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A Review: CRISPR Cas as a Diagnostic Tool

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Introduction

In recent decades, invasive pests have surged, challenging farmers by increasing insect populations and disrupting crop yields and habitats. Traditional Integrated Pest Management (IPM) methods are often slow and labor-intensive, leading to a reliance on pesticides, which fosters resistance and complicates control. Genome editing has emerged as a solution, with CRISPR/Cas systems offering a more efficient alternative. Unlike traditional methods such as mega nucleases and zinc-finger nucleases, CRISPR/Cas systems are simpler, cost-effective, and user-friendly. In entomology, CRISPR/Cas systems are valuable for pest management and vector-borne disease control through genome editing. Presently CRISPR Cas is emerging as a tool for invasive insect species identification. For example, CRISPR-based assays using the CO1 gene have achieved 100% accuracy in identifying invasive moths, and CRISPR-Cas12a assays have proven highly sensitive for detecting fruit pests like Bactrocera zonata and Ceratitis capitata.

Classification of CRISPR Cas system

The CRISPR system has been classified into two major classes. In the Class 1 system, the RNA-guided target cleavage needs several effector proteins, but the Class 2 system requires only one RNA-guided endonuclease to cleave the DNA sequences. The class 1 system of CRISPR is divided into three types I, III, and IV, and the Class 2 system is divided into types II, V, and VI. (Hillary and Ceasar, 2023).

CRISPR as adaptive immunity in bacteria

The history of CRISPR, a revolutionary gene-editing technology, can be traced back to the early 1980s when scientists first observed repetitive DNA sequences in bacteria. These sequences, later named CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), were initially thought to be involved in bacterial metabolism or chromosomal organization. In the early 2000s, researchers began to uncover the biological function of CRISPR. They discovered that CRISPR arrays were accompanied by a set of genes called Cas (CRISPR-

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associated) genes. Together, these components formed a bacterial immune system that could protect bacteria from viral infections.

Components of CRISPR Cas system

- **CRISPR Arrays:** These are segments of DNA that contain a series of repetitive sequences (spacers) interspersed with unique sequences (protospacers). The protospacers are derived from the genomes of invading viruses or other foreign genetic elements.
- **Cas Proteins:** These are a group of enzymes that are associated with CRISPR arrays and are responsible for the various functions of the CRISPR-Cas system. The specific Cas proteins involved can vary depending on the type of CRISPR-Cas system.
- **Protospacer adjacent motif** (**PAM**): 2-6 bp DNA sequence adjacent to a target DNA, which is required for effective target recognition
- **CRISPR-RNA (crRNA):** A mature RNA molecule derived from the pre-crRNA, Complementary to target DNA, guiding the Cas protein to target DNA.
- **Trans-activating crRNA (tracrRNA):** A small RNA with 24 nucleotides complementary to the repeat regions of crRNA.

Mechanism

The CRISPR-Cas system operates in a two-step process:

1. Adaptation: Acquisition of new spacers: When a virus or other foreign genetic element invades a cell, a piece of its DNA is incorporated into the CRISPR array as a new spacer. This process is mediated by Cas1 and Cas2 proteins.
2. Spacer processing: The newly acquired spacer is processed to generate a crRNA (CRISPR RNA). This processing involves the cleavage of the spacer sequence by Cas proteins, such as Cas4.
3. Interference:Target recognition: The crRNA forms a complex with Cas proteins, such as Cas9. This complex binds to a complementary sequence on the target DNA, which is often the DNA of the invading genetic element.
4. DNA cleavage: The Cas protein, in conjunction with other proteins, cleaves the target DNA at specific sites. This cleavage can lead to the inactivation or destruction of the invading genetic element. The mechanism of CRISPR-Cas is highly efficient and can provide bacteria and archaea with a strong defense against a wide range of

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foreign genetic elements. It is also a powerful tool for genetic engineering, as it allows scientists to precisely edit genes in a variety of organisms.

Cas Proteins of the CRISPR System

Cas proteins have gained popularity among the research community for broader genome engineering applications and are currently used in diverse fields, including biotechnology, agriculture, and medical research. There are many cas proteins but, recently discovered programmable Cas proteins like Cas 12, Cas 13, and Cas 14 have improved the precision of the CRISPR/Cas-mediated detection of invasive insect pests.

Why not PCR?

Polymerase chain reaction (PCR) is known as the gold standard for accuracy as a molecular diagnostic test. However, it cannot go out of the laboratory environment due to the time-consuming and complicated thermal cycler device requirement. Isothermal methods provide convenience in their application to on-site diagnostic platforms as these methods do not require complicated devices and equipment. Loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) are mostly preferred for Insect species identification. Because they provide high efficiency in a short time and they are modifiable technologies.

CRISPR-Cas-coupled isothermal amplification is a powerful technique for distinguishing between closely related species. By combining the precision of CRISPR-Cas gene editing with the rapid amplification of isothermal methods, this approach allows for highly specific and sensitive detection of genetic differences. CRISPR-Cas targets specific DNA sequences within a species' genome, while isothermal amplification exponentially amplifies these targeted regions. This combination enables researchers to accurately identify and differentiate between even the most closely related organisms, making it a valuable tool for various fields such as taxonomy, disease diagnosis, and environmental monitoring. Some of the recent works on CRISPR based detection of invasive insect pests (Table 1).

Conclusion

In conclusion, the CRISPR-Cas system, coupled with isothermal amplification techniques, presents a promising approach for the rapid, accurate and user-friendly diagnostic tool for on-site identification of invasive insect species. By leveraging the precision of CRISPR-Cas gene editing and the sensitivity of isothermal amplification, this method offers a

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valuable tool for researchers and practitioners in various fields. The ability to differentiate between closely related species with high specificity and sensitivity can significantly improve our understanding of invasive insect biology, facilitate effective control measures, and ultimately contribute to the protection of agricultural ecosystems and human health

Table 1: List of recent works on CRISPR based detection of invasive insect species

Insect	CRISPR	Year
Locusta migratoria manilensis	CRISPR/Cas13a-lateral flow dipstick	2023
Trogoderma granarium	CRISPR/Cas12a- RPA	2023
Liposcelis bostrychophila	CRISPR/Cas12a-RPA	2023
Bactrocera zonata	CRISPR/Cas12a-RPA	2022

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