

Claims

We claim:

1. A detection method comprising steps of:
contacting a CRISPR-Cas complex comprising:
a Cas protein with collateral cleavage activity that is thermostable at temperatures above at least 50 °C; and
a guide RNA selected or engineered to be complementary to a target nucleic acid sequence; with a sample potentially comprising a target nucleic acid sequence.
2. The method of claim 1, wherein the step of contacting comprises contacting the CRISPR-Cas complex and sample with a reporter susceptible to cleavage by the Cas protein collateral activity.
3. The method of claim 1 or claim 2, wherein the step of contacting comprises incubating for a period of time above the temperature.
4. The method of any one of the preceding claims, further comprising a step of amplifying nucleic acid present in the sample.
5. The method of claim 4, wherein the step of amplifying utilizes a thermostable nucleic acid polymerase.
6. The method of claim 4 or claim 5, wherein the steps of amplifying and contacting are performed in a single vessel.
7. The method of claim 1, wherein the Cas protein is a Cas12 protein.
8. The method of claim 7, wherein the Cas protein has an amino acid sequence having at least 80% sequence identity to any one of SEQ ID Nos. 1-10.
9. In a method of performing a detection assay utilizing a Cas protein with collateral cleavage activity, the improvement that comprises utilizing a Cas protein with thermostable collateral cleavage activity.

10. The improvement of claim 9, wherein the Cas protein is a Cas12 protein.
11. The improvement of claim 10, wherein the Cas protein has an amino acid sequence having at least 80% sequence identity to any one of SEQ ID Nos. 1-10.
12. The improvement of claim 9, wherein a method of performing a detection assay is conducted in a single reaction vessel.
13. The improvement of claim 9, wherein the thermostable collateral cleavage activity is thermostable above a temperature of about 50°C.
14. A non-naturally occurring or engineered composition comprising:
 - (a) a Cas protein with collateral cleavage activity that is thermostable at temperatures above at least 50°C; and
 - (b) at least one guide capable of forming a complex with the Thermostable Cas Protein and directing the complex to bind to a target nucleic acid sequence.
15. The composition of claim 14, wherein the Cas protein has an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NOs: 1-10.
16. The composition of claim 14, wherein the at least one guide comprises:
 - (i) two guide sequences capable of hybridizing to two different target nucleic acid sequences or different regions of a target nucleic acid sequence; or
 - (ii) a plurality of guide sequences capable of hybridizing to a plurality of different target nucleic acid sequences or a plurality of different regions of a target nucleic acid sequence.
17. A non-naturally occurring or engineered composition comprising:
 - (a) a polynucleotide encoding a Cas protein with collateral cleavage activity that is thermostable at temperatures above at least 50°C; and
 - (b) at least one guide capable of forming of complex with the Cas protein and directing the complex to bind to a target nucleic acid sequence.

18. The composition of claim 17, wherein the Cas protein has an amino acid sequence having at least 80%, sequence identity to any one of SEQ ID NOs: 1-10.

19. The composition of claim 17, wherein the at least one guide comprises:

(i) two guide sequences capable of hybridizing to two different target nucleic acid sequences or different regions of a target nucleic acid sequence; or

(ii) a plurality of guide sequences capable of hybridizing to a plurality of different target nucleic acid sequences or a plurality of different regions of a target nucleic acid sequence.

20. A non-naturally occurring or engineered composition for modifying nucleotides in a target nucleic acid comprising a Cas protein with collateral cleavage activity that is thermostable at temperatures above at least 50°C.

21. The composition of claim 20, further comprising at least one guide sequence capable of forming of complex with the Cas protein and directing the complex to bind to a target nucleic acid sequence

22. The composition of claim 20, wherein the Cas protein has an amino acid sequence having at least 80%, sequence identity to any one of SEQ ID NOs: 1-10.

23. The composition of claim 20, wherein the Cas protein has been modified to reduce off-target effects.

24. The composition of claim 20, wherein modification of the nucleotides in the target nucleic acid:

(i) remedies a disease caused by a point mutation;

(ii) inactivates a gene encoded by the target nucleic acid sequence;

(iii) modifies a gene product encoded by the target nucleic acid sequence; or

(iv) modifies expression level of a gene product encoded by the target nucleic acid sequence

25. The composition of claim 21, wherein the at least one guide comprises:

(i) two guide sequences capable of hybridizing to two different target nucleic acid sequences or different regions of a target nucleic acid sequence; or

(ii) a plurality of guide sequences capable of hybridizing to a plurality of different target nucleic acid sequences or a plurality of different regions of a target nucleic acid sequence.

26. A vector system that comprises one or more vectors comprising:

(a) a first regulatory element operably linked to a nucleotide sequence encoding a Cas protein with collateral cleavage activity that is thermostable at temperatures above at least 50°C; and

(b) a second regulatory element operably linked to a nucleotide sequence encoding a guide.

27. The vector system of claim 26, wherein the nucleotide sequence encodes a Cas protein with an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NOs: 1-10.

28. The vector system of claim 26, wherein the nucleotide sequence encoding a Cas protein is codon optimized.

29. The vector system of claim 26, wherein (a) and (b) are comprised in a single vector.

30. The vector system of claim 26, wherein (a) and (b) are comprised in separate vectors.

31. The vector system of claim 26, wherein the vector system comprises a viral vector.

32. A method of cleaving at least one target nucleic acid in a cell comprising contacting a cell with a Cas protein with collateral cleavage activity that is thermostable at temperatures above at least 50°C and at least one guide capable of hybridizing to the at least one target nucleic acid,

wherein the Cas protein is capable of forming a complex with the at least one guide and causing a break in the at least one target nucleic acid.

33. A method of altering expression of at least one target nucleic acid in a cell comprising contacting the cell with a Cas protein with collateral cleavage activity that is thermostable at temperatures above at least 50°C and at least one guide capable of hybridizing to the at least one target nucleic acid, wherein the Cas protein is capable of forming a complex with the at least one guide and causing a break in the at least one target nucleic acid.

34. A method of altering expression of at least one target nucleic acid in a cell comprising contacting the cell with a Cas protein with collateral cleavage activity that is thermostable at temperatures above at least 50°C and at least one guide capable of hybridizing to the at least one target nucleic acid, wherein the Cas protein is capable of forming a complex with the at least one guide and editing the at least one target nucleic acid sequence.

35. A method of modifying at least one target nucleic acid in a cell comprising contacting a cell with a Cas protein with collateral cleavage activity that is thermostable at temperatures above at least 50°C and at least one guide capable of hybridizing to the at least one target nucleic acid, wherein the Cas protein is capable of forming a complex with the at least one guide and editing the at least one target nucleic acid sequence.

36. The method of claim 35, wherein editing the target nucleic acid comprises insertion of a payload nucleic acid at the target nucleic acid sequence.

37. The method of any one of claims 32-36, wherein a nucleotide sequence encodes a Cas protein with an amino acid sequence having at least 80%, sequence identity to any one of SEQ ID NOs: 1-10.

38. The method of any one of claims 32-36, wherein the at least one guide comprises:

(i) two guide sequences capable of hybridizing to two different target nucleic acid sequences or different regions of a target nucleic acid;

(ii) a plurality of different target nucleic acid sequences or a plurality of different regions of a target nucleic acid.

39. A nucleic acid encoding a Cas protein with collateral cleavage activity that is thermostable at temperatures above at least 50°C.

40. The nucleic acid of claim 39, wherein the nucleotide sequence encodes a Cas protein with an amino acid sequence having at least 80% sequence identity to any one of SEQ ID NOs: 1-10.

41. Use of a Cas protein with collateral cleavage activity that is thermostable at temperatures above at least 50°C and at least one guide capable of hybridizing to a target nucleic acid in the manufacture of a medicament for diagnosing or treating a nucleic acid based disease or disorder in a subject.

42. The use of claim 41, wherein the nucleic acid based disease or disorder is a genetic disorder.

43. The use of claim 41, wherein the nucleic acid based disease or disorder is cancer.

44. The use of claim 41, wherein the nucleic acid based disease or disorder is an infectious disease.

45. The use of any one of claims 41-44, wherein the nucleotide sequence encodes a Cas protein with an amino acid sequence having at least 80%, sequence identity to any one of SEQ ID NOs: 1-10.

46. The use of any one of claim 41-45, wherein the at least one guide comprises:

(i) two guide sequences capable of hybridizing to two different target nucleic acid sequences or different regions of a target nucleic acid;

(ii) a plurality of guide sequences capable of hybridizing to a plurality of different target nucleic acid sequences or a plurality of different regions of a target nucleic acid.

47. The use of any one of claims 41-46, wherein the Cas protein is capable of:

(i) forming a complex with the guide and causing a break in the target nucleic acid; or

(ii) forming a complex with the guide and editing the target nucleic acid sequence.

48. The composition of any one of claims 1-25, wherein the Cas protein is associated with a modifying entity.
49. The composition of claim 48, wherein the modifying entity is an adenosine deaminase.
50. The composition of claim 48, wherein the modifying entity is a cytidine deaminase.
51. A pharmaceutical composition of any one of claims 1-25.
52. A method of characterizing a Cas protein comprising assessing one or more of:
 - (a) cis cleavage activity;
 - (b) trans cleavage activity;
 - (c) sensitivity;
 - (d) preference for RNA or DNA target nucleic acid;
 - (e) preference for RNA or DNA non-target nucleic acid; and
 - (f) enzyme stability.