Supporting information

CRISPR Cas12a-enabled biosensors coupled with commercial pregnancy test strips for the visible point-of-care testing of SARS-CoV-2

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Content

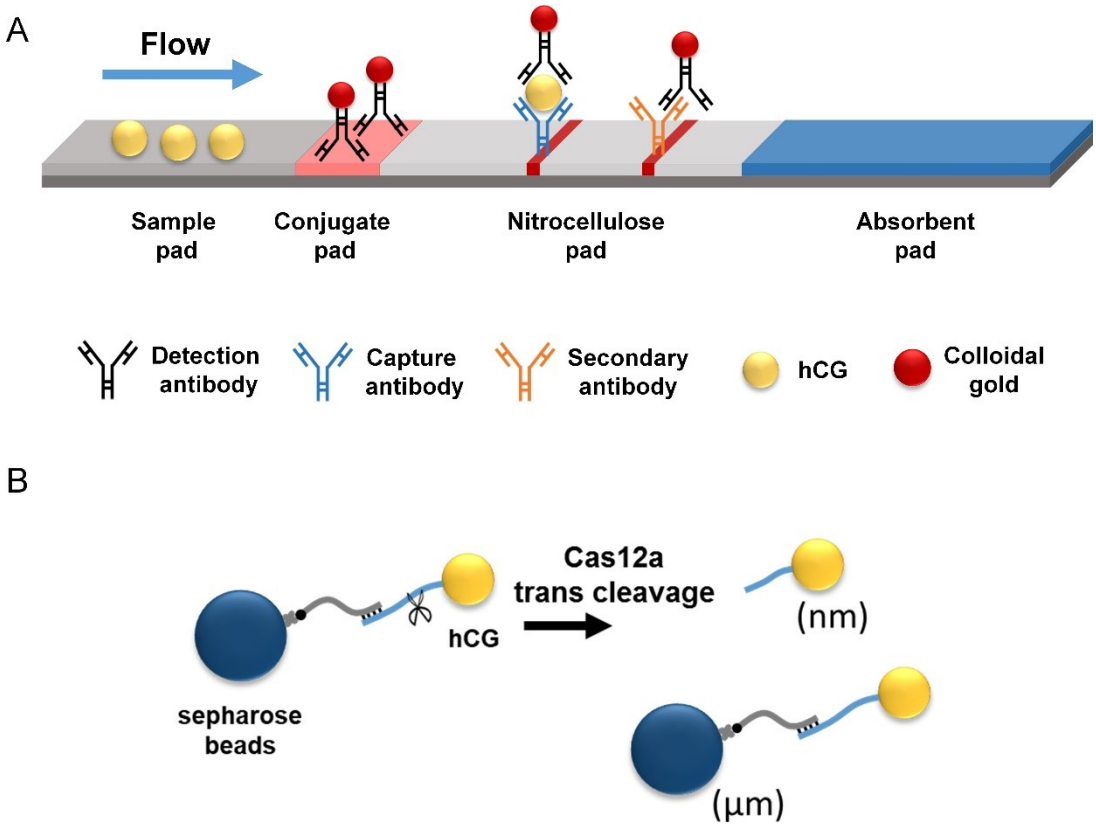

Figure S1. Schematic diagram of CRISPR-PTS assay. (A) Principle of the pregnancy test strip (PTS) assay. (B) Schematic diagram of CRISPR Cas12a-based ssDNA cleavage. The hCG was released from sepharose beads after Cas12a *trans*-cleavage and could be detected by PTS.

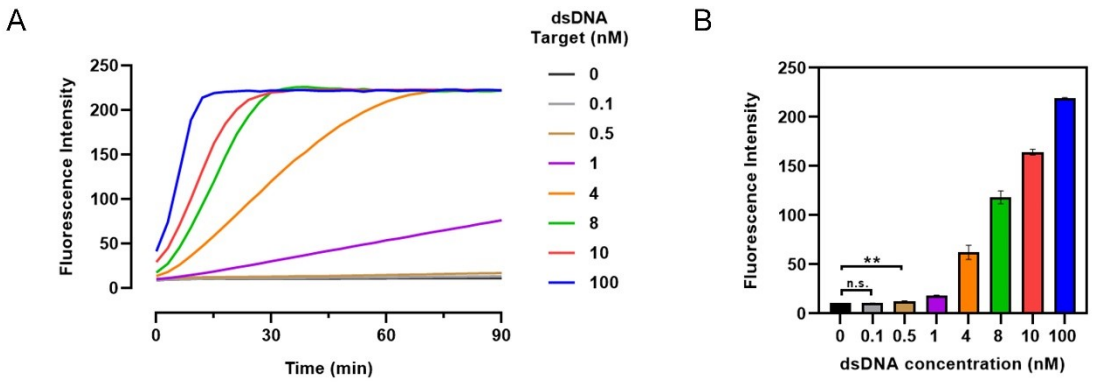

Figure S2. Established CRISPR-fluorescence assay without amplification. (A) Reaction kinetics of SARS-CoV-2 E1 site tested by CRISPR-fluorescence system. The dsDNA was diluted to different final concentrations as targets and the fluorescence was measured every 3 min (the curves showed the mean of three repeated measurements collected every 3 min by a 384-well plate reader). (B) Sensitivity of the CRISPR-fluorescence assay for SARS-CoV-2 E1 site at 15 min (each column was the average of three repeated measurements; error bars represented the mean ± S.D.; **$p < 0.01$ in "Student's t test").

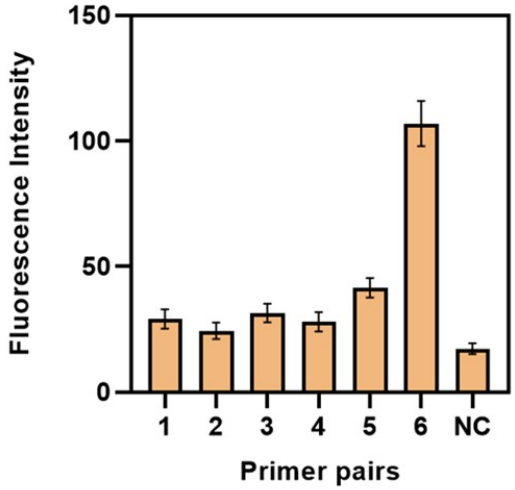

Figure S3. RT-RAA primer optimization. Six different primer combinations were tested in this section and primer pair 6 containing E1-RT-RAA-R2 and E1-RT-RAA-6F showed the highest amplification efficiency (CRISPR-fluorescence assay was used in this part; fluorescence intensity was measured at 15 min; pseudovirus concentration was 5 copies/μL; each column was the average of three repeated measurements; error bars represented the mean ± S.D.; NC: without any primer addition).

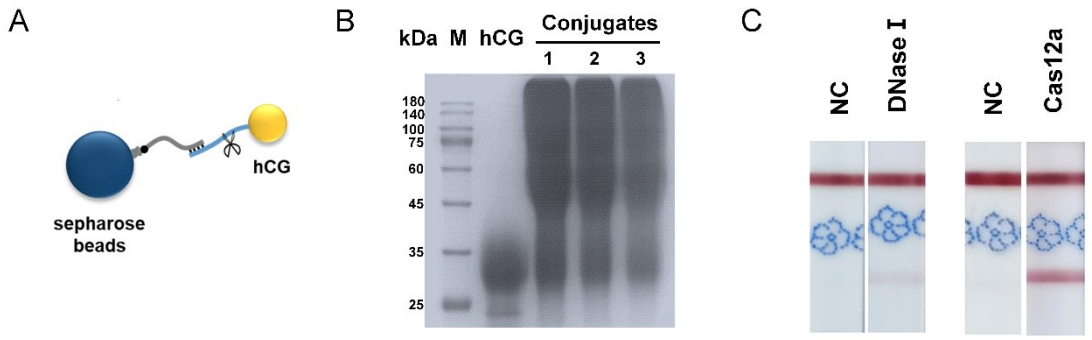

Figure S4. Feasibility of CRISPR-PTS assay. (A) Schematic diagram of CRISPR Cas12a-mediated ssDNA cleavage. (B) SDS-PAGE analysis of hCG and ssDNA conjugate (the theoretical molecular weight of hCG was 36 kDa and he larger molecular weight bands in line 1, 2 and 3 represented the hCG-ssDNA conjugate). (C) Feasibility test of CRISPR-PTS assay. The hCG-modified streptavidin sepharose beads were respectively treated (or not) by DNase I (10 U) and Cas12a (with crRNA, and 100 nM target) followed by immersing the strip into the reaction mixture.

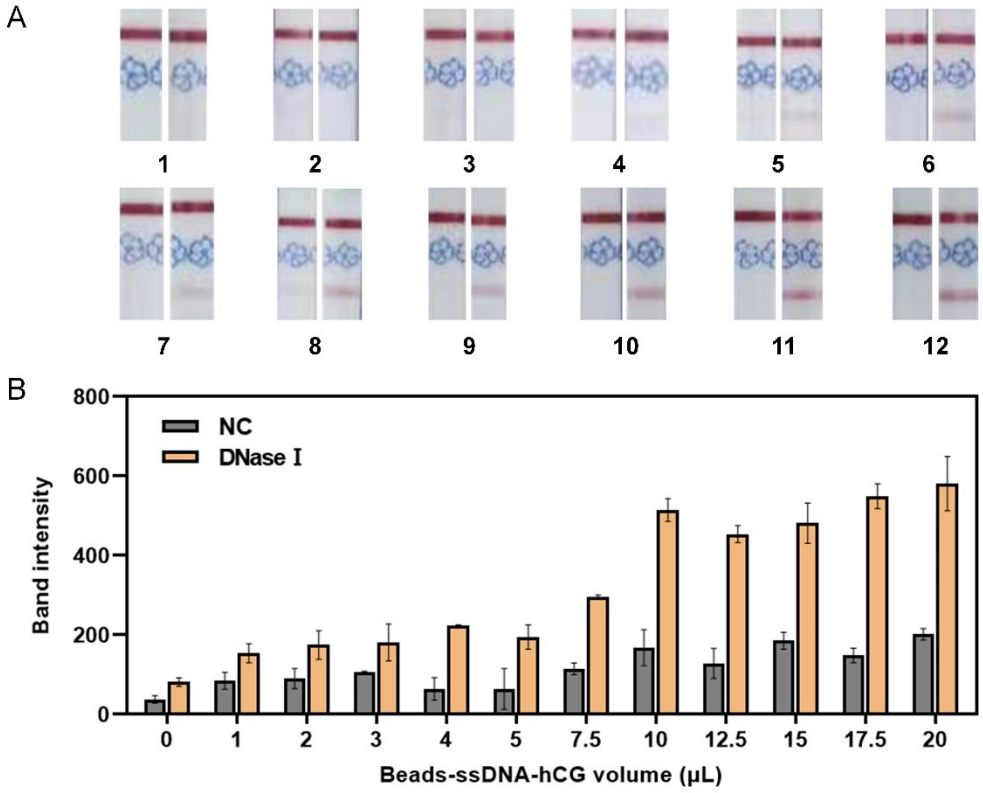

Figure S5. Optimization of hCG-modified beads added volume in CRISPR-PTS assay. (A) Comparison of band intensity observed by unaided eyes between different groups. No. 1 to No. 12 represented experimental groups with beads adding volume ranging from 0 to 20 μL, respectively (left: NC; right: DNase I). (B) Comparison of band intensity observed by a test strip reader between different groups. This cleavage system contained 0.5 U/μL RNase inhibitor, 1×NEBuffer2.1 and different amount of hCG-modified beads, treated by DNase I or not (NC) for 1 hour at 37 ℃ (each column was the average of three repeated measurements; error bars represented the mean ± S.D.).

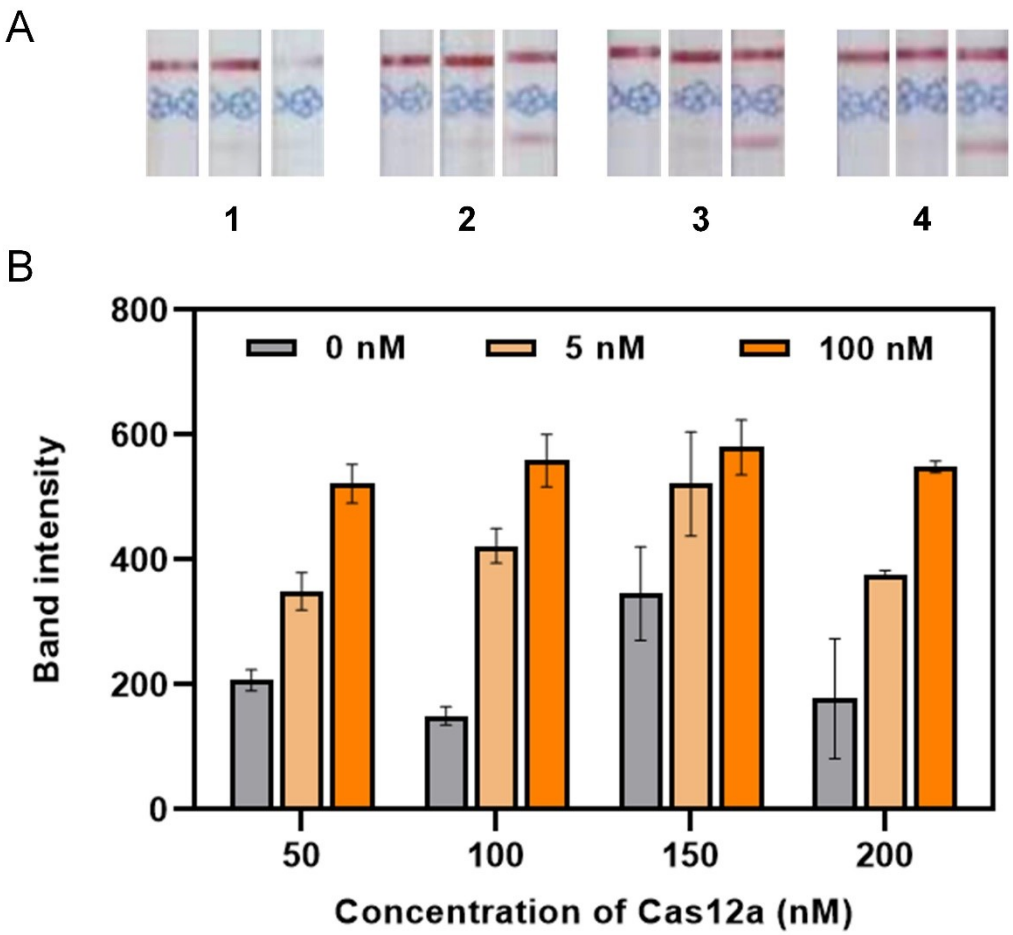

Figure S6. Optimization of Cas12a/crRNA final concentration in CRISPR-PTS assay. (A) Comparison of band intensity observed by unaided eyes between different groups. No. 1 to No. 4 represented experimental groups with Cas12a final concentrations ranging from 50 to 200 nM, respectively (left: 0 nM dsDNA target; middle: 5 nM dsDNA target; right: 100 nM dsDNA target). (B) Comparison of band intensity observed by a test strip reader between different groups. This cleavage system contained 0.5 U/μL RNase inhibitor, 1×NEBuffer2.1 and 20 μL hCG-modified beads, treated by different amount of Cas12a/crRNA (molar ratio=1:2) for 1 hour at 37 ℃. Three different kinds of dsDNA concentrations were tested in this experiment as mocked target (each column was the average of three repeated measurements; error bars represented the mean ± S.D.).

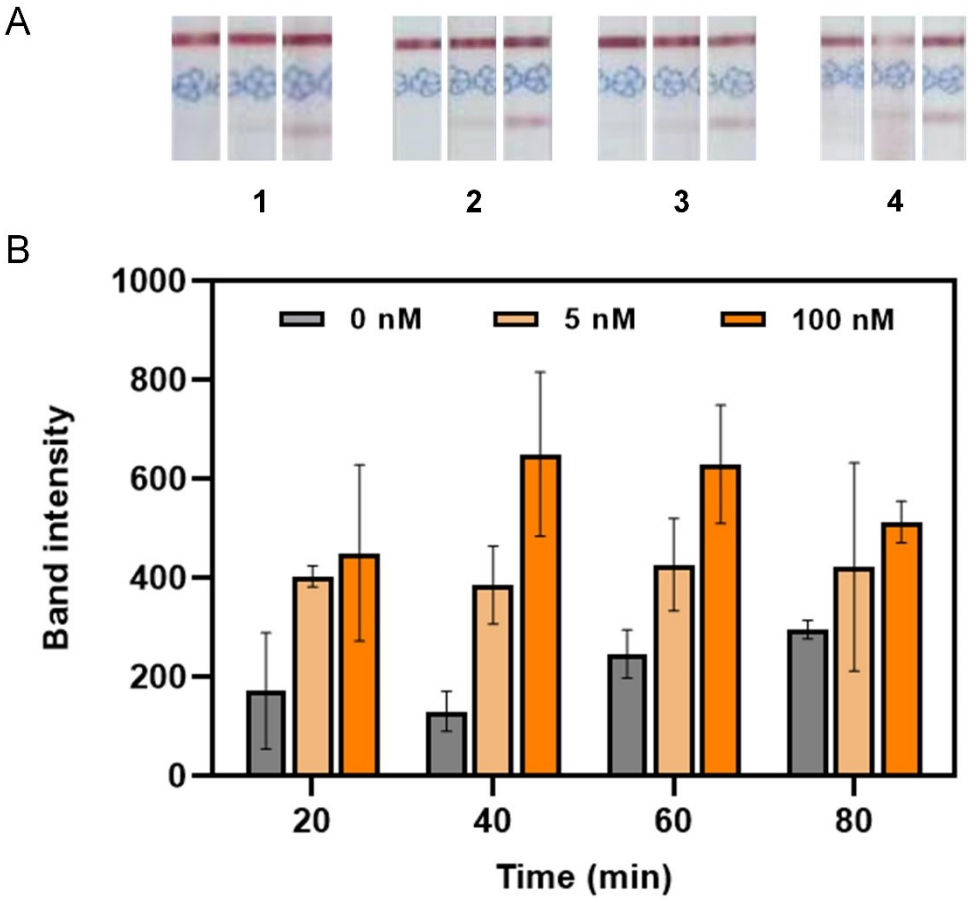

Figure S7. Optimization of cleavage time in CRISPR-PTS assay. (A) Comparison of band intensity observed by unaided eyes between different groups. No. 1 to No. 4 represented experimental groups with cleavage time ranging from 20 to 80 min, respectively (left: 0 nM dsDNA target; middle: 5 nM dsDNA target; right: 100 nM dsDNA target). (B) Comparison of band intensity observed by a test strip reader between different groups. This cleavage system contained 100 nM Cas12a, 200 nM crRNA, 0.5 U/μL RNase inhibitor, 1×NEBuffer2.1 and 20 μL hCG-modified beads, treated at 37 ℃ for different time. Three different kinds of dsDNA concentrations were tested in this experiment as mocked target (each column was the average of three repeated measurements; error bars represented the mean ± S.D.).

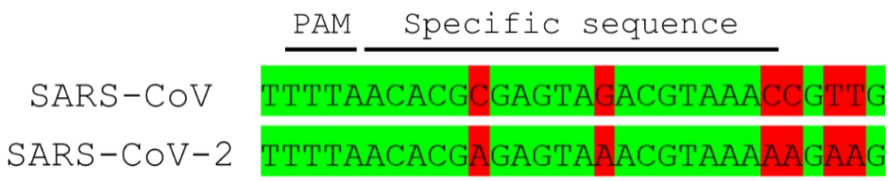

Figure S8. Genome sequence alignment of SARS-CoV and SARS-CoV-2. The protospacer adjacent motif (PAM) and specific sequence for Cas12a/crRNA recognition were both on the E gene of two viral strains. The red part of the sequence represented the significant difference between two viral types and was suitable for CRISPR Cas12a detection system.

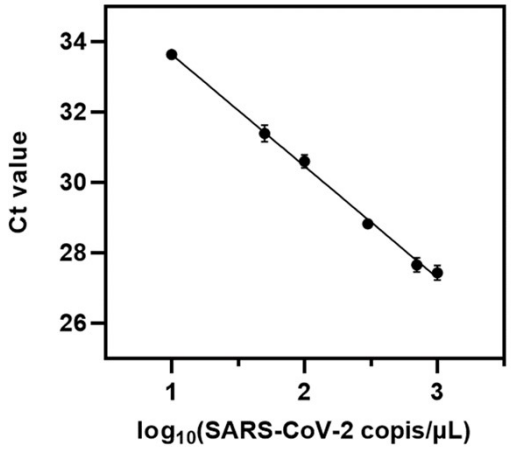

Figure S9. RT-qPCR standard curve of SARS-CoV-2 pseudovirus spiked throat swab samples. The picture showed the linear response of Ct values towards $\log_{10}$ (pseudovirus final concentrations). The data was taken by three biological repetitions and error bars represent the mean ± S.D.; $R^2 = 0.993$.

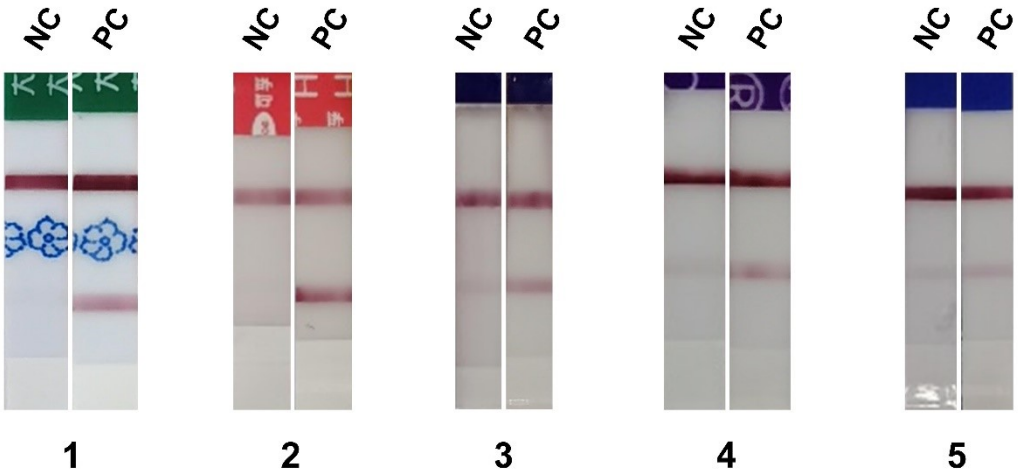

Figure S10. Comparison between strips from different brands using CRISPR-PTS assay (NC: templete was RNase-free ddH_2O; PC: templete was throat swab samples mixed with pseudovirus at a final concentration of 100 copies/μL). The brands of commercial pregnancy test strips from group 1 to 5 were respectively David®, Cofoe®, Wellday®, Yuting® and Hainuo®.

Table S1. Oligonucleotides used in this study.

Name	Sequence (5’ start)
N1-NTS	CTGGTCCCCAAAATTTCCTTGGGTTTGTTCTGGACCAC GTCTGCCGAAAG
N1-TS	CTTTCGGCAGACGTGGTCCAGAACAAACCCAAGGAAA TTTTGGGGACCAG
N2-NTS	CCTCAGCAGCAGATTTCTTAGTGACAGTTTGGCCTTGT TGTTGTTGGCCT
N2-TS	AGGCCAACAACAACAAGGCCAAACTGTCACTAAGAAA TCTGCTGCTGAGG
E1-NTS	AAGAATTCAGATTTTTAACACGAGAGTAAACGTAAAA AGAAGGTTTTACA
E1-TS	TGTAAAACCTTCTTTTTACGTTTACTCTCGTGTTAAAAA TCTGAATTCTT
E2-NTS	TAGCGTACTTCTTTTTCTTGCTTTCGTGGTATTCTTGCT AGTTACACTAG
E2-TS	CTAGTGTAACTAGCAAGAATACCACGAAAGCAAGAAA AAGAAGTACGCTA
N1-crRNA-F	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTA GATCTTGGGTTTGTTCTGGACCA
N1-crRNA-R	TGGTCCAGAACAAACCCAAGATCTACACTTAGTAGAA ATTACCCTATAGTGAGTCGTATTA
N2-crRNA-F	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTA GATTTAGTGACAGTTTGGCCTTG
N2-crRNA-R	CAAGGCCAAACTGTCACTAAATCTACACTTAGTAGAA ATTACCCTATAGTGAGTCGTATTA
E1-crRNA-F	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTA GATACACGAGAGTAAACGTAAAA
E1-crRNA-R	TTTTACGTTTACTCTCGTGTATCTACACTTAGTAGAAAT

	TACCCTATAGTGAGTCGTATTA
E2-crRNA-F	TAATACGACTCACTATAGGGTAATTTCTACTAAGTGTA GATTTGCTTTCGTGGTATTCTTG
E2-crRNA-R	CAAGAATACCACGAAAGCAAATCTACACTTAGTAGAA ATTACCCTATAGTGAGTCGTATTA
E1-RT-RAA-F1	CGGAAGAGACAGGTACGTTAATAGTTAATAGC
E1-RT-RAA-R2	AGACCAGAAGATCAGGAACTCTAGAAGAAT
E1-RT-RAA-F3	ATGTACTCATTCGTTTCGGAAGAGACAGGTA
E1-RT-RAA-R4	GACCAGAAGATCAGGAACTCTAGAAGAATTCAGAT
E1-RT-RAA-F5	TCGGAAGAGACAGGTACGTTAATAGTTAATAGCG
E1-RT-RAA-6F	GTTTCGGAAGAGACAGGTACGTTAATAGTT
SARS-CoV-E-F	CAACGGTTTACGTCTACTCGCGTGTTAAA
SARS-CoV-E-R	TTTAACACGCGAGTAGACGTAAACCGTTG
MERS-ORF1a-F	TACGAGCCCACTACTCCCATTTCGTCAG
MERS-ORF1a-R	CTGACGAAATGGGAGTAGTGGGCTCGTA
MERS-upE-F	CTCTGCGCTACTATGGGTCCCGTGTAGAG
MERS-upE-R	CTCTACACGGGACCCATAGTAGCGCAGAG
MERS-ORF1b-F	TCGATGTTGAGGGTGCTCATGCTTCCCGT
MERS-ORF1b-R	ACGGGAAGCATGAGCACCCTCAACATCGA
Reporter	HEX-TTATTATTATTA-BHQ
SH-DNA	Thiol-$(T)_{74}$-TCAGTGAATCGATCTAGTCAGTCAG
Biotin-DNA	Biotin-$(T)_{74}$-CTGACTGACTAGATCGATTCACTGA
RT-qPCR-F	GGGGAACTTCTCCTGCTAGAAT
RT-qPCR-R	CAGACATTTTGCTCTCAAGCTG
RT-qPCR-probe	FAM-TTGCTGCTGCTTGACAGATT-TARMA

The combination of E1-RT-RAA-R2 and E1-RT-RAA-6F was the primer pair with the highest amplification efficiency.

Table S2. Validation of CRISPR-PTS assay using spiked throat swab samples.

Sample ID	Pseudovirus added final concentration (copies/μL)	CRISPR-PTS		RT-qPCR	
		TL intensity	Conclusion	Ct value	Conclusion
1	Non	301.0	-	Undetermined	-
2	Non	293.0	-	Undetermined	-
3	Non	246.4	-	Undetermined	-
4	Non	278.2	-	Undetermined	-
5	Non	299.3	-	Undetermined	-
6	Non	228.1	-	Undetermined	-
7	Non	343.5	-	Undetermined	-
8[1]	Non	383.9	+	Undetermined	-
9	Non	255.6	-	Undetermined	-
10	Non	191.3	-	Undetermined	-
11	Non	209.9	-	Undetermined	-
12	Non	295.2	-	Undetermined	-
13	Non	290.3	-	Undetermined	-
14	Non	183.3	-	Undetermined	-
15	Non	256.9	-	Undetermined	-
16	Non	306.8	-	Undetermined	-
17	Non	232.7	-	41.93	-
18	Non	238.7	-	Undetermined	-
19	Non	239.6	-	Undetermined	-
20	Non	266.6	-	40.20	-
21[2]	1	388.5	+	36.55	-
22	2.5	429.8	+	34.91	+
23	4	473.2	+	34.34	+

24	5	507.3	+	34.08	+
25	7.5	513.0	+	33.61	+
26	8	545.0	+	33.63	+
27	9	438.5	+	33.13	+
28	12.5	492.4	+	32.71	+
29	15	580.6	+	32.58	+
30	20	634.2	+	32.26	+
31	25	587.0	+	31.50	+
32	30	634.1	+	31.40	+
33	40	648.8	+	30.97	+
34	45	636.3	+	30.71	+
35	50	647.2	+	30.60	+
36	55	574.3	+	30.52	+
37	60	637.6	+	30.34	+
38	65	636.9	+	30.27	+
39	70	660.8	+	30.05	+
40	75	478.6	+	29.94	+
41	80	673.3	+	29.96	+
42	90	655.3	+	29.87	+
43	100	691.8	+	30.41	+
44	150	619.5	+	28.14	+
45	200	806.9	+	27.65	+
46	250	585.7	+	28.25	+
47	500	655.0	+	27.32	+
48	600	832.1	+	26.42	+
49	750	853.9	+	26.82	+
50	1250	751.2	+	26.28	+
51	1300	811.3	+	25.55	+
52	1500	1025.8	+	25.44	+

53	1800	916.2	+	25.30	+
54	2000	762.0	+	26.48	+

(-) Negative result; (+) Positive result.

[1]Sample No. 8 was actually negative control but appeared false-positive result.

[2]Sample No.21 was added with 1 copy/μL pseudovirus at final concentration. Single copy level of pseudovirus was not detected by RT-qPCR but detected by CRISPR-PTS assay established in this study.

Table S3. Comparison between CRISPR-PTS assay and RT-qPCR on various detection parameters

	CRISPR-PTS assay	RT-qPCR
Target	E gene	ORF1ab, N gene
Technological process	1. Sample pretreatment (70 ℃, 10 min) 2. RT-RAA (42°C, 30 min) 3. Cas12a cleavage (37 °C, 40 min) 4. Strip signal readout (37 °C, 2 min)	1. Genome extraction (r.t., 1 h) 2. Reverse transcription (50 °C,15 min) 3. Denature (95 °C, 5 min) 4. Amplification (95 °C, 10 s; 55 °C 40 s; *45)
Sample-to-result time	82 min	117.5 min
LOD	1 copy per μl input	2.5 copies per μl input
Assay results	Qualitative	Quantitative
Material cost	6.08 $	11.04 $
Bulky instrumentation required	No	Yes