Research advances in the CRISPR-Cas13 system in the field of nucleic acid detection

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Abstract. Nucleic acid detection plays an important role in environmental monitoring, food safety, pathogen detection, genetic disease detection and so on. At present, nucleic acid detection methods mainly include nucleic acid molecular hybridization and polymerase chain reaction. Nucleic acid detection of Corona Virus Disease 2019 has become an indispensable part of our daily life. With the establishment of new detection methods, nucleic acid detection is developing towards single molecule sensitivity and single base specificity. Colloidal gold method and magnetic particle chemiluminescence method have been widely used in the autonomous detection of COVID-19, while both approaches have their limitations. There is a more efficient and specific approach: CRISPR-Cas13 system.

Keywords: CRISPR-Cas system; Cas13; nucleic acid detection.

1. Introduction

Over the past decade, CRISPR-Cas technology has developed rapidly. Molecular detection methods based on this technology have the characteristics of fast, sensitive, specific and cheap, and have been widely used in the field of life science, especially in the field of pathogen detection. Currently, CRISPR/Cas12 and CRISPR/Cas13 systems are mainly used to detect respiratory pathogens.

The American team of scientists developed the amplification free CRISPR-Cas13a detection technology for the detection of COVID-19, and the research results were published online in “Cell” on December 4, 2020. The detection technology can directly detect the new coronavirus from the RNA of nasal swab, which can be read through the mobile phone microscope. The method can accurately detect pre extracted RNA in a group of positive clinical samples within 5 minutes. This study combines crRNA targeting novel coronavirus RNA to improve sensitivity and specificity, and uses enzyme kinetics to directly quantify viral load. After integration with the mobile phone based reader device, the detection method can realize rapid and low-cost real-time detection of COVID-19. This essay attempts to research advances in the CRISPR-Cas13 system in the field of nucleic acid detection.

2. CRISPR-Cas 13 system

CRISPR-Cas is a newly emerging gene editing tool. This system was first developed by Jennifer, who is currently at the University of California, Berkeley, and won the 2020 Nobel Prize in Chemistry for it. CRISPR is also known as gene scissors, which is called "Clustered Regularly inter-spaced Palindromic Repeats". Here's an interesting story about the origins of CRISPR: 30 years ago, a young man named Francisco Mojica discovered an archaebacterium on the beach of Santa Pola. When he analyzed the DNA sequence of the archaebacterium, he found many strange palindrome fragments in the genomes of these microorganisms. These fragments are about 30 bases long and are repeated repeatedly without interruption. It is separated by a fragment of about 36 bases in length between

each of the two repeats. So Mojica gave this phenomenon the mouthful name of "frequent palindromic repeat clusters".

It is mainly composed of Cas9 protein and sgRNA, and the sgRNA is composed of tracerRNA and crRNA. The immune effect of CRISPR-Cas system can be divided into the following three steps: Adaptation, Expressionand maturation, and Interference. The first step is to insert the viral DNA or RNA sequence into the CRISPR array, the second step is to form mature crRNA through splicing and other means, and the third part is mainly about the binding of Cas9 protein and sgRNA, sgRNA will recognize the invading viral gene sequence. If complementation can be successfully targeted, the Cas9 protein clefts. There are many families of CRISPR-Cas systems, which are mainly divided into two classes. Among them, Class1 is mainly divided into Type1, Type3 and Type4, and Class2 mainly includes Type2, Type5 and Type6, which are known as Cas9, Cas12 and Cas13. Among them, only Cas13 protein can target complementary RNA in Class2. It is worth mentioning that according to statistics, two thirds of the invasion of the human body are RNA viruses, and only 2.5% of the viral life cycle involves DNA intermediates. Therefore, compared with Cas9 and Cas12 systems targeting complementary DNA, Cas13 system has a wider application scope.

Firstly, the basic structure of CRISPR-Cas13. The CRISPR-Cas13 system belongs to the Class2 Type 6 family, where the functions of the Type A, Type B, Type D, Type X, and Type Y families were identified. Cas13 proteins are single proteins composed of multiple domains, which can recognize crRNA, cleave RNA, and even cleave pre-crRNA. Cas13 protein has two iconic HEPN domains, and two R-XXXX-H conserved motifs are the nuclease active sites of HEPN domains. It also contains the CRISPR array at its loci. CRISPR array is composed of repeats and spacers. CRISPR array is transcribed to form immature pre-crRNA, which is cleaved to form mature crRNA to guide Cas13 protein to function. Cas1 and Cas2 genes may also exist at the Type VI family loci, which are responsible for capturing and integrating exogenous viral gene sequences into Spacer.

Secondly, the advantages of Cas13d. It has the advantages of high efficiency, specificity and portability. Cas13a, Cas13b and Cas13d families are widely used. The Cas13 family can be used against viruses, especially RNA viruses. According to statistics, two-thirds of the viruses that invade humans are single-stranded RNA viruses, and only 2.5% of these viruses involve DNA intermediates in their life cycle, so the Cas13 system is more widely used than the DNA-targeting Cas9 and Cas12 systems.

3. CRISPR-Cas 13 system in nucleic acid detection

Markedly, for CAS13 protein, it has an advantage that other Cas proteins cannot match, which is paractomy activity. Briefly, sgRNA is activated when sgRNA binds to Cas13 protein as well as the substrate, and Cas13 protein forms a ternary complex. At this time, the Cas13 protein cleaves not only the substrate RNA, but also any single-stranded RNA free from the environment. This non-specific cleaving activity is called paracolection activity. In fact, Cas12 has this characteristics, but Cas12 recognizes double-stranded DNA and arbitrarily cuts single-stranded DNA, while Cas13 recognizes and cleaves single-stranded RNA. Based on this feature, many research teams have developed a wide variety of nucleic acid detection methods, but the most widely known technology is the "SHERLOCK" (Specific High-Sensitivity Enzymatic Reporter Unlocking) technology developed by Zhang Feng. Zhang's team added fluorescent probes to the reaction system, which were connected to the quenching group to one end and the fluorescent group to the other end. Normally, molecules do not fluoresce, but when the Cas13 and crRNA complex bind to and recognize the substrate RNA, the Cas13 protein is activated and, in addition to specifically recognizing the substrate RNA, nonspecifically cleaves signal reporters free inside the system. When the intermediate sequence of the fluorescent probe is cut off, the signal molecules release a large amount of fluorescence. This allows the novel coronavirus test. After further optimization, the SHERLOCK platform can also further detect both DNA and RNA, with its extremely high sensitivity, and it has many significant advantages over the traditional detection modalities. In the COVID-19 outbreak, SHERLOCK was

approved for FDA novel coronavirus testing. In addition to SHERLOCK, there are other new detection methods, such as DETECTR and SATOR, most of which rely on sidectomy activity. Although there are some small differences in technical details, there is no difference in principle.

Notably, for nucleic acid detection of COVID-19, few end-to-end solutions can meet the requirements of rapid scalability and low cost without sacrificing sensitivity. Here, Megjun introduces a method for detecting SARS-CoV-2 RNA based on CRISPR-Cas13a protein developed by Dr. Maxwell Z. Wilson of the University of California in the United States. This method has low cost, high sensitivity and is easy to deploy in locations with minimal infrastructure.

Methods based on CRISPR-Cas12 protein and CRISPR-Cas13 protein are revolutionary in pathogen detection. They are sensitive and currently combined with thermostatic amplification method and transverse flow immunochromatographic detection have been used in POCT scenes. These methods are very promising for the detection of COVID-19. However, due to the global demand for testing, the key reagents in these protocols are difficult to obtain. In order to reduce the barriers to covid-19 diagnosis, Dr. Wilson's team designed a method called crest has solved the three main obstacles that restrict those crissr Cas13 based assays, reagent availability, equipment availability and cost, by using universal enzymes, low-cost thermal cycling equipment and easy-to-use fluorescence visualizers. In addition, CREST is equivalent to the gold standard reverse transcription quantitative PCR (RT-qPCR) in sensitivity, and RT-qPCR is most commonly used for COVID-19 detection.

RT-qPCR is the current gold standard for the detection of novel coronavirus. The method has strong specificity and high sensitivity, but because it relies on professional laboratory instruments and professionals, it takes a long time (about 6 hours from sampling to the results), so it is difficult to cope with the amazing transmission speed of the virus. Traditional pathogen detection methods include antigen/antibody detection, PCR amplification detection, isothermal amplification technology, etc., which all have their own characteristics. Qualitative or quantitative PCR, reverse transcription PCR are currently the most common methods of pathogen nucleic acid detection. However,classical PCR testing requires special instruments and is used in a specific experimental environment, and its high testing cost, long sample operation cycle, and high personnel and equipment requirements limit its application. Although many isothermal amplification methods reduce the requirements of hardware facilities and partly reduce the detection time, their sensitivity and specificity are affected in order to achieve the requirements of simple and rapid operation. Although the colloid gold method is simple operation, fast detection speed and low cost, but the detection sensitivity is low.Antigen testing can be self-testing at home, thus reducing the time people spend outside to queue up for nucleic acid testing and reducing the risk of cross-infection. Antigen detection can be faster and easier to operate, and the detectors can get the test results in a very short time. Therefore, antigen detection plays a role in early diversion and rapid management in COVID-19 prevention and control. Compared with antibody serum detection, nucleic acid detection can detect patients in the window period and detect infected people early, but it has high requirements for testing equipment or platform, expensive high sensitivity RT-qPCR instruments, and highrequirements for the cleanliness and operators of the laboratory. In addition, nucleic acid testing is time-consuming, and usually the fastest 24 hours to obtain nucleic acid reports, considering the status of sample transport and sample backlog.Compared with nucleic acid testing, blood specimens from antibody serological testing are easier to obtain and the quality of specimens is guaranteed. The operation is simple and fast, which greatly reduces the risk of infection of medical staff in the process of specimen collection and testing. If say nucleic acid detects the RNA of the virus, it is the direct evidence of the existence of the virus, then what antibody detects is the antibody that is stimulated in the patient blood, it is indirect evidence, have prompt effect to clinical practice. When the nucleic acid test is negative, adding the IgM, IgM and IgG antibody tests can make up for the disadvantage of misdiagnosis caused by the nucleic acid test.

At present, the mainstream antibody detection method in the market is chemiluminescence, but chemiluminescence requires relatively high requirements for equipment, operation and use

environment. Compared with nucleic acid detection, the core advantage of chemiluminescence is easy sampling and operation, but not the advantage of rapid detection. For general screening or for non-developed countries, colloidal gold testing methods have very low requirements for personnel, site, and equipment, which are suitable for large-scale rapid testing.The advantage of nucleic acid testing is that it can be applied directly and early on. After patients infect the novel coronavirus, even if they have no symptoms, they can also detect the virus genes. The disadvantage is that there are more links from sampling to detection, and the results are easy to be affected. Antibody detection is very sensitive, but there is a window period, relatively lag. Antibody IgM is usually detected 3-6 days after onset, antibody IgG can be detected 10-18 days after onset, can not meet the requirements of early diagnosis. In addition, even if the antibody is positive, it is not necessarily the same as that the patient is infected at this time. The qPCR was only opened twice, with little risk of laboratory contamination Compared with the cumbersome operation steps of mNGS, the operation is simpler and easier to promote The amplified DNA was detected while the qPCR reaction occurred, so it does not need to be detected separately later (as on an agarose gel), as in conventional PCR. The qPCR is considered an efficient method, coupled with a liquid handling automation station, which processes large numbers of samples in a short time. The qPCR is able to resolve a 2-fold difference in the number of target DNA molecules, which can detect an initial DNA of molecules as low as only a fewmolecules. Compared to PCR, only 1 / 1000 amount works. Extensive quantification can be performed on several orders of magnitude It is often considered to be highly reproducible. During the process of performing the PCR detection, multiple samples can be tested, and the time of the PCR reaction is about two hours. Different hospitals have different equipment, the quality of the instrument can affect the detection of how many samples, usually a reaction plate can detect 96 samples at the same time. Nucleic virus fast test is mainly the collected samples directly into the lysate, generally does not need to be nucleic acid purification, can directly conduct PCR reaction. Using a special fast detection instrument, the time of nucleic acid detection is much faster, and the general test samples are produced between 0.5 and 1.5 hours. For general hospital nucleic acid testing, PCR is used. Because a batch can be tested for multiple samples at the same time, it can better reduce the waste of hospital resources and reduce the workload of the hospital. Compared with these traditional methods, CRISPR-Cas system has high sensitivity, high specificity, low cost, low time consuming.What' more,it does not need a complex and expensive equipment,or a high level of professional knowledge,and can conduct the characteristics of rapid detection, but it has an off-target problem, may lead to the deviation of detection results and detection efficiency.Therefore, it has broad application value in the prevention and control of infectious diseases, but still more technology is needed to improve it.

4. Conclusion

The application of nucleic acid detection has the potential for off-target risk and the off-target effect is an inherent flaw of the CRISPR system. In comparison to the mature nucleic acid detection method, the detection method based on CRISPR-Cas13 system still has the following issues that need to be resolved. Additionally, the RNA reporter molecule utilized to detect Cas13 as well as crRNA, a crucial part of the CRISPR detection system, are quickly destroyed during usage and storage, which could impair the stability of the detection results. The resolution of these issues will support the development and use of CRISPR nucleic acid detection techniques. The CRISPR-Cas system-based nucleic acid detection approach combines the benefits of high sensitivity, strong specificity, speed and simplicity, etc., and represents an essential development path for the next generation of nucleic acid detection techniques. This method, which primarily relies on fluorescence reading results, is compatible with current nucleic acid detection methods, including those that use real-time fluorescence quantitative cyclers and automatic nucleic acid detection equipment, which helps to promote nucleic acid testing laboratories. While maintaining a constant temperature, its detection process is capable of test strip detection, naked eye reading results mode, low dependence on instruments and equipment, highly suitable for on-site testing and experimental equipment is

relatively scarce in countries and regions to use, and has a wide range of applications in the prevention and control of infectious diseases.

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