Short Communication

Rapid and sensitive detection of COVID-19 using CRISPR/Cas12a-based detection with naked eye readout, CRISPR/Cas12a-NER

Xinjie Wang [a,1], Mingtian Zhong [b,1], Yong Liu [c,d,1], Peixiang Ma [a], Lu Dang [e], Qingzhou Meng [e], Wenwei Wan [f], Xiaodong Ma [b], Jia Liu [a], Guang Yang [a], Zifeng Yang [c,*], Xingxu Huang [f,*], Ming Liu [c,*]

[a] *Shanghai Institute for Advanced Immunochemical Studies, ShanghaiTech University, Shanghai 201210, China*
[b] *Institute for Brain Research and Rehabilitation, Guangdong Key Laboratory of Mental Health and Cognitive Science, Center for Studies of Psychological Application, South China Normal University, Guangzhou 510631, China*
[c] *State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, Guangzhou Institute of Respiratory Health, The First Affiliated Hospital, Guangzhou Medical University, Guangzhou 510120, China*
[d] *Kingmed Virology Diagnostic & Translational Center, Guangzhou Kingmed Center for Clinical Laboratory Co., Ltd., Guangzhou 510005, China*
[e] *Affiliated Cancer Hospital & Institute of Guangzhou Medical University, Guangzhou 510095, China*
[f] *School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China*

The outbreak of the 2019 novel coronavirus disease (COVID-19) has had a significant impact on global health. To advance the diagnostic method of COVID-19, we developed a CRISPR/Cas12a-based assay with a naked eye readout, CRISPR/Cas12a-NER. The assay can detect as few as 10 copies of the virus gene in 45 min without a special instrument and has good consistency with the qPCR assay, providing a simple and reliable on-site diagnostic method suitable for a local hospital or community testing.

The novel coronavirus disease (COVID-19) had caused more than 1,991,562 confirmed cases with 130,885 deaths by 16 April 2020 (World Health Organization Coronavirus disease 2019 situation reports). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection leads to the disease [1]. SARS-CoV-2, a positive-sense virus, belongs to *Betacoronavirus* genus lineage β and is phylogenetically closely related to bat SARS-like coronaviruses [2].

Sensitive and rapid detection of COVID-19 is crucial in controlling the outbreak. Currently, real-time quantitative reverse transcription-polymerase chain reaction (qPCR) assays are recommended as molecular diagnostic methods [3,4]. However, qPCR requires an elaborate instrument and professional operation, which limits its diagnostic application. Recently, a CRISPR/Cas-based nucleic acid detection technology was developed with the advantages of sensitivity, specificity, rapidity, and simplicity [5,6]. To advance the on-site diagnosis of COVID-19, we introduce a CRISPR/Cas12a-based rapid detection assay.

To sensitively and rapidly detect SARS-CoV-2 nucleic acid, CRISPR/Cas12a-based-detection with naked eye readout, termed CRISPR/Cas12a-NER, including the Cas12a protein, SARS-CoV-2 specific CRISPR RNAs (crRNAs) and a single-stranded DNA (ssDNA) reporter, was developed as shown in Fig. 1a. To enable on-site diagnosis, an ssDNA reporter labelled with a quenched green fluorescent molecule was introduced, which will be cleaved by Cas12a when there is nucleic acid of SARS-CoV-2 in the detection system, and the resulting green fluorescence can be seen with the naked eye under 485 nm light (Fig. 1a).

For specific detection of the SARS-CoV-2 genome, a total of 15 crRNAs, which are predicted to distinguish single nucleotide polymorphisms (SNPs) with other SARS or SARS-related viruses, were designed on four domains of the *orf1a*, *orf1b*, *N* and *E* genes over the Wuhan-Hu-1 strain (GenBank accession number MN908947) (Fig. 1b, Fig. S1 online). The results showed that 14 crRNAs, except the E-crRNA1, targeting SARS-CoV-2 were validated, and all of them highly and specifically reacted with synthetic gene fragments (Fig. 1c, Figs. S2 and S3 online). As the CRISPR/Cas12a-NER readout signal relies on the crRNA-dependent targeting cleavage efficiency, which is affected by the secondary structure and spacer sequence of the crRNA [7,8]. The crRNAmix (equal mix work crRNAs targeting at each gene, named *orf1a*-crRNAmix, *orf1b*-crRNAmix, *N*-crRNAmix, *E*-crRNAmix) mediated the strongest fluorescence signal (Fig. S4 online) and was chosen for the detection of COVID-19.

* Corresponding authors.
  E-mail addresses: jeffyah@163.com (Z. Yang), huangxx@shanghaitech.edu.cn (X. Huang), mingliu128@hotmail.com (M. Liu).
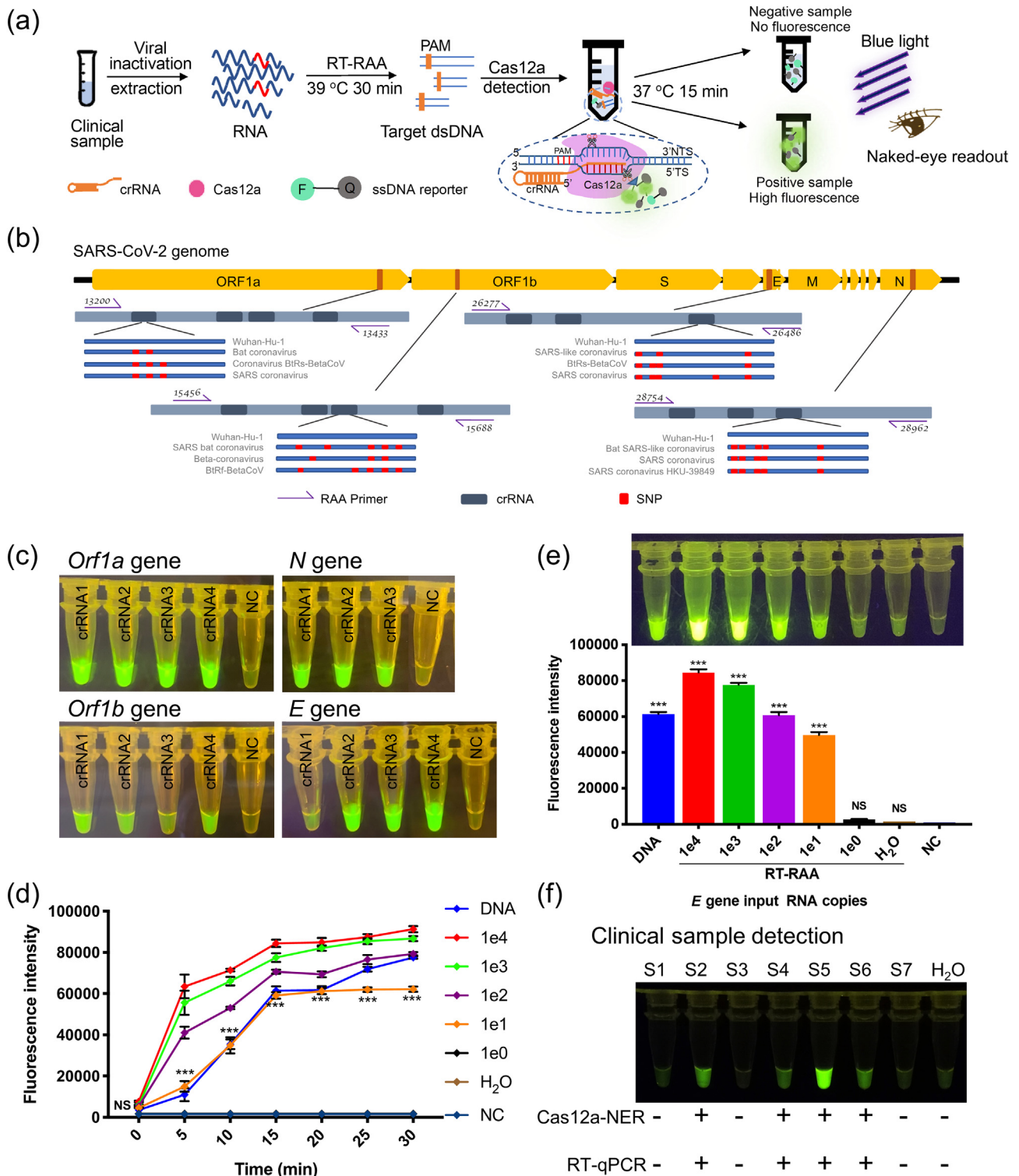 [1] These authors contributed equally to this work.

Fig. 1. CRISPR/Cas12a-based detection with naked eye readout (CRISPR/Cas12a-NER) of SARS-CoV-2. (a) Schematic of Cas12a-based assay for rapid and visual nucleic acid detection. After 30 min of RT-RAA reaction, the Cas12a enzyme targets cleavage for an additional 15 min. The green fluorescent signals can be observed by the naked eye under blue light with a wavelength of 485 nm. (b) Four target gene fragments and SARS-CoV-2-specific crRNAs location on the genome. (c) Validation of crRNA specifically reacts with the target gene. (d) Time course of *E* gene detection with *E*-crRNAmix on gradient diluted input RNAs from 1e4 to 1e0 copies. (e) Sensitivity assay using *E*-crRNAmix to detect gradient diluted *E* gene RNA from 1e4 to 1e0 copies. (f) Diagnostic accuracy of CRISPR/Cas12a-NER on the diagnosis of COVID-19 clinical samples. ***: statistical significance; NS: not significance.

As SARS-CoV-2 is an RNA virus, reverse transcript recombinase-aided amplification (RT-RAA) was combined to obtain enough DNA for Cas12a-mediated detection. The RT-RAA will amplify the target gene fragment in 30 min at 39 °C, followed by a CRISPR/Cas12a reaction at 37 °C (Fig. 1a). Cas12a-mediated detection produces a robust signal in a 15 min reaction (Fig. 1d), and then the assay time is fixed to 15 min. The sensitivity of the CRISPR/Cas12a-NER assay was determined by the fluorescence intensities. The results show that among the four target genes, the *E*-crRNAmix for the target *E* gene is the most sensitive, which can clearly detect 10 copies

of SARS-CoV-2 synthetic nucleic acid (Fig. 1e, Fig. S5 online). Thus, we chose *E*-crRNAmix for a screening assay of SARS-CoV-2, and the remaining target genes can be used for a further confirmed diagnostic assay.

To validate the CRISPR/Cas12a-NER diagnostic accuracy, a total of 31 clinically extracted RNA samples were used for COVID-19 detection with the CRISPR/Cas12a-NER assay and TaqMan qPCR (GenScript, Nanjing, China) for the E gene in parallel. Clinical samples used in this study were collected and treated in strict accordance with the standard operation for COVID-19 recommended by the World Health Organization. All sample treatments were conducted in the State Key Laboratory of Respiratory Disease and Kingmed Virology Diagnostic & Translational Center (Guangzhou). Among these samples, consistent with clinical diagnosis, 16 were determined to be SARS-CoV-2 positive by both CRISPR/Cas12a-NER and qPCR assays and showed 100% agreement (Fig. 1f, Figs. S6 and S7 online), with the kappa value (κ) being 1.0 ($P < 0.001$) (Table S3 online), confirming the outstanding performance of CRISPR/Cas12a-NER.

Overall, we described CRISPR/Cas12a-NER, which provides a novel alternative for the portable, simple, sensitive, and specific detection of the COVID-19 virus, providing a simple and reliable on-site diagnostic method suitable for a local hospital or community testing.

Conflict of interest

The author declares that they have no conflict of interest.

Acknowledgments

Author contributions

Xinjie Wang, Ming Liu, Zifeng Yang and Xingxu Huang conceived the study. Xinjie Wang, Mingtian Zhong, Peixiang Ma and Xingxu Huang designed the experiments. Xiaodong Ma, Guang Yang, Zifeng Yang, Ming Liu and Xingxu Huang supervised the project. Xinjie Wang, Mingtian Zhong and Yong Liu performed the experiments with the assistance of Peixiang Ma. Lu Dang, Qingzhou Meng, Jia Liu, Yong Liu, Zifeng Yang and Ming Liu collected and processed materials. Xinjie Wang, Mingtian Zhong, Wenwei Wan and Lu Dang analyzed the data. Xinjie Wang, Ming Liu and Xingxu Huang wrote the paper.

Appendix A. Supplementary materials

Supplementary materials to this article can be found online at https://doi.org/10.1016/j.scib.2020.04.041.

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Xinjie Wang is a research scientist at Shanghai Institute for Advanced Immunochemical Studies, ShanghaiTech University. His research focuses on the gene-editing tool development and application, including CRISPR-based base editing and nucleic acid detection.

Mingtian Zhong graduated from Guangzhou University of Chinese Medicine with a Ph.D. degree, and now is a postdoctoral researcher of South China Normal University. She studies the molecular and cellular mechanisms of malignancy in respiratory system.

Yong Liu is the director of the Clinical Infectious Disease Laboratory and works in National Clinical Research Center of Respiratory Disease-Research Center for Clinical Virology, Kingmed Virology Diagnostic & Translational Center. His research focuses on the diagnosis of respiratory viral diseases and translational research work.

Zifeng Yang serves as a PI in Clinical Viral Research at Guangzhou Institute of Respiratory Health of Guangzhou Medical University; Co-Executive Director of the Viral Diagnosis and R&D Center of GIRH – KingMed Diagnostics. He mainly engages in drug development and virus disease at the animal-human interface including avian H7N9 and H5N6 influenza virus, as well as the associated viral immunology.

Xingxu Huang is a professor at the School of Life Science and Technology, ShanghaiTech University. His research interests focus on developing genome editing and base editing approaches for linkage of the genotype to phenotype and therapeutic purpose.

Ming Liu is an associate professor of Guangzhou Institute of Respiratory Health, Guangzhou Medical University. His research focuses on CRISPR-based therapeutics and diagnostics of respiratory disease, especially lung cancer.