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Perspective

Advanced Technologies in On-Site Detection of Genetically Modified Products

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Abstract: Transgenic technology is significantly impacting life today. However, with the advancement of genetically modified technologies and the success of genetically modified product commercialization, new challenges have arisen for associated detecting technologies. The need for fast, precise, and portable systems for the on-site detection of genetically modified products has increased dramatically in recent years. This perspective examined the currently available technological support for portable immune biosensing, discussed a portable detection device for ultrafast PCR, and an on-site detection biosensor based on functional nucleic acid and superior detection devices in the field. Moreover, the on-site sequencing of genetically modified organisms was mentioned briefly. Lastly, the future outlook of genetically modified products detection was concluded and discussed in order to provide a comprehensive reference for future research and development in related fields.

Keywords: genetically modified products; on-site detection; portable devices; immune biosensor; ultrafast PCR; functional nucleic acid

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1. Introduction

Transgenic technology can improve traits of receptor organisms by introducing target genes into receptors, and inducing new and superlative forms of the original species [1]. With the increased emergence of new genetically modified (GM) products, the discussion about their safety is also increasing, and people are more and more concerned about whether a product is GM, which makes the demand for transgenic testing higher, and people's requirements for testing is growing as well [2,3]. The cornerstone of breeding, safety evaluation, supervision, and the healthy development of GM technology is the detection of GM substances, and the proteins [4], nucleic acids [5], and metabolite levels of these can be the biomarkers of detection. The testing of GM products has developed greatly with the growing demand for field testing and has become increasingly simple to use, rapid, and portable in recent years, which has aided in the preservation of people's right to know.

An antibody can recognize and bind the protein generated by an exogenous gene directly as an antigen, subsequently transforming the chemical signal into other signals, such as optical signals, for output in order to detect the presence of the foreign gene. The detection of nucleic acid levels is based on signal identification, amplification, and output. The gold standard for genetically modified organism (GMO) detection is polymerase chain reaction (PCR), which is equipment-intensive and time-consuming, making it inconvenient for detection in the field. However, advancements in theoretical PCR research have ascertained that an efficient PCR reaction may be accomplished in a relatively short period

without compromising the sensitivity or specificity of the reaction. Thus, the development of ultra-fast PCR has overcome the time constraints of conventional PCR and made it suitable for on-site detection [6,7].

With the maturation of the technology and extensive study, more transgenic on-site detection approaches based on ultra-fast PCR technology are becoming available. In recent years, for example, isothermal amplification technology has flourished, with loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) being now the most prevalent among rapid detection devices. Isothermal amplification provides benefits that include the non-requirement of temperature changes, minimal instrument needs, significant sensitivity, and rapid consumption, all of which are appropriate for on-site inspections. In LAMP, which was first introduced by Notomi et al. [8] in 2000, four to six precisely designed primers can selectively bind to six to eight areas of the template gene at a constant temperature of 60–65 °C, thanks to the strand displacement activity of DNA polymerase. Complete nucleic acid amplification takes 30–60 min. Recombinase, single-stranded DNA binding protein (SSB), and strand displacement DNA polymerase are the key enzymes used in RPA. The strand exchange process is initiated when the recombinase binds to the primer and locates the template. To prevent repetition, the substituted DNA strand attaches to the SSB. Using a pair of primers and a constant temperature of 37–42 °C, exponential amplification of nucleic acid can be performed in approximately 30 min, which is particularly useful for the quick detection of viral genomes [9].

This perspective focuses on techniques for the on-site detection of GM items. Compared to the laboratory testing, such techniques are capable of producing results in a short period of time in the field, and are usually characterized by simple preparation, simple or automated sample handling, and intuitive interpretation of the results. Hence, they can or have the potential to be applied in the field testing of GM products. First, ultra-fast amplification techniques for on-site detection are introduced. Then, from the viewpoints of portable immune biosensing, portable detection devices for ultra-fast PCR, superior detection devices in the field, and on-site detection biosensors based on functional nucleic acid, methods for the on-site detection of GM products are discussed. Finally, the on-site sequencing of GMOs is briefly mentioned, and debates and future prospects are examined.

2. Portable Immune Biosensors

For the targeted improvement of an original species organism, mainly through the transcription of related proteins by foreign genes, the detection of these proteins is an effective method of GM detection. One of the main detection methods for these proteins is immunoassays, which are capable of the qualitative and quantitative detection of exogenously produced proteins. This technique is relatively quick and easy to execute, is affordable, and is, thus, extensively utilized in field testing of fresh produce.

The protein strip test (PST) is also easy to use, needs little equipment, and requires little knowledge to perform [10]. Van et al. used genuine plant samples to evaluate the traditional PST method and compared the findings to those of PCR methods, revealing the complementary nature of the two techniques [11]. Mutoni et al. investigated the prevalence of GM components in maize food items from Kenyan fields and markets in 2013. This research investigated the possibility of 'gene-flow' in maize fields near the Kenya Agricultural Research Institute (KARI) in Kiboko between 2005 and 2006, where closed field trials (CFT) of Bacillus thuringiensis (Bt) maize were undertaken. This research also used protein crossbands to analyze 120 food samples and confirmed the results using PCR to inform imported food labeling, as well as to assist customers in their purchasing decisions [12]. Zeng et al. created a colloidal gold (Au) immunochromatographic strip (ICS) for the simultaneous detection of numerous transgenic proteins, including CP4 EPSPS, Bt-Cry1Ab, and Bt-Cry1Ac (Figure 1). The strip revealed a single percentage of transgenic content in each of the crops containing Bt-Cry1Ab, Cry1Ac, and CP4 EPSPS, according to parallel examinations of transgenic maize, soybean, sugar beet, and cotton. In crops harboring CP4 EPSPS, at least 0.1 percent transgenic material was identified. High

throughput, simplicity of handling, and visual detection are all advantages of this analysis, which can be completed in approximately 10 min with good accuracy [13]. Lv et al. used a Cry1Ac monoclonal antibody as the coating antibody and a horseradish peroxidase-labeled Cry1Ab monoclonal antibody as the detection antibody to create a double-antibody sandwich ELISA for the measurement of Cry1Ac protein in maize. The results revealed that the constructed double-antibody sandwich ELISA was stable, with a variation coefficient of less than 3% and a detection limit of 9.49 ng/mL, confirming its suitability as a possible detection tool for transgenic product inspection and quarantine [14].

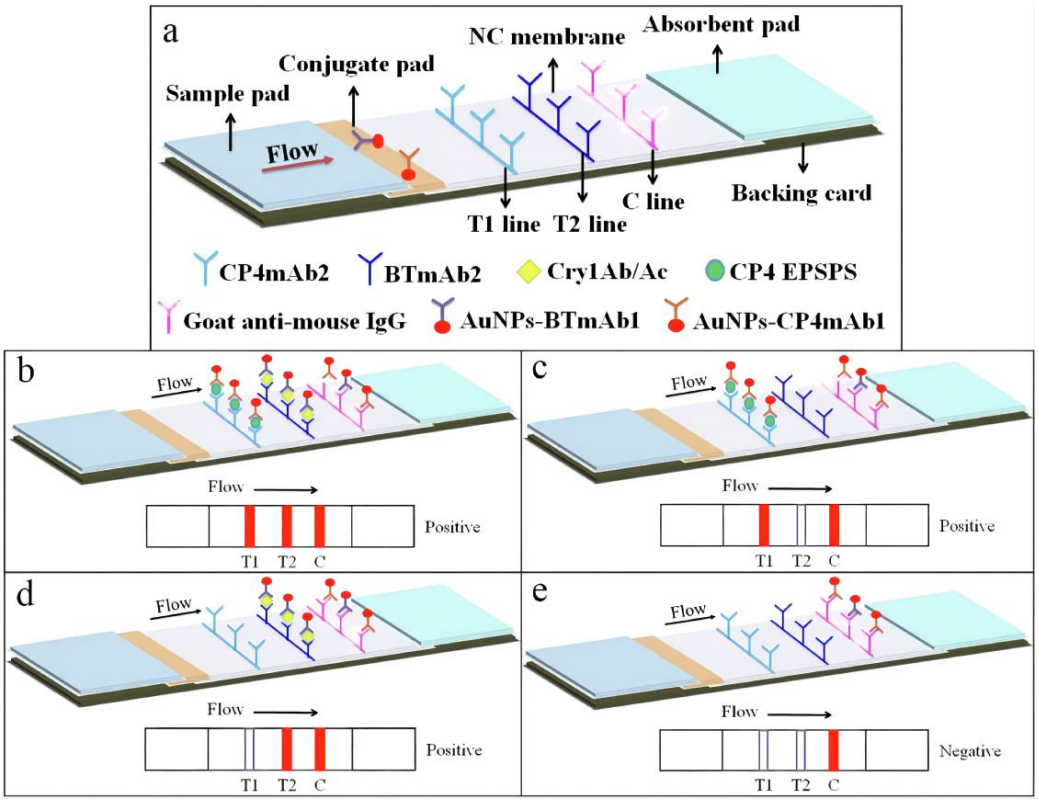

Figure 1. A colloidal Au immunochromatographic strip for the simultaneous detection of numerous transgenic proteins [13]. Structure and composition of the ICS (**a**); Double positive result of the strip (**b**); Single positive result of the strip (**c**,**d**); Negative result of the strip (**e**).

3. Portable Detection Devices for Ultra-Fast PCR

The PCR [15] is not only the gold standard for detecting nucleic acid levels, but the technique is also constantly being improved via optimization. The invention of ultra-fast PCR has drastically shortened reaction times, thereby compensating for the time-consuming and sophisticated equipment requirements of traditional PCR techniques. Its combination with microfluidic chips, lateral flow biosensors, and other technologies provides excellent technological support for quick on-site detection.

Gao et al. employed a combination of Dual Super Polymerase Chain Reaction (DSPCR) and a universal lateral flow biosensor (LFB) to perform ultra-fast visual screening of dual GM elements, with target fragment amplification taking less than 2.5 min. Moreover, in less than 10 min, and without the requirement for large-scale apparatus, the LFB produced visual dual amplification findings via specific antigen–antibody binding with detection limits as low as 0.05 percent in GM maize [16]. For the quick detection of the transgenic maize MON810, Li et al. developed an ultra-fast and extremely stable magnetic test strip based on blocking super PCR (BS-PCR) (Figure 2). The BS-PCR can reduce the time it takes to identify a target gene's signal and amplify it to just 5 min, with the conversion and output of the signal using magnetic chromatography taking another 5 min, bringing total

detection time to 10 min. The novel, entirely portable system was also shown to achieve highly effective sensitivity to a single target [17].

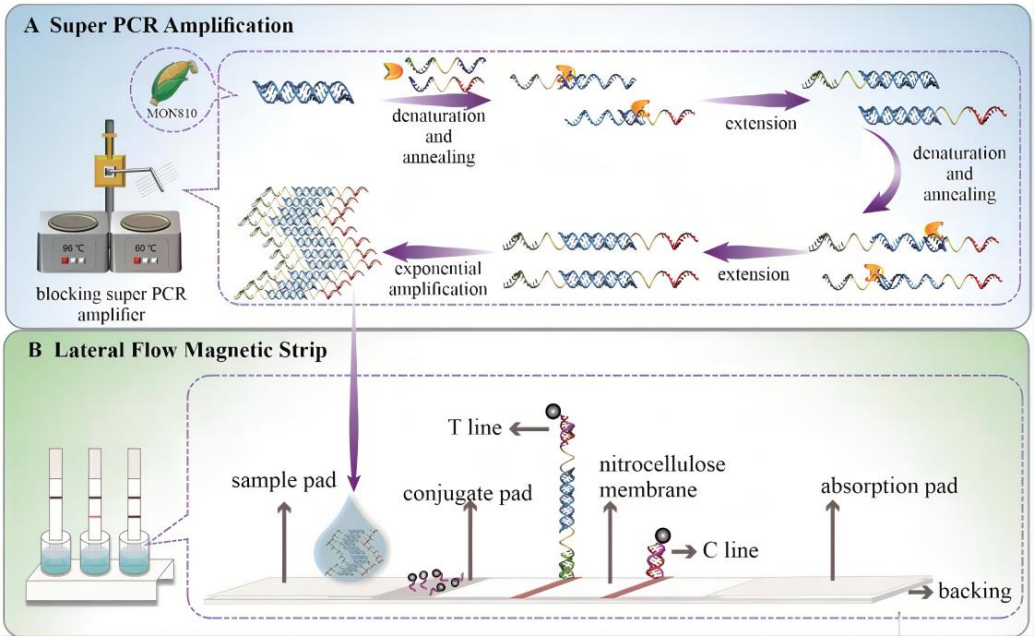

Figure 2. An ultra-fast and extremely stable magnetic test strip based on BS-PCR for the rapid detection of transgenic maize MON810 [17]. The principle of Super PCR amplification (**A**); Structure and composition of the magnetic strip (**B**).

Furthermore, as ultra-fast PCR technology has progressed, a number of ingenious reaction tools for ultra-fast PCR amplification have emerged, all of which are simple, reliable, and affordable. For example, Son et al. suggested a new ultra-high-speed photoelectric PCR technology that uses thin gold films as photothermal converters and light-emitting diodes (LEDs) as heat sources. Heating temperature can be regulated using the difference in the photothermal conversion efficiency of the gold films of various thicknesses. The system was shown to remain stable at temperatures of 94 °C, 60 °C, and 72 °C, and it completes the PCR cycle and performs 30 ultra-high-speed heat cycles in under 5 min [18]. Lee et al. evenly dispersed biconical gold nanoparticles into a PCR tube and employed it as a nanoreactor to absorb the photon energy from infrared LEDs (850 nm), quickly converting it to heat energy to enhance the reaction system and completing 40 PCR cycles in 7.5 min [19]. These abovementioned approaches exhibit significant potential for effective on-site detection, particularly if GM substances change in the future.

4. Superior On-Site Detection Devices

In addition to the PST mentioned above, the combination of nucleic acid-based detection with common on-site detection methods such as microplatforms, microfluidics, microchips, and paper-based devices can achieve the fast, accurate, and stable detection of GM components with high sensitivity and specificity.

Isothermal amplification is quicker, more efficient, more specific, requires fewer items of equipment, and is easier to utilize in field tests than variable-temperature amplification methods. Moreover, it has reportedly shown good results in field tests. Wu et al. created LAMP-based portable equipment for the detection of GM soybean products in the field. Their crude extraction approach took only 5 min and the entire procedure took a total of 30 min, with a detection limit of 0.1 percent. This approach was subsequently used to test five more soybean products, the results of which were similar to the findings obtained via PCR [20]. Li et al. designed a mini-disk capillary (Figure 3) with a detection limit of 25 copies/reaction by combining quick DNA extraction procedures with visualized LAMP

findings to create the mDC-LAMP. Using transgenic maize and transgenic rice for testing, the mDC-LAMP was shown to provide the benefits of high specificity, a lack of cross-contamination, high sensitivity, high throughput, and suitability for field detection [21]. Loo et al. developed a portable and rapid system for the detection of transgenic papaya using LAMP and an integrated microfluidic platform using a microfluidic lab-on-a-disc (LOAD). Both the foreign gene P-35S and the endogenous papaya protease gene were discovered within 15 min, thereby enabling a distinction between the transgenic and non-transgenic papaya. The detection thresholds for the papaya DNA were 10 pg/μL, which enabled the direct testing of papaya juice without genome extraction and required just 0.02 μL for detection. The LOAD was, therefore, shown to be a simple and powerful GM screening tool that can serve as the foundation for field testing GM foods [22].

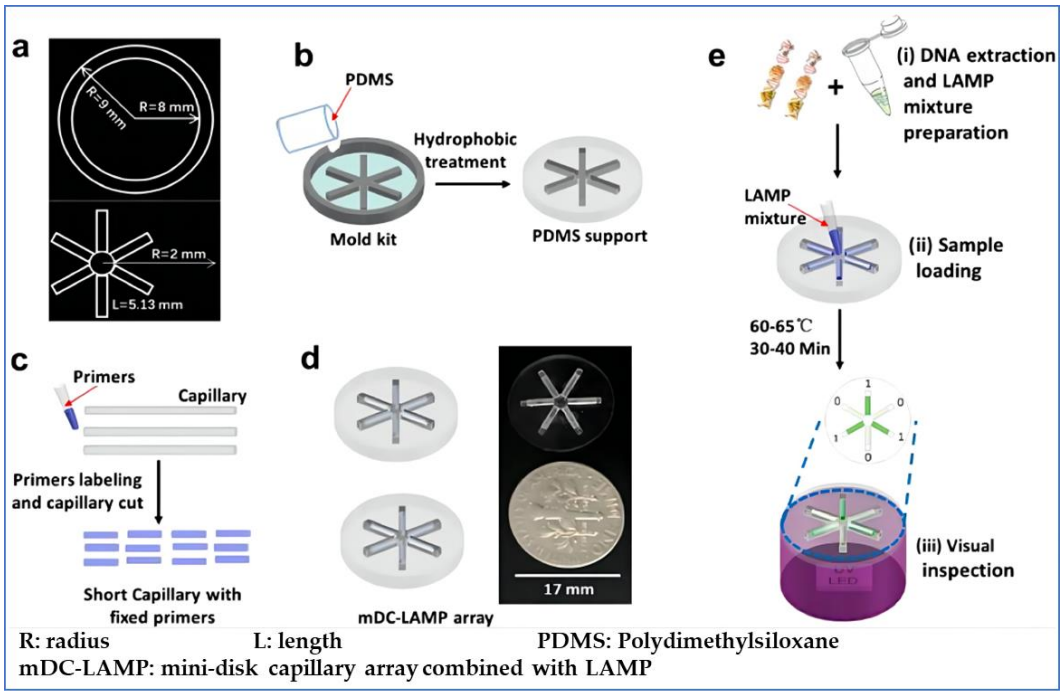

Figure 3. A LAMP-based visualized mini-disk capillary for in field detection [21]. Schematic diagram of the mold kit (**a**); Combination of the circle mold and the actinomorphic star mold for producing the precast PDMS support with hydrophobic treatment (**b**); Short capillaries containing specific LAMP primer sets are cut short for mDC-LAMP array assembly (**c**); Assembled mDC-LAMP array (**d**); Schematic illustration of mDC-LAMP analysis (**e**).

For the multiplex detection of transgenic maize, Li et al. devised a combination of a single universal primer recombinase polymerase amplification (SUP-RPA) and a lateral flow technology isothermal paper-based biosensor. With the use of the lateral flow biosensor (LFB), the particular primer contains the universal sequence at the 5′ end, while the accumulation of Au nanoparticles (AuNPs) forms a distinctive red band that visibly distinguishes the SUP-RPA product. The biosensor can achieve a detection limit of 50 copies, multi-component analysis is possible, and the entire procedure can be completed in less than 30 min and without the use of large additional instruments [23]. There are, in addition, a plethora of gene-editing products currently available. Foreign genes are seldom found in gene-edited organisms and, consequently, several nations have exempted gene-edited agricultural products from GMO regulation [24]. Su et al. created a method for analyzing genome-edited mutants in the germline deletions of tiny genomic fragments that was shown to be both quick and sensitive. With a detection limit of 0.1 ng, the lateral flow nucleic acid biosensor was utilized to visually detect allele-specific PCR products without

the use of other devices, thereby effectively enabling the distinction between gene-edited pigs and wild-type pigs [25].

Cheng et al. proposed a rapid and low-cost method for detecting genome-induced deletions in gene-edited products using rapid multiplex ligation-dependent probe amplification (MLPA) for signal amplification and a dual lateral flow nucleic acid biosensor (LFNAB) for the detection of genome editing-induced deletions, with a detection limit of 0.4 fM and without the need for any additional instrumentation or complexity (Figure 4). In comparison to next-generation sequencing tests, the cascade reaction cuts assay time by at least 20-fold while also dramatically reducing costs (by more than 100 times) [26].

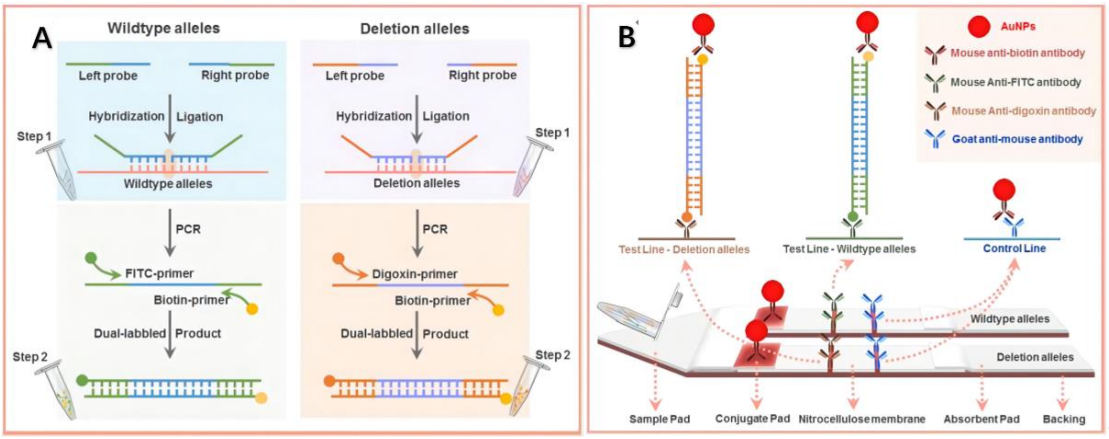

Figure 4. Rapid-MLPA and dual-LFNAB cascade for detecting genome editing-induced deletion [26]. Brief summary of work flow (**A**); Rapid-MLPA procedure (**B**).

Wu et al. created an innovative reaction tube for the detection of GM soybean flour using 3D printing technology, which effectively avoids contamination by omitting the need to lift the lid. The reaction vessel with a pin, a connecting structure with a sealing membrane, and a tube body provides a novel in situ detection solution. The signal amplification approach uses the CRISPR/Cas12a system and LAMP to produce a visual signal that can consistently detect 0.05 percent of transgenic components under UV light (254 nm), making it more sensitive than gel electrophoresis or melting curve methods. The CRISPR/Cas12a system is added to the surface of the sealing membrane at the end of the reaction, which is then poked out by a needle in the lid, and the reaction solution is mixed with the CRISPR/Cas12a system using low-speed centrifugation, ensuring that the reaction solution is always isolated from the external environment [27].

Surface plasmon resonance (SPR) can also be used to identify target genes without the need for amplification. Jang et al. created an Au nanoparticle-coated SPR sensor that can output signals from a laptop computer, thus alleviating the need for bulky and cumbersome equipment that plagues traditional SPR detection methods. The sensor is comprised of a light source, a photodetector, and a cuvette unit with an AuNP-coated sensor chip. The surface of the sensor chip is functionalized with the collected DNA once the sample solution containing the target gene enters the cuvette, thereby enabling the sensitive determination of transgenic crops. After 30 min of target DNA hybridization, the change in the signal is measured at 540 nm, with a response concentration range of 1–100 nM and a detection limit of 1 nM. The sensor chip's benefits include a quick reaction time, simplicity of handling, and portability, making it ideal for in situ detection and testing [28].

These devices provide a unique, rapid, and portable detection which is particularly useful for GMO field testing.

5. On-Site Detection Biosensor Based on Functional Nucleic Acid

In a general sense, functional nucleic acids are nucleic acid molecules that can act as substitutes for standard proteases and antibodies, despite having separate structures and

performing specialized biological activities [29,30]. The biosensor is comprised of three parts, namely, an identification element, a signal amplification element, and a signal output element. It has the capacity to recognize biological components and generate a signal according to varying concentrations for detection. The CRISPR/Cas system, DNAzyme, specially designed primers, hairpin structures, nucleic acid aptamers, and other functional nucleic acids are commonly employed for detection purposes. The use of functional nucleic acids to identify transgenic components on site can significantly enhance detection efficiency and lower detection costs.

The CRISPR/Cas system is generally used in conjunction with nucleic acid amplification. Zhang et al. developed a CRISPR/Cas12-based nucleic acid detection platform and tested its capabilities for rice pathogen diagnosis and GMO identification using the Magnaporthe oryzae gene and the Cry1C gene (Figure 5). Zhang et al. combined the lateral flow assay (LFA) with RPA-Cas12a and also performed DNA extraction using filter paper strips, an optimization that enabled the completion of the assay at body temperature without the need to remove filter paper or other instruments outside of the LFA strip, thus making it suitable for field detection [31].

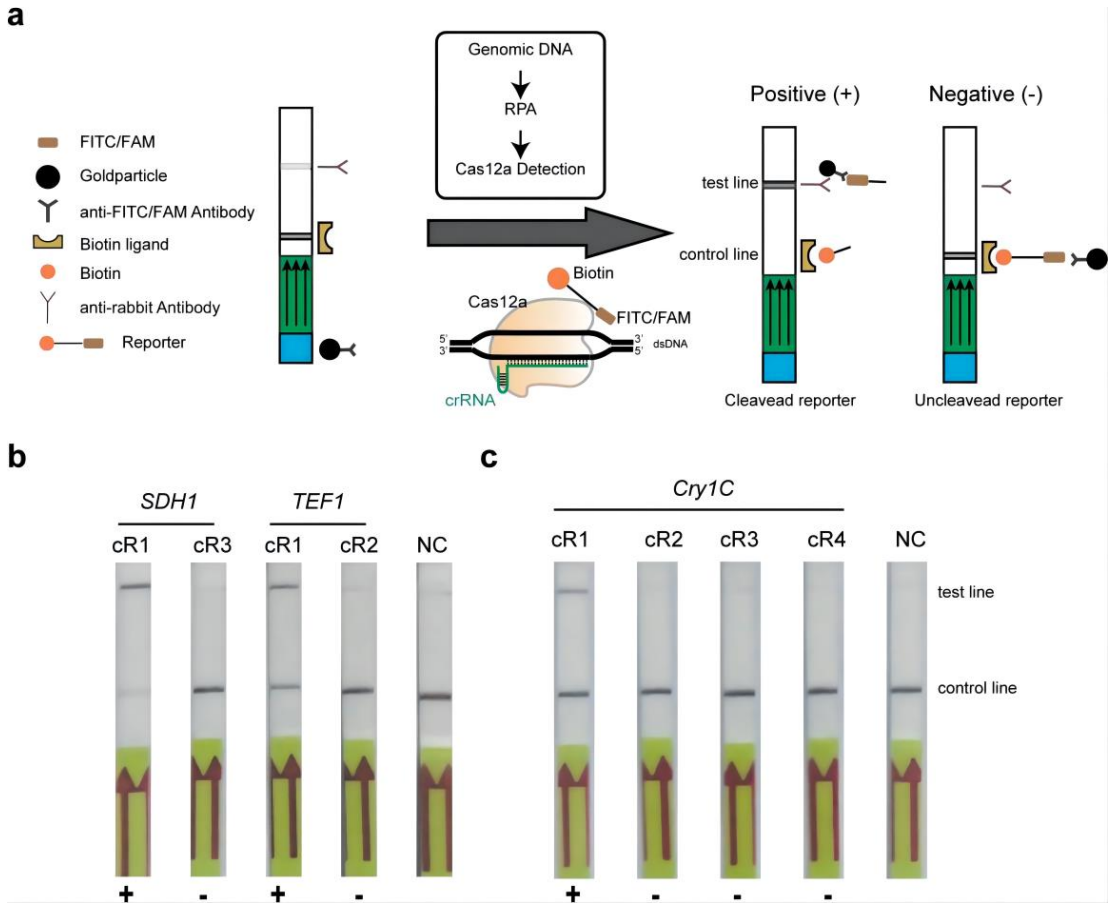

Figure 5. A paper-based RPA-Cas12 nucleic acid detection system that can be completed at body temperature [31]. Schematic diagram of detection principle (**a**); Positive results for different target genes (**b**,**c**).

Tian et al. created a dsDNA/ssDNA switchable isothermal colorimetric biosensor. The universal primer effectively enhanced the RPA, the exonuclease acted as a transducer and mediated the conversion of dsDNA to ssDNA, and finally, a visual signal was obtained using G-quadruplexes with a limit of detection of 3 cfu/mL for *Salmonella* spp. The transgenic maize MON810 enabled a visual semi-quantitative detection limit of 0.1 percent for

Salmonella spp.; based on this finding, Tian et al. created a portable suitcase with a DNA crude extraction kit, an isothermal colorimetric kit, and a portable spectrophotometer, a design that serves as a good model for transgenic field testing [32]. Cheng et al. devised a cascade system combining three sets of primers for multiplex LAMP, a DNAzyme side-stream biosensor, and DNAzyme-enhanced reactions, with the flow measurement biosensor displaying the amplified products without risk of cross-contamination. Following optimization, the detection limit for GM soybeans was approximately 0.1 percent, and the entire procedure was completed in less than 120 min without the use of any big instruments. This approach may also be used to detect GM soybean components in other processed meals in real time [33]. For the identification of P-35S and T-nos in GMOs, Liu et al. established an RPA-based and lateral flow test paper (LFD)-based platform. Following the labeling of forward and reverse primers with various fluorescent groups at the 5′ end, samples were quickly assessed for the presence of P-35S and T-nos genes of GMOs using the RPA-LFD technique at room temperature and, in the 9 GMO samples tested, the detection thresholds of RPA-LFD were found to be 50 and 100 copies [34]. Huang et al. created a cross-primed isothermal amplification assay (CPA) for the rapid detection of the 35S promoter of the plant pathogen cauliflower mosaic virus (CaMV 35S) in the field, which they combined with a biosensor made of anti-contamination test paper. A pair of replacement primers was added at the end of three for the isolation of single-stranded sequences, and one or more cross-primers with extra initiation sites, as well as biotin and FITC double-labeled probes, were inserted throughout each amplification cycle. The technology was then put to the test with nine commercially available samples, with the results' repeatability confirmed using real-time fluorescence quantitative PCR analysis [35].

6. On-Site Sequencing of GMOs

Especially precise identification of GM cultivars is sometimes necessary in the field. This requirement can be easily met using on-site gene sequencing technologies, such as those now employed in plant subspecies identification, microbe and virus identification, and food certification required, for example, in meat adulteration [36,37]. Marcolungo et al. developed a novel all-in-one diagnostic detection approach based on nanopore sequencing and portable equipment, and evaluated it using Xylella fastidiosa as a case study. Pathogen identification and typing were achieved in 13 min, with good specificity and agreement with PCR analysis, thereby enabling the separation of subspecies that are present alone or in combination [38]. The understanding and enhancement of such approaches for GM goods would be extremely beneficial to the advancement of GM product testing as a whole.

7. Conclusions and Perspectives

Depending on the identifying components, the detection of GM products may be classified into one of three categories, namely, protein level detection, nucleic acid level detection, or metabolite level detection, the first two of which are the most extensively utilized (Table 1).

PST has long been recognized as one of the most essential procedures in the detection of GM products, similar to the position of the PCR method in nucleic acid detection. With the advancement of nanomaterials research, their unique catalytic activity and optoelectronic features have provided a significant boost to classical PST. Nanomaterials are used in the combination of the two, while the immunoassay strip is a rising star in the on-site testing of GM products, with excellent results reported. In addition, the invention and use of ultra-fast PCR has breathed fresh life into the previously dubbed 'gold standard' of transgenic detection. Ultra-fast PCR reaction times can be sped up in a variety of ways; on this foundation, it may be utilized in conjunction with a variety of signal output devices, demonstrating a high degree of adaptability to a variety of sensing situations and needs. The quality, efficiency and variety of signal amplifications and signal output linkages have all increased as a result of the development of nucleic acid isothermal amplification technology and functional nucleic acids. Microplatforms, microfluidics, microchips, and

paper-based devices are all employed in conjunction with these two technologies, providing a renewed impetus for the use of on-site testing of GM products. Furthermore, rapid on-site sequencing methods have contributed to the enhancement and enrichment of the transgenics on-site detection system.

Table 1. Advantages and disadvantages of different detection technologies.

Classification		Detection Time	Sensitivity	Advantages	Disadvantage	Source
Portable immune biosensors	Protein crossbands	/	/	Easy to use; low cost; needs little equipment.	Low specificity; not precise enough; not highly sensitive.	[12]
	Au-ICS	10 min	0.1%			[13]
	Double-antibody sandwich ELISA	/	9.49 ng/mL			[14]
Portable detection devices for ultra-fast PCR	DSPCR-based LFB	10 min	single copy	Stable; time-consuming; accurate; sensitive.	Slightly complex instrumentation; specialist requirements for the operator.	[16]
	BS-PCR and magnetic test strip	10 min	single copy			[17]
Superior on-site detection devices	LAMP-based portable equipment	30 min	0.1%	Simple requirements for reaction equipment; short-time consuming; high specificity and sensitivity; easier to utilize in field tests.	Risk of contamination; high requirements for amplification primers.	[20]
	Mini-disk capillary-LAMP	/	25 copies/reaction			[21]
	LAMP-LOAD	15 min	10 pg/μL; 0.02 μL			[22]
	SUP-RPA-based LFB	30 min	50 copies			[23]
	LFB	/	0.1 ng			[25]
	MLPA-LFNAB	/	0.4 fM			[26]
	3D printing reaction tube with CRISPR/Cas12a system and LAMP	/	0.05%			[27]
	Au nanoparticle-coated SPR sensor	30 min	1 nM	No amplification required.	Ambient temperature prone to interference.	[28]
On-site detection biosensor based on functional nucleic acid	RPA-Cas12a-based LFA	/	/	Lower detection costs; highly efficient; high specificity and sensitivity.	Complex design process.	[31]
	Portable suitcase	/	0.1%			[32]
	DNAzyme-enhanced LFB	120 min	0.1%			[33]
	RPA-based LFD	/	50 and 100 copies			[34]
	CPA-based anti-contamination test paper	/	/			[35]

More efficient high-throughput and convenient detection methods should be established in the future to promote and optimize large-scale screening in the field. Furthermore, the rapid pre-treatment of deep-processed foods containing GM ingredients in the market warrants investigation, while effective contamination prevention, visualization, and quantitative detection in the field are also significant and promising directions for future development. Finally, rapid on-site detection findings may be linked with production traceability systems in the future to establish interconnected control and precise traceability that could substantially aid in the identification of GM components.

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