Supporting Information

Light-Start CRISPR−Cas12a Reaction with Caged crRNA Enables Rapid and Sensitive Nucleic Acid Detection

Menglu Hu[†], Ruhan Liu[†], Zhiqiang Qiu, Feng Cao, Tian Tian, Yunxin Lu, Yongzhong Jiang, and Xiaoming Zhou[*]

Abstract: The clustered regularly interspaced short palindromic repeats (CRISPR) system is a promising platform for nucleic acid detection. Regulating the CRISPR reaction would be extremely useful to improve the detection efficiency and speed of CRISPR diagnostic applications. Here, we have developed a light-start CRISPR–Cas12a reaction by employing caged CRISPR RNA (crRNA). When combined with recombinase polymerase amplification, a robust photocontrolled one-pot assay is achieved. The photocontrolled one-pot assay is simpler and is 50-fold more sensitive than the conventional assay. This improved detection efficiency also facilitates the development of a faster CRISPR diagnostic method. The detection of clinical samples demonstrated that 10-20 min is sufficient for effective detection, which is much faster than the current gold-standard technique PCR. We expect this advance in CRISPR diagnostics to promote its widespread detection applications in biomedicine, agriculture, and food safety.

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Experimental Procedures

Fluorescent *Lachnospiraceae* bacterium ND2006 Cas12a (LbCas12a) assay.

Preparation of double-stranded DNA (dsDNA) target. To generate the dsDNA targets, we annealed 10 μM target−F and 10 μM target−R in 1× PCR buffer (TaKaRa). The annealing program was set to 70°C for 5 min followed by gradual cooling until it comes down to 25°C. Finally, the annealed products were stored at −80°C for further use.

Conventional clustered regularly interspaced short palindromic repeats (CRISPR)−LbCas12a assay. Briefly, in a 20 μL reaction solution, 50 nM LbCas12a (Bio-lifesci, Guangzhou) was mixed with 50 nM uncaged CRISPR RNA (crRNA), 800 nM FQ6C probe, 1 nM dsDNA target in 1× LbCas12a reaction buffer (5 mM Tris−HCl (pH9.0), 15 mM $MgCl_2$, 10 mM NaCl, 0.01% IGEPAL CA−630 (v/v) (Sigma)). Finally, the mixture was transferred to a fluorescence plate reader and incubated for 60 min at 37°C, with a fluorescence measurement taken every minute.

Light-start CRISPR−LbCas12a assay. 6-nitropiperonyloxymethyl (NPOM) caged-dT-CE Phosphoramidite for caged crRNA synthesis was purchased from GLEN research. And then the caged crRNA was synthesized by a solid phase synthesis method. The detailed experimental steps of the light-start CRISPR-Cas12a assay are shown below. Briefly, in a 20 μL reaction solution, 50 nM LbCas12a (Bio-lifesci) was mixed with 50 nM caged crRNA, 800 nM FQ6C probe, 1 nM dsDNA target in 1× LbCas12a reaction buffer. And then

the reaction system was irradiated with a UV lamp (λ= 365 nm, 35 W) for 30 s. Finally, the mixture was transferred to a fluorescence plate reader and incubated for 60 min at 37°C, with a fluorescence measurement taken every minute.

PAGE analysis of caged crRNA guided *in vitro cis*-cleavage activity of LbCas12a

To assess whether caged crRNA can be used to regulate the *cis*-cleavage activity of LbCas12a and whether its activity can be terminated by proteinase K, the following experiments were carried out. Briefly, in a total of 20 μL reaction solution, 2 ng/μL template DNA was mixed with 0 μM or 1 μM crRNA (caged crRNA or uncaged crRNA), 0 μM or 1 μM LbCas12a, 0 mg/mL or 2 mg/mL proteinase K (TIANGEN) in 1× LbCas12a reaction buffer. And then the reaction system containing caged crRNA was irradiated with or without a UV lamp (λ= 365 nm, 35 W) for 30 s. Finally, the mixture was incubated at 37°C for 30 min, and subsequent heating to 95°C for 5 min, and then analyzed by 7.5% polyacrylamide gel electrophoresis (PAGE) assay.

Extraction of monkeypox virus (MPXV) DNA samples

The pseudovirus containing MPXV DNA with a concentration of 10^8 copies/mL (Genome location: 164848-165798, GeneBank: NC_063383.1) was purchased from Tsingke Biotechnology Co., Ltd. and treated with TIANamp Virus DNA/RNA Kit (TIANGEN) following the manufacturer's protocol to obtain the MPXV DNA sample. Then the products were stored at −80°C for further use.

Collection and extraction of Epstein−Barr virus (EBV) clinical samples

Clinical EBV samples were collected from symptomatic patients with suspected EBV infection at Sun Yat−Sen University Cancer Center. All nucleic acid extractions were performed using the QIAamp DNA Blood Mini Kit (Qiagen) following the manufacturer's protocol. And the final products were stored at −80°C for further use.

Clinical EBV samples detection by qPCR

The qPCR reaction was performed using the EBV DNA Quantitative Fluorescence Diagnostic Kit (Sansure Biotech). Briefly, a 12.5 μL qPCR reaction solution consisted of 9.5 μL PCR reaction solution, 0.5 μL enzymes mixture, and 2.5 μL template. The mixture was incubated on a qPCR instrument and the PCR thermal cycling program was set as follows: 50°C for 2 min, 94°C for 5 min and followed by 45 cycles of amplification reaction at 94°C for 15 s, 57°C for 30 s. The fluorescence signal of each cycle was monitored with the CFX96 Touch Deep Well Real-Time PCR Detection System (Bio-rad).

Preparation of RNA samples for sensitivity and specificity analysis

The RNA reference material used for sensitivity analysis was purchased from the National Institute of Metrology, China, which included three key characteristic genes of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), namely the E gene (full length), N gene (full length), and Orf1ab gene (Genome location: 13201−15600, GenBank No. NC_045512). These three key characteristic genes were obtained by *in vitro* transcription, respectively, and quantified using a digital PCR method. RNA samples from other related viral strains were used for specificity determination. RNA samples of six pseudoviruses (human coronavirus 229E (hCoV-229E), respiratory syncytial virus type A (RSV-A), respiratory syncytial virus type B (RSV-B), influence A virus (IVA), influence B virus (IVB), middle east respiratory syndrome coronavirus (MERS-CoV)) were purchased from the National Standard Material Platform (Henan, China). These pseudoviruses were processed with the MagZol Reagent Kit (Magen) according to the manufacturer's protocol to obtain RNA samples. The detailed steps for obtaining the RNA sequences of SARS-CoV and bat SARS-like coronavirus (bat-SL-CoVZC45) are shown below. First, DNA plasmids (shown in table S2) containing SARS-CoV and bat-SL-CoVZC45 O-gene fragments were synthesized and cloned into the pUC57 vector. Then, a PCR was performed to amplify the genome fragments of SARS-CoV and bat-SL-CoVZC45 in the plasmids. TaKaRa Taq HS Perfect Mix (TaKaRa) was used to perform PCR, and the detailed experimental steps and program settings followed the instruction manual. And then the PCR products were used as templates for *in vitro* transcription. A total of 50 μL of the *in vitro* transcription system contained 2 mM NTP mixture (Sangon), 1 U/μL recombinant RNase inhibitor (TaKaRa), 2.5 U/μL T7 RNA polymerase (TaKaRa), and 2 μL DNA template in 1× T7 RNA polymerase Buffer (TaKaRa). The reaction system was incubated at 37°C for 4 h and the final product was stored at −80°C for further use.

Collection and extraction of SARS-CoV-2 clinical samples

The clinical samples of SARS-CoV-2 RNA used in this study were extracted from nasopharyngeal and throat swab samples by QIAamp Viral RNA Mini Kit (Qiagen) according to the manufacturer's protocol. These nasopharyngeal and throat swab samples were collected by trained medical professionals in Wuhan, Hubei Province, China, during 2020.

SARS-CoV-2 RNA detection by reverse transcription quantitative PCR (RT–qPCR)

The RT−qPCR reaction for the detection of SARS-CoV-2 RNA was carried out with the COVID-19 (SARS-CoV-2) Nucleic Acid Test Kit (Wuhan Easy Diagnosis Biomedicine). Briefly, a 12.5 μL reaction system was prepared with 10 μL PCR solution and 2.5 μL RNA samples. The PCR thermal cycling program was set as follows: reverse transcription at 50°C for 15 min, thermal denaturation at 95°C for 30 s and followed by 40 cycles of amplification reaction at 95°C for 3 s, 60°C for 40 s. Fluorescence signals were recorded every cycle by a CFX96 Touch Deep Well Real−Time PCR Detection System. According to the manufacturer's protocol of the kit, samples with C_t values less than 38 were defined as positive samples, samples with C_t values between 38 and 40 were defined as suspiciously positive samples, and samples with C_t values greater than 40 were defined as negative samples.

Conventional one-pot recombinase polymerase amplification (RPA) (reverse-transcription RPA (RT-RPA))-LbCas12a assay

The TwistAmp Basic Kit (TwistDx) was used for RPA (RT-RPA), which contains Primer-free rehydration buffer, RPA pellet, and magnesium acetate. The detailed experimental steps of the conventional one-pot RPA-LbCas12a assay used for the detection of DNA

are shown below. First, a resuspended RPA mixture was made by resuspending one RPA pellet with 29.5 μL Primer-free rehydration buffer. Then, 1 μL 10× NEBuffer r2.1 (TaKaRa), 1 μL 2 μM LbCas12a, 1 μL 2 μM uncaged crRNA, 1 μL 20 μM FQ6C probe, and 1 μL RNase-free water (TaKaRa) were mixed to prepare solution A. And 9.5 μL of solution B was prepared with 0.48 μL 10 μM forward primers, 0.48 μL 10 μM reverse primers, 5.9 μL resuspended RPA mixture, target, and RNase-free water. Finally, solution B was added to the bottom of the EP tube, solution A was placed on the wall of the EP tube, and 0.5 μL 280 mM magnesium acetate was placed on the other location of the wall of the EP tube. These solutions were mixed by centrifugation, and the mixture was then incubated at 37°C for 90 min, with fluorescence measured every minute.

Detection of RNA samples followed the same protocol, except that 0.1 μL 200 U/μL SuperScript IV reverse transcriptase (Thermo Fisher Scientific) and 0.2 μL 5,000 U/mL RNase H (NEB) needs to be added to solution B.

Photocontrolled one-pot RPA (RT-RPA)-LbCas12a assay

The TwistAmp Basic Kit was used for RPA (RT-RPA), which contains Primer-free rehydration buffer, RPA pellet, and magnesium acetate. The detailed experimental steps of the photocontrolled one-pot RPA-LbCas12a assay used for the detection of DNA are shown below. First, a resuspended RPA mixture was made by resuspending one RPA pellet with 29.5 μL Primer-free rehydration buffer. Then, 1 μL 10× NEBuffer r2.1, 1 μL 2 μM LbCas12a, 1 μL 2 μM caged crRNA, 1 μL 20 μM FQ6C probe, and 1 μL RNase-free water were mixed to prepare solution A. And 9.5 μL of solution B was prepared with 0.48 μL 10 μM forward primers, 0.48 μL 10 μM reverse primers, 5.9 μL resuspended RPA mixture, target, and RNase-free water. Finally, solution B was added to the bottom of the EP tube, solution A was placed on the wall of the EP tube, and 0.5 μL 280 mM magnesium acetate was placed on the other location of the wall of the EP tube. These solutions were mixed by centrifugation. Finally, the mixture was incubated at 37°C for 10 min, 20 min, or 30 min for RPA, irradiated with a UV lamp (λ=365 nm, 35 W) for 30 s to activate the LbCas12a system, and followed incubating at 37°C for another 80 min, 70 min or 60 min for CRISPR detection. The fluorescence signal of the mixture was monitored with a fluorescence plate reader. Detection of RNA samples followed the same protocol, except that 0.1 μL 200 U/μL SuperScript IV reverse transcriptase and 0.2 μL 5,000 U/mL RNase H needs to be added to solution B.

Ethical approval

The clinical trial conducted for EBV samples in this study was approved by the ethics committee of the Sun Yat-sen University Cancer Center (B2022-769-01). And the clinical trial conducted for SARS-CoV-2 RNA samples in this study was approved by the ethics committee of the Hubei Provincial Center for Disease Control and Prevention/Academy of Preventive Medicine (2020−061−01).

SUPPORTING INFORMATION

Supplementary Figures and Tables.

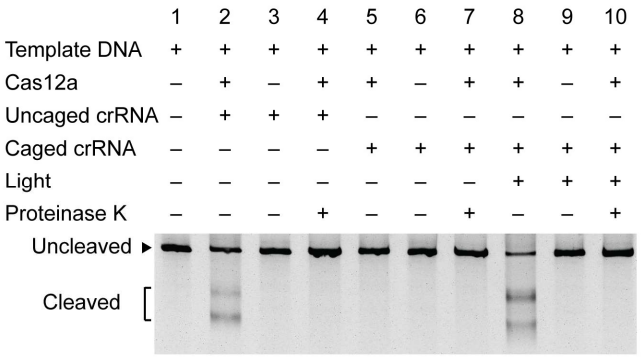


Figure S1. Optical control of the *cis*-cleavage activity of LbCas12a. The caged crRNA was used to guide the Cas12a protein for investigating the performance of optical control of the cis-cleavage activity of LbCas12a.

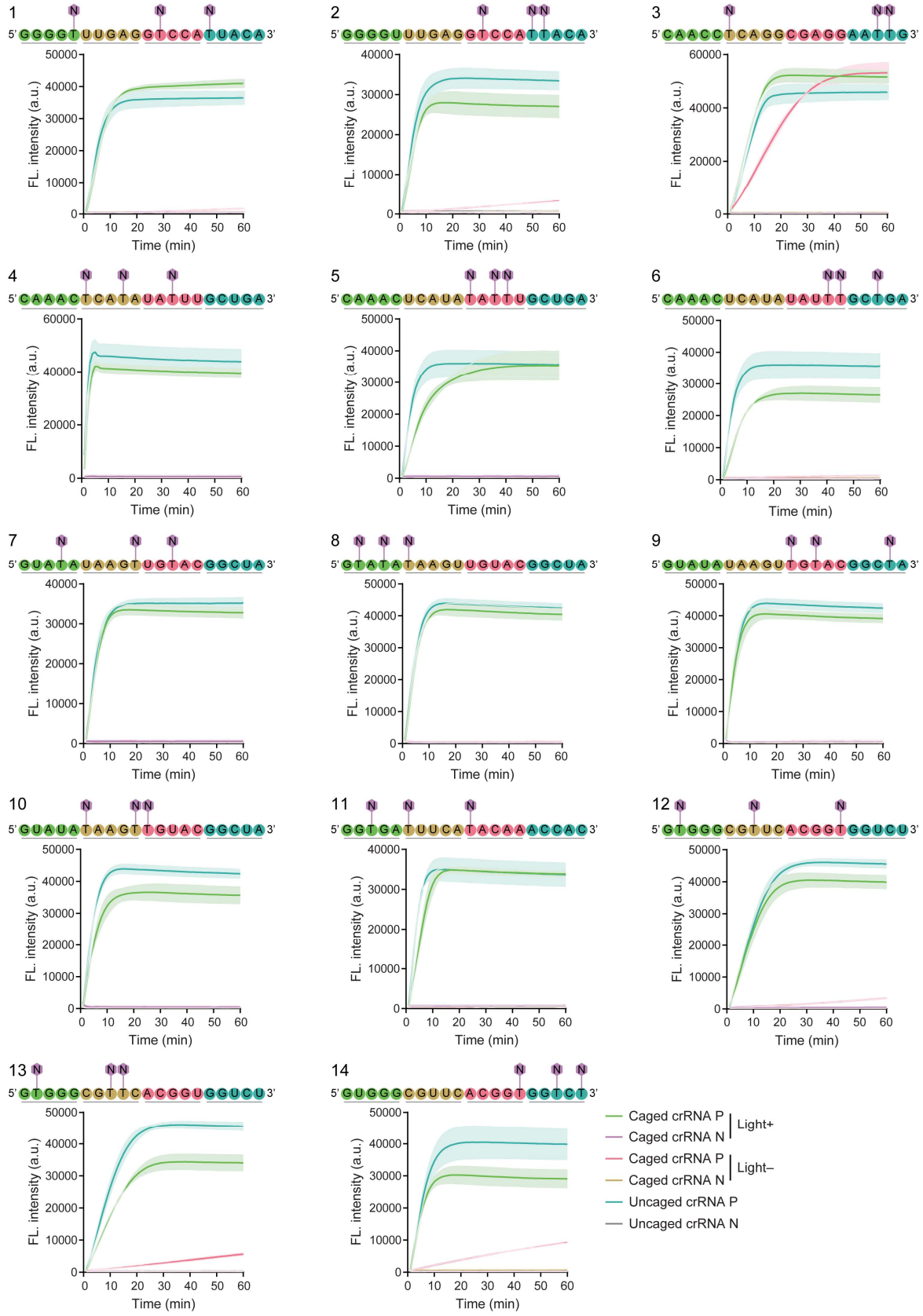


Figure S2. Investigating the influence of modification position of NPOM-dt on crRNA activity. The activity of fourteen crRNAs, which are modified with three NPOM-dts at different positions in the spacer region of crRNA, were evaluated. The "N" on the sequence represents the NPOM-dt modification. Bases are numbered 1-20 from the 5′ to the 3′. "Light +" and "Light -" represent with or without UV irradiation for 30 s. P represents the detection of the dsDNA target. N represents the blank control with RNase-free water as the target. The dsDNA target is formed by annealing two single strands of DNA.

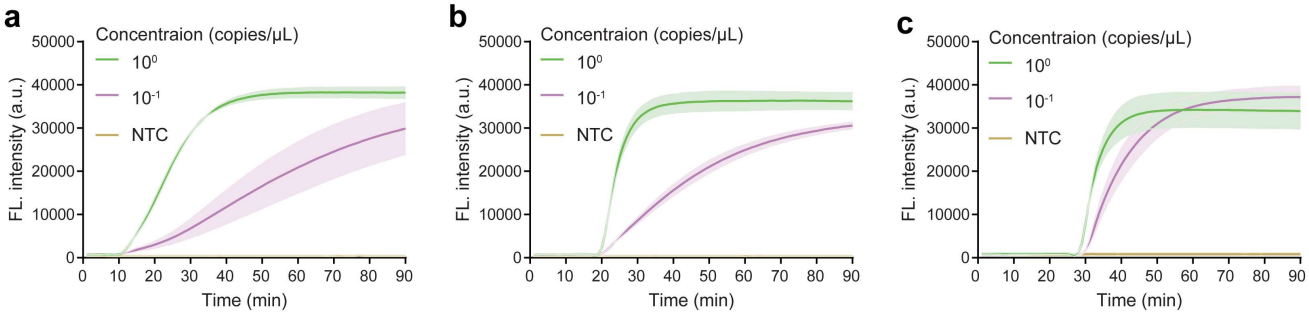


Figure S3. Evaluation of the effect of photoactivation timing on the detection efficiency and speed. The real-time fluorescence analysis of the photocontrolled one-pot assay for the detection of different concentrations of MPXV DNA (10^0, 10^{-1}, and 0 copies/μl) at the photoactivation time of 10 min (a), 20 min (b), and 30 min (c). NTC represents no-template control.

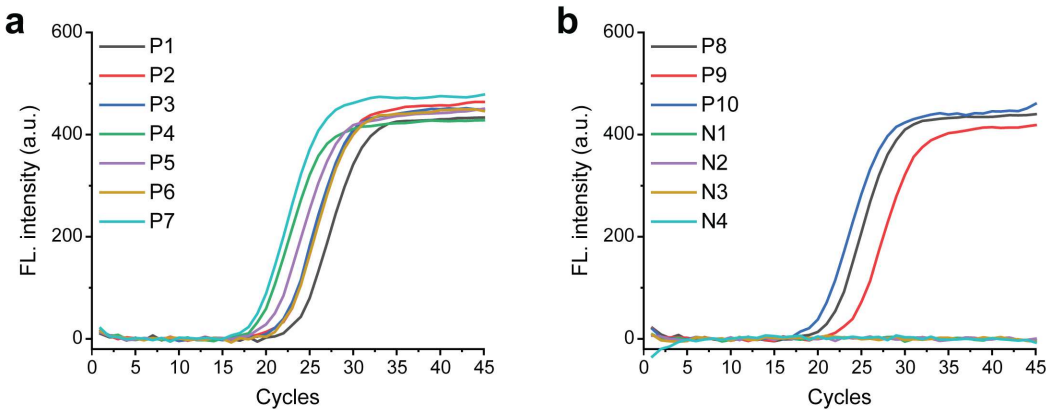


Figure S4. qPCR assay for detection of clinical EBV DNA samples. P1−P10 represent positive samples, N1−N4 represent negative samples.

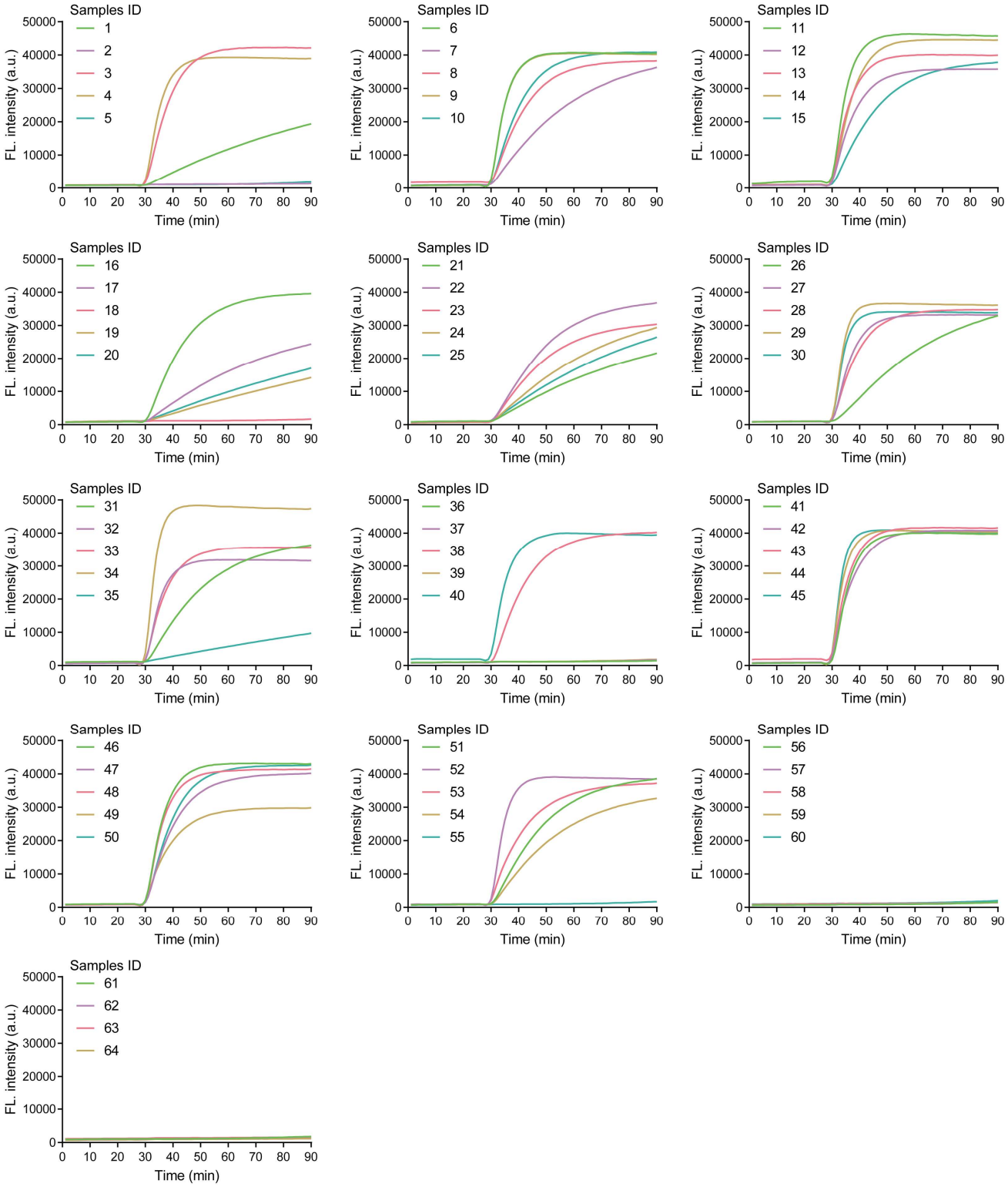


Figure S5. Photocontrolled one-pot RT-RPA-CRISPR-Cas12a assay for detection of clinical SARS-CoV-2 RNA samples (Samples ID: 1−64).

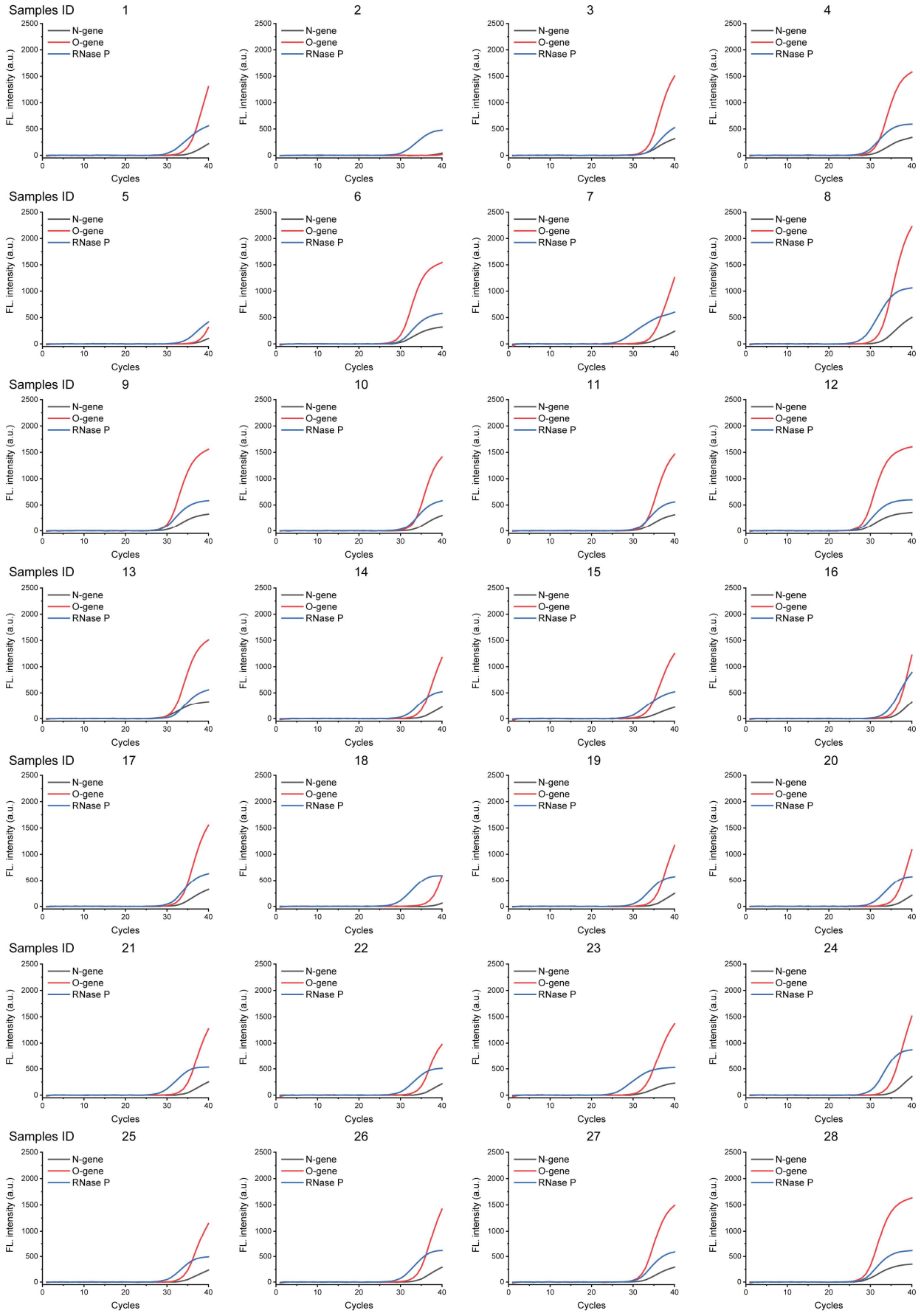


Figure S6. RT−qPCR assay for detection of clinical SARS-CoV-2 RNA samples (Samples ID: 1−28).

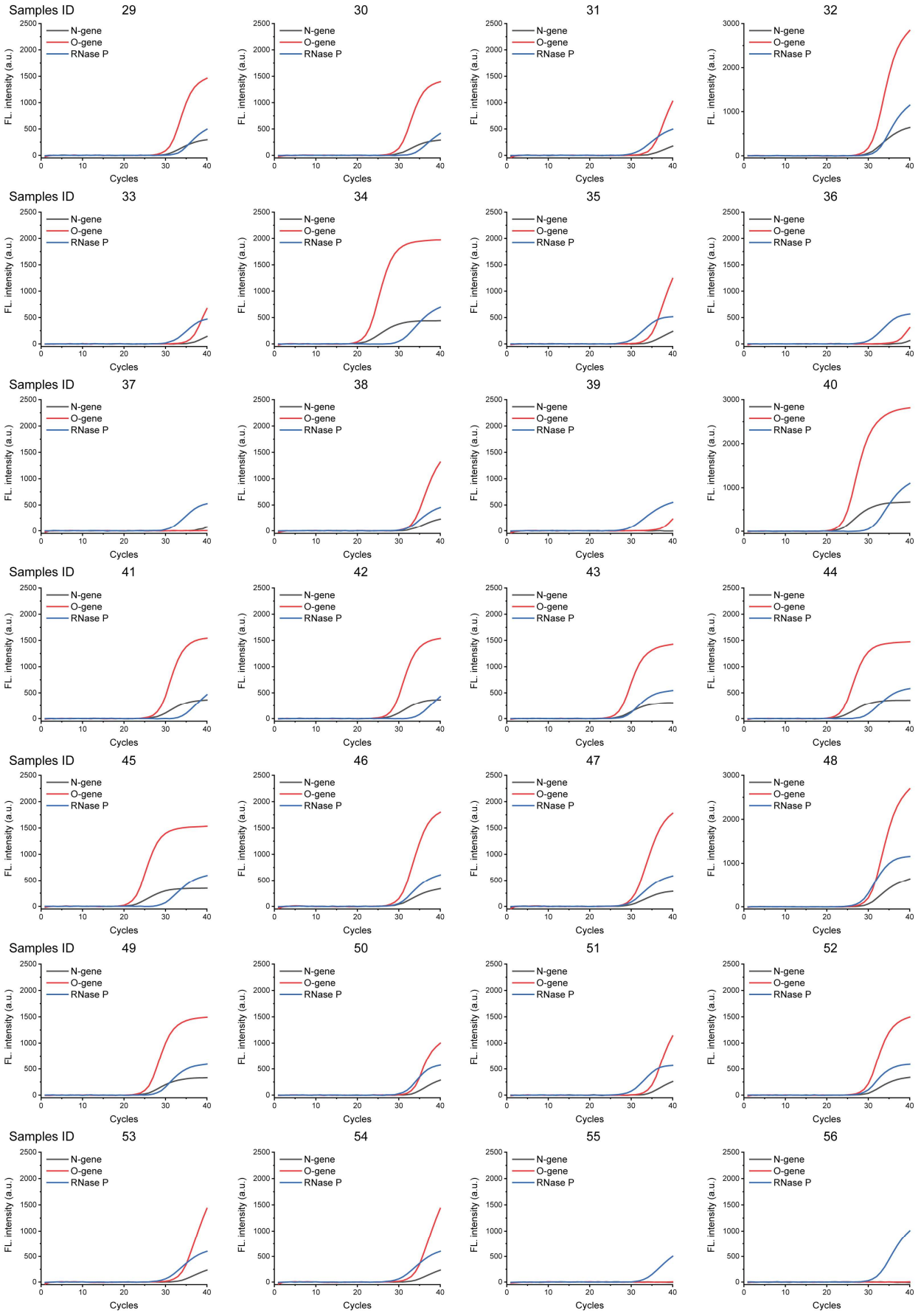


Figure S7. RT−qPCR assay for detection of clinical SARS-CoV-2 RNA samples (Samples ID: 29-56).

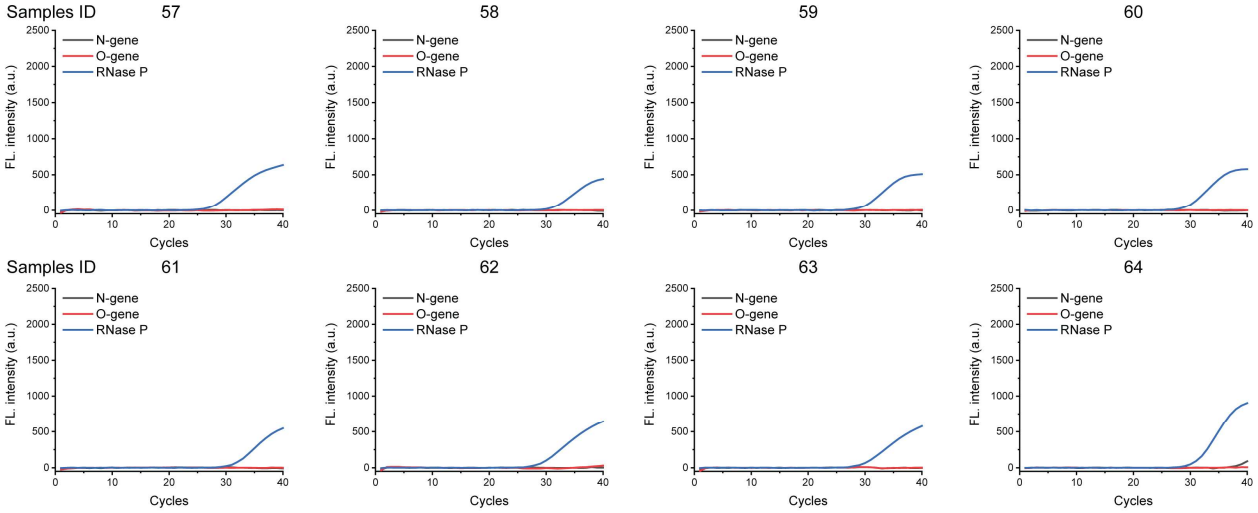


Figure S8. RT−qPCR assay for detection of clinical SARS-CoV-2 RNA samples (Samples ID: 57-64).

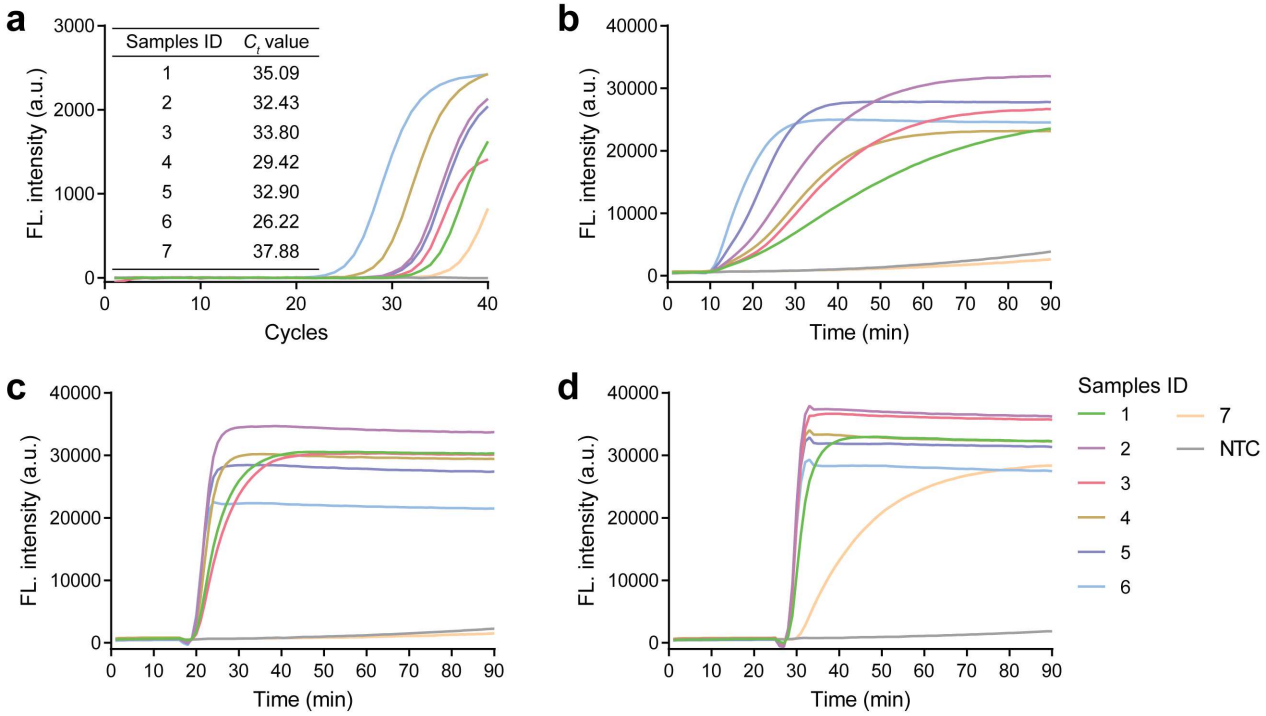


Figure S9. Evaluation of the effect of photoactivation timing on the sensitivity and speed of the photocontrolled one-pot assay in clinical samples detection. (a) RT−qPCR assay for detection of clinical SARS-CoV-2 RNA samples. The real-time fluorescence analysis of the photocontrolled one-pot assay for the detection of SARS-CoV-2 RNA samples at the photoactivation timing of 10 min (b), 20 min (c), and 30 min (d). NTC represents no-template control. Samples ID 1-6 has a C_t value of less than 36, and Samples ID 7 has a C_t value of 37.88.

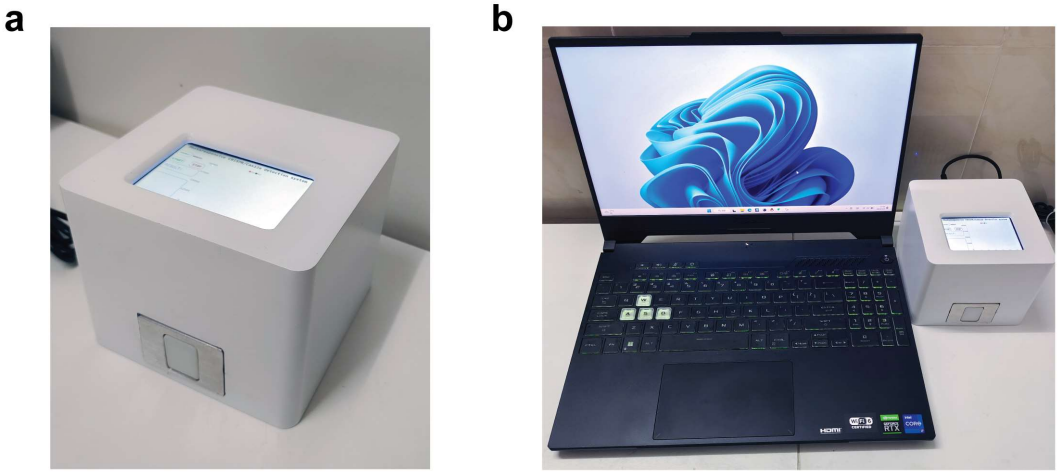


Figure S10. Design of a portable fluorescence detection device equipped with a UV photoregulatory module. (a) A photograph of the fluorescence detection device. (b) A picture showing the size of the fluorescence detection device compared to a notebook computer.

SUPPORTING INFORMATION

Table S1. Sequences used for assay development.

Name	Sequences (from 5′ to 3′)
ASFV uncaged crRNA	UAAUUUCUACUAAGUGUAGAU**GGGGUUUGAGGUCCAUUACA**[a]
ASFV caged crRNA (2)	UAAUUUCUACUAAGUGUAGAU**GGGGdt*UUGAGGdt***[b]**CCAUUACA**
ASFV caged crRNA (3)	UAAUUUCUACUAAGUGUAGAU**GGGGdt*UUGAGGdt*CCAdt*UACA**
ASFV caged crRNA (4)	UAAUUUCUACUAAGUGUAGAU**GGGGdt*Udt*GAGGdt*CCAdt*UACA**
ASFV caged crRNA (5)	UAAUUUCUACUAAGUGUAGAU**GGGGdt*Udt*GAGGdt*CCAdt*dt*ACA**
ASFV caged crRNA (6)	UAAUUUCUACUAAGUGUAGAU**GGGGdt*dt*dt*GAGGdt*CCAdt*dt*ACA**
ASFV RPA F	GCCGAAGGGAATGGATACTGAGGGAATAGCAA
ASFV RPA R	AGGGAACCAGTGGCCCTCTCCTATGCAACA
ASFV Target F	CTGATAGTATTTAGGGGTTTGAGGTCCATTACAGCTGTAATGAACATTACGTCTTATGT
ASFV Target R	ACATAAGACGTAATGTTCATTACAGCTGTAATGGACCTCAAACCCCTAAATACTATCAG
ASFV *cis* template DNA	TATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCA ACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGC GATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTCGAGCTCGGTACC TCGCGAATGCATCTAGATAAGTCCAGGAAATTCATTCACCAAATCCTTTTGCGATGCAAGCTTTATGGTGATAAAGCGC TCGCCGAAGGGAATGGATACTGAGGGAATAGCAAGGTTCACGTTCTCGTTAAACCAAAAGCGCAGCTTAATCCAGAG CGCAAGAGGGGGCTGATAGTATTTAGGGGTTTGAGGTCCATTACAGCTGTAATGAACATTACGTCTTATGTCCAGATA CGTTGCGTCCGTGATAGGAGTGATATCTTGTTTACCTGCTGTTTGGATATTGTGAGAGTTCTCGGGAAAATGTTGTGA AAGAAATTTCGGGTTGGTATGGCTGCACGTTCGCTGCGTATCATTTTCATCGGTAAGAATAGGTTTGCTTTGGTGCGG CTTGTGCAAATCATGAATGTTGCATAGGAGAGGGCCACTGGTTCCCTCCACCGATACCTCCTGGCCGACCAAGTGCT TATATCCATCGGATCCCGGGCCCGTCGACTGCAGAGG
MPXV uncaged crRNA	UAAUUUCUACUAAGUGUAGAU**GUAUAUAAGUUGUACGGCUA**
MPXV caged crRNA	UAAUUUCUACUAAGUGUAGAU**GUAdt*AUAAGdt*UGdt*ACGGCUA**
MPXV Target F	GGATATGAGGTTATTGGTGTTTCGTATATAAGTTGTACGGCTAATTCTTGGAATGTTAT
MPXV Target R	ATAACATTCCAAGAATTAGCCGTACAACTTATATACGAAACACCAATAACCTCATATCC
MPXV RPA F	GGAATGTCAACCTCTTCAATTAGAACACGG
MPXV RPA R	GATAGAAAATGTAGATCCGGAAATTAATCC
EBV uncaged crRNA	UAAUUUCUACUAAGUGUAGAU**CAAACUCAUAUAUUUGCUGA**
EBV caged crRNA	UAAUUUCUACUAAGUGUAGAU**CAAACdt*CAdt*AUAdt*UUGCUGA**
EBV RPA F	GACCCGGCCCACAACCTGGCCCACTAAGGG
EBV RPA R	ACTCCATCGTCAAAGCTGCACACAGTCACC
EBV Target F	ATGGTCTTTTTACAAACTCATATATTTGCTGAGGTTTTGAAGGATGCGATTAAGGACCT
EBV Target R	AGGTCCTTAATCGCATCCTTCAAAACCTCAGCAAATATATGAGTTTGTAAAAAGACCAT
O gene uncaged crRNA	UAAUUUCUACUAAGUGUAGAU**GGUGAUUUCAUACAAACCAC**
O gene caged crRNA	UAAUUUCUACUAAGUGUAGAU**GGdt*GAdt*UUCAdt*ACAAACCAC**
O gene RPA F	TGATGCCATGCGAAATGCTGGTATTGTTGG
O gene RPA R	TTAATGTAAGGCTTTGTTAAGTCAGTGTCA
O gene Target F	TGGTAACTGGTATGATTTCGGTGATTTCATACAAACCACGCCAGGTAGTGGAGTTCCTG
O gene Target R	CAGGAACTCCACTACCTGGCGTGGTTTGTATGAAATCACCGAAATCATACCAGTTACCA
O gene plasmid PCR primer F	AATTCTAATACGACTCACTATAGGGTGACATGGTACCACATATATC
O gene plasmid PCR primer R	TCCCAATATTTAAAATAACGGTC
FQ6C probe	FAM−CCCCCC−BHQ1

Table Footnote: [a] The bolded font represents the spacer region of crRNA. [b] dt* represents dt−NPOM modification.

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Table S2. Sequences used for evaluating the effect of caging position on crRNA activity.

Name	Sequences (from 5′ to 3′)
crRNA1 (ASFV)	UAAUUUCUACUAAGUGUAGAU**GGGGdt*[b]UUGAGGdt*CCAdt*UACA**[a]
crRNA2 (ASFV)	UAAUUUCUACUAAGUGUAGAU**GGGGUUUGAGGdt*CCAdt*dt*ACA**
Target F (ASFV)	CTGATAGTATTTAGGGGTTTGAGGTCCATTACAGCTGTAATGAACATTACGTCTTATGT
Target R (ASFV)	ACATAAGACGTAATGTTCATTACAGCTGTAATGGACCTCAAACCCCTAAATACTATCAG
crRNA3 (EBV1)	UAAUUUCUACUAAGUGUAGAU**CAACCdt*CAGGCGAGGAAdt*dt*G**
Target F (EBV1)	GTAAGACCTCCCTTTACAACCTCAGGCGAGGAATTGCCCTTGCTGTTCCACAATGTCGT
Target R (EBV1)	ACGACATTGTGGAACAGCAAGGGCAATTCCTCGCCTGAGGTTGTAAAGGGAGGTCTTAC
crRNA4 (EBV2)	UAAUUUCUACUAAGUGUAGAU**CAAACdt*CAdt*AUAdt*UUGCUGA**
crRNA5 (EBV2)	UAAUUUCUACUAAGUGUAGAU**CAAACUCAUAdt*Adt*dt*UGCUGA**
crRNA6 (EBV2)	UAAUUUCUACUAAGUGUAGAU**CAAACUCAUAUAUdt*dt*GCdt*GA**
Target F (EBV2)	ATGGTCTTTTTACAAACTCATATATTTGCTGAGGTTTTGAAGGATGCGATTAAGGACCT
Target R (EBV2)	AGGTCCTTAATCGCATCCTTCAAAACCTCAGCAAATATATGAGTTTGTAAAAAGACCAT
crRNA7 (MPXV)	UAAUUUCUACUAAGUGUAGAU**GUAdt*AUAAGdt*UGdt*ACGGCUA**
crRNA8 (MPXV)	UAAUUUCUACUAAGUGUAGAU**Gdt*Adt*Adt*AAGUUGUACGGCUA**
crRNA9 (MPXV)	UAAUUUCUACUAAGUGUAGAU**GUAUAUAAGUdt*Gdt*ACGGCdt*A**
crRNA10 (MPXV)	UAAUUUCUACUAAGUGUAGAU**GUAUAdt*AAGdt*dt*GUACGGCUA**
Target F (MPXV)	GGATATGAGGTTATTGGTGTTTCGTATATAAGTTGTACGGCTAATTCTTGGAATGTTAT
Target R (MPXV)	ATAACATTCCAAGAATTAGCCGTACAACTTATATACGAAACACCAATAACCTCATATCC
crRNA11 (O-gene)	UAAUUUCUACUAAGUGUAGAU**GGdt*GAdt*UUCAdt*ACAAACCAC**
Target F (O-gene)	TGGTAACTGGTATGATTTCGGTGATTTCATACAAACCACGCCAGGTAGTGGAGTTCCTG
Target R (O-gene)	CAGGAACTCCACTACCTGGCGTGGTTTGTATGAAATCACCGAAATCATACCAGTTACCA
crRNA12 (HBV)	UAAUUUCUACUAAGUGUAGAU**Gdt*GGGCGdt*UCACGGdt*GGUCU**
crRNA13 (HBV)	UAAUUUCUACUAAGUGUAGAU**Gdt*GGGCGdt*dt*CACGGUGGUCU**
crRNA14 (HBV)	UAAUUUCUACUAAGUGUAGAU**GUGGGCGUUCACGGdt*GGdt*Cdt***
Target F (HBV)	CTCTGCACGTCGCATGGAGACCACCGTGAACGCCCACCAAATATTGCCCAAGGTCTTAC
Target R (HBV)	GTAAGACCTTGGGCAATATTTGGTGGGCGTTCACGGTGGTCTCCATGCGACGTGCAGAG

Table Footnote: [a] The bolded font represents the spacer region of crRNA. [b] dt* represents NPOM−dt modification.

SUPPORTING INFORMATION

Table S3. Plasmids used in this study.

Name	Sequences (from 5′ to 3′)
ASFV plasmid	AAGTCCAGGAAATTCATTCACCAAATCCTTTTGCGATGCAAGCTTTATGGTGATAAAGCGCTCGCCGAAGGGAATGGATACTGAGG GAATAGCAAGGTTCACGTTCTCGTTAAACCAAAAGCGCAGCTTAATCCAGAGCGCAAGAGGGGGCTGATAGTATTTAGGGGTTTG AGGTCCATTACAGCTGTAATGAACATTACGTCTTATGTCCAGATACGTTGCGTCCGTGATAGGAGTGATATCTTGTTTACCTGCTGT TTGGATATTGTGAGAGTTCTCGGGAAAATGTTGTGAAAGAAATTTCGGGTTGGTATGGCTGCACGTTCGCTGCGTATCATTTTCAT CGGTAAGAATAGGTTTGCTTTGGTGCGGCTTGTGCAAATCATGAATGTTGCATAGGAGAGGGCCACTGGTTCCCTCCACCGATAC CTCCTGGCCGACCAAGTGCTTATATCC
SARS CoV O gene plasmid	AGGCATACTATGTCTAACTACCAACATGAAGAGACTATTTATAACTTGGTTAAAGATTGTCCAGCGGTTGCTGTCCATGACTTTTTC AAGTTTAGAGTAGATGGTGACATGGTACCACATATATCACGTCAGCGTCTAACTAAATACACAATGGCTGATTTAGTCTATGCTCTA CGTCATTTTGATGAGGGTAATTGTGATACATTAAAAGAAATACTCGTCACATACAATTGCTGTGATGATGATTATTTCAATAAGAAGG ATTGGTATGACTTCGTAGAGAATCCTGACATCTTACGCGTATATGCTAACTTAGGTGAGCGTGTACGCCAATCATTATTAAAGACTG TACAATTCTGCGATGCTATGCGTGATGCAGGCATTGTAGGCGTACTGACATTAGATAATCAGGATCTTAATGGGAACTGGTACGAT TTCGGTGATTTCGTACAAGTAGCACCAGGCTGCGGAGTTCCTATTGTGGATTCATATTACTCATTGCTGATGCCCATCCTCACTTTG ACTAGGGCATTGGCTGCTGAGTCCCATATGGATGCTGATCTCGCAAAACCACTTATTAAGTGGGATTTGCTGAAATATGATTTTAC GGAAGAGAGACTTTGTCTCTTCGACCGTTATTTTAAATATTGGGACCAGACATACCATCCCAATTGTATTAACTGTTTGGATGATAG GTGTATCCTTCATTGTGCAAACTTTAATGTGTTATTTTCTACTGTGTTTCCACCTACAAGTTTTGGACCACTAGTAAGAAAAATATTT GTAGATGGTGTTCCTTTTGTTGTTTCAACTGGATACCATTTTCGTGAGTTAGGAGTCGTACATAATCAGGATGTAAACTTACATAGC TCGCGTCTCAGTTTCAAGGAACTTTTAGTGTATGCTGCTGATCCAG
Bat-SL-CoVZC45 O-gene plasmid	AGGCACACAATGTCCAACTACCAACATGAAGAGACTATTTACAACTTGGTTAAAGGGTGTCCAGCCGTTGCTGTTCATGACTTTTTC AAGTTTAGAGTAGATGGTGACATGGTACCACATATATCACGTCAACGTCTAACTAAATACACAATGGCTGATTTAGTCTATGCTCTA CGTCATTTTGACGAGGGCAATTGTGATACATTAAAGGAAATACTTGTCACATACAAATGTTGTGATGACAACTATTTCAATAAGAAG GATTGGTATGACTTTGTAGAAAATCCTGATGTTCTACGCGTATACGCGAACCTAGGTGAGCGTGTACGTCAAGCCTTATTGAAAAC TGTGCAATTCTGCGATGCTATGCGCGATGCGGGTATCGTAGGTGTACTGACACTAGACAATCAGGATCTGAATGGGAATTGGTAC GACTTCGGAGATTTCGTACAGGTAGCACCAGGCTGCGGAGTCCCTATTGTGGATTCATACTATTCTTTGCTGATGCCTATTCTGAC ACTCACAAGGGCTTTGGCTGCTGAGTCCCATATGGACGCTGATCTCGCGAAGCCACTTATTAAGTGGGATTTGCTGAAATATGATT TCACGGAAGAGAGACTATGTCTTTTCGACCGTTATTTTAAATATTGGGACCAGACGTACCATCCTAATTGTATTAATTGTTTGGATG ACAGGTGTATCCTTCATTGTGCAAACTTTAATGTGTTATTTTCTACTGTGTTTCCACCTACGAGTTTTGGACCACTAGTAAGAAAGAT ATTTGTAGATGGTGTACCTTTTGTTGTTTCAACGGGATACCATTTCCGTGAGCTAGGGGTTGTACATAATCAGGATGTAAACTTACA TAACTCGCGTCTCAGTTTTAAGGAACTTTTAGTGTACGCTGCTGATCCAG

Table S4. C_t values obtained from the detection of 64 clinical SARS-CoV-2 RNA samples by RT−qPCR.

Samples ID	1	2	3	4	5	6	7	8
N-gene (C_t)	34.65	38.61	31.73	30.10	36.81	29.31	32.99	32.04
O-gene (C_t)	35.21	/	33.65	31.34	39.07	30.10	34.75	33.22
Samples ID	9	10	11	12	13	14	15	16
N-gene (C_t)	29.40	32.38	30.87	27.33	28.22	34.42	32.27	35.49
O-gene (C_t)	30.46	33.26	33.08	28.32	31.67	35.11	33.81	37.14
Samples ID	17	18	19	20	21	22	23	24
N-gene (C_t)	32.20	38.21	35.12	34.29	33.58	34.63	31.10	35.02
O-gene (C_t)	33.48	37.77	36.03	35.32	34.54	35.11	32.96	35.56
Samples ID	25	26	27	28	29	30	31	32
N-gene (C_t)	33.51	33.23	31.85	27.94	30.13	29.03	34.45	29.53
O-gene (C_t)	34.55	34.23	32.28	29.11	31.11	30.39	35.28	31.06
Samples ID	33	34	35	36	37	38	39	40
N-gene (C_t)	36.02	20.63	33.87	38.33	37.41	32.19	/	22.94
O-gene (C_t)	37.02	22.12	34.80	38.75	/	33.51	39.41	24.47
Samples ID	41	42	43	44	45	46	47	48
N-gene (C_t)	26.33	26.66	26.22	22.18	21.75	29.02	29.02	29.38
O-gene (C_t)	28.44	28.44	27.33	23.82	22.72	30.29	30.38	30.86
Samples ID	49	50	51	52	53	54	55	56
N-gene (C_t)	24.55	32.95	33.38	28.87	26.29	33.05	/	/
O-gene (C_t)	26.05	34.05	34.88	29.67	27.68	33.81	/	/
Samples ID	57	58	59	60	61	62	63	64
N-gene (C_t)	/	/	/	/	/	/	/	38.80
O-gene (C_t)	/	/	/	/	/	/	/	/

SUPPORTING INFORMATION

Author Contributions

Menglu Hu, Ruhan Liu, and Xiaoming Zhou conceived the project, interpreted the data, discussed the results, and commented on the study, Xiaoming Zhou supervised the study. Menglu Hu and Ruhan Liu performed the experiments and contributed the data analysis and management. Menglu Hu and Xiaoming Zhou wrote the manuscript. Zhiqiang Qiu designed and constructed the portable fluorescence detection device. Feng Cao and Tian Tian assisted in conducting the CRISPR experiment. Yunxin Lu and Yongzhong Jiang collected clinical samples. Menglu Hu and Ruhan Liu contributed equally to this work.