Elsevier has created a Monkeypox Information Center in response to the declared public health emergency of international concern, with free information in English on the monkeypox virus. The Monkeypox Information Center is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

CRISPR-Cas12a-based detection of monkeypox virus

Dear Editor,

We read with interest the recently published research in *Journal of Infection* by Dr. Usman Ayub Awan et al., who emphasized that the remerging monkeypox is a new threat to the world [1]. Monkeypox has been declared a public health emergency of international concern by the World Health Organization (WHO) on 23 July 2022. Between 1 January and 25 August 2022, it has resulted in more than 46,700 laboratory confirmed cases of monkeypox and 12 deaths worldwide in 75 countries [2]. Public health authorities are proactively identifying cases and tracing their contacts to contain its spread. As with COVID-19, PCR is the current method capable of being deployed at sufficient speed to provide timely feedback on any public health interventions [3]. However, standard RT-qPCR methods that require laboratory-based testing instruments such as a thermal cycler for DNA amplification have constraints, including high cost, detection time, and the need for trained experts, limiting their application in point-of-care (POC) testing and resource-limited areas. Thus, the development of novel detection strategies for monkeypox is still urgent needed.

CRIPSR-based detection strategies have been widely used to detect of various viruses [4,5]. In this study, we report the development and initial validation of a CRIPSR-Cas12a-based assay for detection of monkeypox virus. We designed the system that

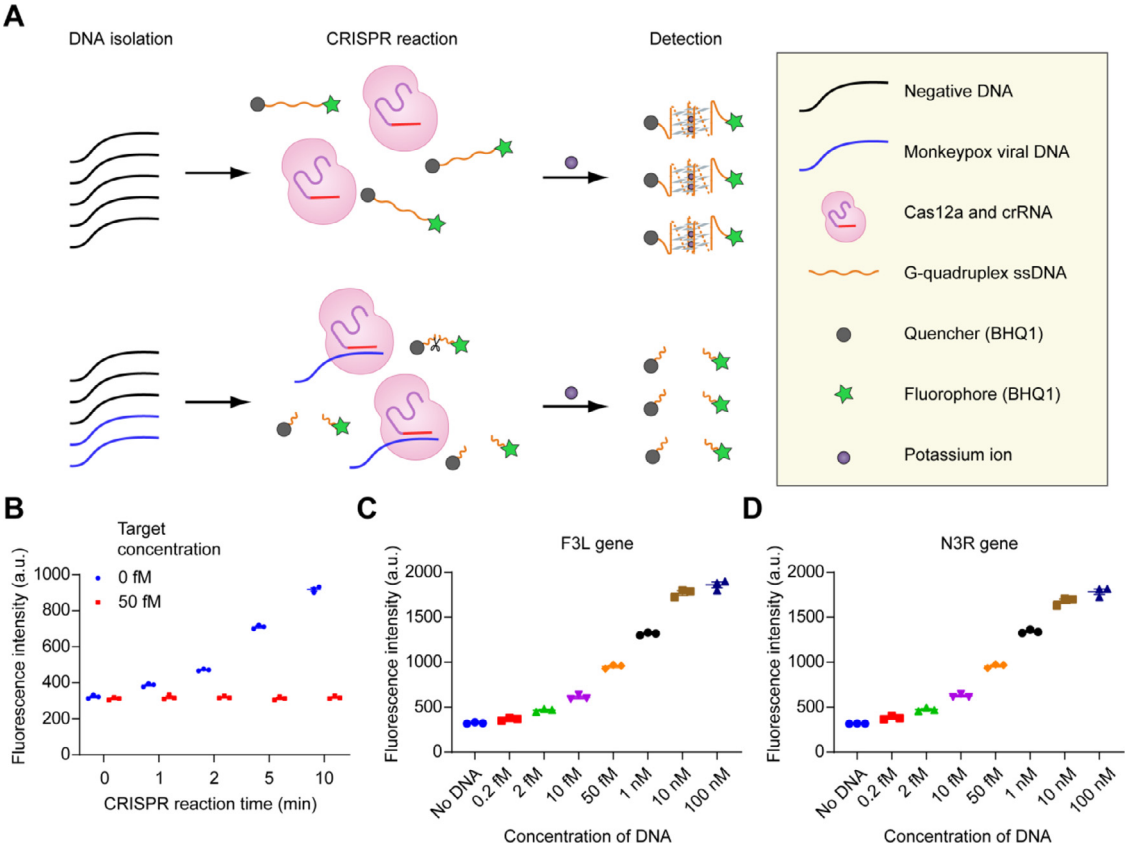

Fig. 1. The CRISPR-Cas12a-based system for detection of monkeypox virus. (A) Schematic of CRISPR-Cas12a-based system proposed in this study. (B) Fluorescence signal of CRISPR-Cas12a-based assay for target DNA saturates within 10 min. (C and D) The fluorescence intensity (λ_{em} = 520 nm) for the detection of the monkeypox viral F3L (C) and N3R (D) genes at various concentrations. The assay revealed a statistically significant detection of F3L and N3R DNAs, at a concentration as low as 2 fM ($^{**}P < 0.01$ vs. no RNA).

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Table 1
Oligonucleotides in this study.

Name	Sequences (5′→3′)
Fluorescently-labeled G-quadruplex oligonucleotides	
P4-WT	BHQ-GGGTTAGGGTTAGGGTTAGGG-FAM
CRISPR Cas12a gRNA sequences	
F3L-gRNA	TCACAATGAAATATTATGTT
N3R-gRNA	TAACGGCGACGAATATACTG

detect the monkeypox viral DNA by using fluorescence readout. In the presence of the DNA target analyte, the G-quadruplex oligonucleotide (Table 1), which is labeled with 6-fluorescein (6-FAM) on the 3′-end and black hole-1 quencher (BHQ-1) on the 5′-end, gets degraded through Cas12a-mediated collateral cleavage resulting in fluorescent signal. While in the absence of the DNA target analyte, Cas12a cannot cleave the fluorescently-labeled G-quadruplex oligonucleotides and the oligonucleotides can formation G-quadruplex structures in 100 mM K^+condition, resulting in the fluorescence quenching (Fig. 1A). We found that the FAM fluorescent signal was detectable in 2 min and a strong signal was achieved within 10 min (Fig. 1B), suggesting the system is a potential rapid detection technology. In addition, we used the system to detect the F3L and N3R genes (Table 1). DNA samples of F3L and N3R genes in the same concentration range (from 0.2 fM to 100 nM) were subjected to the system, and the enhanced FAM fluorescence signal with monkeypox DNA was statistically significant ($P < 0.01$) at concentrations as low as 2 fM (Fig. 1C and 1D), indicating the high sensitivity of our system for monkeypox virus detection.

In summary, we develop a rapid, easy-to-implement and accurate CRISPR-Cas12a-based system for detection of monkeypox virus. Our CRISPR-based assay provides a visual and faster alternative to current PCR-based diagnosis for monkeypox virus.

Author contributions

J.Y.L. was the principal investigator who conceived and designed the study, obtained financial supports and approved the final version of the manuscript. Y.T.S. conducted the functional, mechanism and partial biophysical experiments, performed the statistical analyses, interpreted the results and drafted manuscript. Q.X. helped to conduct partial functional and mechanism experiments. M.S.L. and K.Y.Z. conducted data management. All the authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Declaration of competing interest

The authors declare no conflict of interests.

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Not applicable.

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Yutong Sui[#]
Qi Xu[#]
Mingsheng Liu
Kuiyang Zuo
Xiaomei Liu[*]
Jinyu Liu[*]
Department of Toxicology, School of Public Health, Jilin University, Changchun, China
[*]Corresponding authors at: 1163 Xinmin Street.
E-mail addresses: liuxiaom@jlu.edu.cn (X. Liu), jy_liu@jlu.edu.cn (J. Liu)
[#] These authors contributed equally to this work.