Supplementary Information

A simple and rapid method to assay SARS-CoV-2 RNA based on primer exchange reaction

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Reagents and materials

All the DNA oligonucleotides used in this work were obtained from Sangon Biotech. Co. Ltd. (Shanghai, China). The RNA sequence was synthesized by Genscript. Bio Tech (Nanjing China) (Table S1). The target RNA used in this study is the artificially synthesized conserved regions (ORF1ab fragment) of SARS-CoV-2 RNA

according to GenBank, SARS-CoV-2 NC_045512. Large Fragment Bst DNA polymerase, 10x Bst Reaction Bufffer (200mM Tris-HCl, 100mM KCl, 100mM $(NH_4)_2SO_4$, 20mM $MgSO_4$, 1% Triton X-100) and 100mM $MgSO_4$ were bought from Beyotime Biotech. (Shanghai China). dATP (100 mM), dTTP (100 mM), dCTP (100 mM) were bought from Sangon Biotech. Co. Ltd. (Shanghai, China). RNase free water and RNase inhibitor were obtained from Takara Bio. (Dalian China). EnGen® Lba Cas12a (Cpf1) and 10×NEbuffer 2.1 were purchased from New England Biolabs (Beijing China). Ultra-pure water used in all experiments were purified by a Millipore purification system. Human serum was provided by the First Affiliated Hospital of Nanjing Medical University. Our research has been approved by the ethics committee of Nanjing Medical University and Nanjing University.

Native polyacrylamide gel electrophoresis

The products of the PER cascade were verified by 12% non-denaturing gel electrophoresis. Specifically, we mixed 100nM target RNA with 100nM HP and 500nM primers, and then added Bst DNA polymerase (0.4U/μL) and 200μM dNTPs (A, T, C) to initiate the PER cascade. After two hours of reaction at 37°C, 10μL of the reaction solution was incubated with nucleic acid dye for 1 minute. Subsequently, the reaction solution was injected into the 12% PAGE. After running for 1 hour at 100V in 1 × TBE buffer, the gel is finally scanned by the gel imaging system.

Detection protocol for SARS-COV-2 RNA

Firstly, different concentrations of target RNA are added to the reaction solution with a total volume of 20μL, which contains 2nM HP and 10nM primers ,0.2U/μL Bst DNA polymerase, 4μL1mM dNTP (A, T, C), 2μL 10×Bst reaction Buffer and 3μL100mM $MgSO_4$. Then, the mixed solution was reacted at 37°C for 30 min. Next, 2μL 1μM cas12a, 2μL 1μM CrRNA, 2μL 10μM fluorescence reporter, 5μL 10×NEbuffer 2.1, and 19μL RNase free water were added into the solution to reach a total volume of 50μL. After reacting for 10 minutes at 37℃, the solution was finally

transferred to a black 96-well plate to measure its fluorescence value through Infinite 200Pro (Excitation 485nm, Emission 525 nm). When detecting target RNA in complex biological samples of serum and saliva, except for the need to add RNase inhibitor to prevent RNA degradation, the other steps are the same as above.

Table S1. DNA and RNA sequences used in this work

Name	Sequence (5' to 3')
HP (15bp)	ACTAAATTCAGGGCCTTTTGGCCCTGAATTTAGTAATA AGAGATCAACTACTGAAGCATGGGTTCGCGGAGTTGA TCTCTTATT-Inverted dT
HP (17bp)	ACTAAATTCAGGGCCTTTTGGCCCTGAATTTAGTAATA AGAGATCAACTCCACTGAAGCATGGGTTCGCGGAGTT GATCTCTTATT-Inverted dT
HP (19bp)	ACTAAATTCAGGGCCTTTTGGCCCTGAATTTAGTAATA AGAGATCAACTCCGCACTGAAGCATGGGTTCGCGGAG TTGATCTCTTATT-Inverted dT
HP (21bp)	ACTAAATTCAGGGCCTTTTGGCCCTGAATTTAGTAATA AGAGATCAACTCCGCGACTGAAGCATGGGTTCGCGGA GTTGATCTCTTATT-Inverted dT
primer	ATCTC TTAT
Target virus RNA	UUUUUGAUCAACUCCGCGAACCCAUGCUUCAGUUUU UU
Single-base mismatched RNA (1M)	UUUUUGAUCAAGUCCGCGAACCCAUGCUUCAGUUUU UU
Two-base mismatched RNA (2M)	UUUUUGAUCAAGUCCGCGAACCCAUGCUACAGUUUU UU
Three-base mismatched RNA (3M)	UUUUUGAUCAAGUCCGCGGACCCAUGCUACAGUUUU UU
random DNA	TCAACATCAGTTGGCACCCATGTGATATCCTTTTT
CrRNA	UAAUUUCUACUAAGUGUAGAUUGAAUUUAGUAAUA AGAGAU
excitation DNA	ATCTCTTATTACTAAATTCA
Fluorescence reporter (FQ)	FAM-TTATT-BHQ1

Red sequences represent conserved regions of the SARS-CoV-2 RNA.

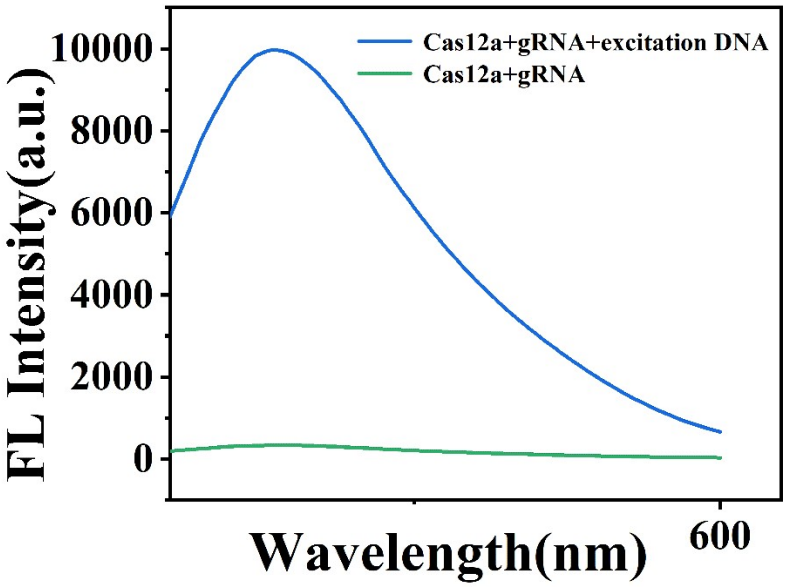

Fig. S1. The verification of trans-cleavage activity of cas12a protein (cpf1). Excitation DNA is 5nM, cas12a is 40nM, CrRNA is 40nM and fluorescence reporter (FQ) is 400nM. Reaction time is 15min.

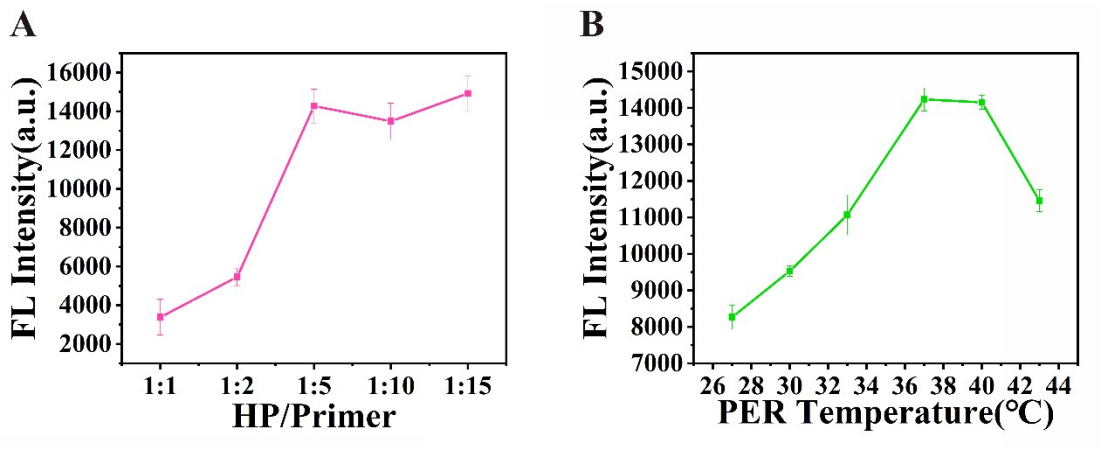

Fig. S2. Influence of the concentration ratio of HP to primer (A) and the PER temperature (B) on the detection performance of the PER-based method. Target RNA is 5nM and HP is 2nM.

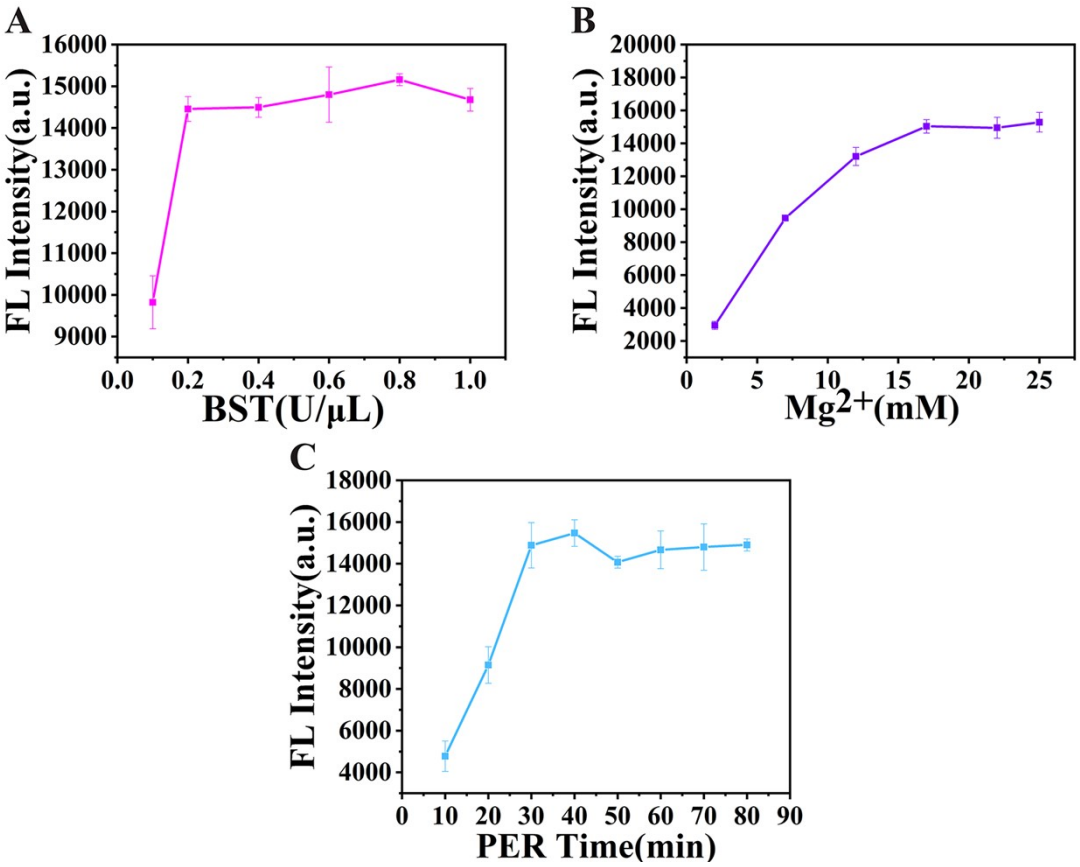

Fig. S3. Influence of the concentration of Bst DNA polymerase (A), Mg^{2+} concentration (B) and the PER reaction time (C) on the final detection performance of the biosensor. Target RNA is 5nM, HP is 2nM and primer is 10nM.

Table S2. Comparison with some reported detection strategies for SARS-CoV-2 RNA.

Signal output mode	LOD	Detection time	Tested mutated SARS-CoV-2	References
Fluorescence	500 pM	60min	No	[1]
Colorimetry	140 pM	90min	No	[2]
Colorimetry	30.3 fM	141min	Yes	[3]
Electrochemistry	26 fM	185min	Yes	[4]
Fluorescence	1.3 pM	40min	Yes	This work

Table S3. Results for the detection of target RNA in 1xBst reaction buffer, 10% human serum and 10% saliva samples.

Sample number	**Samples**	**Added(nM)**	**Found(nM)**	**Recovery (%)**	**RSD (%) n=3**
1	Buffer	0.2	0.214	107	5.3
2	Buffer	2	1.993	99.6	2.1
3	10% Serum	0.2	0.214	107	3.4
4	10% Serum	2	2.04	102	4.1
5	10% Saliva	0.2	0.207	103.5	3.9
6	10% Saliva	2	1.992	99.6	3.0

References

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