Supplementary Material

CRISPR/Cas12a-mediated ultrasensitive and on-site monkeypox viral testing

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Expression and purification of cas12a

The pMBP-LbCas12a was a gift from Jennifer Doudna (Addgene plasmid # 113431; http://n2t.net/addgene:113431; RRID: Addgene_113431). The plasmid pMBP-LbCas12a was transformed into *Escherichia coli* (*E. coli*) Rosetta (DE3) by heat shock. Totally 1 L aliquot of liquid Terrific-Broth medium with 100 μg/mL ampicillin was inoculated with 10 mL overnight cultures containing activated bacteria in the Luria-Bertani medium for the expansion of cultivation. Growth medium plus inoculant was grown at 220 rpm and 37 ℃ for 2 h and then at 200 rpm and 20 ℃ for another 20 min until the cell density at 600 nm (OD_{600}) reached 0.6. Then, 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added and the cells were cultured at 20 °C for 18 h. Cells were harvested by centrifugation, resuspended in lysis buffer (50 mM Tris-HCl, 500 mM NaCl, 0.25 mg/mL lysozyme, pH 8.0), and lysed by sonication. Protein purification included separation via high-affinity Ni-NTA resin (Genescript, China), MBP-digestion via TEV protease (Beyotime, China), and final acquisition via Dextrin Beads 6FF (Smart-Lifesciences, China). The purified LbCas12a was quantified by BCA protein determination reagent and stored at -80 °C.

Sensitivity evaluation of PCR-Cas12a-MPXV fluorescence assay

The PCR reaction system was carried out in a 10 μL solution containing 400 nM forward and reverse primers (F2 and R2), 1 × Taq Master Mix, 1 μL serial 10-fold dilution (10^{10-0} copies/μL) of DNA templates, and ultrapure water. The temperature procedure of PCR was 95 °C for 3 min, 30 circles at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min, and final extension at 72 °C for 5 min. Then, the PCR-Cas12a-MPXV

fluorescence assay was executed using the same condition as the RAA-Cas12a-MPXV fluorescence assay, except for PCR products in place of RAA products.

Agarose gel electrophoresis

For agarose gel electrophoresis, all of the reaction samples were incubated the same as the RAA-Cas12a-MPXV fluorescence assay procedures, and a 5 μL aliquot of the reaction solution was subsequently mixed with 1 μL of 6 × loading buffer (Sangon Biotech, China) containing 1 % GelRed (Biosharp, China). Then, agarose gel electrophoresis was performed with 3 % agarose gel at a constant voltage of 150 V for 30 min using 1 × TAE (40 mmol/L Tris-Acetate; 1 mmol/L EDTA; pH 8.0) as the running buffer, followed by imaging with an automatic digital gelatin image analysis system (Tanon, China).

Table S1. DNA sequences used in this study.

Name	Sequence (5'→3') *
F1.1	CAGCTCCAACGATACTCCTCCT
F1.2	TCTACGACAATGGATGCTGATACACGGC
F2	TACAGCTCCAACGATACTCCTCC
R1	GACAGGGTTAACACCTTTCCAATAAAT
R2	TTCCGTCAATGTCTACACAGGCA
crRNA1	UAAUUUCUACUAAGUGUAGAUA*CAGUACUCAUUAAUAACG*
crRNA2	UAAUUUCUACUAAGUGUAGAUA*UGAUGUUAUUCCGGUUAA*
crRNA3	UAAUUUCUACUAAGUGUAGAUA*CCGGAAUAACAUCAUCAA*
FQ reporter	FAM-TTATTT-BHQ1
FB reporter	FAM-TTATTT-biotin

* The italicized sequences in crRNAs are complementary to partial sequences of the target.

Table S2. Cost analysis of the RAA-Cas12a-MPXV assay.

Component	Amount	Cost ($)	Number of uses	Cost/ reaction ($)
RAA nucleic acid amplification kit	48 tubes	204.5	240	1
Cas12a (Self-expression)	1 nmol	7.3	1000	0.0073
crRNA	1 OD	35	2630	0.0133
F-Q reporter	1 OD	52.2	595	0.0877
F-B reporter	1 OD	31.7	658	0.0481
Lateral flow strip	40 tests	43.8	40	1.095
Total cost of RAA-Cas12a-MPXV fluorescence assay per reactions ($)				**1.1083**
Total cost of RAA-Cas12a-MPXV lateral flow strip assay per reaction ($)				**2.1637**

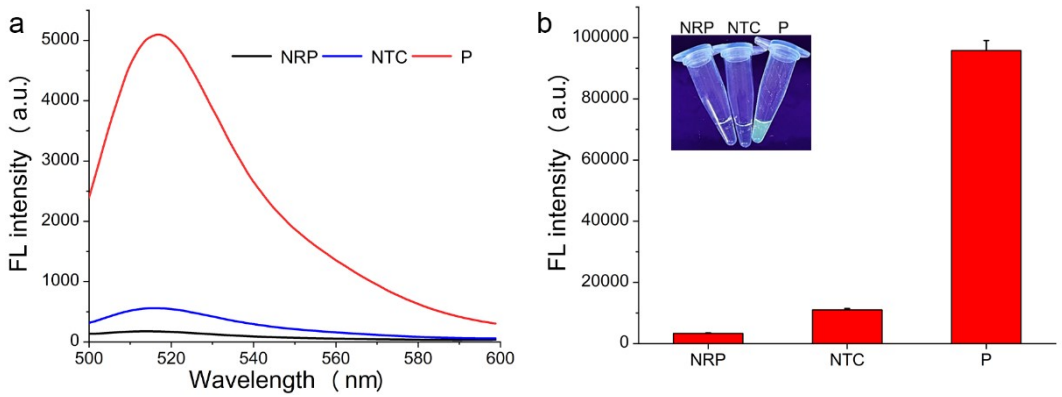

Fig. S1 Verification of RAA-Cas12a-MPXV testing strategy. (**a**) Fluorescence spectra of feasibility verification. (**b**) Fluorescence intensity of feasibility verification. The inset was photographed by a smartphone under UV light. RAA was carried out under 42 °C for 30 min using F2 and R2, then 1 μL RAA product was added to 20 μL CRISPR/Cas12a reaction under 37 °C for 40 min (50 nM Cas12a, 50 nM crRNA, 1 μM FQ reporter, 1 × Cas Buffer). NRP, no RAA product; NTC, no template control; P, positive. Error bars represent the standard deviation from three independent experiments.

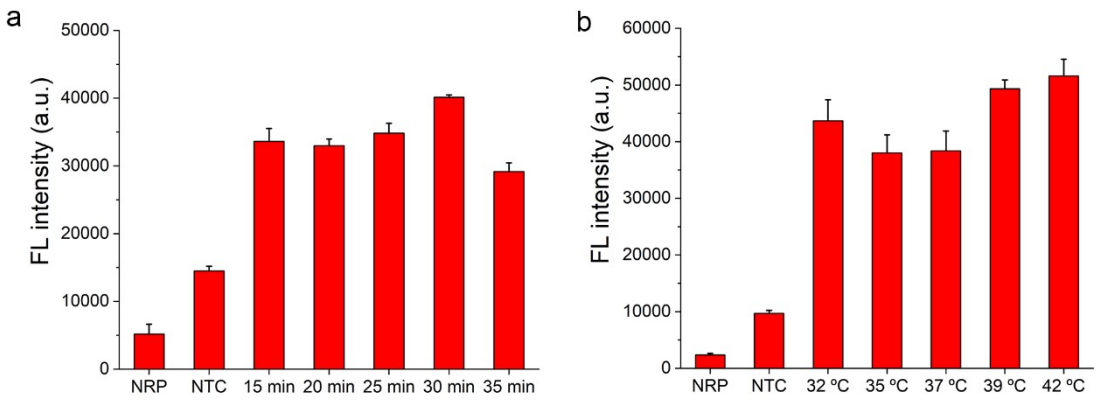

Fig. S2 Optimization of RAA reaction conditions. (**a**) Fluorescence intensity of RAA-Cas12a-MPXV assay using RAA amplicons under different reaction times (15 min, 20 min, 25 min, 30 min, 35 min). (**b**) Fluorescence intensity of RAA-Cas12a-MPXV assay using RAA amplicons under different reaction temperatures (42 ℃, 39 ℃, 37 ℃, 35 ℃, 32 ℃). NRP, no RAA product. NTC, no template control. Error bars represent the standard deviation from three independent experiments.

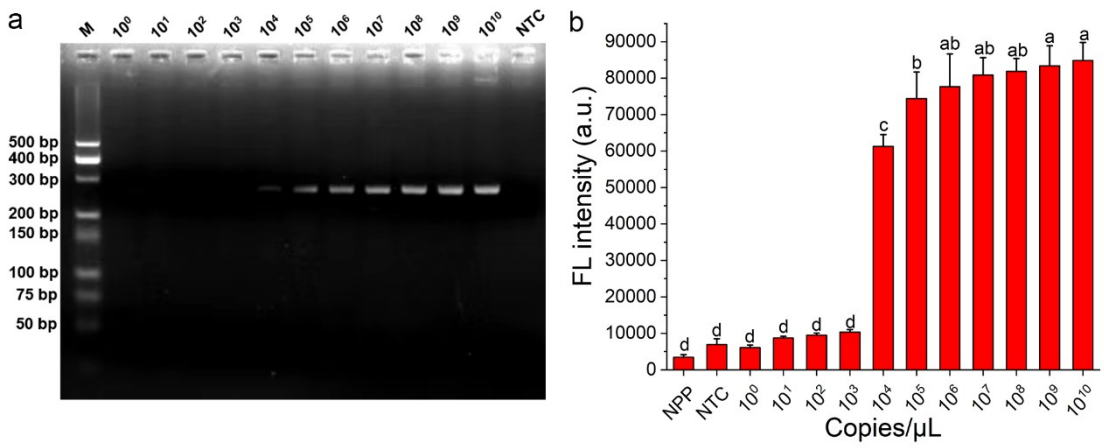

Fig. S3 The evaluation of PCR sensitivity. (**a**) The agarose gel image of PCR products using serial 10-fold dilution (10^{10-0} copies/μL) of DNA templates. M, DNA marker. (**b**) Fluorescence intensity of CRISPR/Cas12a assay with PCR products using serial 10-fold dilution (10^{10-0} copies/μL) of DNA templates. NPP, no PCR product. NTC, no template control. Significance analysis results are labeled with different letters above the error bars ($p < 0.05$). Error bars represent the standard deviation from three independent experiments.