroxymethyl cytosine; a xanthine; a hypoxanthine; a 2-aminoadenine; a 6-methyl derivative of adenine; a 6-methyl derivative of guanine; a 2-propyl derivative of adenine; a 2-propyl derivative of guanine; a 2-thiouracil; a 2-thiothymine; a 2-thiocytosine; a 5-propynyl uracil; a 5-propynyl cytosine; a 6-azo uracil; a 6-azo cytosine; a 6-azo thymine; a pseudouracil; a 4-thiouracil; an 8-haloadenin; an 8-aminoadenin; an 8-thioladenin; an 8-thioalkyladenin; an 8-hydroxyladenin; an 8-haloguanin; an 8-aminoguanin; an 8-thiolguanin; an 8-thioalkylguanin; an 8-hydroxylguanin; a 5-halouracil; a 5-bromouracil; a 5-trifluoromethyluracil; a 5-halocytosine; a 5-bromocytosine; a 5-trifluoromethylcytosine; a 5-substituted uracil; a 5-substituted cytosine; a 7-methylguanine; a 7-methyladenine; a 2-F-adenine; a 2-amino-adenine; an 8-azaguanine; an 8-azaadenine; a 7-deazaguanine; a 7-deazaadenine; a 3-deazaguanine; a 3-deazaadenine; a tricyclic pyrimidine; a phenoxazine cytidine; a phenothiazine cytidine; a substituted phenoxazine cytidine; a carbazole cytidine; a pyridoindole cytidine; a 7-deazaguanosine; a 2-aminopyridine; a 2-pyridone; a 5-substituted pyrimidine; a 6-azapyrimidine; an N-2, N-6 or O-6 substituted purine; a 2-aminopropyladenine; a 5-propynyluracil; and a 5-propynylcytosine.

42. The composition of any one of claims 28 to 41, wherein the DNA-targeting RNA is

conjugated to a moiety selected from: a polyamine; a polyamide; a polyethylene glycol; a polyether; a cholesterol moiety; a cholic acid; a thioether; a thiocholesterol; an aliphatic chain; a phospholipid; an adamantane acetic acid; a palmityl moiety; an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety; a biotin; a phenazine; a folate; a phenanthridine; an anthraquinone; an acridine; a fluorescein; a rhodamine; a dye; a coumarin; a moiety that improves uptake, enhances resistance to degradation, and/or strengthens sequence-specific hybridization; and a moiety that improves uptake, distribution, metabolism or excretion.

43. The composition of any one of claims 28 to 41, wherein the DNA-targeting RNA comprises two RNA molecules, each of which comprises one of the two complementary stretches of nucleotides that hybridize to form the dsRNA, wherein said two RNA molecules are chemically linked to one or more moieties or conjugates.

44. The composition of any one of claims 28 to 42, wherein the DNA-targeting RNA comprises two RNA molecules, each of which comprises one of the two complementary stretches of nucleotides that hybridize to form the dsRNA, wherein said two RNA molecules are not covalently linking by intervening nucleotides.

45. The composition of any one of claims 28 to 44, wherein one or more of:
the polynucleotide encoding said Cas9 polypeptide, and
the one or more DNA polynucleotides encoding said DNA-targeting RNA,
is a recombinant expression vector.

46. The composition of claim 45, wherein the recombinant expression vector is a viral vector.

47. The composition of claim 46, wherein the viral vector is selected from the group consisting of retroviral, lentiviral, adenoviral, adeno-associated, and herpes simplex virus vectors.

48. The composition of claim 45, wherein the recombinant expression vector is selected from the group consisting of plasmids, cosmids, minicircles, phage, and viral vectors.

49. The composition of any one claims 28 to 48, wherein the Cas9 polypeptide comprises one or more mutations in a RuvC domain and/or a HNH domain.

50. The composition of any one claims 28 to 49, wherein the Cas9 polypeptide has reduced nuclease activity compared to a corresponding wild-type Cas9 protein.

51. A composition comprising a DNA-targeting RNA, or a DNA polynucleotide encoding said DNA-targeting RNA, wherein the DNA-targeting RNA comprises:
(a) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a target sequence in a target DNA, wherein the target DNA is present in a plant cell, an animal cell, or a single-cell eukaryotic organism, and
(b) a protein-binding segment that interacts with a Cas9 protein,
wherein the protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA),
wherein said dsRNA comprises complementary nucleotides of a tracrRNA and a CRISPR RNA (crRNA),
wherein the composition is for guiding said Cas9 polypeptide to a target DNA by contacting the target DNA in a eukaryotic cell with a complex comprising said Cas9 polypeptide and said DNA-targeting RNA.

52. A DNA-targeting RNA, or a DNA polynucleotide encoding said DNA-targeting RNA, wherein the DNA-targeting RNA comprises:
(a) a DNA-targeting segment comprising a nucleotide sequence having 100% complementarity to an 18-25 nucleotide long target sequence in a target DNA, wherein the target DNA is present in a plant cell, an animal cell, or a single-cell eukaryotic organism, and
(b) a protein-binding segment that interacts with a Cas9 protein,
wherein the protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA),
wherein said dsRNA comprises complementary nucleotides of a tracrRNA and a CRISPR RNA (crRNA) .

53. A DNA-targeting RNA, or a DNA polynucleotide encoding said DNA-targeting RNA, wherein the DNA-targeting RNA comprises:
(a) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a target sequence in a target DNA, wherein the target DNA is present in a plant cell, an animal cell, or a single-cell eukaryotic organism, and
(b) a protein-binding segment that interacts with a Cas9 protein,
wherein the protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA),
wherein said dsRNA comprises complementary nucleotides of a tracrRNA and a CRISPR RNA (crRNA),
wherein the DNA-targeting RNA, or a DNA polynucleotide encoding said DNA-targeting RNA is characterized by at least one of the following:
(A) said two complementary stretches of nucleotides hybridize to form 8 to 15 base pairs or hybridize to form 15 to 18 base pairs; and
(B) the DNA polynucleotide encoding said DNA-targeting RNA is a viral expression vector.

54. The composition of claim 51 or DNA-targeting RNA, or a DNA polynucleotide encoding said DNA-targeting RNA of claims 52 and 53 wherein said two complementary stretches of nucleotides hybridize to form 8 to 15 base pairs or hybridize to form 15 to 18 base pairs.

55. The composition of claim 51 or 54, or DNA-targeting RNA, or a DNA polynucleotide encoding said DNA-targeting RNA of any one of claims 52 to 54, wherein the percent complementarity between the nucleotides that hybridize to form the dsRNA of the protein-binding segment is greater than 70%.

56. The composition of any one of claims 51 and 54 to 55, or DNA-targeting RNA, or a DNA polynucleotide encoding said DNA-targeting RNA of any one of claims 52 to 55, wherein the target DNA is chromosomal DNA.

57. The composition of any one of claims 51 and 54 to 56, or DNA-targeting RNA of any one of claims 52 to 56, wherein the DNA-targeting RNA comprises one or more of: a modified

nucleobase, a modified backbone or non-natural internucleoside linkage, a modified sugar moiety, a Locked Nucleic Acid, and a Peptide Nucleic Acid.

58. The composition of any one of claims 51 and 54 to 56, or DNA-targeting RNA of any one of claims 52 to 56, wherein the DNA-targeting RNA comprises a non-natural internucleoside linkage that comprises one or more of: a phosphorothioate, a phosphoramidate, a non-phosphodiester, a heteroatom, a chiral phosphorothioate, a phosphorodithioate, a phosphotriester, an aminoalkylphosphotriester, a 3'-alkylene phosphonates, a 5'-alkylene phosphonate, a chiral phosphonate, a phosphinate, a 3'-amino phosphoramidate, an aminoalkylphosphoramidate, a phosphorodiamidate, a thionophosphoramidate, a thionoalkylphosphonate, a thionoalkylphosphotriester, a selenophosphate, and a boranophosphate.

59. The composition of any one of claims 51 and 54 to 56, or DNA-targeting RNA of any one of claims 52 to 56, wherein the DNA-targeting RNA comprises one or more of: (i) a non-natural internucleoside linkage selected from a phosphorothioate, an inverted polarity linkage, and an abasic nucleoside linkage; (ii) a locked nucleic acid (LNA); and (iii) a modified sugar moiety selected from 2'-O-methoxyethyl, 2’-O-methyl, and 2’-fluoro.

60. The composition of any one of claims 51 and 54 to 56, or DNA-targeting RNA of any one of claims 52 to 56, wherein the DNA-targeting RNA comprises one or more modified sugar moieties selected from: 2'-O-(2-methoxyethyl), 2'-dimethylaminooxyethoxy, 2'-dimethylaminoethoxyethoxy, 2’-O-methyl, and 2′ -fluoro.

61. The composition of any one of claims 51 and 54 to 56, or DNA-targeting RNA of any one of claims 52 to 56, wherein the DNA-targeting RNA comprises a nucleobase comprising one or more of: a 5-methylcytosine; a 5-hydroxymethyl cytosine; a xanthine; a hypoxanthine; a 2-aminoadenine; a 6-methyl derivative of adenine; a 6-methyl derivative of guanine; a 2-propyl derivative of adenine; a 2-propyl derivative of guanine; a 2-thiouracil; a 2-thiothymine; a 2-

thiocytosine; a 5-propynyl uracil; a 5-propynyl cytosine; a 6-azo uracil; a 6-azo cytosine; a 6-azo thymine; a pseudouracil; a 4-thiouracil; an 8-haloadenin; an 8-aminoadenin; an 8-thioladenin; an 8-thioalkyladenin; an 8-hydroxyladenin; an 8-haloguanin; an 8-aminoguanin; an 8-thiolguanin; an 8-thioalkylguanin; an 8-hydroxylguanin; a 5-halouracil; a 5-bromouracil; a 5-trifluoromethyluracil; a 5-halocytosine; a 5-bromocytosine; a 5-trifluoromethylcytosine; a 5-substituted uracil; a 5-substituted cytosine; a 7-methylguanine; a 7-methyladenine; a 2-F-adenine; a 2-amino-adenine; an 8-azaguanine; an 8-azaadenine; a 7-deazaguanine; a 7-deazaadenine; a 3-deazaguanine; a 3-deazaadenine; a tricyclic pyrimidine; a phenoxazine cytidine; a phenothiazine cytidine; a substituted phenoxazine cytidine; a carbazole cytidine; a pyridoindole cytidine; a 7-deazaguanosine; a 2-aminopyridine; a 2-pyridone; a 5-substituted pyrimidine; a 6-azapyrimidine; an N-2, N-6 or O-6 substituted purine; a 2-aminopropyladenine; a 5-propynyluracil; and a 5-propynylcytosine.

62. The composition of any one of claims 51 and 54 to 61, or DNA-targeting RNA of any one of claims 52 to 61, wherein the DNA-targeting RNA is conjugated to a moiety selected from: a polyamine; a polyamide; a polyethylene glycol; a polyether; a cholesterol moiety; a cholic acid; a thioether; a thiocholesterol; an aliphatic chain; a phospholipid; an adamantane acetic acid; a palmityl moiety; an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety; a biotin; a phenazine; a folate; a phenanthridine; an anthraquinone; an acridine; a fluorescein; a rhodamine; a dye; a coumarin; a moiety that improves uptake, enhances resistance to degradation, and/or strengthens sequence-specific hybridization; and a moiety that improves uptake, distribution, metabolism or excretion.

63. The composition of any one of claims 51 and 54 to 61, or DNA-targeting RNA of any one of claims 52 to 61, wherein the DNA-targeting RNA comprises two RNA molecules, each of which comprises one of the two complementary stretches of nucleotides that hybridize to form the dsRNA, wherein said two RNA molecules are chemically linked to one or more moieties or

conjugates.

64. The composition of any one of claims 51 and 54 to 62, or DNA-targeting RNA of any one of claims 52 to 62, or a DNA polynucleotide encoding said DNA-targeting RNA of any one of claims 52 to 56, wherein the DNA-targeting RNA comprises two RNA molecules, each of which comprises one of the two complementary stretches of nucleotides that hybridize to form the dsRNA, wherein said two RNA molecules are not covalently linking by intervening nucleotides.

65. One or more nucleic acids comprising:
(a) one or more nucleotide sequences encoding a DNA-targeting RNA, wherein the DNA-targeting RNA comprises:
(i) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a target sequence in a target DNA, and
(ii) a protein-binding segment that interacts with a Cas9 polypeptide,
wherein the protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA),
wherein said dsRNA comprises complementary nucleotides of a tracrRNA and a CRISPR RNA (crRNA),
wherein said one or more nucleotide sequences are operably linked to one or more promoters that are functional in a eukaryotic cell; and, optionally,
(b) a nucleotide sequence encoding a Cas9 polypeptide, wherein the nucleotide sequence encoding said Cas9 polypeptide is operably linked to a promoter functional in a eukaryotic cell.

66. The one or more nucleic acids of claim 65, wherein said nucleic acids are one or more recombinant expression vectors.

67. The one or more nucleic acids of claim 66, wherein the one or more recombinant expression vectors are one or more viral vectors.

68. The one or more nucleic acids of claim 67, wherein the one or more viral vectors are selected from the group consisting of retroviral, lentiviral, adenoviral, adeno-associated, and

herpes simplex virus vectors.

69. The one or more nucleic acids of claim 66, wherein the one or more recombinant expression vectors are selected from the group consisting of plasmids, cosmids, minicircles, phage, and viral vectors.

70. The one or more nucleic acids of any one of claims 65 to 69, wherein the nucleotide sequence encoding the Cas9 polypeptide encodes a protein transduction domain that is covalently linked to the carboxyl terminus or the amino terminus of the Cas9 polypeptide, wherein said protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of the eukaryotic cell.

71. The one or more nucleic acids of any one of claims 65 to 70, wherein said two complementary stretches of nucleotides hybridize to form 8 to 15 base pairs or hybridize to form 15 to 18 base pairs.

72. The one or more nucleic acids of any one of claims 65 to 71, wherein the percent complementarity between the nucleotides that hybridize to form the dsRNA of the protein-binding segment is greater than 70%.

73. The one or more nucleic acids of any one of claims 65 to 72, wherein the target DNA is chromosomal DNA.

74. The one or more nucleic acids of any one of claims 65 to 73, wherein the Cas9 polypeptide comprises one or more mutations in a RuvC domain and/or a HNH domain.

75. The one or more nucleic acids of any one of claims 65 to 74, wherein the Cas9 polypeptide has reduced nuclease activity compared to a corresponding wild-type Cas9 protein.

76. A kit comprising
(a) a Cas9 polypeptide, or a nucleic acid encoding said Cas9 polypeptide; and
(b) a DNA-targeting RNA, or one or more nucleic acids encoding said DNA-targeting RNA,

wherein the DNA-targeting RNA comprises:
(i) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a sequence in a target DNA, wherein the target DNA is present in a plant cell, an animal cell, or a single-cell eukaryotic organism, and
(ii) a protein-binding segment that interacts with said Cas9 polypeptide,
wherein the protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA),
wherein said dsRNA comprises complementary nucleotides of a tracrRNA and a CRISPR RNA (crRNA), and
wherein (a) and (b) are in the same or separate containers,
wherein the kit is for guiding said Cas9 polypeptide to a target DNA by contacting the target DNA in a eukaryotic cell with a complex comprising said Cas9 polypeptide and said DNA-targeting RNA.

77. A kit comprising
(a) a Cas9 polypeptide, or a nucleic acid encoding said Cas9 polypeptide; and
(b) a DNA-targeting RNA, or one or more nucleic acids encoding said DNA-targeting RNA,

wherein the DNA-targeting RNA comprises:
(i) a DNA-targeting segment comprising a nucleotide sequence having 100% complementarity to an 18-25 nucleotide long target sequence in a target DNA, wherein the target DNA is present in a plant cell, an animal cell, or a single-cell eukaryotic organism, and
(ii) a protein-binding segment that interacts with said Cas9 polypeptide,
wherein the protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA),
wherein said dsRNA comprises complementary nucleotides of a tracrRNA and a CRISPR RNA (crRNA), and
wherein (a) and (b) are in the same or separate containers.

78. A kit comprising
(a) a Cas9 polypeptide, or a nucleic acid encoding said Cas9 polypeptide; and
(b) a DNA-targeting RNA, or one or more nucleic acids encoding said DNA-targeting RNA,
wherein the DNA-targeting RNA comprises:
(i) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a sequence in a target DNA, wherein the target DNA is present in a plant cell, an animal cell, or a single-cell eukaryotic organism, and
(ii) a protein-binding segment that interacts with said Cas9 polypeptide,
wherein the protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA),
wherein said dsRNA comprises complementary nucleotides of a tracrRNA and a CRISPR RNA (crRNA), and
wherein (a) and (b) are in the same or separate containers,
wherein the kit is characterized by at least one of the following:
(A) one or more of:
the polynucleotide encoding said Cas9 polypeptide, and
the one or more DNA polynucleotides encoding said DNA-targeting RNA,
is a viral expression vector; and
(B) a protein transduction domain is covalently linked to the carboxyl terminus or the amino terminus of the Cas9 polypeptide, wherein said protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of a cell.

79. The kit of any one of claims 76 to 78, wherein the target DNA is chromosomal DNA.

80. The kit of any one of claims 76 to 79, wherein one or more of:
the nucleic acid comprising a nucleotide sequence encoding said Cas9 polypeptide, and
the one or more nucleic acids comprising a nucleotide sequence encoding said DNA-targeting RNA, is a recombinant expression vector.

81. The kit of claim 80, wherein the recombinant expression vector is a viral vector.

82. The kit of claim 81, wherein the viral vector is selected from the group consisting of retroviral, lentiviral, adenoviral, adeno-associated, and herpes simplex virus vectors.

83. The kit of claim 80, wherein the recombinant expression vector is selected from the group consisting of plasmids, cosmids, minicircles, phage, and viral vectors.

84. The kit of any of claims 76 to 83, wherein a protein transduction domain is covalently linked to the carboxyl terminus or the amino terminus of the Cas9 polypeptide, wherein said protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of a cell.

85. The kit of any of claims 76 to 84 wherein the DNA-targeting RNA comprises one or more of: a modified nucleobase, a modified backbone or non-natural internucleoside linkage, a modified sugar moiety, a Locked Nucleic Acid, and a Peptide Nucleic Acid.

86. The kit of any of claims 76 to 84, wherein the DNA-targeting RNA comprises a non-natural internucleoside linkage that comprises one or more of: a phosphorothioate, a phosphoramidate, a non-phosphodiester, a heteroatom, a chiral phosphorothioate, a phosphorodithioate, a phosphotriester, an aminoalkylphosphotriester, a 3'-alkylene phosphonates, a 5'-alkylene phosphonate, a chiral phosphonate, a phosphinate, a 3'-amino phosphoramidate, an aminoalkylphosphoramidate, a phosphorodiamidate, a thionophosphoramidate, a thionoalkylphosphonate, a thionoalkylphosphotriester, a selenophosphate, and a boranophosphate.

87. The kit of any of claims 76 to 84, wherein the DNA-targeting RNA comprises one or more of: (i) a non-natural internucleoside linkage selected from a phosphorothioate, an inverted polarity linkage, and an abasic nucleoside linkage; (ii) a locked nucleic acid (LNA); and (iii) a modified sugar moiety selected from 2'-O-methoxyethyl, 2’-O-methyl, and 2’-fluoro.

88. The kit of any of claims 76 to 84, wherein the DNA-targeting RNA comprises one or more modified sugar moieties selected from: 2'-O-(2-methoxyethyl), 2'-

dimethylaminooxyethoxy, 2'-dimethylaminoethoxyethoxy, 2’-O-methyl, and 2′ -fluoro.

89. The kit of any of claims 76 to 84, wherein the DNA-targeting RNA comprises a nucleobase comprising one or more of: a 5-methylcytosine; a 5-hydroxymethyl cytosine; a xanthine; a hypoxanthine; a 2-aminoadenine; a 6-methyl derivative of adenine; a 6-methyl derivative of guanine; a 2-propyl derivative of adenine; a 2-propyl derivative of guanine; a 2-thiouracil; a 2-thiothymine; a 2-thiocytosine; a 5-propynyl uracil; a 5-propynyl cytosine; a 6-azo uracil; a 6-azo cytosine; a 6-azo thymine; a pseudouracil; a 4-thiouracil; an 8-haloadenin; an 8-aminoadenin; an 8-thioladenin; an 8-thioalkyladenin; an 8-hydroxyladenin; an 8-haloguanin; an 8-aminoguanin; an 8-thiolguanin; an 8-thioalkylguanin; an 8-hydroxylguanin; a 5-halouracil; a 5-bromouracil; a 5-trifluoromethyluracil; a 5-halocytosine; a 5-bromocytosine; a 5-trifluoromethylcytosine; a 5-substituted uracil; a 5-substituted cytosine; a 7-methylguanine; a 7-methyladenine; a 2-F-adenine; a 2-amino-adenine; an 8-azaguanine; an 8-azaadenine; a 7-deazaguanine; a 7-deazaadenine; a 3-deazaguanine; a 3-deazaadenine; a tricyclic pyrimidine; a phenoxazine cytidine; a phenothiazine cytidine; a substituted phenoxazine cytidine; a carbazole cytidine; a pyridoindole cytidine; a 7-deazaguanosine; a 2-aminopyridine; a 2-pyridone; a 5-substituted pyrimidine; a 6-azapyrimidine; an N-2, N-6 or O-6 substituted purine; a 2-aminopropyladenine; a 5-propynyluracil; and a 5-propynylcytosine.

90. The kit of any of claims 76 to 89, wherein the DNA-targeting RNA is conjugated to a moiety selected from: a polyamine; a polyamide; a polyethylene glycol; a polyether; a cholesterol moiety; a cholic acid; a thioether; a thiocholesterol; an aliphatic chain; a phospholipid; an adamantane acetic acid; a palmityl moiety; an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety; a biotin; a phenazine; a folate; a phenanthridine; an anthraquinone; an acridine; a fluorescein; a rhodamine; a dye; a coumarin; a moiety that improves uptake, enhances resistance to degradation, and/or strengthens sequence-specific hybridization; and a moiety that improves uptake, distribution, metabolism or excretion.

91. The kit of any one of claims 76 to 89, wherein the DNA-targeting RNA comprises two RNA molecules, each of which comprises one of the two complementary stretches of nucleotides that hybridize to form the dsRNA, wherein said two RNA molecules are chemically linked to one or more moieties or conjugates.

92. The kit of any one of claims 76 to 90, wherein the DNA-targeting RNA comprises two RNA molecules, each of which comprises one of the two complementary stretches of nucleotides that hybridize to form the dsRNA, wherein said two RNA molecules are not covalently linking by intervening nucleotides.

93. The kit of any of claims 76 to 92, wherein the Cas9 polypeptide comprises one or more mutations in a RuvC domain and/or a HNH domain.

94. The kit of any of claims 76 to 93, wherein the Cas9 polypeptide has reduced nuclease activity compared to a corresponding wild-type Cas9 protein.

95. The composition of any one of claims 28 to 64, or the DNA-targeting RNA of any one of claims 52 to 64, or DNA polynucleotide encoding said DNA-targeting RNA of claims 52 to 56 and 64 , or the one or more nucleic acids of any one of claims 65 to 75, or the kit of any one of claims 76 to 94, for use in a method of therapeutic treatment of a patient.

96. A genetically modified eukaryotic cell, comprising one or more of:
(a) a DNA-targeting RNA, and/or a nucleic acid that encodes the DNA-targeting RNA, wherein the DNA-targeting RNA comprises:
(i) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a sequence in the target DNA, and
(ii) a protein-binding segment that interacts with a Cas9 polypeptide,
wherein the protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA),
wherein said dsRNA comprises complementary nucleotides of a tracrRNA and a CRISPR RNA (crRNA), and

(b) the Cas9 polypeptide and/or a nucleic acid that encodes the Cas9 polypeptide,
wherein the cell is not a human cell in vivo, not a human germ cell, and not a human embryonic cell.

97. The genetically modified eukaryotic cell of claim 96, wherein said two complementary stretches of nucleotides hybridize to form 8 to 15 base pairs or hybridize to form 15 to 18 base pairs.

98. The genetically modified eukaryotic cell of claim 96 or 97, wherein the percent complementarity between the nucleotides that hybridize to form the dsRNA of the protein-binding segment is greater than 70%.

99. The genetically modified eukaryotic cell of any of claims 96 to 98, wherein the target DNA is chromosomal DNA of said eukaryotic cell.

100. The genetically modified eukaryotic cell of any one of claims 96 to 99, wherein a protein transduction domain is covalently linked to the carboxyl terminus or the amino terminus of the Cas9 polypeptide, wherein said protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of the cell.

101. The genetically modified eukaryotic cell of any one of claims 96 to 100, wherein the DNA-targeting RNA comprises one or more of: a modified nucleobase, a modified backbone or non-natural internucleoside linkage, a modified sugar moiety, a Locked Nucleic Acid, and a Peptide Nucleic Acid.

102. The genetically modified eukaryotic cell of any one of claims 96 to 100, wherein the DNA-targeting RNA comprises a non-natural internucleoside linkage that comprises one or more of: a phosphorothioate, a phosphoramidate, a non-phosphodiester, a heteroatom, a chiral phosphorothioate, a phosphorodithioate, a phosphotriester, an aminoalkylphosphotriester, a 3'-alkylene phosphonates, a 5'-alkylene phosphonate, a chiral phosphonate, a phosphinate, a 3'-amino phosphoramidate, an aminoalkylphosphoramidate, a phosphorodiamidate, a thionophosphoramidate, a thionoalkylphosphonate, a thionoalkylphosphotriester, a selenophosphate, and a boranophosphate.

103. The genetically modified eukaryotic cell of any one of claims 96 to 100, wherein the

DNA-targeting RNA comprises one or more of: (i) a non-natural internucleoside linkage selected from a phosphorothioate, an inverted polarity linkage, and an abasic nucleoside linkage; (ii) a locked nucleic acid (LNA); and (iii) a modified sugar moiety selected from 2'-O-methoxyethyl, 2’-O-methyl, and 2’-fluoro.

104. The genetically modified eukaryotic cell of any one of claims 96 to 100, wherein the DNA-targeting RNA comprises one or more modified sugar moieties selected from: 2'-O-(2-methoxyethyl), 2'-dimethylaminooxyethoxy, 2'-dimethylaminoethoxyethoxy, 2’-O-methyl, and 2′ -fluoro.

105. The genetically modified eukaryotic cell of any one of claims 96 to 100, wherein the DNA-targeting RNA comprises a nucleobase comprising one or more of: a 5-methylcytosine; a 5-hydroxymethyl cytosine; a xanthine; a hypoxanthine; a 2-aminoadenine; a 6-methyl derivative of adenine; a 6-methyl derivative of guanine; a 2-propyl derivative of adenine; a 2-propyl derivative of guanine; a 2-thiouracil; a 2-thiothymine; a 2-thiocytosine; a 5-propynyl uracil; a 5-propynyl cytosine; a 6-azo uracil; a 6-azo cytosine; a 6-azo thymine; a pseudouracil; a 4-thiouracil; an 8-haloadenin; an 8-aminoadenin; an 8-thioladenin; an 8-thioalkyladenin; an 8-hydroxyladenin; an 8-haloguanin; an 8-aminoguanin; an 8-thiolguanin; an 8-thioalkylguanin; an 8-hydroxylguanin; a 5-halouracil; a 5-bromouracil; a 5-trifluoromethyluracil; a 5-halocytosine; a 5-bromocytosine; a 5-trifluoromethylcytosine; a 5-substituted uracil; a 5-substituted cytosine; a 7-methylguanine; a 7-methyladenine; a 2-F-adenine; a 2-amino-adenine; an 8-azaguanine; an 8-azaadenine; a 7-deazaguanine; a 7-deazaadenine; a 3-deazaguanine; a 3-deazaadenine; a tricyclic

pyrimidine; a phenoxazine cytidine; a phenothiazine cytidine; a substituted phenoxazine cytidine; a carbazole cytidine; a pyridoindole cytidine; a 7-deazaguanosine; a 2-aminopyridine; a 2-pyridone; a 5-substituted pyrimidine; a 6-azapyrimidine; an N-2, N-6 or O-6 substituted purine; a 2-aminopropyladenine; a 5-propynyluracil; and a 5-propynylcytosine.

106. The genetically modified eukaryotic cell of any one of claims 96 to 105, wherein the DNA-targeting RNA is conjugated to a moiety selected from: a polyamine; a polyamide; a polyethylene glycol; a polyether; a cholesterol moiety; a cholic acid; a thioether; a thiocholesterol; an aliphatic chain; a phospholipid; an adamantane acetic acid; a palmityl moiety; an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety; a biotin; a phenazine; a folate; a phenanthridine; an anthraquinone; an acridine; a fluorescein; a rhodamine; a dye; a coumarin; a moiety that improves uptake, enhances resistance to degradation, and/or strengthens sequence-specific hybridization; and a moiety that improves uptake, distribution, metabolism or excretion.

107. The genetically modified eukaryotic cell of any one of claims 96 to 105, wherein the DNA-targeting RNA comprises two RNA molecules, each of which comprises one of the two complementary stretches of nucleotides that hybridize to form the dsRNA, wherein said two RNA molecules are chemically linked to one or more moieties or conjugates.

108. The genetically modified eukaryotic cell of any one of claims 96 to 106, wherein the DNA-targeting RNA comprises two RNA molecules, each of which comprises one of the two complementary stretches of nucleotides that hybridize to form the dsRNA, wherein said two RNA molecules are not covalently linking by intervening nucleotides.

109. The genetically modified eukaryotic cell of any one of claims 96 to 108, wherein the Cas9 polypeptide comprises one or more mutations in a RuvC domain and/or a HNH domain.

110. The genetically modified eukaryotic cell of any one of claims 96 to 109, wherein the Cas9 polypeptide has reduced nuclease activity compared to a corresponding wild-type Cas9 protein.

111. The genetically modified eukaryotic cell of any one of claims 96 to 110, wherein said

genetically modified eukaryotic cell is not a human cell in vivo.

III. Parties' Arguments and Means of Proof

1. Petitioner's Arguments and Means of Proof

Petitioner has made the arguments in (1) to (3) below and presented the documentary evidence in (4) below as the means of proof, seeking a judgment to the following effect: "The patent for the invention as in claims 1, 2, 6, 15-19, 21, 22, 25, 28-30, 33-36, 45-48, 51-53, 56, 65-70, 73, 76-84, 95, 96, 99, 100, and 111 of Patent No. 6692856 is invalid. Trial costs shall be borne by the Respondents."

(1) The application documents of the first and second priority basis applications do not disclose using the CRISPR-Cas9 system (note by Judicial Panel: understood to mean a complex including the Cas9 polypeptide and DNA-targeting RNAs) to cleave target genes, and therefore the Patent cannot enjoy the benefit of the priority claim, and should be limited to what was filed on January 28, 2013, which is the filing date of the third priority basis application.

(2) Ground for Invalidation 1 (Expanded Prior Application/Petitioner's Exhibit No. 1)

The invention as in claims 1, 2, 6, 15-19, 21, 22, 25, 28-30, 33-36, 45-48, 51-53, 56, 65-70, 73, 76-84, 95, 96, 99, 100, and 111 of the Patent is the same as the invention disclosed in the specification, claims or drawings of a foreign-language patent application from before the Patent's filing date (i.e., January 28, 2013, the filing date of the third priority basis application, as noted in Section (1) above), which is the filed internationally on the international filing date of PCT/US2013/074667 (International Publication Nol 2014/093622, JP 2016-501531 A (Japanese translation of the international application)Petitioner's Exhibit No. 1), whose international publication date is after the filing date of the Patent. Furthermore, at the time of filing, the applicant was not the same as the applicant of the aforementioned foreign-language patent application, and therefore, under Patent Act Article 29-2, as applied mutatis mutandis under Article 184-13, the Patent cannot be granted a patent and is to be found invalid as it falls under Patent Act Article 123 (1) (2).

(3) Ground for Invalidation 2 (Expanded Prior Application/Petitioner's Exhibit No. 2)

The invention as in claims 1, 2, 6, 15-19, 21, 22, 25, 28-30, 33-36, 45-48, 51-53, 56, 65-70, 73, 76-84, 95, 96, 99, 100, and 111 of the Patent is the same as the invention disclosed in the specification, claims or drawings of a foreign-language patent application from before the Patent's filing date (i.e., January 28, 2013, the filing date of the third priority basis application, as noted in Section (1) above), which is the filed internationally on the international filing date of PCT/US2013/074667 (International Publication Nol 2014/093622, JP 2016-501531 A (Japanese translation of the international application)Petitioner's Exhibit No. 1), whose international publication date is after the filing date of the Patent. Furthermore, at the time of filing, the applicant was not the same as the applicant of the aforementioned foreign-language patent application, and therefore, under Patent Act Article 29-2, as applied mutatis mutandis under Article 184-13, the Patent cannot be granted a patent and is to be found invalid as it falls under Patent Act Article 123 (1) (2).

(4) Means of Proof

Petitioner's Exhibit No. 1: JP 2016-501531 A (Japanese translation of PCT application)
Petitioner's Exhibit No. 2: JP 2016-500003 A (Japanese translation of PCT application)
Petitioner's Exhibit No. 3: US Patent Application 61/652086, application documents
Petitioner's Exhibit No. 4: US Patent Application 61/716526, application documents
Petitioner's Exhibit No. 5: US Patent Application 61/736527, application documents
Petitioner's Exhibit No. 6: US Application 61/717324, application documents
Petitioner's Exhibit No. 7: Decision on Motions of Patent Interference No. 106,115
Petitioner's Exhibit No. 8: DECLARATION OF TECHNICAL EXPERT CHAD MIRKIN, PH.D., IN SUPPORT OF BROAD
Petitioner's Exhibit No. 9: Catalyst Magazine 18-20 <https://berkeley.app.box.com/v/catalyst-9-1>
Petitioner's Exhibit No. 10: eLIFE; Vol. 2, e00471 (2013) <https://elifesciences.org/articles/00471>
Petitioner's Exhibit No. 11: "BROAD OPPOSITION 2" <https://acts.uspto.gov/ifiling/PublicView.jsp> submitted by Broad in US Patent Interference No.106,115
Petitioner's Exhibit No. 12: Decision on Priority of Patent Interference No. 106,115 <https://acts.uspto.gov/ifiling/PublicView.jsp>
Petitioner's Exhibit No. 13: DECLARATION OF BRYAN R. CULLEN, PH.D. UNDER 37 C.F.R. §1.132 <https://acts.uspto.gov/ifiling/PublicView.jsp> Interference No. 106,115), Broad Ex. 3451
Petitioner's Exhibit No. 14: PLoS ONE; Vol. 3 (9), e3121, 1-15 (2008)
Petitioner's Exhibit No. 15: J.Mol.Biol.; Vol. 223, 67-78 (1992)

Petitioner's Exhibit No. 16: Genes & Development; Vol. 7, 2016-2032 (1993)
Petitioner's Exhibit No. 17: EMO J.; Vol. 15 (3), 569-580 (1996)
Petitioner's Exhibit No. 18: Journal of Virology; Vol. 73 (3), 1868-1877 (1999)
Petitioner's Exhibit No. 19: Gene Therapy; Vol. 16, 1189-1201 (2009)
Petitioner's Exhibit No. 20: US Patent Application 14/054,414, NOTICE OF ALLOWANCE
Petitioner's Exhibit No. 21: "OPENING BRIEF FOR CROSS-APPELLANTS" submitted by Broad during CAFC appeal in US Patent Interference No.106,115
Petitioner's Exhibit No. 22: Genone Biology; Vol. 18, 218 (2017)
Petitioner's Exhibit No. 22-3: Addgene: p3s-Cas9-HN Sequences https://www.addgene.org/104171/sequences/>
Addene p3s-Cas9-HN Sequencing Result - Sequence Analyzer <https://www.addgene.org/browse/sequence/199282/>
Petitioner's Exhibit No. 22-4: NCBI Blast: Cas9-WT (p3s-Cas9-HN) vs PCT KR 2013 009488 <https://blast.ncbi.nlm.nih.gov/Blast.cgi>
Petitioner's Exhibit No. 23: Decision - Motions of Patent Interference No. 106,132
Petitioner's Exhibit No. 24: Verdict in CAFC Appeal No. 2017-1907 in Patent Interference No.106,048
Petitioner's Exhibit No. 25: Wikipedia, "細胞膜透過ペプチド" [Cell-penetrating peptide], <https://ja.wikipedia.org/wiki/細胞膜透過ペプチド>
(Henceforth, "Petitioner's Exhibit No. 1," "Petitioner's Exhibit No. 2," etc.)

2. Respondents' Arguments and Means of Proof

Respondents have argued that the grounds for invalidation set forth by Petitioner are groundless and submitted the following documentary evidence as a means of proof in seeking a judgment to the following effect: "The claim is not established. Trial costs shall be borne by the Petitioner."

Respondents' Exhibit No. 1: PRESS RELEASE, The Nobel Prize in Chemistry 2020
Respondents' Exhibit No. 2-1: Professor Doyon opinion
Respondents' Exhibit No. 2-2: Translation of Respondents' Exhibit No. 2-1
Respondents' Exhibit No. 2-3: Reference documents for Respondents' Exhibit No. 2-1 and correspondence beetween Petitioner's and Respondents' exhibits
Respondents' Exhibit No. 2-4: Professor Doyon CV
Respondents' Exhibit No. 3-1: Professor Randou opinion
Respondents' Exhibit No. 3-2: Translation of Respondents' Exhibit No. 3-1
Respondents' Exhibit No. 4-1: Professor Wahle opinion
Respondents' Exhibit No. 4-2: Translation of Respondents' Exhibit No. 4-1
Respondents' Exhibit No. 5-1: Professor Zamore opinion

Respondents' Exhibit No. 5-2: Translation of Respondents' Exhibit No. 5-1
Respondents' Exhibit No. 6-1: Professor Songheimer opinion
Respondents' Exhibit No. 6-2: Translation of Respondents' Exhibit No. 6-1
Respondents' Exhibit No. 7-1: Professor Barrangou opinion
Respondents' Exhibit No. 7-2: Translation of Respondents' Exhibit No. 7-1
Respondents' Exhibit No. 8: Ruling on Opposition (Opposition 2020-700867)
Respondents' Exhibit No. 9: Decision (Appeal 2017-13795)
Respondents' Exhibit No. 10: Decision (Appeal 2017-019510)
Respondents' Exhibit No. 11: Decision (Appeal 2020-000013)
Respondents' Exhibit No. 12-1: Science; Vol. 337 (6096), 816-821 and Supplementary Information
Respondents' Exhibit No. 12-2: Translation of Respondents' Exhibit No. 12-1
Respondents' Exhibit No. 13: Nature Methods; Vol. 8 (1), 74-79 (2010)
Respondents' Exhibit No. 14: Nature; Vol. 482 (7385), 331-338 (2012)
Respondents' Exhibit No. 15: Annu. Rev. Genet.; Vol. 45, 273-297 (2011)
Respondents' Exhibit No. 16: Nature Reviews Microbiology; Vol. 9, 467-477 (2011)
Respondents' Exhibit No. 17: Nature; Vol. 463 (7280), 568-571 (2010)
Respondents' Exhibit No. 18: Nature; Vol. 468 (7320), 67-72 (2010)
Respondents' Exhibit No. 19: Science; Vol. 327 (5962), 167-170 (2010)
Respondents' Exhibit No. 20: Microbiology; Vol. 155 (3), 733-740 (2009)
Respondents' Exhibit No. 21: Nucleic Acids Research; Vol. 39 (21), 9275-9282 (2011)
Respondents' Exhibit No. 22: Journal of Bacteriology; Vol. 190 (4), 1401-1412 (2008)
Respondents' Exhibit No. 23: Nature; Vol. 471 (7340), 602-607 and Supplementary Information (2011)
Respondents' Exhibit No. 24: Journal of Bacteriology; Vol. 190 (4), 1390-1400 (2008)
Respondents' Exhibit No. 25: Curr. Opin. Microbiol.; Vol. 14 (3), 321-327 (2011)
Respondents' Exhibit No. 26: The Journal of Cell Biology; Vol. 137 (2), 291-303 (19 97)
Respondents' Exhibit No. 27: Molecular and Cellular Biology; Vol. 20 (1), 213-223 (2000)
Respondents' Exhibit No. 28: Nature Protocols; Vol. 2 (10), 2325-2331 (2007)
Respondents' Exhibit No. 29: Proc. Natl. Acad. Sci. USA; Vol. 105 (50), 19821-19826 (2008)
Respondents' Exhibit No. 30: PLoS ONE; Vol. 3 (9), e3121, 1-15 (2008)
Respondents' Exhibit No. 31: Journal of Genetics and Genomics; Vol. 39 (5), 209-215 (2012)

Respondents' Exhibit No. 32: Methods in Molecular Biology: Molecular Embryology Methods and Protocols; Vol. 97, 487-490 (1999)

Respondents' Exhibit No. 33: Methods in Molecular Biology: Molecular Methods in Developmental Biology Xenopus and Zebrafish; Vol. 127, 125-132 (1999)

Respondents' Exhibit No. 34: Developmental Biology; Vol. 217 (2), 394-405 (2000)

Respondents' Exhibit No. 35: Nature Biotechnology; Vol. 26 (6), 695-701 (2008)

Respondents' Exhibit No. 36: Nature Biotechnology; Vol. 26 (6), 702-708 and Supplementary Information (2008)

Respondents' Exhibit No. 37: Nature Protocols; Vol. 4 (12), 1855-1868 (2009)

Respondents' Exhibit No. 38: PLoS ONE; Vol. 4 (2), e4348, 1-13 (2009)

Respondents' Exhibit No. 39: Proc. Natl. Acad. Sci. USA; Vol. 77 (12), 7380-7384 (1980)

Respondents' Exhibit No. 40: Developmental Biology; Vol. 221 (2), 337-354 (2000)

Respondents' Exhibit No. 41: Nature Cell Biology; Vol. 2, 70-75 (2000)

Respondents' Exhibit No. 42: Reproduction, Fertility and Development; Vol. 17 (1,2), 316 "Abstract 331" (2005)

Respondents' Exhibit No. 43: Science; Vol. 325 (5939), 433 and Supplementary Material (2009)

Respondents' Exhibit No. 44: PLoS ONE; Vol. 5 (1), e8870, 1-7 (2010)

Respondents' Exhibit No. 45: Nature Biotechnology; Vol. 29 (8), 695-696 and Supplementary Information (2011)

Respondents' Exhibit No. 46: Nature; Vol. 299 (5882), 456-458 (1982)

Respondents' Exhibit No. 47: Nethods in Cell Biology; Vol. 48, 451-482 (1995)

Respondents' Exhibit No. 48: Journal of Invertebrate Pathology; Vol. 66, 293-296 (1995)

Respondents' Exhibit No. 49: RNA; Vol. 9 (7), 881-891 (2003)

Respondents' Exhibit No. 50: Methods in Enzymology: RNA Inter ference; Vol. 392, 36-55 (2005)

Respondents' Exhibit No. 51: Methods Mol. Biol.; Vol. 518, 123-133 (2009)

Respondents' Exhibit No. 52: Science; Vol. 333 (6040), 307 and Supplementary Material (2011)

Respondents' Exhibit No. 53: Biochem. Biophys. Res. Commun.; Vol. 116 (3), 1160-1167 (1983)

Respondents' Exhibit No. 54: Mol. Endocrinol.; Vol. 5 (12), 1897-1902 (1991)

Respondents' Exhibit No. 55: Proc. Natl. Acad. Sci. USA; Vol. 88 (7), 2702-2706 (1991)

Respondents' Exhibit No. 56: Cancer Research; Vol. 55 (16), 3490-3494 (1995)

Respondents' Exhibit No. 57: Zebrafish, Methods in Molecular Biology; Vol. 546, 117-130 (2009)

Respondents' Exhibit No. 58: Mechanisms of Development; Vol. 118 (1-2), 91-98 (2002)

Respondents' Exhibit No. 59: Molecluar Biotechnology; Vol. 46 (3), 287-300 (2010)
Respondents' Exhibit No. 60: Nucleic Acids Research; Vol. 21 (9), 2025-2029 (1993)
Respondents' Exhibit No. 61: Molecluar Biotechnology; Vol. 28 (1), 21-31 (2004)
Respondents' Exhibit No. 62: J.Lipid Res.: Vol. 46 (2), 356-365 (2005)
Respondents' Exhibit No. 63: Short. Protocols in Molecular Biology; 9-1 to 9-57, Third Edition (1995)
Respondents' Exhibit No. 64: Exp. Dermatol.; Vol. 17 (5), 405-411 (2008)
Respondents' Exhibit No. 65: Biochimica et Biophysica Acta; Vol. 1466 (1-2), 11-15 (2000)
Respondents' Exhibit No. 66: Biotechniques; Vol. 19 (1), 72-76,78 (1995)
Respondents' Exhibit No. 67: Curr. Opin. Biotechnol.; Vol. 19 (5), 506-510 (2008)
Respondents' Exhibit No. 68: Exp. Dermatol.; Vol. 14 (4), 315-320 (2005)
Respondents' Exhibit No. 69: Nucleic Acids Research; Vol. 39 (21), 9283-9293 (2011)
Respondents' Exhibit No. 70: Nature Biotechnology; Vol. 28 (8), 839-847 (2010)
Respondents' Exhibit No. 71: Molecular and Cellular Biology; Vol. 8 (12), 5495-5503 (1988)
Respondents' Exhibit No. 72: Nonviral Vectors for Gene Therapy; Chapter 7, 139-142, 147-153 (1999)
Respondents' Exhibit No. 73: Nucleic Acids Research; Vol. 20 (7), 1785-1791 (1992)
Respondents' Exhibit No. 74: IUBMS Life; Vol. 62 (3), 183-193 (2010)
Respondents' Exhibit No. 75: Principles of Gene Manipulation and Genomics; 7th Ed ition, 233-235 (2006)
Respondents' Exhibit No. 76: US Patent Application Publication No. 2010/0076057
Respondents' Exhibit No. 77: Nature Medicine; Vol. 8 (12), 1427-1432 (2002)
Respondents' Exhibit No. 78: Nucleic Acids Research; Vol. 39 (12), e82, 1-11 (2011)
Respondents' Exhibit No. 79: Trends in Biotechnology; Vol. 22 (7), 346-353 (2004)
Respondents' Exhibit No. 80: J. Ind. Microbiol. Biotechnol.; Vol. 32 (3), 115-123 (2005)
Respondents' Exhibit No. 81: Gene Therapy; Vol. 12 (10), 795-802 (2005)
Respondents' Exhibit No. 82: The Plant Journal; Vol. 61 (1), 176-187 (2010)
Respondents' Exhibit No. 83: Nature Biotechnology; Vol. 29 (2), 143-148 and Supplementary Information (2010)
Respondents' Exhibit No. 84: Nature; Vol. 459 (7245), 437-441 and Supplementary Information (2009)
Respondents' Exhibit No. 85: eLIFE; Vol. 2, e00563 (2013)
Respondents' Exhibit No. 86: Prices jointly awarded to Dr. Doudna and Dr. Charpentiert (selection)
Respondents' Exhibit No. 87: Genome Research; Vol. 22 (7), 1327-1333 (2012)

Respondents' Exhibit No. 88-1: Nature Biotechnology; Vol. 31 (3), 230-232 and Supplementary Information (2013)

Respondents' Exhibit No. 88-2: Translation of Respondents' Exhibit No. 88-1

Respondents' Exhibit No. 89: Nature Methods; Vol. 8 (9), 753-755 and Supplementary Information (2011)

Respondents' Exhibit No. 90: US Provisional Patent Application No. 61/734,256, specification

Respondents' Exhibit No. 91: Nature Biotechnology; Vol. 29 (2), 149-153 and Supplementary Information (2011)

Respondents' Exhibit No. 92: Nature Protocols; Vol.7 (1), 171-192 and Supplementary Materials (2012)

Respondents' Exhibit No. 93: Manuscript submitted to Science

Respondents' Exhibit No. 94: Nucleic Acids Research; Vol. 40(15), e117, 1-10 (2012)

Respondents' Exhibit No. 95: Science; Vol. 339 (6121), 823-826 (2013)

Respondents' Exhibit No. 96: Nature Biotechnology; Vol. 29 (8), 697-698 and Supplementary Information (2011)

Respondents' Exhibit No. 97: Nature Biotechnology; Vol. 31 (3), 227-229 and Supplementary Information (2013)

Respondents' Exhibit No. 98-1: eLIFE; Vol.2, e00471 (2013)

Respondents' Exhibit No. 98-2: Translation of Respondents' Exhibit No. 98-1

Respondents' Exhibit No. 99-1: Science; Vol. 339 (6121), 819-823 and Supplementary Material (2013)

Respondents' Exhibit No. 99-2: Translation of Respondents' Exhibit No. 99-1

Respondents' Exhibit No. 100-1: Genome Research; Vol. 24 (6), 1012-1019 and Supplementary Information (2014)

Respondents' Exhibit No. 100-2: Translation of Respondents' Exhibit No. 100-1

Respondents' Exhibit No. 101-1: Nature Biotechnology; Vol. 33 (1), 73-80 and Supplementary Information (2014)

Respondents' Exhibit No. 101-2: Translation of Respondents' Exhibit No. 101-1

Respondents' Exhibit No. 102: Gene Therapy; Vol.15, p. 1463-1468 (2008)

Respondents' Exhibit No. 103: Methods; Vol. 53, p.339-346 (2011)

Respondents' Exhibit No. 104: WO 2012/012738 A1

Respondents' Exhibit No. 105: JP 2001-503971 A (Japanese translation of PCT application)

Respondents' Exhibit No. 106: JP H10-80274 A

Respondents' Exhibit No. 107: JP 2002-538842 A (Japanese translation of PCT application)

Respondents' Exhibit No. 108: WO 2011/130346 A1

Respondents' Exhibit No. 109: Cell; Vol. 82 (4), 545-554 (1995)

Respondents' Exhibit No. 110: Nature; Vol. 381 (6580), 332-336 (1996)

Respondents' Exhibit No. 111: Genes & Development; Vol.14(5), 559-573 (2000)

Respondents' Exhibit No. 112: Cold Spring Harbor Prospectives in Biology; Vol. 3 (8), 1-19 (2011)

Respondents' Exhibit No. 113: US Patent No. 4,952,496

Respondents' Exhibit No. 114: J. Mol. Biol.; Vol. 223 (1), 67-78 (1992)

Respondents' Exhibit No. 115: Genes & Development; Vol. 7 (10), 2016-2032 (1993)

Respondents' Exhibit No. 116: Translation of priority basis application 1 (US Patent Application 61/652086)

Respondents' Exhibit No. 117: Translation of priority basis application 2 (US Patent Application 61/716256)

Respondents' Exhibit No. 118: Gene Therapy; Vol. 6 (2), 263-270 (1999)

Respondents' Exhibit No. 119: Journal of Virology; Vol. 73 (3), 1868-1877 (1999)

Respondents' Exhibit No. 120: Gene Therapy; Vol. 16, 1189-1201 (2009)

Respondents' Exhibit No. 121: Methods; Vol. 56 (3), 351-357 (2012)

Respondents' Exhibit No. 122-1: Grounds for ruling in opposition petition case relating to EU Patent Application EP18152360.2/EU Patent EP3401400 :

Respondents' Exhibit No. 122-2: Preliminary claim in opposition petition case relating to EU Patent Application EP18152360.2/EU Patent EP3401400 :

Respondents' Exhibit No. 123: Grounds for ruling in opposition petition case relating to EU Patent Application EP13793997.1/EU Patent EP2800811

Respondents' Exhibit No. 124: Intellectual Property High Court, verdict delivered on December 21, 2022, judgment text for trial decision revocation request case no. 10129 Reiwa 3 (Gyo Ke)

Respondents' Exhibit No. 125: Proc. Natl. Acad. Sci. U.S.A; Vol. 85, 166-5170 (1988)

Respondents' Exhibit No. 126: PloS One; 1:e162, 1-4 (2007)

Respondents' Exhibit No. 127: MOLECULAR AND CELLULAR BIOLOGY; Vol. 7 (6), 2087-2096 (1987)

Respondents' Exhibit No. 128: Proc. Nat. Acad. Sci. U.S.A; Vol. 97 (11), 5995-6000 (2000)

Respondents' Exhibit No. 129: MOLECULAR AND CELLULAR BIOLOGY; Vol. 8 (19), 4204-4211 (1988)

Respondents' Exhibit No. 130: Methods in Molecular Biology; Vol. 113, 465-471 (1999)

Respondents' Exhibit No. 131: Nucleic Acids Res.; Vol. 39 (21), 9275-9282, Supplementary Figures (2011) (Addition of cited sections relating to Respondents' Exhibit No. 21)

Respondents' Exhibit No. 132: CRISPR 2012: 5th Annual CRISPR research meeting Program & Conference Logistics

Respondents' Exhibit No. 133: Science; Vol. 339 (6121), 823-826 (2013)

Respondents' Exhibit No. 134: Proc. Natl. Acad. Sci. U.S.A.; Vol. 93, 3094-3098 (1996)

Respondents' Exhibit No. 135: Molecular and Cellular Biology; Vol. 21 (1), 289-297

(2001)

Respondents' Exhibit No. 136: Mechanisms of Development; Vol. 118, pp. 91-98 (2002)

Respondents' Exhibit No. 137: Nature Biotechnology; Vol. 29, 143-148 and Supplementary Information (2010)

Respondents' Exhibit No. 138: Life; Vol. 62 (3), 183-193 (2010)

Respondents' Exhibit No. 139: THE JOURNAL OF BIOLOGICAL CHEMISTRY; Vol. 269 (14), 10444-10450 (1994)

Respondents' Exhibit No. 140: DIABETES; Vol. 52, 1732-1737 (2003)

Respondents' Exhibit No. 141: Circulation Journal Vol. 66, 1155-1160 (2002)

Respondents' Exhibit No. 142: RNA; Vol. 9, 493-501 (2003)

Respondents' Exhibit No. 143: THE JOURNAL OF GENE MEDICINE; Vol. 8, 433-441 (2006)

Respondents' Exhibit No. 144: Genesis, The Journal of Genetics and Development; Vol. 51 (12); 835-843 (2013)

Respondents' Exhibit No. 145: Cell Research; Vol. 23, 720-723 (2013)

Respondents' Exhibit No. 146: JP 2016-505256 A (Japanese translation of PCT translation)

Respondents' Exhibit No. 147: THE JOURNAL OF BIOLOGICAL CHEMISTRY; Vol. 276 (14), 11323-11334 (2001)

Respondents' Exhibit No. 148: Nucleic Acids Research; Vol. 29, 4826-4833 (2001)

Respondents' Exhibit No. 149: Biochimie; Vol. 92, 1715-1721 (2010)

Respondents' Exhibit No. 150: Proceedings of the National Academy of Sciences; Vol. 85, 5166-5170 (1988)

Respondents' Exhibit No. 151: Nature; Vol. 456, 357-362 (2008)

Respondents' Exhibit No. 152: Streptococcus-bacterial genus-Microbiology Dictionary, Hardy Diagnostics URL:https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/Streptococcus.htm

Respondents' Exhibit No. 153: Nature Review Genetics; Vol. 11, 636-646 (2010)

Respondents' Exhibit No. 154: Nature; Vol. 457, 405-412 (2009)

Respondents' Exhibit No. 155: Nature; Vol. 411, 494-498 (2001)

Respondents' Exhibit No. 156: Nature; Vol. 471, 602-609 (2011), and Supplementary Information

Respondents' Exhibit No. 157: Biology of the Cell; Vol. 100, 125-138 (2008)

Respondents' Exhibit No. 158: Proceedings of the Royal Society of London. Series A, Containing Papers of a Mathematical and Physical Character; Vol. 106, 724-749 (1924)

Respondents' Exhibit No. 159: Science; Vol. 351 (6268), 84-88 (2016), and Supplementary materials

Respondents' Exhibit No. 160: Nature; Vol. 529, 490-495 (2016)
Respondents' Exhibit No. 161: Curr. Biol.; Vol. 26; 15 (2), R61-64 (2016)
Respondents' Exhibit No. 162: GENES & DEVELOPMENT; Vol. 16, 948-958 (2002)
(Henceforth, "Respondents' Exhibit No. 1," "Respondents' Exhibit No. 2," etc.)

IV. Court's Ruling
1. Details of the evidence
(1) Details of Petitioner's Exhibit No. 1

Petitioner's Exhibit No. 1 (JP 2016-501531 A) contains the following matter. (Emphasis added by the Court; thus hereafter.)

WHAT IS CLAIMED IS:
2. A method of modifying an organism or a non-human organism by manipulation of a target sequence in a genomic locus of interest comprising
delivering a non-natural ly occurring or engineered composition comprising:
A) – I. a CRISPR-Cas system chimeric RNA (chiRNA) polynucleotide sequence, wherein the polynucleotide sequence comprises:
(a) a guide sequence capable of hybridizing to a target sequence in a eukaryotic cell,
(b) a tracr mate sequence, and
(c) a tracr sequence, and
II. a polynucleotide sequence encoding a CRISPR enzyme, optionally comprising at least one or more nuclear localization sequences,
wherein (a), (b) and (c) are arranged in a 5' to 3' orientation,
wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR comple to the target sequence, and
wherein the CRISPR complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized to the target sequence, and (2) the tracr mate sequence that is hybridized to the tracr sequence and the polynucleotide sequence encoding a CRISPR enzyme is DNA or RNA,
or
(B) I. polynucleotides comprising:
(a) a guide sequence capable of hybridizing to a target sequence in a eukaryotic cell, and
(b) at least one or more tracr mate sequences,
II. a polynucleotide sequence encoding a CRISPR enzyme, and
III. a polynucleotide sequence comprising a tracr sequence,
wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide

sequence directs sequence-specific binding of a CRISPR complex to the target sequence, and wherein the CRISPR. complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized to the target sequence, and (2) the tracr mate sequence that is hybridized to the tracr sequence, and the polynucleotide sequence encoding a CRISPR enzyme is DNA or RNA.

2. The method of claim 1, wherein any or all of the polynucleotide sequence encoding a CRISPR enzyme, guide sequence, tracr mate sequence or tracr sequence, is/are RNA.

3. The method of claim 1 or 2, wherein the polynucleotides encoding the seque ce encoding a CRISPR enzyme, the guide sequence, tracr mate sequence or tracr sequence is/are RNA and are delivered via liposomes, nanoparticies, exosomes, microvesicles, or a gene-gun.

4. The method of any of claims 1 to 3, wherein the polynucleotides are comprised within a vector system comprising one or more vectors.

5. A method of modifying an organism or a non -human organism by manipulation of a target sequence in a genomic locus of interest comprising

delivering a non-naturally occurring or engineered composition comprising a viral vector system comprising one or more viral vectors operably encoding a composition for expression thereof, wherein the composition comprises:

(A) a non-naturally occurring or engineered composition comprising a vector system comprising one or more vectors comprising

I. a first regulatory element operably linked to a CRISP R-Cas system chimeric RNA (chiRNA) polynucleotide sequence, wherein the polynucleotide sequence comprises

(a) a guide sequence capable of hybridizing to a target sequence in a eukaryotic cell,

(b) a tracr mate sequence, and

(c) a tracr sequence, and

II. a second regulatory element operably linked to an enzyme-coding sequence encoding a CRISPR enzyme, optionally comprising at least one or more nuclear localization sequences, wherein (a), (b) and (c) are arranged in a 5' to 3' orientation,

wherein components I and II are located on the same or different vectors of the system, wherein

when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the target sequence, and wherein the CR ISPR complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized to the target sequence, and (2) the tracr mate sequence that is hybridized to the tracr sequence,
or
(B) a non-naturally occurring or engineered composition comprising a vector system comprising one or more vectors comprising
I. a first regulator}? element operably linked to
(a) a guide sequence capable of hybridizing to a target sequence in a eukaryotic cell, and
(b) at least one or more tracr mate sequences,
II. a second regulatory element operably linked to an enzyme-coding sequence encoding a CRISPR enzyme, and
IÏÏ. a third regulatory element operably linked to a tracr sequence,
wherein components L II and I II are located on the same or different vectors of the system, wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the target sequence, and wherein the CRISPR. complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized to the target sequence, and (2) the tracr mate sequence that is hybridized to the tracr sequence.
6. The method of claim 5, wherein one or more of the viral vectors are delivered via liposomes, nanoparticles, exosomes, microvesicles, or a gene-gun.
7. A method of treating or inhibiting a condition caused by a defect in a target sequence in a genomic locus of interest in a subject or a non-human subject in need thereof comprising modifying the subject or a non-human subject by manipulation of the target sequence and wherein the condition is susceptible to treatment or inhibition by manipulation of the target sequence comprising providing treatment comprising:
delivering a non-naturally occurring or engineered composition comprising an AAV or lenti virus

vector system, comprising one or more AAV or lentivirus vectors operably encoding a composition for expression thereof, wherein the target sequence is manipulated by the composition when expressed, wherein the composition comprises:
(A) a non-natural Sy occurring or engineered composition comprising a vector system comprising one or more vectors comprising
]. a first regulatory element operably linked to a CRISPR-Cas system chimeric RNA (chiRNA) polynucleotide sequence, wherein the polynucleotide sequence comprises
(a) a guide sequence capable of hybridizing to a target sequence in a eukaryotic ceil,
(b) a tracr mate sequence, and
(c) a tracr sequence, and
II. a second regulatory element operably linked to an enzyme-coding sequence encoding a CRISPR enzyme comprising at least one or more nuclear localization sequences,
wherein (a), (b) and (c) are arranged in a 5' to 3' orientation,
wherein components I and II are located on the same or different vectors of the system, wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR comple to the target sequence, and wherein the CRISPR complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized to the target sequence, and (2) the tracr mate sequence that is hybridized to the tracr sequence,
or
(B) a non-naturally occurring or engineered composition comprising a vector system comprising one or more vectors comprising
I. a first regulatory element operably linked to
(a) a guide sequence capable of hybridizing to a target sequence in a eukaryotic cell, and
(b) at least one or more tracr mate sequences,
II. a second regulatory element operably linked to an enzyme-coding sequence encoding a CRISPR enzyme, and
III. a third regulatory element operably linked to a tracr sequence,
wherein components 1, II and III are located on the same or different vectors of the system, wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide

sequence directs sequence-specific binding of a CRISPR complex to the target sequence, and wherein the CRISPR complex comprises the CRISPR enzyme eomplexed with (1) the guide sequence that is hybridized to the target sequence, and (2) the tracr mate sequence that is hybridized to the tracr sequence.
8. The method of any preceding claim, wherein the method is carried out in vitro, and/or ex vivo,
9. The method of any preceding claim including inducing expression.
10. The method of any preceding claim wherein the organism or subject is a eukaryote.
1 1. The method of claim 10 wherein the organism or subject is a non-human eukaryote.
12. The method of any of claims 1 to 1 1 wherein the organism or subject is a mammal or a non~hurnan mammal.
13. The method of any of claims 4 to 8 wherein the viral vector is an AAV or lenti viral vector.
14. The method according to any preceding claim wherein the CRISPR enzyme is a Cas9.
15. The method according to any preceding claim wherein expression of the guide sequence is under the control of the T7 promoter and is driven by the expression of T7 polymerase.
16. A method of delivering a CRISPR enzyme of any preceding claim, comprising delivering to a cell mRNA encoding the CRISPR enzyme.
17. The method of any one of claims 1 to 16, wherein the polynucleotide or enzyme coding sequence encoding the CRISPR enzyme is delivered to the ceil by delivering mRNA encoding the CRISPR enzyme to the cell.

18. A method of preparing the AAV or lentivirus vector of claim 7 comprising transfecting piasmid(s) containing or consisting essentially of nucleic acid moleeulc(s) coding for the AA.V or lenti virus into AAV~mfected or lentivirus-infected cells, and supplying AAV AAV or lentiviras rep and/or cap and/or helper nucleic acid molecules obligatory for replication and packaging of the AAV or lentivirus.
19. A method of preparing an AAV or lentivirus vector for use in the method of claim 7, comprising transfecting plasmid(s) containing or consisting essentially of nucleic acid molecule(s) coding for the AAV or lentivirus into AAV-infected or lentivirus-infected cells, and supplying AAV AAV or lentivirus rep and/or cap and/or helper nucleic acid molecules obligatory for replication and packaging of the AAV or lentivirus.
20. The method of claim 18 or 19 wherein the AAV or lentivirus rep and/or cap obligatory for replication and packaging of the AAV or lentivirus are supplied by transfecting the ceils with helper plasmid(s) or helper virus(es).
21. The method of claim 20 wherein the helper vims is a poxvirus, adenovirus, lentivirus, herpesvirus or bacuio virus.
22. The method of claim 21 wherein the poxvirus is a vaccinia virus.
23. The method of any of claims 18 to 22 wherein the cells are mammalian ceils.
24. The method of any of claims 18 to 22 wherein the cells are insect cells and the helper virus (where present) is baeulovirus.
25. The method of any of claims 1 to 15 wherein the target sequence is flanked at its 3' end or followed by 5' -NRG (where N is any Nucleotide), or where the CR iSPR enzyme is (or is derived from) a genus belonging to the group consisting of Corynebacter, Sutterella, Legionella,

Treponema, Filifactor, Eubacterium, Streptococcus, Lactobacillus, Mycoplasma, Bacteroides, Flaviivola, Flavobacterium, Sphaerochaeta, Azospirillum, Gluconacetobacter, Neisseria, Roseburia, Parvibaculum, Staphylococcus, Nitratifractor, Mycoplasma and Campylobacter. ,
26. A composition as defined in any of claims 1 -25 for use in medicine or in therapy.
27. A composition as defined in any of claims 1-25 for use in a method of modifying an organism or a non-human organism by manipulation of a target sequence in a genomic locus of interest or in a method of treating or inhibiting a condition caused by a defect in a target sequence in a genomic locus of interest.
28. Use of a composition as defined in any of claims 1-25 in ex vivo gene or genome editing.
29. Use of a composition as defined in any of claims 1-25 in the manufacture of a medicamen t for ex vivo gene or genome editing or for use in a method of modifying an organism or a non-human organism by manipulation of a target sequence in a genomic locus of interest or in a method of treating or inhibiting a condition caused by a defect in a target sequence in a genomic locus of interest.
30. A composition comprising:
A) - I. a CRISPR-Cas system chimeric RNA (chiRNA) polynucleotide sequence, wherein the polynucleotide sequence comprises:
(a) a guide sequence capable of hybridizing to a target sequence in a eukaryotic cell,
(b) a tracr mate sequence, and

(c) a tracr sequence, and
II. a polynucleotide sequence encoding a CRISPR enzyme, optionally comprising at least one or more nuclear localization sequences,
wherein (a), (b) and (c) are arranged in a 5' to 3' orientation,
wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the target sequence, and wherein the CRISPR complex comprises the CRISPR enzyme complexed with (1) the guide sequence that is hybridized to the target sequence, and (2) the tracr mate sequence that is hybridized to the tracr sequence and the polynucleotide sequence encoding a CRISPR enzyme is DNA or RNA,
or
(B) I. poly ucleotides comprisi g:
(a) a guide sequence capable of hybridizing to a target sequence in a eukaryotic cell, and (b) at least one or more tracr mate sequences,
II. a polynucleotide sequence encoding a CRISPR enzyme, and
III. a polynucleotide sequence comprising a tracr sequence,
wherein when transcribed, the tracr mate sequence hybridizes to the tracr sequence and the guide sequence directs sequence-specific binding of a CRISPR complex to the target sequence, and wherein the CR ISPR complex comprises the CRISPR enzyme complexed wit (1) the guide sequence that is hybridized to the target sequence, and (2) the tracr mate sequence that is hybridized to the tracr sequence, and the polynucleotide sequence encoding a CRISPR enzyme is DNA or RNA;
for use in medicine or therapy; or for use in a method of modifying an organism or a non-human organism by manipulation of a target sequence in a genomic locus of interest; or for use in a method of treating or inhibiting a condition caused by a defect in a target sequence in a genomic locus of interest; or for use in ex vivo gene or genome editing.
31. The composition of claim 30, wherein the polynucleotides are comprised within a vector system comprising one or more vectors.

(2) Matter in Petitioner’s Exhibit No. 2

The following matter is described in Petitioner’s Exhibit No. 2 (JP 2016-500003 A).

Claims

[Claim 1]

A composition for cleaving target DNA in eukaryotic cells or organisms comprising a guide RNA specific for target DNA or DNA that encodes the guide RNA, and Cas protein-encoding nucleic acid or Cas protein.

[Claim 2]

The composition of claim 1, wherein the target DNA is an endogenous target DNA.

[Claim 3]

The composition of claim 1, wherein the guide RNA is a dualRNA comprising a crRNA and a tracrRNA.

[Claim 4]

The composition of claim 1, wherein the guide RNA is a single-chain guide RNA (sgRNA).

[Claim 5]

The composition of claim 4, wherein the single-chain guide RNA comprises portion of a crRNA and a tracrRNA.

[Claim 6]

The composition of claim 1, wherein the guide RNA further comprises one or more additional nucleotides at the 5' end of the single-chain guide RNA or the crRNA of the dualRNA.

[Claim 7]

The composition of claim 1, wherein the guide RNA further comprises 2 additional guanine nucleotides at the 5' end of the single-chain guide RNA or the crRNA of the dualRNA.

[Claim 8]

The composition of claim 1, which induces targeted mutagenesis in eukaryotic cells or organisms.

[Claim 9]

The composition of claim 1, for use in the genotyping of a genome in the eukaryotic cells or organisms in vitro.

[Claim 10]

The composition of claim 1, whereinthe guide RNA and the Cas protein function as a pair, and wherein the pair comprises two guide RNAs which induce two nicks on different strands.

[Claim 11]

The composition of claim 1, wherein the guide RNA is in the form of an isolated RNA, or is encoded in a vector, wherein the vector is a viral vector, plasmid vector, or agrobacterium vector.

[Claim 12]

The composition of claim 1, comprising a guide RNA specific for the target DNA and Cas9

protein.
[Claim 13]
The composition of claim 1, for cleaving the target DNA in eukaryotic cells or organisms ex vivo or in vivo.
[Claim 14]
The composition of claim 1, wherein the Cas protein-encoding nucleic acid or the Cas protein is derived from the genus Streptococcus.
[Claim 15]
The composition of claim 14, wherein the genus Streptococcus is Streptococcus pyogenes.
[Claim 16]
The composition of claim 15, wherein the Cas protein recognizes the NGG trinucleotide.
[Claim 17]
The composition of claim 1, wherein the Cas protein is Cas9 protein or variant thereof.
[Claim 18]
The composition of claim 1, wherein the Cas protein is linked to a protein transduction domain.
[Claim 19]
The composition of claim 17, wherein the variant of Cas9 protein is a mutant form of Cas9 in which the catalytic aspartate residue is changed to any other amino acid.
[Claim 20]
The composition of claim 19, wherein the amino acid is an alanine.
[Claim 21]
The composition of claim 1, wherein the Cas protein-encoding nucleic acid comprises a nucleotide sequence of SEQ ID No. 1 or a nucleotide sequence having at least 50% homology to SEQ ID NO. 1.
[Claim 22]
Use of a composition of any one of claims 1 to 21 for targeted mutagenesis in eukaryotic cells or organisms.
[Claim 23]
A kit for cleaving a target DNA in eukaryotic cells or organisms comprising the composition according to any one of claims 1 to 21.
[Claim 24]
A method for preparing a eukaryotic cell or organism comprising Cas protein and a guide RNA comprising a step of co-transfecting or serial-transfecting the eukaryotic cell or organism with a

Cas protein-encoding nucleic acid or Cas protein, and a guide RNA or DNA that encodes the guide RNA.
[Claim 25]
The method of claim 24, wherein the guide RNA is a dualRNA comprising a crRNA and a tracrRNA.
[Claim 26]
The method of claim 24, wherein the guide RNA is a single-chain guide RNA.
[Claim 27]
The method of claim 26, wherein the single-chain guide RNA comprises portion of a crRNA and a tracrRNA.
[Claim 28]
The method of claim 24, wherein the guide RNA further comprises one or more additional nucleotides at the 5' end of the single-chain guide RNA or the crRNA of the dualRNA.
[Claim 29]
The method of claim 24, wherein the guide RNA further comprises 2 additional guanine nucleotides at the 5' end of the single-chain guide RNA or the crRNA of the dualRNA.
[Claim 30]
The method of claim 24, wherein the eukaryotic cell or organism is co-transfected or serial-transfected with a Cas9 protein and a guide RNA.
[Claim 31]
The method of claim 24, wherein the serial-transfection is performed by transfection with Cas protein-encoding nucleic acid first, followed by second transfection with naked guide RNA.
[Claim 32]
The method of claim 24, wherein the Cas protein is Cas9 protein or variant thereof.
[Claim 33]
The method of claim 24, wherein the Cas protein-encoding nucleic acid or the Cas protein is derived from the genus Streptococcus.
[Claim 34]
The method of claim 33, wherein the genus Streptococcus is Streptococcus pyogenes.
[Claim 35]
The method of claim 32, wherein the variant of Cas9 protein is a mutant form of Cas9 in which the catalytic aspartate residue is changed to any other amino acid.
[Claim 36]
The method of claim 35, wherein the amino aicd is an alanine.

[Claim 37]
The method of claim 24, wherein the guide RNA and Cas protein function as a pair, and wherein the pair comprises two guide RNAs which induce two nicks on different DNA strands.
[Claim 38]
The method of claim 37, wherein the two nicks are separated by at least 100 bps.
[Claim 39]
The method of claim 24, wherein the transfection is performed by the method selected from the group consisting of microinjection, electroporation, DEAE-dextran treatment, lipofection, nanoparticle-mediated transfection, protein transduction domain mediated transduction, virus-mediated gene delivery, and PEG-mediated transfection in protoplast.
[Claim 40]
A eukaryotic cell or organism comprising Cas protein and a guide RNA prepared by the method according to any one of claims 24 to 39.
[Claim 41]
A method for cleaving a target DNA in eukaryotic cells or organisms comprising a step of transfecting the eukaryotic cells or organisms comprising a target DNA with the composition according to any one of claims 1 to 21.
[Claim 42]
The method of claim 41, wherein the eukaryotic organisms are mammals or plants.
[Claim 43]
The method of claim 41, wherein the guide RNA and Cas protein function as a pair , and wherein the pair comprises two guide RNAs which induce two nicks on different DNA strands.
[Claim 44]
The method of claim 43, wherein the two nicks are separated by at least 100 bps.
[Claim 45]
The method of claim 41, wherein the transfection is co-transfection or serial-transfection.
[Claim 46]
The method of claim 45, wherein the serial-transfection is performed by transfection with Cas protein-encoding nucleic acid first, followed by second transfection with naked guide RNA.
[Claim 47]
The method of claim 41, further comprising a step of analysing the pattern of a cleavage, wherein the pattern indicates the detection of mutation or variation in the genome.

[Claim 48]
An embryo comprising a genome edited by the composition according to any one of claims 1 to 21.
[Claim 49]
A genome-modified animal obtained by transferring the embryo of claim 48 into the oviducts.
[Claim 50]
A plant regenerated from the genome-modified protoplasts prepared by the method of any one of claims 24 to 39.
[Claim 51]
A method of preparing a genome-modified animal comprising a step of introducing the composition according to any one of claims 1 to 21 into an embryo of an animal; and a step of transferring the embryo into an oviduct of pseudopregnant foster mother to produce a genome-modified animal.
[Claim 52]
A composition for genotyping mutations or variations in an isolated biological sample, comprising a guide RNA specific for the target DNA sequence Cas protein.
[Claim 53]
The composition of claim 52, wherein the mutations or variations are induced by engineered nucleases in cells.
[Claim 54]
The composition of claim 52, wherein the mutations or variations are a naturally-occurring mutations or variations.
[Claim 55]
A composition for genotyping nucleic acid sequences in pathogenic microorganisms in an isolated biological sample, comprising a guide RNA specific for the target DNA sequence and Cas protein.
[Claim 56]
A kit for genotyping mutations or variations in an isolated biological sample, comprising the composition of any one of claims 52 to 55.
[Claim 57]
A method of genotyping mutations or variations in an isolated biological sample, using the composition of any one of claims 52 to 55.

(3) Matter in Petitioner's Exhibit No. 3

Petitioner's Exhibit No. 3 (application documents of first priority basis application) describes the following matter. Note that since Petitioner's Exhibit No. 3 is in English, we excerpt here Respondents' Exhibit No. 116, which is a translation of it. (The respondents submitted the translation of Petitioner's Exhibit No. 3 and on page 6 of the Oral Hearing Brief

dated April 7, 2023, it is stated that the content of the translation in Respondents' Exhibit No. 116 submitted by the respondents is not disputed.)

A. Title of the invention

"METHODS AND COMPOSITIONS FOR RNA-DIRECTED SITE-SPECIFIC DNA MODIFICATION"

B. Claims

54. A method of site-specific modification at target DNA, the method comprising:
contacting the target DNA with a complex comprising:
(i) a DNA-targeting RNA, or a DNA polynucleotide encoding the same, wherein the DNA-targeting RNA comprises a nucleotide sequence that is complementary to a sequence in the target DNA; and
(ii) a site-directed modifying polypeptide, or a polynucleotide encoding the same, wherein the site-directed modifying polypeptide exhibits site-directed enzymatic activity.

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58. The method of Claim 54, wherein the target DNA is part of a chromosome in a cell.

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61. The method of Claim 58, wherein the cell is a eukaryotic single-cell organism.

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65. The method of Claim 58, wherein the cell is a plant cell.

66. The method of Claim 58, wherein the cell is an animal cell.

...

73. The method of Claim 54, wherein the enzymatic activity modifies the target DNA.

74. The method of Claim 73, wherein the enzymatic activity is nuclease activity, methyltransferase activity, demethylase activity, DNA repair activity, DNA damage activity, deamination activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity, transposase activity, recombinase activity, polymerase activity, ligase activity, helicase activity, photolyase activity or glycosylase activity.

75. The method of Claim 74, wherein the DNA-modifying enzymatic activity is nuclease activity.

76. The method of Claim 75, wherein the nuclease activity introduces a double strand break in the target DNA.

C. Background

[0001] In recent years, engineered nuclease enzymes designed to target specific DNA sequences have attracted considerable attention as powerful tools for the genetic manipulation of cells and whole organisms, allowing targeted gene deletion, replacement and repair, as well as the insertion of exogenous sequences (transgenes) into the genome. Two major technologies for engineering site-specific DNA nucleases have emerged, both of which are based on the

construction of chimeric endonuclease enzymes in which a sequence non-specific DNA endonuclease domain is fused to an engineered DNA binding domain. However, targeting each new genomic locus requires the design of a novel nuclease enzyme, making these approaches both time consuming and costly. In addition, both technologies suffer from limited precision, which can lead to unpredictable off-target effects.
[0002] There is need in the field for a technology that allows precise targeting of nuclease activity (or other protein activities) to distinct locations within a target DNA in a manner that does not require the design of a new protein for each new target sequence. The present disclosure addresses this need.

D. Detailed description
[0072] The present disclosure provides a DNA-targeting RNA that comprises a targeting sequence and, together with a modifying polypeptide, provides for site-specific modification of a target DNA and/or a polypeptide associated with the target DNA. The present disclosure further provides modifying polypeptides. The present disclosure further provides methods of site-specific modification of a target DNA and/or a polypeptide associated with the target DNA; and kits and compositions for carrying out the methods.
DNA-targeting RNA
[0073] The present disclosure provides a DNA-targeting RNA that directs the activities of an associated polypeptide (e.g., a site-directed modifying polypeptide) to a specific target sequence within a target DNA. A subject DNA-targeting RNA comprises: a first segment (also referred to herein as a "DNA-targeting segment" or a "DNA-targeting sequence") and a second segment (also referred to herein as a "protein-binding segment" or a "protein-binding sequence").
DNA-targeting segment of a DNA-targeting RNA
[0074] The DNA-targeting segment of a subject DNA-targeting RNA comprises a nucleotide sequence that is complementary to a sequence in a target DNA. In other words, the DNA-targeting segment of a subject DNA-targeting RNA interacts with a target DNA in a sequence-specific manner via hybridization (i.e., base pairing). As such, the nucleotide sequence of the DNA-targeting segment may vary and determines the location within the target DNA that the DNA-targeting RNA and the target DNA will interact. The DNA-targeting segment of a subject DNA-targeting RNA can be modified (e.g., by genetic engineering) to hybridize to any desired sequence within a target DNA.
Protein-binding segment of a DNA-targeting RNA
[0076] The protein-binding segment of a subject DNA-targeting RNA interacts with a site-directed modifying polypeptide. The subject DNA-targeting RNA guides the bound polypeptide to a specific nucleotide sequence within target DNA via the above-mentioned DNA-targeting segment. The protein-binding segment of a subject DNA-targeting RNA comprises two stretches of nucleotides that are complementary to one another. The complementary nucleotides of the protein-binding segment hybridize to form a double stranded RNA duplex (dsRNA) (see Figures

1A and 1B).

…

[0078] A subject double-molecule DNA-targeting RNA comprises two separate RNA molecules. Each of the two RNA molecules of a subject double-molecule DNA-targeting RNA comprises a stretch of nucleotides that are complementary to one another such that the complementary nucleotides of the two RNA molecules hybridize to form the double stranded RNA duplex of the protein-binding segment (Figure 1A).

[0079] A subject single-molecule DNA-targeting RNA comprises two stretches of nucleotides (a targeter-RNA and an activator-RNA) covalently linked by intervening nucleotides ("linkers" or "linker nucleotides") that are complementary to one another and hybridize to form the double stranded RNA duplex (dsRNA duplex) of the protein-binding segment, thus resulting in a stem-loop structure (Figure 1B). The targeter-RNA and the activator-RNA can be covalently linked via the 3' end of the targeter-RNA and the 5' end of the activator-RNA. Alternatively, targeter-RNA and the activator-RNA can be covalently linked via the 5' end of the targeter-RNA and the 3' end of the activator-RNA.

…

[0083] An exemplary double-molecule DNA-targeting RNA comprises a crRNA-like (CRISPR RNA or "targeter-RNA" or crRNA repeat) molecule and a corresponding tracrRNA-like (trans-acting CRISPR RNA or "activator-RNA" or tracrRNA) molecule (see Figure 1A). A crRNA-like molecule (targeter-RNA) comprises both the DNA-targeting segment (single stranded) of the DNA-targeting RNA and a stretch ("duplex-forming segment") of nucleotides that forms one half of the dsRNA duplex of the protein-binding segment of the DNA-targeting RNA. A corresponding tracrRNA-like molecule (activator-RNA) comprises a stretch of nucleotides (duplex-forming segment) that forms the other half of the dsRNA duplex of the protein-binding segment of the DNA-targeting RNA (see Figure 1A). In other words, a stretch of nucleotides of a crRNA-like molecule are complementary to and hybridize with a stretch of nucleotides of a tracrRNA-like molecule to form the dsRNA duplex of the protein-binding domain of the DNA-targeting RNA. As such, each crRNA-like molecule can be said to have a corresponding tracrRNA-like molecule. The crRNA-like molecule additionally provides the single stranded DNA-targeting segment. Thus, a crRNA-like and a tracrRNA-like molecule (as a corresponding pair) hybridize to form a DNA-targeting RNA (see Figure 1A). The exact sequence of a given crRNA or tracrRNA molecule is characteristic of the species in which the RNA molecules are found. Various crRNAs and tracrRNAs are depicted individually and in corresponding complementary pairs in Figures 6-9. A subject double-molecule DNA-targeting RNA can comprise any corresponding crRNA and tracrRNA pair.

Site-directed modifying polypeptide

[0089] A subject DNA-targeting RNA and a subject site-directed modifying polypeptide form a complex. The DNA-targeting RNA provides target specificity to the complex by comprising a nucleotide sequence that is complementary to a sequence of a target DNA (as noted above). The site-directed modifying polypeptide of the complex provides the site-specific activity. In other words, the site-directed modifying polypeptide is guided to a DNA sequence (e.g. a chromosomal sequence or an extrachromosomal sequence, e.g. an episomal sequence, a minicircle sequence, a mitochondrial sequence, a chloroplast sequence, etc.) by virtue of its association with at least the protein-binding segment of the DNA-targeting RNA (described above).

…

[0091] In some cases, the site-directed modifying polypeptide is a naturally-occurring modifying polypeptide. In other cases, the site-directed modifying polypeptide is not a naturally-occurring polypeptide (e.g., a chimeric polypeptide as discussed below or a naturally-occurring polypeptide that is modified, e.g., mutation, deletion, insertion).

[0092] Exemplary naturally-occurring site-directed modifying polypeptides are provided in Figure 12 as a non-limiting and non-exhaustive list of naturally occurring Cas9/Csn1 endonucleases. These naturally occurring polypeptides, as disclosed herein, bind a DNA-targeting RNA, are thereby directed to a specific sequence within a target DNA, and cleave the target DNA to generate a double strand break. A subject site-directed modifying polypeptide comprises two portions, an RNA-binding portion and an activity portion. In some embodiments, a subject site-directed modifying polypeptide comprises: (i) an RNA-binding portion that interacts with a DNA-targeting RNA, wherein the DNA-targeting RNA comprises a nucleotide sequence that is complementary to a sequence in a target DNA; and (ii) an activity portion that exhibits site-directed enzymatic activity (e.g., activity for DNA methylation, activity for DNA cleavage, activity for histone acetylation, activity for histone methylation, etc.), wherein the site of enzymatic activity is determined by the DNA-targeting RNA.

Exemplary site-directed modifying polypeptides

[0096] In some cases, the site-directed modifying polypeptide comprises an amino acid sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99%, or 100%, amino acid sequence identity to amino acids 7-166 or 731-1003 of the Cas9/Csn1 amino acid sequence depicted in Figure 2, or to the corresponding domains in any of the amino acid sequences depicted in Figure 12.

Nucleic acids encoding a subject DNA-targeting RNA and/or a subject site-directed modifying polypeptide

[00120] The present disclosure provides a nucleic acid comprising a nucleotide sequence encoding a subject DNA-targeting RNA and/or a subject site-directed modifying polypeptide. In some embodiments, a subject DNA-targeting RNA-encoding nucleic acid is an expression

vector, e.g., a recombinant expression vector.

[00121] In some embodiments, a subject method involves contacting a target DNA or introducing into a cell (or a population of cells) one or more nucleic acids comprising nucleotide sequences encoding a DNA-targeting RNA and/or a site-directed modifying polypeptide. In some embodiments a cell comprising a target DNA is in vitro. In some embodiments a cell comprising a target DNA is in vivo. Suitable nucleic acids comprising nucleotide sequences encoding a DNA-targeting RNA and/or a site-directed modifying polypeptide include expression vectors, where an expression vector comprising a nucleotide sequence encoding a DNA-targeting RNA and/or a site-directed modifying polypeptide is a "recombinant expression vector."

[00124] Numerous suitable expression vectors are known to those of skill in the art, and many are commercially available. The following vectors are provided by way of example; for eukaryotic host cells: pXT1, pSG5 (Stratagene), pSVK3, pBPV, pMSG, and pSVLSV40 (Pharmacia). However, any other vector may be used so long as it is compatible with the host cell.

[00126] In some embodiments, a nucleotide sequence encoding a DNA-targeting RNA and/or a site-directed modifying polypeptide is operably linked to a control element, e.g., a transcriptional control element, such as a promoter. The transcriptional control element may be functional in either a eukaryotic cell, e.g., a mammalian cell; or a prokaryotic cell (e.g., bacterial or archaeal cell). In some embodiments, a nucleotide sequence encoding a DNA-targeting RNA and/or a site-directed modifying polypeptide is operably linked to multiple control elements that allow expression of the nucleotide sequence encoding a DNA-targeting RNA and/or a site-directed modifying polypeptide in both prokaryotic and eukaryotic cells.

[00127] Non-limiting examples of suitable eukaryotic promoters (promoters functional in a eukaryotic cell) include those from cytomegalovirus (CMV) immediate early, herpes simplex virus (HSV) thymidine kinase, early and late SV40, long terminal repeats (LTRs) from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. The expression vector may also contain a ribosome binding site for translation initiation and a transcription terminator. The expression vector may also include appropriate sequences for amplifying expression. The expression vector may also include nucleotide sequences encoding protein tags (e.g., 6xHis tag, hemagglutinin tag, green fluorescent protein, etc.) that are fused to the site-directed modifying polypeptide, thus resulting in a chimeric polypeptide.

…

[00129] Methods of introducing a nucleic acid into a host cell are known in the art, and any known method can be used to introduce a nucleic acid (e.g., an expression construct) into a stem cell or progenitor cell. Suitable methods include, e.g., infection, lipofection, electroporation, calcium phosphate precipitation, polyethyleneimine (PEI)-mediated transfection, DEAE-dextran mediated transfection, liposome-mediated transfection, and the like.

[00157] In some cases, the site-directed modifying polypeptide exhibits nuclease activity that cleaves target DNA at a target DNA sequence defined by the region of complementarity between the DNA-targeting RNA and the target DNA. The nuclease activity cleaves target DNA to produce double strand breaks. These breaks are then repaired by the cell in one of two ways: non-homologous end joining, and homology-directed repair (Figure 4). In non-homologous end joining (NHEJ), the double-strand breaks are repaired by direct ligation of the break ends to one another. As such, no new nucleic acid material is inserted into the site, although some nucleic acid material may be lost, resulting in a deletion. In homology-directed repair, a donor polynucleotide with homology to the cleaved target DNA sequence is used as a template for repair of the cleaved target DNA sequence, resulting in the transfer of genetic information from the donor polynucleotide to the target DNA. As such, new nucleic acid material may be inserted/copied into the site. In some cases, a target DNA is contacted with a subject donor polynucleotide. In some cases, a subject donor polynucleotide is introduced into a subject cell. The modifications of the target DNA due to NHEJ and/or homology-directed repair lead to, for example, gene correction, gene replacement, gene tagging, transgene insertion, nucleotide deletion, gene disruption, gene mutation, etc.

Target cells of interest

[00165] In some of the above applications, the subject methods may be employed to induce DNA cleavage and DNA modification in mitotic or post-mitotic cells in vitro and/or ex vivo and/or in vitro (e.g., to produce genetically modified cells that can be reintroduced into an individual). Because the DNA-targeting RNA provide specificity by hybridizing to target DNA, a mitotic and/or post-mitotic cell of interest in the disclosed methods may include a cell from any organism (e.g. a bacterial cell, an archaeal cell, a cell of a single-cell eukaryotic organism, a plant cell, an animal cell, a cell from an invertebrate animal (e.g. fruit fly, cnidarian, echinoderm, nematode, etc.), a cell from a vertebrate animal (e.g., fish, amphibian, reptile, bird, mammal), a cell from a mammal, a cell from a rodent, a cell from a human, etc.). Any type of cell may be of interest (e.g. a stem cell, e.g. an embryonic stem (ES) cell, an induced pluripotent stem (iPS) cell, a germ cell; a somatic cell, e.g. a fibroblast, a hematopoietic cell, a neuron, a muscle cell, a bone cell, a hepatocyte, a pancreatic cell etc.). Cells may be from established cell lines or they may be

primary cells, where “primary cells”, “primary cell lines”, and “primary cultures” are used interchangeably herein to refer to cells and cells cultures that have been derived from a subject and allowed to grow in vitro for a limited number of passages, i.e. splittings, of the culture. For example, primary cultures are cultures that may have been passaged 0 times, 1 time, 2 times, 4 times, 5 times, 10 times, or 15 times, but not enough times go through the crisis stage. Typically, the primary cell lines of the present invention are maintained for fewer than 10 passages in vitro.

[00173] In some embodiments, a DNA-targeting RNA may be provided directly as RNA. In such cases, the DNA-targeting RNA may be produced by direct chemical synthesis or may be transcribed in vitro from a DNA encoding the DNA-targeting RNA. Methods of synthesizing RNA from a DNA template are well known in the art. In some cases, the DNA-targeting RNA will be synthesized in vitro using an RNA polymerase enzyme (e.g., T7 polymerase, T3 polymerase, SP6 polymerase, etc.). Once synthesized, the RNA may directly contact a target DNA or may be introduced into a cell by any of the well-known techniques for introducing nucleic acids into cells (e.g., microinjection, electroporation, transfection, etc).

[00178] A subject site-directed modifying polypeptide may instead be provided to cells as a polypeptide. Such a polypeptide may optionally be fused to a polypeptide domain that increases solubility of the product. The domain may be linked to the polypeptide through a defined protease cleavage site, e.g. a TEV sequence, which is cleaved by TEV protease. The linker may also include one or more flexible sequences, e.g. from 1 to 10 glycine residues. In some embodiments, the cleavage of the fusion protein is performed in a buffer that maintains solubility of the product, e.g. in the presence of from 0.5 to 2 M urea, in the presence of polypeptides and/or polynucleotides that increase solubility, and the like. Domains of interest include endosomolytic domains, e.g. influenza HA domain; and other polypeptides that aid in production, e.g. IF2 domain, GST domain, GRPE domain, and the like. The polypeptide may be formulated for improved stability. For example, the peptides may be PEGylated, where the polyethyleneoxy group provides for enhanced lifetime in the blood stream.

[00179] Additionally or alternatively, the subject site-directed modifying polypeptide may be fused to a polypeptide permeant domain to promote uptake by the cell. A number of permeant domains are known in the art and may be used in the non-integrating polypeptides of the present invention, including peptides, peptidomimetics, and non-peptide carriers. For example, a permeant peptide may be derived from the third alpha helix of Drosophila melanogaster transcription factor Antennapaedia, referred to as penetratin, which comprises the amino acid sequence RQIKIWFQNRRMKWKK. As another example, the permeant peptide comprises the HIV-1 tat basic region amino acid sequence, which may include, for example, amino acids 49-57 of naturally-occurring tat protein. Other permeant domains include poly-arginine motifs, for example, the region of amino acids 34-56 of HIV-1 rev protein, nona-arginine, octa-arginine, and

the like. (See, for example, Futaki et al. (2003) Curr Protein Pept Sci. 2003 Apr; 4(2): 87-96; and Wender et al. (2000) Proc. Natl. Acad. Sci. U.S.A 2000 Nov. 21; 97(24):13003-8; published U.S. Patent applications 20030220334; 20030083256; 20030032593; and 20030022831, herein specifically incorporated by reference for the teachings of translocation peptides and peptoids). The nona-arginine (R9) sequence is one of the more efficient PTDs that have been characterized (Wender et al. 2000; Uemura et al. 2002). The site at which the fusion is made may be selected in order to optimize the biological activity, secretion or binding characteristics of the polypeptide. The optimal site will be determined by routine experimentation.
[00198] Cells that have been genetically modified in this way may be transplanted to a subject for purposes such as gene therapy, e.g. to treat a disease or as an antiviral, antipathogenic, or anticancer therapeutic, for the production of genetically modified organisms in agriculture, or for biological research. The subject may be a neonate, a juvenile, or an adult. Of particular interest are mammalian subjects. Mammalian species that may be treated with the present methods include canines and felines; equines; bovines; ovines; etc. and primates, particularly humans. Animal models, particularly small mammals, e.g. murine, lagomorpha, etc. may be used for experimental investigations.

D. Examples
[00248] Streptococcus pyogenes
A recombinant DNA-targeting polypeptide based on the sequence of Streptococcus pyogenes Cas9/Csn1 endonuclease was heterologously expressed in Escherichia coli and purified by a combination of affinity, ion exchange and gel filtration chromatographic steps according to standard procedures in the art. The protein was eluted and stored in 20 mM HEPES 7.5 150 mM potassium chloride and 1 mM TCEP. Targeting RNAs were obtained either by chemical synthesis (for the targeter and activator segments of the double molecule DNA-targeting RNA) or by in vitro transcription using T7 RNA polymerase (single-molecule DNA-targeting RNAs) according to standard protocols. Target DNAs were obtained by chemical synthesis of the individual single strands. One of the strands (the strand complementary to the targeting sequence in the DNA-targeting RNA) was subsequently [32P]-labeled at its 5' end using T4 polynucleotide kinase and [gamma-32P]-ATP as per standard procedures. The two strands were subsequently annealed by mixing equimolar amounts of the two DNA strands, heating to 95oC for 3 min and slow-cooling to room temperature.
[00249] DNA cleavage was performed by incubating the above DNA-targeting polypeptide (500 nM final concentration) together with the DNA-targeting RNA (500 nM concentration) and the target DNA (10 nM) in a total volume of 10 microliter, in a cleavage buffer containing 20 mM

HEPES pH 7.5, 100 mM potassium chloride, 5 mM magnesium chloride, 1mM dithiothreitol and 5% (v/v) glycerol. For reactions guided by double-molecule DNA-targeting RNA, the targeter and activator RNAs were mixed in equimolar amounts and annealed by heating to 95oC for 1 min and slow-cooling to room temperature, prior to addition to the DNA-targeting polypeptide. The DNA-targeting RNA/polypeptide complexes were assembled by incubation in the cleavage buffer for 15 min at room temperature. Subsequently, the assembled complex was added to target DNA and incubated for 1 hr at 37 oC. Reactions were quenched by the addition of 20 microliter of formamide quench buffer (5% glycerol, 1 mM EDTA, 0.025% sodium dodecyl sulfate in formamide) and subsequently resolved on a 12% polyacrylamide, 7M urea denaturing gel. Clevage products were visualized by phosphorimaging according to standard procedures.
[00250] The results are depicted in Figures 3 and 5.
[00251] Figures 3A-C depict target DNA cleavage by a site-directed modifying polypeptide (exemplified by the Cas9/Csn1 protein of Streptococcus pyogenes), which is directed by a DNA-targeting RNA. (A) Radiolabeled target DNAs were incubated in the presence of Cas9/Csn1 and various DNA-targeting RNA species (as indicated). Cleavage products were resolved using denaturing polyacrylamide gel electrophoresis and visualized by phosphorimaging. (B) Schematic diagrams of the DNA-targeting RNAs used in conjunction with the Cas9/Csn1 site-directed modifying polypeptide. Note that while one of the tested single-molecule DNA-targeting RNAs (RNA chimera A) supported efficient target DNA cleavage, the other tested single-molecule DNA-targeting RNA (RNA chimera B) did not. (C) Schematic representations of DNA-targeting RNA sequences and DNA targets.
[00252] Figures 5A and 5B depict target DNA cleavage. Figure 5 A. Target DNA cleavage using a Cas9/Csn1 site-directed modifying polypeptide from a variety of different species (refer to sequences in Figure 12) and a DNA-targeting RNA. This experiment demonstrates that the various Cas9/Csn1 site-directed modifying polypeptides can utilize the same DNA-targeting RNA. B. A schematic representation of the DNA-targeting RNA used in Figure 5A.

F. Brief description of the drawings
[0004] Figures 1A and 1B provides a schematic drawing of two exemplary subject DNA-targeting RNAs, each associated with a site-directed modifying polypeptide and with a target DNA. A DNA-targeting RNA comprises a single stranded “DNA-targeting segment” and a “protein-binding segment,” which comprises a stretch of double stranded RNA. (A) A DNA-targeting RNA can comprise two separate RNA molecules (referred to as a “double-molecule” or “two-molecule” DNA-targeting RNA). A double-molecule DNA-targeting RNA comprises a “targeter-RNA” and an “activator-RNA.” (B) A DNA-targeting RNA can comprise a single RNA molecule (referred to as a “single-molecule” DNA-targeting RNA). A single-molecule DNA-targeting RNA comprises “linker nucleotides.”

[0005] Figure 2 depicts the amino acid sequence of a Cas9/Csn1 protein from Streptococcus pyogenes. Domains 1 and 2 are conserved to varying degrees among the Cas9/Csn1 proteins of many different species (also see Figures 10-12). The presence of domains 1 and 2 is a characteristic of Cas9/Csn1 proteins. The depicted domain 1 is 1 of 3 motifs present in Cas9/Csn1 that define a RuvC endonuclease domain. The depicted domain 2 is a combination of motifs 2 and 3 of the RuvC endonuclease domain as well as an HNH endonuclease domain.
[0006] Figures 3A-C depict target DNA cleavage by a site-directed modifying polypeptide (exemplified by the Cas9/Csn1 protein of Streptococcus pyogenes), which is directed by a DNA-targeting RNA. (A) Radiolabeled target DNAs were incubated in the presence of recombinant Cas9/Csn1 and various DNA-targeting RNA species (as indicated). Cleavage products were resolved using denaturing polyacrylamide gel electrophoresis and visualized by phosphorimaging. (B) Schematic diagrams of the DNA-targeting RNAs used in conjunction with the Cas9/Csn1 site-directed modifying polypeptide. Note that while one of the tested single-molecule DNA-targeting RNAs (RNA chimera A) supported efficient target DNA cleavage, the other tested single-molecule DNA-targeting RNA (RNA chimera B) did not. (C) Schematic representations of DNA-targeting RNA sequences and DNA targets.
[0007] Figure 4 depicts target DNA editing through double-stranded DNA breaks introduced using a Cas9/Csn1 site-directed modifying polypeptide and a DNA-targeting RNA.
[0008] Figures 5A and 5B depict target DNA cleavage. Figure 5 A. Target DNA cleavage using a Cas9/Csn1 site-directed modifying polypeptide from a variety of different species (refer to sequences in Figure 12) and a DNA-targeting RNA. Target cleavage was carried out under the same conditions as in Figure 3. This experiment demonstrates that the various Cas9/Csn1 site-directed modifying polypeptides can utilize the same DNA-targeting RNA. B. A schematic representation of the DNA-targeting RNA used in Figure 5A.

G. Drawings

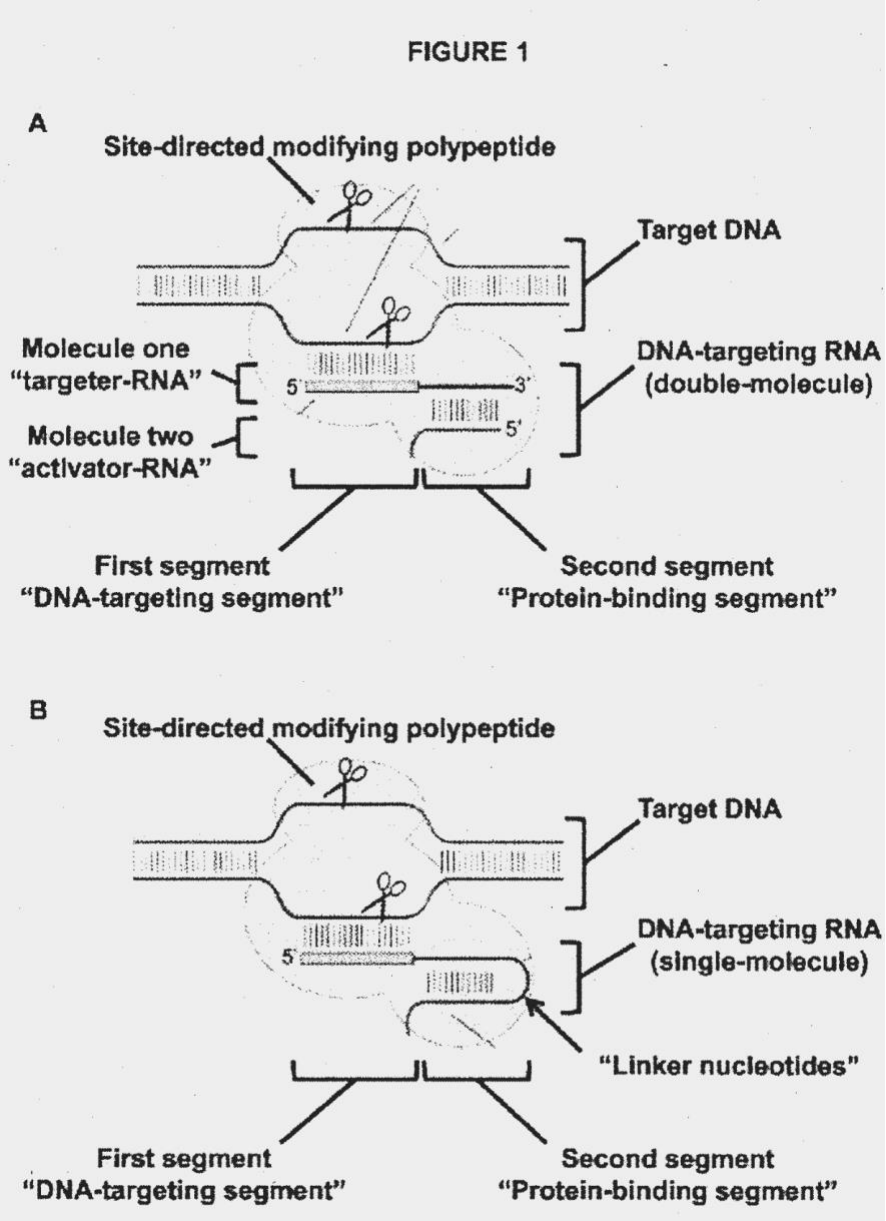


FIGURE 1
A
Site-directed modifying polypeptide
Target DNA
Molecule one
"targeter-RNA"
Molecule two
"activator-RNA"
5'
3'
5'
DNA-targeting RNA
(double-molecule)
First segment
"DNA-targeting segment"
Second segment
"Protein-binding segment"
B
Site-directed modifying polypeptide
Target DNA
5'
DNA-targeting RNA
(single-molecule)
"Linker nucleotides"
First segment
"DNA-targeting segment"
Second segment
"Protein-binding segment"

(Fig. 1)


FIGURE 2
Cas9/Csn1 Streptococcus pyogenes (Total length = 1368)
Domain 1: RuvC endonuclease domain motif 1 (7-166)
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALL
FDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEE
SFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIY
LALAHMIKFRGHFLIEGDLNPDNDVDKLFIQLVQTYNQLFEENPINASGVDA
KAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAED
AKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITK
APLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGG
ASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGEL
HAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEE
TITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYN
ELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIEC
FDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFED
REMIEERLKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTI
LDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSGQGDSLHEHIANLAGS
PAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNSRER
MKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDIN
RLSDYDVDHIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKN
YWRQLLNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVA
QILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKVREINNYHH
AHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATA
KYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVL
SMPQVNIVKKTEVQTGGFSKESILPKRNDKLIARKKDWDPKKYGGFDSPTV
AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKK
DLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEK
LKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNK
HRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQ
SITGLYETRIDLSQLGGD
Domain 2: RuvC/HNH/RuvC endonuclease domain motifs (731-1003)

(Fig. 2)

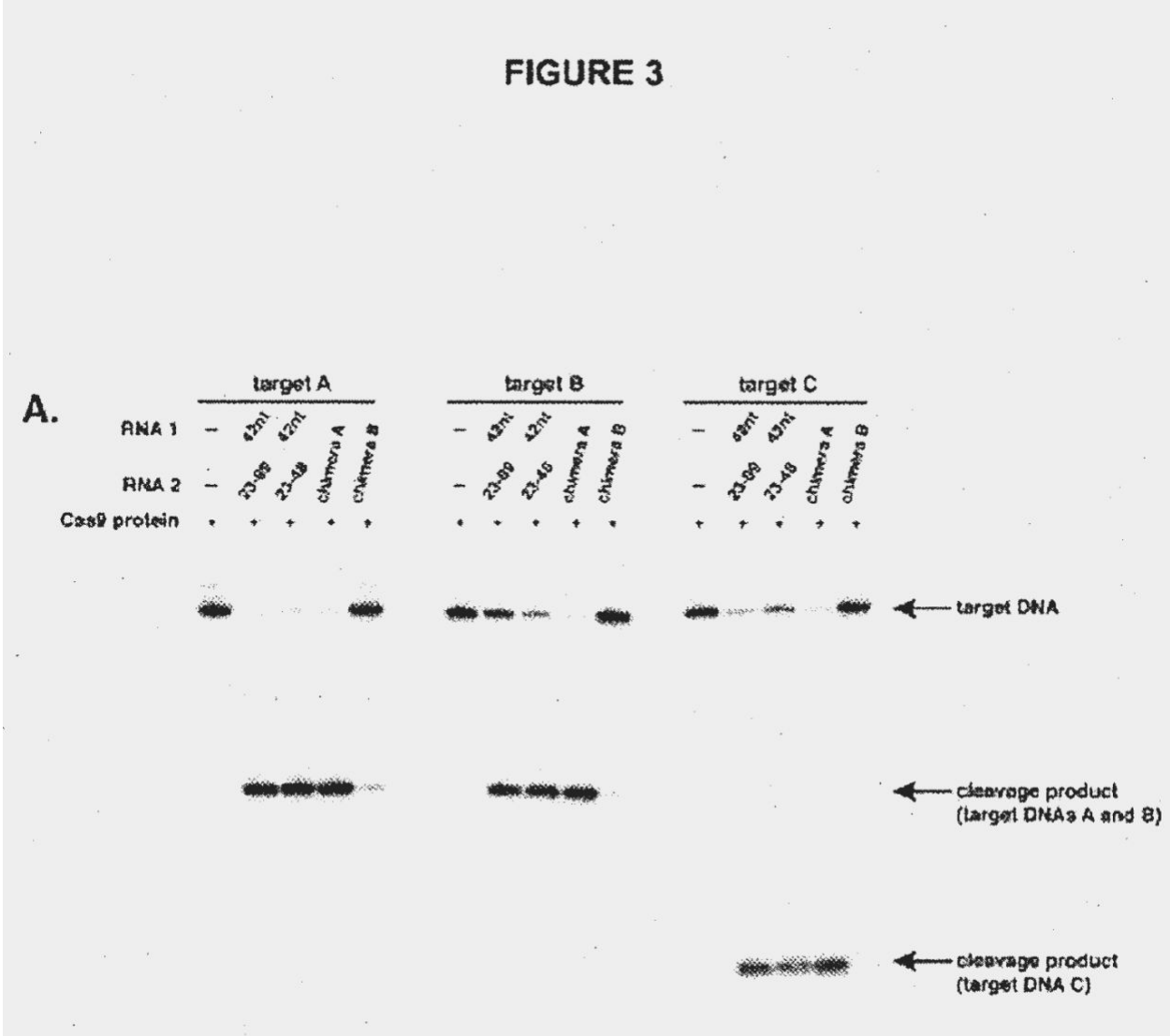


FIGURE 3
A.
target A
target B
target C
RNA 1
RNA 2
Cas9 protein
42nt
23-99
23-46
chimera A
chimera B
target DNA
cleavage product
(target DNAs A and B)
cleavage product
(target DNA C)

(Fig. 3A)

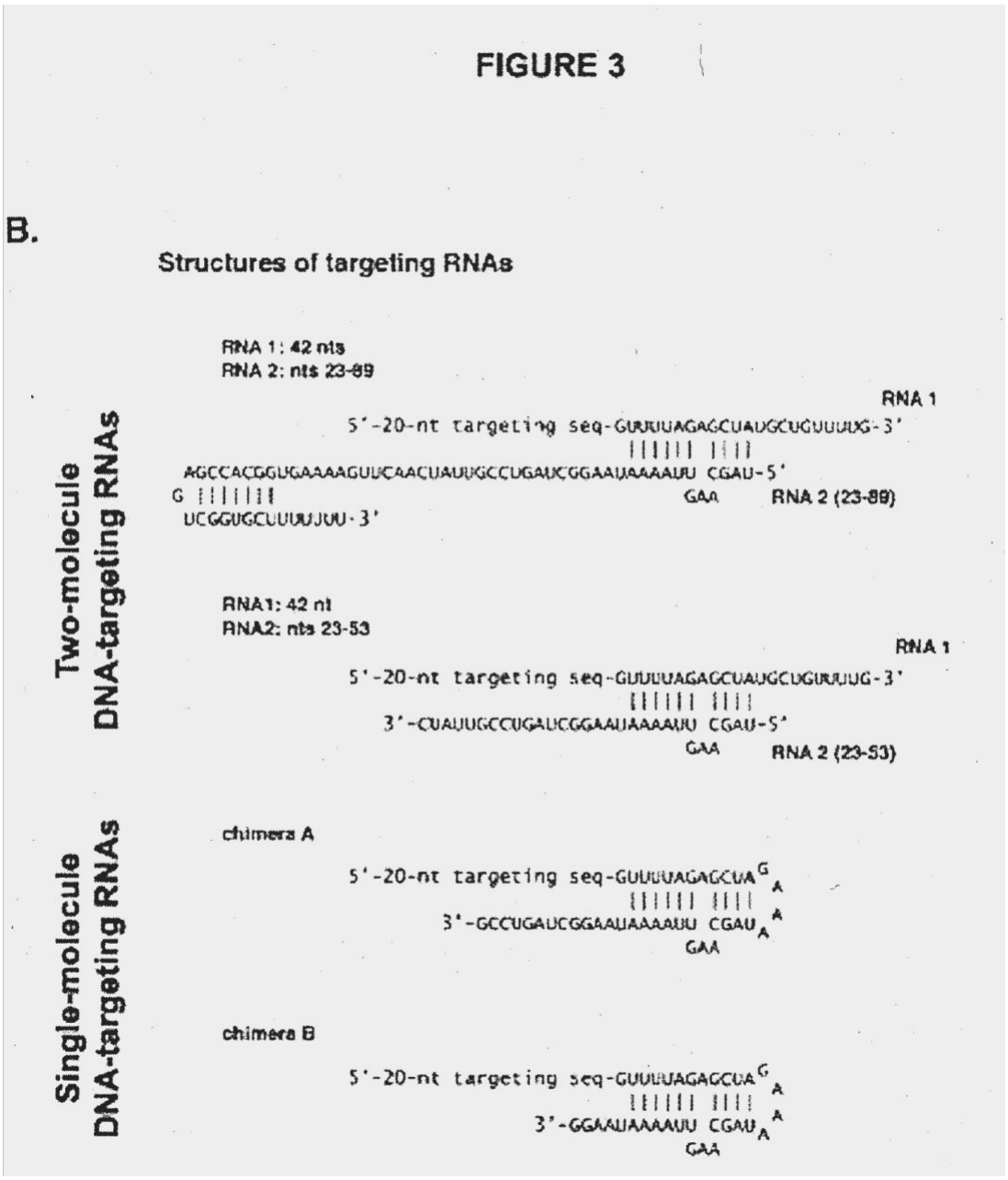


FIGURE 3
B.
Structures of targeting RNAs
Two-molecule DNA-targeting RNAs
RNA 1: 42 nts
RNA 2: nts 23-89
RNA 1
5'-20-nt targeting seq-GUUUUAGAGCUAUGCUGUUUUG-3'
AGCCACGGUGAAAAGUUCAACUAUUGCCUGAUCGGAAUAAAAUU CGAU-5'
G
GAA
RNA 2 (23-89)
UCGGUGCUUUUJUU-3'
RNA1: 42 nt
RNA2: nts 23-53
RNA 1
5'-20-nt targeting seq-GUUUUAGAGCUAUGCUGUUUUG-3'
3'-CUAUUGCCUGAUCGGAAUAAAAUU CGAU-5'
GAA
RNA 2 (23-53)
Single-molecule DNA-targeting RNAs
chimera A
5'-20-nt targeting seq-GUUUUAGAGCUA G A
3'-GCCUGAUCGGAAUAAAAUU CGAU A A
GAA
chimera B
5'-20-nt targeting seq-GUUUUAGAGCUA G A
3'-GGAAUAAAAUU CGAU A A
GAA

(Fig. 3B)

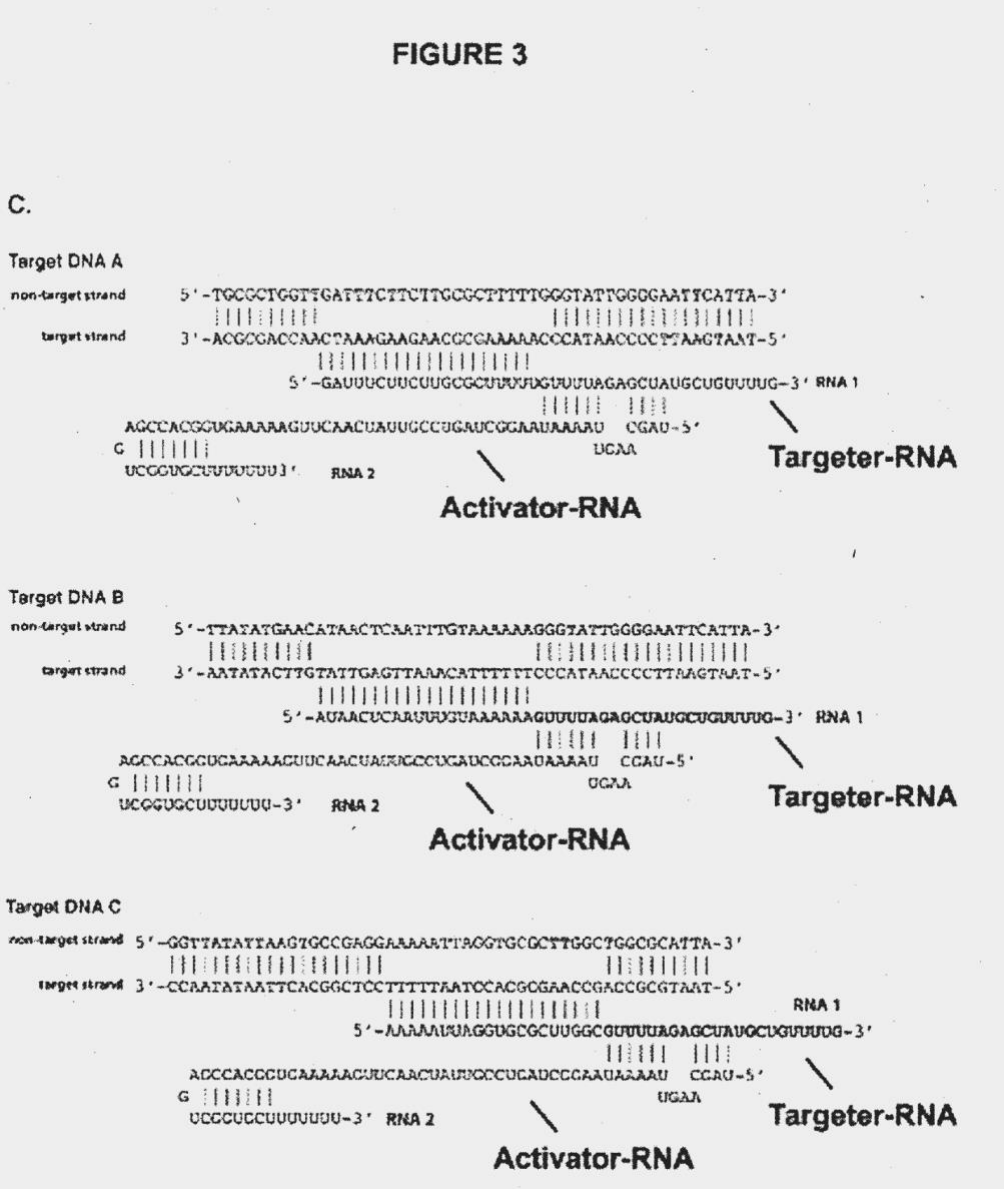


FIGURE 3
C.
Target DNA A
non-target strand
target strand
RNA 1
RNA 2
Targeter-RNA
Activator-RNA
Target DNA B
non-target strand
target strand
RNA 1
RNA 2
Targeter-RNA
Activator-RNA
Target DNA C
non-target strand
target strand
RNA 1
RNA 2
Targeter-RNA
Activator-RNA

(Fig. 3C)

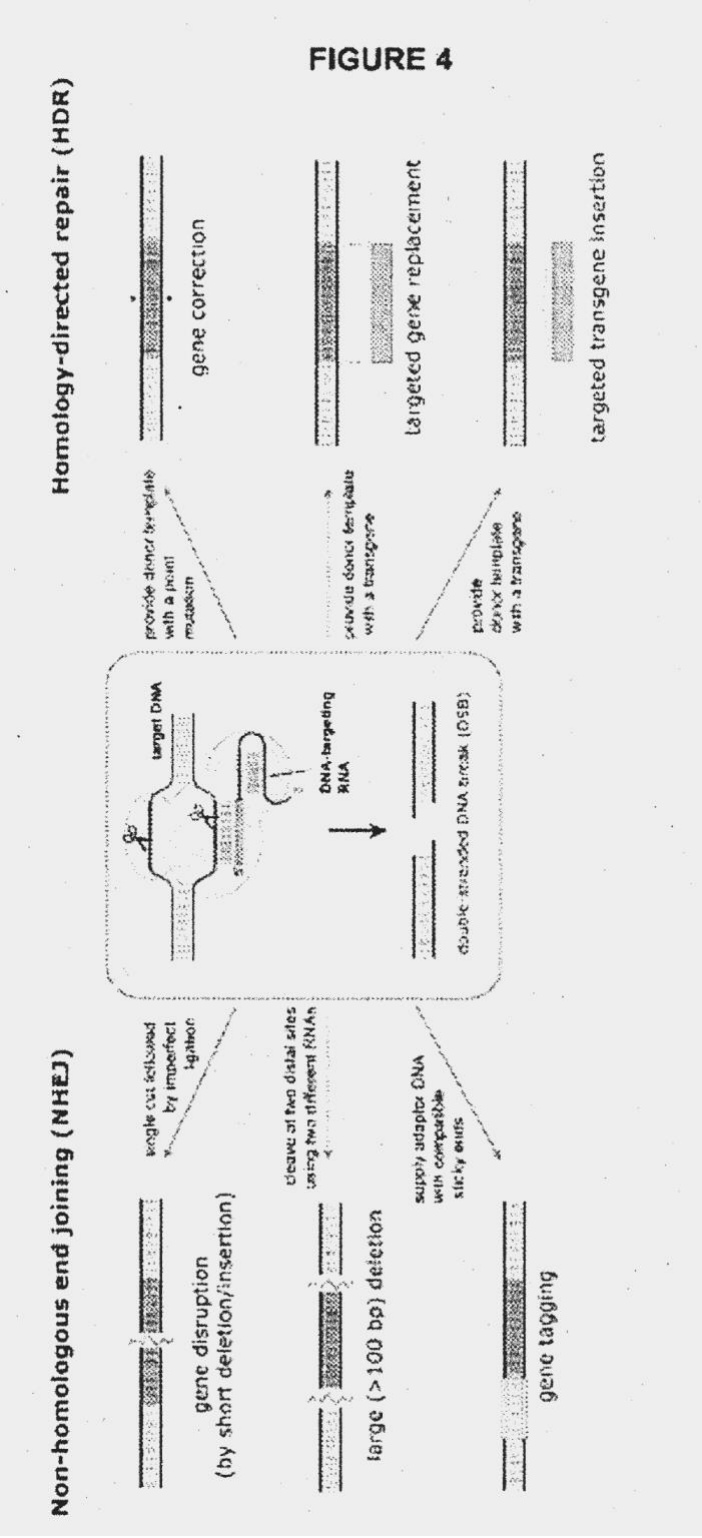
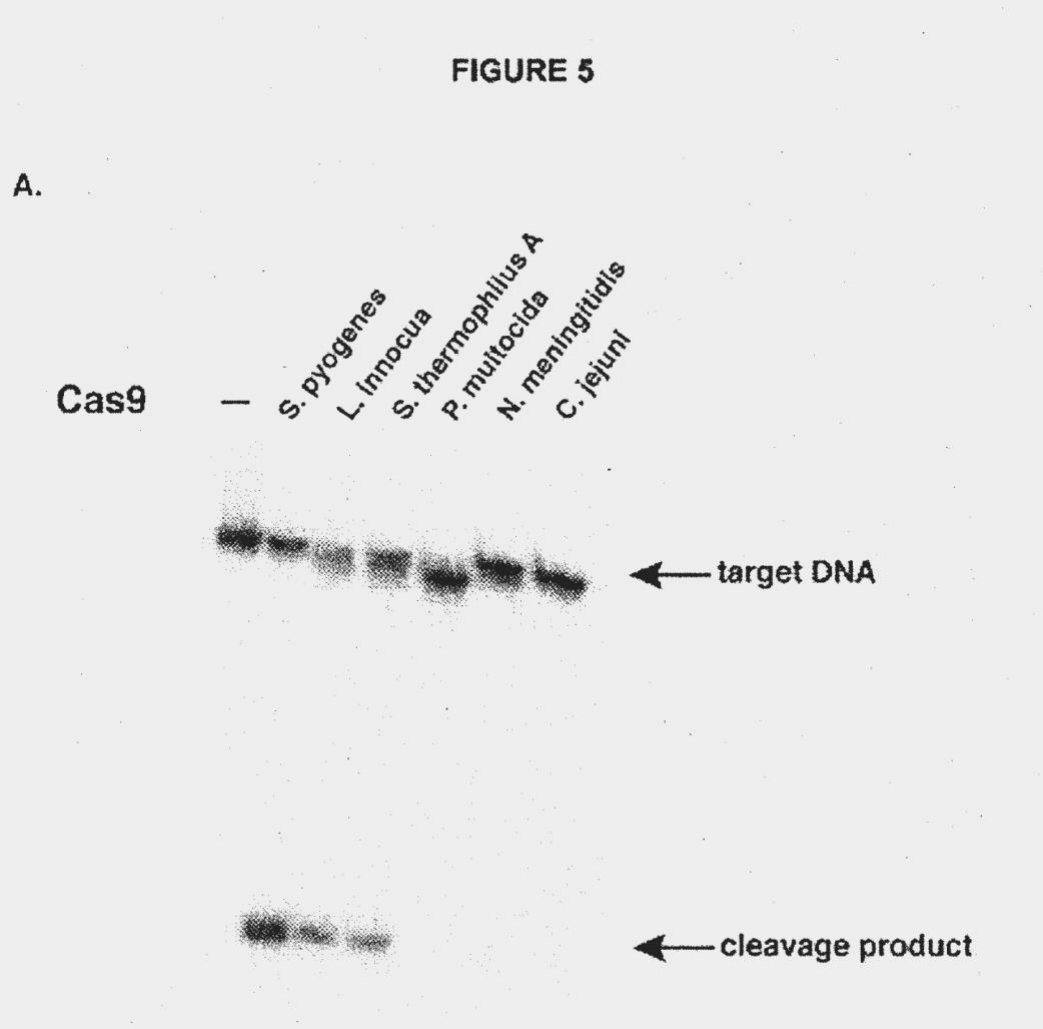
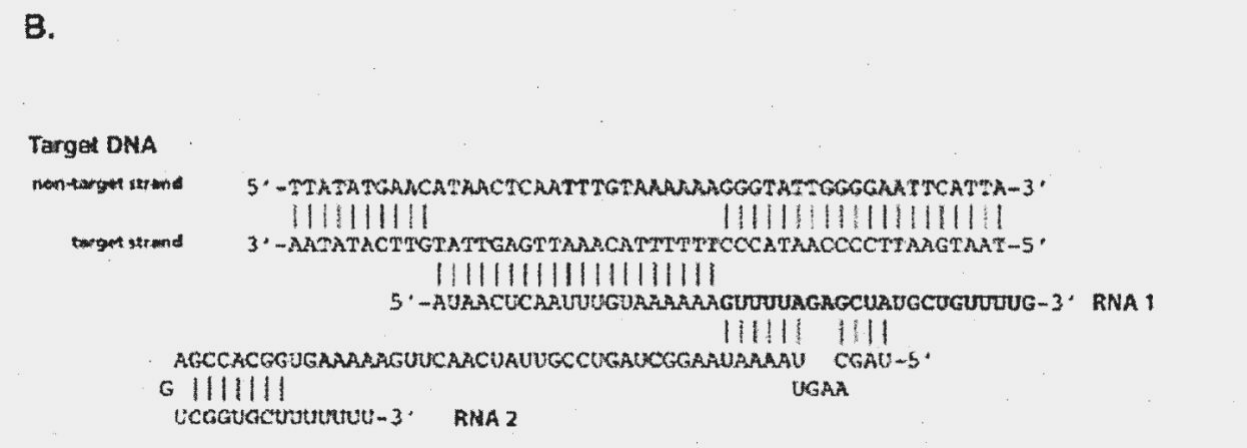


FIGURE 4
Homology-directed repair (HDR)
Non-homologous end joining (NHEJ)
target DNA
DNA-targeting RNA
double-stranded DNA break (DSB)
gene correction
targeted gene replacement
targeted transgene insertion
gene disruption
(by short deletion/insertion)
large (>100 bp) deletion
gene tagging

(Fig. 4)



(Fig. 5A)



(Fig. 5B)

(4) Matter in Respondents' Exhibit No. 15

Matter in Respondents' Exhibit No. 15 (Annu. Rev. Genet.; Vol. 45, 273?97 (2011)) is a

general discussion titled “CRISPR-Cas Systems in Bacteria and Archaea: Versatile Small RNAs for Adaptive Defense and Regulation” and published before the filing date of the first priority basis application. It has the following.

“Mature crRNA, together with Cas9, interferes with matching invasive ds-DNA by homology-driven cleavage within the protospacer sequence, in the direct vicinity of the PAM. Mismatches at the 3’ end of the protospacer and/or in the PAM allow phages and plasmids to circumvent CRISPR-encoded immunity.” (Page 281, right column, lines 13-20.)

(5) Matter in Respondents’ Exhibit No. 19

Respondents’ Exhibit No. 19 (Science; vol. 327 (5962), 167-170 (2010)) is a general discussion titled “CRISPR/Cas, the Immune System of Bacteria and Archaea” and published before the filing date of the first priority basis application. It has the following.

“Analysis of phage sequences adjacent to proto-spacers revealed the presence of conserved sequences, called CRISPR motifs, or proto-spacer adjacent motifs (PAMs). Phages may also circumvent the CRISPR/Cas system by mutating the CRISPR motif (18), which indicates that it is involved in CRISPR- encoded immunity. Additionally, CRISPR motif mutation can result in loss of phage resistance despite the presence of a matching CRISPR spacer. The absence of this motif in the CRISPR locus likely allows the system to act on the invading target DNA specifically and precludes an “autoimmune” response on the host chromosome.” (Page 169, right column, lines 15-30.)

(6) Matter in Respondents’ Exhibit No. 25

Respondents’ Exhibit No. 25 (Curr. Opin. Microbiol.; vol. 14(3), 321-327 (2011)) is a general discussion titled “CRISPR-Based Adaptive Immune Systems” and published before the filing date of the first priority basis application. It has the following.

“For DNA targeting systems, it is critical that the pathway not target the corresponding spacer in its own CRISPR (which matches the protospacer in the invader). PAMs provide one mechanism for distinction of self vs. non-self. PAMs are critical for silencing by several systems [6,7,51], and the PAM recognized by the CRISPR-Cas system in the invader is not present in the repeat sequence that flanks the potential target in the CRISPR.” (Page 4, lines 25-29.)

(7) Matter in Respondents’ Exhibit No. 72

Respondents’ Exhibit No. 72 (Nonviral Vectors for Gene Therapy; Chapter 7, 139-142, 147-153 (1999)) is part of a book titled “Nonviral Vectors for Gene Therapy” and published before the filing date of the first priority basis application. “Chapter 7 - Nuclear Transport of Exogenous DNA” has the following.

"Larger molecules need a nuclear localization signal (NLS) sequence in order to be actively trans- ported through the central channel of the pore by the help of different transport factors. ... However, the pathway for which the most detailed information is available today is the so-called classical transport pathway of proteins carrying lysine- and arginine-rich (basic) NLSs." (Page 141, lines 21-29.)

IV. Attempts to Increase the Efficiency of Nuclear Uptake

It is well established that the attachment of NLSs to proteins not normally karyophilic enables their efficient nuclear targeting." (Page 148, lines 37-40.)

(8) Matter in Respondents' Exhibit No. 79

Respondents' Exhibit No. 79 (Trends in Biotechnology; vol. 22(7), 346-353 (2004)) is a general discussion titled "Codon bias and heterologous protein expression" and published before the filing date of the first priority basis application. It has the following.

"In general, the more codons that a gene contains that are rarely used in the expression host, the less likely it is that the heterologous protein will be expressed at reasonable levels. ... A common strategy to improve expression is therefore to alter the rare codons in the target gene so that they more closely reflect tile codon usage of the host, without modifying the amino acid sequence of the encoded protein." (Page 348, right column, lines 39-48.)

2. Priority date of the Patent

(1) Court's judgment

As noted in Section 1(3) above, the first priority basis application is the US provisional application describing the invention titled "Methods and Compositions for RNA-Directed Site-Specific DNA Modification" which discloses an invention of a new technology capable of replacing the two main technologies for manipulating site-specific DNA nucleases which had garnered attention as powerful tools for genetic engineering of cells and entire organisms (Section 1(3)C).

The claims describe a method for site-specifically modifying target DNA by causing a complex including DNA-targeting RNA and a site-specific modifying polypeptide to come into contact with the target DNA (Section 1(3)B, claim 54), and indicate that the target DNA is part of the chromosomes inside eukaryotic, plant, and animal cells (Section 1(3)B, claims 58, 61, 65, and 66) and that the enzyme activity of the site-specific modifying polypeptide is nuclease activity and the DNA modification is DNA double-strange cleavage (Section 1(3)B, claims 73-76).

In this regard, the detailed description indicates that the DNA-targeting RNA used in the invention described in the claims includes a DNA-targeting segment and protein-binding

segment (Section 1(3)D, paragraph [0073]), that the DNA-targeting segment includes a nucleotide sequence which complements the sequence in the target DNA (Section 1(3)D, paragraph [0074]), that the protein binding segment interacts with the site-specific modifying polypeptide and includes a range of two mutually complementary nucleotides which hybridize to form double-stranded RNA (Section 1(3)D, paragraph [0076]) and includes crRNA and corresponding tracrRNA (Section 1(3)D, paragraph [0083]). Furthermore, the site-specific modifying polypeptide used in the invention described in the claims forms a complex with the DNA-targeting RNA, and is the natural Cas9/Csn1 protein derived from the various bacteria whose amino acid sequences are given in FIGs. 2 and 12 (the application documents of the first priority basis application describe "Cas9/Csn1 endonuclease" and "Cas9/Csn site-specific modifying polypeptide," jointly referred to herebelow as "Cas9 polypeptide" to match the language in the specification of the Patent) and their modified forms (Section 1(3)D, paragraphs [0089], [0091], and [0092], and Section 1(3)F, paragraph [0005]). It is also indicated that the complex used in the invention described in the claims operates by a mechanism whereby the DNA-targeting segment base-pairs specifically with the target DNA sequence, and the site-specific modifying polypeptide modifies (e.g., cleaves) the target DNA at that location (Section (3)D, paragraph [0089], Section (3)F, paragraph [0004], and Section (3)G, FIG. 1).

Thus, the detailed description describes concretely and in detail the structure and mechanism of action of the Cas9 polypeptide and the DNA-targeting RNA used in the invention set forth in the claims.

Moreover, the examples indicate in two experiments, in FIGs. 2 and 5, that target DNA was successfully cleaved using the method set forth in the claims. Of these, the experiment in FIG. 3 was an experiment in a buffer solution using a Streptococcus pyogenes-derived Cas9 polypeptide and DNA-targeting RNA on three types of target DNA (FIG. 3C), describing using a DNA-targeting RNA which included a DNA-targeting segment and a protein-binding segment (FIG. 3B), and indicating that cleavage occurred in all the target DNA (FIG. 3A) (Section (3)E, paragraphs [0249] and [0251], Section (3)F, paragraph [0006], and FIGs. A to C). The experiment in FIG. 5 is an experiment in a buffer solution using six Cas9 polypeptides having different derivations and the same DNA-targeting RNA on the same target DNA, indicating that a DNA-targeting RNA which included a DNA-targeting segment and a protein binding segment was used (FIG. 5B), and that the target DNA was cleaved by all of the Cas9 polypeptides (FIG. 5A) (Section (3)E, paragraphs [0249] and [0251], Section (3)F, paragraph [0008], and Section (3)G, FIGs. 5A and 5B).

Accordingly, the "method for site-specific cleavage in target DNA, comprising a step of causing target DNA to come in contact with a complex including a site-specific modifying

polypeptide displaying nuclease activity and a DNA-targeting RNA which includes a nucleotide sequence which is complementary to the sequence in the target DNA" can be said to be described in the application documents of the first priority basis application in detail and accompanied by the structure of the complex, the mechanism of action, and concrete data.

At the same time, as noted above, the claims also describe a method for cleaving target DNA using part of the chromosomes in eukaryotic single cells, plant cells, animal cells, and other cells as the target (Section 1(3)B, claims 58, 61, 65, and 66).

In this regard, the detailed description indicates that the priority basis invention can be applied not only to in vitro cells but also in vivo cells (Section 1(3)D, paragraph [00121]) and to DNA of any type of cell derived from any organism, with specific biological species and cell types given as examples (Section 1(3)D, paragraph [00165]), describing two technical means for realizing these applications: a means for introducing a complex including DNA-targeting RNA and a site-specific modifying polypeptide into a cell in the form of a nucleic acid encoding the components, and a means for introducing the complex into the cell as-is (Section 1(3)D, paragraph [00121]). A known, specific example is given of the former - an expression vector applied to expression of an exogenous nucleic acid in a eukaryotic cell (Section 1(3)D, paragraph [00124]); it is indicated that in the expression vector, the nucleic acid can be operably linked to various regulatory elements suitable to its expression (the promoter, ribosome binding site, transcription terminator, sequences for enhancing expression), with specific, known examples of the regulatory elements (Section 1(3)D, paragraph [00126]) and many examples of known means for introducing nucleic acids such as expression vectors into the cell given (Section 1(3)D, paragraph [00129]). Regarding the latter, it is indicated that the DNA-targeting RNA, which is a component of the complex, can be made using chemical synthesis or in vitro synthesis, and can be introduced into the cell using known techniques (e.g., micro-injection, electroporation, and transfection) (Section 1(3)D, paragraph [00173]); and it is indicated that the site-specific modifying polypeptide, such as the Cas9 polypeptide, which is another component of the complex, can fuse with domains such as a domain for enhancing solubility, a domain for improving stability, and a permeable domain for promoting cellular uptake, with specific examples of each domain given (Section 1(3)D, paragraphs [00178] and [00179]). In addition to the production methods for the Cas9 polypeptide and the DNA targeting RNA (Section 1(3)E, paragraph [0284]), the examples indicate that the complex of the Cas9 polypeptide and the DNA-targeting RNA were obtained by incubating the chemically synthesized DNA-targeting RNA and the Cas9 polypeptide obtained through recombinant expression of E. coli in a buffer solution for 15 min (Section 1(3)E).

It is also indicated that genetically modified cells obtained by applying the priority basis invention to the DNA in eukaryotic cells, plant cells, and animal cells can be widely used for

purposes such as gene therapy as an antiviral, antipathogenic, and anticancer drug, for producing genetically modified organisms in agriculture, and for biological research (Section 1(3)D, paragraph [00198]).

Thus, the application documents for the first priority basis application primarily focuses on providing a complex which includes a site-specific modifying polypeptide and a DNA-targeting RNA as a new technology to replace the two principal technologies for manipulating site-specific DNA nucleases, and discloses not just a method for producing this complex, but also shows that the complex site-specifically cleaves target DNA based on concrete data. The application documents disclose that the complex which includes a site-specific modifying polypeptide and a DNA-targeting RNA is also applicable to target DNA inside in vivo cells (e.g., eukaryotic cells) and not just in vitro cells, disclosing the utility of such applications and disclosing that such applications can be realized concretely by use of known technical means by persons skilled in the art.

Accordingly, applying the priority basis invention to target DNA in eukaryotic cells such as plant cells, animal cells, and single-celled eukaryotic organisms is a technical matter which can be derived by generalizing everything set down in the application documents for the first priority basis application, and the Invention provided with this technical matter can enjoy the benefit of a priority claim based on the first priority basis application.

(2) Petitioner's argument

A. Petitioner's argument

(A) Lack of disclosure in the application documents for the first and second priority base applications

The application documents for the first and second priority base applications do not describe specific modes for carrying out the CRISPR/Cas9 system in eukaryotic cells, merely presenting a list of names of various eukaryotic cells as a future challenge. The application documents indicate nothing regarding the role of PAM sequences and the CRISPR/Cas9 system adapted to work in eukaryotic cells. Moreover, at the time the first and second priority basis applications were filed, it was unknown whether codon optimization would be required for the CRISPR/Cas9 system to work in eukaryotic cells.

In contrast, Petitioner's Exhibits No. 1 and 2, which described implementation of the CRISPR/Cas9 system in eukaryotic cells, demonstrate ingenuity not described in the application documents for the first and second priority basis applications (e.g., binding of a nuclear localization signal NLS to a specific location of the Cas9 polypeptide, and codon optimization in the nucleic acid encoding the Cas9 polypeptide), which goes beyond the prior art.

(B) Barriers to introduction of the CRISPR/Cas9 system into eukaryotic cells

At the time the first and second priority basis applications were filed, persons skilled in the art considered a. to e. below to be barriers to introduction of the CRISPR/Cas9 system in

eukaryotic cells, and were unaware that the CRISPR/Cas9 complex could function completely in eukaryotic cells.

a. RNA degradation

At the time the first and second priority basis applications were filed, it was known that eukaryotic cells expressed ribonuclease (an enzyme which cleaves RNA) not expressed in prokaryotic cells, and that introduction of double-stranded RNA into a eukaryotic cell would trigger an interferon response and RNA interference, degrading the RNA. Accordingly, persons skilled in the art understood the possibility of experiencing barriers relating to stability of the RNA component of the CRISPR/Cas9 system.

b. Folding and formation of the complex

At the time the first and second priority basis applications were filed, persons skilled in the art understood that there was a possibility that the cellular temperature, ion concentration (e.g., magnesium ion concentration) and pH in eukaryotic cells could differ from those of prokaryotic cells, and that the eukaryotic cell environment could affect the formation and functioning of the CRISPR/Cas9 system, which originated in prokaryotic cells, in unpredictable ways.

c. Eukaryotic cell resistance

At the time the first and second priority basis applications were filed, persons skilled in the art were aware of the possibility that the CRISPR/Cas9 system, which is a bacterial adaptive immune system, could trigger toxicity or cell death in eukaryotic cells.

d. Colocalization

Unlike prokaryotic cells, eukaryotic cells include chromosomal DNA which is a target of the CRISPR/Cas9 system in the nucleus separated by a membrane structure. At the time the first and second priority basis applications were filed, persons skilled in the art did not know whether there were conditions necessary for localizing the CRISPR/Cas9 system components simultaneously inside the nucleus.

e. Chromatin

Eukaryotic cell genomic DNA has a chromatin structure, which is a complex and tightly packed structure which combines with proteins (principally histones), which is different from the small DNA molecules which are the natural target of the CRISPR/Cas9 system. At the time the first and second priority basis applications were filed, it was unknown whether it would be possible for the CRISPR/Cas9 system to edit the chromosomes of the eukaryotic cell which is made up of chromatin.

(C) Cases of failure to transfer from a prokaryotic host to a eukaryotic host

Persons skilled in the art were aware of the cases a. to e. Where there was a failure to transfer from prokaryotic cell host to eukaryotic cell hosts, and therefore could have anticipated that special conditions would be needed to use the CRISPR/Cas9 system to edit the eukaryotic cell genome, and could not reasonably have expected success in the absence of experimental results.

a. Group II introns

Group II introns are RNA-derived endonuclease is originating in prokaryotic cells and

requiring an RNA component. They are the closest precedent to the CRISPR/Cas9 system. In order to apply these two eukaryotic cells, a large quantity of magnesium ions needs to be injected, but such a high magnesium ion concentration is harmful to eukaryotic cells.

Moreover, it had been reported that the CRISPR/Cas9 system was magnesium-dependent, so persons skilled in the art would have anticipated the same barriers as with group II introns when applying the CRISPR/Cas9 system to eukaryotic cells.

b. T7RNA polymerase

Although the bacteriophage-derived T7RNA polymerase was expected to work with almost all DNA, it has not been used in the majority of advanced eukaryotic cells.

c. Hammerhead ribozyme

Like the CRISPR/Cas9 system, the hammerhead ribozyme contributes to nucleic acid cleavage and might depend on the RNA molecule. The most important factor determining ribozyme activity in vivo is the colocalization of the ribozyme in the target nucleic acid, but do to the ease with which RNA molecules degrade inside eukaryotic cells, a significant portion of the ribozyme will undoubtedly degrade during transport to the target location. Therefore, persons skilled in the art believed that in vitro ribozyme activity could not predict its in vivo activity.

d. Riboswitches

Riboswitches are RNA which bind to specific chemical substances and change their shape in response. There are present in bacteria, but efforts to make riboswitches in test tubes function inside eukaryotic cells have failed. The different way RNA folds in test tubes and in cells is thought to be a likely cause.

e. pAgo

The prokaryotic pAgo nucleic acid-derived nuclease can cleave bear DNA using a nucleic acid-derived method in a test tube, but is unable to cleave DNA wrapped around a histone. Moreover, since pAgo originates in thermophilic bacteria, it does not function at 37°C, the temperature needed for gene editing in mammalian cells and it precipitates when expressed in E. coli. It is also difficult to add the correct guide nucleic acid. For these reasons, no pAgo capable of editing the genome in a programmable way in mammalian cells has been obtained.

(D) Interference ruling by the USPTO

In interference no. 106,115 at the US PTO PTAB, the same arguments as an (B) and (C) above were considered, and it was determined that the inventions by The Broad Institute, Inc. relating to Petitioner’s Exhibit No. 1, etc., were the senior party, and the invention of the present

case was the junior party (Petitioner's Exhibit No. 7).

(E) Differences between ZFNs/TALENs and the CRISPR/Cas9 system

The respondents the CRISPR/Cas9 system can be implemented in eukaryotic cells based on existing techniques in eukaryotic cells using zinc finger nuclease as (ZFNs) and transcription activity-like effector nuclease is (TALENs).

However, ZFNs and TALENs are techniques which were originally developed for eukaryotic cells, whereas the CRISPR/Cas9 system was originally developed for prokaryotic cells. Moreover, ZFNs and TALENs artificial, created by artificially binding a DNA-binding domain and a DNA cleavage domain, whereas the CRISPR/Cas9 system is structurally different in being the Cas9 polypeptide combined with a DNA-targeting RNA produced in natural prokaryotic cells. The mechanism of action of the sequence-specific binding to the target DNA also differs in each.

Accordingly, ZFNs and TALENs cannot be a reference for application of the CRISPR/Cas9 system to eukaryotic cells.

B. Judgment of the Court

(A) Lack of disclosure in the application documents for the first and second priority basis applications

It is found that at the time the first priority basis application was filed it was widely known that proto-space adjacent motif (PAM) sequences were needed downstream of the target DNA sequence in order for the CRISPR/Cas9 system to cleave the target DNA sequence (Section 1(4) to (6)). The CRISPR/Cas9 system has a mechanism of action whereby a DNA-targeting segment pair-binds sequence-specifically to the target DNA, and the Cas9 polypeptide cleaves the DNA there (Section 1(3)D, paragraph [0089], Section 1(3)F, paragraph [0004], Section 1(3)G, fig.1), and it would be obvious to a person skilled in the art that a Pam should be present downstream of the target DNA sequence, as is widely known, even when the target DNA is inside the eukaryotic cell. It was widely known at the time the first priority basis application was filed that in order to express an exogenous gene efficiently inside the host eukaryotic cell, a nuclear localization signal (MLS) for active transportation of the exogenous gene into the nucleus to the appropriate location had to be added (Section 1(7)) and that the codon had to be optimized according to the host cell (Section 1(8)). Therefore, it would have naturally occurred to a person skilled in the art that adding a nuclear localization signal (NLS) and optimizing the code on would be useful when expressing the CRISPR/Cas9 system in a eukaryotic cell.

The petitioner argues that binding the nuclear localization signal (MLS) to a specific location of the Cas9 polypeptide and codon optimization in the nucleic acid encoding the Cas9 polypeptide were innovations in Petitioner's Exhibit No. 1 and 2 which describe implementing

the CRISPR/Cas9 system in the eukaryotic cell and go beyond the prior art. However, the description relating to the nuclear localization signal (MLS) in Petitioner's Exhibit No. 1 is limited to the simple optional additional matter of "including at least one or more nuclear localization sequences depending on the case UNDERLYING" in claim 1 (Section 1(1)), and Petitioner's Exhibit No. 2 makes no mention whatsoever in the claims (Section 1(2)). Therefore it cannot be said that binding the nuclear localization signal (MLS) to a specific location of the Cas9 polypeptide is either a characteristic configuration or an innovation when applying the CRISPR/Cas9 system to eukaryotic cells. As regards codon optimization, it is not mentioned in the claims in either Petitions Exhibit No. 1 or 2 (Section 1(1) and (2)), and therefore this, too, cannot be said to be either a characteristic configuration or innovation when applying the CRISPR/Cas9 system to eukaryotic cells.

Accordingly, just because the application documents of the first priority basis application lack any explicit description of proto-space or adjacent motifs (PAM), Nuclear Localization Signals (MLS), or codon optimization does not hinder the application of the CRISPR/Cas9 system, i.e., the priority basis invention, and eukaryotic cells.

Hence, in light of the known art on the filing date of the first priority basis application, applying the priority basis invention to target DNA in eukaryotic cells such as plant cells, animal cells, and single celled eukaryotes can be found to be described in the application documents for the first priority basis application to a degree allowing implementation by a person skilled in the art. Therefore, The petitioner's argument that the application documents for the first and second priority basis applications merely list names of various types of eukaryotic cells as a future task SOURCE" fails.

(B) Barriers to introduction of the CRISPR/Cas9 system into eukaryotic cells

As noted in Section A (B) above, the petitioner argues that RNA degradation (a), folding and complex formation (b), eukaryotic cell resistance (c), colocalization (d), and chromatin (e) are barriers to introduction of the CRISPR/Cas9 system into eukaryotic cells.

However, arguments a. to e. By the petitioner merely describe general and abstract possibilities or concerns relating to things that could happen when applying the CRISPR/Cas9 system to a eukaryotic cell in a buffer solution as described in the examples in the application documents of the first priority basis application, based on differences between prokaryotic and eukaryotic cells. The arguments do not indicate that it has been confirmed that these things actually occur, and therefore it is not found that they would be barriers to be overcome when introducing the CRISPR/Cas9 system into eukaryotic cells.

(C) Cases of failure to transfer from a prokaryotic host cell to a eukaryotic host cell.

As noted in Section A(C) above, the petitioner argues that persons skilled in the art knew about cases of failure to transfer from a prokaryotic cell host to a eukaryotic cell host, such as

group II introns (a), T7RNA polymerase (b), hammerhead ribozymes (c), riboswitches (d), and pAgo (e), and therefore would not have reasonably expected success in editing eukaryotic cell genes using the CRISPR/Cas9 system in the absence of experimental results.

However, these cases only have low relevance to the CRISPR/Cas9 system, being related to the CRISPR/Cas9 system only in being derived from prokaryotic cells. Even if there were papers reporting cases of failure to transfer these technologies to eukaryotic cells, they could not be said to hinder application of the CRISPR/Cas9 system to eukaryotic cells or to obviate the expectation of the success of such application.

(D) Decision in USPTO interference

Japanese and US patent law are based on different legal systems. Japanese patent law lacks any provisions relating to the type of interference procedure used under a first-to-invent system or the decisions produced by such a system.

As regards the EU patents corresponding to the Patent under review here, the EPO's Opposition Division has made the same judgment as this Court in Section 2(1) above, indicating that the technical barriers mentioned by the petitioner do not raise doubts about the ability to implement the Patent, and that the first priority basis application contains adequate disclosure to implement the CRISPR/Cas9 system in eukaryotic cells (Respondents' Exhibit No. 122-1).

Thus, there is no reason for the details of the USPTO interference decision (Petitioner's Exhibit No. 7) to affect the judgment in Section 2(1).

(E) Differences between ZFNs/TALENs and the CRISPR/Cas9 system

This Court's judgment that the Patent is described in the application documents of the first priority basis application is based on the matter set down in the application documents and the known art at the time of filing, as noted in Section 2(1) above. The application documents do not describe the application of the CRISPR/Cas9 system to eukaryotic cells by its similarity to ZFNs or TALENs, nor is the common technical knowledge considered in the judgment in Section 2(1) above related to ZFNs or TALENs. This Court's judgment in Section 2(1) is therefore unaffected by any differences between the CRISPR/Cas9 system and ZFNs/TALENs, or the degree thereof.

(F) Summary

The petitioner's arguments are therefore not to be accepted, and the Patent is found to be described in the application documents for the first priority basis application and can therefore enjoy the benefit of priority claim based on the first priority basis application.

3. Ground for invalidation 1 (Expanded Prior Application/Petitioner's Exhibit No. 1)

As laid out in Section 1 above, the Patent can enjoy the benefit of priority claim based on the first priority basis application, and therefore in applying the provisions of Patent Law Article 29-1 the Patent is deemed to have been filed on May 25, 2012, the filing date of the first priority basis application.

In contrast, the filing date of the first priority basis application in Petitioner's Exhibit No. 1 (December 12, 2012) is later than the filing date of the first priority basis application of the Patent (May 25, 2012), and therefore the application in Petitioner's Exhibit No. 1 does not qualify as "another patent application" under Patent Law Article 29-2 with respect to the Patent, even if it could enjoy the benefit of priority claim based on the first priority basis application.

Accordingly, ground for invalidation 1 argued by the petitioner is groundless.

4. Ground for invalidation 2 (Expanded Prior Application/Petitioner's Exhibit No. 2)

As laid out in Section 1 above, the Patent can enjoy the benefit of priority claim based on the first priority basis application, and therefore in applying the provisions of Patent Law Article 29-1 the Patent is deemed to have been filed on May 25, 2012, the filing date of the first priority basis application.

In contrast, the filing date of the first priority basis application in Petitioner's Exhibit No. 2 (October 23, 2012) is later than the filing date of the first priority basis application of the Patent (May 25, 2012), and therefore the application in Petitioner's Exhibit No. 2 does not qualify as "another patent application" under Patent Law Article 29-2 with respect to the Patent, even if it could enjoy the benefit of priority claim based on the first priority basis application.

Accordingly, ground for invalidation 2 argued by the petitioner is groundless.

V. Conclusion

Thus, the patent for the invention as in claims 1, 2, 6, 15-19, 21, 22, 25, 28-30, 33-36, 45-48, 51-53, 56, 65-70, 73, 76-84, 95, 96, 99, 100, and 111 cannot be deemed invalid due to the petitioner's arguments and means of proof.

Trial costs shall be borne by the petitioner as set down in Civil Procedure Law Article 61 applied mutatis mutandis under Patent Law Article 169(2).

We therefore issue a judgment as stated in the Conclusion.

August 30, 2023

Chief Justice	JPO Administrative Judge	Satoru Fukui
	JPO Administrative Judge	Keiko Nagai
	JPO Administrative Judge	Hajime Kamijo

(Instruction under Administrative Case Litigation Law Article 46)

Appeals against this decision cn be brought within 30 days of when the certified copy of the decision is mailed (with any additional days of an extension period added) with the counterparty to this decision as the defendant.

Administrative Judge Satoru Fukui

90 days is added for parties residing outside Japan as the filing deadline.

[Decision category] P1123.161-Y (C12N)