

Claims

We claim:

1. A detection method comprising steps of:
 - (a) contacting a CRISPR-Cas complex comprising:
 - a Cas protein with collateral cleavage activity that is thermostable at temperatures above at least 52 °C, wherein the Cas protein has an amino acid sequence of any one of SEQ ID NOs: 15, 1, 11, 18, 37, 48, 73, 74, 76, 83, 84, 90, 92, 95, 99, 101, 141, 274, or 275; and
 - a guide RNA selected or engineered to be complementary to a target sequence; with
 - (i) a sample potentially comprising a nucleic acid of the target sequence; and
 - (ii) a reporter susceptible to cleavage by the Cas protein collateral activity, wherein the step of contacting comprises incubating for a period of time above at least 52°C whereby said Cas protein exhibits the collateral cleavage activity that is thermostable in the presence of said nucleic acid of the target sequence; and
 - (b) amplifying the nucleic acid of the target sequence present in the sample, wherein the steps of amplifying and contacting are performed in a single vessel.
2. The method of claim 1, wherein the step of amplifying utilizes a thermostable nucleic acid polymerase.
3. The method of claim 1, wherein the thermostable collateral cleavage activity is thermostable above a temperature of about 60°C.
4. The method of claim 1, wherein the thermostable collateral cleavage activity is thermostable above a temperature of about 65°C.

5. The method of claim 1, wherein the reporter is a detectably labeled nucleic acid reporter.
6. An engineered composition for use in the detection method of claim 1, comprising:
 - (a) a Cas protein having collateral cleavage activity that is thermostable at temperatures above at least 52°C, wherein the Cas protein has an amino acid sequence of any one of SEQ ID NOs: 15, 1, 11, 18, 37, 48, 73, 74, 76, 83, 84, 90, 92, 95, 99, 101, 141, 274, or 275;
 - (b) at least one guide capable of forming a complex with the thermostable Cas protein; and
 - (c) a reporter susceptible to cleavage by the Cas protein collateral activity.
7. The engineered composition of claim 6, wherein the thermostable collateral cleavage activity is thermostable above a temperature of about 60°C.
8. The engineered composition of claim 6, wherein the thermostable collateral cleavage activity is thermostable above a temperature of about 65°C.
9. The engineered composition of claim 6, wherein the reporter is a detectably labeled nucleic acid reporter.
10. The engineered composition of claim 6, wherein the guide comprises or is guide RNA.