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Principle and applications of CRISPR/Cas system

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Abstract. With the continuous in-depth research and exploration of the CRISPR/Cas9 system by researchers, people's ability to edit biological genes is also constantly improving. In recent years, gene editing technology as a hot technology has been paid more and more attention, it has played an important role in crop breeding, disease treatment, molecular diagnosis and other aspects. Among the gene editing technologies that have been developed, the emerging CRISPR/Cas technology gene editing system has become the most widely used gene editing technology at present. Compared with the first two generations of gene-editing technologies: ZFN and TALEN, CRISPR/Cas system has the advantages of short cycle, high efficiency, low cost, and has great potential for the development of animal and plant breeding. The development of CRISPR/Cas9 technology has promoted the progress of modern life sciences. With these tools for efficient, simple, and precise genome modification, transcriptional regulation, and epigenetic editing, CRISPR systems have the potential to revolutionize genetics, medicine, and agriculture. In this review, the basic principle of CRISPR/Cas technology and its application will be introduced and illustrated.

Key words: CRISPR/Cas system, gene editing, application.

1. Introduction

Gene editing is a genetic manipulation technology that involves the site-specific modification of specific DNA sequences in the genome. The basic principle is to replace, cut, add or insert exogenous DNA sequences into the target gene of the cell genome to produce heritable changes [1]. Artificial construction can specifically cut the DNA sequence of nuclease. The target gene segment is precisely targeted to break the DNA double strand, and thus activating the DNA repair mechanism in the cell, resulting in gene modification.

CRISPR-Cas system is the most widely used gene-editing technique. The basic principle is to achieve accurate gene editing by identifying target gene target sites, damaging double-stranded DNA and activating DNA damage repair pathways [2]. CRISPR/Cas9 is now widely used in important crops such as rice, corn and peanuts. In addition, it can be applied to polyploid crops such as wheat, cotton and soybeans [3].

2. CRISPR/Cas system

The CRISPR/Cas system was first discovered in bacteria and archaea (bacteria with unique structures that live in extreme environments such as deep-sea craters, terrestrial thermal vents, and saline lakes).

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CRISPR is a repeating sequence in the genome of a prokaryote. CRISPR stands for Clustered Regularly Interspersed Short Palindromic Repeats. CRISPR is found in 40% of sequenced bacteria and 90% of sequenced archaea.

2.1. Classification

The leader, repeat, and spacer make up the majority of the CRISPR gene sequence. The proteins expressed by the Cas gene, which is positioned nearby the CRISPR gene or dispersed throughout the genome, can interact with the CRISPR sequence area. Therefore, the CAS gene has been referred to as a CRISPR-associated (Cas) gene. "Many different types of Cas genes, including Cas1-Cas10, have been discovered. The Cas protein expressed by the Cas gene is vital in the defense mechanism. The CRISPR-Cas system is now split into two main categories based on the function of the Cas protein in bacterial immune defense (Figure 1). First group: Complexes made up of numerous Cas proteins, including types I, III, and IV, serve as their effectors for chopping foreign nucleic acids.

Second, they act on relatively single Cas proteins, such as type II Cas9 and type V Cpf proteins [4].

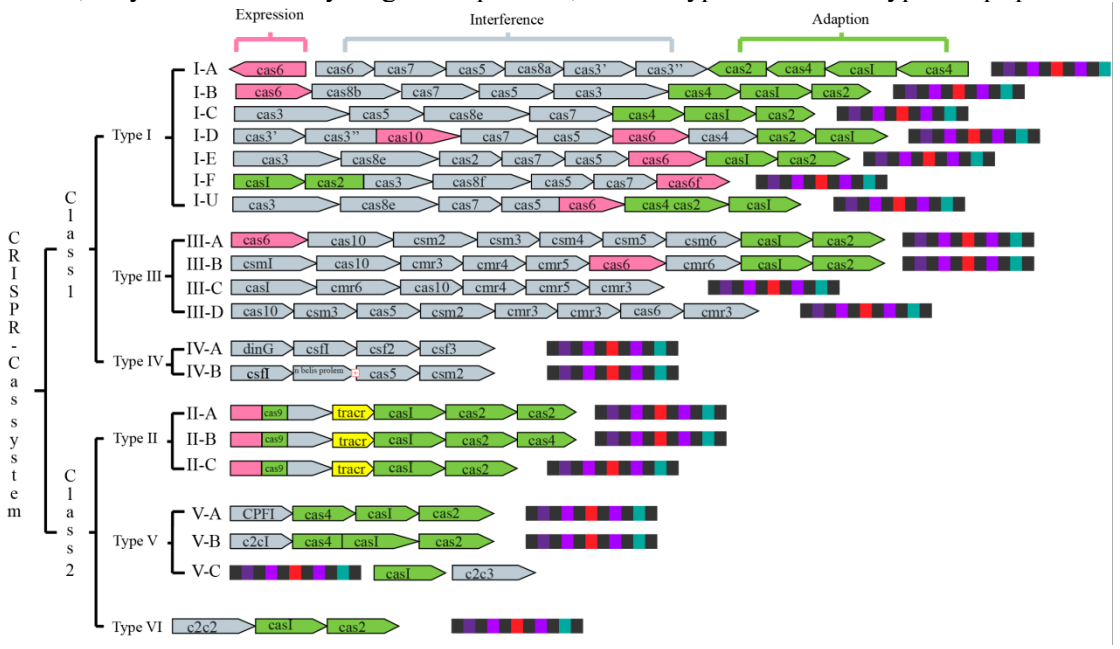

Figure 1. CRISPR-CAS system.

2.2. Mechanism of CRISPR/Cas system

The most prevalent CRISPR system now in use is Type II CRISPR-CAS, also referred to as CRISPR-Cas9. The three stages of the CRISPR-Cas9 mechanism are targeted interference, expression of CRIPSR loci (including transcription and post-transcriptional maturation processing), and the capture of foreign DNA by highly variable spacer sections of the system (Figure 2).With the development and improvement of new technologies, CRISPR/Cas9 gene editing system has become the most widely used gene editing technology due to its advantages of short cycle, high efficiency and cost compared with previous technologies. It has great development potential in plant and animal breeding, and also provides a new way for gene directed modification.

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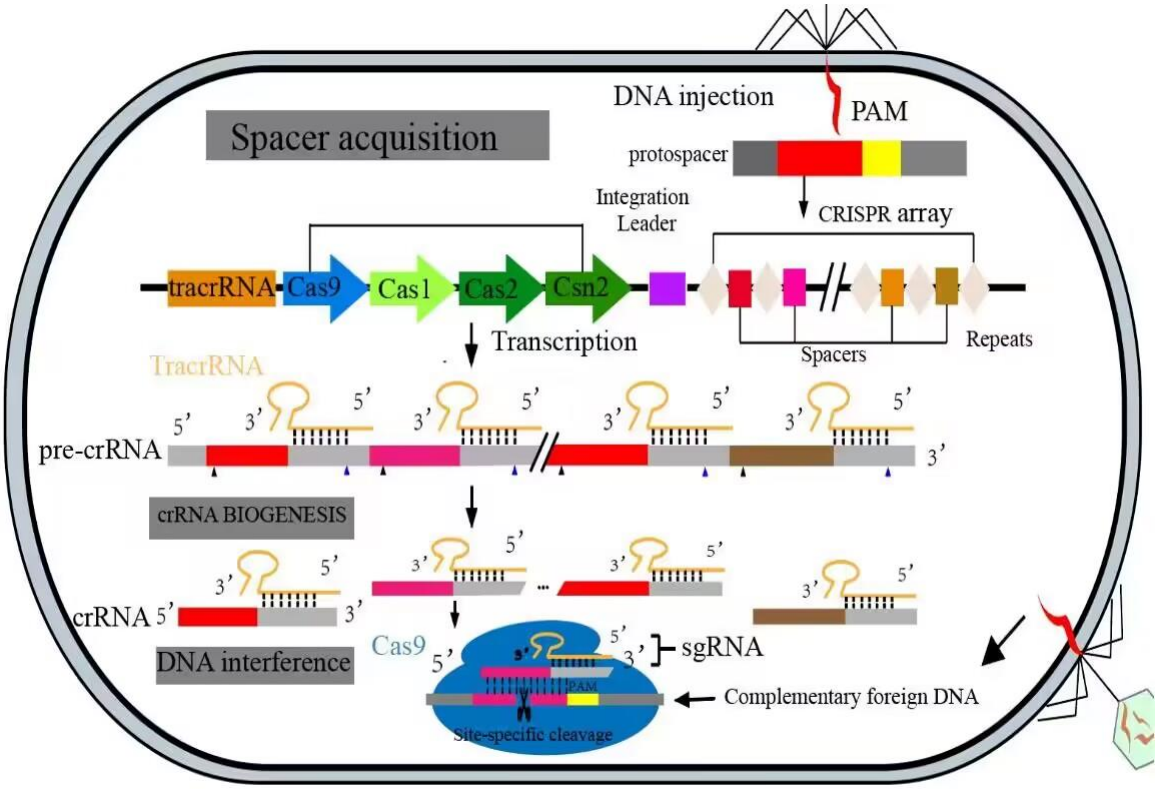

Figure 2. The mechanism of CRISPR-Cas9.

3. Application

CRISPR/Cas9 gene editing technology covers a wide range of fields, not only limited to gene mutation and breed breeding of animals and plants, but also has significant application effect in gene expression regulation, chromosome structure research, gene function screening and identification, genome epigenetics and other fields [5].

3.1. Crop cultivation

The CRISPR technology was used to knock out S-RNase gene, which controls potato self-incompatibility. The self-compatible diploid potato plant with the S-RNase gene edited is shown in Figure 3 together with the phenotype of the wild-type plant [6].

High yield, an important agronomic goal for polyploid rapeseed farming, depends in part on unbreakable fruit pods. Although the JAGGED (JAG) gene is a significant player in pod cracking, it is unknown how it affects rapeseed's ability to resist pod cracking. The CRISPR/Cas9 gene editing method was used to do multi-gene editing on five JAG genes in rape, and it was discovered that the deletion of five homologous genes would significantly influence the shape, size, and other aspects of the fruit pod [7].

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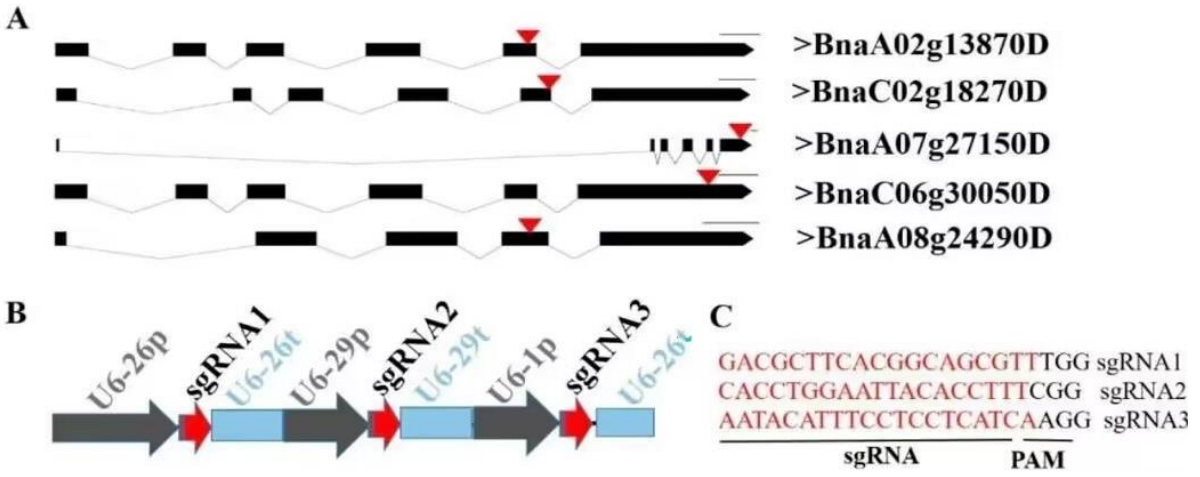

Figure 3. Structure of five homologous genes of JAG and schematic diagram of multi genome editing vector.

3.2. Treatment detection of diseases

CRISPR technology has the advantages of being fast, simple, single copy and can be combined with high technology in molecular diagnostics, making it rapidly popular in the field of IVD. In particular, in 2020, Zhang Feng's team used Cas13 combined with thermostatic amplification to efficiently detect COVID-19, indicating that CRISPR technology will lead a new generation of molecular diagnostics.

Zhang Feng's team developed a new technology called SHERLOCK to detect COVID-19. This technology is based on the alternative method of combining isothermal amplification and CRISPR-mediated detection and uses Cas13a protease and its binding guide RNA as the core to accurately identify COVID-19 RNA [8]. At the same time, the researchers add a special molecule to the sample, which is linked to the RNA. Activation of Cas13a will cut the RNA on this special molecule. By confirming whether these molecules are cut off, we can know whether COVID-19 is present in the original sample. The R&D team has developed a more convenient detection method that allows the isothermal amplification step to amplify RNA numbers and the chemical reaction to detect the Cas enzyme to be carried out in the same test tube. For this reason, they chose loop-mediated isothermal amplification technology as the method for amplifying RNA. The reagents required for LAMP are not difficult to obtain, have high practicality and reduce the need for RT-qPCR technology, simplifying the detection steps.Zhang Feng's team named it 'STOP' [9].

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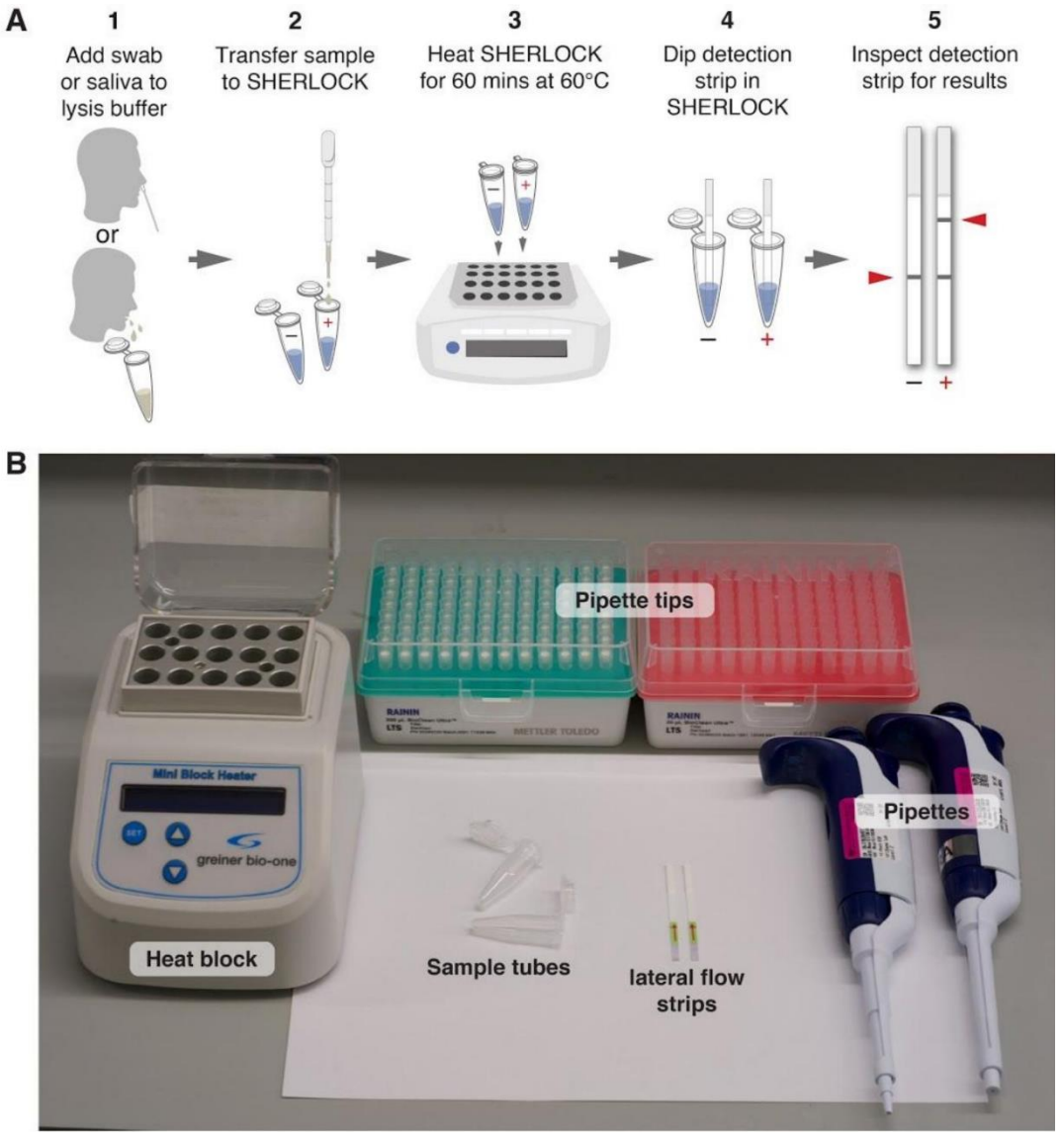

Figure 4. Overview of the STOP Covid method [10].

STOPCovid has the sensitivity equivalent to the level of RT-qPCR, and it also simplifies the steps of RNA extraction. It only needs to add reagents for splitting the virus and releasing RNA to the throat swabs or saliva samples containing COVID-19. The released viral RNA can be used for detection without purification and separation of RNA. The whole process can be completed in only one test tube.

4. Conclusions

With the development and improvement of the new technology, CRISPR/Cas9 gene editing system has become the most widely used gene editing technology due to its advantages such as short cycle, high efficiency and low cost compared with previous technologies, and has great potential for the development of plant and animal breeding. CRISPR/Cas9 gene editing technology covers a wide range of aspects, not only limited to plant and animal gene mutation, breed breeding, etc. It is also effective in gene expression regulation, chromosome structure research, gene function screening and identification, genome epigenetics and other fields. CRISPR/Cas9 technology has been widely used and applied in gene function research and variety breeding of polyploid crops. It has successfully achieved the simultaneous knockout of multiple copies of target genes, and through the design of multiple g RNA

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can be used to edit associated genes, effectively helping to solve the breeding dilemma of the complex genome structure of polyploid crops such as wheat, cotton, soybean and sugarcane.

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