Supplementary Information

A functional RNA/DNA circuit for one-pot detection of SARS-CoV-2 RNA

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Materials and Reagents.

All DNA sequences used in this work including FQ fluorescent reporters were synthesized by Sangon Biotech. Co. Ltd. (Shanghai, China). The RNA nucleotides were obtained from Genscript. Bio Tech (Nanjing China). Refer to Table S1 for the detailed sequence. The target RNA used in this research is a synthetic gene according to the ORF1ab fragment of the SARS-CoV-2 RNA (GenBank, NC_045512). EnGen® Lba Cas12a (M0653T) and 10 × NEBuffer r2.1 reaction buffer (500 mM NaCl, 100 mM Tris-HCl, 100 mM MgCl2, 1000 μg/ml Recombinant Albumin, pH 7.9@25°C) were obtained from New England Biolabs (Beijing China). RNase inhibitor was bought from Takara Bio. (Dalian China). DEPC-H2O was obtained from Sangon Biotech. Co. Ltd. (Shanghai, China), and all reagents and solutions were prepared in an RNase-free environment with DEPC-treated deionized water. The Millipore purification system was used to prepare the ultrapure water required for the experiments. Human serum samples and the total RNA clinical specimens were provided by the Center for Disease Control and Prevention of Wuhu, China. The research was ratified by the scientific ethical committee of the Wuhu Hospital of East China Normal University (the project number is 2020dx2-1), and informed consent was procured in all cases.

Apparatus.

Fluorescent analysis was operated on a TECAN Infinite 200Pro sunrise microplate reader. Native polyacrylamide gel electrophoresis was operated on a Tanon EPS300 electrophoresis machine. The gel imaging was operated on a CLNX GenoSens 2100 imaging system.

Native polyacrylamide gel electrophoresis.

The proposed gRNA-based HCR was validated by using 12% native gel electrophoresis. The 20μL reaction system contains 100nM DNA activator (DH), 100nM gRNA (RH), 10nM target RNA and 1×NEBuffer r2.1 reaction buffer. After mixing all the components, react at 37℃ for 150min. After the reaction, 10μl of the reaction solution was incubated with nucleic acid dye for 3min, and then injected into

12% PAGE. Finally, PAGE was run at 120V for 1 hour in the electrophoresis machine (Tanon EPS300), and the result was visualized by an imaging system (CLNX GenoSens 2100).

Specific protocol for detection of SARS-CoV-2 RNA.

First, different concentrations of target RNA were added to a reaction system with a total volume of 50μL, which contained 4μl of 1μM DH, 2μL of 1μM RH, 40nM, 3μL of 1μM LbCas12a(cpf1), 300nM of fluorescent probe FQ Reporter, and 1×NEBuffer r2.1 reaction buffer (50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl2, 100 μg/ml Recombinant Albumin, pH 7.9@25°C). Next, the reaction was carried out at 37°C for 60 minutes. After the reaction, the fluorescence intensity at 525 nm (Excitation 485nm) was measured by using a microplate reader (TECAN Infinite 200Pro). In addition, it is worth noting that when detecting target RNA in human serum and saliva samples, it is necessary to add RNase inhibitor for treatment to avoid RNA degradation. Other than that, the operation steps are the same as those described above. All solutions involved in the experiments were prepared with DEPC-H2O, and all nucleotides were diluted with DEPC-treated PBS.

Extraction and RT-qPCR of SARS-COV-2 RNA from clinical samples.

Total RNA was extracted from each nasopharyngeal swab sample using a nucleic acid extraction kit according to the manufacturer's instructions (Tianlong Technologies, Xian, China) and stored in nuclease-free environment at -80℃ for next use. The RT-q-PCR verification of clinical samples is carried out with the 2019-nCoV nucleic acid detection kit (Daangene, Guangzhou China).

Table S1. DNA and RNA sequences used in this study.

Name	Sequence (5' to 3')
DH (stem 13bp)	ACTGAAGCATGGGTTCGCGGAGTTGCAAACTTGAGCAACTCCGCGAA
DH (stem 14bp)	ACTGAAGCATGGGTTCGCGGAGTTGCAAACTTGAGCAACTCCGCGAAC
DH (stem 15bp)	ACTGAAGCATGGGTTCGCGGAGTTGCAAACTTGAGCAACTCCGCGAACC
DH (stem 16bp)	ACTGAAGCATGGGTTCGCGGAGTTGCAAACTTGAGCAACTCCGCGAACCC
RH (stem 12bp)	UAAUUUCUACUAAGUGUAGAUCAACUCCGCGAACCCAUGCUUCUUCGCGGAGUUGCUCAAGUUUG
RH (stem 13bp)	UAAUUUCUACUAAGUGUAGAUCAACUCCGCGAACCCAUGCUUCGUUCGCGGAGUUGCUCAAGUUUG
RH (stem 14bp)	UAAUUUCUACUAAGUGUAGAUCAACUCCGCGAACCCAUGCUUCGGUUCGCGGAGUUGCUCAAGUUUG
RH (stem 15bp)	UAAUUUCUACUAAGUGUAGAUCAACUCCGCGAACCCAUGCUUCGGGUUCGCGGAGUUGCUCAAGUUUG
Target virus RNA	CAACUCCGCGAACCCAUGCUUCAGU
Single-base mismatched RNA (M1)	CAAGUCCGCGAACCCAUGCUUCAGU
Two-base mismatched RNA (M2)	CAAGUCCGCGAACACAUGCUUCAGU
Three-base mismatched RNA (M3)	CAAGUCCGCGAACACAUGCCUCAGU
miR-21	UAGCUUAUCAGACUGAUGUUGA
Fluorescence reporter (FQ)	FAM-TTATT-BHQ1

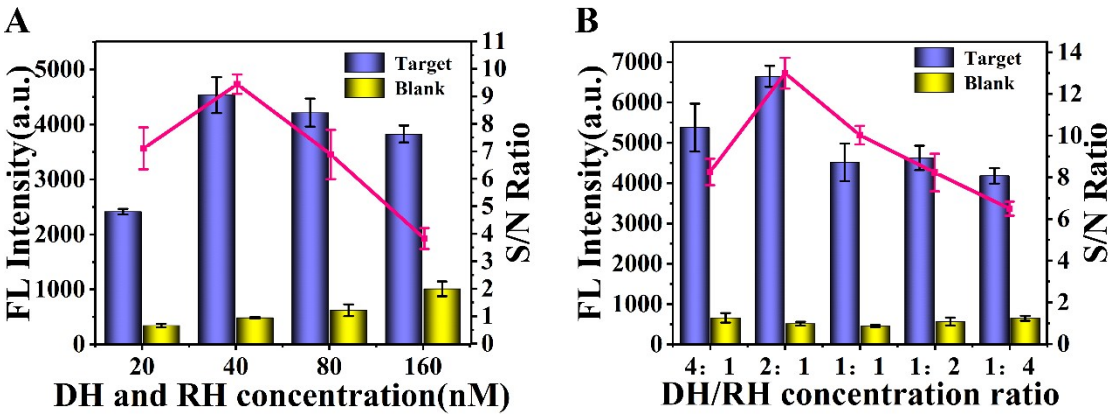

Figure S1. Influence of the concentration of DH and RH (A) and the ratio of DH and RH (B) on the performance of the method. Error bars: SD, n = 3.

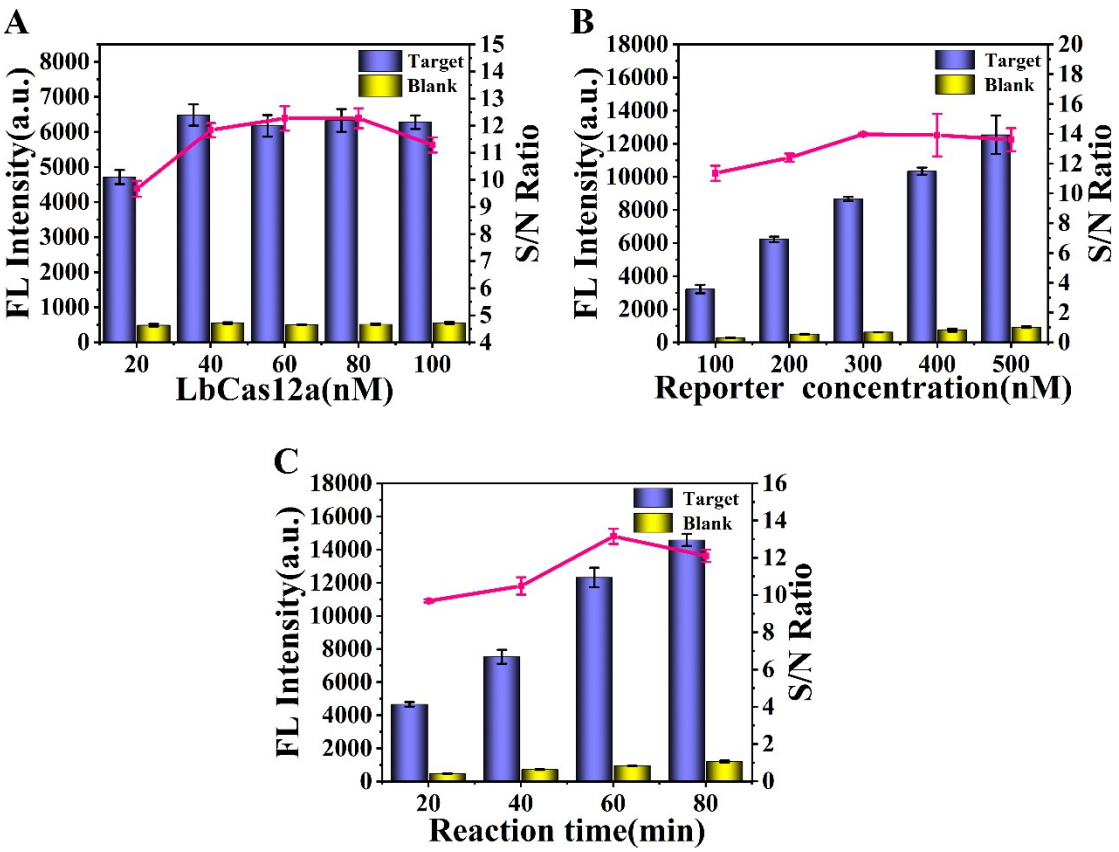

Figure S2. Optimization of LbCas12a protein concentration (A), FQ reporter concentration (B), and reaction time (C). The concentration of target RNA is 1nM. Error bars: SD, n = 3.

Table S2. The performance of the method in 1xNEbuffer r2.1 reaction buffer, 10% human serum and 10% saliva samples.

Sample number	Samples	Added(nM)	Found(nM)	Recovery (%)	RSD (%)
1	10% Serum	0.1	0.111	111	3.6
2	10% Serum	1	0.999	99.9	3.4
3	10% Saliva	0.1	0.105	105	4.4
4	10% Saliva	1	1.01	101	6.1
5	Buffer	0.1	0.107	107	6.1
6	Buffer	1	0.994	99.4	5.1

Table S3. The results of RT-qPCR for clinical samples

Positive sample	Ct value (RT-qPCR)	Normal sample	Ct value (RT-qPCR)
1	25.83	1	undetected
2	24.61	2	undetected
3	22.95	3	undetected
4	17.09	4	undetected
5	24.35	5	undetected
6	28.96	6	undetected
7	27.94	7	undetected
8	28.15	8	undetected
9	27.90	9	undetected
10	27.78	10	undetected
11	16.82	11	undetected
12	27.65	12	undetected
13	14.3	13	undetected
14	30.62	14	undetected
15	31.57	15	undetected
16	23.89	16	undetected
17	17.53	17	undetected
18	21.14	18	undetected
19	28.05	19	undetected
20	28.27	20	undetected
21	33.63	21	undetected
22	28.52	22	undetected
23	28.82	23	undetected
24	24.15	24	undetected
25	23.89	25	undetected
26	23.12	26	undetected

27	31.47	27	undetected
28	21.55	28	undetected
29	23.55	29	undetected
30	24.71	30	undetected
31	25.79	31	undetected
32	36.39	32	undetected
33	24.97	33	undetected
34	26.15	34	undetected
35	20.15	35	undetected
36	18.25	36	undetected
37	22.63	37	undetected
38	22.08	38	undetected
39	19.77	39	undetected
40	24.22	40	undetected

Ct value ≤40: positive; Ct value = undetected: negative

Table S4. Comparison with some reported detection strategies for SARS-CoV-2 RNA.

Method	One-pot detection	Enzymes involved	Time	LOD	References
Colorimetry	No	5	50min	50 copies	[1]
Fluorescence	No	2	40min	1.3 pM	[2]
Fluorescence	No	5	50min	2 copies	[3]
Colorimetry	No	4	141min	30.3 fM	[4]
Electrochemistry	No	1	185min	26 fM	[5]
Fluorescence	Yes	1	60min	544fM	This work

References

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