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Application/Patent No.: 201380038920.6 Issued Document Serial No.: 2024010200840550

Case No.: 4W115088

Title of Invention: Methods and Compositions for RNA-guided Target DNA Modification and for RNA-guided Transcription Regulation

Applicant/Patentee: Emmanulle Charpentier, Board of Directors of the University of California, University of Vienna

Petitioner for Invalidation: Wang Xiaoyang

Examination Decision on the Request for Invalidation

(No. 562593)

In accordance with the provision of Paragraph 1, Article 46 of the Chinese Patent Law, the China National Intellectual Property Administration has examined the request for invalidation filed by the petitioner for invalidation in respect of the aforesaid patent right, and hereby decides as follows:

☐ Declare all the patent rights invalid.

☐ Declare some of the patent rights invalid.

☒ Maintain the validity of the patent rights.

If not satisfied with this Decision, in accordance with the provision of Paragraph 2, Article 46 of the Chinese Patent Law, the party concerned may, within three months from the date of receipt of this Decision, institute legal proceedings in the Beijing Intellectual Property Court, and the other party shall participate in the lawsuit as a third party.

Attachment: 36 pages of the text (starting from page 2) for this Decision.

201019 For applications in written form, the corresponding documents shall b Filing Office, The China National Intellectual Property Administration, 6 Xitucheng Bridge, Haidian, Beijing, 100088

2022.10 For electronic applications, the corresponding documents shall be submitted to the electronic patent application system in the form of electronic documents. Documents submitted in forms other than electronic format shall be deemed not have been submitted unless otherwise specified.

Chairman of the Panel: Zou Kai

First Member: Wu Wenying

Second Member: Han Shiwei Cui Yang Shi Jing

(CHINA NATIONAL INTELLECTUAL PROPERTY ADMINISTRATION

Reexamination and Invalidation Department of China National Intellectual Property Administration

Seal for Patent reexamination and invalidation review)

Examination Decision on the Request for Invalidation (No. 562593)

by China National Intellectual Property Administration

Case No.	No. 4W115088
Decision Date	January 5, 2024
Title of Invention	Methods and Compositions for RNA-guided Target DNA Modification and for RNA-guided Transcription Regulation
Int. Cl. No.	C12N 15/11
Petitioner for Invalidation	Wang Xiaoyang
Patentee	Emmanuelle Charpentier, Board of Directors of the University of California, University of Vienna
Patent No.	201380038920.6
Filing Date	March 15, 2013
Earliest Priority Date	May 25, 2012
Date of Announcement of Grant of Patent	July 14, 2017
Date of Request for Invalidation	September 30, 2022
Legal Basis	Paragraphs 2 and 3, Article 22 of the Chinese Patent Law, Paragraphs 3 and 4, Article 26 of the Chinese Patent Law, Article 33 of the Chinese Patent Law, Paragraph 2, Article 20 of the Rules for the Implementation of the Chinese Patent Law

Main Points of Decision:

An invention or a utility model for the same subject matter as referred to in Article 29 of the Chinese Patent Law means an invention or a utility model with the same technical field, technical problem to be solved, technical solution, and prospective effect.

In the process of judging whether a prior application had contained the same subject matter, the prior application should be analyzed and studied as a whole. If the prior application as a whole had disclosed the technical solution for which a later application claimed protection in its claims, and demonstrated by examples that the system composed of three elements could achieve the cleavage of target nucleic acid, the feasibility of a CRISPER/Cas9 system as a gene editing tool was proved, providing the foundation and direction for the long-term desire to find new gene editing tools and methods in the art. The technical solution claimed in the claims is an application extension made by those skilled in the art to follow the trend of technological development around the example solution, and the inventive concept that adopts three main elements is also the same as that of the prior application. On this basis, it is determined that the technical solution claimed in the claims can play the same role and solve the same technical problem as the example solution in the prior application, which is technically reasonable. The technical solution claimed in the claims belongs to the same subject matter as the prior application.

Documents whose publication date is between the priority date and the filing date of the patent are not prior art and cannot be used for judging the novelty and inventiveness of the corresponding patents.

The language used in the claims should not be mechanically interpreted based solely on the literal meaning expressed in the claims, but should be interpreted from a technical perspective by those skilled in the art, combined with the teachings in the Description and the common general knowledge in the art. If those skilled in the art can clearly understand the structure of sgRNA as defined in the claims based on the Description and its accompanying drawings, then the terms will not lead to unclear protection scope of the claims.

If a claim summarizes one or several technical features, and the scope of the summary is reasonably available to those skilled in the art based on the expected technical effect contained in the Description, the corresponding summary will not result in that the claim is not being supported by the Description.

The scope of the original application document includes the information clearly recorded by those skilled in the art according to the original Description and Claims, as well as the technical information set that is objectively and accurately expressed in the application documents determined “directly and unambiguously” from the perspective of the applicant’s true meaning. In the process of determining the technical information set objectively and accurately expressed in the application documents, it should not be mechanically understood only literally, but should be comprehensively judged from the perspective of those skilled in the art. If the modified technical solution does not exceed the above-mentioned technical information set, the modification does not exceed the scope of the original Description and Claims.

In the absence of sufficient evidence to indicate that the lack of a certain technical feature not defined by the claims will lead to a failure of the corresponding technical solution achieving its invention purpose, it will be difficult to directly determine that the corresponding technical feature is a necessary technical feature, and it will also be impossible to further draw the conclusion that the corresponding claim has the defect of lacking the necessary technical feature based on this.

I. Brief of the Case

This patent has the patent No. of 201380038920.6, of which the earliest priority date is May 25, 2012, the filing date is March 15, 2013, and the date of announcement of grant of patent is July 14, 2017. The Claims at the time of this patent being granted a patent right are as follows:

"1. A method for modifying target DNA, comprising contacting the target DNA with a complex, wherein the complex comprises:

(a) Cas9 peptides, and

(b) single-molecule DNA-targeting RNA, comprising:

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

(ii) a protein binding segment, interacting with the Cas9 peptides, wherein the protein binding segment comprises two complementary nucleotide segments hybridized to form a double-stranded RNA (dsRNA) duplex,

wherein the dsRNA duplex comprises complementary nucleotides of tracrRNA and CRISPR RNA (crRNA),

wherein the two complementary nucleotide segments are covalently linked by an intervening nucleotide,

wherein the contacting is in vitro or in an ex vivo cell; and

wherein the modifying is to cleave the target DNA.

2. The method according to claim 1, wherein the length of the dsRNA duplex is 8 base pairs (bp) to 30 bp.

3. The method according to claim 1, wherein the complementary percentage of the nucleotides hybridized to form the dsRNA duplex in the protein binding segment is 70% or above.

4. The method according to claim 2, wherein the complementary percentage of the nucleotides hybridized to form the dsRNA duplex in the protein binding segment is 70% or above.

5. The method according to any one of claims 1-4, wherein the target DNA is present in bacterial cells, archaeal cells, single-cell eukaryotes, plant cells, invertebrate cells, or vertebrate cells.

6. The method according to any one of claims 1-4, wherein the target DNA is chromosomal DNA.

7. The method according to any one of claims 1-4, wherein the contacting comprises introducing the followings into cells: (a) the Cas9 peptides or a polynucleotide encoding the Cas9 peptides, and (b) the DNA-targeting RNA or a DNA polynucleotide encoding the DNA-targeting RNA.

8. The method according to claim 5, wherein the contacting comprises introducing the followings into cells: (a) the Cas9 peptides or a polynucleotide encoding the Cas9 peptides, and (b) the DNA-targeting RNA or a DNA polynucleotide encoding the DNA-targeting RNA.

9. The method according to claim 6, wherein the contacting comprises introducing the followings into cells: (a) the Cas9 peptides or a polynucleotide encoding the Cas9 peptides, and (b)

the DNA-targeting RNA or a DNA polynucleotide encoding the DNA-targeting RNA.

10. The method according to claim 7, wherein the method further comprises introducing a donor polynucleotide into the cells.

11. The method according to claim 8, wherein the method further comprises introducing a donor polynucleotide into the cells.

12. The method according to claim 9, wherein the method further comprises introducing a donor polynucleotide into the cells.

13. The method according to any one of claims 1-4 and 8-12, wherein a protein transduction domain is covalently linked to the amino terminal of the Cas9 peptides, wherein the protein transduction domain facilitates crossing of the Cas9 peptides from cytoplasm into organelles of the cells.

14. The method according to claim 5, wherein a protein transduction domain is covalently linked to the amino terminal of the Cas9 peptides, wherein the protein transduction domain facilitates crossing of the Cas9 peptides from cytoplasm into organelles of the cells.

15. The method according to claim 6, wherein a protein transduction domain is covalently linked to the amino terminal of the Cas9 peptides, wherein the protein transduction domain facilitates crossing of the Cas9 peptides from cytoplasm into organelles of the cells.

16. The method according to claim 7, wherein a protein transduction domain is covalently linked to the amino terminal of the Cas9 peptides, wherein the protein transduction domain facilitates crossing of the Cas9 peptides from cytoplasm into organelles of the cells.

17. The method according to any one of claims 1-4 and 8-12, wherein a protein transduction domain is linked to the carboxyl terminal of the Cas9 peptides, wherein the protein transduction domain facilitates crossing of the Cas9 peptides from cytoplasm into organelles of the cells.

18. The method according to claim 5, wherein a protein transduction domain is linked to the carboxyl terminal of the Cas9 peptides, wherein the protein transduction domain facilitates crossing of the Cas9 peptides from cytoplasm into organelles of the cells.

19. The method according to claim 6, wherein a protein transduction domain is linked to the carboxyl terminal of the Cas9 peptides, wherein the protein transduction domain facilitates crossing of the Cas9 peptides from cytoplasm into organelles of the cells.

20. The method according to claim 7, wherein a protein transduction domain is linked to the carboxyl terminal of the Cas9 peptides, wherein the protein transduction domain facilitates crossing of the Cas9 peptides from cytoplasm into organelles of the cells.

21. A composition, comprising: (a) Cas9 peptides, or a polynucleotide encoding the Cas9 peptides, and

(b) single-molecule DNA-targeting RNA, or a DNA polynucleotide encoding the single-molecule DNA-targeting RNA, wherein the single-molecule DNA-targeting RNA comprises: (i) a DNA targeting segment, comprising a nucleotide sequence complementary to a sequence in the target DNA; and

(ii) a protein binding segment, interacting with the Cas9 peptides, wherein the protein

binding segment comprises two complementary nucleotide segments hybridized to form a double-stranded RNA (dsRNA) duplex,

wherein the dsRNA duplex comprises complementary nucleotides of tracrRNA and CRISPR RNA (crRNA), and

wherein the two complementary nucleotide segments are covalently linked by an intervening nucleotide.

22. The composition according to claim 21, wherein the length of the dsRNA duplex is 8 base pairs (bp) to 30 bp.

23. The composition according to claim 21, wherein the complementary percentage of the nucleotides hybridized to form the dsRNA duplex in the protein binding segment is 70% or above.

24. The composition according to claim 22, wherein the complementary percentage of the nucleotides hybridized to form the dsRNA duplex in the protein binding segment is 70% or above.

25. The composition according to any one of claims 21-24, wherein the target DNA is present in bacterial cells, archaeal cells, single-cell eukaryotes, plant cells, invertebrate cells, or vertebrate cells.

26. The composition according to any one of claims 21-24, wherein the target DNA is chromosomal DNA.

27. The composition according to any one of claims 21-24, wherein a protein transduction domain is covalently linked to the amino terminal of the Cas9 peptides, wherein the protein transduction domain facilitates crossing of the Cas9 peptides from cytoplasm into organelles of the cells.

28. The composition according to claim 25, wherein a protein transduction domain is covalently linked to the amino terminal of the Cas9 peptides, wherein the protein transduction domain facilitates crossing of the Cas9 peptides from cytoplasm into organelles of the cells.

29. The composition according to claim 26, wherein a protein transduction domain is covalently linked to the amino terminal of the Cas9 peptides, wherein the protein transduction domain facilitates crossing of the Cas9 peptides from cytoplasm into organelles of the cells.

30. The composition according to any one of claims 21-24, wherein a protein transduction domain is linked to the carboxyl terminal of the Cas9 peptides, wherein the protein transduction domain facilitates crossing of the Cas9 peptides from cytoplasm into organelles of the cells.

31. The composition according to claim 25, wherein a protein transduction domain is linked to the carboxyl terminal of the Cas9 peptides, wherein the protein transduction domain facilitates crossing of the Cas9 peptides from cytoplasm into organelles of the cells.

32. The composition according to claim 26, wherein a protein transduction domain is linked to the carboxyl terminal of the Cas9 peptides, wherein the protein transduction domain facilitates crossing of the Cas9 peptides from cytoplasm into organelles of the cells.

33. The composition according to any one of claims 21-24, wherein the Cas9 peptides and the single-molecule DNA-targeting RNA are present inside in vitro cells or ex vivo cells.

34. The composition according to claim 25, wherein the Cas9 peptides and the

single-molecule DNA-targeting RNA are present inside in vitro cells or ex vivo cells.

35. The composition according to claims 26, wherein the Cas9 peptides and the single-molecule DNA-targeting RNA are present inside in vitro cells or ex vivo cells.

36. The composition according to claim 27, wherein the Cas9 peptides and the single-molecule DNA-targeting RNA are present inside in vitro cells or ex vivo cells.

37. The composition according to any one of claims 28-29 and 31-32, wherein the Cas9 peptides and the single-molecule DNA-targeting RNA are present inside in vitro cells or ex vivo cells.

38. The composition according to claim 30, wherein the Cas9 peptides and the single-molecule DNA-targeting RNA are present inside in vitro cells or ex vivo cells.

39. A single-molecule DNA-targeting RNA, or a DNA polynucleotide encoding the single-molecule DNA-targeting RNA, wherein the single-molecule DNA-targeting RNA comprises:

(a) a DNA targeting segment, comprising a nucleotide sequence complementary to a target sequence in the target DNA; and

(b) a protein binding segment, interacting with Cas9 peptides, wherein the protein binding segment comprises two complementary nucleotide segments hybridized to form a double-stranded RNA (dsRNA) duplex, wherein the dsRNA duplex comprises complementary nucleotides of tracrRNA and CRISPR RNA (crRNA), and the two complementary nucleotide segments are covalently linked by an intervening nucleotide.

40. The single-molecule DNA-targeting RNA according to claim 39, wherein the length of the dsRNA duplex is 8 base pairs (bp) to 30 bp.

41. The single-molecule DNA-targeting RNA according to claim 39, wherein the complementary percentage of the nucleotides hybridized to form the dsRNA duplex in the protein binding segment is 70% or above.

42. The single-molecule DNA-targeting RNA according to claim 40, wherein the complementary percentage of the nucleotides hybridized to form the dsRNA duplex in the protein binding segment is 70% or above.

43. The single-molecule DNA-targeting RNA according to any one of claims 39-42, wherein the target DNA is present in bacterial cells, archaeal cells, single-cell eukaryotes, plant cells, invertebrate cells, or vertebrate cells.

44. The single-molecule DNA-targeting RNA according to any one of claims 39-42, wherein the target DNA is chromosomal DNA.

45. One or more nucleic acids, comprising:

(a) a first nucleotide sequence, encoding a single-molecule DNA-targeting RNA, wherein the single-molecule DNA-targeting RNA comprises:

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

(ii) a protein binding segment, interacting with Cas9 peptides, wherein the protein binding

segment comprises two complementary nucleotide segments hybridized to form a double-stranded RNA (dsRNA) duplex, wherein the dsRNA duplex comprises complementary nucleotides of tracrRNA and CRISPR RNA (crRNA), and the two complementary nucleotide segments are covalently linked by an intervening nucleotide;

wherein the first nucleotide sequence encoding the DNA-targeting RNA is operatively linked to a promoter; and, optionally,

(b) a second nucleotide sequence, encoding a Cas9 polynucleotide, wherein the nucleotide sequence encoding the Cas9 peptides is operatively linked to the promoter.

46. The one or more nucleic acids according to claim 45, wherein the nucleic acids are one or more recombinant expression vectors.

47. The one or more nucleic acids according to claim 45, wherein the length of the dsRNA duplex is 8 base pairs (bp) to 30 bp.

48. The one or more nucleic acids according to claim 46, wherein the length of the dsRNA duplex is 8 base pairs (bp) to 30 bp.

49. The one or more nucleic acids according to any one of claims 45-48, wherein the complementary percentage of the nucleotides hybridized to form the dsRNA duplex in the protein binding segment is 70% or above.

50. The one or more nucleic acids according to any one of claims 45-48, wherein the target DNA is present in bacterial cells, archaeal cells, single-cell eukaryotes, plant cells, invertebrate cells, or vertebrate cells.

51. The one or more nucleic acids according to claim 49, wherein the target DNA is present in bacterial cells, archaeal cells, single-cell eukaryotes, plant cells, invertebrate cells, or vertebrate cells.

52. The one or more nucleic acids according to any one of claims 45-48, wherein the target DNA is chromosomal DNA.

53. The one or more nucleic acids according to claim 49, wherein the target DNA is chromosomal DNA.

54. A kit, comprising:

(a) Cas9 peptides, or a nucleic acid comprising a nucleotide sequence encoding the Cas9 peptides, and

(b) single-molecule DNA-targeting RNA, or a nucleic acid comprising a nucleotide sequence encoding the single-molecule DNA-targeting RNA, wherein the single-molecule DNA-targeting RNA comprises:

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

(ii) a protein binding segment, interacting with the Cas9 peptides, wherein the protein binding segment comprises two complementary nucleotide segments hybridized to form a double-stranded RNA (dsRNA) duplex,

wherein the dsRNA duplex comprises complementary nucleotides of tracrRNA and CRISPR

RNA (crRNA), and

wherein the two complementary nucleotide segments are covalently linked by an intervening nucleotide; and

wherein (a) and (b) are in the same or separate containers.

55. The kit according to claim54, wherein the target DNA is present in bacterial cells, archaeal cells, single-cell eukaryotes, plant cells, invertebrate cells, or vertebrate cells.

56. The kit according to claim 54, wherein the target DNA is chromosomal DNA.

57. Use of the composition according to any one of claims 21-38, or the single-molecule DNA-targeting RNA according to any one of claims 39-44, or the one or more nucleic acids according to any one of claims 45-53 in preparation of medicines for treating diseases."

For this patent right, the petitioner submitted a request for invalidation to the China National Intellectual Property Administration on September 30, 2022, requesting that claims 1-57 of this patent be declared invalid.

On 28 October, 2022, the petitioner submitted statement of supplementary opinions and the following Evidences 1-34:

Evidence 1: US61652086 and its Chinese translation (i.e., the priority document P1 of this patent);

Evidence 2: US61716256 and its Chinese translation (i.e., the priority document P2 of this patent);

Evidence 3: CN104968784A, with the earliest priority date of October 23, 2012 and the publication date of October 7, 2015;

Evidence 4: CN105658796A, with the earliest priority date of December 12, 2012 and the publication date of June 8, 2016;

Evidence 5: RNA Guided Human Genome Engineering via Cas9, Prashant Mali et al., Science, with the publication date of January 3, 2013, and its Chinese translation;

Evidence 6: Multiplex Genome Engineering Using CRISPR/Cas Systems, Le Cong et al., Science, with the publication date of January 3, 2013, and its Chinese translation;

Evidence 7: A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity, Martin Jinek, et al., Science, with the publication date of June 28, 2012, and its Chinese translation;

Evidence 8: Cheap and easy technique to snip DNA could revolutionize gene therapy, Robert Sanders, Berkeley News, with the publication date of January 7, 2013, and its Chinese translation;

Evidence 9: RNA-programmed genome editing in human cells, Martin Jinek et al., eLIFE, pp. 1-9, with the publication date of January 29, 2013, and its Chinese translation;

Evidence 10: A CRISPR Approach to Gene Targeting, Dana Carroll, Molecular Therapy, with the publication date of September 2012, and some of its Chinese translation;

Evidence 11: Cas9-crRNA ribonucleoprotein complex mediatespecific DNA cleavage for adaptive immunity in bacteria, Gasiunas, et al., PNAS, with the publication date of September 4, 2012, and some of its Chinese translation;

Evidence 12: Documents submitted by the applicant in response to the examination opinions in case EP3071695, and some of its Chinese translation;

Evidence 13: Identifying and Visualizing Functional PAM Diversity across CRISPR-Cas Systems, Leena et al., Molecular Cell, with the publication date of April 7, 2016, and some of its Chinese translation;

Evidence 14: crRNA and tracrRNA guide Cas9-mediated DNA interference in Streptococcus thermophilus, Karvelis, et al., Landes Bioscience, with the publication date of May 2013, and some of its Chinese translation;

Evidence 15: Efficient genome engineering in eukaryotes using Cas9 from Streptococcus thermophilus, Xu et al., Cellular and Molecular Life Sciences, with the publication date of July 20, 2014, and some of its Chinese translation;

Evidence 16: DNA targeting specificity of RNA-guided Cas9 nucleases, Hsu et al., Nature biotechnology, with the publication date of September 2013, and some of its Chinese translation;

Exhibits 17: Genome engineering using the CRISPR-Cas9 system, Ran F A et al., Nature Protocol, with the publication date of October 24, 2013, and some of its Chinese translation;

Evidence 18: Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9, Doench J G et al., Nature biotechnology, with the publication date of February 2016, and some of its Chinese translation;

Evidence 19: Identification of potential drug targets for tuberous sclerosis complex by synthetic screens combining CRISPR-based knockouts with RNAi, Housden B E et al., SCIENCE SIGNALING, with the publication date of September 8, 2015, and some of its Chinese translation;

Evidence 20: Enhanced Specificity and Efficiency of the CRISPR/Cas9 System with Optimized sgRNA Parameters in Drosophila, Ren X et al., Cell Reports, with the publication date of November 6, 2014, and some of its Chinese translation;

Evidence 21: Documents in which the applicant responds to the examination opinions in the substantive examination procedure of this patent;

Evidence 22: Groupll Intron-Based Gene Targeting Reactions in Eukaryotes, Mastroianni et al., PLoS ONE, with the publication date of September 2008, and some of its Chinese translation;

Evidence 23: Regulated processive transcription of chromatin by T7 RNA polymerase in Trypanosoma brucei, Wirtz et al., Nucleic Acids Research, with the publication date of 1998, and some of its Chinese translation;

Evidence 24: Factors Governing the Activity In Vivo of Ribozymes Transcribed by RNA Polymerase II, Shiori Koseki et al., JOURNAL OF VIROLOGY, with the publication date of March 1999, and some of its Chinese translation;

Evidence 25: Engineering ligand-responsive gene-control elements: lessons learned from natural riboswitches, Link et al., Gene Therapy, with the publication date of July 9, 2009, and some of its Chinese translation;

Exhibits 26: The interference procedural decisions of the USPTO, and some of its Chinese translation;

Evidence 27: JENNIFER DOUDNA, CRISPR CODE KILLER, Pandic et al., RISING STARS, with the publication date of January 7, 2014, and some of its Chinese translation;

Evidence 28: The CRISPR revolution, Doudna et al., Catalyst Magazine, with the publication date of July 9, 2014, and some of its Chinese translation;

Evidence 29: Testimony of Dr. Fuqiang Chen, and its Chinese translation;

Evidence 30: Letter from the inventor in October 2012, and some of its Chinese translation;

Evidence 31: Letter from the inventor in December 2012, and some of its Chinese translation;

Evidence 32: CN104854241A of the disclosed text of this patent;

Evidence 33: CN104854241B of the authorized text of this patent; and

Evidence 34: Expert testimony.

Based on the above evidences, the petitioner has the following viewpoints:

1. Claims 1-57 cannot possess the priority of P1 or P2. If the priority is not established, claims 1-9, 17-26, 30-53, 57 with respect to Evidence 3 or Evidence 8 or Evidence 9, claims 1-53 with respect to Evidence 4, claims 1-12, 17-20, 21-26, 30-53, 57 with respect to Evidence 5, claims 1-53 and 57 with respect to Evidence 6, and claims 1-4, 21-24, 39-42, 45-49 with respect to Evidence 7 do not comply with the novelty as prescribed in Paragraph 2, Article 22 of the Chinese Patent Law. Claims 1-57 do not comply with the inventiveness as prescribed in Paragraph 3, Article 22 of the Chinese Patent Law with respect to one of the Evidences 5-9 or a combination of one of the Evidences 5-9 and common general knowledge.

2. Claims 1-57 have unclear scope of protection and do not receive support from the Description, which does not comply with the provision of Paragraph 4, Article 26 of the Chinese Patent Law.

3. The modification of claims 1-57 goes beyond the scope of the original Description and Claims, and does not comply with the provision of Article 33 of the Chinese Patent Law.

4. Independent claims 1, 21, 39, 45, 54, 57 lack necessary technical features, and thus do not comply with the provision of Paragraph 2, Article 22 of the Rules for the Implementation of the Chinese Patent Law.

After passing the formal examination, the China National Intellectual Property Administration accepted the above request for invalidation on December 29, 2022 and transferred the above request for invalidation, the statement of supplementary opinions and the copies of evidences to the patentee. At the same time, a panel was set up for hearing.

On April 18, 2023, the patentee submitted a Statement of Opinions and an evidence list of Counter-Evidences CE1-129 in response to the above request for invalidation. On April 21, 2023, the patentee also submitted the following Counter-Evidences CE1-129 and references 1-111. Based on the submitted counter-evidences and references, the patentee believes that this patent complies with the relevant provisions of the Chinese Patent Law.

Counter-Evidences CE1-129 are specifically as follows:

反证编号	标题和相关说明
CE1	Jinek 等，A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity Science,公开日为 2012 年 8 月 17 日，及其部分中文译文
CE2	Cong 等，Multiplex Genome Engineering Using CRISPR/Cas Systems,Science,2013 年 1 月 3 日在 线公开，及其部分中文译文
CE3	Mali 等，RNA-Guided Human Genome Engineering via Cas9,Science,2013 年 1 月 3 日在线公开， 及其部分中文译文
CE4	Jinek 等，RNA-programmed genome editing in human cells, eLife,公开日为 2013 年 1 月 29 日，及

	其部分中文译文
CE5	Deltcheva 等，CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III, Nature,公开日为 2011 年 3 月 31 日，及其部分中文译文
CE6	WO 2014/093595 A1,公开日为 2014 年 6 月 19 日，及其部分中文译文
CE7	Makarova 等，Evolution and classification of the CRISPR-Cas systems. Nat Rev Microbiology,公开日 为 2011 年 6 月，及其部分中文译文
CE8	Sapranauskas 等，The Streptococcus thermophilus CRISPR/Cas system provides immunity in Escherichia coli, Nucleic Acid Research,公开日为 2011 年 8 月 3 日，及其部分中文译文
CE9	Lambowitz 等，Group II Introns: Mobile Ribozymes that Invade DNA, Cold Spring Harb Perspect Biol, 2011,及其部分中文译文
CE10	Garneau 等，The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA Nature,公开日为 2010 年 11 月 4 日，及其部分中文译文
CE11	Mastroianni 等，Group II Intron-Based Gene Targeting Reactions in Eukaryotes, PLoS One,公开日 为 2008 年 9 月 1 日，及其部分中文译文
CE12	Zimmerly 等，Group II Intron Mobility Occurs by Target DNA-Primed Reverse Transcription, Cell, 公开日为 1995 年 8 月 25 日，及其部分中文译文
CE13	Jenuwein 等，The immunoglobulin μ enhancer core establishes local factor access in nuclear chromatin independent of transcriptional stimulation, Genes &Development,1993,及其部分中文译文
CE14	Koseki 等，Factors Governing the Activity In Vivo of Ribozymes Transcribed by RNA Polymerase II Virology,公开日为 1999 年 3 月，及其部分中文译文
CE15	Link 等，Engineering ligand-responsive gene-control elements: lessons learned from natural riboswitches, Gene Therapy,公开日为 2009 年 7 月 9 日，及其部分中文 译文
CE16	Raymond 等，High-efficiency FLP and ΦC31 Site-Specific Recombination in Mammalian Cells, PLoS One,2007,及其部分中文译文

CE17	Cho 等，Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease, Nat Biotechnology,公开日为 2013 年 1 月 29 日，及其部分中文译文
CE18	Hwang 等，Eficient In Vivo Genome Editing Using RNA-Guided Nucleases, Nal Biotechnol,公开日 为 2013 年 3 月，及其部分中文译文
CE19	JENNIFER DOUDNA,CRISPR CODE KILLER, Pandika, Rising Stars,公开日为 2014 年 1 月 7 日，及其部分中文译文
CE20	Catalyst Magazine,公开日为 2014 年 7 月 9 日，及其部分中文译文
CE21	Kim 等，In vivo genome editing with a small Cas9 orthologue derived from Campylobacter jejuni, Nat Communications,公开日为 2017 年 2 月 21 日，及其部分中文译文
CE22	US 611652,086,及其部分中文译文
CE23	US 61/757,640,及其部分中文译文
CE24	Xu 等，Efficient genome engineering in eukaryotes using Cas9 from Streptococcus thermophiles Cellular and Molecular Life Sciences,公开日为 2014 年 7 月 20 日，及其部分中文译文
CE25	Karvelis 等，crRNA and tracr RNA guide Cas9-mediated DNA interference in Streptococcus thermophiles, RNA Biology,2013,及其部分中文译文
CE26	Reply to Restriction Requirement (appl.no.US 12/556,589)Prosecution
CE27	Graham and Root, Resources for the design of CRISPR gene editing experiments, Genome Bio,2015, 及其部分中文译文
CE28	Hsu 等，DNA targeting specificity of RNA-guided Cas9 nucleases, Nat Biotechnology,公开日为 2013 年 8 月 28 日，及其部分中文译文

CE29	Barrangou, RNA-mediated programmable DNA Cleavage, Nat Biotechnology,2012,及其部分中文译 文
CE30	Wiedenheft 等，RNA-guided genetic silencing systems in bacteria and archaea, Nature,公开日为 2012 年 2 月 16 日，及其部分中文译文
CE31	Horvath and Barrangou, CRISPR/Cas, the Immune System of Bacteria and Archaea, Science,公开日 为 2010 年 1 月 8 日，及其部分中文译文
CE32	Deveau 等，Phage Response to CRISPR-Encoded Resistance in Streptococcus thermophiles, J of Bacteriology,公开日为 2008 年 2 月，及其部分中文译文
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CE129	专家意见

On April 24, 2023, the panel of the China National Intellectual Property Administration for this case transferred the Statement of Opinions submitted by the patentee on April 18, 2023 and its annexes to the petitioner, and on April 28, 2023, transferred the documents submitted by the patentee on April 21, 2023 to the petitioner.

On May 17, 2023, the patentee resubmitted the Chinese translations of the relevant parts in Counter-Evidences CE1-129.

The panel transferred the Statement of Opinions submitted by the patentee on May 17, 2023 and its annexes to the petitioner on May 22, 2023.

The panel sent the Notification of Oral Proceedings to both parties on May 4, 2023, and the oral proceedings were scheduled to be held on June 20, 2023.

The oral proceedings were held as scheduled, and both parties entrusted agents to attend the oral proceedings. During the oral proceedings, the confirmed facts are as follows:

(1) The petitioner submitted the following Evidence 35-37 in court:

Evidence 35: Appeal. Nos. 2022-1594, 2022-1653, United States Court of Appeals for the Federal Circuit, and some of its Chinese translation;

Evidence 36: Interference No.106127, United States Patent and Trademark Office, and some of its Chinese translation;

Evidence 37: *Essential Cell Biology (Third Edition)*, Science Press, B. Alberts et al., 1st Edition in March 2012, 7th Printing in March 2016.

The petitioner asserts that Evidences 35 and 36 are derived from the proceedings of the same family of this case in the United States, and are raised against Counter-Evidences CE11, 9, 111, and Evidence 37 is used as a common sense evidence.

The patentee acknowledges the authenticity and public disclosure of Evidences 1-37 and has no objection to the Chinese translations of Evidences 1-31, 35 and 36.

(2) The patentee waived Counter-Evidences: CE 12, 19-24, 26, 33, 34, 39, 41, 46, 48, 49, 58, 66, 68, 71-73, 75, 76, 82, 86-88, 91, 93, 99, 103, 107, 109, 112, 114, 115, 125, 127, 128. The

petitioner accepted the authenticity of the remaining Counter-Evidences.

The petitioner supplemented the Chinese translations of the relevant paragraphs in Counter-Evidences CE 7, 32, 37, 38, and 86 in court.

Both parties agreed that the Chinese translation of Counter-Evidence CE7 “in type II systems, Cas9 loaded with crRNA probably directly targets invading DNA, in a process that requires the PAM” would be judged by the panel. If the Chinese translations of other Counter-Evidences are submitted by the petitioner, the ones submitted by the petitioner shall prevail. If the petitioner has not submitted, the ones submitted by the patentee shall prevail.

(3) For the specific reasons why claims 1-57 do not comply with the provision about clearness as prescribed in Paragraph 4, Article 26 of the Chinese Patent Law, the petitioner waived the reasons for the unclear expression of “in vitro” and “Cas9 peptides”, and only insisted on the reason for the unclearness of “how the DNA targeting segment and the protein binding segment forms sgRNA”. The other reasons and scopes on invalidation confirmed by the petitioner were consistent with the written opinions.

(4) Both parties confirmed that the PAM sequence of *Streptococcus pyogenes* was known before P1, which was NGG.

At this point, the panel believes that the facts of this case are clear and can make an examination decision.

II. Causes of the Decision

1. Examination basis

The examination text of this request for invalidation is the granted text of this patent.

2. Evidence identification

The patentee has no objection to the authenticity, legality, and public disclosure of Evidences 1-37, as well as the accuracy of the relevant Chinese translations; and the panel confirms Evidences 1-37.

The petitioner has no objection to the authenticity and public disclosure of Counter-Evidences 1-11, 13-18, 25, 27-32, 35-38, 40, 42-45, 47, 50-57, 59-65, 67, 69, 70, 74, 77-81, 83-85, 89, 90, 92, 94-98, 100-102, 104-106, 108, 110, 111, 113, 116-124, 126 and 129, and the panel confirms these Counter-Evidences.

As for the Chinese translations of Counter-Evidences, both parties have disputes over the Chinese translation of the “in type II systems, Cas9 loaded with crRNA probably directly targets invading DNA, in a process that requires the PAM” in Counter-Evidence 7. For the determination of the disputed part, please refer to the following comments. For the Chinese translations of other Counter-Evidences and the Chinese translation of other parts of Counter-Evidence 7, if the petitioner submits the Chinese translations of the corresponding parts or makes a supplementary translation, the submission of the petitioner shall prevail; and if the petitioner fails to submit, the submission of the patentee shall prevail.

3. Regarding priority

Paragraph 1, Article 29 of the Chinese Patent Law stipulates that where, within twelve

months from the date on which any applicant first filed in a foreign country an application for a patent for invention, he or it files in China an application for a patent for the same subject matter, he or it may, in accordance with any agreement concluded between the said foreign country and China, or in accordance with any international treaty to which both countries are party, or on the basis of the principle of mutual recognition of the priority, enjoy a priority.

An invention or a utility model for the same subject matter as referred to in Article 29 of the Chinese Patent Law means an invention or a utility model with the same technical field, technical problem to be solved, technical solution, and prospective effect.

In the process of judging whether a prior application had contained the same subject matter, the prior application should be analyzed and studied as a whole. If the prior application as a whole had disclosed the technical solution for which a later application claimed protection in its claims, and demonstrated by examples that the system composed of three elements could achieve the cleavage of target nucleic acid, the feasibility of a CRISPER/Cas9 system as a gene editing tool was proved, providing the foundation and direction for the long-term desire to find new gene editing tools and methods in the art. The technical solution claimed in the Claims is an application extension made by those skilled in the art to follow the trend of technological development around the example solution, and the inventive concept that adopts three main elements is also the same as that of the prior application. On this basis, it is determined that the technical solution claimed in the Claims can play the same role and solve the same technical problem as the example solution in the prior application, which is technically reasonable. The technical solution claimed in the Claims belongs to the same subject matter as the prior application.

3.1.1 The present invention

The present invention relates to a novel programmable DNA targeting and cleavage tool, which includes Cas9 and single-molecule DNA-targeting RNA (sgRNA). Claims 1-20 relate to a method for modifying target DNA, claims 21-38 relate to a composition, claims 39-44 relate to single-molecule DNA-targeting RNA, claims 45-53 relate to one or more nucleic acids, claims 54-56 relate to a kit, and claim 57 relates to use of the composition, the single-molecule DNA-targeting RNA, and the one or more nucleic acids in preparation of medicines for treating diseases; and the core of claims 1-57 is Cas9 and single-molecule DNA-targeting RNA (sgRNA).

3.1.2 Priority text

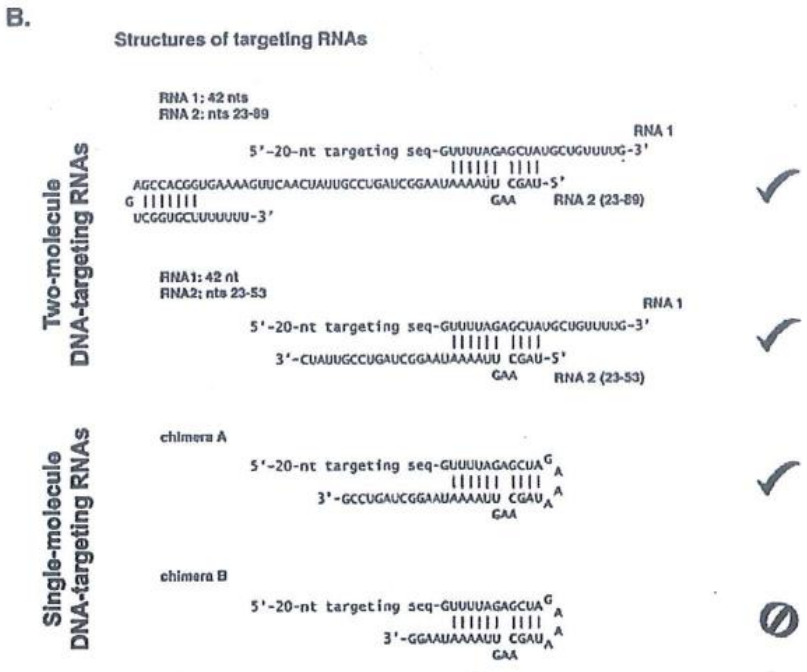
The US provisional application US61652086 (P1), which serves as the basis of priority, describes the invention entitled “Methods and Compositions for RNA-guided Site-Specific DNA Modification”, and specifically discloses the invention of a new technology that can replace the two main technologies used to manipulate site-specific DNA nucleases; and these two techniques have attracted attention as powerful tools for genetic engineering of cells and whole organisms (see paragraphs 0001-0002 of P1). The claims describe a method for site-specific modification of target DNA by contacting a complex comprising DNA-targeting RNA and site-specific modified peptides with the target DNA (see claim 54 of P1), and states that the target DNA is a portion of chromosomes within eukaryotic, plant, and animal cells (see claims 58, 61, 65, and 66 of P1), the

enzyme activity of the site-specific modified peptides is nuclease activity, and DNA modification is DNA double-stranded break (see claims 73-76 of P1).

Regarding DNA-targeting RNA, paragraph 0073 of the Description of P1 states that "the present disclosure provides DNA-targeting RNA, which guides the activity of related peptides (such as site-specific modified peptides) against specific target sequences within the target DNA. The DNA-targeting RNA according to the present invention includes a first segment (also referred to as a "DNA targeting segment" or "DNA targeting sequence" herein) and a second segment (also referred to as a "protein binding segment" or "protein binding sequence" herein). Regarding the DNA targeting segment of the DNA-targeting RNA, paragraph 0074 of the Description states that "The DNA targeting segment of the DNA-targeting RNA according to the present invention includes nucleotide sequences complementary to the sequences in the target DNA. In other words, the DNA targeting segment of the DNA-targeting RNA according to the present invention is hybridized (i.e., subjected to base pairing) to interact with the target DNA in a sequence-specific manner. Therefore, the nucleotide sequence of the DNA targeting segment can be altered, and the location within the target DNA where the DNA-targeting RNA and the target DNA will interact can be determined. The DNA targeting segment of the DNA-targeting RNA according to the present invention can be modified (e.g., through genetic engineering) to hybridize any desired sequence within the target DNA". Regarding the protein binding segment of the DNA-targeting RNA, paragraph 0076 of the Description states that "The protein binding segment of the DNA-targeting RNA according to the present invention interacts with site-specific modified peptides. The DNA-targeting RNA according to the present invention guides the bound peptides to a specific nucleotide sequence within the target DNA through the DNA targeting segment mentioned above. The protein binding segment of the DNA-targeting RNA according to the present invention includes two segments of complementary nucleotides. The complementary nucleotides of the protein binding segment are hybridized to form a double-stranded RNA duplex (dsRNA) (see FIG. 1A and FIG. 1B)"; and in paragraph 0083, it is recorded that "Exemplary two-molecule DNA-targeting RNA includes crRNA-like ("CRISPR RNA" or "target-RNA" or "crRNA repeat") molecules and corresponding tracrRNA-like ("trans-acting CRISPR RNA" or "activator-RNA" or "tracrRNA") molecules (see FIG. 1A). The crRNA-like molecules (target-RNA) include a DNA targeting segment (single-stranded) of the DNA-targeting RNA, and a segment of nucleotides ("duplex forming segment") that form half of a dsRNA duplex of the protein binding segment of the DNA-targeting RNA. The corresponding tracrRNA-like molecules (activator-RNA) include a segment of nucleotides (duplex forming segment) that form the other half of the dsRNA duplex of the protein binding segment of the DNA-targeting RNA (see FIG. 1A). In other words, the segment of nucleotides of the crRNA-like molecules are complementary to and hybridized with the segment of nucleotides of the tracrRNA-like molecules to form the dsRNA duplex of a protein binding domain of the DNA-targeting RNA. Thus, each of the crRNA-like molecules may be referred to as having corresponding tracrRNA-like molecule. The crRNA-like molecules additionally provide a single-stranded DNA targeting segment. Hence, the

crRNA-like and tacrRNA-like molecules (as a corresponding pair) are hybridized to form the DNA-targeting RNA. The precise sequence of a given crRNA or tracrRNA molecule is the characteristic of the species in which RNA molecules are discovered. The various crRNAs and tractRNAs are depicted individually and in the form of corresponding complementary pairs in FIGS. 6-9 of P1...". That is, the Description of P1 indicates that the DNA-targeting RNA used in the invention described in the Claims comprises a DNA targeting segment and a protein binding segment, the DNA targeting segment comprises a nucleotide sequence complementary to a sequence in the target DNA, and the protein binding segment interacts with the site-specific modified peptides and comprises two mutually complementary nucleotide segments that can be hybridized to form double-stranded RNA, i.e., comprises crRNA and corresponding tracrRNA. In addition, the site-specific modified peptides used in the invention described in the Claims form a complex with the DNA-targeting RNA, the peptides are natural Cas9/Csn1 proteins derived from various bacteria, and the amino acid sequences thereof (P1 recites "Cas9/Csn1 endonuclease" and "Cas9/Csn site-specific modified peptides", which are hereinafter collectively referred to as "Cas9 peptides" to match the expression in the Description of this patent) and their modification forms (see paragraphs 0089-0092, and 0005) are given in FIG. 2 and FIG. 12. It is also indicated that the complex used in the present invention as described in the Claims works through such a mechanism, in which the DNA targeting segment pairs with specific bases of the sequence of the target DNA, and the site-specific modified peptides modify the target DNA (e.g. cleavage) at that position (see paragraphs 0004 and 0089, FIG. 1).

Therefore, the Description of P1 clearly describes and explains the specific mechanism of action of site-specific DNA modification methods, as well as the structural elements of Cas9 peptides and DNA-targeting RNA required to achieve the modification. Furthermore, the examples also indicate that in the two experiments as shown in FIG. 3 and FIG. 5, it was specifically verified that the methods described in the Claims were successfully used for cleaving the target DNA. The experiment as shown in FIG. 3 was conducted in a buffer solution using Cas9 peptides derived from *S. pyogenes* and the DNA-targeting RNA (FIG. 3C). In FIG. 3A, it was specifically tested that four different RNA constructors targeting DNA combined with Cas9 were applied for cleavage of three different target DNAs. FIG. 3B shows that the four RNA constructors for testing are either two-molecule DNA-targeting RNA or single-molecule DNA-targeting RNA with different lengths.



The two-molecule DNA-targeting RNA includes a crRNA element (RNA 1) and a tracrRNA element (RNA 2). The single-molecule DNA-targeting RNA (including chimera A and chimera B) contains a crRNA element covalently linked to the tracrRNA element. The crRNA elements are identical in the two-molecule DNA-targeting RNAs. Two single-molecule DNA-targeting RNAs may be constructed by using different shorter crRNA elements. The tracrRNA elements are different in the four RNA constructors. The following table shows the comparison on the sequences of crRNA elements and tracrRNA elements:

Sequences of tested tracrRNA elements:	
RNA-$2_{23\text{-}89}$	5′ - UAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAGUGGCACCGAGUCGGUGCUUUUUUU - 3′
RNA-$2_{23\text{-}53}$	5′ - UAGCAAGUUAAAAUAAGGCUAGUCCGUUAUC - 3′
tracr of Ch. A	5′ - UAGCAAGUUAAAAUAAGGCUAGUCCG - 3′
tracr of Ch. B	5′ - UAGCAAGUUAAAAUAAGG - 3′
Sequences of tested crRNA elements:	
RNA-1:	5′ - GUUUUAGAGCUAUGCUGUUUUG - 3′
Chimera A/B	5′ - GUUUUAGAGCUA - 3′

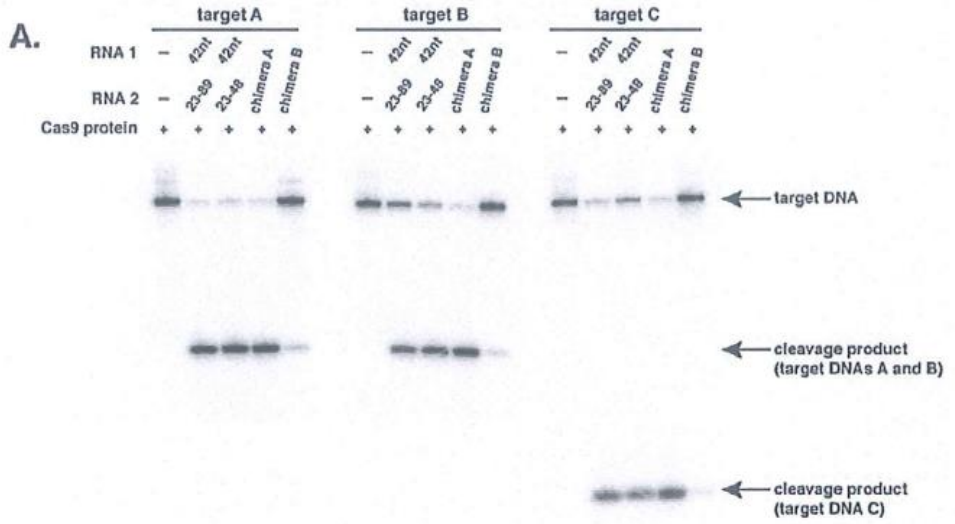
Each of the four RNA constructors contains a DNA targeting segment, which is individually selected to be complementary to each of three different targets. FIG. 3C depicts the sequences of three DNA targeting segments for one of the two two-molecule DNA-targeting RNAs, and the specific sequences of the three target DNAs are as follows:

Target DNA A 5′ – TGCGCTGGTTGATTTCTTCTTGCGCTTTTTGGGTATTGGGGAATTCATTA - 3′
Target DNA B 5′ – TTATATGAACATAACTCAATTTGTAAAAAAGGGTATTGGGGAATTCATTA - 3′
Target DNA C 5′ – GGTTATATTAAGTGCCGAGGAAAAATTAGGTGCGCTTGGCTGGCGCATTA - 3′

The gray shaded sequences are identical to the sequence of the three DNA targeting segments for each of the four RNA constructors. The underlined sequences (GGG and TGG) adjacent to the targeting sequence represent PAM.

In FIG. 3A, the target DNA cleavage results show that the combination of Cas9 with crRNA and tracrRNA elements is necessary and sufficient for DNA cleavage. If all components are

present, target DNA cleavage is observed. Both chimera A and chimera B are capable of cleaving the target DNA.



The experiments as shown in FIG. 5 were conducted on the same target DNA, performed in buffer solutions using six different Cas9 peptides from different sources and the same DNA-targeting RNA. It was shown that various Cas9/Csn1 site-specific modified peptides can utilize the same DNA-targeting RNA (FIG. 5A) (see paragraphs 0008, 0249, and 0251-252, as well as FIG. 5A and FIG. 5B).

Therefore, P1 specifically describes “A method for site-specific cleavage in target DNA, comprising the step of contacting the target DNA with a complex, wherein the complex comprises site-specific modified peptides showing nuclease activity and DNA-targeting RNA, the DNA-targeting RNA comprises a nucleotide sequence complementary to the sequence in the target DNA”, with the structure, mechanism of action and specific data of the complex being attached. That is, P1 has demonstrated through examples that a system composed of three elements can achieve the cleavage of target nucleic acid, which proves the feasibility of a CRISPER/Cas9 system as a gene editing tool, providing the foundation and direction for the long-term desire to find new gene editing tools and methods in the art. Meanwhile, as described above, the Claims of P1 also recite a method of using a portion of chromosomes from eukaryotic single cells, plant cells, animal cells, and other cells as a target DNA for the target to be cleaved (see claims 58, 61, 65, and 66). At the same time, the Description states that the invention can be applied not only to cells in vitro but also to cells in vivo (see paragraph 00121), and to the DNA of any type of cells derived from any organism. Taking the given specific biological species and cell type as an example (see paragraph 00165), two technical means for achieving these applications are described. Also described are a method for introducing a complex including DNA-targeting RNA and site-specific modified peptides into cells in the form of nucleic acids encoding the components, and a method for introducing the complex into the cells as is (see paragraph 00121). A known specific example of the former is given as applied to an expression vector for the expression of exogenous nucleic acids in eukaryotic cells (see paragraph 00124). It is pointed out

that in the expression vector, the nucleic acids can be operatively linked to various regulatory elements (such as a promoter, a ribosome binding site, a transcription terminator, sequences for enhancing expression) suitable for their expression, and specific known examples (see paragraph 00126) of the regulatory elements and many examples (see paragraph 00129) of the known methods for introducing nucleic acids (e.g., expression vectors) into cells are given. With regard to the latter, it is pointed out that the DNA-targeting RNA as a component of the complex can be prepared by means of chemical synthesis or in vitro transcription, and can be introduced into cells using known techniques (e.g., microinjection, electroporation, and transfection) (see paragraph 00173); it is pointed out that the site-specific modified peptides (e.g., Cas9 peptides) as another component of the complex can be fused with domains (such as the domains for enhancing solubility, the domains for improving stability, and the permeable domains for promoting cell uptake); and the specific examples (see paragraphs 00178-00179) of all the domains are given. In addition to the production methods (see paragraph 0248) of the Cas9 peptides and the DNA-targeting RNA, these examples indicate that the complex including the Cas9 peptides and the DNA-targeting RNA can be obtained by incubating chemically synthesized DNA-targeting RNA and Cas9 peptides obtained through *Escherichia coli* recombinant expression in a buffer solution for 15 minutes (see paragraph 0249).

The Description also indicates that the genetically modified cells obtained by applying the invention to DNA in eukaryotic cells, plant cells and animal cells can be widely used for various purposes, e.g., gene therapy for the treatment of diseases, or as antiviral, antipathogenic and anticancer drugs, for the production of genetically modified organisms in agriculture or for transplantation to subjects in biological research (see paragraph 00198).

Therefore, P1 provides and explains the site-specific DNA modification method and its mechanism of action, and verifies through specific experiments that the complex including the site-specific modified peptides and the DNA-targeting RNA required for achieving the modification can be used as a new technology to replace the two main technologies used for manipulating site-specific DNA nucleases. Specifically, not only is a method for producing this complex disclosed, but it has also been demonstrated through cleavage experiments that the complex can achieve the purpose of site-specific cleavage of target DNA. P1 has disclosed that the complex including the site-specific modified peptides and the DNA-targeting RNA is also applicable to target DNA in the in vivo cells (such as eukaryotic cells), not just to the target DNA in the in vitro cells. The applicability of this application has been disclosed, and this application can be achieved by those skilled in the art using known technical methods.

Therefore, although P1 had not provided the specific experimental data for the site-specific DNA modification method in all application scenarios, including eukaryotic cells, it had been clearly documented in P1 that the complex could be applied to target DNA in eukaryotic cells (such as plant cells, animal cells, and single-cell eukaryotes). The technical solution of applying the complex specifically to eukaryotic cells (such as plant cells, animal cells, and single-cell eukaryotes) for specific gene cleavage and editing is an application extension made by those

skilled in the art around the example solution in P1 and its descriptions of application scenarios in accordance with the trend of technological development. The inventive concept of the three main elements used in the method is the same as that recorded in P1. On this basis, it is determined that the technical solution claimed in the Claims can play the same role and solve the same technical problem as the example solution in the prior application, which is technically reasonable. That is, based on the in vitro cleavage experiment of the complex described in the priority document P1, which reveals the related knowledge of cleavage principles such as the roles of the components in the complex, and the principle of nucleic acid hybridization matching known in the art, it is possible to determine that once the complex is in contact with the nucleic acids in cells after being introduced into the corresponding cells, it is technically reasonable to realize the cleavage of intracellular nucleic acids based on the complementary hybridization of nucleic acid bases and the cleavage activity of the enzyme. This cleavage activity can be obtained by those skilled in the art from the original contents of the priority document in combination with the prior art; and because the complementary hybridization of the nucleic acids targeting DNA and the target nucleic acid bases in the complex mainly depends on the complementarity of the nucleic acids targeting DNA and the target nucleic acid bases, the nucleic acid cleavage activity is also the inherent activity of Cas9 enzyme. To sum up, the subject matter claimed in claims 1-57 can be obtained by the overall content described in P1, and claims 1-57 can possess the priority of P1. The US provisional application US61716256 (P2), which serves as the basis of priority, covers the content of P1, so that claims 1-57 can also possess the priority of P2 for similar reasons.

3.2 Regarding the proposition of petitioner

3.2.1 The petitioner claims that: claims 1-57 all relate to single-molecule DNA-targeting RNA (sgRNA), but P1 does not clearly or completely describe the sgRNA, and especially does not state that the dsRNA duplex of the sgRNA contains complementary nucleotides of tracrRNA and crRNA.

In this regard, the panel has the following viewpoints: first of all, the terms “DNA-targeting RNA”, “DNA targeting segment”, “protein binding segment”, and “single-molecule DNA-targeting RNA” are introduced in paragraphs 0045-0047 of the Description of P1. The Description provides a detailed description of the DNA-targeting RNA of this patent starting from paragraph 0073: the RNA includes a first segment and a second segment, where the first segment is also called the DNA targeting segment, and the second segment is also called the protein binding segment. The Description then describes these two segments separately, with paragraphs 0074-0075 describing the DNA targeting segment and paragraph 0076 beginning to describe the protein binding segment, and with explicit reference to FIG. 1A and FIG. 1B. Paragraph 0079 specifically states that the sgRNA includes two segments of nucleotides, i.e., a target RNA and an activator RNA, which are complementary to each other, covalently linked by an intervening nucleotide, and hybridized to form a double-stranded RNA duplex of the protein binding segment, resulting in a stem-loop structure, and with reference to FIG. 1B. FIG. 1A and FIG. 1B of P1 illustrate that the DNA targeting/protein binding segment and the target RNA/activator RNA are

both structural descriptions of the same DNA-targeting RNA from different perspectives, which can be in the two-molecule or single-molecule form. Those skilled in the art will not consider that the sgRNA containing the DNA targeting segment and the protein binding segment and the sgRNA containing the target RNA and the activator RNA are two different technical solutions. Secondly, paragraph 0084 describes the activator RNA and the target RNA in the context of two-molecule DNA-targeting RNA. However, it will be appreciated from FIG. 1 and the overall disclosure of the Description, for example, paragraph 0079, that the descriptions of the target RNA (crRNA) and the activator RNA (tracrRNA) also apply to sgRNA. Paragraphs 0087-0088 provide a more specific introduction to the dsRNA duplex of the sgRNA, which clearly indicates that the activator RNA is tracrRNA and the target RNA is crRNA, and provides specific sequences of the tracrRNA and the crRNA. Therefore, paragraphs 0076 to 0088 are specific descriptions of the protein binding segment of the DNA-targeting RNA in this patent, and are not another technical solution claimed by the petitioner. In addition, all DNA-targeting RNAs used in the examples and shown in the drawings have protein binding segments formed by the sequences of the crRNA and the tracrRNA. Therefore, the Description of P1 clearly describes the sgRNA as defined in the Claims of this patent, where the dsRNA duplex contains the complementary nucleotides of the tracrRNA and the crRNA.

3.2.2 The petitioner claims that, based on P1, the technical solutions of claims 1-57 cannot be expected to achieve the technical effect of cleaving target DNA in eukaryotic cells. The specific reasons are as follows:

1) P1 did not perceive the requirements of the sgRNA-Cas9 system for PAM sequences, and common general knowledge cannot make up for this defect: P2 and Evidence 7 indicate that PAM sequence-mediated double-stranded DNA melting, strand invasion and R-loop formation are also required to generate the cleavage of target DNA. The experiments in P1 were designed under the condition of not recognizing the role of PAM. The common general knowledge in the art cannot compensate for the defect of P1 regarding PAM. Evidences 7, 11-13 indicate that the role of PAM in the sgRNA-Cas9 system is gradually understood in the art after the disclosure of P1.

2) P1 also contains many other incomplete, insufficient, and erroneous disclosure information, resulting in unpredictable technical effects of claims 1-57 when applied to cleave target DNA in eukaryotic cells (see Evidence 6-9 and 14-21): the examples based on P1 cannot predict other dsRNA duplexes in protein binding segments that interact with Cas9 other than chimera A; the protein binding segment in P1 needs to have other defining characteristics such as the length of tracrRNA in addition to the dsRNA duplexes, and Evidence 15 suggests that for *Streptococcus thermophilus*, protrusions formed in natural gRNA are essential; it is impossible to anticipate any length of DNA targeting segments based on P1; and it is unclear to what extent the complementarity between the DNA targeting segment and the DNA target sequences is acceptable.

3) Based on P1, those skilled in the art can at best expect that the sgRNA-Cas9 system has a 50% success rate in cleaving its natural target sequence in vitro without cells; Evidences 26 and 34 indicate that the result of chimera A in P1 may be an accidental event or experimental

contamination; and P1 had not provided experimental examples of cleavage in eukaryotic cells or even experimental examples of cleavage in cells.

4) Unknown or unpredictable factors faced when applying CRISPR-Cas9 modification to eukaryotic cells are as follows: The CRISPR-Cas9 system may be degraded or disturbed in eukaryotic cells; the environment in eukaryotic cells may affect the expression of Cas9 and sgRNA, Cas9 folding and sgRNA-Cas9 complex formation; the tolerance of eukaryotic cells to CRISPR-Cas9 system; the co-localization of CRISPR-Cas9 system and target DNA; and whether chromatinized DNA can be accessed.

5) Failures encountered by other systems during transformation: Other similar systems in the process of transformation applied to eukaryotic cells encountered failures, such as type II intron (Evidence 22), T7 RNA polymerase (Evidence 23), hammerhead ribozyme (Evidence 24), riboswitch (Evidence 25), and pAgo.

6) The inventors also experienced failures in the application to eukaryotic cells (Evidence 8-9, 26-28, 30, 31).

7) Others also experienced failures after trying the methods in P1 or P2 (Evidences 29 and 34).

In this regard, the panel believes that,

1.1) First of all, the understanding of the contents of the application documents should not depart from the general understanding of the technical development status by those skilled in the art. Although P1, which serves as the basis of priority, is the first application of the technical solution, the formation and development of relevant technical terms and concepts have their context. For example, Counter-Evidence CE32 had proved in 2008 that *S. thermophilus* carrying a type II system after being subjected to phage attack was incorporated into new spacers in a CRISPR array, where it was disclosed that the counterparts of these spacers in foreign target DNA, i.e., prototypical spacers, were always located near a short motif referred to as a CRISPR1 motif (see the Chinese translation of Counter-Evidence CE32). In 2009, Counter-Evidence CE40 used bioinformatics to comprehensively analyze the motifs flanking the sequences of the prototypical spacers in foreign DNA and coined the term “PAM”. By comparing the spacers and repeat sequences of CRISPR arrays of different species and strains, common PAM sequences of CRISPR systems from different species were determined (see the Chinese translation of Counter-Evidence CE40). The Counter-Evidence CE31, published in January 2010, revealed the existence of conserved sequences, known as CRISPR motifs or protospacer adjacent motifs (PAMs), by analyzing phage sequences adjacent to the prototypical spacers. Furthermore, CRISPR motif mutations may result in loss of phage resistance despite the presence of a matching CRISPR spacer. The absence of this motif in the CRISPR locus may allow the system to act specifically on the invading target DNA and preclude an “autoimmune” response on the host chromosomes (FIG. 2)” (see the Chinese translation of Counter-Evidence CE31). In June 2010, another review of Counter-Evidence CE38 pointed out that: “Finally, despite the presence of matching CRISPR spacers, mutations in PAM may lead to loss of phage resistance, as shown by *S. thermophilus*. The

presence of a complete motif only in the targeted foreign nucleic acid provides a simple way for the CRISPR/Cas system to distinguish the target and the host spacer, as the latter does not contain this motif. Therefore, the absence of PAM in the CRISPR locus avoids autoimmune response" (see the Chinese translation of Counter-Evidence CE38). In November 2010, Counter-Evidence CE10 demonstrated that the CRISPR/Cas system in *S. thermophilus* cleaves foreign double-stranded DNA in the spacer sequence at three adjacent nucleotides upstream of PAM; and this applies whether phage DNA or plasmid DNA is used as the target DNA (see the Chinese translation of Counter-Evidence CE10). The Counter-Evidence CE37, published in April 2011, relates to a CRISPR Cas system targeting invader DNA, where it is disclosed that "the crRNA is incorporated into an effector complex and guides the complex to invade the nucleic acids (through base-pairing interactions). Silencing may occur at the DNA or RNA level, and DNA targeting requires at least one subset of the CRISPR-Cas system for PAM in DNA targets... PAM provides a mechanism to distinguish self from non-self. PAM is essential for the silencing of several systems, and the PAM recognized by the CRISPR-Cas system in the invader does not exist in the repeated sequences on both sides of the potential target in CRISPR", i.e., the Counter-Evidences CE31, CE38, CE10 and CE37 published before the priority date of P1 all indicate the importance of the PAM in the target DNA.

Counter-Evidence CE7, published in June 2011, introduced a new nomenclature for the CRISPR/Cas system, establishing the terms type I, type II, and type III used since then, where the type II system is defined by the presence of Cas9 in the CRISPR/Cas locus. It can be inferred that the CRISPR/Cas systems of *S. thermophilus* involved in Counter-Evidences CE32 and CE10 both belong to type II CRISPR/Cas systems. CE8 published in August 2011 transformed the entire CRISPR-Cas9 locus of Gram-positive *S. thermophilus* into only a distantly related Gram-negative bacterium, i.e., *E. coli* (which generally does not have a type II system). The transformed strain was then attacked with two plasmids, where the target DNA (i.e., the two protospacer sequences corresponding to the intermediate spacer sequences of the CRISPR array) was artificially integrated, and a genuine PAM specific for *S. thermophilus* was deliberately placed at the 3' terminal of the target DNA. Counter-Evidence CE8 also analyzed the complementarity requirements between the spacer-derived sequences of crRNA and the prototypical spacers, and found that mismatches close to PAM were intolerable, while mismatches farther from the 5' terminal of the prototypical spacer sequences were tolerable.

The review of Counter-Evidence CE36 published in December 2011 revealed the same classification of CRISPR/Cas systems as Counter-Evidence CE7, citing Counter-Evidences CE10, CE32, CE40, and CE8. CE36 disclosed that "The processed crRNA forms CRISPR ribonucleoprotein (crRNP) complexes with specific Cas proteins, which promotes spacer bases pairing with targets or matching invasive nucleic acids. The crRNA serves as a guide (hence also referred to as the term guide RNA) to allow for specific base pairing between the exposed crRNA in ribonucleoprotein interference complexes and the corresponding prototypical spacer on exogenous DNA. The crRNA is likely to directly interact with complementary sequences in the

target. The unique presence of PAM sequences on invading foreign DNA (on the contrary, they are absent in host spacer sequences) may play a dual-role: firstly, in the selection and acquisition of the spacer, and secondly, in the process of distinguishing self interference from non-self interference, highlighting their importance. In fact, it has been proven that despite a perfect match between the spacer and prototypical spacer sequences, mutations in PAM can still evade CRISPR encoded immunity". FIG. 2 in the review of Counter-Evidence CE30 published in February 2012 also indicates that the target DNA cleaved by the type II system is contained in the PAM downstream of the prototypical spacer sequences.

Those skilled in the art should understand the general status of technical development in this field. In a relatively short period of time, multiple reviews and/or papers on research in the field of CRISPR/Cas systems that are particularly actively reported by professional publishing houses can reflect the understanding of PAM by technicians in this field before submitting P1 to some extent, that is, those skilled in the art can clearly know, based on the above-mentioned publications published before P1, that in order to enable the type II system to cleave foreign target DNA, a PAM adjacent to the prototypical spacer is required, the PAM is closely adjacent to the 3' terminal of the prototypical spacer sequences, and double-stranded DNA cleavage occurs at upstream of the PAM within the prototypical spacer. The contents of many documents published after P1 have once again confirmed this point.

Secondly, as mentioned above, the sequences of the three DNA targeting segments shown in FIG. 3C in P1 all contain the PAM sequence of *S. pyogenes*, indicating that the previously disclosed role of PAM has been taken into account in the experimental design of P1.

In addition, Evidence 7 has clearly stated that recognition of PAM by Cas9 is a prerequisite, and Evidence 11 further confirms this point. The information recorded in Evidence 13 indicates the diversity of PAM, but does not negate the role of PAM, i.e., the more detailed analysis of the mechanism by which PAM is required for cleavage by Evidences 7, 11 and 13 disclosed after P1 will not affect the content disclosed in the prior art before P1. As for Evidence 12, it mainly relates to the selection of more preferred PAM specific sequences capable of achieving more efficient cleavage in specific bacteria, which is a statement of the applicant's opinion as to whether the selection of specific sequences can bring about novelty and inventiveness. In the case where the contribution of the present invention does not lie in the selection and determination of specific PAM sequences, the content related to the specific selection of PAM in Evidence 12 does not affect the analysis of the general role of PAM mentioned above.

1.2) For the Chinese translation of "in type II systems, Cas9 loaded with crRNA probably directly targets invading DNA, in a process that requires the PAM" in Counter-Evidence CE 7, the Chinese translation submitted by the patentee is: "在Ⅱ型系统中，装载 ctRNA 的 Cas9 可能直接靶向侵入的 DNA，这个过程需要 PAM", and the Chinese translation submitted by the petitioner is: "在Ⅱ型系统中，装载 crRNA 的 Cas9 可能在一个需要 PAM 的过程中直接靶向侵入的 DNA".

In this regard, the panel believes that the term "probably" modifies "directly targets" instead

of whether this process requires PAM, that is, PAM is determined to be required for the process of targeting invasive DNA. Therefore, the Chinese translation of this sentence should be "在Ⅱ型系统中，装载 crRNA 的 Cas9 在一个需要 PAM 的过程中可能直接靶向侵入的 DNA". It also corroborates that the type II system requires PAM sequences to cleave foreign target DNA is known in the art.

2.1) Firstly, FIG. 8 and FIG. 9 of P1 provide examples of approximately 30 different crRNA and tracrRNA sequences, with FIG. 9D and FIG. 9F (*Streptococcus thermophilus* and *Neisseria meningitidis*) revealing dsRNA duplexes as short as 9 base pairs, and illustrate how they form the dsRNA duplexes. Due to the natural existence of these sequences, those having ordinary skill in the art will appreciate that these sequences are capable of forming functional CRISPR-Cas9 complexes in the native bacteria. Moreover, FIG. 9 of P1 illustrates that sgRNAs of different species include duplexes of various lengths for the natural bimolecular form and unimolecular form, and those skilled in the art will appreciate in light of this teaching that the lengths of the duplexes are not absolutely fixed, but can be routinely adjusted within the scope of its disclosure depending on the desired effect, thus having some flexibility. Secondly, the example data of P1 demonstrate that those skilled in the art will be able to identify the relatively minimal crRNA and tacrRNA elements required for DNA cleavage. As described above, chimera B is also capable of cleaving double-stranded target DNA, but with less efficiency than chimera A, indicating that retaining more natural tracrRNA elements at the 3' terminal of the tracrRNA sequence can provide more effective DNA cleavage. Therefore, those skilled in the art will further understand how to design effective tracrRNA within the scope defined by the Claims based on the disclosure of the above laws. Thirdly, Example 1 of P1 provides a cell-free in vitro assay, which provides a simple tool for those skilled in the art for rapidly assessing whether a single-molecule DNA-targeting RNA designed in accordance with the disclosure of P1 provides DNA cleavage, i.e., allows those skilled in the art to test their predictions through conventional experiments. In addition, the Evidences 14-16 cited by the petitioner does not question this point, and the contents thereof also indicate that only when the length of tracrRNA in the protein binding segment is within a certain range can the target DNA be cleaved. As for the protrusion structure described in Evidence 15, FIG. 3B of P1 shows that dsRNA duplexes contain protrusions formed by non-complementary nucleotides, and FIG. 9D also discloses protrusions formed by non-complementary nucleotides. which will be considered by those skilled in the art when reading P1.

2.2) Firstly, Example 1 of P1 was conducted using a DNA targeting segment with the length of 20 nt, and each of two-molecule and single-molecule DNA-targeting RNAs described in FIG. 9 of P1 includes a DNA targeting segment with the length of 20 nt. It is known that nucleic acid hybridization specificity increases with the increasing of length. It will also be appreciated by those skilled in the art from ZFN and TALEN related technologies that a DNA targeting segment with the length of about 20 nt should be sufficient to achieve specific targeting of genes in, for example, human cells. Secondly, it is known that a spacer in the CRISPR array of the type II system (at the DNA level, i.e., before processing) has a length of about 30 nucleotides in *S.*

pyogenes and *S. thermophilus*. Thus, those skilled in the art can design a DNA targeting segment with an appropriate length in combination with this knowledge. In addition, the cell-free in vitro assay provided by P1 allows those skilled in the art to quickly and directly assess and adjust the functions of DNA targeting segments with different lengths. In the case of failure, for example, where cleavage is impossible because the DNA targeting segment is too short, those skilled in the art will know how to adjust (i.e., increase the length).

2.3) Firstly, those skilled in the art are able to recognize the impact of complementarity of the DNA targeting segment and the target DNA A, B and C. Those skilled in the art are aware of the general principles of complementarity between nucleic acids, and thus must know that complete complementarity between a DNA targeting segment and its counterpart in the target DNA is the best way to ensure that Cas9 is indeed guided to the target DNA by the DNA-targeting RNA. Reducing the complementarity between the DNA targeting segment and its counterpart in the target DNA will lead to a decrease in the hybridization strength, which in turn may reduce the efficiency and/or specificity of the DNA-targeting RNA in guiding Cas9 to the target DNA. Secondly, as stated in paragraph 0075 of the Description of P1, the complementarity percentage between the DNA targeting segment and the nucleotide sequence of the target DNA may be at least 65%. Those skilled in the art have the ability to make routine adjustments and improvements, and thus test their recorded limit boundary, such as 65%. Even if it is found that a specific DNA targeting segment has no function, the Description will not be considered misleading or the present invention cannot be repeated, and the degree of complementarity is usually increased further only based on the close correlation of the degree of complementarity with the hybridization strength.

Finally, Evidences 6-9 and 14-20 are documents published after the priority date of P1, which relate to detailed analysis of the specific effects of dsDNA length, tractRNA length and DNA targeting segment length in the protein binding segment on the cleavage activity of the complex, and will not affect the contents contained in P1 and documents published before P1. Evidence 21 is the reply comment of the applicant at the substantive examination stage of this patent, and does not affect the above conclusion.

3) As mentioned above, FIG. 3A of P1 shows that chimera B is also able to achieve cleavage of the target DNA, which is not a 50% success rate, and the Claims do not define the specific extent to which the cleavage or disruption needs achieve. In addition, although the cell-free experiment in a test tube is different from the specific environment in eukaryotic cells, only the specific application environment of the target DNA modification method changes, and the three core elements contained therein are not substantially changed. That is to say, the overall inventive concept is still the same, and the targeting to DNA is also implemented based on base complementary hybridization between sgDNA and target DNA. This targeting effect depends on the complementarity of base pairs; and although the hybridization between nucleic acids is related to the factors of external environment in which the nucleic acids are located, there is no evidence to suggest that complementary nucleic acids cannot be hybridized within cells based on the

existing research on nucleic acid hybridization conditions. Therefore, based on the feasibility of in vitro cleavage, it can be preliminarily determined that sgDNA in a complex should also be able to achieve the targeting function in cells through complementary hybridization with target nucleic acids, which is technically reasonable. The further cleavage activity after targeted localization is inherent to the Cas9 protein. Whether it is in a cell-free environment, prokaryotic cells, or eukaryotic cells, the above-mentioned function of sgRNA guiding Cas9 protein to cleave a target gene is essentially the same, there are only different requirements for expressing the above CRISPR/Cas9 system in different environments or selectively transferring it into the nuclei of target cells, especially eukaryotic cells, to be in contact with DNA. Those skilled in the art understand that in order to achieve the desired genome cleavage, the functional units of the CRISPR/Cas9 system must be present in the nucleus of the target cell so as to be in contact with the DNA to be cleaved, which can be achieved by a variety of delivery vectors. Therefore, the absence of specific experimental examples of cleavage in cells or eukaryotic cells in the prior application does not mean that the complex cannot achieve cleavage in cells or eukaryotic cells. From the perspective of technical rationality, its cleavage activity in cells can be concluded from the overall contents of the priority documents based on its inherent properties. In Evidence 34, “success in eukaryotic cells” refers to whether the target DNA can be effectively cleaved in eukaryotic cells, rather than whether the target DNA can be cleaved. Evidence 26 is a decision in the US interference procedure, which is aimed at the question of who invented it first and does not affect the above conclusion.

4) Although there are differences in the extracellular and intracellular environments, as well as structural differences between prokaryotic cells and eukaryotic cells, RNA degradation, folding and complex formation, eukaryotic cell tolerance, co-localization and other issues need to be considered when introducing exogenous RNAs into cells. However, those skilled in the art can reasonably understand and recognize that, in order to apply the CRISPR/Cas9 system to different specific environments, it is necessary to carry out adjustment according to the characteristics of different application environments in the premise of taking the common effects of different factors into account, thus effectively playing the role of cleavage, which does not conflict with the mechanism of the CRISPR/Cas9 system to play the role of cleavage itself. On the basis that the prior application has demonstrated through in vitro cell-free experimental examples that a system composed of three elements can realize the cleavage of target nucleic acids, which provides a foundation and direction for the long-term desire to find new gene editing tools and methods in the art, it is technically reasonable to use the same concept to expand the application in accordance with the trend of technological development to play the same role and solve the same technical problems. Issues such as RNA degradation, folding and complex formation, eukaryotic cell tolerance, and co-localization mainly affect the delivery efficiency of the CRISPR/Cas9 system successfully delivered to the site in contact with the target DNA, and further affect the overall efficiency and activity of cleavage. The petitioner has no evidence to suggest that these factors will affect the inherent cleavage activity of the CRISPR/Cas9 system that has successfully reached

the target DNA contact site, resulting in its complete inability to perform the function of DNA cleavage. The level of cutting efficiency is not exactly the same as whether cleavage can be performed.

5) The correlation between systems involved in failure cases encountered by other systems during the transformation process and the CRISPR/Cas9 system only lies in originating from prokaryotic cells, and have a less correlation with the CRISPR/Cas9 system. Even though there are papers (Evidences 22-25) reporting the failure cases of applying these technologies to eukaryotic cells, the core of these papers is still to apply the CRISPR/Cas9 system of this patent to different cells, and to explore the specific effects brought by the adjustments and changes in various components, which shows from the side that those skilled in the art have the consensus of applying it to eukaryotic cells, but new technical problems may need to be solved in the process of application. It does not mean that they hinder the application of the CRISPR/Cas9 system to eukaryotic cells or the CRISPR/Cas9 system cannot be applied to eukaryotic cells.

6) Firstly, Evidences 27 and 28 suggest that there may be difficulties and frustrations in the embodiments during application to eukaryotic cells, but the difficulties encountered during the specific implementation process only indicate that the implementation still needs to match the appropriate conditions, and do not deny the technical rationality of its role in eukaryotic cells, that is, it only expresses the concern for the efficiency of the system. The efficiency of the system is different from the reasonable expectation of its implementation. Secondly, Evidences 26, 30 and 31 involve the inventor's e-mails, which are internal communication e-mails formed long after P1 was submitted. Those having ordinary skill in the art cannot obtain these non-public internal communications, so the internal communication e-mails will not affect the understanding of P1 and the contents disclosed before P1 by those having ordinary skill in the art. Thirdly, Evidences 8 and 9 are also documents published after the filing date of P1, which states that "it is not yet known whether such a bacterial system can function in eukaryotic cells". It is only an objective record of the results of experiments that have not been conducted from a technical rigor perspective, which does not indicate that the system cannot achieve cleavage in eukaryotic cells.

7) Firstly, the petitioner submitted the data shown in Evidence 29 to demonstrate that at the filing date of P1, the use of Cas9 and DNA-targeting RNA for cleavage (in eukaryotic cells) was not fully disclosed. In fact, some of the successful experiments in Evidence 29 employed Cas9 and single-molecule DNA-targeting RNA as revealed by P1, which were necessary for realizing DNA cleavage. There is no contradiction or conflict between the contents of experimental process and results and the content of P1, which demonstrates from the side that the complex can achieve cleavage in eukaryotic cells, the failure of some experiments only indicates that suitable conditions may need to be matched in the process of specific implementation. In just a few months after the disclosure of P1, several other teams successfully used the complex claimed by the present invention as the core component of a tool for gene editing of eukaryotic cells with reference to the off-the-shelf technology, and their experimental process and results were not in conflict with the contents revealed in the priority document. The attempts and verifications by

other teams had once again demonstrated that it is technically reasonable for those skilled in the art to preliminarily conclude that the complex should also be able to achieve cleavage in eukaryotic cells based on the inherent properties of the complex and the common general knowledge. Secondly, Evidence 34 is only a personal opinion expressed by the expert based on the technological development progress, and its nature belongs to the testimony of witnesses, but the expert himself did not appear in court to accept cross-examination on the specific content of the testimony. In addition, as mentioned above, the difficulties in the specific implementation process only indicate that appropriate conditions still need to be matched at the time of implementation, which is not inconsistent with the preliminary conclusion that the complex should also be able to achieve cleavage in eukaryotic cells based on the inherent properties of the complex.

Evidence 10 is a document published after the priority date of P1, which states that "there is no assurance that Cas9 will function effectively on chromosomal targets, nor that the desired DNA-RNA hybrids will be stable in this environment. ...These problems can only be solved by working attempts to apply the system to eukaryotes". This content is also an objective expression of the uncertainty of the results of experiments that have not been carried out. It is also concerned about the cleavage efficiency of the system in eukaryotic cells, and does not negate the rationality of the application of the system to eukaryotes.

Evidences 35 and 36 are part of the rulings in the US interference procedure. The legal system of the patent law in China is different from that in the United States. The Chinese patent law does not contain any provisions related to the interference procedure adopted under the prior invention system or the judgment generated by this system. The judgment idea of the prior invention system is different from the criteria for judging whether the priority is established. In addition, the contents cited therein relate to the type II intron, ZFN/TALEN, which are different from CRISPR/Cas9, so it will not affect the determination of the above conclusions. Evidence 37 records information about protein folding and nuclear composition in eukaryotic cells, only indicating that eukaryotic cells are very different from prokaryotic cells. The objective differences between prokaryotic cells and eukaryotic cells are well-known, and thus the existence of the objective differences will not affect the improvement of those skilled in the art in the direction of eukaryotic cells, nor will it affect the above conclusions.

4. Regarding Paragraphs 2 and 3, Article 22 of the Chinese Patent Law

A document whose publication date is between the priority date and filing date of a patent is not considered as prior art and thus cannot be used for determining the novelty and inventiveness of the corresponding patent.

As mentioned above, claims 1-57 can possess the priority of P1, while the publication date of Evidences 3-9 is later than the priority date of P1 on May 25, 2012, so they cannot be used for commenting on the novelty and inventiveness of the Claims. On this basis, the petitioner's reason that this patent does not comply with Paragraphs 2 and 3, Article 22 of the Chinese Patent Law cannot be established.

5. Regarding Paragraph 4, Article 26 of the Chinese Patent Law

Paragraph 4, Article 26 of the Chinese Patent Law stipulates that claims shall be based on the Description and clearly and concisely define the protection scope of a patent claimed.

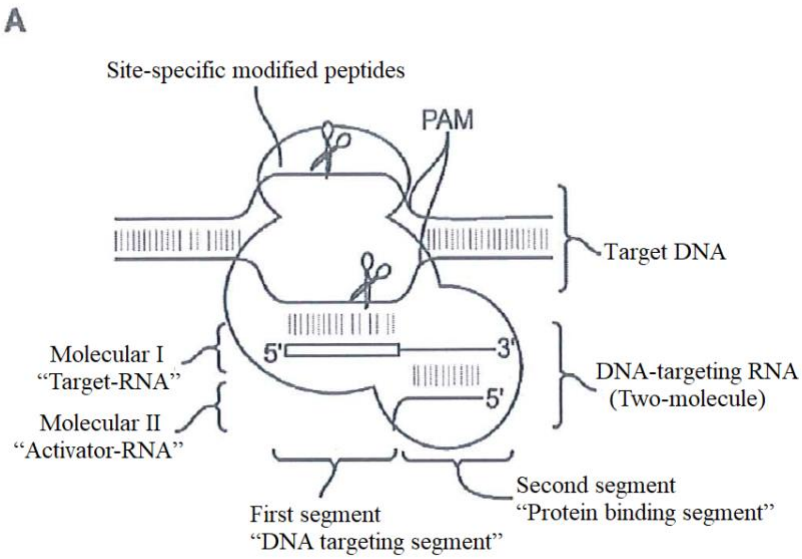
The language used in the Claims should not be mechanically interpreted based solely on the literal meaning expressed in the Claims, but should be interpreted from a technical perspective by those skilled in the art, combined with the teachings in the Description and the common general knowledge in the art. If those skilled in the art can clearly understand the structure of sgRNA as defined in the Claims based on the Description and its accompanying drawings, then the terms will not lead to unclear protection scope of the Claims.

If a claim summarizes one or several technical features, and the scope of the summary is reasonably available to those skilled in the art on the basis of the content contained in the Description and based on the expected technical effect, the corresponding summary will not result in the claim not being supported by the Description.

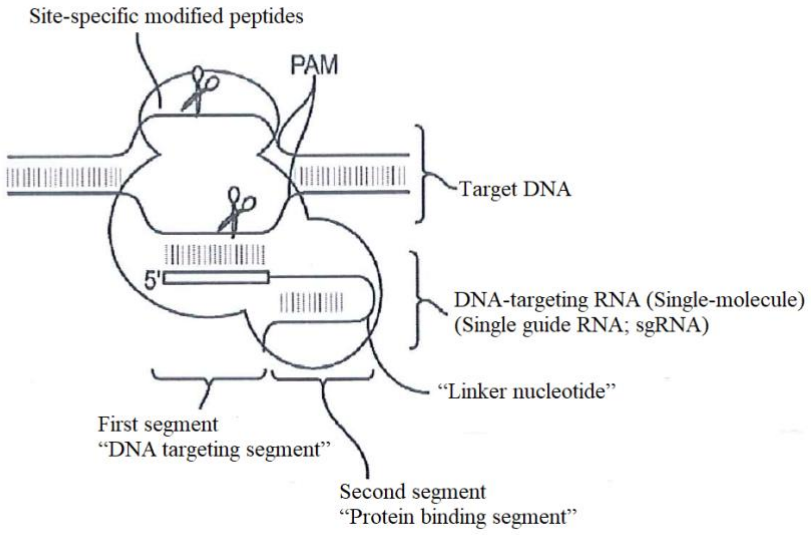
5.1 The petitioner believes that it is unclear how the DNA targeting segment and the protein binding segment forms the sgRNA, resulting in unclear scope of protection of claims 1-57.

In this regard, the panel believes that, according to the Description of this patent as well as FIG. 1 and FIG. 9, those skilled in the art can clearly know how the DNA targeting segment and the protein binding segment forms the sgRNA, for the following reasons:

FIGS. 1A and 1B are as follows:



B



It can be clearly seen from these figures that there are two different ways (two different sets of terms) to describe the same RNA components. In one set of terms, from top to bottom in the above figure (FIG. 1A), the term "target RNA" denotes the crRNA component, and the term "activator RNA" denotes the tracrRNA component (see paragraph [0145] of the Description). As shown in the above figure, the 5' portion of the target RNA is hybridized with the target DNA, and the 3' portion is hybridized with the activator RNA. The target RNA and the activator RNA may be linked together by means of an optional "linker nucleotide" so as to form a single RNA molecule, i.e., sgRNA (compare FIG. 1A and FIG. 1B). As shown in the figures above, the term "DNA-targeting RNA" refers collectively to two kinds of RNAs with duplexes, i.e., with or without the optional "linker nucleotide" as shown in FIG. 1B.

In the one set of terms, from left to right in the above figure (FIG. 1A) this time, the DNA-targeting RNA as a whole can be described as having two segments, referred to as a "DNA targeting segment" and a "protein binding segment". The "DNA targeting segment" is the 5' terminal of the crRNA that will be hybridized with the depicted target DNA. The "protein binding segment" includes a dsRNA duplex formed by hybridization of the target RNA (crRNA) with the activator RNA (tracrRNA). Cas9 interacts with the protein binding segment of the DNA-targeting RNA, and the DNA targeting segment is hybridized with the sequence of the target DNA.

Thus, crRNA (target RNA) includes a DNA targeting segment (single-stranded) and a segment of nucleotides ("duple- forming segment") forming half of a dsRNA duplex of a protein binding segment, while tracrRNA (activator RNA) includes a segment of nucleotides (duplex-forming segment) forming the other half of the dsRNA duplex of the protein binding segment; and the crRNA and the tracrRNA may be linked by a linker (i.e., an intervening nucleotide).

In addition, FIG. 9 shows exemplary sequence combinations of protein binding segments of two-molecule DNA-targeting RNA and single-molecule DNA-targeting RNA. The exemplary single-molecule DNA-targeting RNA shown in FIG. 9 includes complementary nucleotides of

tracrRNA and of crRNA (see paragraph 0048 of the Description). Therefore, those skilled in the art can recognize that any disclosed two-molecule DNA-targeting RNA can be transformed into a single-molecule DNA-targeting RNA form, and vice versa (by adding or removing linkers, respectively); and these aspects are generally applicable to DNA-targeting RNAs. Hence, those skilled in the art are aware of the structural relationship between the DNA targeting segment and the protein binding segment, and the invalidation reasons that the protection scope of claim 1-57 is unclear proposed by the petitioner are untenable.

5.2 The petitioner believes that the specific reasons why claims 1-57 are not supported by the Description are as follows: 1) The most supported scope of the disclosure of P1 is that the wild-type Cas9 proteins of *S. pyogenes*, *Listeria innocua* and *S. thermophilus* form a complex with chimera A, which can cleave the natural target DNA in a cell-free system. However, Evidence 7 published on June 28, 2012, after P1 and before P2, mentioned the function of PAM, the PAM sequence of *S. pyogenes* and the minimal prime area of natural crRNA and natural tracrRNA derived from *S. pyogenes* used for forming sgRNA, and disclosed the content of Example 1 of this patent, and Evidence 7 was a prior art at the time of filing of this patent (i.e. March 15, 2013), therefore, claim 1 should be limited to the scope supported by P1 mentioned above. 2) In the examples of this patent, only several specific variants of wild-type Cas9 derived from five kinds of bacteria (*S. pyogenes* SF370, *S. thermophilus* LMD-9, *L. innocua* Clip11262, *Campylobacter jejuni subsp* NCTC 11168 and *N. meningitidis* Z2491) and wild-type Cas9 derived from one kind of bacteria (*S. pyogenes* SF370) were prepared, making it difficult for those skilled in the art to anticipate that other Cas9 peptides can achieve the cleavage of target DNA. 3) “Target DNA” covers any DNA sequence, and some of which cannot be cleaved by Cas9 peptides in the absence of PAM sequences. 4) “DNA targeting segment” contains a nucleotide sequence complementary to the sequences in the target DNA. However, subsequent evidences show that not all length of DNA targeting segments that have any complementarity with the target can achieve the cleavage of target DNA. 5) “Protein binding segment” contains two complementary nucleotide fragments that hybridize to form dsRNA duplexes, and the dsRNA duplexes contain complementary nucleotides of tracrRNA and crRNA. However, this patent only verified the cleavage effect of dsRNA formed by partial sequences of tracrRNA and crRNA derived from *S. pyogenes*, and subsequent evidences show that in addition to dsRNA duplexes, protein binding segments must also have the definition of other structural features. For example, Evidences 14-16 show that the length of tracrRNA is with requirements, and the bulge formed in the natural gRNA of *S. thermophilus* is essential. 6) There is species specificity between the sources of Cas9 and sgRNA, and not all Cas9 derived from any source can cooperate with sgRNA derived from any source to play the role of cleavage. 7) Claim 1 does not define the promoter of sgRNA, but subsequent Evidence 26 indicates that not all promoters can achieve the expression and function of sgRNA, resulting in them not being supported by the Description.

In this regard, the panel believes that,

1) The reason why the petitioner claims to limit claim 1 to the scope supported by P1 above

is the reason whether it can enjoy the priority of P1, and as mentioned above, claims 1-57 enjoy the priority of P1 and P2, so the reason proposed by the petitioner for not supporting is not established.

2) The bacterial immune system type II CRISPR system and Cas9 peptides were known to those skilled in the art before the filing date of this patent, and the structure of Cas9 peptides is also known to those skilled in the art. Meanwhile, paragraphs 0201 and 0202 of the Description of this patent disclose 255 sequences of Cas9. This patent also discloses RuvC and HNH structural domains, and the Counter-Evidence CE8 has determined that they are important for activity, because the mutations in the HNH and RuvC structural domains will reduce or eliminate the DNA cleavage activity of the type II system in *S. pyogenes*. Therefore, it is clear to those skilled in the art that natural Cas9 peptides actually do have the function of cleaving target DNA in cells. Although the experimental evidence of the disclosure of this patent is only an example, those skilled in the art can reasonably understand, in combination with the prior art, that the disclosure and teachings of this patent are not limited to Cas9 of *S. pyogenes* and single-molecule DNA-targeting RNA used in the examples. In addition, those skilled in the art also know that the type II CRISPR system can cleave various foreign DNA in different environments. For example, Counter-Evidence CE8 proves that the type II CRISPR-Cas9 system of gram-positive *S. thermophilus* can be transferred to distant gram-negative bacteria - *Escherichia coli* (which naturally does not have the type II CRISPR-Cas9 system) and cleave the target DNA, and also proves that the type II CRISPR-Cas9 system can target non-natural target sequences. According to the guidance given in this patent and common general knowledge in the art, those skilled in the art can reasonably expect that Cas9 derived from other sources can achieve similar cleavage functions when combined with the corresponding sgRNA based on the structural similarity of Cas9 derived from different sources to a certain extent.

3) The terms used in the claims should be interpreted from a technical perspective by those skilled in the art, combined with the teachings in the Description and the common general knowledge in the art, and should not be mechanically interpreted based solely on the literal meaning expressed in the claims. The bacterial immune system type II CRISPR system (including that Cas9 is the symbolic protein of type II system and the requirement that the target DNA contains PAM sequences in the type II system) is well known in the art. As mentioned above, the target DNA is the object of cleavage and processing in the specific application of the system, and it does not directly affect the structure and composition of the CRISPR system. Therefore, those skilled in the art will understand that the target DNA in the method claims, as the action object of the method, should be DNA that can be cleaved, not any DNA. The fact that the claims to a product do not specifically define the features that will not have a material effect on its protection scope will not lead to that the claims cannot be supported by the Description.

4) It is understood by those skilled in the art that based on the knowledge of the CRISPR/Cas system in the prior art and the teachings in this patent, the spacer sequence in the CRISPR/Cas system usually corresponds to the DNA targeting segment of the claims. For example, it is well

known that the length of the spacer sequence in the CRISPR/Cas system is about 26 to 72 bp (at the DNA level, i.e. before processing). According to the Counter-Evidence CE5, it is known that the length of the spacer part (i.e. the processed form of RNA actually used for cleaving the complex) of the mature crRNA obtained by pre-crRNA processing is 20 nucleotides. The length of many DNA targeting segments in the Description is 20 nucleotides and demonstrates a complete complementarity with the target DNA. It is known that the specificity of hybridization increases with the increase of length, and the core requirement for DNA targeting segments is to be complementary to the target DNA based on the principle of complementary base pairing, and reducing the complementarity between the DNA targeting segments and the corresponding part of the target DNA will reduce the hybridization intensity, so it may further reduce the efficiency and/or specificity of Cas9 targeting to the target DNA. Moreover, it is known in the prior art that the requirements for complementarity between DNA targeting segments and target sequences are more stringent at the 3' terminal of the DNA targeting segment, while mismatches can be tolerated at the 5' terminal of the DNA targeting segment. Therefore, when determining the degree and region of complementarity, it is obvious that those skilled in the art will start screening from DNA targeting segments that have 100% sequence complementarity with the target DNA and sequences with mismatches at the 5' terminal. Whether the DNA targeting segments can achieve the final cleavage is closely related to the complementary binding of the segments and the target sequences and the existence of PAM sequences on the target sequence. Although not all DNA targeting segments can realize cleavage, those skilled in the art can select and determine appropriate DNA targeting segments based on the principles of playing the role of CRISPR/Cas system, the relationship between hybridization intensity and complementarity, and the impact of different terminal mismatches on hybridization. Therefore, the expression “DNA targeting segments” in claim 1 can be supported by the Description.

5) According to the knowledge of CRISPR/Cas system in the prior art and the disclosure in this patent about the duplexes formed by tracrRNA and crRNA, those skilled in the art will understand that the repetitive sequences in crRNA usually correspond to the nucleotide segments hybridized with complementary nucleotides of tracrRNA in crRNA. For example, see the supplementary FIG. 11 of Counter-Evidence CE5, which discloses the sequences and alignments of tracrRNA derived from different bacterial species. tracrRNAs derived from different species have different lengths, but they all contain an “anti-repetitive region”, which is taught in the Counter-Evidence CE5 as a region that hybridizes with the repetitive region of crRNA during the processing/maturation of crRNA. Therefore, it is known in the art that tracrRNA sequences and crRNA sequences (such as crRNA repetitive regions) can vary between different bacterial species (i.e., there may be a certain degree of sequence conservatism when comparing tracrRNA sequences or crRNA sequences derived from certain related bacterial species, but the level of conservatism will decrease when comparing tracrRNA sequences or crRNA sequences derived from more distant bacterial species). Moreover, tracrRNA sequences and crRNA sequences are known in the art. In view of the known differences in sequences between different bacterial

species, those skilled in the art know that matching crRNA and tracrRNA sequences should usually be used to form the protein binding segments of sgRNA.

With regard to the disclosure of this patent, although in the examples, the protein binding segments contained in the single-molecule DNA-targeting RNA are constructed with the sequences of *S. pyogenes*, the disclosure of this patent distinctly provides guidance on how to design the DNA-targeting RNA (including longer and shorter fragment forms, and single-molecule forms) from the crRNA and tracrRNA elements of other organisms. For example, FIG. 6 provides tracrRNA sequences derived from different organisms. FIG. 7 provides the sequences of repetitive sequences of crRNAs derived from nine different bacteria, and these sequences are involved in the formation of dsRNA duplexes that form part of the protein binding segments. FIG. 8 summarizes these information and highlights examples of nucleotide segments derived from different bacteria in crRNA-derived sequences and tracrRNA-derived sequences to show how they are involved in the formation of dsRNA duplexes. The description of FIG. 8 (see paragraph [0047]) also states that: “See SEQ ID NO: 431-562 for more exemplary activator-RNA sequences. See SEQ ID NO: 563-679 for more exemplary duplex formation segment target-RNA sequences.” FIG. 9 shows the examples that how to further shorten each sequence at the 3' terminal of the crRNA component and at the 5' and 3' terminals of the tracrRNA component, and further illustrates how they are covalently linked to obtain single-molecule DNA-targeting RNAs. The description of FIG. 9 (see paragraph 0048) states that: “The sequences provided are non-limited examples and are intended to illustrate how single-molecule DNA-targeting RNA and two-molecule DNA-targeting RNA can be designed based on natural sequences derived from various species. Various examples of appropriate sequences derived from various species are listed below...”.

Evidence 14 reveals that tracrRNA with the length of more than 58 nt supports the effective cleavage of pSP1 plasmid by St-Sas9, Evidence 15 reveals that the raised motif is essential in StCas9, and the mutant with the length of less than 18 bp of sgRNA is almost completely inactivated, and Evidence 16 reveals that the length and mismatches of crRNA-tracrRNA duplexes have an effect on the activity of SpCas9. Evidences 14-16 are further research on the mechanism of action and structural optimization of CRISPR/Cas system after the priority date, which can only show that the length of tracrRNA or the hump of 3' terminal is crucial to whether a specific Cas9 can cleave the target DNA. However, this patent provides sufficient guidance on how to obtain a large number of single-molecule DNA-targeting RNAs, and FIG. 24 of this patent proves that the activity of single-molecule DNA-targeting RNA is not limited to Cas9 derived from the same organism. In combination with the teachings about protein binding segments in the Description and the knowledge about crRNA and tracrRNA in the prior art, those skilled in the art will select appropriate length and structural design of tracrRNA when implementing the method of claim 1, and exclude the protein binding segments that cannot be bound to Cas9. Therefore, the limitation can be supported by the Description.

6) It is known to those skilled in the art that the bacterial immune system type II CRISPR

system has the activity of cleaving DNA in cells. Although the components of the DNA cleavage complex of the type II CRISPR system are unknown in the prior art, the components of the complex identified by the inventor of this patent are known in the prior art. It is known that the crRNA sequence is consisted of a variable 5' region (called a “spacer region”) that hybridizes with the target DNA and a 3' region (called a “repetitive region”). The repetitive sequences are the same as the corresponding sequences in bacterial species, but it is known that there are significant differences between different bacterial species. It is also known from the art that the tracrRNA sequence contains an “anti-repetitive” region, and the region can form a duplex with the repetitive sequence of crRNA (see supplementary FIG. 11 of the Counter-Evidence CE5). Although the tracrRNA sequence can vary among different bacterial species, it is also stated in Counter-Evidence CE5 that “The analysis shows the potential co-evolution of tracrRNA anti-repetitive sequences and CRISPR repetitive sequences”. In other words, crRNA and tracrRNA sequences evolve together to continue to match each other. Therefore, those skilled in the art certainly have known that matching crRNA and tracrRNA sequences should be used to replicate the activity of natural systems.

Similarly, it is known that Cas9 sequences vary with bacterial species, but it is also known that Cas9 has specific domain structure (for example, including RuvC/HNH domain), and Cas9 proteins can be identified by their sequences and their positions in the bacterial genome (as part of the type II CRISPR system) (see Counter-Evidence CE7, Counter-Evidence CE8 and Counter-Evidence CE5). It is known that for different bacterial species, the type II CRIPSR system with components derived from the corresponding species has the function of cleaving the target DNA in cells. The experiments disclosed in this patent prove that *S. pyogenes* crRNA, tracrRNA and Cas9 are necessary and sufficient components of DNA cleavage complexes, and are widely applicable to other sequences, such as various different crRNA, tracrRNA and Cas9 sequences known in the prior art or recognizable according to the teachings of the prior art. Those skilled in the art will understand that, as in the case of most orthologous sequences in nature, using sequences derived from the same origin of species will provide more effective activity than using sequences from more distant species. Such understanding is confirmed in the Description of this patent, where the data in FIG. 5 of P1 (corresponding to FIG. 24 of this patent) support the following conclusion: if Cas9, crRNA and tracrRNA components derived from the same bacterial species, DNA cleavage is particularly effective, and when these sequences are not derived from the same species, the efficiency of DNA cleavage will decrease. In addition, with these information from the prior art and the teachings of this patent, those skilled in the art will exclude the combination of Cas9, crRNA and tracrRNA that cannot achieve DNA cleavage.

7) Firstly, the promoter is a DNA sequence with transcriptional initiation specificity and is not a necessary element for implementing the claimed methods. In fact, the components of the complex that cleaves the target DNA in this patent, i.e. Cas9 peptides and sgRNA, complexes of Cas9 and sgRNA, or mRNA encoding peptides and sgRNA, can be directly introduced (such as injection or other well-known methods in the art) to the position where DNA cleavage is required

(see paragraphs 0248 and 0303 of the Description of this patent).

Secondly, as for claims 21-56, which relate to the compositions of cleavage complexes, sgRNA or nucleic acids encoding components of cleavage complexes, those skilled in the art know how to prepare these products. For example, all components of cleavage complexes can be synthesized de novo without using any promoter.

However, if those skilled in the art want to express sgRNA with DNA vectors, the conventional promoters and common methods for achieving such expression are also well known in the prior art. Vectors suitable for driving RNA expression in eukaryotic cells are well-known. These vectors include vectors with U6 promoters and pSUPER vectors (H1 promoter), etc. Therefore, these vectors have been developed to allow the stable expression of small RNAs in the nucleus of eukaryotic cells and are easy for those skilled in the art to use.

In addition, the E-mail of Evidence 26 only reflects the inventor's analysis of the problems during the implementation of the system in eukaryotic cells, and reflects that the inventor has plans to solve these problems at all stages with conventional solutions. In the case that the functions of promoters, the types and specific performance of common promoters are relatively well known to those skilled in the art, those skilled in the art can select whether to use promoters according to specific implementation, and when selecting promoters, they will also refer to the known performance of existing promoters for selection. Therefore, the failure to define the promoters in claim 1 will not lead to that the claim cannot be supported by the Description. Those skilled in the art can determine whether the commonly used promoters can play a role during the process of specific use and select them through conventional technical means.

5.3 The petitioner believes that claims 2, 22, 40, 47 and 48 define the length of dsRNA duplexes to 8 bp to 30 bp. However, the Description of this patent only proves that dsRNA derived from *S. pyogenes* is able to achieve the cleavage of target DNA at specific positions of tracrRNA and crRNA with the necessary minimum length, and there is no evidence to prove that tracrRNA and crRNA derived from different sources can achieve the cleavage of target DNA within the dsRNA length of 8 bp to 30 bp at any position. Therefore, claims 2, 22, 40, 47 and 48 cannot be supported by the Description.

In this regard, the panel believes that, FIGS. 10, 12, 14, 23, 27, 28 and 31 of this patent show the results of DNA cleavage experiments, and all tested RNAs can guide DNA cleavage (although shorter forms cannot work "effectively") (see paragraph 0747 of the Description). Although the results of FIGS. 10, 12 and 23 are obtained by using two-molecule guide RNA, those skilled in the art will recognize that any disclosed two-molecule DNA-targeting RNA can be transformed into single-molecule DNA-targeting RNA by adding linkers. From FIGS. 14 and 27, it can be seen that the duplex length of chimeras A and B is 11 bp. The duplex length of sgRNA in FIG. 28 is 12 bp. The duplex length of guide RNA in FIGS. 10, 12 and 23 is 21 bp. As shown in FIG. 31, sgRNAs with RNA duplex lengths of 11, 15 and 21 were successfully used for cleavage. FIGS. 8 and 9 provide many examples of different crRNA and tracrRNA sequences derived from different species, which illustrates how they form dsRNA duplexes of different lengths. Due to the natural

existence of these sequences, those skilled in the art will understand that these sequences form functional CRISPR-Cas9 complexes in natural bacteria. For example, *N. meningitidis* in FIG. 8 forms a duplex of about 30 bp. FIG. 9 also discloses examples of shorter crRNA elements and shorter tracrRNA elements in the protein binding segments of single-molecule DNA-targeting RNA derived from different organisms of non-*S. pyogenes* (such as *L. innocua*), which can be extrapolated to other species. Those skilled in the art can select the crRNA and tracrRNA of dsRNA duplexes according to the prior art about the knowledge of type II CRISPR-Cas system. Based on the above specific examples and teachings on sgRNA in the Description, those skilled in the art can reasonably summarize the corresponding scope.

5.4 The petitioner believes that claims 3, 4, 23, 24, 41, 42 and 49 define the complementary percentage between dsRNA duplex nucleotides to be 70% or above; however, the Description of this patent only proves that dsRNA derived from *S. pyogenes* can achieve the cleavage of target DNA when having a specific length and the necessary minimum length (resulting in a specific complementary percentage).

In this regard, the panel believes that, the definition is a reasonable summary based on specific examples in this patent. For example, FIG. 9 shows the structure of single-molecule DNA-targeting RNA used in the examples (also see FIGS. 14, 27 and 28). The duplex is 11 bps and there is a 3 nt mismatch. Therefore, the complementarity between nucleotides of dsRNA duplexes is about 70%. Those skilled in the art can select crRNA and tracrRNA according to knowledge in the prior art about the type II CRISPR-Cas system, determine the length and complementarity of dsRNA duplexes, and determine positions where non-complementary regions can exist based on the influence of different regions. Therefore, based on the specific examples and teachings on sgRNA in the Description, those skilled in the art can reasonably summarize the complementary percentage of the two.

To sum up, the reason that this patent does not comply with the provision of Paragraph 4, Article 26 of the Chinese Patent Law proposed by the petitioner cannot be established.

6. Regarding Article 33 of the Chinese Patent Law

Article 33 of the Chinese Patent Law stipulates that the applicant may modify its patent application documents, but the modification of the application documents for invention and utility model patents shall not exceed the scope recorded in the original Description and Claims.

The scope of the original application document includes the information clearly recorded by those skilled in the art according to the original Description and Claims, as well as the technical information set that is objectively and accurately expressed in the application documents determined “directly and unambiguously” from the perspective of the applicant’s true meaning. In the process of determining the technical information set objectively and accurately expressed in the application documents, it should not be mechanically understood only literally, but should be comprehensively judged from the perspective of those skilled in the art. If the modified technical solution does not exceed the above-mentioned technical information set, the modification does not exceed the scope of the original Description and Claims.

The petitioner believes that, 1) the expression “protein binding segment” in claim 1 is beyond the scope, as neither the original Description nor the Claims of this patent records that sgRNA contains dsRNA duplexes of complementary nucleotides of tracrRNA and crRNA; paragraph 0190 of the Description records that crRNA and tracrRNA are “complementary to each other” and form a duplexes without any single-stranded region, that is, sgRNA only includes the duplex (the protein binding segment). 2) The original disclosure of this patent only records that the inserting nucleotides link the target RNA and the activator RNA, rather than the complementary nucleotide segments of tracrRNA and crRNA.

In this regard, the panel believes that,

1) Paragraph 0190 of the Description of this patent records that “The single-molecule DNA-targeting RNA of the present invention contains two segments of nucleotides (target-RNA and activator-RNA), and the two segments of nucleotides are complementary to each other, covalently linked by inserting nucleotides (“linkers” or “linker nucleotides”), and hybridized to form a double-stranded RNA duplex (dsRNA duplex) with the protein binding segment, thus generating a stem-loop structure.” Meanwhile, paragraphs 0192 and 0193 record “activator -RNA (tracrRNA)” and “target-RNA (crRNA)”; in other words, the activator RNA is tracrRNA, and the target RNA is crRNA. Therefore, the structure of the protein binding segment in claim 1 has been explicitly recorded in the original Description. As for the reason that the sgRNA only includes the duplex proposed by the petitioner, it is recorded in paragraph 0138 of the Description that RNA molecules that bind to site-specific modified peptides and target the peptides to specific positions within the target DNA are referred to as “DNA-targeting RNA” or “DNA-targeting RNA polynucleotides” (also referred to as “guide RNA” or “gRNA” herein). The DNA-targeting RNA of the present invention comprises two segments: a “DNA targeting segment” and a “protein binding segment”; paragraph 0139 records that the DNA targeting segment (or “DNA targeting sequence”) comprises nucleotide sequences complementary to a specific sequence in the target DNA (a complementary strand of the target DNA); paragraph 0144 records that the terms “DNA-targeting RNA” or “gRNA” are included, which refer to two-molecule DNA-targeting RNA and single-molecule DNA-targeting RNA (i.e. sgRNA); and paragraph 0190 records that sgRNA comprises two segments of nucleotides (target-RNA and activator-RNA), and the two segments of nucleotides are complementary to each other, covalently linked by inserting nucleotides (“linkers” or “linker nucleotides”), and hybridized to form a double-stranded RNA duplex (dsRNA duplex) with the protein binding segment, thus generating a stem-loop structure (FIG. 1B). FIG. 1B and FIG. 9 also provide graphical representations of sgRNA. It can be distinctly seen from the above description of sgRNA that sgRNA comprises both the protein binding segment and the DNA targeting segment, and the complementary nucleotides of crRNA and tracrRNA only form the dsRNA duplex with the protein binding segment. Those skilled in the art can reasonably understand that sgRNA comprises the dsRNA duplex and single-stranded nucleotide segments that form the DNA targeting segment.

2) As mentioned above, the activator RNA is tracrRNA, and the target RNA is crRNA.

Paragraph 0190, FIG. 1B and FIG. 9 of the Description distinctly show that the two nucleotide segments linked by the inserting nucleotides are the two complementary nucleotide fragments of crRNA and tracrRNA. It has also been proved in the examples (see FIGS. 14, 27, 28 and 31). Therefore, the reason that the modifications of claims 1-57 go beyond the scope proposed by the petitioner is not established.

7. Regarding Paragraph 2, Article 20 of the Rules for the Implementation of the Chinese Patent Law

Paragraph 2, Article 20 of the Rules for the Implementation of the Chinese Patent Law stipulates that independent claims shall reflect the technical solutions of the invention or utility model as a whole, and record the necessary technical features for solving technical problems.

In the absence of sufficient evidence to indicate that the lack of a certain technical feature not defined by the claims will inevitably lead to a failure of the corresponding technical solution achieving its invention purpose, it is usually difficult to directly determine that the corresponding technical feature is a necessary technical feature, and it will also be impossible to further draw the conclusion that the corresponding claim has the defect of lacking the necessary technical feature based on this.

7.1 The petitioner believes that independent claims 1, 21, 39, 45, 54 and 57 lack necessary technical features: the definition of target DNA lacks PAM, and the relationship of binding between PAM and target is not common general knowledge before the priority date of P1 (see Evidences 7, 11-13), but is determined by the inventor through long-term experiments and research, and it is necessary.

In this regard, the panel believes that,

7.1 As mentioned above, PAM is located on the target DNA to be cleaved, which belongs to the technical features of defining the object to be cleaved rather than the technical features of the cleavage tool, and it is common general knowledge in the art that PAM sequences must be present in the target DNA of type II CRISPR system. Those skilled in the art will select sequences with PAM as target sequences according to the prior art and relevant teachings of this patent when designing DNA targeting segment for guiding Cas9 to cleave the target DNA and implementing the specific cleavage methods. PAM sequence requirements in the target DNA targeted by the type II CRISPR system are not the technical contribution of this patent. Therefore, the reason that the independent claims 1, 21, 39, 45, 54 and 57 lack necessary technical features proposed by the petitioner is not established.

7.2 The petitioner believes that the Counter-Evidences submitted by the patentee are all natural immunology research, which is different from the field of gene editing involved in this patent, and there are differences in terms of research purposes, research means, research methods and the like between the two.

In this regard, the panel believes that, as mentioned above, the gene editing related to CRISPR-Cas9 is essentially derived from an immune defense mechanism in bacteria and archaea that responds to the invasion of extraneous DNA. The Counter-Evidences submitted by the

patentee also belong to the category of natural immunology research and are not unrelated to gene editing. The actual mechanism of action is to cleave exogenous nucleic acids, and CE8 also transforms the CRISPR-Cas9 locus of gram-positive *S. thermophilus* into gram-negative bacteria - *E. coli*, which is only distantly related (such bacteria usually do not have the type II system). Then, two plasmids were used to attack the transformed strain, which verified that CRISPR-Cas9 can also play a role in cleaving exogenous nucleic acids in *E. coli*, that is, the substantive mechanism is the same as gene editing in this patent. Therefore, although there are some differences between the two in terms of research purposes, research means, research methods and the like, it does not hinder those skilled in the art from proving that the CRISPR-Cas9 system can cleave the target DNA adjacent to PAM based on the Counter-Evidences.

7.3 The petitioner believes that according to the Description, in order to realize the cleavage of target DNA, in addition to the "DNA targeting segment" and the "protein binding segment", a third segment such as a stability control segment is also required. Therefore, claim 1 lacks the necessary technical features.

In this regard, the panel believes that, as for the stability control sequence, those skilled in the art can reasonably understand that the sequences is not necessary for achieving cleavage, but it is described in this patent as optional sequence that can be used for increasing the stability of RNA, such as enhancing efficiency. For example, paragraphs 0493-0494 of the Description provide a list of example sequences that can be used as the stability control sequence, and record "SEQ ID NO: 683-696". The first sequence listed, SEQ ID NO: 683, is AUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU, which is only the 3' region of tracrRNA in *S. pyogenes* depicted in FIG. 12C. Chimera A ends with the 48th G at the 3' terminal and does not include the stability control sequence, but chimera A is obviously functional. Therefore, the term stability control sequence is used as a broader term in this patent to describe sequences that can be included in DNA-targeting RNA (e.g., sgRNA, such as chimera A) to provide additional stability. Other examples of the stability control sequence are referred to as "transcription termination segment" in this patent. These other example sequences are not included as part of the DNA-targeting RNA, and these RNAs have been proved to be effective in Example 1 or Example 2. Obviously, the transcription termination segment is not necessary to perform the function. Therefore, although the stability control sequence mentioned in this patent may provide higher efficiency, it is not necessary to perform the cleavage function. Moreover, the petitioner has not provided any other evidence to prove that the third segment is essential for realizing the cleavage of target DNA. Therefore, the reason that claim 1 lacks necessary technical features proposed by the petitioner is not established.

On the basis of the above facts and reasons, the panel has made the following examination decision.

III. Decision

The patent No. 201380038920.6 remains valid.

If the party concerned is not satisfied with the decision, in accordance with the provision of

Paragraph 2, Article 46 of the Chinese Patent Law, the party concerned may, within three months from the date of receipt of the decision, institute legal proceedings in the Beijing Intellectual Property Court. According to the provision of this paragraph, after one party brings a lawsuit, the other party shall participate in the lawsuit as a third party.

Chairman of the Panel: Zou Kai

First Member: Wu Wenying

Second Member: Han Shiwei, Cui Yang, Shi Jing

Reexamination and Invalidation

(CHINA NATIONAL INTELLECTUAL PROPERTY ADMINISTRATION Seal for Patent reexamination and invalidation review)