In one aspect, the present disclosure refers to a method for detecting a target nucleic acid molecule, comprising the steps of: adding a guide RNA, a Cas protein, a nucleic acid probe and a buffer solution into a system comprising the target nucleic acid molecule, and then detecting cleavage of the nucleic acid probe, wherein the Cas protein is a Cas12 protein, the Cas12 protein has collateral single-stranded DNA cleavage activity and the nucleic acid probe is a single-stranded DNA, the guide RNA guides the Cas protein to specifically bind to the target nucleic acid molecule.

Preferably, the Cas protein is Cas12a or Cas protein having a collateral single-stranded DNA cleavage activity similar to that of the Cas12a.

Preferably, the Cas protein is Cas12a.

The Cas12a is preferably one of FnCas12a, AsCas12a, LbCas12a, Lb5Cas12a, HkCas12a, OsCas12a, TsCas12a, BbCas12a, BoCas12a, or Lb4Cas12a.

Preferably, the Cas12a is LbCas12a.

Preferably, the guide RNA refers to an RNA that guides the Cas protein to specifically bind to a target DNA.

In another preferred example, the nucleic acid probe is a single-stranded DNA; the single-stranded DNA is preferably a fluorescently-labeled single-stranded DNA; the single-stranded DNA is preferably a fluorescent probe that is labeled with a fluorescent group HEX at a 5’ terminal and labeled with a quenching group BHQ1 at a 3' terminal.

In another preferred example, the method for detecting the nucleic acid probe is preferably a fluorescence detection method; and the fluorescence detection method is preferably a detection method using a microplate reader or a fluorescence spectrophotometer.

Preferably, the target nucleic acid molecule to be detected in the reaction system of the target nucleic acid molecule to be detected is obtained after amplification.

Preferably, the detection method of the present disclosure can be used for detecting a pathogenic microorganism, gene mutation, or a specific target DNA.

In another preferred example, the Cas protein includes Cas12b (C2c1).

In a second example of the present disclosure, provided is use of a Cas protein in a method for detecting a target nucleic acid molecule, or in preparation of a formulation for detecting a target nucleic acid molecule.

In another preferred example, when a target DNA, a guide RNA and a Cas protein form a ternary complex, the complex will cleave other single-stranded DNA molecules in the system.

Preferably, the guide RNA refers to an RNA that guides the Cas protein to specifically bind to a target DNA.

In a third example of the present disclosure, provided is a kit including a guide RNA, a Cas protein, and a nucleic acid probe.

In another preferred example, the kit further includes a buffer solution.

In a fourth example of the present disclosure, provided is a detection system for detecting a target nucleic acid molecule, the system including:

(a) a Cas protein, which is Cas12a or a Cas protein having a collateral single-stranded DNA cleavage activity similar to that of the Cas12a;

(b) a guide RNA that guides the Cas protein to specifically bind to the target nucleic acid molecule; and

(c) a nucleic acid probe which is a single-stranded DNA;

wherein the target nucleic acid molecule is a target DNA.

In another aspect, the present disclosure refers to a detection system comprising:

(a) a Cas protein;

(b) a target nucleic acid molecule;

(c) a guide RNA that guides the Cas protein to specifically bind to the target nucleic acid molecule; and

(d) a nucleic acid probe ~~comprising~~ is a single-stranded DNA;

wherein the Cas protein is a Cas12 protein, the Cas12 protein has collateral single-stranded DNA cleavage activity.

In another preferred example, the detection system further includes (d) a buffer solution.

In another preferred example, the detection system further includes a target nucleic acid molecule to be detected.

In another preferred example, the concentration of the target nucleic acid molecule to be detected in the detection system is 1-100 copies/microliter or 10^{15} copies/microliter, preferably 1-10 copies/microliter, and more preferably 1-5 copies/microliter.

In another preferred example, in the detection system, the molar ratio of the nucleic acid probe to the target nucleic acid molecule is 10^3: 1 to 10^{14}: 1, and preferably 10^4: 1 to 10^7: 1.

In another preferred example, the detection site of the target nucleic acid molecule is located at positions 1-12 downstream of the PAM sequence of the guide RNA.

In another preferred example, the length of the guide RNA is 15-30 nt, and preferably 15-18 nt.

In another preferred example, the target DNA includes a cDNA.

In another preferred example, the target DNA is selected from a group consisting of a single-stranded DNA, a double-stranded DNA, or a combination thereof.

In another preferred example, the single-stranded DNA is a fluorescent-labeled and biotin-labeled single-stranded DNA.

In another preferred example, the single-stranded DNA is a fluorescent-labeled single-stranded DNA.

In another preferred example, the single-stranded DNA is a fluorescent probe that is labeled with a fluorescent group HEX at a 5’ terminal and labeled with a quenching group BHQ1 at a 3' terminal.

In a fifth example of the present disclosure, provided is a kit for detecting a target nucleic acid molecule, the kit including:

i) a first container and a Cas protein in the first container, the Cas protein being Cas12a or a Cas protein having a collateral single-stranded DNA cleavage activity similar to that of the Cas12a;

ii) an optional second container and a guide RNA in the second container, the guide RNA guiding the Cas protein to specifically bind to the target nucleic acid molecule;

iii) a third container and a nucleic acid probe in the third container; and

iv) an optional fourth container and a buffer solution located in the fourth container;

wherein the target nucleic acid molecule is a target DNA.

In another aspect, the present disclosure refers to a kit, comprising

i) a first container comprising a Cas protein, wherein the Cas protein has collateral single-stranded DNA cleavage activity;

ii) an optional second container comprising a guide RNA, wherein the guide RNA guides the Cas protein to specifically bind to a target nucleic acid molecule;

iii) a third container comprising a nucleic acid probe, the nucleic acid probe is a single-stranded DNA;

iv) an optional fourth container comprising a buffer;

wherein the Cas protein is a Cas12 protein.

In another preferred example, any two, three, or four (or all) of the first, second, third, and fourth containers may be the same or different containers.

In another preferred example, the nucleic acid probe carries a fluorescent group and a quenching group.

In a sixth example of the present disclosure, provided is a method for detecting whether a target nucleic acid molecule exists in a sample, including the following steps:

(a) providing the detection system for detecting a target nucleic acid molecule according to the fourth example of the present disclosure, wherein the detection system further has a sample to be detected; and

(b) detecting whether the nucleic acid probe in the detection system is cleaved by a Cas protein, wherein the cleavage is a trans-cleavage of a collateral single-stranded DNA;

P3 is a 3' segment sequence that is located at the 3' terminal and is complementary to the sequence of the target nucleic acid molecule.

In another preferred example, the PAM primer specifically binds to upstream or downstream of the target nucleic acid molecule.

In another preferred example, P1 has a length of 0-20 nt.

In another preferred example, P3 has a length of 5-20 nt.

In another preferred example, the PAM primer has a length of 18-50 nt, and preferably 20-35 nt.

In another preferred example, the complementation includes complete complementation and partial complementation.

In another preferred example, at least one primer containing the PAM sequence is used in the nucleic acid amplification.

In another preferred example, when the upstream and downstream (in a range of -20 nt to +20 nt, preferably in a range of -15 nt to +15 nt, and more preferably in a range of -10 nt to +10 nt) of the target site contain a PAM sequence, then a primer containing or not containing the PAM sequence can be used, and the amplified amplification product contains the PAM sequence.

In another preferred example, the detection in step (b) includes a fluorescence detection method.

In another preferred example, the fluorescence detection method uses a microplate reader or a fluorescence spectrophotometer for detection.

In a seventh example of the present disclosure, provided is use of a Cas protein in preparation of a detection reagent or kit for detecting a target nucleic acid molecule based on collateral single-stranded DNA cleavage, where the Cas protein is Cas12a or a Cas protein having a collateral single-stranded DNA cleavage activity similar to that of the Cas12a.

In another aspect, the present disclosure refers to use of a Cas protein in preparation of a detection reagent or a kit for detecting a target nucleic acid molecule, wherein the Cas protein is Cas12~~a or a Cas protein having a collateral single-stranded DNA cleavage activity similar to that of the Cas12a~~.

In another preferred example, the Cas12a is selected from a group consisting of FnCas12a, AsCas12a, LbCas12a, Lb5Cas12a, HkCas12a, OsCas12a, TsCas12a, BbCas12a, BoCas12a, Lb4Cas12a, or a combination thereof; and more preferably, the Cas12a is LbCas12a.

CLAIMS

1. A method for detecting a target nucleic acid molecule, comprising the steps of:

adding a guide RNA, a Cas protein, a nucleic acid probe and a buffer solution into a system comprising the target nucleic acid molecule, and then detecting cleavage of the nucleic acid probe, wherein the Cas protein is a Cas12 protein, the Cas12 protein has collateral single-stranded DNA cleavage activity and the nucleic acid probe is a single-stranded DNA, the guide RNA guides the Cas protein to specifically bind to the target nucleic acid molecule.

~~2. The method of claim 1, wherein the Cas protein has collateral single-stranded DNA cleavage activity and the nucleic acid probe comprises a single-stranded DNA.~~

~~3. The method of claim 1 or 2, wherein the guide RNA guides the Cas protein to specifically bind to the target nucleic acid molecule.~~

~~4~~2. The method of ~~any one of~~ claim~~s~~ 1, wherein the nucleic acid probe comprises a single-stranded DNA carrying a detectable label.

~~5~~3. The method of ~~any one of~~ claim~~s~~ 1 or ~~4~~2, wherein the target nucleic acid molecule is amplified.

~~6~~4. The method of any one of claims 1-~~5~~3, wherein the Cas protein is a type V-A enzyme in CRISPR system classification or a type V-B enzyme in CRISPR system classification~~Cas12 protein~~.

~~7~~5. The method of claim ~~6~~4, wherein the Cas12 protein is selected from the group consisting of FnCas12a, AsCas12a, LbCas12a, Lb5Cas12a, HkCas12a, OsCas12a, TsCas12a, BbCas12a, BoCas12a, Lb4Cas12a and Cas12b (i.e., C2c1).

~~8~~6. The method of any one of claim~~s~~ 1-~~7~~5, wherein the method for detecting the nucleic acid probe comprises a fluorescence detection method.

~~9~~7. The method of any one of claims 1-~~8~~6, wherein the nucleic acid probe comprises a fluorescent label.

~~10~~8. The method of any one of claims 1-~~8~~6, wherein the nucleic acid probe comprises a fluorescent group HEX at the 5' end and a quenching group BHQ1 at the 3' end.

9~~11~~. The method of any one of claims 1-~~10~~8, further comprising a step of amplifying the target nucleic acid molecule.

~~12~~10. The method of claim ~~11~~9, wherein the step of amplifying the target nucleic acid molecule comprises PCR amplification, LAMP amplification, RPA amplification, ligase chain reaction, branched DNA amplification, NASBA, SDA, transcription-mediated amplification, rolling circle amplification, HDA, SPIA, NEAR, TMA or SMAP2.

~~13~~11. The method of claim ~~11~~ 9 or ~~12~~10, wherein the target nucleic acid molecule is amplified using primers, wherein at least one primer comprises a PAM sequence.

~~14~~12. The method of any one of claims 1-~~13~~11, wherein the target nucleic acid molecule comprises a sequence found only in a pathogen.

~~15~~13. The method of any one of claims 1-~~14~~12, wherein the target nucleic acid molecule comprises a single nucleotide polymorphism (SNP) site in human or other species.

~~16~~14. The method of any one of claims 1-~~15~~13, wherein the target nucleic acid molecule comprises a gene mutation.

~~17~~15. The method of any one of claims 1-~~16~~14, wherein the guide RNA comprises a crRNA of 15 nt, 16 nt or 17 nt in length.

~~18~~16. A detection system comprising:

(a) a Cas protein;

(b) a target nucleic acid molecule;

(c) a guide RNA that guides the Cas protein to specifically bind to the target nucleic acid molecule; and

(d) a nucleic acid probe ~~comprising~~ is a single-stranded DNA;

wherein the Cas protein is a Cas12 protein, the Cas12 protein has collateral single-stranded DNA cleavage activity.

~~19. The detection system according to claim 18, wherein the Cas protein has collateral single-stranded DNA cleavage activity.~~

~~20~~17. The detection system according to claim ~~18~~ 16~~or 19~~, wherein the nucleic acid probe comprises a detectable label.

~~21~~18. The detection system according to any one of claims ~~18~~16-17~~20~~, wherein the Cas protein is a type V-A enzyme in CRISPR system classification or a type V-B enzyme in CRISPR system classification ~~a Cas12 protein~~.

~~22~~19. The detection system according to claim ~~21~~18, wherein the Cas12 protein is selected from the group consisting of FnCas12a, AsCas12a, LbCas12a, Lb5Cas12a, HkCas12a, OsCas12a, TsCas12a, BbCas12a, BoCas12a, Lb4Cas12a and Cas12b (i.e., C2c1).

~~23~~20. The detection system according to claim ~~21~~18, wherein the Cas12 protein is LbCas12a.

~~24~~21. The detection system according to any one of claims ~~18-23~~16-20, wherein the nucleic acid probe comprises a fluorescent label.

~~25~~22. The detection system according to any one of claims ~~18-23~~16-20, wherein the nucleic acid probe comprises a fluorescent group HEX at the 5' end and a quenching group BHQ1 at the 3' end.

~~26~~23. The detection system according to any one of claims ~~18-25~~16-22, wherein the target nucleic acid molecule is a target DNA.

~~27~~24. The detection system according to claim ~~26~~ 23, wherein the target nucleic acid molecule is amplified.

~~28~~25. The detection system according to claim ~~27~~24, wherein the target nucleic acid molecule is amplified by a method selected from the group consisting of: PCR amplification, LAMP amplification, RPA amplification, ligase chain reaction, branched DNA amplification, NASBA, SDA, transcription-mediated amplification, rolling circle amplification, HDA, SPIA, NEAR, TMA and SMAP2.

~~29~~26. The detection system according to claim ~~27 or 28~~24 or 25, wherein the target nucleic acid molecule is amplified using primers, and wherein at least one primer comprises PAM sequence.

~~30~~27. The detection system according to any one of claims ~~18 29~~ 16-26, wherein the target nucleic acid molecule comprises a PAM sequence upstream or downstream of a binding site of the guide RNA.

~~31~~28. The detection system according to claim ~~30~~27, wherein the PAM sequence is -20nt to +20nt upstream or downstream of ~~the~~ a target site of the guide RNA.

~~32~~29. The detection system according to any one of claims ~~18 31~~16-28, wherein the target nucleic acid molecule comprises a sequence found only in a pathogen.

~~33~~30. The detection system according to any one of claims 16-28~~18 31~~, wherein the target nucleic acid molecule comprises a single nucleotide polymorphism (SNP) site in human or other species.

~~34~~31. The detection system according to any one of claims ~~18 31~~16-28, wherein the target nucleic acid molecule comprises a gene mutation.

~~35~~32. The detection system according to any one of claims ~~18 34~~16-31, wherein the guide RNA comprises a crRNA of 15 nt, 16 nt or 17 nt in length.

~~36~~33. The detection system according to any one of claims ~~18 35~~16-32, further comprising a buffer.

~~37~~34. A kit, comprising

i) a first container comprising a Cas protein, wherein the Cas protein has collateral single-stranded DNA cleavage activity;

ii) an optional second container comprising a guide RNA, wherein the guide RNA guides the Cas protein to specifically bind to a target nucleic acid molecule;

iii) a third container comprising a nucleic acid probe, the nucleic acid probe is a single-stranded DNA;

iv) an optional fourth container comprising a buffer;

wherein the Cas protein is a Cas12 protein.

~~38~~35. The kit according to claim ~~37~~34, wherein the Cas protein is a type V-A enzyme in CRISPR system classification or a type V-B enzyme in CRISPR system classification ~~a Cas12 protein~~.

~~39~~36. The kit according to claim ~~38~~35, wherein the Cas12 protein is selected from the group consisting of FnCas12a, AsCas12a, LbCas12a, Lb5Cas12a, HkCas12a, OsCas12a, TsCas12a, BbCas12a, BoCas12a, Lb4Cas12a and Cas12b (i.e., C2c1).

~~40~~37. The kit according to claim ~~38~~35, wherein the Cas12 protein is LbCas12a.

~~41~~38. The kit according to any one of claims ~~38-40~~35-37, wherein the nucleic acid probe comprises a fluorescent label.

~~42~~39. The kit according to any one of claims ~~38-40~~35-37, wherein the nucleic acid probe comprises a fluorescent group HEX at the 5' end and a quenching group BHQ1 at the 3' end.

~~43~~40. The kit according to any one of claims ~~38-42~~35-39, wherein the target nucleic acid molecule is a target DNA.

~~44~~41. The kit according to any one of claims ~~38-42~~ 35-39, wherein the nucleic acid probe comprises a single-stranded DNA.

~~45~~42. The kit according to any one of claims ~~38-44~~35-41, wherein the guide RNA comprises a crRNA of 15 nt, 16 nt or 17 nt in length.

~~46~~43. Use of a Cas protein in preparation of a detection reagent or a kit for detecting a target nucleic acid molecule, wherein the Cas protein is Cas12~~a or a Cas protein having a collateral single-stranded DNA cleavage activity similar to that of the Cas12a~~.

~~47. The use according to claim 46, wherein the Cas protein is a Cas12 protein.~~

4844. The use according to claim 4743, wherein the Cas12 protein is selected from the group consisting of FnCas12a, AsCas12a, LbCas12a, Lb5Cas12a, HkCas12a, OsCas12a, TsCas12a, BbCas12a, BoCas12a, Lb4Cas12a and Cas12b (i.e., C2c1).

4945. The use according to claim 4844, wherein the Cas12 protein is LbCas12a.

5046. The use according to any one of claims 46-4944-46, wherein the target nucleic acid molecule comprises a sequence found only in a pathogen.

5147. The use according to any one of claims 46-4943-45, wherein the target nucleic acid molecule comprises a single nucleotide polymorphism (SNP) site in human or other species.

5248. The use according to any one of claims 46-4943-45, wherein the target nucleic acid molecule comprises a gene mutation.