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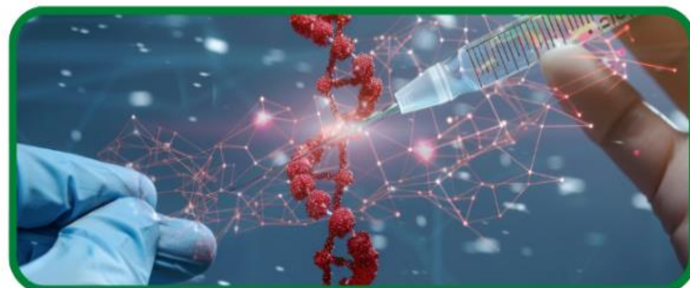

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CRISPR-Cas Technology as a Next-Generation Tool for Food Safety and Pathogen Detection

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1. ABSTRACT

Foodborne pathogens like Salmonella, Escherichia coli, and Listeria monocytogenes are significant contributors to foodborne diseases globally, causing serious threats to public health and food safety. Rapid and accurate detection of these pathogens is required to avoid outbreaks and ensure food quality. Conventional detection methods, such as culture-based and biochemical approaches, are time-consuming and often non-sensitive. Recently, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated (Cas) systems have been recognized as novel tools for rapid, sensitive, and specific detection of microbial pathogens. CRISPR-based detection assays, especially those involving Cas12 and Cas13 enzymes, allow specific identification of pathogen-specific nucleic acid sequences within a short period. Methods like SHERLOCK and DETECTR have shown high efficiency in the detection of foodborne microorganisms. These approaches have several advantages, including rapid detection, high specificity, and the possibility of on-site analysis. This review discusses the mechanism of CRISPR-Cas systems, their application in foodborne pathogen detection, advantages over conventional methods, limitations, and future prospects in food safety and microbiological diagnostics. CRISPR-based technologies are a promising next-generation approach for enhancing food safety monitoring and safeguarding public health.

2. INTRODUCTION

Food safety has become a global concern due to the rising number of cases of foodborne diseases, which are caused by microbial contaminants. Foodborne pathogens such as Salmonella, Escherichia coli, Listeria monocytogenes, and Campylobacter are known to cause millions of cases of infection and death worldwide every year. These microorganisms can easily contaminate food at different levels, such as production, processing, storage, and distribution. The consumption of contaminated food can cause serious health problems, including diarrhea, fever, vomiting, and even death.

It is important to detect foodborne pathogens early and accurately to avoid outbreaks and ensure food safety. Traditional detection methods, such as culture-based methods, biochemical tests, and molecular techniques like Polymerase Chain Reaction (PCR), have been used extensively. Although these methods are accurate, they have some drawbacks. Culture-based methods are time-consuming and take several days to provide results, while molecular techniques require specialized equipment and expertise.
In recent years, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins have been recognized as game-changers in molecular biology. Initially identified as components of the bacterial immune response to viral infections, CRISPR-Cas systems possess the ability to target and cut specific nucleic acid sequences with high specificity. This specificity has made it possible to develop CRISPR-based diagnostic platforms for the rapid and accurate detection of pathogens.

CRISPR-based detection assays, especially those using Cas12 and Cas13 enzymes, have proven to be highly promising for the rapid detection of foodborne pathogens. Methods such as SHERLOCK (Specific High-Sensitivity Enzymatic Reporter UnLOCKing) and DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter) have been shown to possess high sensitivity and specificity for the detection of bacterial DNA and RNA.

These methods possess a number of advantages over the conventional detection methods, including faster detection, higher accuracy, and the possibility of point-of-care testing.
The purpose of this review is to present an overview of CRISPR-Cas systems and their use in the rapid detection of foodborne pathogens.

3. CRISPR-Cas System

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins are a prokaryotic defense system. This system protects bacteria and archaea from invading genetic elements like bacteriophages and plasmids. The CRISPR loci are composed of short repeat sequences interspaced with unique spacer sequences. These spacer sequences are derived from the DNA of previously invaded viral particles. These spacers act as a molecular memory that helps the bacteria defend themselves against future attacks by the same viruses.

The CRISPR-Cas system has three main steps: adaptation, expression, and interference. In the adaptation step, pieces of foreign DNA are added to the CRISPR locus in the form of spacers. In the expression step, the CRISPR locus is transcribed into a precursor CRISPR RNA (pre-crRNA), which is later cleaved into mature CRISPR RNAs (crRNAs). In the interference step, the crRNA directs the Cas protein to the complementary target nucleic acid sequence, which is then cleaved and degraded.
The CRISPR-Cas systems can be divided into two major classes and several types depending on their structural and functional properties. Of these, the Class 2 systems, which have a single effector protein, are commonly used in biotechnology and diagnostics. The most well-studied Cas proteins are Cas9, Cas12, and Cas13.
Cas9 is a DNA-targeting endonuclease that causes double-stranded breaks in specific DNA sequences guided by a single-guide RNA (sgRNA). Cas12 is a DNA-targeting endonuclease that has collateral cleavage activity, which means that after being activated by binding to its specific target sequence, it can cleave single-stranded DNA molecules in the vicinity. This special property of Cas12 makes it very useful for diagnostics. Cas13, on the other hand, targets RNA and has collateral cleavage activity for single-stranded RNA molecules after activation. This property allows for sensitive detection of RNA viruses and bacterial transcripts.

The identification of the CRISPR-Cas system and its development as a gene editing and diagnostic platform has brought a revolution in molecular biology. The high specificity, programmability, and speed of action of the CRISPR-Cas system have enabled the development of novel diagnostic platforms. In food microbiology, CRISPR diagnostic platforms have been developed based on the ability of Cas proteins to recognize specific DNA/RNA sequences.

4. CRISPR-Based Detection Methods for Foodborne Pathogens

The ability to recognize sequences specifically, which is a unique feature of CRISPR-Cas systems, has made it possible to create highly sensitive and fast diagnostic tools for the detection of pathogens. Recently, CRISPR-based tools have attracted considerable attention in the field of food microbiology because of their speed, specificity, and potential for point-of-care testing. Among the different CRISPR-Cas systems, Cas12 and Cas13-based detection platforms are most commonly used for the detection of foodborne pathogens.

4.1 Cas12-Based Detection Systems

Cas12 is a DNA-targeting endonuclease that possesses both specific cleavage of target double-stranded DNA and collateral cleavage of single-stranded DNA in the vicinity after activation. This collateral cleavage function is the principle behind many CRISPR-based diagnostic assays.
A general Cas12-based detection scheme involves the extraction and amplification of nucleic acids from the target food sample using methods such as recombinase polymerase amplification (RPA) or loop-mediated isothermal amplification (LAMP). The amplified DNA is then detected by a guide RNA-guided Cas12 enzyme. After binding to the specific target sequence, the Cas12 enzyme is activated and randomly cleaves single-stranded DNA reporter molecules conjugated with a fluorescent or chromogenic probe in the vicinity. The cleavage of the reporter molecules generates a detectable signal, confirming the presence of the pathogen.

Cas12-based assays have been successfully used for the rapid detection of several foodborne bacteria, such as Salmonella enterica, Escherichia coli, and Listeria monocytogenes. These assays are highly sensitive and can detect pathogens within 30-60 minutes.

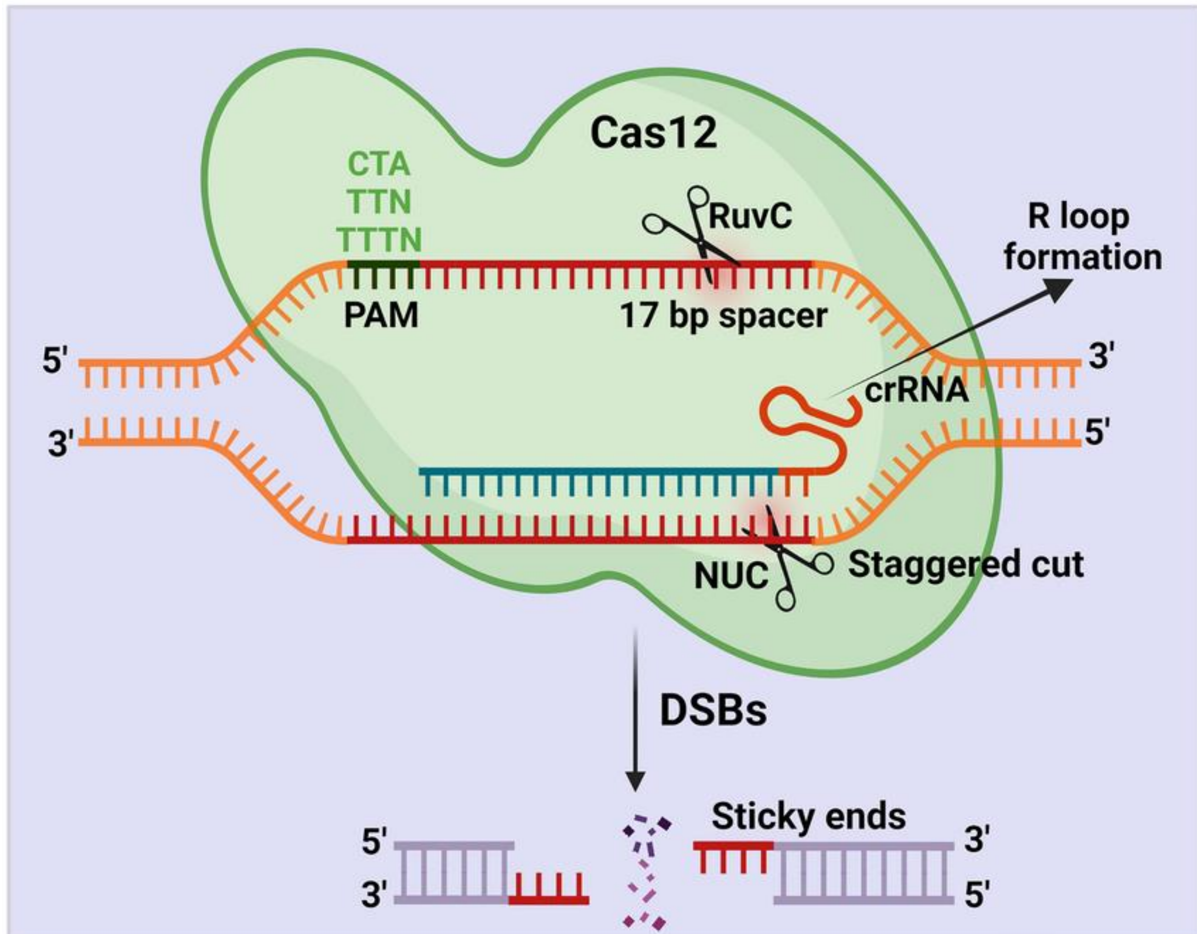

Figure: CRISPR Cas12 mechanism.

4.2 Cas13-Based Detection Systems

In contrast to Cas12, Cas13 is an RNA-targeting enzyme. After binding to its specific target RNA, Cas13 performs collateral cleavage of single-stranded RNA. This feature of Cas13 makes it an excellent tool for the detection of RNA viruses and bacterial messenger RNA.

The detection assay generally includes reverse transcription of RNA (if required), isothermal amplification, and the CRISPR-Cas13 reaction. The activation of Cas13 leads to the cleavage of RNA reporters, producing a detectable fluorescent or visual output. Cas13-based systems are recognized for their outstanding sensitivity, which sometimes goes beyond the attomolar level.

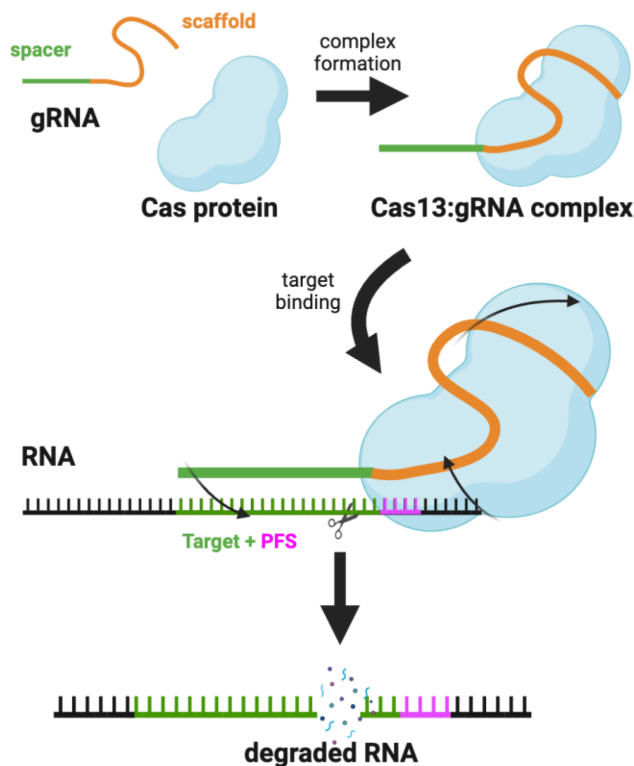

Image:- CRISPR Cas13 mechanism.

4.3 SHERLOCK Technology

Specific High-Sensitivity Enzymatic Reporter UnLOCKing (SHERLOCK) is one of the most popular CRISPR-based diagnostic systems and mainly relies on the Cas13 enzyme. SHERLOCK integrates isothermal amplification and CRISPR-based detection to provide highly sensitive nucleic acid detection.

The SHERLOCK system involves the initial amplification of the target nucleic acid by recombinase polymerase amplification. The amplified target activates the Cas13 enzyme guided by a specific crRNA. Activated Cas13 then cleaves reporter RNA molecules, which provides a fluorescent or lateral flow signal. SHERLOCK has been shown to have the capability to detect very low concentrations of pathogens and has been used for the detection of foodborne bacteria and viral contaminants.

4.4 DETECTR Technology

DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) is another significant CRISPR-based diagnostic tool that usually employs Cas12 enzymes. DETECTR is a combination of isothermal amplification, usually LAMP, and Cas12-based detection.

In this technique, the target DNA activated by amplification triggers the activation of Cas12, which subsequently cuts the single-stranded DNA reporters that are labeled. The result can be analyzed by fluorescence or lateral flow strips, making it a suitable technique for rapid and portable diagnostics. DETECTR has been successfully employed for the detection of different bacterial pathogens of significance to food safety.

Table 1. Various CRISPR-Cas systems have been successfully applied for detection of major foodborne pathogens

Target Pathogen	CRISPR System Used	Target Molecule	Amplification Method	Detection Format	Typical Limit of Detection	Key Advantage

Salmonella enterica	Cas12a	DNA	RPA / LAMP	Fluorescence / Lateral flow	~10–100 CFU/mL	Rapid detection in food samples
Escherichia coli O157:H7	Cas12a	DNA	RPA	Fluorescence	~1–10 CFU/mL	High specificity for pathogenic strains
Listeria monocytogenes	Cas12a	DNA	LAMP	Fluorescence	~10 CFU/mL	Suitable for dairy monitoring
Vibrio parahaemolyticus	Cas12a	DNA	RPA	Fluorescence	~10 CFU/mL	Effective for seafood testing
Campylobacter jejuni	Cas12a	DNA	RPA	Fluorescence	~10–100 CFU/mL	Rapid poultry screening
Norovirus	Cas12a	RNA	RT-RPA	Fluorescence	Attomolar level	Ultra-sensitive RNA detection
Hepatitis A virus (foodborne)	Cas13a	RNA	RT-LAMP	Fluorescence	Very low copies	Suitable for viral surveillance
Generic foodborne bacteria	SHERLOCK (Cas13)	RNA/DNA	RPA	Fluorescence / Lateral flow	Ultra-low	Field-deployable
Multiple pathogens	DETECTR (Cas12)	DNA	LAMP	Visual / Fluorescence	Very low	Point-of-care potential

4.5 Comparison with Conventional Detection Methods

In comparison to conventional culture and PCR-based methods, the CRISPR-based detection methods have some advantages. They are faster, with the ability to provide results within an hour, and have high specificity because of the guide RNAs. Moreover, most of the CRISPR-based methods are isothermal, which means that they do not require thermal cyclers, and thus they are very promising for use in the food industry.
Despite the advantages, there are still some challenges that exist in the CRISPR-based detection methods. These include the need for sample preparation, the risk of contamination during amplification, and the need for further standardization.

Table 2. Comparison of conventional and CRISPR-based methods for detection of foodborne pathogens.

Method	Principle	Time Required	Sensitivity	Equipment Needed	Advantages	Limitations
Culture-Based Method	Growth of microorganisms on selective media	2–5 days	Moderate	Incubator, culture media	Gold standard, confirms viable cells	Very slow, labor-intensive
Biochemical Tests	Metabolic and enzymatic profiling	1–3 hours	Moderate	Biochemical kits	Simple, inexpensive	Less specific, time-consuming
PCR	Amplification of target DNA	3–5 hours	High	Thermal cycler	Highly sensitive and specific	Requires expensive equipment

Real-Time PCR (qPCR)	Fluorescent monitoring of DNA amplification	1–3 hours	Very high	Real-time PCR machine	Quantitative, rapid	High cost, skilled personnel
CRISPR-Cas12 Detection	Target DNA recognition + collateral ssDNA cleavage	30–60 min	Very high	Basic incubator, fluorescence/lateral flow	Ultra-specific, rapid, portable	Usually needs pre-amplification
CRISPR-Cas13 Detection	Target RNA recognition + collateral ssRNA cleavage	30–60 min	Ultra-high	Basic incubator, fluorescence reader	Extremely sensitive, RNA detection	Multi-step workflow
SHERLOCK Platform	RPA + Cas13 reporter cleavage	~1 hour	Ultra-high	Minimal portable setup	Field-deployable, highly sensitive	Amplification contamination risk
DETECTR Platform	LAMP + Cas12 reporter	~1 hour	Ultra-high	Minimal portable setup	Rapid, visual readout possible	Optimization required

5. Applications of CRISPR-Based Detection in Food Safety

The development of CRISPR-based diagnostic platforms has contributed greatly to the advancement of food safety diagnostics. The ability of these platforms to detect foodborne pathogens quickly, sensitively, and specifically has made them valuable tools in the prevention of foodborne outbreaks. These platforms are currently being investigated for their use in monitoring microbial contamination in different food matrices, such as dairy products, meat, seafood, produce, and processed foods.

5.1 Detection of Bacterial Pathogens in Dairy Products

Milk and dairy products are very prone to contamination by pathogenic bacteria like Listeria monocytogenes, Salmonella enterica, and pathogenic Escherichia coli. The traditional culture-based detection method for these bacteria in dairy products takes several days, which is a drawback. CRISPR-Cas12-based assays have shown the capability to detect these bacteria in milk within an hour with high sensitivity. Rapid detection methods can be incorporated into dairy processing units for quality monitoring.

5.2 Monitoring of Meat and Poultry Contamination

Meat and poultry products are known to be a source of foodborne illnesses. Bacteria like Salmonella, Campylobacter, and Listeria are often found to be present in raw and processed meat products. CRISPR-based detection techniques have been successfully used to detect these bacteria in meat samples. The use of isothermal amplification and CRISPR-based detection techniques enables the detection of these bacteria in slaughterhouses and processing units.

5.3 Detection in Fresh Produce and Ready-to-Eat Foods

Raw fruits, vegetables, and ready-to-eat foods are commonly consumed without additional cooking, which increases the risk of disease transmission. CRISPR-Cas tools have been employed for the detection of E. coli O157:H7 and Salmonella in leafy vegetables, sprouts, and cut produce. The short turnaround time of CRISPR-based diagnostics facilitates the early detection of contaminated samples, thus preventing large-scale foodborne disease outbreaks.

6. Application in Seafood Safety

Seafood products are prone to contamination by pathogens like Vibrio species and Listeria monocytogenes. CRISPR-based tests, especially those employing Cas12, have proved to be effective in identifying these pathogens in fish and shellfish samples. The high sensitivity of these tests makes them particularly effective in identifying contamination levels that may not be detected by other tests.

6.1 Role in Outbreak Prevention and Surveillance

The rapid detection of food-borne pathogens is essential for the prevention of outbreaks and for epidemiological monitoring. CRISPR-based detection methods have the potential to greatly reduce the time interval between sampling and the generation of results, thus enabling the rapid application of control measures, including product recalls and tracing the source of contamination. CRISPR-based diagnostics could thus become a crucial part of food safety surveillance systems.

7. Advantages and Limitations of CRISPR-Based Detection Methods

CRISPR-based diagnostic systems have been recognized as promising tools for the rapid detection of foodborne pathogens. The distinct molecular process of CRISPR-based systems provides several advantages over traditional methods of detection. Nevertheless, despite their great potential, there are still some limitations that require attention before they can be fully adopted in industry.

7.1 Advantages of CRISPR-Based Detection

i. High Sensitivity and Specificity

One of the most important benefits of CRISPR-based detection systems is their high sensitivity and specificity. The programmable guide RNA allows for the specific identification of target nucleic acid sequences, even for strains of microorganisms that are very similar. In many cases, the detection limits of CRISPR-based assays have been found to be equal to or better than traditional PCR assays.

ii. Rapid Detection

Conventional culture-based techniques may take 2-5 days to confirm the presence of pathogens, whereas CRISPR-based techniques can provide results in 30-60 minutes after amplification. This is a very useful aspect of CRISPR-based techniques for food safety monitoring, as early detection can prevent large-scale contamination.

iii. Isothermal Reaction Conditions

Most of the CRISPR-based diagnostic platforms are isothermal when combined with amplification techniques like LAMP or RPA. This reduces the need for thermal cyclers, which are expensive in PCR-based platforms. This makes CRISPR-based diagnostics more suitable for field applications.

iv. Potential for Point-of-Care Testing

CRISPR systems can be combined with lateral flow strips and portable fluorescence readers to develop user-friendly point-of-care detection kits. Portable platforms are very useful for inspections in the food industry, border control testing, and real-time surveillance.

v. Programmability and Flexibility

CRISPR-Cas systems are highly adaptable. By merely altering the guide RNA sequence, the same system can be repurposed to target a different pathogen. This adaptability makes CRISPR-based diagnostics highly flexible for multiplex food safety diagnostics.

vi. Cost-Effectiveness

Although the initial setup costs may be moderate, the potential for cost-effectiveness of CRISPR-based assays lies in the reduced equipment needs, short reaction time, and simplicity of the process.

7.2 Limitations of CRISPR-Based Detection

i. Requirement for Nucleic Acid Amplification

However, most of the existing CRISPR detection systems also require a pre-amplification step like RPA or LAMP to be performed to reach a high level of sensitivity. This introduces an additional step in the process and also poses a risk of contamination if not managed carefully.

ii. Challenges in Sample Preparation

Food samples can be quite complex and may also contain inhibitors that can affect the process of nucleic acid extraction and amplification. Effective and standardized sample preparation techniques are still required for effective performance.

iii. Risk of Contamination

Since most CRISPR detection systems involve an amplification step, there is also a risk of cross-contamination that can result in obtaining a false-positive result.

iv. Limited Commercial Standardization

Although CRISPR-based diagnostics have proven to be highly effective in a research environment, large-scale commercial validation and approval are still underway. Standard operating procedures for commercial application in the food industry have not yet been fully developed.

v. Technical Expertise Requirement

Although easier than other molecular techniques, CRISPR-based tests still require technical expertise for handling nucleic acids, preparing the assay, and interpreting the results, particularly in early laboratory applications.

vi. Multiplexing Limitations

The ability to detect multiple pathogens within a single reaction (multiplexing) remains difficult in most CRISPR platforms and is an area of ongoing research.

8. Future Outlook

CRISPR-based diagnostic tools are progressing at a fast pace and are likely to have a revolutionary impact on food safety and microbiological monitoring in the years to come. With the rapid progress being made in the field of molecular biology and bioengineering, the next generation of CRISPR diagnostic tools is being designed to address the existing limitations and improve their usability in the field.

One of the most promising areas for future development is the design of amplification-free CRISPR diagnostic systems. The removal of the nucleic acid amplification step would greatly simplify the process, minimize the risk of contamination, and make it possible to achieve one-step detection. Scientists are actively engaged in efforts to improve the sensitivity of Cas enzymes to make it possible to directly detect low levels of pathogens in food samples.

Another area of significant development is the integration of CRISPR diagnostic tools with microfluidics and lab-on-a-chip technology. These miniaturized systems could make it possible to develop fully automated sample-to-result systems that could be used for food safety testing in the field. The use of CRISPR diagnostic tools in combination with smartphone-based fluorescence readers and portable biosensors could further improve accessibility, especially in developing countries.

Multiplex detection is also likely to see improvements in the coming years. More advanced CRISPR tools that can detect multiple foodborne pathogens in one test would be very useful for overall food safety surveillance. Moreover, the availability of lyophilized CRISPR reagents and ready-to-use kits could make their commercialization and industrialization easier.

Artificial intelligence and bioinformatics approaches could also help in CRISPR diagnostics by providing rapid guide RNA design, predictive modeling of test performance, and real-time data analysis. As more validation experiments are conducted and regulatory guidelines are refined, CRISPR-based detection systems are likely to become essential parts of overall food safety testing.

Conclusion

Foodborne pathogens continue to pose a significant threat to public health and the food industry globally, thereby creating a pressing need for rapid, sensitive, and reliable detection techniques. Conventional detection methods, although popular, have been known to possess certain drawbacks such as a long processing time, need for advanced equipment, and decreased sensitivity in complex food samples.

CRISPR-Cas systems have been identified as highly advanced molecular platforms that provide extremely high specificity and rapid detection of microbial pathogens. Cas12 and Cas13-based platforms, such as SHERLOCK and DETECTR, have been found to possess exceptional potential for the detection of foodborne bacteria with high sensitivity and rapidity.

The general workflow of the CRISPR-Cas genome editing process, from target identification to validation, is shown in figure.

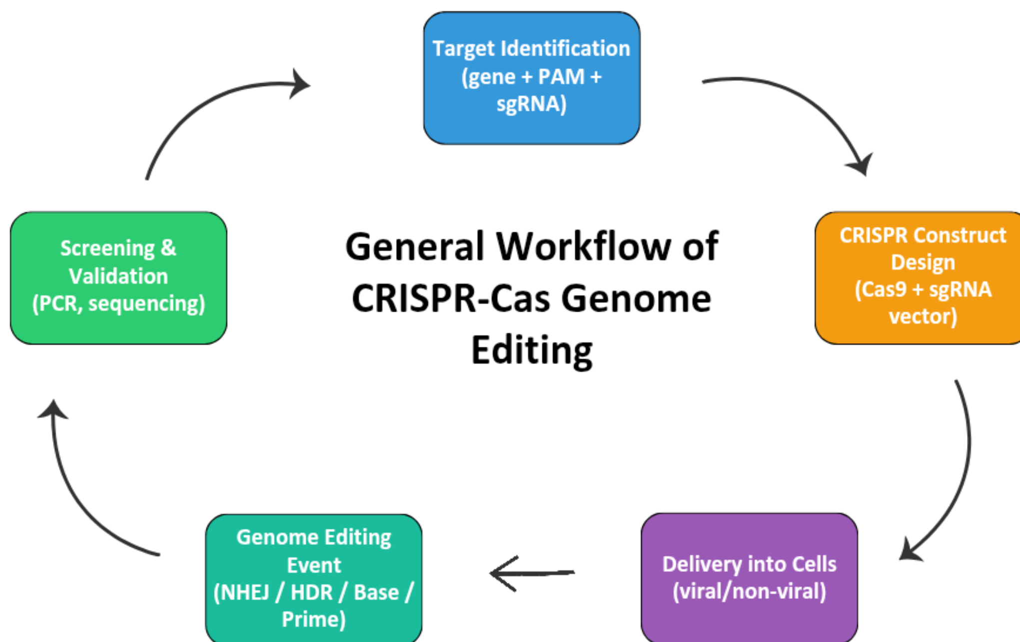

Figure:- General workflow of CRISPR-Cas genome editing showing the major steps involved in the genome modification process.

However, despite some challenges, such as the requirement for nucleic acid amplification and standardization for industrial applications, research is currently underway to address these challenges. Future applications with microfluidics, biosensors, and portable devices will further improve the applicability of CRISPR diagnostics. In conclusion CRISPR-based detection technologies are a next-generation approach for enhancing food safety surveillance. With ongoing technological development, CRISPR-based detection technologies have great potential for becoming a common tool for the rapid detection of foodborne pathogens in the food industry and public health sectors.

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