Supporting Information

Isothermal amplification and ambient visualization in a single tube for the
detection of SARS-CoV-2 using loop-mediated amplification and CRISPR
technology

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Additional Information on the Preparation of SARS-CoV-2 and its Viral RNA

The original SARS-CoV-2 virus strain (SARS-CoV-2/CANADA/VIDO 01/2020) was obtained from the University of Saskatchewan, Canada. SARS-CoV-2 was produced from the infection of Vero-E6 cells at a multiplicity of infection (MOI) of 0.01 for 48 h, followed by harvesting the supernatant. The amount of virus in the supernatant was assessed by a plaque assay and by RT-quantitative PCR (RT-qPCR). Plaque assays were performed as follows: Vero-E6 cells were infected with serially diluted supernatant in infection medium consisting of Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 1× non-essential amino acids (Gibco), 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 2% fetal bovine serum, 50 IU/mL penicillin, 50 IU/mL streptomycin. After 1h, the infecting medium was removed and monolayers were overlaid with Minimum Essential Medium (MEM) supplemented with 10mM HEPES and 1.2% avicel RC-591 (DuPont). After 48 h, cells were fixed in 10% formaldehyde and stained by 0.5% (w/v) crystal violet. Plaques were counted. The amount of viral RNA was quantified as follows: Cell supernatants (140 μL) were collected at various time points after infection, and RNA was isolated by using the QIAmp Viral RNA Mini kit (Qiagen). Reverse transcription was carried out by using Superscript IV Vilo master mix (Invitrogen). RT-qPCR for detecting the N gene of RNA was performed using Taqman fast Master mix containing 2 μL of cDNA. The primers and Taqman probe for the N gene (N2 primers) were designed by the United States Center for Disease Control and Prevention (CDC). A standard curve was generated using dilutions of positive control standards from CDC (IDT Cat # 10006625).

Additional Information on Reverse Transcription Loop-Mediated Exponential Amplification (RT-LAMP)
Sequences of the amplified regions of the N gene and E gene of SARS-CoV-2, along with sequences of the primer sets for the RT-LAMP reactions, are summarized in Supporting Information Table S1. The matching colors and underlines denote complementary sequences. The primer set designed for amplifying each gene (N or E) recognizes eight regions in the target sequence. All primers were synthesized by Integrated DNA Technologies (IDT, San Diego, CA).

The procedures of RT-LAMP were based on the protocol of New England BioLabs (NEB, www.neb.com/protocols/2014/10/09/typical-rt-lamp-protocol) with minor modifications. Briefly, a 25 μL RT-LAMP reaction solution contained 1.4 mM deoxynucleotide (dNTP, NEB), 1× Isothermal Amplification Buffer (NEB), 0.2 μM each of the outer primers (F3 and B3), 1.6 μM each of the inner primers (FIP and BIP), 0.8 μM each of the loop primers (LF and LB), 4 units of RNase inhibitor (Invitrogen), 7.5 units of WarmStart® RTx reverse transcriptase (NEB), 8 units of Bst 2.0 DNA polymerase (NEB), and 1-10 μL of sample or nuclease-free water (as negative control). RT-LAMP reactions were performed at 62 ℃ for 30 min.

In the experiments of real-time fluorescence detection of the LAMP products, 0.5× SYBR Green I dye (Invitrogen) was also added to the 25 μL of RT-LAMP reaction solution. Fluorescence signals were monitored and recorded every 1 min using a StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific).

Additional Information on CRISPR Cas12a-mediated Fluorescence Detection

Sequences of the guide RNAs (gRNAs), recognizing the specific sequences of the RT-LAMP amplicons, are listed in Supporting Information Table S2. The ssDNA (8 nt) reporter was dually labeled with a fluorophore (6-carboxyfluorescein, 6-FAM) at the 5’ end and a quencher (Iowa Black® fluorescence quencher, IABk FQ) at the 3’ end. The ssDNA reporter served as a substrate
for the trans-cleavage activity of Cas12a. For a few optimization experiments, a dsDNA activator was used, instead of the RT-LAMP amplicon. The dsDNA activator has the same sequence as the amplicon of the N gene. The gRNAs, the ssDNA reporter labeled with a fluorophore/quencher pair, and the dsDNA activator were all obtained from IDT (San Diego, CA).

Cas12a-mediated trans-cleavage of the ssDNA reporter, for the purpose of fluorescence detection, was carried out at room temperature (approximately 23 ℃) for 10 min. The EnGen® Lba Cas12a enzyme (NEB) at 1 μM concentration was pre-incubated with 1.25 μM of gRNA in 1× NEBuffer 2.1 (NEB) to form the ribonucleoprotein (RNP) complex. An aliquot of the RNP complex solution was placed inside the cap of a PCR tube. The optimum concentrations of the Cas12a reaction reagents were 400 nM of the RNP complex, 40 mM MgSO₄, and 10 μM ssDNA reporter in 50 mM Tris-HCl buffer (pH=7.9).

Additional Information on Assay Kit for RT-LAMP and CRISPR Cas12a-mediated Ambient Visualization in a Single Tube

The assay kit, consisting of a 0.2-mL PCR tube and a vial of rehydration buffer solution containing primers and the reporter, was convenient to be shipped to other locations for field applications. The PCR tube was prepared to contain dried (lyophilized) reagents for the assay. The mixture of the RT-LAMP reaction reagents, except the primers, was added at the bottom of the PCR tube. This mixture of the RT-LAMP reaction reagents was composed of 35 nmol of dNTP, 1× Isothermal Amplification Buffer, 150 nmol of MgSO₄, 4 units of RNase inhibitor, 7.5 units of WarmStart® RTx reverse transcriptase, 8 units of Bst 2.0 DNA polymerase and approximate 5 μmol of D-(+)-trehalose dihydrate (Sigma). The RNP complex (22 pmol) for the Cas12a-mediated reaction, 400 nmol of MgSO₄, and 500 nmol of Tris-HCl were added inside the cap of the tube.
The tube was placed in a vacuum desiccator for 2 h to dry the reagents. After the reagents were dry, the tube was capped and stored at 4 °C until use. The vial of rehydration buffer contained the primers for RT-LAMP and 5 μM of ssDNA reporter for the Cas12a-mediated reaction. One vial of rehydration buffer contained the primer set for the N gene and the other vial of rehydration buffer contained the primer set for the E gene.

An aliquot (2-5 μL) of RNA extract sample and 20-23 μL buffer solution containing the primers and reporter were added into the bottom of the tube, mixing with and rehydrating the RT-LAMP reagents. The tube was capped and placed in a temperature controller and the bottom of the tube was maintained at 62 °C for 30 min. After 30 min of the RT-LAMP reactions, the tube was removed from the temperature controller, and left at the room temperature. Inverting and wrist-flicking of the tube mixed the RT-LAMP reaction product with the Cas12a reagents. After 10 min at the room temperature, green fluorescence was visualized under the excitation of a handheld UV lamp and a photo was recorded using a personal smartphone. The analysis of a RNA extract was complete in 40 min, including 30 min for RT-LAMP and 10 min for the Cas12a-mediated detection.

**Additional Information on RT-qPCR Assay Conducted in Parallel with the RT-LAMP and CRISPR Cas12a-mediated Assay**

An RT-qPCR assay for the N2 target, according to the U.S. Center for Disease Control and Prevention (CDC) protocol, was used to compare and confirm the results from analyses using the RT-LAMP and CRISPR Cas12a-mediated assay. A 2-5 μL aliquot of RNA extract samples was mixed with the RT-qPCR master mix that was comprised of 1.5 μL of N2 primer-probe mix from the U.S. CDC EUA kit (IDT cat#10006606) and 1× TaqPath™ 1-Step RT-qPCR Master Mix.
(Thermo Fisher) to a final volume of 20 µL. The sequences of primers and probes are listed in Table S4. The reverse transcription and PCR thermal cycling parameters are summarized in Table S5, consistent with the U.S. CDC instruction of use (https://www.fda.gov/media/134922/download). An DNA standard, the N-gene plasmid purchased from IDT (IDT Cat#10006625), was used to calibrate and quantify synthetically transcribed RNA. The RNA samples with known copy numbers were used to generate standard curves for subsequent quantitation.
**Supplementary Tables**

**Table S1.** Sequences of the amplified regions of the N gene and E gene of SARS-CoV-2 as well as the primers for the RT-LAMP reactions used in this study. The complementary sequences are highlighted with matching colors and underlined. The forward internal primer (FIP) consists of an F1c region at the 5’ end and an F2 region at the 3’ end. The backward internal primer (BIP) consists of a B1c region at the 5’ end and a B2 region at the 3’ end.

| Description               | Sequences (5’ - 3’)                                                                                                                                 |
|---------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| Amplified region of the N | AACACAAGCTTTTCGGCAGACGTGGTCCAGAACAACACCCAAGGAAATTTTGGGGACGAGGAACGAATCTAGACACAGAAGTCTGATTACAAAAATTTTGGGGACGAGGAACGAATCTAGACACAGAAGTCTGATTACAACCATTGGCGCAATTTGGGATCTTTGTCATCCCTTTGTCTGATTAGTTC |
Amplified region of the E gene of SARS-CoV-2

CCGACGACGACTACTAGCGTGCTTTGTAAGCACAAGCCTGATGAG
TACGAACTTTATGTACTCATCGTTTCGGAAGAGACAGGTACGTGA
ATAGTTAATAGCGTACTTCTTTTTTCTGGCTTCGTTGCTTATTCTTGCT
AGTTACACTAGCCATCTCCCTACTGCGCTTCGATTGTGTGCGTACTGC
TGCAATATTGTTAACGTGAGTTCTTGTAACACCTTCTTTTTACGTTC
ACTCT

E gene-F3 CCGACGACGACTACTAGC

E gene-B3 AGAGTTAAACGTAAAAAGAAGGTT

E gene-FIP ACCTGTCTCTTCCGAAACGAA

E gene-BIP CTAAGCCATCCTACTGCGCT-ACTCACGTTAACAATATTGCA

E gene-LB TGAGTACATAAGTTCGTAC

E gene-LF TCGATTGTGTGCCTACTGC
**Table S2.** Sequences of the RT-LAMP amplicons, gRNAs, DNA activator, and ssDNA reporter labeled with a fluorophore/quencher pair. The 20-nt spacer sequences of the gRNAs, which recognize and complement the target, are labeled in red color (for the N gene) and green color (for the E gene). The recognized region in the amplicon is labeled in the matching color. The protospacer adjacent motifs (PAMs) in the N gene and E gene are labeled in blue and purple respectively.

| Description                                      | Type  | Sequences (5’- 3’)                                                                 |
|--------------------------------------------------|-------|-----------------------------------------------------------------------------------|
| RT-LAMP amplicon of the N gene                   | dsDNA | AATTGCACAA**TTTGCCCCCAGCGCTTCAGCGTTCT** TCGGAATGTCG                              |
| gRNA for the N gene                               | RNA   | UAAUUUCUACUAAGUGUAGAUCCCCCAGCGCUUC AGCGUUC                                        |
| Activator for the N gene                          | dsDNA | GCAAATTGCACAA**TTTGCCCCCAGCGCTTCAGCGTTCT** TCTTCGGAATGTCG                         |
| RT-LAMP amplicon of the E gene                   | dsDNA | ACGTTAATTAGTTAATAGCGTACTTTCTTTTTCTTCTGCT **TTCGTGGTATTTCTTTGCTAGTTACA**           |
| gRNA for the E gene                               | RNA   | UAAUUUCUACUAAGUGUAGAU**GUGGUAAUCUUUG** CUAGUUAC                                   |
| Reporter labeled with fluorophore and quencher    | ssDNA | /6-FAM/TTATTATT/IABk/                                                              |

6-FAM: 6-carboxyfluorescein; IABk: Iowa Black® fluorescence quencher
Table S3. Results from the detection of SARS-CoV-2 in 100 human samples. The analyses of the first 82 samples, from sample #1 to sample #82, used 2 µL aliquots of each sample extract for the detection of both the N gene and the E gene. The analyses of the last 18 samples, from sample #83 to sample #100, used 10 µL of each sample extract for the detection of the N gene. There was no sample remaining for the detection of the E gene in the last 18 samples (#83–#100). Typically, 5-10 samples were analyzed in a batch; and each batch included a pair of negative control and positive control (not all control images are shown). Both the negative controls and the positive controls contained all reaction reagents, except that nuclease-free water was used as the sample in the negative control and 3750 copies of SARS-CoV-2 RNA was used as the sample in the positive control. All the samples were analyzed by a collaborating public health laboratory using the standard RT-PCR method targeting the E gene. The Ct values from the RT-PCR analyses of the E gene are included for information.

| Samples          | Our assay | Ct value of RT-PCR | Diagnosis   |
|------------------|-----------|--------------------|-------------|
|                  | N-gene    | E-gene             |             |
| Negative control |           | N/A                | Negative    |
| Positive control |           | N/A                | Positive    |
| Sample #1        |           | Undetectable       | Negative    |
| Sample #2        |           | 23.0               | Positive    |
| Sample #3        |           | 28.1               | Positive    |
| Sample #4        |           | Undetectable       | Negative    |
| Sample #5        |           | 33.0               | False Negative |
| Sample #  | Value   | Status  |
|----------|---------|---------|
| #6       | 28.9    | Positive|
| #7       | Undetectable | Negative |
| #8       | Undetectable | Negative |
| #9       | 11.7    | Positive|
| #10      | 19.6    | Positive|
| #11      | 17.8    | Positive|
| #12      | Undetectable | Negative |
| #13      | Undetectable | Negative |
| #14      | Undetectable | Negative |
| #15      | 19.6    | Positive|
| #16      | 19.0    | Positive|
| #17      | Undetectable | Negative |
| #18      | Undetectable | Negative |
| #19      | Undetectable | Negative |
| Sample # | Value | Status   |
|----------|-------|----------|
| #20      | 24.5  | Positive |
| #21      | 24.3  | Positive |
| #22      | Undetectable | Negative |
| #23      | Undetectable | Negative |
| #24      | 30.3  | Positive |
| #25      | 23.7  | Positive |
| #26      | Undetectable | Negative |
| #27      | Undetectable | Negative |
| #28      | 25.9  | Positive |
| #29      | Undetectable | Negative |
| #30      | Undetectable | Negative |
| #31      | Undetectable | Negative |
| #32      | 26.6  | Positive |
| #33      | 28.3  | Positive |
| Sample # | Result   |
|----------|----------|
| #34      | Undetectable  Negative |
| #35      | Undetectable  Negative |
| #36      | Undetectable  Negative |
| #37      | 27.2  Positive |
| #38      | 28.2  Positive |
| #39      | Undetectable  Negative |
| #40      | Undetectable  Negative |
| #41      | Undetectable  Negative |
| #42      | Undetectable  Negative |
| #43      | 23.6  Positive |
| #44      | Undetectable  Negative |
| #45      | Undetectable  Negative |
| #46      | Undetectable  Negative |
| #47      | Undetectable  Negative |
| Sample #   | Result           | Status | Color  |
|-----------|------------------|--------|--------|
| #48       | 23.5             | Positive | Green  |
| #49       | Undetectable     | Negative | Blue   |
| #50       | Undetectable     | Negative | Blue   |
| #51       | Undetectable     | Negative | Blue   |
| #52       | Undetectable     | Negative | Blue   |
| #53       | Undetectable     | Negative | Blue   |
| #54       | Undetectable     | Negative | Blue   |
| #55       | Undetectable     | Negative | Blue   |
| #56       | Undetectable     | Negative | Blue   |
| #57       | Undetectable     | Negative | Blue   |
| #58       | Undetectable     | Negative | Blue   |
| #59       | Undetectable     | Negative | Blue   |
| #60       | Undetectable     | Negative | Blue   |
| #61       | Undetectable     | Negative | Blue   |
| Sample #   | Result          |
|-----------|----------------|
| Sample #62| Undetectable    |
| Sample #63| 23.4 Positive   |
| Sample #64| Undetectable    |
| Sample #65| Undetectable    |
| Sample #66| Undetectable    |
| Sample #67| Undetectable    |
| Sample #68| Undetectable    |
| Sample #69| Undetectable    |
| Sample #70| Undetectable    |
| Sample #71| Undetectable    |
| Sample #72| 22.4 Positive   |
| Sample #73| 21.2 Positive   |
| Sample #74| 22.2 Positive   |
| Sample #75| 23.1 Positive   |
| Sample #  | Value | Result |
|----------|-------|--------|
| #76      | 24.0  | Positive |
| #77      | 25.0  | Positive |
| #78      | 19.6  | Positive |
| #79      | 26.1  | Positive |
| #80      | 27.0  | Positive |
| #81      | 29.1  | Positive |
| #82      | 28.2  | Positive |
| #83      | N/A   | 31.9   | Positive |
| #84      | N/A   | 33.1   | Positive |
| #85      | N/A   | 30.9   | Positive |
| #86      | N/A   | 35.0   | Positive |
| #87      | N/A   | 33.3   | Positive |
| #88      | N/A   | 34.1   | Positive |
| #89      | N/A   | 31.1   | Positive |
| Sample #   | Result | Value | Status       |
|-----------|--------|-------|--------------|
| #4        | N/A    | Undetectable | Negative     |
| #90       | N/A    | 30.6  | Positive     |
| #91       | N/A    | 39.2  | False Negative |
| #92       | N/A    | 30.8  | Positive     |
| #93       | N/A    | 30.9  | Positive     |
| #94       | N/A    | 33.6  | Positive     |
| #95       | N/A    | 33.6  | False Negative |
| #96       | N/A    | 33.7  | Positive     |
| #97       | N/A    | 31.1  | Positive     |
| #98       | N/A    | 30.2  | Positive     |
| #99       | N/A    | 32.6  | Positive     |
| #100      | N/A    | 32.9  | Positive     |

N/A: not available.
Table S4. Sequences of the primers and the probes for the RT-qPCR assay. The complementary sequences are highlighted in matching colors.

| Description               | Sequences (5’- 3’)                                                                 |
|---------------------------|-----------------------------------------------------------------------------------|
| Amplified fragments of SARS-CoV-2 (N2) | TTACAAACATTGGCCGCAAA TTGCACAATTGCCCCCAGG GCTTCAGCGTCTTGGAAATGTCGCGC |
| N2-Forward primer         | TTACAAACATTGGCCGCAAA                                                             |
| N2-Reverse primer         | GCGGACATTCCGAAGAA                                                                |
| N2-Probe                  | 6-FAM/AACAATTGCCCCCAGCGTTTCAG/BHQ_1                                            |

6-FAM: 6-carboxyfluorescein; BHQ_1: Black Hole Quencher®-1
Table S5. Thermal cycling conditions for RT-qPCR conducted in parallel with the RT-LAMP CRISPR-Cas12a assay.

| Steps                      | Temperature | Duration | Cycles |
|----------------------------|-------------|----------|--------|
| UNG activation             | 25 °C       | 2 min    | N/A    |
| Reverse Transcription      | 50 °C       | 15 min   |        |
| Polymerase activation      | 95 °C       | 2 min    |        |
| Denaturation               | 95 °C       | 3 sec    | 50     |
| Extension and detection    | 60 °C       | 30 sec   |        |
Table S6. Results of detecting the N gene of SARS-CoV-2 at very low copy numbers (0, 1, 5, 10, 15 and 30 copies/μL). Conditions were shown in Figure S8.

| Concentration | No target | 1 copy/μL | 5 copies/μL | 10 copies/μL | 15 copies/μL | 30 copies/μL |
|---------------|-----------|-----------|-------------|--------------|--------------|--------------|
| # of tests    | 12        | 12        | 12          | 12           | 12           | 12           |
| # of positives| 0         | 3         | 3           | 8            | 10           | 12           |
| Positive rate | N/A       | 25%       | 25%         | 67%          | 83%          | 100%         |
Table S7. RT-qPCR results of detecting the N gene of SARS-CoV-2 at very low copy numbers (0, 1, 2, 5, 10, 15 and 30 copies/μL). For comparison, RT-qPCR was conducted for the detection of the N gene of SARS-CoV-2 at very low copy numbers. The experiment was repeated in five batches. In each batch, samples containing different concentrations of the viral RNA (0, 1, 2, 5, 10, 15 and 30 copies/μL) were analyzed in triplicate. Five microliters (5 μL) of sample was used for each reaction.

| Concentration | No target | 1 copy/μL | 2 copy/μL | 5 copies/μL | 10 copies/μL | 15 copies/μL | 30 copies/μL |
|---------------|-----------|-----------|-----------|-------------|--------------|--------------|--------------|
| # of tests    | 15        | 15        | 15        | 15          | 15           | 15           | 15           |
| # of positives| 0         | 5         | 7         | 14          | 15           | 14           | 15           |
| Average Ct value | N/A | 36.0±1.1 | 35.6±0.6 | 35.1±1.0 | 33.7±0.9 | 33.1±0.4 | 32.3±0.6 |
| Positive rate | N/A       | 33%       | 47%       | 93%         | 100%         | 93%          | 100%         |
Supplementary Figures

Figure S1. Thermal image of a 0.2-mL PCR reaction tube that was heated at the bottom to 62 ºC. The PCR tube contained 25 μL RT-LAMP reagent mixture at the bottom of the tube and 10 μL of Cas12a reagent mixture inside the cap of the tube. The bottom of the tube was heated and maintained at 62 ºC for 30 min, and the image was obtained by using a TG165 Imaging IR Thermometer (FLIR). The image indicates approximately 31 ºC near the cap of the tube. The ε stands for emissivity.
Figure S2. Visualization and assay setup. (A) An example of visualizing the RT-LAMP and CRISPR Cas12a reaction products of two samples, a negative and a SARS-CoV-2 positive. (B) A picture showing a heating block and relative positions of the sample vials, handheld UV lamp, and personal smartphone. The UV lamp (UVP UVGL-58, Analytik Jena AG) was approximately 5 cm from the sample vials. The smartphone (iPhone1Pro, Apple) camera was approximately 16 cm from the sample vials. The picture was taken under the indoor ambient light.
Figure S3. Testing different concentrations of SARS-CoV-2 RNA using RT-LAMP and SYBR Green I dye detection. (A) RT-LAMP amplification curves from the analyses of the N gene at different concentrations. (B) Time to reach threshold fluorescence from the analysis of the N gene at different concentrations. The threshold fluorescence was set at 100,000 (arbitrary unit). (C) RT-LAMP amplification curves from the analyses of the E gene at different concentrations. (D) Time to reach threshold fluorescence from the analysis of the E gene at different concentrations. The threshold fluorescence was set at 200,000 (arbitrary unit). The RNA samples were extracted from Vero-E6 cell cultures infected with SARS-CoV-2, and the concentrations were previously determined using an RT-qPCR assay.
**Figure S4.** Amplification of the N gene using RT-LAMP at (A) 62 °C, (B) 57 °C, (C) 52 °C, (D) 47 °C, (E) 42 °C, and (F) 37 °C. The starting sample contained 5 μL of viral RNA at a concentration of 75,000 copies/μL. SYBR Green was used for real-time fluorescence monitoring. The 25 μL of RT-LAMP reaction solution contained 1.4 mM dNTP, 1× Isothermal Amplification Buffer, 8 mM MgSO₄ (including 2 mM MgSO₄ in 1× Isothermal Amplification Buffer), 0.2 μM each of the outer primers (F3 and B3), 1.6 μM each of the inner primers (FIP and BIP), 0.8 μM each of the loop primers (LF and LB), 4 units of RNase inhibitor, 7.5 units of WarmStart® RTx reverse transcriptase, 8 units of Bst 2.0 DNA polymerase, and 5 μL of 75,000 copies/μL viral RNA (as target) or nuclease-free water (as negative control).
**Figure S5.** The dNTP inhibits the trans-cleavage activity of Cas12a by interacting with the Mg²⁺. After supplementing additional Mg²⁺ into the reaction, trans-activity of Cas12a was recovered. In Reaction (i), 25 μL of solution A was prepared to simulate the components of RT-LAMP reagent, containing 1× Isothermal Amplification Buffer, 8 mM MgSO₄ (including 2 mM MgSO₄ in 1× Isothermal Amplification Buffer), 0.2 μM each of the outer primers (N gene-F3 and B3), 1.6 μM each of the inner primers (N gene-FIP and BIP), 0.8 μM each of the loop primers (N gene-LF and LB), 4 units of RNase inhibitor, 7.5 units of WarmStart® RTx reverse transcriptase, 8 units of Bst 2.0 DNA polymerase, and 5 nM of N gene dsDNA activator. Solution A was mixed with 10 μL Cas12a reagent, containing 400 nM of RNP complex and 10 μM of ssDNA reporter in 50 mM Tris-HCl buffer (pH=7.9). Reaction (ii) was the same as Reaction (i) except adding additional 1.4 mM of dNTP in the solution A. Reaction (iii) was the same as Reaction (ii) except adding additional 10 mM of MgSO₄ in the Cas12a reagent. These 35 μL mixtures were incubated at 23 °C and their fluorescence was monitored in real-time at 1-min interval.
Figure S6. Optimization of the Mg\(^{2+}\) concentration in the Cas12a reagent mixture. 10 μL of Cas12a reagent was prepared and added to the cap, containing 400 nM of RNP complex, 10 μM ssDNA reporter, and various concentrations of Mg\(^{2+}\) (10, 20, 40 and 80 mM) in 50 mM of Tris-HCl buffer (pH=7.9). These Cas12a reagents were then mixed with 25 μL of RT-LAMP amplification products. The RT-LAMP reaction was designed for targeting the N gene. The positive samples contained 3750 copies of SARS-CoV-2 RNA before the start of RT-LAMP. The negative controls contained all the reagents but no target; the input sample was nuclease-free water instead of the viral RNA. These mixtures were left at room temperature. These results show that Mg\(^{2+}\) at 40 mM is optimum for the activity of Cas12a.
Figure S7. Optimization of the ssDNA reporter concentration in the Cas12a reagent mixture. Ten microliters (10 μL) of Cas12a reagent was prepared and added on the cap, containing 400 nM of RNP complex, 40 mM MgSO₄, and various concentrations of ssDNA reporter (2.5, 5, 10 and 20 μM) in 50 mM of Tris-HCl buffer (pH=7.9). These Cas12a reagents were then mixed with 25 μL of RT-LAMP amplification product. The RT-LAMP reaction was designed for targeting the N gene. The positive samples contained 3750 copies of SARS-CoV-2 RNA before the start of RT-LAMP. The negative controls contained all the reagents but no target; the input sample was nuclease-free water instead of the viral RNA. These mixtures were left at room temperature. The pictures of the reaction tubes were recorded under excitation of UV lamp at 2 min, 4 min, 6 min, 8 min, 10 min and 60 min (Part of result is shown in Figure 4B). The pictures captured at 10 min are shown in this figure. The fluorescence increases when the ssDNA reporter is increased from 2.5 to 10 μM. Little increase is observed when ssDNA reporter is further increased from 10 to 20 μM. We also noticed marginal increases in background signal of negative controls along with an increase of the concentration of the ssDNA reporter, particularly from 10 to 20 μM. Therefore, we chose to use 10 μM reporter, to achieve the brightest fluorescence and relatively low background.
Figure S8. Representative images obtained from the detection of the N gene of SARS-CoV-2 at very low copy numbers. The experiment was repeated in four batches. In each batch, samples containing different concentrations of the viral RNA (0, 1, 5, 10, 15 and 30 copies/μL) were analyzed in triplicate. Five microliters (5 μL) of sample was used for each reaction. Results are summarized in Table S6.
Figure S9. Representative images obtained from the detection of the E gene at a range of concentrations (0, 8, 15, 30, 45, 60, 75, 750, 7500, 75000 and 750000 copies/μL). Five microliters (5 μL) of sample was used for each reaction.
**Figure S10.** Twelve replicate analyses showing reproducible detection of the N gene of SARS-CoV-2. The positive sample contained 750 copies/μL of RNA extracted from supernatants of infected Vero-E6 cell cultures. The negative sample contained 5 μL of nuclease-free water as the sample input. (A) Pictures taken using a personal smartphone camera. (B and C) Color intensity obtained using the Image J software (NIH). Operation procedures for measuring color intensity were: (1) Open picture in the Image J software; (2) Click the Image-Color-Split Channels; (3) Choose the green channel; (4) Select the entire fluorescent area; (5) Click Analyze-Measure. The measured mean value is referred to as color intensity. The overall color intensities (arbitrary unit) were $232 \pm 8$ from the 12 positive tests and $127 \pm 13$ from the 12 negative tests. (D and E) Fluorescence intensity measured using a fluorescence detector (built in with the Thermo Fisher Scientific StepOnePlus™ Real-Time PCR System). Fluorescence intensity was measured after 30 min of RT-LAMP and 10 min of CRISPR Cas12a-mediated reaction. The overall fluorescence intensities (arbitrary unit) were $(783 \pm 47) \times 10^3$ from the 12 positive tests and $(75 \pm 6) \times 10^3$ from the 12 negative tests.
Figure S11. Images obtained from the analyses of 5 μL RNA extract from non-infected people (sample#: 1, 19, 39, 51, 61, 71). The N gene of SARS-CoV-2 was detected. The positive control (PC) contained 3750 copies of the N gene at the start of the amplification. The negative control (NC) contained all the reagents but no target, nuclease-free water was used as the input sample instead of the N gene.
References

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