Supporting Information

CRISPR-Cas12a-based ultrasensitive assay for visual detection of SARS-Co-2 RNA

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Experimental Section

Reagents and Materials.

CRISPR RNA (crRNA) and DNA oligonucleotides were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). The plasmids containing SARS-CoV-2, MERS-CoV, Ebola and Zika gene fragment were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). RPA kit was purchased from AMP future. Ferric chloride anhydrous (≥ 97%), sodium acetate, trisodium citrate dihydrate (≥ 99%), and ethylene glycol were purchased from China National Pharmaceutical Group Corp (Shanghai, China). Streptavidin, N-hydroxysuccinimide (NHS) (98%), 1-(3-diaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (98.5%), N, N'-methylenebisacrylamide (MBA) (99%), methacrylic acid (MAA) (99%), N-ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline, sodium salt (TOOS) and 4-aminoantipyrine (4-AA) were purchased from Heowns Biochemical Technology Co., Ltd (Tianjin, China). Glucose oxidase (GOx) and horseradish peroxidase were obtained from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Cas12a nuclease, reverse transcriptase and COVID-19-pseudovirus were purchased from Novoprotein (Shanghai, China). Tris(2-carboxyethyl) phosphine, hydrochloride (TCEP) (98%+) was obtained from Adamas-beta. Sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC), 2, 2'-azobis(2-methylpropionitrile) (AIBN) (98%), allylamine hydrochloride (98%) were purchased from Macklin (Shanghai, China). T7 RNA polymerase was obtained from Beyotime Biotechnology Co. Ltd. (Shanghai, China). NTPs Mixture was purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). FastDigest EcoRI and FastDigest HindIII were purchased from Thermos Scientific. Plasmid extraction kit, agarose gel DNA recovery kit and RNA cleanup kit were purchased from Tiangen Biotech Co., Ltd. (Beijing, China) Other aqueous solutions used in experiments were prepared using deionized water (18.2 MΩ·cm).

Instruments.

The concentrations of DNA were determined on NanoDrop One (Thermo Scientific,

USA). Fluorescence assay was tested on a fluorescence spectrometer (FLS-980, Edinburgh, UK). UV-vis absorption spectra were recorded on NanoDrop One (Thermo Scientific, USA). Native polyacrylamide gel electrophoresis analysis and agarose gel electrophoresis were carried out on gel electrophoresis apparatus (Bio-Rad, USA) and imaged with JS-680D automatic digital gel imaging analysis system.

Experimental details

Native polyacrylamide gel electrophoresis (native-PAGE). DNA sample (20 μL) was mixed with DNA loading buffer (2 μL) and then injected into the notch of gel (15%)。The gel was run in 1×TBE buffer for 80 min at a constant voltage of 120 V. After that, the gel was immersed in GelRed dye solution for 30 min. The image of gel was obtained by the JS-680D automatic digital gel imaging analysis system.

Agarose gel electrophoresis. The DNA sample was added with DNA loading buffer and then injected into the notch of agarose gel (1.5%). The gel was run at constant voltage of 100 V in 1×TAE buffer for 60 min. After that, the image of gel was acquired by the JS-680D automatic digital gel imaging analysis system.

Preparation for colorimetric substrate. A mixture of 2 mL of glucose solution (15%), 40 μL of TOOS solution (100 mM), 5 μL of 4-AA solution (10%), 100 μL of horseradish peroxidase (1 mg/mL) and 10 mL of MES buffer (100 mM, pH 5.7) was prepared.

Preparation of Luria-Bertani (LB) culture medium. Tryptone (10 g), yeast extract (5 g) and NaCl (10 g) was dissolved into deionized water (1 L). The pH of the mixture was adjusted to 7. The prepared culture medium was sterilized at high pressure for 20 min.

Synthesis of ORF1ab RNA by in vitro transcription. ORF1ab DNA fragment containing T7 promotor was obtained according to abovementioned procedure. The synthesis of ORF1ab RNA was realized by in vitro transcription. A mixture of 3 μL of 10×transcription buffer, 4 μL of NTPs, 10 μL of ORF1ab DNA, 0.5 μL of RNase inhibitor (40 U/μL), 1 μL of T7 RNA polymerase, 11.5 μL of water was prepared. The mixture was incubated at 37 °C for 12 h. After that, 3 μL of DNase I (10 mg/mL) was added into the mixture. The solution was incubated at 37 °C for another 4 h to

digest the DNA template. The generated ORF1ab RNA was purified by RNA cleanup kit. And the concentration of ORF1ab RNA was determined by NanoDrop One.

Detection of ORF1ab RNA gene fragment by the developed strategy. ORF1ab RNA was diluted into different concentrations and then amplified by RPA kit according to the operation procedure. Specifically, 29.4 μL of buffer A was added into the tube containing the lyophilized RPA reagents. Then, 2 μL of the forward primer (10 μM) and 2 μL of the reverse primer (10 μM) was added into above mixture. After that, 10 μL of solution containing certain concentration of ORF1ab was added into the tube. Then, 4.1 μL of deionized water containing 100 U of reverse transcriptase was added into the tube. Finally, 2.5 μL buffer B was added into the lid of the tube. The amplification reaction was started by turning the tube upside down. The mixture was incubated at 42 °C for 30 min and then heated at 80 °C for 10 min. 1 μL of the amplified product was taken out and mixed with 70 μL of reaction buffer (20 mM Tris−HCl (pH 7.5), 100 mM KCl, 30 mM $MgCl_2$, 0.05% Tween 20) containing CRISPR-Cas12a and MNPs-GOx. The mixture was incubated for 20 min at 37 °C. After that, the MNPs were removed by magnetic separation. The supernatant was taken out and mixed with 180 μL of colorimetric substrate. The mixture was incubated at 37 °C for 10 min to obtain the visual result.
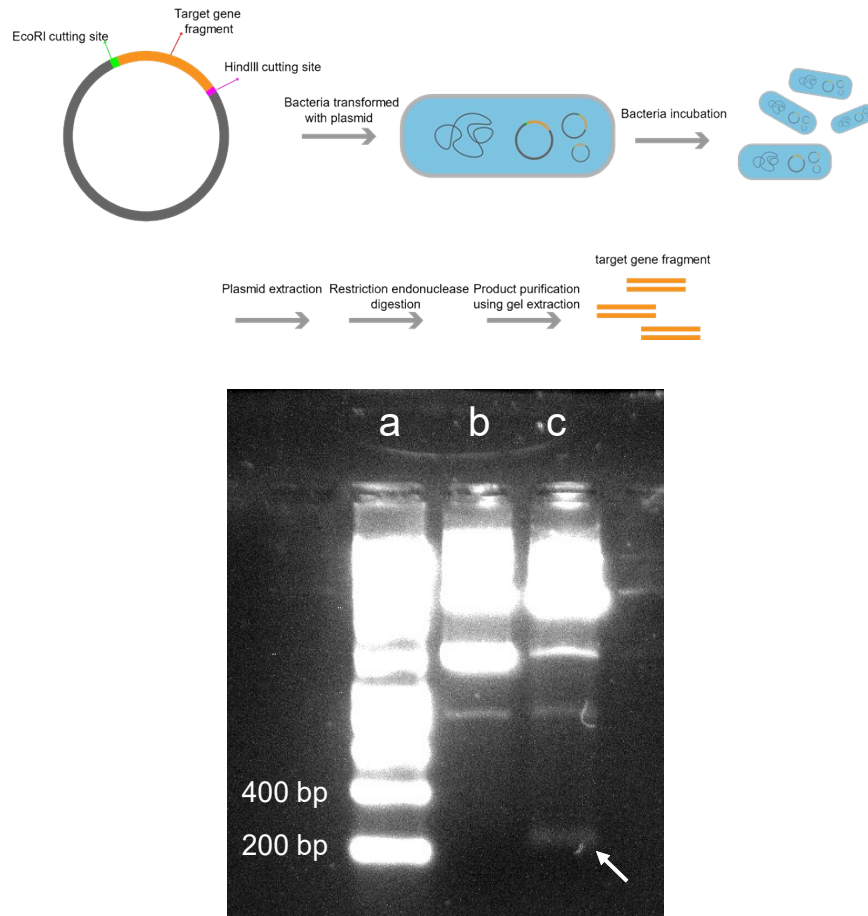
Supporting Figures and Table.

Fig. S1. (A) The schematic illustration of acquisition of target gene fragment. (B) Agarose gel electrophoresis image of the target gene fragment. a. marker; b. plasmid; c. plasmid+EcoRI+HindIII.
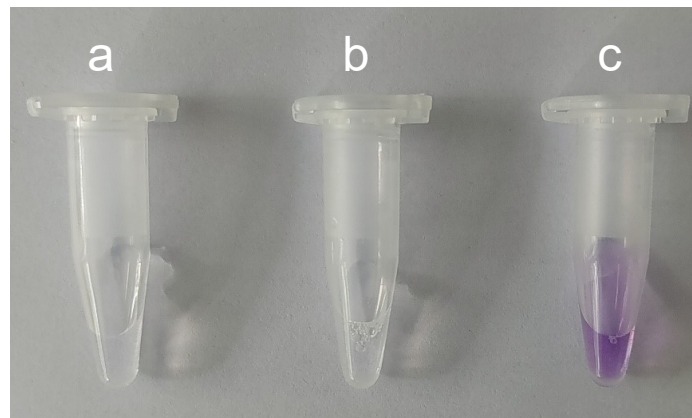

Fig. S2. Colour change of solution after target gene fragment and its amplicons were introduced to activate CRISPR-Cas12a for cleavage of MNPs-GOx. a. control; b. 100 fM of target gene fragment; c. RPA amplicons of 100 fM of target gene fragment.
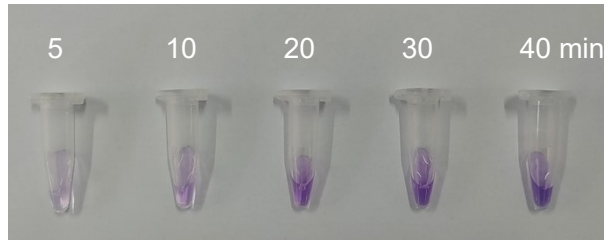
Fig. S3. Color change of solution with the increasing incubation time of CRISPR-Cas12a for cleavage of MNPs-GOx.
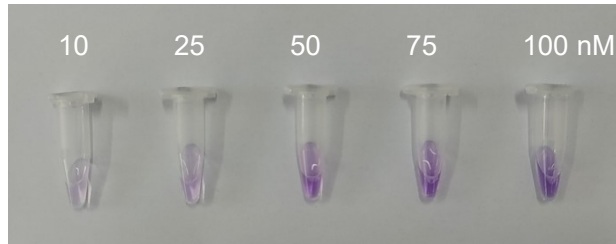

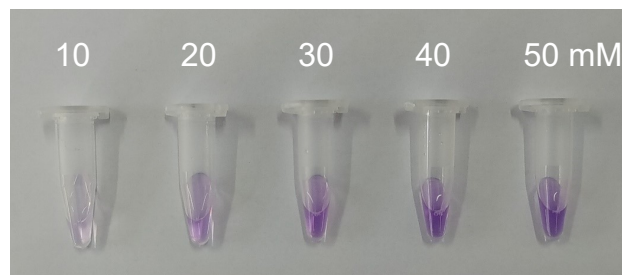**Fig. S4.** Color change of solution with the increasing concentration of CRISPR-Cas12a for cleavage of MNPs-GOx.

Fig. S5. Color change of solution with the increasing concentration of Mg^{2+} for cleavage of MNPs-GOx.
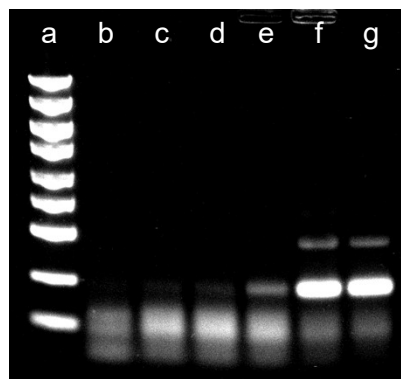

Fig. S6. Agarose gel electrophoresis image of the RPA amplicons of different concentrations of target gene fragment. a. marker; b. 0; c. 10 aM; d. 100 aM; e. 1 fM; f. 10 fM; g. 100 fM.
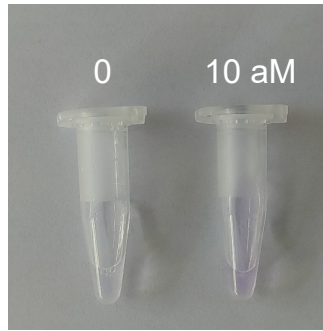
Fig. S7. Colour change of solution when the developed method was used to detect 0 and 10 aM of target gene fragment.
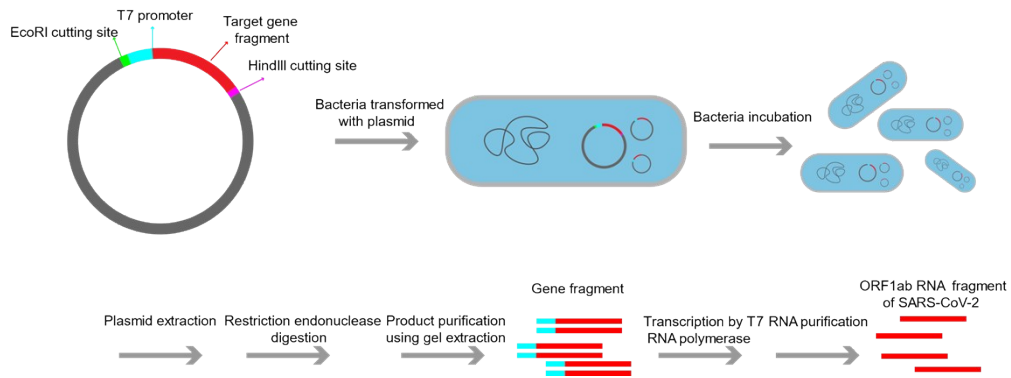

Fig. S8. The schematic illustration of acquisition of ORF1ab RNA gene fragment of SARS-CoV-2.
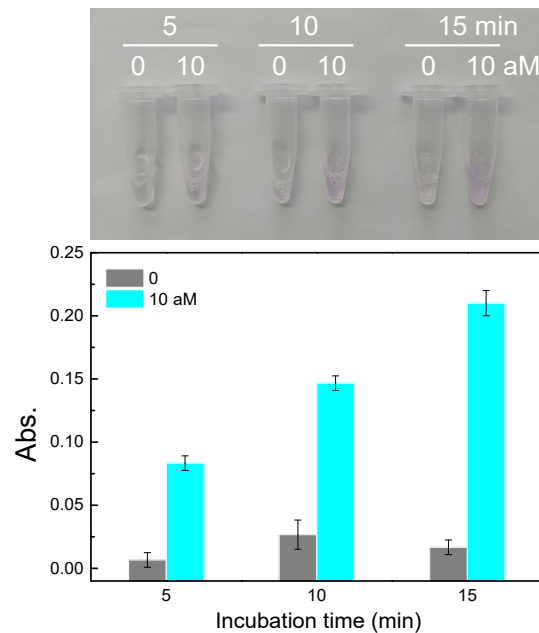

Fig. S9. Colour change of solution when the RT-RPA amplicons of 0 and 10 aM of RNA gene fragment were incubated with MNPs-GOx for different times.
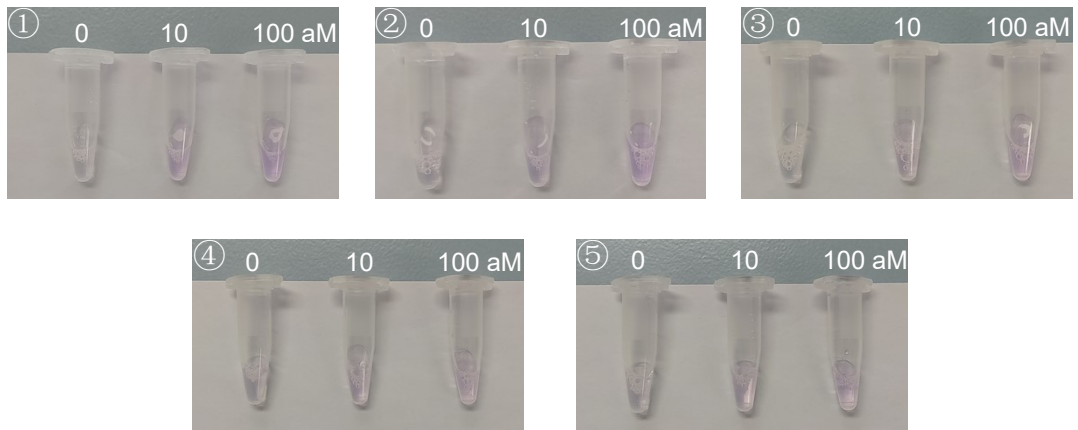
Fig. S10. Repeatability of the developed strategy for 0, 10, 100 aM of SARS-CoV-2 RNA gene fragment at different time periods.
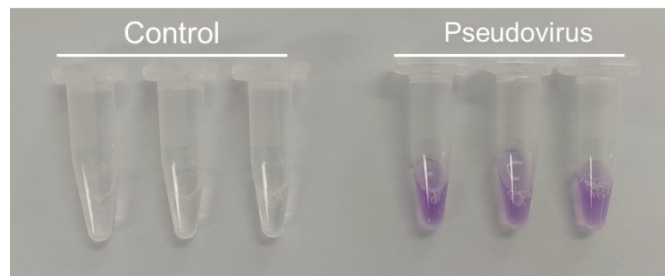

Fig. S11. Detection of SARS-CoV-2 pseudovirus using the developed strategy.

Table S1. Nucleic acid sequences used in the experiment.

nucleic acid	sequence (5'-3')
model target	TTCCCTCGAGGACAAGGCGTTCCAATTAACACCTTTGATGAAGGAAGAGCTCATCCG GTAATGTAAACAGATTTAATGTTGCTATTACCAGAGCAAAAGTAGGCATACTTTGCA TAATGTCTGATAGAGACCTTTATGACAAGTTGCAATTTACAAGTCTTGAAATTCCAC GTAGGAATGTGGCAACTAACCACAATCATATGGGTTGCAACTGAGGGAGCCTTGAA
ebola gene fragment	TAATA CGACT CACTA TAGGA CGCCC ACGAG CTGCC A GACA GCATT CAAGG GATGG ACACG ACCAC CATGT TCGAG CAC GA TCATC ATCCA GAGAG AATTA TCGAG GTGAG TACCG TCAAT CA AGG AGCGC CTCAC AAGTG CGCGT TCCTA CTGTA TTTCA TAAGA AGAGA GTTGA ACCAT TAACA GTTCC TCCAG CACCT AAAGA CAT AT GTCCG ACCTT GAAAA AAGGA TTTTT GTGTG ACAGT AGTTT TT GCA AAAAA GATCA CCAGT TGGAG AGTTT AACTG ATAGG GAATT ACTCC TACTA ATCGC CCGTA AGACT TGTGG ATCAG TAGAA CAA CA ATTAA AT
MERS-CoV gene fragment	CACCTTTTTGACACTTTTCTTGTTGCCTGTGGCTATTTGTTTGAC TTATGCAAACATAGTCTACGAGCCCACTACTCCCATTTCGTCAG CGCTGATTGCAGTTGCAAATTGGCTTGCCCCCACTAATGCTTAT ATGCGCACTACACATACTGATATTGGTGTCTACATTAGTATGTCA CTTGTATTAGTCATTGTAGTGAAGAGATTGTACAACCCATCACT TTCTAACTTTGCGTTAGCATTGTGCAGTGGTGTAATGTGGTTGT ACACTTATAGCAT
zika gene fragment	GGGCCAGCACAGTGGGATGATCGTTAATGACACAGGACATG AAACTGATGAGAATAGAGCGAAAGTTGAGATAACGCCCAAT TCACCAAGAGCCGAAGCCACCCTGGGGGGGGTTTGGAAGCCT AGGACTTGATTGTGAACCGAGGACAGG
SARS-CoV-2 gene fragment with T7 promotor	TTAATACGACTCACTATAGGGTAATGTAAACAGATTTAATGTTGCTATTACCAGAGC AAAAGTAGGCATACTTTGCATAATGTCTGATAGAGACCTTTATGACAAGTTGCAATT TACAAGTCTTGAAATTCCACGTAGGAATGTGGCAACTTTACAAGCTGAAAATGTAAC AGGACTCTTTAAAGATTGTAGTAAGGTAATCACTGGGTTACATCCTACACAGGCACC TACACACCTCAGTGTTGACACTAAATTCAAAACTGAAGGTTTATGTGTTGACATACC TGGCATACCTAAGGACATGACCTATAGAAGACTCATCTCTATGATGGGTTTTAAAAT GAATTATCAAGTTAATGGTTACCCTAACATGTTTATCACCCGCGAAGAAGCTATAAG ACATGTACGTGCATGGATTGGCTTCGATGTCGA
crRNA (model target)	UAAUU UCUAC UAAGU GUAGA U A UGAAG GA AGAG CUCAU CCG
RT-RPA forward primer (model target)	TTCCCTCGAGGACAAGGCGTTCCAATTAACAC
RT-RPA reverse primer (model target)	TTCAAGGCTCCCTCAGTTGCAACCCATATGAT
crRNA(ORF1ab)	UAAUU UCUAC UAAGU GUAGA U AAGAUUGUAGUAAGGUA AUCACUG
RT-RPA forward primer (ORF1ab)	CTTGAAATTCCACGTAGGAATGTGGCAACTTTAC
RT-RPA reverse primer (ORF1ab)	GTATGCCAGGTATGTCAACACATAAACCTTCAG

crRNA(pseudovirus)	UAAUU UCUAC UAAGU GUAGA U CA GGUAA CCUAC AAAGC AAC
RT-RPA forward primer (pseudovirus)	ATGAT TGATG TTCAA CAATG GGGTT TTACA GG
RT-RPA reverse primer (pseudovirus)	GTC AAC ACG CTT AAC AAA GCA CTC GTG GAC AG
ssDNA linker	biotin-TTATTATTATTATTATTATTATTATTATTATTA TTAT-SH C6