



Short Communication

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

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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.

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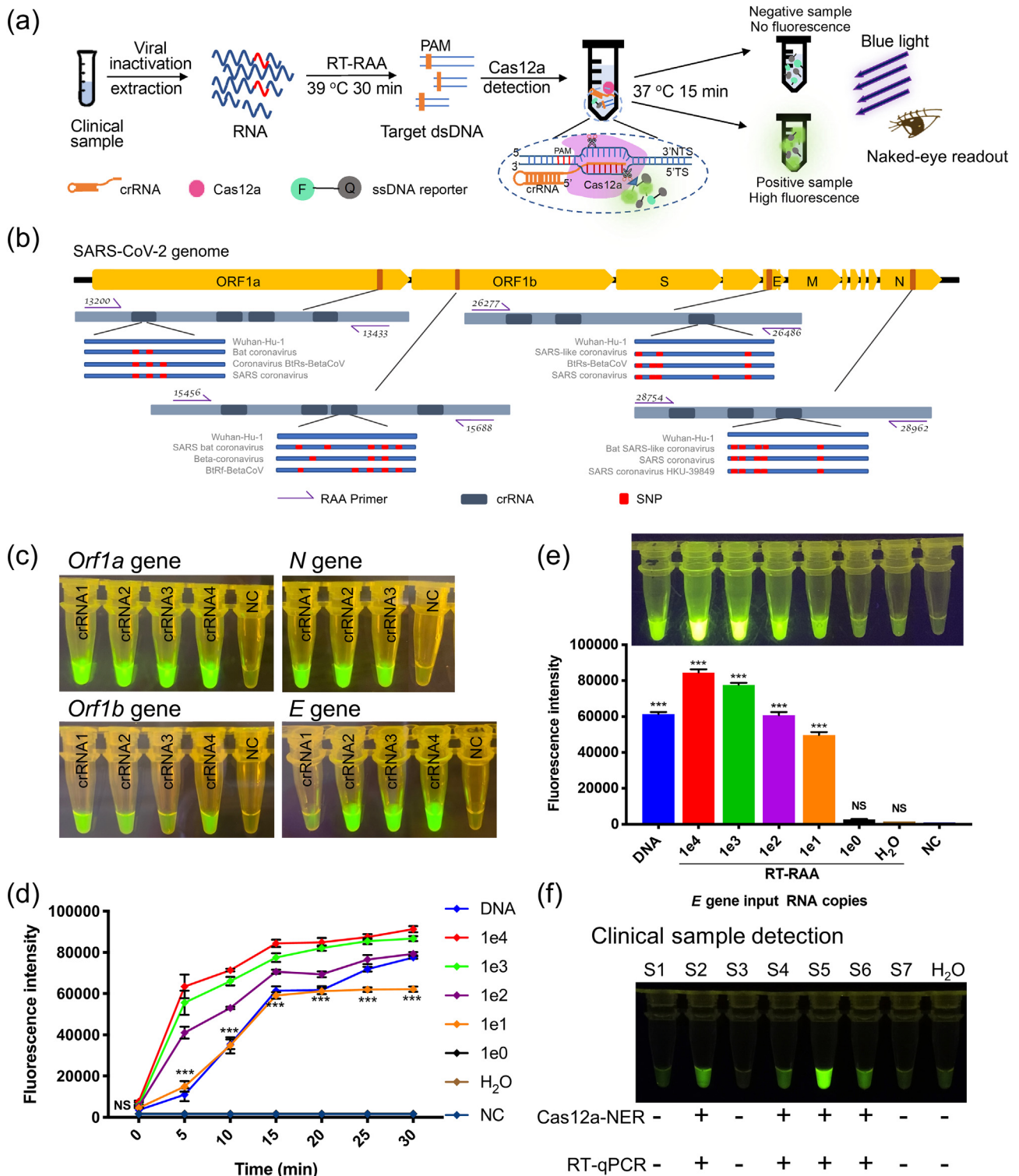


Fig. 1. CRISPR/Cas12a-based detection with naked eye readout (CRISPR/Cas12a-NER) of SARS-CoV-2. (a) Schematic of Cas12a-based assay for rapid and visual nucleic acid detection. After 30 min of RT-RAA reaction, the Cas12a enzyme targets cleavage for an additional 15 min. The green fluorescent signals 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 recombinaise-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.

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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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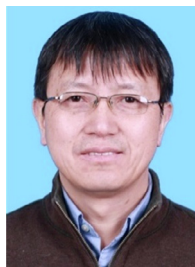
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