Supplementary Information

CRISPR/Cas12a-mediated gold nanoparticle aggregation for colorimetric detection of SARS-CoV-2

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1. Experiments

Preparation of SARS-CoV-2 Viral RNA

We obtained the original SARS-CoV-2 virus strain from the University of Saskatchewan, Canada (SARS-CoV-2/CANADA/VIDO 01/2020). The virus was generated from the infection of Vero-E6 cells. RT-qPCR was used to detect the N gene and quantify the amount of viral RNA.

Swab samples and RNA extraction

All respiratory swab samples used in this study were collected and treated by Alberta Precision Laboratories, Canada. Three extraction platforms, NUCLISENS easyMAG (BioMerieux), KingFisher Flex System (Thermo Fisher Scientific), and STARlet automated extractor (Hamilton), were used.

RT-LAMP reaction

Table S1 summarizes the sequences of the target region in the N and E genes. Primers for both genes were synthesized by Integrated DNA Technologies (IDT).

The same RT-LAMP protocols reported previously were used in this study.[1] The reaction solution (25 µL) contained 1.4 mM deoxynucleotide (dNTP), 1x isothermal amplification buffer, 8 mM $MgSO_4$ (including 2 mM $MgSO_4$ in 1x isothermal amplification buffer), 5 µM outer primers F3 and B3, 40 µM inner primers FIP and BIP, 20 µM loop primers LF and LB, 0.3 U/µL WarmStart RTx reverse transcriptase, 0.32 U/µL Bst2.0 polymerase, and 0.16 U/µL RNase inhibitor. All reagents were purchased from New England BioLabs (NEB) except for the RNAse inhibitor (Invitrogen). The tube was placed in a myBlock Mini Digital Dry Bath (Benchmark Scientific) and the reaction was performed at 62 °C for 30 min.

Functionalization of AuNPs

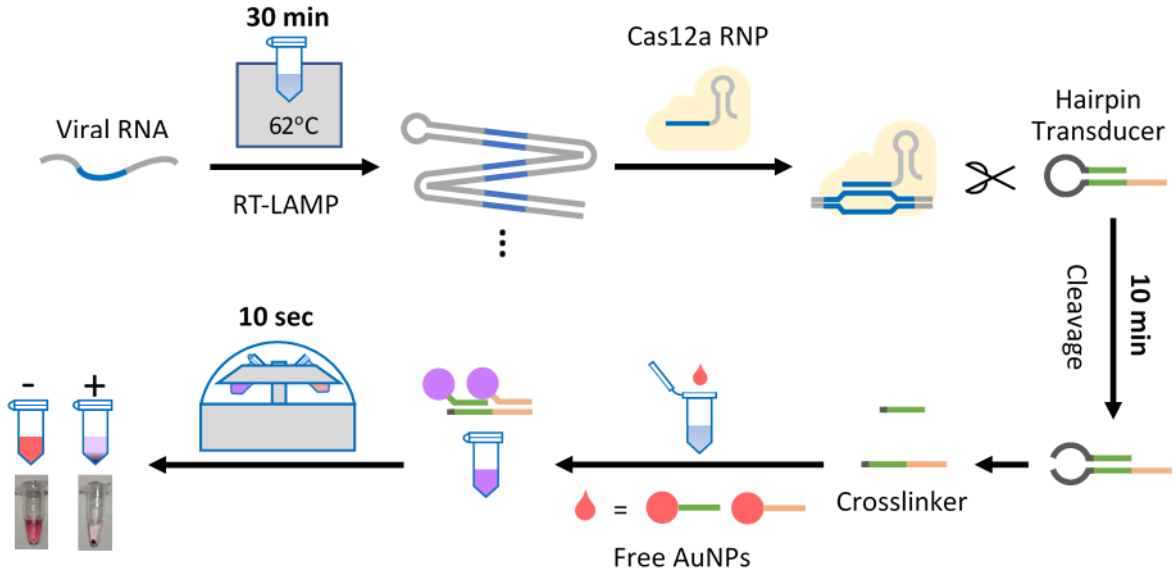
AuNPs 20 nm in size were used in this study because 20-nm AuNPs have been commonly used in colorimetric assays.[2] The AuNPs were functionalized with thiolated DNA sequences (Table S3). For functionalization of AuNPs with two DNA strands, 1 mL of 20 nM AuNP (7.0 x10^{11}/mL, Ted Pella) was first incubated with 100 µL 20% Tween 80 (Sigma-Aldrich) for 5 min in a 2 mL micro test tube. Thirteen microlitres of 100 µM thiolated DNA (AuNP-DNA1 or AuNP-DNA2) were then added into the tube, followed by incubation for another 5 min. The molar ratio of DNA to AuNP was approximately 1000:1. Afterward, 400 µL of 5 M NaCl was added and the solution was incubated for 3 h for salt aging. After salt aging, the mixture was centrifuged at 21,100 g for 11 min to remove the supernatant solution that contained unconjugated DNA. The washing step, which consisted of (i) addition of 1 mL of washing buffer (10 mM Tris-HCl + 0.05% Tween 20) to the precipitate, (ii) resuspension of the precipitate in the washing buffer, (iii) centrifugation at 21,100 g for 11 min, and (iv) removal of the supernatant solution, was repeated three times. Finally, the functionalized AuNPs were resuspended in 50 µL of washing buffer to a final concentration of 20 nM.

Cas12a-mediated AuNP aggregation

Table S2 lists the sequences of two guide RNAs (gRNAs) that specifically recognize the RT-LAMP amplicons of the N gene and the E gene, respectively. Table S3 lists the sequences of hairpin transducers (HT) of different designs. HT18-InvdT was used to perform the colorimetric detection of the viral RNA target.

Two µM ribonucleoprotein (RNP) was first prepared by incubating 2 µM EnGen Lba Cas12a protein (NEB) with 3 µM gRNA in 1x NEBuffer 2.1 (NEB) at room temperature for 30 min. Thirty microliters of reaction solution contained 200 nM RNP, 1 µM linker, 20 nM DNA activator (Table S2, or nuclease-free water as negative control) and 1x NEBuffer 2.1. This solution was incubated at room temperature for 10 min for cleavage of HT. Afterward, 30 µL of the AuNP solution containing 2 nM of each AuNP and 1.67 M NaCl was added to initiate the aggregation. The final concentrations of AuNP and NaCl were 1 nM and 0.83 M in 60 µL of mixture. After 1 min of incubation, the mixture was centrifuged for 10 s in the spinner at 3,000 rpm. A photograph of the test tube was taken using a smartphone camera. Finally, 50 µL of the supernatant of the centrifuged mixture was loaded on the 96-well plate to record the UV-Vis spectrum from 400 to 700 nm.

2. Schematics showing the overall operation of the assay



Scheme S1. Overall process of CRISPR/Cas12a-mediated AuNP aggregation for colorimetric detection of viral RNA. The viral RNA is first amplified by the RT-LAMP at 62 °C for 30 min. Cas12a-gRNA RNP is added into the RT-LAMP reaction solution for binding to the specific region of amplicons. Cas12a-gRNA RNP is then activated to cleave the DNA loop of the hairpin transducer. The *trans*-cleavage reaction is performed at room temperature for 10 min. Consequently, the RNA crosslinker in the hairpin transducer is released from the lock. The crosslinker brings AuNPs together, generating AuNP aggregates and causing the color change from red to purple. The solution is centrifuged using a portable spinner to precipitate the aggregates, resulting in a clear color change of the solution.

3. Investigation of hairpin transducers of toehold design

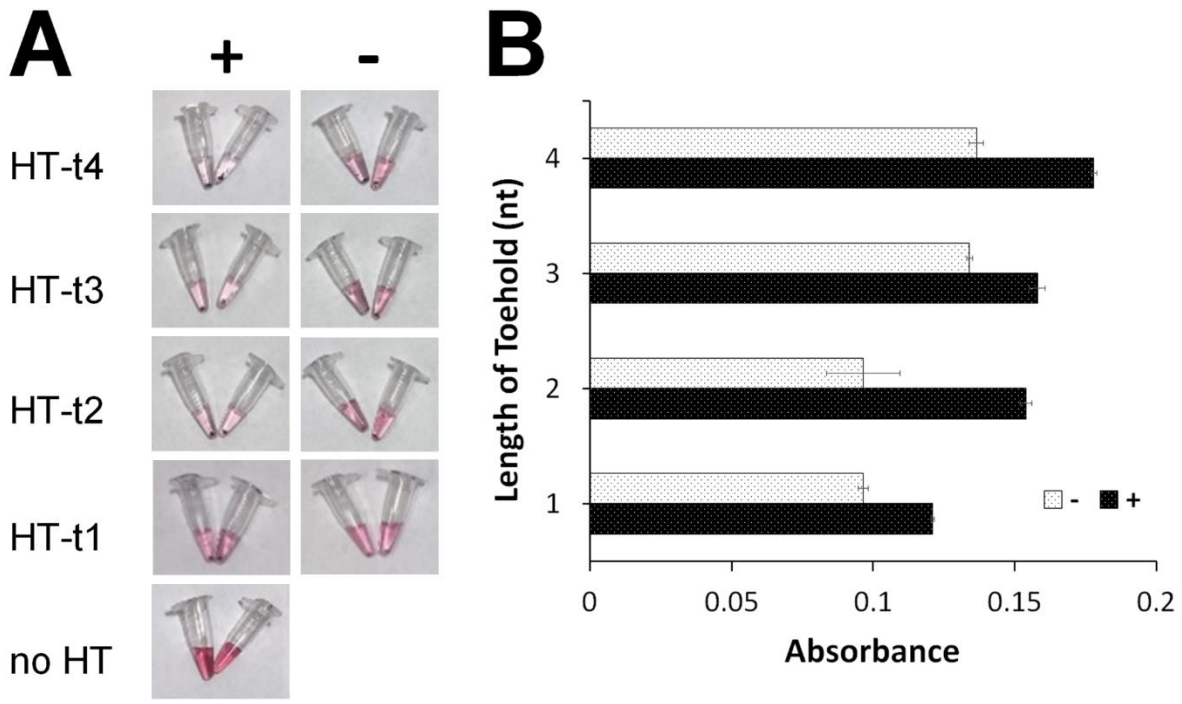
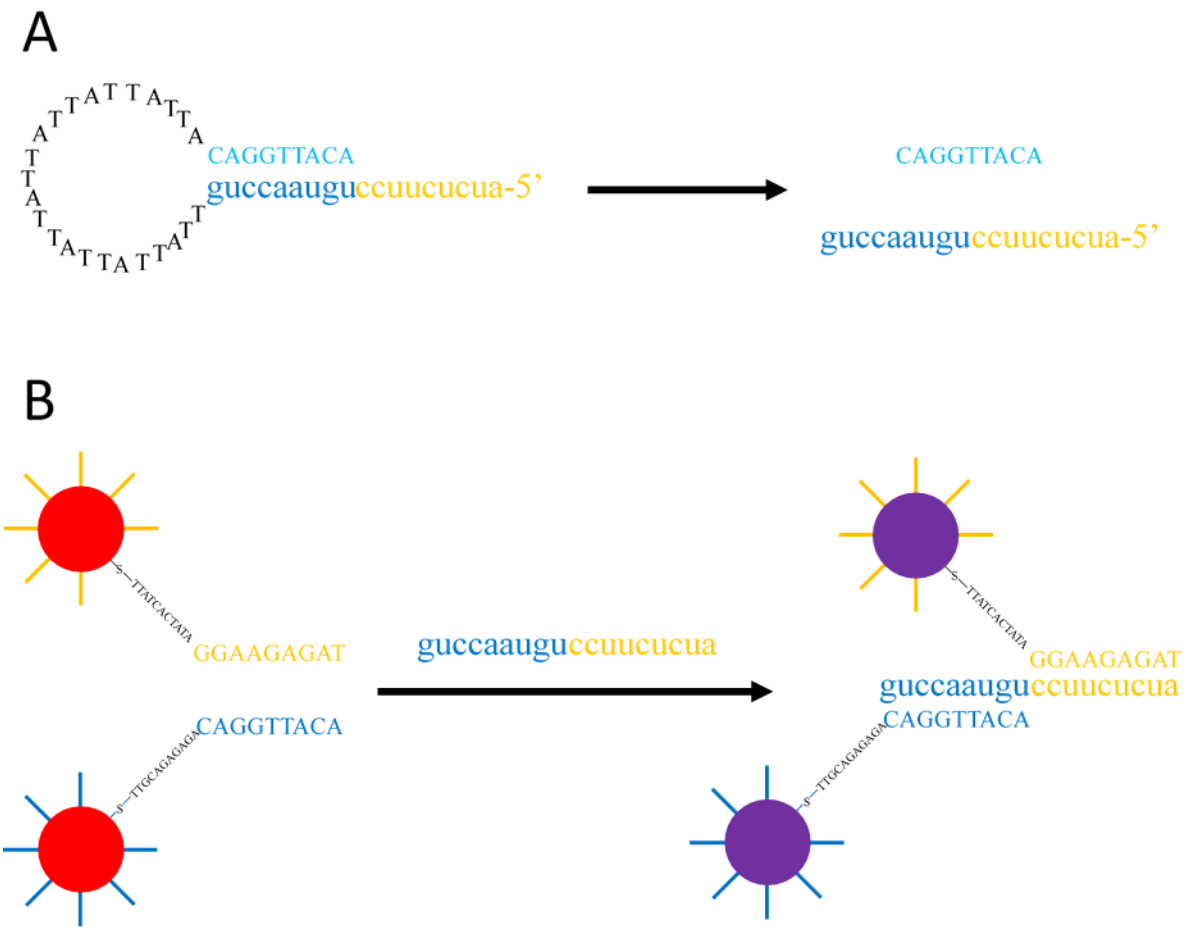


Figure S1. Comparison of hairpin transducer containing different toehold lengths by colorimetric assay (A) and UV-Vis absorbance (B). '+' indicates samples containing 20 nM DNA activator for Cas12a-gRNA. '-' indicates samples containing all reagents but no DNA activator. 'no HT' indicates the negative control, containing all reagents except the hairpin transducer. 'Absorbance' is the difference of UV-Vis absorbance values at 530 nm (Figure S2) between the samples and the negative control.

4. Sequence design



Scheme S2. Sequence designs of the hairpin transducer HT18 (sequence shown in Table S3) and the DNAs functionalized on AuNPs. The sequences in uppercase are DNA and the sequences in lowercase are RNA. (A) Before the cleavage, the blue sequence is stably hybridized to the light-blue sequence (T_m = 48 °C). Because the *trans*-cleavage activity of Cas12a only cleaves ssDNA, not RNA, the ssDNA loop region of the hairpin transducer is cleaved by the activated Cas12a. The cleavage of the ssDNA loop changes the intra-molecular hybridization to inter-molecular hybridization and leads to a substantial decrease in the stability of the hybridization. The estimated melting temperature (T_m) of the hybrid before the cleavage of the loop is 48 °C, but decreases to 25.5 °C after the cleavage of the ssDNA loop. Thus, the DNA lock spontaneously dissociates from the RNA crosslinker after the cleavage of the ssDNA loop. (B) Hybridization of the RNA crosslinker to the two DNAs conjugated on two AuNPs assembles AuNPs and results in the color change from red to purple. The conjugated DNAs have a much higher local concentration than the released DNA locks, thus displacing the DNA locks from the RNA crosslinker.

Table S1. Sequences of the N and E gene target regions of SARS-CoV-2 and RT-LAMP primers. For each gene target, six primers were used: F3 (forward outer primer), B3 (backward outer primer), FIP (forward inner primer), BIP (backward inner primer), BL (backward loop primer), and FL (forward loop primer). The underlined sequences of the primers can hybridize with the target region with the same labels.

Name	Sequence (5' → 3')
N gene target region	AAC ACA AGC TTT CGG CAG ACG TGG TCC AGA ACA AAC CCA AGG AAA TTT TGG GGA C CAG GAA CTA ATC AGA CAA GGA A CTG ATT ACA AAC ATT GGC CGC A AAT TGC ACA ATT TGC CCC CAG CGC TTC AGC GTT CTT CGG AAT GTC G *CGC ATT GGC ATG GAA GTC AC ACC TTC GGG AAC GTG GTT GAC CTA CAC AGG TGC CAT CAA A* TT *GGA TGA CAA AGA TCC AAA TTT C*
N-F3	AAC ACA AGC TTT CGG CAG
N-B3	*G AAA TTT GGA TCT TTG TCA TCC*
N-FIP	T GCG GCC AAT GTT TGT AAT CAG CCA AGG AAA TTT TGG GGA C
N-BIP	*CGC ATT GGC ATG GAA GTC AC T TTG ATG GCA CCT GTG TAG*
N-BL	T TCC TTG TCT GAT TAG TTC
N-FL	*ACC TTC GGG AAC GTG GTT*
E gene target region	CCG ACG ACG ACT ACT AGC GTG CC TTT GTA AGC ACA AGC TGA TG A GTA CGA ACT TAT GTA CTC A TTC GTT TCG GAA GAG ACA GGT ACG TTA ATA GTT AAT AGC GTA CTT CTT TTT CTT GCT TTC GTG GTA TTC TTG CTA GTT ACA *CTA GCC ATC CTT ACT GCG CT TCG ATT GTG TGC GTA CTG C TGC AAT ATT GTT AAC GTG AGT* CTT GTA A *AAC CTT CTT TTT ACG TTT ACT CT*
E-F3	CCG ACG ACG ACT ACT AGC
E-B3	*AG AGT AAA CGT AAA AAG AAG GTT*
E-FIP	ACC TGT CTC TTC CGA AAC GAA TTT GTA AGC ACA AGC TGA TG
E-BIP	*CTA GCC ATC CTT ACT GCG CT ACT CAC GTT AAC AAT ATT GCA*
E-BL	T GAG TAC ATA AGT TCG TAC
E-FL	*TCG ATT GTG TGC GTA CTG C*

Table S2. Sequences of Cas12a gRNA and DNA activator. In two gRNAs, the sequences in red are the spacer regions. The N gene activator is dsDNA composed of two strands, 1 and 2. The red sequence of strand 2 is the protospacer region and the blue sequence of strand 1 is the protospacer adjacent motif (PAM) region.

Name	Sequence (5' → 3')
N-gRNA	UAA UUU CUA CUA AGU GUA GAU CCC CCA GCG CUU CAG CGU UC
E-gRNA	UAA UUU CUA CUA AGU GUA GAU GUG GUA UUC UUG CUA GUU AC
N-activator strand 1	GCA AAT TGC ACA A TTT G CCC CCA GCG CTT CAG CGT TC TTC GGA ATG TCG C
N-activator strand 2	G CGA CAT TCC GAA GA ACG CTG AAG CGC TGG GGG C AAA T TGT GCA ATT TGC

Table S3. Sequences of hairpin transducers (HT) and thiolated DNAs. In the sequences of HT, RNAs in blue and green are subdomains I and II of the RNA crosslinker that can respectively hybridize with complementary sequences of 'AuNP-DNA1' in bold and 'AuNP-DNA2' in italics. The underlined sequences of 'AuNP-DNA1' and 'AuNP-DNA2' are the complementary regions of 'HT18-invdT'. Activated Cas12a RNP cleaves the DNA loop domain in black. The DNA lock domain of HT in bold and light blue can form a hairpin structure by binding to subdomain I. 'HT-t1' indicates the HT of toehold design has a toehold of 1 nt. 'HT20' means the HT of no-toehold design has an RNA crosslinker domain of 20 nt (10 + 10). 'HT18-InvdT' has the same sequence of 'HT18' except that its 3'-end is modified with the *inverted* dT. 'AuNP-DNA1' and 'AuNP-DNA2' are conjugated with the thiol group at the 5'-end for the functionalization of AuNP.

Name	Sequence (5' → 3')
HT-t1	rArUrC rUrCrU rUrCrC rUrArU rArGrU rUrGrU rArArC rCrUrG rUrCrU rCrUrC TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TT **GAG AGA CAG GTT AC**
HT-t2	rArUrC rUrCrU rUrCrC rUrArU rArGrU rUrGrU rArArC rCrUrG rUrCrU rCrUrC TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TT **GAG AGA CAG GTT A**
HT-t3	rArUrC rUrCrU rUrCrC rUrArU rArGrU rUrGrU rArArC rCrUrG rUrCrU rCrUrC TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TT **GAG AGA CAG GTT**
HT-t4	rArUrC rUrCrU rUrCrC rUrArU rArGrU rUrGrU rArArC rCrUrG rUrCrU rCrUrC TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TTA TT **GAG AGA CAG GT**
HT20	rArUrC rUrCrU rUrCrC rU rUrGrU rArArC rCrUrG rU TTA TTA TTA TTA TTA TTA TTA TTA **A CAG GTT ACA**
HT18	rArUrC rUrCrU rUrCrC rUrGrU rArArC rCrUrG TTA TTA TTA TTA TTA TTA TTA TTA **CAG GTT ACA**
HT16	rArUrC rUrCrU rUrC rUrGrU rArArC rCrU TTA TTA TTA TTA TTA TTA TTA TTA **AG GTT ACA**
HT18-InvdT	rArUrC rUrCrU rUrCrC rUrGrU rArArC rCrUrG TTA TTA TTA TTA TTA TTA TTA TTA **CAG GTT ACA** -InvdT
AuNP-DNA1	HS-TTA TC **ACT ATA GGA AGA GAT**
AuNP-DNA2	HS-TTG CA *GAG AGA CAG GTT ACA*

Table S4. Sequences of the crosslinker used for the optimization of AuNP aggregation. Sequences in orange and blue are subdomains I and II that can respectively hybridize with complementary sequences of 'AuNP-DNA1' in bold and 'AuNP-DNA2' in italics (Table S3).

Name	Sequence (5' → 3')
L20	ATC TCT TCC T TGT AAC CTG T
L18	ATC TCT TCC TGT AAC CTG
L16	ATC TCT TC TGT AAC CT
L14	ATC TCT T TGT AAC C
L12	ATC TCT TGT AAC

5. UV-Visible absorption spectra

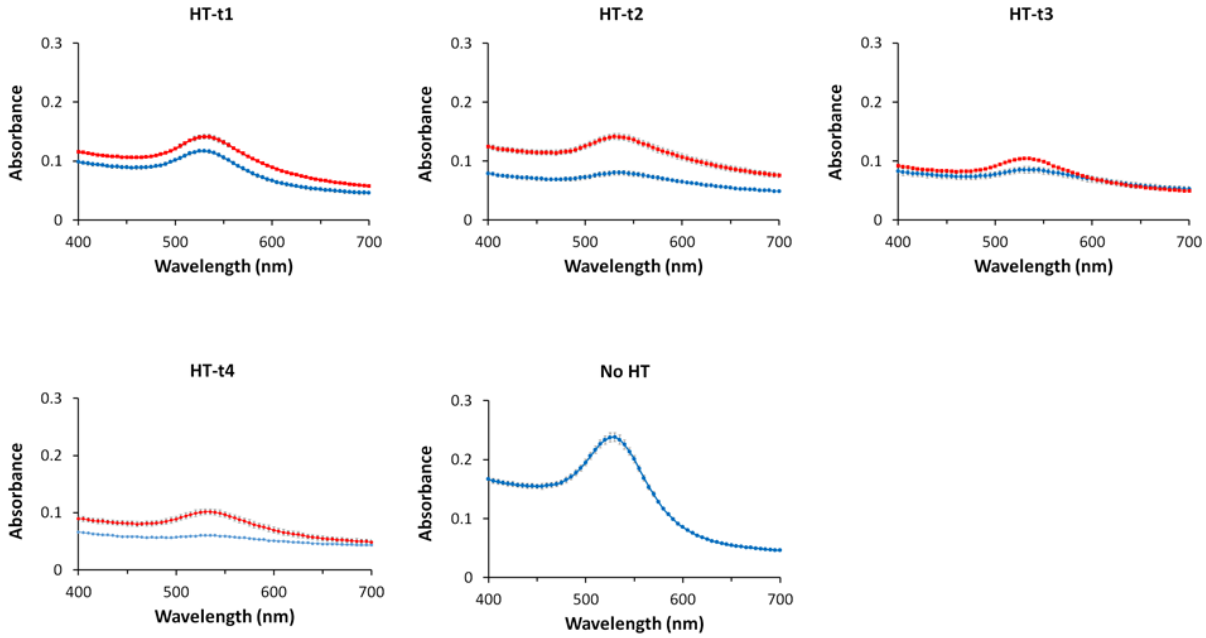


Figure S2. Absorption spectra obtained from measuring positive (blue curve) and negative (red) samples using hairpin transducers (HT) of different toehold lengths. 'HT-t4' indicates that the HT has a 4-nt toehold. The blue curves result from positive samples containing 20 nM DNA activator and the red curves result from negative samples without the activator. The negative control contained all reagents but no HT. After *trans*-cleavage for 10 min and addition of DNA-functionalized AuNPs, the samples were centrifuged for 10 s using a portable spinner. The supernatants were loaded on a 96-well plate to record the UV-Vis spectra from 400 to 700 nm.

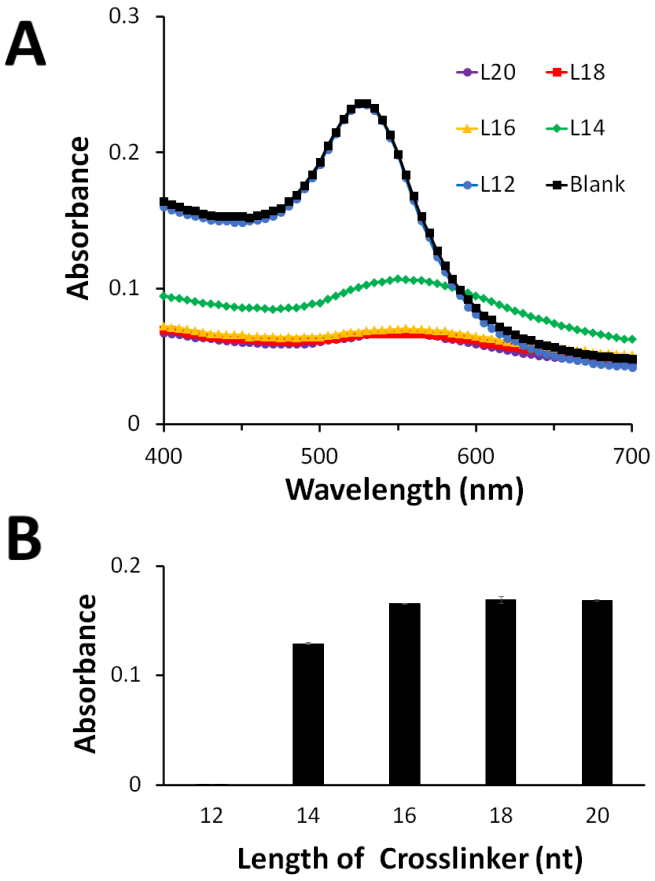


Figure S3. Determination of the shortest length of crosslinker for sufficient aggregation of AuNPs. (A) UV-Vis absorption spectra of samples containing 1 nM of each DNA-functionalized AuNP, 100 nM of crosslinker of different lengths, 800 mM NaCl and 1x NEBuffer 2.1. After addition of the crosslinker, the solutions were centrifuged for 10 s in the spinner. The supernatants were loaded on a 96-well plate to record the UV-Vis spectra from 400 to 700 nm. (B) The difference of absorbance between the samples and the blank at 530 nm.

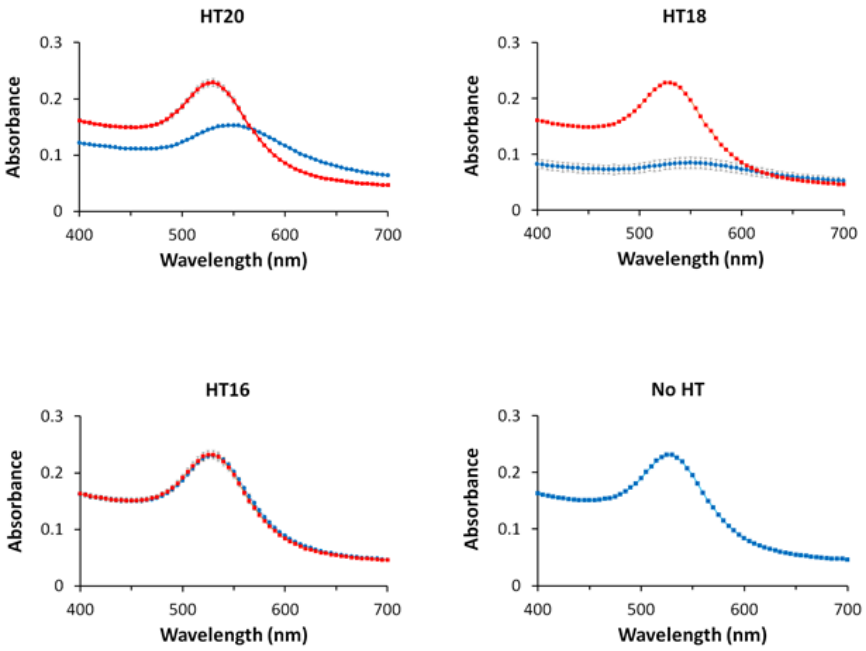


Figure S4. Absorption spectra obtained from measuring positive (blue curve) and negative (red) samples using hairpin transducers (HT) of different lengths of RNA crosslinker. 'HT20' indicates that the HT has an RNA crosslinker domain of 20 nt. The blue curves result from positive samples containing 20 nM DNA activator for Cas12a-gRNA and the red curves are from negative samples without the activator. The negative control contained all reagents but no HT. After *trans*-cleavage for 10 min and addition of DNA-functionalized AuNPs, the samples were centrifuged for 10 s in the spinner. The supernatants were loaded on a 96-well plate to record the UV-Vis spectra from 400 to 700 nm.

6. A 10-second spinning enhances visual differentiation

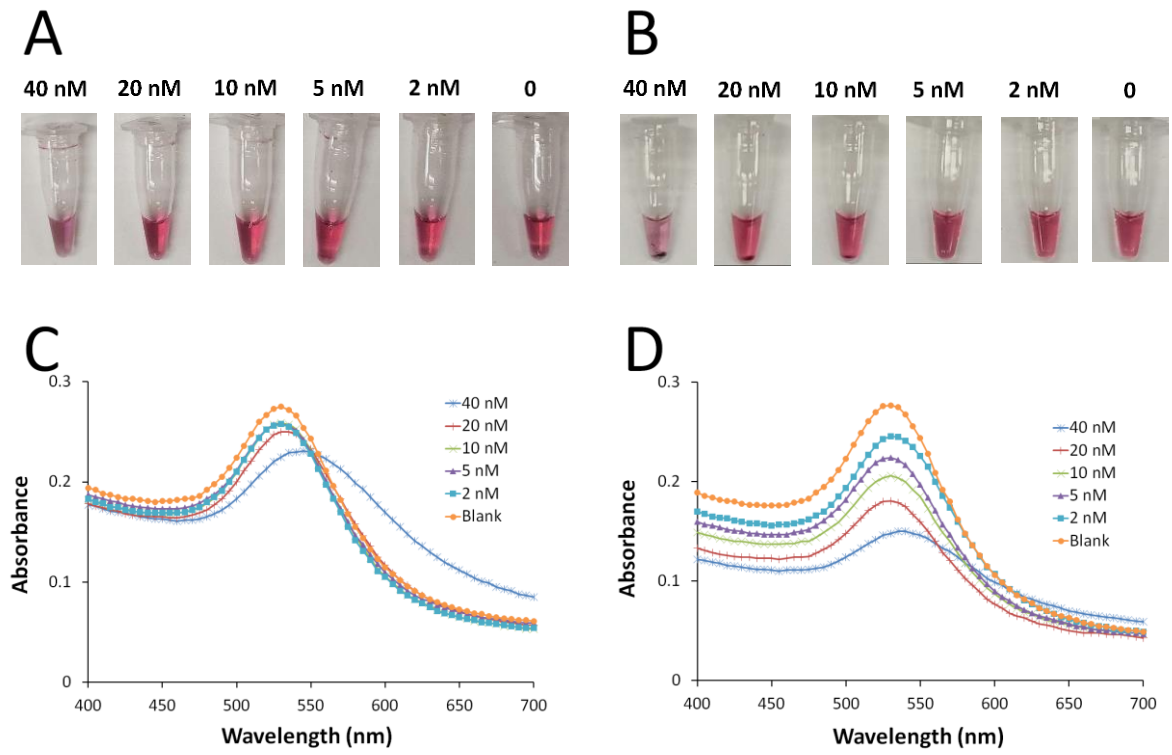


Figure S5. Improvement of the sensitivity by spinning aggregated AuNPs at 3,000 rpm for 10 s. (A) and (C) are the photos and UV-Vis spectra of AuNPs assembled by 0–40 nM L18 before centrifugation. (B) and (D) are the photos and UV-Vis spectra of AuNPs after centrifugation. Note that the changes in absorbance values are much larger than the red shifts in maximal absorption wavelength (λ_{max}) in response to low concentrations of the target. Moreover, a short (10 s) spin of the reaction mixture enables rapid detection of the changes in absorbance value and color intensity within 1 min. Therefore, detection based on the reduction of absorbance value provides a lower limit of detection and faster analysis.

7. Detection of viral RNA of SARS-CoV-2

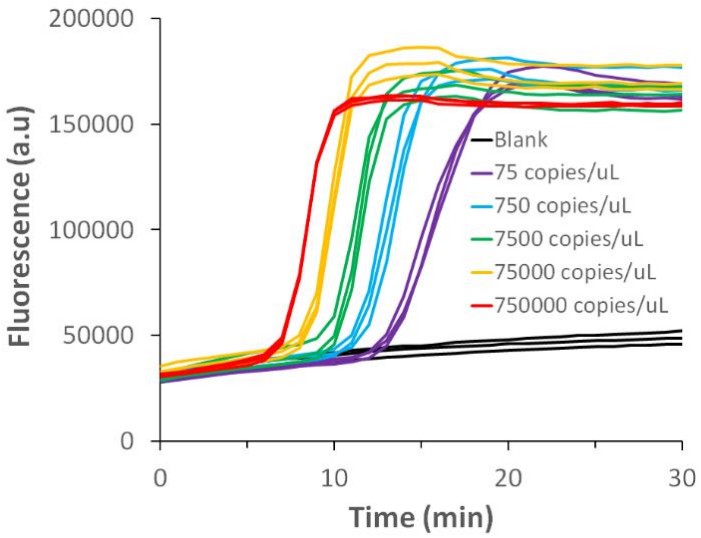


Figure S6. Real-time detection of SARS-CoV-2 viral N gene (75–750,000 copies/μL) using RT-LAMP. SYBR Green was used for detection and 5 μL of the viral RNA sample was used for each reaction. Each curve stands for one of the triplicate analysis.

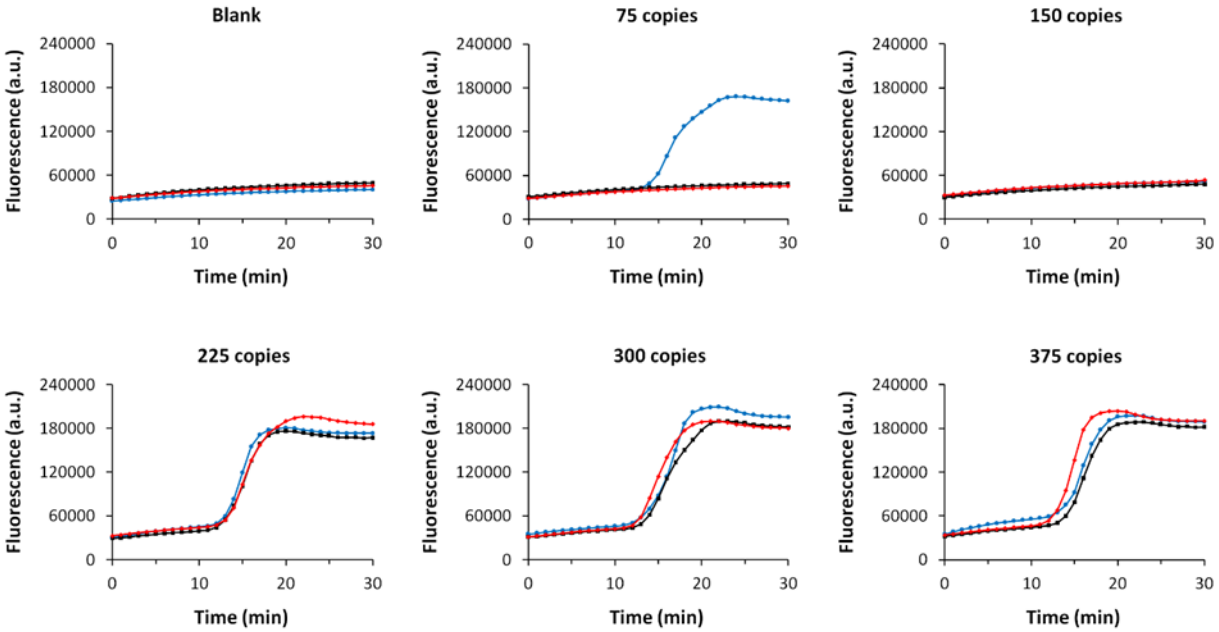


Figure S7. Real-time detection of the N gene of viral RNA at low copy numbers using RT-LAMP. Three curves in three different colors represent triplicate analysis.

Figure S8. Detection of the N gene at low copy numbers using the colorimetric method. The same RT-LAMP samples shown in Figure S6 were used. Cas12a-gRNA RNP and hairpin transducer were added to the RT-LAMP samples to perform the *trans*-cleavage reaction for 10 min. DNA-functionalized AuNPs were added, and AuNP aggregation was induced by the crosslinker. Each tube corresponded to each curve in Figure S6.

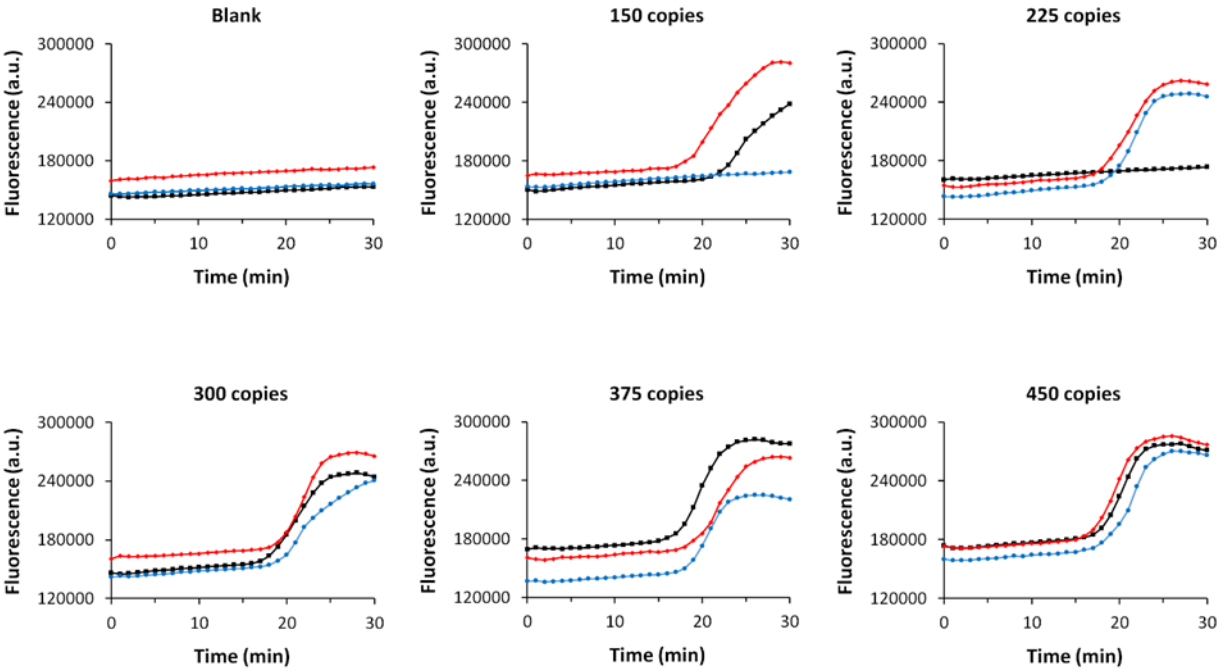


Figure S9. Real-time detection of the E gene at low copy numbers using RT-LAMP. Three curves labeled in three different colors represent triplicate analysis.

Figure S10. Detection of the E gene at low copy numbers using the colorimetric method. The same RT-LAMP samples shown in Figure S8 were analyzed. Cas12a RNP and hairpin transducer were added to the RT-LAMP samples, and the *trans*-cleavage reaction was allowed for 10 min. DNA-functionalized AuNPs were then added. The available crosslinker from the positive samples induced AuNP aggregation and the color change. Each tube corresponded to each curve in Figure S8.

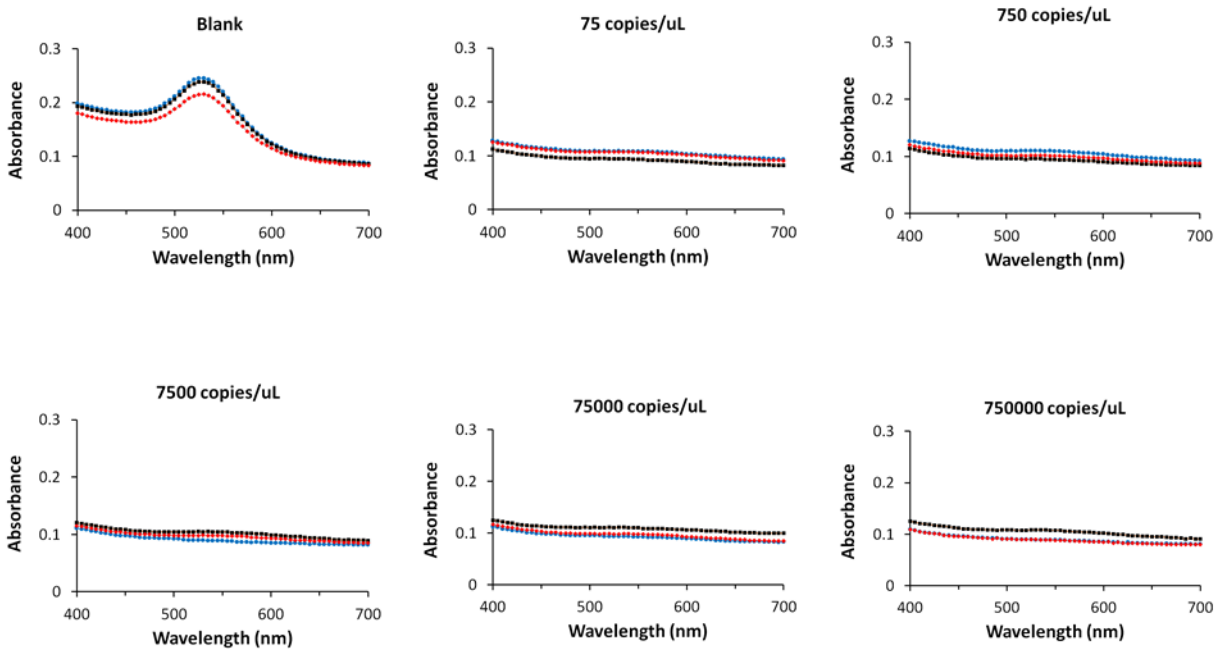


Figure S11. UV-Vis absorption spectra from the analysis of the samples as shown in Figure 3. Three curves in three different colors represent triplicate analysis.

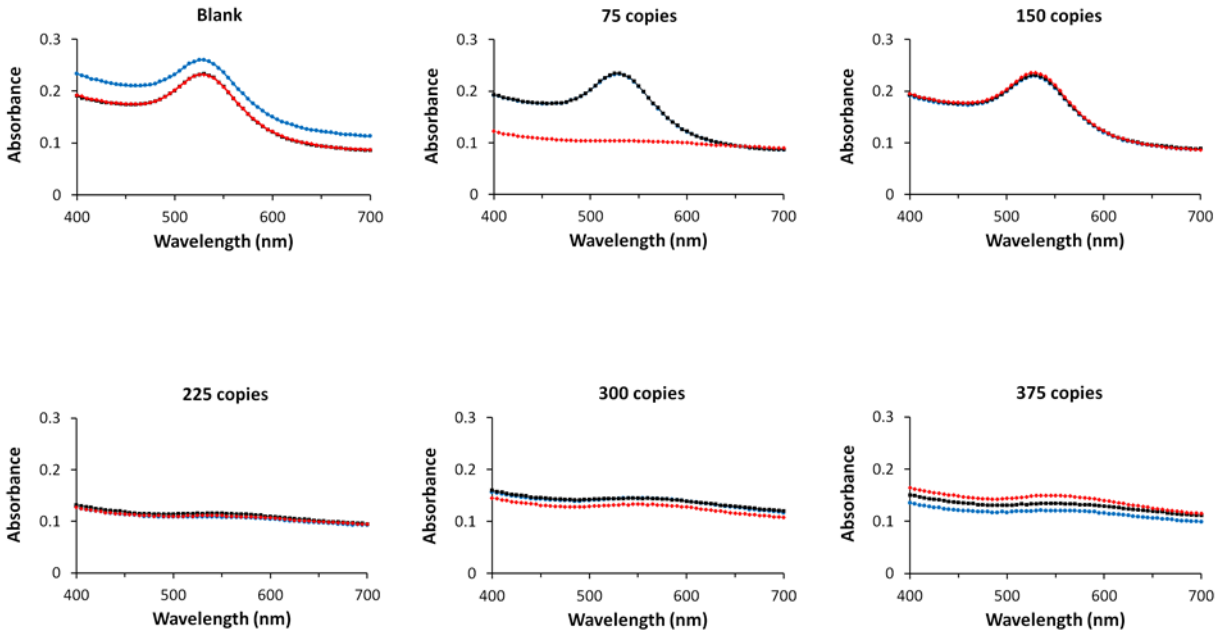


Figure S12. UV-Vis absorption spectra from the analysis of samples for detection of the N gene at low copy numbers. The same samples as shown in Figure S7 were analyzed. Three curves in three different colors represent triplicate analysis.

8. Analysis of clinical samples

Table S5. Summary of the analysis of SARS-CoV-2 N gene in 54 clinical samples.

	Positive samples	Negative samples
Positive results	25/27, 92.6%	0/27, 0%
Negative results	2/27, 7.4%	27/27, 100%

Table S6. Analysis of the N gene of SARS-CoV-2 in patient samples. Each set of tests contained a positive control, a negative control, and 4–6 samples. The positive control was 3750 copies of SARS-CoV-2 viral RNA and the negative control was nuclease-free water. Our collaborating public health laboratory also detected all the samples by using RT-qPCR targeting the E gene of SARS-CoV-2. The results from RT-qPCR are listed in the table.

Set of tests	Sample#	Ct value	Visualized detection
1st	Positive control	N/A	
	Negative control	N/A	
	#57	25.2	
	#59	25.2	
	#1	Negative	
	#2	Negative	
2nd	Positive control	N/A	
	Negative control	N/A	
	#72	20.1	
	#90	26.4	
	#3	Negative	

	#12	Negative	
3rd	Positive control	N/A	
	Negative control	N/A	
	#106	23.5	
	#143	23.9	
	#151	Negative	
	#152	Negative	
4th	Positive control	N/A	
	Negative control	N/A	
	#125	31.1	
	#134	30.2	
	#137	32.6	
	#159	Negative	
	#160	Negative	
	#161	Negative	
5th	Positive control	N/A	
	Negative control	N/A	
	#123	24.3	

	#144	24.9	
	#148	24.3	
	#162	Negative	
	#163	Negative	
	#164	Negative	
6th	Positive control	N/A	
	Negative control	N/A	
	#110	25.8	
	#122	24.9	
	#132	25.7	
	#165	Negative	
	#166	Negative	
	#167	Negative	
7th	Positive control	N/A	
	Negative control	N/A	
	#107	27.6	
	#114	27.4	
	#146	27.6	

	#168	Negative	
	#169	Negative	
	#170	Negative	
8th	Positive control	N/A	
	Negative control	N/A	
	#103	26.6	
	#124	26.7	
	#150	26.4	
	#171	Negative	
	#172	Negative	
	#173	Negative	
9th	Positive control	N/A	
	Negative control	N/A	
	#115	32.6	
	#127	31.7	
	#129	28.2	
	#174	Negative	
	#175	Negative	

	#176	Negative	
10th	Positive control	N/A	
	Negative control	N/A	
	#126	35.4	
	#130	37.0	
	#145	25.3	
	#177	Negative	
	#178	Negative	
	#179	Negative	

Table S7. Summary of the analysis of SARS-CoV-2 E gene in 8 clinical samples.

	Positive samples	Negative samples
Positive results	8/8, 100%	0/8, 0%
Negative results	0/8, 0%	8/8, 100%

Table S8. Analysis of the E gene of SARS-CoV-2 in patient samples. Each set of tests contained a positive control, a negative control, and 4 samples. The positive and negative controls were the same as noted in Table S6.

Set of tests	Sample#	Ct value	Visualized detection
1st	Positive control	N/A	
	Negative control	N/A	
	#57	25.2	
	#59	25.2	

	#1	Negative	
	#2	Negative	
2nd	Positive control	N/A	
	Negative control	N/A	
	#72	20.1	
	#90	26.4	
	#3	Negative	
	#12	Negative	

9. Comparison of this assay with other reported assays

Table S9. Analytical performance and equipment required for our method and other reported assays for the SARS-CoV-2 viral RNA.

Method	Reagent for signal generation	Specificity	Sensitivity gene	Time (min)	Equipment for signal generation	Ref.
Colorimetric	CRISPR/Cas12 AuNP	High	225 copies N	45	No	This
Fluorescence	CRISPR/Cas12 reporter	High	225 copies N	40	LED/UV light	1
Colorimetric	pH indicator	Low	10 copies ORF1ab	20	No	3
Fluorescence	Fluorescent calcein	Low	20 copies ORF1ab-4	20	No	4
Lateral Flow	CRISPR/Cas13 AuNP	High	10-100 copies/µL S/ORF1ab	60	HybriDetect Dipstick	5
Lateral Flow	CRISPR/Cas12 AuNP	High	20 copies	40	Milenia HybriDetect 1	6
Fluorescence	CRISPR/Cas12 reporter	High	5 copies N	40	LED/UV light	7
Colorimetric	CRISPR/Cas12 AuNP	High	1 copy N/ORF1ab	50-80	No	8

References

1. B. Pang, J. Xu, Y. Liu, H. Peng, W. Feng, Y. Cao, J. Wu, H. Xiao, K. Pabbaraju, G. Tipples, M. A. Joyce, H. A. Saffran, D.L. Tyrrell, H. Zhang and X. C. Le, *Anal. Chem.*, 2020, **92**, 16204.

2. (a) N. L. Rosi, D. A. Giljohann, C. S. Thaxton, A. K. R. Lytton-Jean, M. S. Han and C. A. Mirkin, Science, 2006, 312, 1027. (b) J. S. Lee, P. A. Ulmann, M. S. Han and C. A. Mirkin, Nano Lett., 2008, 8, 529. (c) S. K. Kailasa, J. R. Koduru, M. L. Desai, T. J. Park, R. K. Singhal and H. Basu, *TRAC-Trend. Anal. Chem.*, 2018, **105**, 106.

3. L. Yu, S. Wu, X. Hao, X. Dong, L. Mao, V. Pelechano, W. H. Chen and X. Yin, *Clin. Chem.*, 2020, **66**, 975.

4. C. Yan 1, J. Cui 1, L. Huang, B. Du, L. Chen, G. Xue, S. Li, W. Zhang, L. Zhao, Y. Sun, H. Yao, N. Li, H. Zhao, Y. Feng , S. Liu, Q. Zhang, D. Liu and J. Yuan, *Emerg. Microbes Infect.*, 2020, **26**, 773.

5. F. Zhang, O. O. Abudayyeh and J. S. Gootenberg, A protocol for detection of COVID-19 using CRISPR diagnostics, https://www.broadinstitute.org/files/publications/special/COVID-19%20detection%20(updated).pdf

6. J. P. Broughton, X. Deng, G. Yu, C. L. Fasching, V. Servellita, J. Singh, X. Miao, J. A. Streithorst, A. Granados, A. S. Gonzalez, K. Zorn, A. Gopez, E. Hsu, W. Gu, S. Miller, C. Y. Pan, H. Guevara, D. A. Wadford, J. S. Chen and C. Y. Chiu, *Nat. Biotechnol.*, 2020, **38**, 870.

7. X. Ding, K. Yin, Z. Li, R. V. Lalla, E. Ballesteros, M. M. Sfeir and C. Liu, *Nat. Commun.*, 2020, **11**, 1.

8. W. S. Zhang, J. Pan, F. Li, M. Zhu, M. Xu, H. Zhu, Y. Yu and G. Su, *Anal. Chem.*, 2021, **93**, 4126.