Sensing platform for nucleic acid detection based on 2-Aminopurine probe sheared by trans-cleavage activity of CRISPR/Cas12a system

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S1: Detection of goat pox virus

The method which combines PCR and 2-AP probe-mediated CRISPR/Cas12a system was used to detect GTPV samples to verify its practical application potential. The detailed preparation process of the mock samples was as follows: 30 parts of healthy bovine blood were taken, and 400 μL of EDTA was added to each part of bovine blood. Then, different concentrations of GTPV nucleic acid were added to EDTA anticoagulation (Among the 30 samples, there were 12 strong positive samples, 9 weak positive samples, and 9 negative samples). After mixing well, the nucleic acids in these mixtures were extracted with a Trace RNA/DNA Extraction Kit. Simultaneously, these samples were first tested by real-time PCR, and then they were detected by the method we build. The real-time PCR reaction system was: 12.5 μL 2×Probe qPCR Mix (with UNG), 0.5 μL real-time PCR probe (10 μM), 1 μL real-time PCR forward primer (10 μM), 1 μL real-time PCR reverse primer (10 μM), 5 μL of nucleic acid extracts from different clinical samples. The reaction process of real-time PCR was as follows: 25 °C for 10 min; 95 °C for 2 min; 95 °C for 10 s, 60 °C for 30 s, 40 cycles.

Table 1S All nucleic acids used in this article.

Projects	**Sequence (5'→3')**
Poly adenine probe (A-pro)	AAAAAAAAA/i2-Amp/AAAAAA
Poly guanine probe (G-pro)	GGGGGGGGG/i2-Amp/GGGGGG
Poly cytosine probe (C-pro)	CCCCCCCCC/i2-Amp/CCCCCC
Poly thymine probe (T-pro/T-pro 1)	TTTTTTTTT/i2-Amp/TTTTTT
Poly thymine probe (T-pro 2)	TTTTTT/i2-Amp/TTT/i2-Amp/TTTTTT
Poly thymine probe (T-pro 3)	TTTTTT/i2-Amp/TTT/i2-Amp/TTT/i2-Amp/TTT
Poly thymine probe (T-pro 4)	TTT/i2-Amp/TTT/i2-Amp/TTT/i2-Amp/TTT/i2-Amp/TTT
ssDNA	CATCTTGTAAAATGAACGGGAAGTAC
crRNA for ssDNA	UAAUUUCUACUAAGUGUAGAUUUCCCG UUCAUUUUACAAGAUG
Forward primer for RPA/PCR	ACTACCTAGAGTACTATCAGGAAAGACG GTATCAA
Reverse primer for RPA/PCR	AGACAGCCCGATATGCATTAGCTTGGAT GCTGGTC
crRNA for GTPV detection	UAAUUUCUACUAAGUGUAGAUUGAAGU GUUAGAAAGUGUAAACUC
ssDNA-FQ	HEX-TATTATT-BHQ1
Forward primer for dPCR/real-time PCR	TGAATTAGTGTTGTTTCTTC
Reversed primer for dPCR/real-time PCR	GGGAATCCTCAAGATAGTTCG
Probe for dPCR/real-time PCR	6-FMA/TGCCGCAAAATGTCGA/MGB

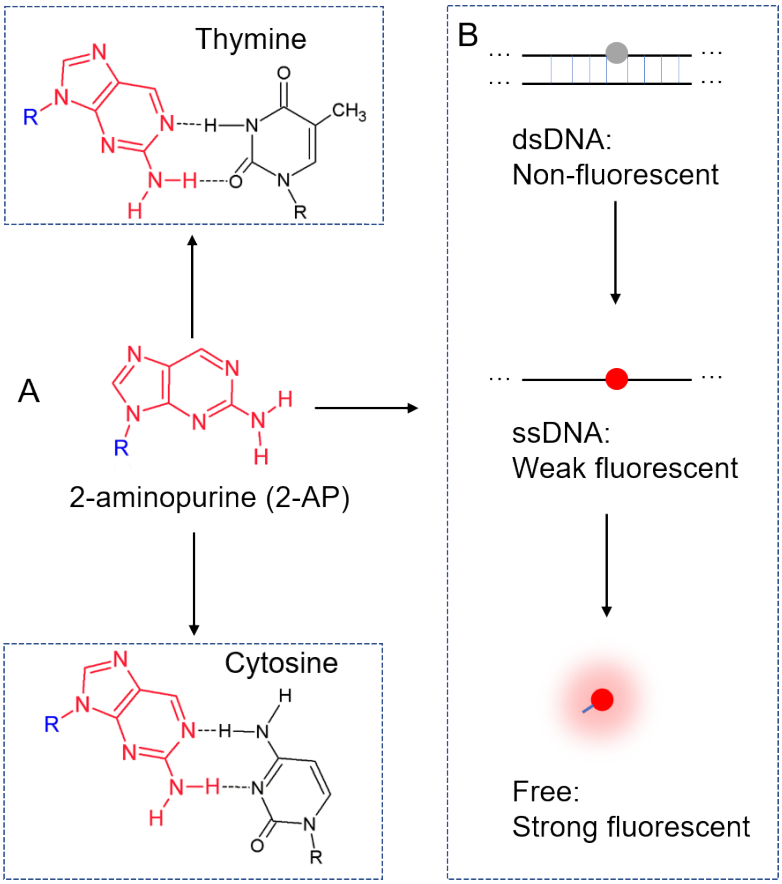

Figure S1 (A) Binding of 2-AP to thymine or cytosine; (B) Fluorescence of 2-AP in different micro-environments.

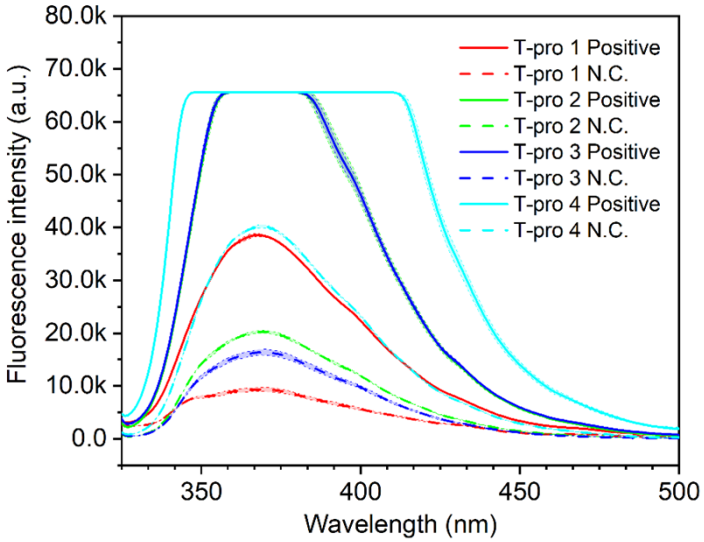

Figure S2 Signal changes of T-pro 1, T-pro 2, T-pro 2, and T-pro 4 in the activated CRISPR/Cas12a system when their final concentrations were controlled at 10 μM.

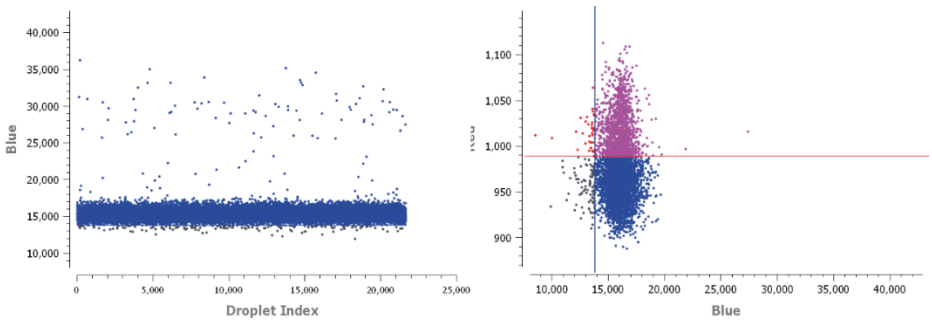

Figure S3 Detection of GTPV by dPCR.

Figure S4 The time optimization for the cleavage of 2-AP probe.