SCIENTIA
AGRICULTURAE
BOHEMICA

Article

Next Generation Sequencing used for Identification of Genetically Modified Food: Review

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Abstract: This paper summarizes the current status of genetically modified (GM) crops and classifies and summarizes several techniques, comparing their strengths and limitations. This article discusses the current state, benefits, problems, and future possibilities of high-throughput sequencing for GM food detection. It aims to guide future research and development in this subject. Next-generation sequencing (NGS) provides unprecedented depth, precision, and breadth for detecting genetically modified organisms (GMOs) in food. Traditional approaches such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) can only identify known genetic sequences, whereas NGS can examine the entire genome, allowing for the discovery of both known and unknown genetic alterations. This paper gives a thorough overview of NGS technologies, their use in GM food identification, benefits over existing detection methodologies, obstacles, and compatibility with current regulatory frameworks. In summary, NGS provides a diverse technique to detect GM foods that can solve present and upcoming food safety and regulatory concerns. Its application in regular analysis is predicted to increase as technology becomes more affordable and user-friendly.

Keywords: next-generation sequencing, DNA sequencing, GM food, food safety, detection methods.

Received for publication on May 19, 2025

Accepted for publication on October 24, 2025

1. Introduction

Genetically modified organisms (GMOs) and their products have been on the market for over decades (James, 2010). GMOs are live organisms that have had their genetic composition changed using gene technology. It may be a plant, animal, or microbe. To integrate numerous pieces of DNA into an organism's genome, recombinant DNA (rDNA) is often inserted into the host (Jensen, 2001). A genetically modified (GM) food is one with intentionally inserted genes, rather than gaining them naturally through crossbreeding or recombination. Transgenes, or inserted gene sequences, might originate from the same species, another species within the same kingdom, or even a different kingdom. For example, genetically modified Bt maize contains a bacterium-derived gene.

In 1973, biochemists Herbert Boyer and Stanley Cohen developed the first genetically modified organism by inserting DNA from one bacterium into another (Ballian, 2005). Since 1996, when the first GMO reached the global market, the use of GMOs in food items has been the topic of public controversy (Bright et al., 1996). A variety of GM plants have been developed and produced throughout the last two decades (Hails, 2000). Many nations have commercialized genetically modified plants and their derivative products for use in food/feed (Kalaitzandonakes et al., 2014).

The laws regulating genetically modified (GM) crops and feed products vary by country, leading to asynchronous approval processes. For instance, while the United States permits 195 GM varieties, the European Union (EU) allows 55 GM varieties (excluding stacks) for food or feed products as of 2017 (Turnbull et al., 2021). In the EU, the introduction of new GMOs into food and feed supply chains is mainly governed by European Commission (EC) Regulation 1828/2003 and EC Regulation 1830/2003. According to EC Regulation 1827/2003, authorized GMOs must undergo food/feed and environmental safety testing before being marketed and unauthorized genetically modified organisms (UGMOs) are prohibited in the European market (Kalaitzandonakes et al., 2014).

Over the past 25 years, GM crop output has increased more than 100 times (Branford, 2013; Mathur et al., 2017). Farmers currently grow 190 million hectares of transgenic crops, comparable to Mexico's whole surface area (ISAAA, 2018). The four principal farmed crops are soybeans (49%), maize (31%), cotton (12%), and canola (8%) (ISAAA, 2020). Australia and New Zealand prohibit the cultivation of genetically modified crops and strongly oppose the use of gene editing technology. The Environmental Protection Authority (EPA) administers the 1996 Hazardous Substances and New Organisms Act (HSNO) rules, which are among the most thorough in the world and have high requirements for approval evaluation (Fritsche et al., 2018).

In the last few decades, molecular methods have become increasingly commonly used in everyday settings to determine the composition of food items (Staats et al., 2016; Holst-Jensen et al., 2003). Currently, PCR using DNA markers is used to identify GM food, followed by Sanger sequencing, the most widely used molecular biotic process to identify a specific product (Eurlings et al., 2013; Ramos-Gómez et al., 2016). Crop-specific and GMO-specific TaqMan PCRs are commonly used to detect the presence of a GMO in a sample (Scholtens et al., 2017). Impenetrable targets in GMO screening may indicate the existence of unauthorized GMOs. A genome walking technique, in which known GM targets are employed to 'read' into the unknown area, is commonly used in conjunction with Sanger sequencing to locate the unknown region of a UGMO (Arulandhu et al., 2016).

In recent years, the area of molecular biology has progressively used next-generation sequencing (NGS) technologies to solve difficulties related to the identification of species, variations, and so on (Holst-Jensen et al., 2016). NGS enables enormous parallel sequencing of specific DNA, possibly providing an overview of a product's genetic content in a single examination (Su et al., 2011). This progress may be seen not just in genomics but also in medicine and other fields such as agriculture and environmental research (Shokralla et al., 2012). At present, the detection of unknown GMOs remains challenging, as no standardized approach is available without pre-existing knowledge of the transgene. Therefore, our literature review attempted to summarize what has been published regarding NGS for the identification of genetically modified foods.

2. Literature Review

2.1. What are genetically modified foods?

Genetically modified foods have become a central topic in agriculture, food production, and even global health debates. With the progress of biotechnology, scientists can now adjust the genetic makeup of living organisms to introduce useful traits, such as better resistance to pests or improved nutritional content (Benbrook, 2012). In practice, the process usually has three steps: first, find and

separate the gene that has the trait you want; second, use a vector, like a virus or bacterium, to put that gene into the DNA of a host organism; and third, reproduce the modified organism to make sure that the new trait is always there (National Research Council, 2010).

2.2. The history of genetically modified food

The foundation for modern genetic engineering was established through centuries of selective breeding and hybridization aimed at enhancing agricultural yield, flavor, and insect resistance (Zhang et al., 2016). In 1973, Cohen and his team created recombinant DNA technology. This was a huge stride forward since it made it possible to transmit genetic material between living beings (Oakes et al., 1991). Ten years later, scientists made the first plants whose genes had been modified so that they could not be harmed by antibiotics (Bevan & Chilton, 1982; Fraley, 1983; Herrera-Estrella et al., 1983). The United States had given the go-ahead for field experiments of pest-resistant tomatoes and tobacco by 1986 (Fraley, 1983). In 1994, the FDA approved the Flavr Savr tomato as the first genetically engineered product for people to eat (Herrera-Estrella et al., 1983). Monsanto came out with Roundup Ready Soybeans in 1996. These soybeans were made to be resistant to glyphosate herbicides (Bawa and Anilakumar, 2013). Because more people were worried about GMOs, the European Union made it mandatory to label all GMO foods, including animal feed, in 1997 (Woolsey, 2013). The rapid spread of technology is shown by the fact that by 1999, more than 100 million acres of land throughout the world were planted with genetically modified seeds (Woolsey, 2013) (Figure 1).

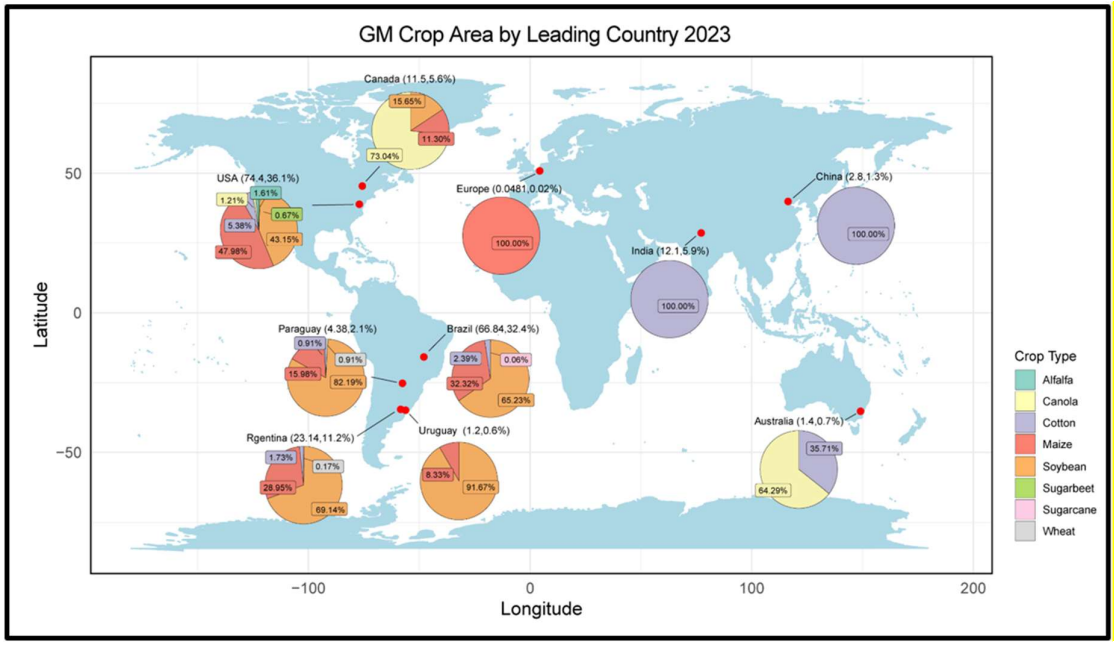

Figure 1: An overview of the nations where genetically modified crops have been grown (AgbioInvestor, 2023).

2.3. Genetically modified foods: Hopes and Issues

Crop yields have increased because GM crops are more resistant to pests, diseases, and harsh environmental conditions (James, 2014). Pest-resistant GM crops minimize the need for chemical pesticides, which benefit both farmers and the environment. Some GM foods are designed to have better nutritional characteristics. Golden rice, for example, has been engineered to include beta-carotene, a precursor to vitamin A, to treat vitamin A deficiency in underdeveloped nations (Brookes

& Barfoot, 2014). Genetic alterations can reduce the ripening process, resulting in less food loss (Potrykus, 2001). From 2007 to 2013, the global rise in agricultural revenue from GM food reached $116 billion, nearly double that of the preceding decade (Smyth, 2020; Al-Mahmood, 2021). Research estimated that enhanced genetics and resistance to pests and weeds contributed to 42% of the economic gain from higher output. Reduced production expenses, including pesticide and herbicide consumption, accounted for the remaining 59% (Smyth, 2020).

Potential cons of genetically modifying food are a relatively new activity; therefore, the long-term consequences of safety are unclear. Several issues might concern human health. Scientists have yet to demonstrate that GMO foods are hazardous to health, although a study is underway (Baulcombe et al., 2014). The argument over genetically modified foods mostly concerns the possible negative impact on human health and environmental safety. Consumers' anxiety about GM foods stems from four sources: the scientific community's difficulty in explaining the biological techniques involved, concerns about improperly disseminating GM foods, ethical principles inherent in traditional food processing, and concerns about the evaluation of GM foods (Gibson et al., 2013).

The main issue associated with GM foods are Allergic reactions: There is a slight possibility that GM foods will cause an allergic reaction, but only if the genetic modification causes the creation of an allergen. For example, if scientists link a Brazil nut gene with a soybean, a person with a nut allergy may experience an allergic reaction to soybean-based goods (Bawa and Anilakumar, 2013). Cancer: There have been worries that consuming GM foods may contribute to the development of cancer by increasing the quantities of possibly carcinogenic chemicals in the body. According to the American Cancer Society Trusted Source, there is no evidence that commercially available GM foods enhance or decrease cancer risk. While cancer rates in the United States have fluctuated over time, there is no evidence that these fluctuations are related to the introduction of GM foods. If there is a relationship, it may take several years before a trend appears (Touyz, 2013). Antibacterial resistance: Some genetically modified foods have been changed such that they don't work with particular medications. Theoretically, the genes of these plants may enter the bodies of individuals or animals that consume them. People or animals may acquire resistance to antibiotics as a result (Ray et al., 2012; Rasheed and Jwher, 2022). Gene flow to wild species: Cross-pollination could transmit genes from GM crops to wild relatives. This might have unforeseen impacts on the environment and lead natural populations to get new features (Ellstrand, 2001).

3. Methods for Detecting Genetically Modified Foods

3.1. Detecting GM foods using phenotypic technique

Phenotypic characterization can identify the presence or absence of a certain trait. So yet, only testing for characteristics like herbicide tolerance is available. Herbicide bioassays are ways for detecting herbicide-resistant genetically modified crops (He et al., 2024). Phenotype-based identification, also known as biological testing, is used for differentiation between GM and non-GM crops by looking for specific transgenic features in samples (Kamle et al., 2017). This approach, despite not needing costly equipment, is mostly used to identify viable transgenic crops.

3.2. Protein-based detection technique

Protein-based approaches, such as ELISA, Western blot, and the immunochromatographic test strip method, are commonly employed in GM crop identification. ELISA uses antigen-antibody reactions, which are useful in identifying target proteins carrying foreign genes in GM crops (Gampala et al., 2019; Zhang et al., 2016). The ELISA test yields quantitative findings in hours, with detection limits below 0.09%. However, other producers have somewhat greater quantification limits, such as 0.2%. ELISAs are developed to identify new genetically modified proteins or traits (Tripathi, 2005).

3.3. Chromatography:

Chromatographic approaches are being developed to detect fatty acids, triglycerides, and other compounds in GMO that are only identifiable qualitatively. These methods for detecting triglycerides in GM canola were created using high-performance liquid chromatography (HPLC) and atmospheric pressure chemical ionization mass spectrometry (APCI-MS) (Byrdwell et al., 2001). Quantification was performed with a flame ionizer detector (FID) combined with HPLC, revealing that GM canola oils did not contain a higher proportion of triglycerides for enhanced oxidative stability in canola and soybean (Jin et al., 2016).

3.4. Techniques for detecting genetically modified food using nucleic acids: (Table 1)

3.4.1. Polymerase chain reaction (PCR):

PCR is a technique for producing many copies of a given DNA region in vitro. PCR uses a thermostable DNA polymerase called Taq polymerase and DNA primers tailored especially for the DNA area of interest (Taha et al., 2024). PCR can be qualitative or quantitative. Qualitative PCR includes standard PCR and reverse transcription (RT-PCR), whereas quantitative PCR refers to real-time PCR (qPCR). Standard PCR is used to detect GM foods qualitatively; it can identify less than 0.01% modified content in raw materials and can only confirm or reject the existence of GM foods. Quantitative real-time PCR is utilized to determine the precise percentage of targeted DNA sequences unique to GM foods in the total sample (Trkulja, 2015; Sheet et al., 2023) Furthermore, contemporary GM food detection and identification methods rely on sequence-specific amplification (TaqMan PCR) of GMO-related sequences, followed by event-specific GMO identification. Because of the growing number of GM foods and their related targets, many TaqMan PCR assays are necessary to undertake an informative screening. Several enrichment approaches (LAM PCR, LT-RADE, Site-Finding-PCR, A-T linker, and LF PCR) have been developed to identify undiscovered adjacent sequences of known GM elements (Garcia-Cañas et al., 2024; Fraiture et al., 2015).

3.4.2. Microarrays Technique:

To identify GM foods using microarray technology, GM targets are amplified by PCR using target-specific or universal primers before being hybridized on the array (Nakaya et al., 2007). This enables the simultaneous detection of over 250,000 targets in a single test. Although microarray technique has a better throughput than qPCR, it has a somewhat poorer sensitivity (Dobnik et al., 2010). One approach, known as multiplex quantitative DNA array-based PCR (MQDA-PCR), was evaluated on transgenic maize events. The signal is then detected by hybridizing the PCR products with fluorescently tagged probes on the DNA array (Shao et al., 2014; Rudi et al., 2003).

3.4.3. Loop-Mediated Isothermal Amplification (LAMP): LAMP is the best method for finding GM foods since it is fast, accurate, sensitive, and easy to use. This method uses four primers that each target a different part of the target. This creates a loop structure that lets the reaction start and grow quickly at a constant temperature. The amplification may be seen in the tube using fluorescent dyes. This application has led to the creation of several LAMP markers (Kiddle et al., 2012).

3.4.4. Southern Blotting Techniques:

Southern blotting is a reliable way to find GMOs in food samples. It works by moving pieces of DNA from a gel to a membrane and then using designated probes to find specific sequences. One of the main advantages of Southern blotting is that it can find larger DNA pieces, which can provide more information about the genetic changes in the samples. Southern blotting can also be used to find specific gene sequences and where they are in the genome. This is a more extensive analysis than PCR (Adugna and Mesfin, 2008).

3.4.5. DNA Biosensors:

These are scientific tools that can find both biological things, like DNA, and physical and chemical things. These biosensors seek certain DNA patterns that are connected to changes in genes to discover GMOs. DNA probes are put on the surface of the sensor during the process. After that, these probes attach to the target DNA in the sample. The sensor picks up and measures the signal that the hybridization event sends out, which shows that GMOs are present (Drummond et al., 2003; Anjum and Pundir, 2007).

Table 1: Comparison of Detection Methods

Method	Advantages	Limitations	Applications
PCR	Sensitive, specific, widely established.	Needs prior sequence data; less effective with degraded DNA.	Basic GMO detection and confirmation.
qPCR	Highly sensitive and quantitative; event-specific detection; accepted in regulations.	Requires reference material and known sequences; equipment cost.	Gold standard for routine GMO screening and quantification.
ELISA	Rapid, low-cost; detects proteins directly.	Only detects expressed proteins; reduced accuracy in processed foods.	Field monitoring; screening raw materials.
Microarrays	High throughput; can test many targets at once.	Expensive; lower sensitivity than PCR; relies on known sequences.	Research and multiplex detection of GMO traits.
LAMP	Fast, simple, no thermocycler needed; tolerant to inhibitors.	Complex primer design; limited multiplexing.	On-site or field testing.
NGS	Comprehensive; detects known and unknown modifications; structural insights.	Costly, bioinformatics-intensive; not yet standardized for regulation.	Characterization of GMOs; detection of unauthorized events.

3.4.6. Next-Generation Sequencing: - Principles:

Setting up the library is a key step in getting the complementary DNA (cDNA) or genomic DNA (gDNA) ready to be sequenced. Typically, the first step is fragmentation, which can be accomplished by machines (such as sonication or shearing) or enzymes (such as Nextera transposase or DNase I). Enzymes may also introduce adapters during fragmentation. This is followed by the addition of one adenine, which modifies and repairs the 3′ end of the DNA and facilitates the addition of adapters. In addition to binding to primers and flow cells, these adapters may also contain index sequences that allow samples to be combined. Following ligation, the fragments are arranged according to size, typically ranging from 200 to 500 bp. Magnetic beads or gel electrophoresis can be used for this. To ensure there is sufficient material for sequencing, PCR on the library may occasionally be amplified using high-fidelity polymerases (Marx, 2012).

Clonal amplification is necessary for the majority of next-generation sequencing technologies because it increases the signal from extremely low DNA concentrations. Illumina systems typically use bridge amplification for this. Dense clusters formed by repetitive synthesis cycles originate from a single molecule. The flow cell is connected to short oligonucleotides that form bonds with fragments. By inserting the complementary strand back into the sequencing process, you may produce paired end reads from these groups, which may have hundreds of copies. Some platforms, such as Roche 454 or Ion Torrent, use a different method. This approach attaches microscopic beads to DNA strands. After that, the beads are packed into tiny drops that operate like their own microreactors. After several rounds of amplification, each bead becomes coated with identical DNA fragments, producing clonal templates for sequencing (Shendure and Ji, 2008).

Signal Detection and Base Calling is the process of transforming fluorescence or current signals into base calls with Phred-scale quality ratings using base calling algorithms. Errors can occur as substitutions (Illumina), insertions/deletions (Ion Torrent, Nanopore), or random errors (PacBio raw). Illumina employs real-time imaging with four-channel fluorescence detection. Ion Torrent identifies protons in each well depending on pH variations. Nanopore uses machine learning models such as Guppy or Bonito to interpret current signals (Table 2), often known as "squiggle plots" (Poplin et al., 2018).

Platform Comparison

Table 2: Next generation sequencing platform comparison (63).

Feature	Illumina	Ion Torrent	PacBio SMRT	Oxford Nanopore
Read length	50-300 bp	200-400 bp	10-100	>1 Mb
Error type	Substitution	Insertion/ deletion	Random	Indel-dominant
Output per run	Up to 6 Tb	Up to 10 Gb	30-60 Gb	>100 Gb
Time per run	1 day	2-4 hours	10-30 hours	Real time
Cost per Gb	Low	Moderate	Moderate to high	Moderate

3.4.7. NGS Applications for Identifying Genetically Modified Foods:

Next-generation sequencing is an excellent technique to find and talk about GMOs in food. Some of the most important ways that NGS can help find genetically engineered foods are shown in Table 3. NGS can find genetic parts that have been added to GMOs, like transgenes (like Bt or herbicide resistance genes), selectable markers (like nptII and bar), and promoters (like CaMV 35S). Researchers can find out if certain genetic changes are present by sequencing and comparing readings to a database of known GMO components (Fraiture et al., 2015). WGS stands for whole-genome sequencing. It looks at all the genes in a sample of food or plants. Finds areas where genes can be added, how many copies there are, and the unanticipated changes that happen when genes are changed. Appropriate for new GMOs that do not have any previous sequencing data (Byrdwell et al., 2001). Even if the DNA is broken up, metagenomic analysis can find genetically modified (GM) material in processed meals. Shotgun sequencing can find very small amounts of GMO DNA in very complicated combinations (Pauwels et al., 2017). Illumina (sequencing by synthesis), Oxford Nanopore (long-read sequencing), and PacBio are some of the most common sequencing systems. They can find alterations in the genome, like where transgenes are added and where mutations happen by chance (Sun et al., 2024). Authentication of Non-GMO Products: NGS can identify even trace levels of transgenic DNA, ensuring the absence of GMOs in "non-GMO" goods. This great sensitivity protects the validity of non-GMO claims and aids in the prevention of cross-contamination (He et al., 2024).

Table 3: Summary of NGS Applications in GMO Detection (case studies).

Study	Sample Type	Platform	Key Findings
Zhang et al. (2012)	Transgenic cattle	Illumina	Identified integration sites & copy numbers
Pauwels et al. (2017)	Processed foods	Shotgun sequencing	Detected GMO DNA fragments in complex food matrices
Moon et al. (2024)	GM crops	Nanopore	Characterized transgene stability across generations

3.5. The relevance of NGS in identifying GM foods:

NGS also enables the detection of unknown GMOs without prior knowledge of the transgene. However, Sanger sequencing was a breakthrough in DNA sequencing, allowing for both DNA sequencing and decoding (Kumar et al., 2020). However, its shortcomings, such as high costs, poor throughput, and time-consuming procedures, have become more evident. The Human Genome Project, which relied mainly on Sanger sequencing, cost around $100 million and over ten years to finish the sequencing process, making it impracticable for whole-genome or transcriptome sequencing (Al-Shuhaib and Hashim, 2023).

On the other hand, unlike NGS, other methods such as Southern blotting required designing and hybridizing particular backbone probes to validate the absence of a backbone, which may be determined by the lack of hybridization. To evaluate Transfer-DNA (T-DNA) stability across generations, further blots containing T-DNA-specific probe sets are required (Moon et al., 2024). NGS does not need the creation of probes. NGS does not require the development of probes. Instead, the entire plasmid sequence is aligned with the target's WGS data set. The mapped measurements are shown and processed to provide data for visualization. With just one mapping and visualization, NGS lets users find T-DNA, copy number, undesirable genes, and the absence of a backbone. This means that they don't have to make extra data for each item (Moon et al., 2024). It makes it easy to quickly check the locations of junctions, the number of copies, and the depth of coverage. NGS's standardized designs make it easy to repeat, accurate, and fast employing automated software tools for data processing (Chen et al., 2020).

In 2012, Zhang et al. used NGS to find out how well transgenes were introduced to transgenic cattle. They worked out how to always find transgene sequences, integration locations, and the quantity of copies. But it could be hard to switch from traditional to NGS because of rules or what people think. To avoid these problems, the rules and steps for sending in and reviewing NGS data need to be clear and the same every time. Standardizing the quantity of NGS data and sequencing coverage depth is essential for the molecular characterization of GM foods (Sims et al., 2014; Yang et al., 2013). There is still some debate regarding the best range for discovering inserts, although many studies have shown that deeper sequencing depths are better for finding GM foods more precisely. But having more coverage could cost more; therefore, it's important to define the right standards for plants and pathogens (Ajay et al., 2011; Wang et al., 2008). Recent studies indicate that utilizing NGS data enhances the speed and accuracy of genetic characterization of GM crops. This could be used instead of or in addition to current methods. With NGS technology, it's easy and rapid to figure out how many copies of inserted genes there are, where they are in the genome, if vector backbones are present, and if T-DNA stays stable over generations. NGS is also accurate enough to discover changes in nucleotide bases that aren't single nucleotide polymorphisms (SNPs), such as minor insertions and deletions. This enables researchers to compare events and reference genomes. The use of new

technologies like NGS based on scientific data is expected to considerably increase customer confidence (Moon et al., 2024). As long as NGS remains more cost-effective and quicker than whole genome or shotgun sequencing, DNA walking enrichment combined with long-read sequencing is a sensible choice for detecting and characterizing GM foods, especially unauthorized ones, by clarifying the ambiguous signals from routine qPCR screenings. However, analyzing a large number of sequencing amplicons can be a challenging task that demands significant time and expertise (Fraiture et al., 2018).

3.6. Advantages of using NGS to identify genetically modified foods:

Next-generation sequencing (NGS) is becoming increasingly important for studying GMOs because it offers so many advantages. One of its best qualities is that it can accurately find genetic changes, even when they are very small or in complicated food matrices. This is because it is incredibly accurate and sensitive. NGS can detect GMOs that are either unknown or not approved because it doesn't need to know the target sequence ahead of time, unlike PCR-based approaches. It shows accidental mutations, structural alterations, and even epigenetic modifications that can arise in genetically designed organisms, which gives a more complete picture than just basic detection. Another important bonus is that high-throughput NGS platforms can process a lot of samples at once, which cuts down on the time and expense of analysis (Holst-Jensen, 2009).

3.7. Challenges and prospects of Next-Generation Sequencing in regulatory GMO detection

Although next-generation sequencing (NGS) has reshaped many areas of molecular biology, its use in regulatory GMO monitoring remains limited. A major barrier is the cost of sequencing instruments and reagents, which can be prohibitive for many routine testing laboratories (Leimanis et al., 2008). Added to this is the demand for advanced bioinformatics capacity and well-trained staff who are able to process and interpret the enormous data sets generated by sequencing. Another complication is the absence of common standards between laboratories, which often leads to inconsistent results and creates obstacles for international harmonization (Kok et al., 2014). Regulatory frameworks also remain largely centered on PCR-based testing, and the process of adapting legal requirements to include NGS has been slow and complex (Hasan, 2025).

When you compare NGS to qPCR, it's easier to see what each one is good at and what it's not good at. qPCR remains the gold standard in GMO analysis due to its high precision, speedy results, and relatively low cost, particularly in food matrices where DNA is often damaged (Kok et al., 2014). But qPCR can't find genetic changes that are both known and unknown; NGS can look at the genome in more detail (Leimanis et al., 2008). The quality of library preparation, the complexity of the food matrix being analyzed, and sequencing depth all have a large effect on how effectively NGS works, which isn't always the same. Because of this, NGS is better understood as a technique to make more full genetic profiles, while qPCR is often more reliable for focused routine detection (Hasan, 2025). The appropriateness of sequencing systems for regulatory applications also evolves. Illumina technology is currently the most widely employed due to its precision, scalability, and cost-effectiveness per base. It is especially beneficial for laboratories requiring reliable high-throughput data (Kok et al., 2014). Long-read technologies such as Oxford Nanopore and PacBio provide distinct advantages for elucidating structural variations and complex insertions (Leimanis et al., 2008); nonetheless, their elevated error rates and operational costs render them challenging for routine application. A hybrid approach may serve as a viable alternative: NGS, specifically short-read Illumina sequencing, can be utilized for comprehensive characterization or the detection of restricted genetic elements, while qPCR would continue to be the preferred method for routine screening (Hasan, 2025).

4. Conclusions

The investigation of GM food characterization has been more important, particularly as these foods become more extensively commercialized. Traditional detection techniques, including Southern blotting, PCR, Sanger sequencing, and ELISA, have proven efficient for determining transgene copy counts and insertion locations. However, these approaches have drawbacks, such as long experimental timeframes and technical limits, and they may be influenced by unanticipated events like sequence replacement. NGS, which allows for 'detection-by-sequencing' of GMOs in food and feed matrices, has been proposed as an alternative to designing specific primers to amplify target sequences for each GM food. Furthermore, NGS has emerged as a transformative technology in the field of GMO detection because of its high-resolution capabilities, which allow for the complete analysis of both known and undiscovered GM foods in a variety of food matrices. While standardization, cost, and data interpretation remain issues, continuous technology improvements and regulatory harmonization are paving the road for NGS to be used routinely in food safety and bio-surveillance applications. Integrating NGS into global regulatory standards promises to make the food system more open, informed, and secure.

Author Contributions: Conceptualization, A.O. methodology, A.F. software, A.O.; validation, A.O. formal analysis, A.F.; investigation, A.M. resources, A.M. data curation, A.O.; writing—original draft preparation, A.O. writing—review and editing, A.F; visualization, A.M.; supervision, A.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: We'd like to thank the colleagues who contributed to this review.

Conflicts of Interest: According to the authors of the manuscript, no conflicts of interest existed during the writing or data analysis.

Data availability statement: The manuscript includes all the data for the study.

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