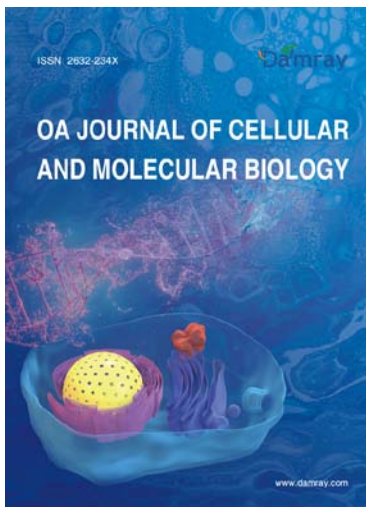


Application of CRISPR/Cas-based Biosensing Platform in Molecular Diagnostics

F. Apendi*, J. Ibara

Department of Public Health, Marien Ngouabi University, Brazzaville, Congo.



<https://cmb.damray.com/>

OPEN ACCESS

DOI:

10.26855/oajrcmb.2019.12.001

Received: October 16, 2019

Accepted: November 15, 2019

Published: December 12, 2019

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Abstract

The CRISPR/Cas system is widely used in genome editing. The CRISPR/Cas system can accurately cut and recognize DNA and RNA sequences. In the process of recognizing target sequences, the CRISPR/Cas system can not only recognize the target sequence to start the targeted cleavage activity, and also show non-specific cleavage activity in DNA, RNA molecules and other single strands, add fluorescent group reporter nucleic acid molecules to the reaction system, and observe through fluorescent signals to the location of the target nucleic acid molecule, and with the help of the different cutting preferences of the Cas protein, it can also detect multiple target molecules at the same time.

Keywords

Molecular diagnostics, CRISPR/Cas, Biosensing platform, Application value

Introduction

As early as 2012 CRISPR technology has been applied to biological DNA sequence mutation, knockout, addition, replacement, with the help of CRISPR technology for gene editing is not only easier and more convenient to build, fast, ready, and can reduce costs, relatively low dependence on equipment, expand the scope of adaptation, therefore, CRISPR technology has "gene magic shear" It has been awarded the Nobel Prize for Chemistry in 2020, opening up even better prospects for biotechnology development. Currently, the CRISPR/Cas system has more methods for nucleic acid molecular diagnostics, and this study discusses and analyses different Cas protein nucleic acid molecules based on CRISPR diagnostics, as follows.

1. Introduction to CRISPR/Cas systems

1.1 Structural analysis of the CRISPR/Cas system

The CRISPR/Cas system, which is present in archaea and bacteria, is an acquired immune system with good resistance to exogenous genetic material such as plasmids and phages. CRISPR/Cas, also known as clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated proteins, the CRISPR/Cas system contains many conserved, short, repetitive sequence regions and spacer regions, with the CRISPR sequence promoter being the upstream lead region [1]. The common action between the protein encoded in the upstream polymorphic family of genes and the CRISPR sequence is the Cas gene, and the opposite direction of the Cas gene and the leading sequence-repeat-spacer sequence is the trans-activated crRNA, which forms the crRNA, or guide RNA, when mediating the maturation of the non-coding precursor crRNA.

1.2 Mechanistic analysis of the CRISPR/Cas system

Bacteria and archaea are different and there are some differences in CRISPR/Cas systems. Taking the CRISPR/Cas9 system in *Streptococcus pyogenes*, after viral invasion, the bacteria will integrate the viral invasion fragment DNA sequence and fuse the rest of its own CRISPR spacer region. If the virus appears to invade again, after the leading region is regulated, it will transcribe the CRISPR sequence into a long pre-crRNA, by pairing with tracrRNA, and RNaseIII enzyme processing, forming a gRNA structure. gRNA structure is then fused with Cas9 protein to form Cas9-gRNA. gRNA is influenced by Cas9 protein to produce nucleic acid endonuclease activity, which can degrade and recognize the target sequence [2]. The target sequence can be recognised by the PAMCas9 protein based on the motif around the original spacer sequence.

1.3 Classification of CRISPR/Cas systems

CRISPR/Cas systems can be divided into two categories according to the number of effector proteins: complex systems that require the help of multiple effector proteins to function, and systems in which a single effector protein will function. However, with reference to the new classification criteria, CRISPR/Cas systems are divided into 6 types, 33 subtypes and 2 categories. One category system covers types I, III and IV, with a total of 16 subtypes, and 2 category system covers types II, V and VI, with a total of 17 subtypes [3].

1.4 Principles of molecular diagnostics with CRISPR/Cas systems

Compared to Cas9, Cas12 and Cas13 subtypes are guided by guide RNA, which not only can initiate targeted cleavage activity for recognition of target sequences, but also have indirect non-specific cleavage activity, which can degrade and shear ssRNA and ssDNA. This provides a good basis for molecular diagnostics in the CRISPR/Cas system. Currently used more reporter nucleic acid molecules are oligonucleotides, the two ends have dark quencher, fluorescein, respectively, restricted by the burst effect, the reporter nucleic acid molecules are non-fluorescent, when the target sequence, Cas protein combination, activation of non-specific cleavage activity, the reporter nucleic acid molecules are sheared, not restricted by the burst effect, will produce fluorescence [4].

2. Molecular diagnostic platform for CRISPR/Cas systems

2.1 CRISPR/Cas9 molecular diagnostic platform

Cas9 protease has no non-specific cleavage activity and is mainly used in combination with PCR and Cas9 for specific diagnostics. The CRISPR/Cas9 system has been used in nucleic acid molecular diagnostics as early as 2016 and has led to the derivation of techniques for Zika virus lineage identification. 2019 FLASH technology using Cas9sgRNA is applied to pathogenic antimicrobial resistance sequences, by which FLASH technology can cut sequences of interest to form Illumina sequencing fragments. Phosphatases block DNA, cDNA input genes, and the Cas9 enzyme cleaves and digests sgRNA, so that the cleavage product can be ligated against a universal sequencing bridging substrate [5]. The target sequences are amplified and then used in flow pool sequencing, a diagnostic approach that is already far superior to other CRISPR diagnostics and can be applied in thousands of target diagnostics.

2.2 CRISPR/Cas13a molecular diagnostic platform

As early as 2016 CRISPR/Cas13a mechanism, function has been validated, Cas13a can perform specific shearing of target RNA, but also non-specific degradation of RNA function. 2017 SHERLOCK technology was introduced, con-

firmed the formation of a new detection system containing recombinase polymerase isothermal amplification technology, in the process of nucleic acid molecular testing, for DNA RPA amplification is required, while RNA is first amplified by RT-RPA before the DNA product is transformed into an RNA molecule. The SHERLOCK technology has high single base specificity and aM sensitivity, but only one nucleic acid sequence can be tested at a time and fluorescence data needs to be collected by other equipment. In 2018 after updating the SHERLOCK technology to form SHERLOCKv2, the assay ended up with substantially increased sensitivity and the ability to select specific fluorescent reporter groups according to cutting preference, allowing for the detection of multiple types of viral nucleic acids at once. The technology has now been applied to lung cancer blood samples, during which free tumour DNA can be accurately observed at concentrations $>2\text{amol/L}$ ZIKA, DENV single-stranded RNA can be detected in just 90 minutes, and the operation is more convenient and faster [6-7].

2.3 CRISPR/Cas12a molecular diagnostic platform

In 2015, a team discovered that when Cas12a-crRNA single- and double-stranded DNA recognition is activated, the single-stranded DNA is subject to non-specific shearing and degradation by Cas12a with the help of RPA. The team referred to the SHERLOCK technique and introduced the RPA step to form the RPA+Cas12a nucleic acid molecular test technique, or DETECTR, which directly omits the process of transcribing the DNA product to RNA after amplification, enabling rapid and accurate detection of HPV types 16 and 18 in cervical cancer and accurate differentiation of HPV virus subtypes. Later, DETECTR was used to develop a loop-mediated isothermal amplification technique and various signal output forms of the test [8-10].

3. Conclusion

Effective diagnostic tools in disease treatment are important for patient recovery, and clinical tests have been continuously modified and optimized by diagnostic methods related to the CRISPR/Cas system, further improving the accuracy and speed of various pathogen detection and providing more convenience for clinical laboratory disease testing. However, there is still much room for improvement in the various assays in the CRISPR/Cas system, and improvements should be made continuously in the future to promote the further development of the CRISPR/Cas system for molecular nucleic acid diagnosis.

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