

## 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 60-65 °C; and

a guide RNA selected or engineered to be complementary to a target sequence; with

a sample potentially comprising a nucleic acid of the target 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 that is at least 80% identical to that of SEQ ID NO: 15.
9. 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. 3-21, 33-47, 51-56, 68-178, and 274-283.
10. The method of claim 1, wherein the Cas protein has an amino acid sequence having 80%, sequence identity to any one of SEQ ID Nos. 1-283.
11. 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.

12. The improvement of claim 11, wherein the Cas protein is a Cas12 protein.

13. The improvement of claim 12, wherein the Cas protein has an amino acid sequence that is at least 80% identical to that of SEQ ID NO: 15.

14. The improvement of claim 12, wherein the Cas protein has an amino acid sequence having at least 80%, sequence identity to any one of SEQ ID Nos. 3-21, 33-47, 51-56, 68-178, and 274-283.

15. The improvement of claim 11, wherein a method of performing a detection assay is conducted in a single reaction vessel.

16. The improvement of claim 11, wherein the thermostable collateral cleavage activity is thermostable above a temperature of about 60°C.

17. The improvement of claim 11, wherein the thermostable collateral cleavage activity is thermostable above a temperature of about 65°C.

18. The improvement of claim 11, wherein the Cas protein has an amino acid sequence having at least 80% sequence identity to any one of SEQ ID Nos. 1-283.