Review Article

Application of CRISPR/Cas12a for SARS-CoV-2 Nucleic Acid Detection

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Abstract

SARS-CoV-2 spans 3 years, and although the virus has been adjusted from "Category B, Tube A" to "Category B, Tube B" it is still present in the environment, causing widespread epidemic infections and great impact on human health and the environment. Currently, there are various nucleic acid detection methods for this virus, and CRISPR methods are widely used to construct biosensors for accurate and rapid specific detection of nucleic acid sequences due to their powerful gene-editing capabilities. In this article, we introduce the application of different forms of sensors such as fluorescent paper strips built with CRISPR/Cas12a for SARS-CoV-2 nucleic acid detection.

Keywords: CRISPR/Cas12a, rapid detection technology, SARS-CoV-2, sensor, virus

Introduction

The WHO announced the existence of a new type of coronavirus disease in 2019.[1] The epidemic spread over 3 years, and on January 8, 2023, it was downgraded from "Class B and A" to "B and B" and no longer included in the management of quarantine infectious diseases. At the end of 2019, pneumonia of unclear cause appeared in Wuhan, China. Although the level of the virus has been reduced, it still exists in the environment and has a great impact on the environment and human health.

The sense single-stranded RNA virus SARS-CoV-2 is encased in an envelope. The base number of the virus genome is about 29,800 bp, and it contains 14 open reading frames (ORFs). According to the function, it can be divided into: the ORFs region, structural protein region (S, M, N, and E), and accessory factors region.[2] The main manifestations of the SARS-CoV-2 infection are dry cough, fever, nasal congestion, and fatigue, which may be accompanied by changes in the lung images. Some severe patients may experience symptoms such as shortness of breath, respiratory failure, heart damage, shock, and organ failure, eventually leading to death.[3,4] As of February 2023, further than 700 million people around the world have been infected, with over 6.8 million deaths, which makes an accurate and rapid-fire discovery of the SARS-CoV-2 particularly important. At present, there are colorful discovery styles for the SARS-CoV-2; fluorescent reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is presently the most routine system for detecting SARS-CoV-2 and is called the gold-standard system for laboratory and clinical opinion of new coronaviruses.[5,6] This system has the advantages of high selectivity and high sensitivity; however, it requires large-scale instruments and professional testing personnel, and the detection results are lagging. As a gene-editing tool, the CRISPR/Cas system and its associated protein (Cas) play a pivotal role in gene editing and disease treatment. With the discovery of a series of Cas effector protein cleavage activities, the CRISPR/Cas system provides a new way for the rapid and accurate discovery of nucleic acid sequences.[7]

CRISPR/Cas12a

CRISPR was originally discovered in prokaryotes (bacteria and archaea), which can resist the invading acquired immune systems such as viruses.[8]

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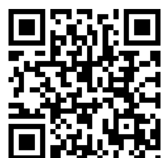

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The CRISPR/Cas system is made up of two parts; the first part is the gene encoding the Cas-related protein; the alternate part is the CRISPR array, which contains a series of 20–50 base pairs of repeat sequences and a similar length unique spacer, there will be an AT-rich leader sequence before CRISPR array.[7]

Since the development of CRISPR/Cas, there are various types of CRISPR/Cas systems. CRISPR/Cas12a (Cpf1) is a nuclease guided by RNA for the targeted cutting of DNA. The basic principle of CRISPR detection is to release or bind the detector of the CRISPR-Cas system through the target, which leads to the activation or restriction of the collateral fractionalization exertion in the Cas protein, independently, finally releasing the detection signal.[9,10] When CRISPR/Cas12a distinguishes and cuts the target sequence, it will cut the surrounding linear and circular ssDNA molecules.[11] Cas12a can also serve as a powerful genome-editing tool.

CRISPR-based detection technology has been successfully applied to descry a variety of contagions, including Zika virus,[12] dengue virus, human papillomavirus, and SARS-CoV-2.[13] Li *et al*.[14] applied CRISPR/Cas12a to nucleic acid detection for the first time, developed a rapid and sensitive detection technology that can distinguish homozygous and heterozygous genotype mutations in human 293T cells, and combined with RT-PCR technology to realize the sensitive detection of encephalitis virus. Rapid detection technology grounded on CRISPR/Cas12a system is one of the important research directions in nucleic acid detection. This article mainly focuses on the application of fluorescent sensors and test strip colorimetric sensors grounded on the CRISPR/Cas12a combined with different signal reading styles in the discovery of SARS-CoV-2 nucleic acids.

Fluorescence Sensor Based on Crispr/Cas12a

Fluorescent signal-based sensors are one of the commonly used methods for CRISPR/Cas12a detection. Target detection can be achieved through a simple fluorescent signal reading device, which has the advantages of convenient detection and low background value. Chen *et al*.[15] used the characteristics of CRISPR/Cas12a to create a nucleic acid testing method, which is DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR), introducing a single-stranded DNA linked to a fluorescent reporter group into the reaction; when Cas12a cuts the target sequence, it will trigger its nonspecific ability to cut ssDNA, the reporter group. Therefore, fluorescence is emitted, and the presence of the target is judged by the fluorescent.

It is common to combine CRISPR/Cas12a technology with amplification technology to improve the sensitivity of detection by increasing the content of the target sequence.[16-18] Wang *et al*.[19] constructed a CRISPR/Cas12a-based visual detection method – CRISPR/Cas12a-NER. This method does not require special instruments. Viral SARS-CoV-2 genes were detected within 10 copies. This strategy introduces an ssDNA reporter gene labeled with a fluorescent group into the reaction system, and green fluorescence can be observed by the eyes under 485 nm blue light behind responding to the target. Combining this method with RT and recombinase-mediated strand-replacement nucleic acid amplification (RT-RAA) technology can obtain enough DNA to increase the detection rate of positive results. Ding *et al*.[20] proposed an all-in-one dual CRISPR-Cas12a which is used for the detection of SARS-CoV-2. We verified the detection method using clinical tar models of SARS-CoV-2, and the final detection result was consistent with the result of the RT-PCR assay, and the detection result could be observed within 20 min. It is worth noting that this strategy does not require the transfer of a separate amplification product, and the detected target sequence is not limited by the PAM sequence of Cas12a.[21] Most particularly, when the reaction is strong, the color change in the response tube can be directly observed by the eyes indeed under ambient light conditions without excitation. In addition, the fluorescent sensor constructed by CRISPR/Cas12a can not only detect nucleic acid sequences but also be applied to the detection of small molecules and proteins.[22]

Test Strip Colorimetric Sensor Based on CRISPR-Cas12a

Broughton *et al*.[23] reported a fast (<40 min), convenient lateral flow analysis method based on DETECTR. The RNA of SARS-CoV-2 was uprooted from the case's tar sample for RT and isothermal amplification as the detection target, and the release of the reporter molecule was observed on the test strip to confirm the existence of the contagion. Compared with RT-PCR detection, this positive detection rate of the method can reach 95%. Lucia *et al*.[24] used the characteristics of CRISPR-Cas12a to construct a fast, accurate, and movable SARS-CoV-2 nucleic acid discovery system, which can not only realize visual detection by fluorescence spectrophotometer but also realize convenient detection by test strips. It is worth noting that this method does not reduce the detection sensitivity (10 copies/μL) while gaining convenience. Ali *et al*.[25] constructed a rapid-fire, high-sensibility, and largely specific system for detecting SARS-CoV-2 nucleic acid. The method and RT-loop-mediated isothermal amplification (RT-LAMP) combined to improve the specific discovery capability of SARS-CoV-2. The combination of the RT-LAMP-CRISPR/Cas12a method and test strips and the sensitive (10 copies/μL) and efficient instant discovery of SARS-CoV-2 nucleic acid can be realized within 40 min. The SARS-CoV-2 detection method is helpful for the effective detection and control of novel coronavirus.

In addition to directly constructing the colorimetric sensor of the test strip, the discovery of nucleic acid can also be realized by modifying the pregnancy test strip. Tang *et al*.[26] reported a pregnancy test paper target detection – CRISPR and Large DNA assembly Induced Pregnancy strips for signal-ON (CLIPON), the target existence; the crRNA specifically binds to the target and activates Cas12a in the

complex and nonspecifically cleaves the DNA marker on the single-stranded nucleic acid-HCG probe (NHP) so that the NHP is detached from the large DNA. The migration of the assembly (CLD) on the test strip causes the detection line to appear, realizing the discovery of SARS-CoV-2. Compared with other methods of modifying pregnancy test paper, the CLIPON method uses crRNA to recognize DNA to activate Cas12a, which is more specific than the strand-displacement method, and the efficient enzymatic reaction can achieve the purpose of signal amplification; CLIPON, in addition to detecting ssDNA and dsDNA, can also be detected; NHP, as a reporter molecule, is completely independent of DNA targets, and there is no need to design a variety of different NHPs according to different DNA targets.

Summary and Prospect

The high application of CRISPR/Cas12a rapid detection technology based on two different signal outputs in nucleic acid detection of SARS-CoV-2 proves that this technology can not only be used as a powerful tool of gene editing but also has important application value in nucleic acid detection. The easy programmability of the CRISPR/Cas system makes it not only suitable for nucleic acid discovery but also for the rapid detection of small molecules and proteins by designing different crRNA recognition sequences.[22] There have been articles on the improvement of detection sensitivity that, in addition to perfecting the discovery sensitivity through amplification, discovery sensitivity can also be bettered by activating more Cas through the combination of multiple crRNAs.[27] However, in the process of using CRISPR/Cas12a to detect nucleic acids, improving the detection sensitivity and target preamplification operations are still two aspects that need to be improved to further increase the detection throughput and the degree of automation. With the development of the CRISPR/Cas12a rapid diagnostic platform, it will occupy a more pivotal role in the fields of nucleic acid detection, clinical diagnosis, and food safety in the future.

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Conflicts of interest

There are no conflicts of interest.

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