Supplementary Information

A specific and low background nucleic acids sensing strategy based on rolling circle amplification coupled with a magnetic DNA machine†

Xiangde Lai,‡[a,b] Xuan Zhao,‡[a,b] Yanan Peng,[a,b] Rui Zhang,[a,b] Huajie Pang,[a,b] Zhijun Gao,[b] Dongxia Li,[b] Peipei Cao,[c] Qiumei Pu,[a,b] Bin Qiao,*[b,d] Hua Pei*[b] and Qiang Wu*[a,b,d]

[a] *The First Affiliated Hospital, Hainan Medical University, Haikou 570102, China. E-mail: wuqiang001001@aliyun.com*

[b] *The Second Affiliated Hospital, School of Tropical Medicine, Hainan Medical University, Haikou 570311, China. E-mail: hy0211085@hainmc.edu.cn, phzmh61@aliyun.com*

[c] *School of Basic Medicine and Life Sciences, Hainan Medical University, Haikou 571199, China.*

[d] *Key Laboratory of Emergency and Trauma of Ministry of Education, Research Unit of Island Emergency Medicine, Chinese Academy of Medical Sciences (No. 2019RU013), Hainan Medical University, Haikou 571199, China.*

‡ These authors contributed equally to this work.

Experimental sections

Materials and reagents

All the nucleotide sequences used in this experiment were synthesized and processed by Sangon Biotech Co., Ltd. (Shanghai, China), and the nucleotide sequences have been listed in Table S1. T4 DNA ligase, exonuclease I (Exo I, E. coli), exonuclease III (Exo III, E. coli), bst DNA polymerase, and nicking endonuclease (Nb.BbvCI) were purchased from New England Biolabs (Beijing) Ltd. (Beijing, China). Heat labile double-stranded DNA-specific nuclease (HL dsDNase) was acquired from HaiGene Biotech Co., Ltd. (Harbin, China). Deoxynucleotide (dNTP) solution mixture was obtained from Thermo Fisher Scientific (China) Co., Ltd (Shanghai, China). Streptavidin magnetic beads (SA-MBs, average diameter 1 μm) were purchased from Beaver Biomedical Engineering Co., Ltd. (Suzhou, China). Ultrapure water (≥18.2 MΩ) was obtained from the Millipore-Q ultrapure water system. All other chemicals used for this experiment were of analytical grade without further purification. The serum samples were obtained from the Second Affiliated Hospital of Hainan Medical University, which was approved by the Ethics Committee of Hainan Medical University (Approval number: HYLL-2022-419).

Apparatus

Amplification reaction was performed using a Biometra TAdvanced 96 G (Jena, Germany). A thermostatic mixer (Kylin-Bell BE-3500) was used for thermostatic shaking (Jiangsu, China). The 24-well magnetic separation rack was purchased from Shenzhen Borxi Technology Development Co., Ltd (Shenzhen, China). Constant temperature reaction was used with the thermostatic metal bath HB-100 from Shanghai Kehuai Instruments Co., Ltd (Shanghai, China). Fluorescence detection was performed using a BioTek Synergy H1 multifunctional microplate detector (California, USA).

Preparation of circular DNA template

The ligation of G-padlock probe into circular DNA was performed in 30 μL of reaction mixture, including 16 μL of G-padlock probe (10 μM L^{-1}), 8 μL of target (10 μM L^{-1}), 5 μL of 10 × T4 DNA ligase buffer and 1 μL of T4 DNA ligase (400 U μL^{-1}), all reagents were added and mixed thoroughly. The reaction mixture was placed in the PCR instrument and incubated for 2 h at 25 °C. After the ligation reaction was completed, the T4 DNA ligase was inactivated by incubation at 65 °C for 15 min. Then added 2.5 μL Exo I (30 U μL^{-1}), 2.5 μL Exo III (200 U μL^{-1}), 5 μL 10 × Exo I reaction buffer, and 5 μL 10 × NEBuffer 1 (10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM DTT) to the reaction mixture, then incubated for 1 h at 37 °C in PCR instrument to excise the remaining excess nucleotide sequences beyond the circular DNA template, after that, the reaction solution was incubated for 20 min at 85 °C to inactivate Exo I and

Exo III. Finally, the prepared solution was stored at 4 °C for further use.

RCA reaction

A total of 25 μL of RCA reaction mixture was created, including 10 μL of target, 5 μL of circular DNA template, 2 μL of 10 × ThermoPol reaction buffer pack (20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100), 3 μL $MgSO_4$ (100 mM L^{-1}), 1 μL dNTP Mix (10 mM) and 4 μL bst DNA polymerase (8 U μL^{-1}), all reagents were added and mixed fully. The reaction mix was incubated in a PCR instrument at 65 °C for 1.5 h. After the amplification reaction was completed, the reaction was terminated by inactivating bst DNA polymerase at 85 °C for 10 min.

HL dsDNase degrades RCA product and produces initiator

10 μL of RCA product was added to a total of 20 μL of reaction mixture to produce the initiator, which also included 1 μL of ssDNA (20 μM L^{-1}), 3 μL of HL dsDNase (2 U μL^{-1}), and 6 μL of 10 × dsDNase buffer. The reaction mixture was quickly mixed and placed on ice, then the reaction was carried out at 30 °C for 45 min, and the reaction was terminated by inactivating HL dsDNase at 65 °C for 10 min.

Assembling magnetic DNA machine

10 μL of SA-MBs solution (10 mg mL^{-1}) were taken and vortex shaken for 20 s, magnetically separated using magnetic separation rack, and the supernatant was removed. Then added 1 mL of wash buffer I (10 mM Tris-HCl, 1 mM EDTA, 1 M NaCl, 0.1% Tween-20), vortex shook for 20 s, magnetic separation for 1 min, and removed the supernatant. Repeat the washing twice. Then added 45 μL of wash buffer I and 5 μL of capture probe (10 μM L^{-1}), vortex shook to mix well. Subsequently, the mixture was placed in a constant temperature mixing instrument and incubated at 37 °C 500 rpm min^{-1} for 30 min to fully bind the capture probe to SA-MBs. After that, the mixture was magnetically separated and the supernatant was removed. Wash buffer I and TE buffer containing 12.5 mM (pH 7.0) were used once each to remove the excess unbound capture probe. Finally, 50 μL of TE buffer containing 12.5 mM (pH 7.0) was added and vortex shaken for 30 s, then the reaction solution was kept at 4 °C until use.

Fluorescence detection of DENV nucleic acids based on magnetic DNA machine triggered by initiator

The prepared magnetic DNA machine was vortexed and shaken 30 s to mix well before use. Then 5 μL of magnetic DNA machine, 10 μL of initiator, 1.5 μL of Nb.BbvCI (10 U μL^{-1}), 3.5 μL of 10 × rCutSmart buffer (50 mM Potassium Acetate, 20 mM Tris-acetate, 10 mM Magnesium Acetate, 100 μg ml^{-1} Recombinant Albumin), and 80 μL of ultrapure water were added to a 1.5 mL EP tube. The reaction mixture was then placed in a constant temperature metal bath at 37 °C for 45 min. After completion of the reaction, the supernatant was magnetically separated by magnetic separation rack and aspirated for follow-up fluorescence detection. The fluorescence detection was performed with a BioTek Synergy H1 multifunctional microplate detector with an excitation wavelength of 480 nm, an emission wavelength of 518 nm, a gain of 100 and a height of 5 mm.

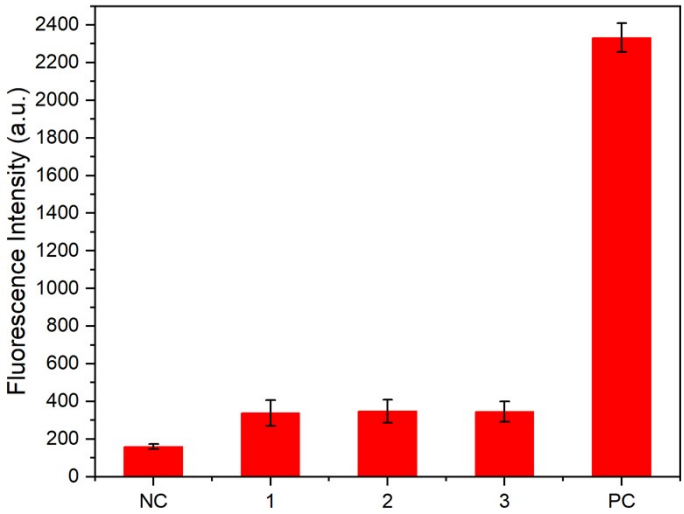

Fig.S1 Functional verification of ssDNA and HL dsDNase. NC: negative control; 1: in the absence of ssDNA and HL dsDNase (initial RCA products); 2: in the absence of ssDNA; 3: in the absence of HL dsDNase; PC: positive control (in the presence of ssDNA and HL dsDNase).

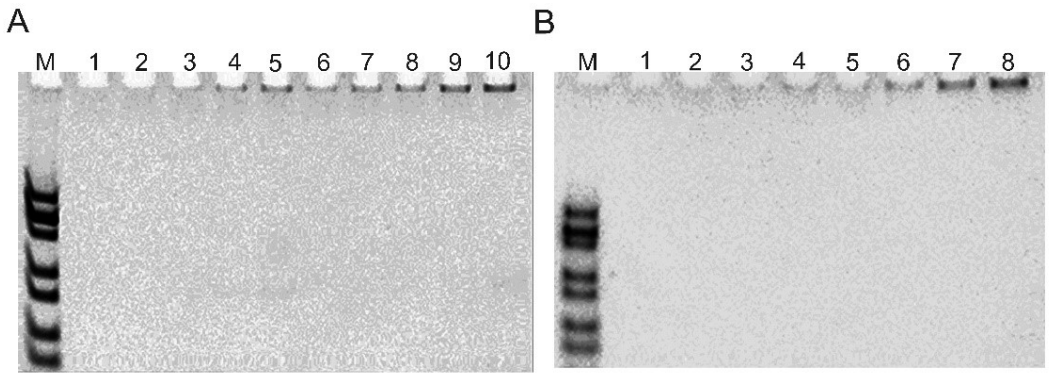

Fig.S2 Native-PAGE (8%) of the optimization for RCA. (A) Reaction time of RCA:1-5 are the negative of 0.5 h, 1 h, 1.5 h, 2 h, 2.5h and 6-10 are the positive of 0.5 h, 1 h, 1.5 h, 2 h, 2.5h. (B) Quantity of bst DNA polymerase: 1-4 are the negative of 8 U, 16 U, 24 U, 32 U and 5-8 are the positive of 8 U, 16 U, 24 U, 32 U.

Table S1 Nucleotide sequences used in this paper

Name	Sequence (from 5′ to 3′)	Note
DENV-1	CATTGGATTTTGAACTGATAAAGA	Target
Padlock	Phos-CAAAATCCAATGTGGTTGGTTGGTTGGTGCTGAGGTTATTATTATTATTTATTATTAGCTGAGGTCTTTATCAGTT	5′-end phosphorylated padlock probe
ssDNA	TCTTTATCAGTTCAAAATCCAATG	Single-stranded DNA
Initiator	CCTCAGCTAATAATAAATAATAATAATAATAACCTCAGCACCAACCAACCAACCA	Initiator sequence
Biotin-S-FAM	Biotin-TGGTTGGTTGGTTGGTGCTGAGGTT-FAM	Capture probe
Mis-1	CATTGGATTTTGAACTGATAGAGA	One base mismatch sequence
Mis-2	CATTGGATTTTGAACTGATAGGGA	Two base mismatch sequence
Mis-3	CATTGGATTTTGAACTGATAGGTA	Three base mismatch sequence
Random	GAGAAAAAACTCCAGTATTATTGA	Complete mismatch sequence

Table S2 Comparison with reported DENV nucleic acid sensing methods

Methods	Output signal	LOD	Linear range	Ref.
Cu-based zwitterionic metal organic framework	Fluorescence	3.3×10^{-10} M	1.0×10^{-9} - 6.0×10^{-8} M	1
ZnO/Pt-Pd nanocomposites	Electrochemistry	4.3×10^{-5} M	1.0×10^{-6} - 1.0×10^{-4} M	2
Silver nanocluter	Fluorescence	1.0×10^{-7} M	-	3
Fluorescent aptasensor	Fluorescence	2.5×10^{-9} M	4.0×10^{-9} - 5.1×10^{-7} M	4
Label-free electrochemical detection	Electrochemistry	3.0×10^{-9} M	1.0×10^{-8} - 1.0×10^{-7} M	5
RCA coupled with magnetic DNA machine	Fluorescence	7.5×10^{-12} M	2.0×10^{-11} - 5.0×10^{-8} M	This work

Notes and references

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Table S3 Serum recovery results of DENV nucleic acids sensing strategy

Spiked concentration (× 10^{-9} M)	Detected [a] (× 10^{-9} M)	Recovery [b] (%)	RSD [c] (%)
20	21.01	105.08	5.22
10	10.22	102.21	3.31
5	4.98	99.73	1.73

[a] sensing strategy a Results are mean (n = 3).
[b] Recovery (%) = (Detected concentration / spiked concentration) × 100.
[c] RSD (%) = (Standard deviation / mean) × 100%.