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# Electronic Supplementary Material

## CRISPR/Cas12a Technology Combined with RT-ERA for Rapid and Portable SARS-CoV-2 Detection

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**Supplementary Materials and Methods**

**Human clinical sample collection and RNA preparation**

Clinical samples RNA used in this study were recruited from the Chinese Academy of Inspection and Quarantine (Beijing). Clinical samples used in this study were collected and treated in strict accordance with the standard operation for SARS-CoV-2 recommended by the World Health Organization. All sample treatments were conducted in the Laboratory of China, Beijing. According to the requirements of National Health Commission of China, SARS-CoV-2 was inactivated in BSL-3 laboratory at 56°C for 30 minutes. And the viral RNA was extracted in BSL-2 laboratory by QIAamp Viral RNA Kit, according to the manufacturer's instructions (QIAGEN, cat. no.52906).

**Nucleic acid preparation**

The *N* (28700-29300) and *ORF1ab* (13000-13600) gene fragment of SARS-CoV-2 (GenBank: MN908947.3) was synthesized by GenScript (Nanjing, China) and cloned into the pUC57 vector. The RNAs of *N* and *ORF1ab* gene were transcribed with pUC57-*N*/pUC57-*ORF1ab* as a template using the T7 Transcription Kit (VIEWSOLID BIOTEH, cat. no. VK010) according to the manufacturer's instructions. Concentration was determined with Nanodrop and stored at −80 °C until use. The crRNA and primer were also synthesized by GenScript. The

sequences are listed in Supplementary Table S1.

Take 100 μL pseudovirus (from SARS-CoV-2 nucleic acid detection kit) and 140 μL $10^4$ PFU/mL influenza virus PR8 strain, $10^6$ PFU/mL human Enterovirus D68 (EV-D68) and human Enterovirus 71 (EV71) to extract RNA. 40 μL DEPC water was used for washing, and 4 μL RNA was added to each detection system for specificity test.

**Quantitative Real-time PCR**

The quantitative real-time PCR detection of the SARS-CoV-2 was carried out using SARS-CoV-2 nucleic acid test kit approved for market in China according to the manufacturer's instructions (DA AN GENE, cat. no. DA0931). Briefly, single-tube PCRs were prepared containing 17 μL of PCR reaction solution A, 3 μL of PCR reaction solution B and 2 μL sample RNA. The amplification conditions used were an initial denaturation step of 50°C for 15 min and 94°C for 15 min, followed by 45 cycles of 94°C for 15 s, 55°C for 45 s. The cycle value (Ct) ≤ 40 was judged as SARS-CoV-2 positive.

**CRISPR/Cas12a detection reaction**

The isothermal amplification of the *N* gene and *ORF1ab* gene was performed with a commercial Enzyme Recombinant Application (RT-ERA) kit (GenDX, cat. no. KS102) according to the manufacturer's instructions. Briefly, 4 μL RNA sample, 2.5 μL forward prime, 2.5 μL reverse primer (10 μmol/L), 20 μL reaction buffer, 19 μL DNase/RNase-Free water and 2 μL activator were incubated at 37 °C for 30 min. 4 μL crRNA (1 μmol/L) and 3 μL buffer was incubated with 1 μL Cas12a (1 μmol/L) (New England Biolabs, cat. no. M0653S) at 25°C for 20 min and fully combined. Then, 20 μL RT-ERA reaction system and 2 μL ssDNA FQ probe (10 μmol/L) were transferred to the CRISPR/Cas12a cleavage assay (total detection system: 30 μL). Reactions were incubated in GS8 Isothermal Cycler (GenDX, cat.no. GS8) for 10 min at 37°C with fluorescence measurements taken every 30 seconds.

For immunochromatographic strips detection reactions, the 20 μL ERA reaction system and 2 μL ssDNA FB probe (100 μmol/L, labelled with FAM and biotin) were transferred to the CRISPR/Cas12a cleavage assay. The CRISPR/Cas12a detection reaction was diluted 1:2 in detection buffer, and then the strips (Milenia biotec, cat. no. MGDH1) were inserted and incubated at room temperature for 1 min. The strips were then removed and photographed with a camera. The band density was analyzed by ImageJ for visualization and quantification.

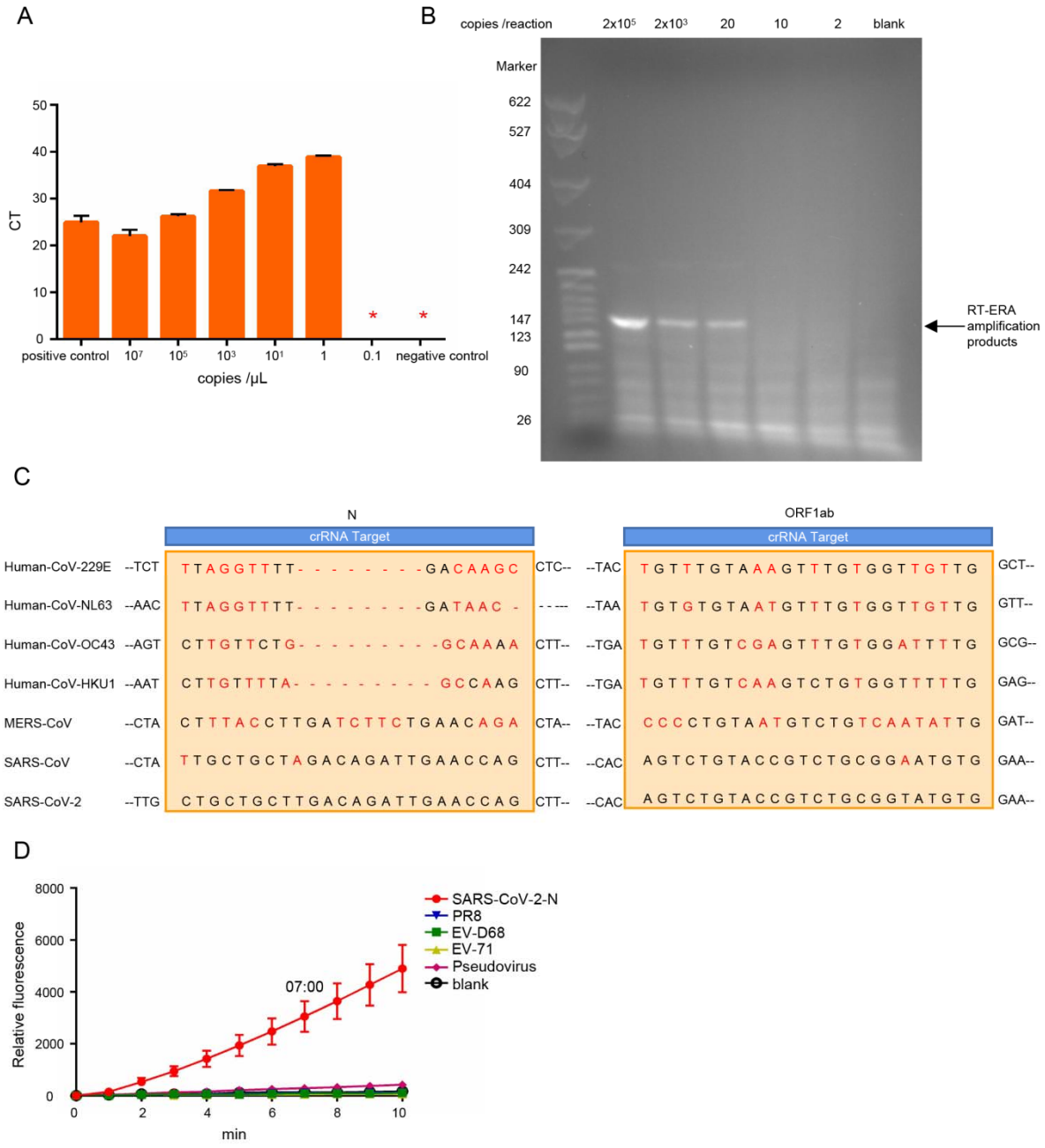


**Fig. S1** **A** Serially diluted synthetic *N* gene was taken as the template, and qRT-PCR detection sensitivity was tested according to the description in the manual (Red * indicates no signal). **B** The results of agarose gel electrophoresis of RT-ERA amplified products (the arrow points to the amplified band). **C** The genome map of crRNA target sequences of *N* and *ORF1ab* genes of human coronavirus. **D** The RNA of SARS-CoV-2 (100 copies/μL, *ORF1ab* gene), influenza virus PR8 strain, EV-D68, EV71, and pseudoviridae were amplified by RT-ERA and detected by CRISPR/Cas12a system.

**Table S1** List of primers and crRNA

| Names | | | Sequences |
|---|---|---|---|
| N | crRNA | | UAAUUUCUACUAAGUGUAGAUCUGCUGCUUGACAGAUUGAACCAG |
| | Primer | F | GCAATGGCGGTGATGCTGCTCT |
| | | R | TGTGTTACATTGTATGCTTTAG |
| ORF1ab | crRNA | | UAAUUUCUACUAAGUGUAGAUCACAUACCGCAGACGGUACAGACU |
| | Primer | F | CCGTTGCCACATAGATCATCCA |
| | | R | TGGGTTCGCGGAGTTGATCACA |