

Review

Applications and Prospects of CRISPR Molecular Diagnostics in the Detection of Genetically Modified Crops

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CITATION

Hou K., Dai Y., Hu F., Ni H., Ding T., Yang C., Zhang J., Qin R., Zhang C. Applications and Prospects of CRISPR Molecular Diagnostics in the Detection of Genetically Modified Crops. *Agriculture and Biology*. 2026, 2(3): 1–20. <https://doi.org/10.70737/416nr930>

ARTICLE INFO

Received: 20 August 2026

Accepted: 19 October 2026

Available online: 21 October 2026

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Abstract: Genetically modified (GM) technology plays a pivotal role in improving crop traits, such as enhancing yields and stress resistance. However, as the cultivation area of GM crops continues to expand, biosafety supervision faces increasingly severe challenges, demanding innovative advancements in detection technologies. This review traces the evolutionary trajectory of representative molecular detection techniques and systematically dissects the molecular recognition and signal amplification mechanisms underlying Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based molecular diagnostics. Crucially, we highlight the application potential of CRISPR technology in GM element screening, multiplex detection, and on-site rapid testing. On this basis, future optimization directions and application prospects are discussed, aiming to provide a solid theoretical foundation and technical reference for the biosafety supervision of GM crops.

Keywords: genetically modified crops; molecular detection; CRISPR; on-site rapid detection; biosafety

1. Introduction

Genetically modified crops are produced by introducing exogenous genes into crop genomes through recombinant DNA technology, thereby altering genetic traits and generating genetically stable plants and progeny [1–4]. They also provide abundant research materials for investigating plant life processes at the molecular level. With the expansion of commercial GM crop cultivation and the continued iteration of research and development technologies, new crop varieties integrating desirable traits such as insect and disease resistance, herbicide tolerance, stress resistance, and high yield have continued to emerge [5,6], and their application scope has steadily broadened. While GM products bring substantial economic benefits and social convenience [5,7], their safety has attracted extensive attention. Therefore, it is urgent to develop precise detection methods for GM components and improve regulatory efficiency [1,8]. Establishing simple and accurate quantitative detection methods can promote the steady development of GM agriculture and has far-reaching practical value for protecting domestic agricultural trade interests, meeting biosafety regulatory standards, and optimizing labeling management systems [9,10]. At present, GM component detection methods can generally be divided into two categories [11]. The first comprises strategies targeting exogenous nucleic acids [12–14], including Polymerase Chain Reaction (PCR), quantitative real-time PCR

(qPCR), isothermal amplification, and gene chips; the second comprises methods based on exogenous proteins [15–17], such as enzyme-linked immunosorbent assay, Western blot, and strip tests [8,18]. In addition, CRISPR-based nucleic acid detection has emerged as a revolutionary diagnostic paradigm capable of bridging the gap between laboratory-grade precision and field-deployable simplicity [19]. Owing to its programmable targeting and signal-amplifying mechanics, this technology offers unprecedented analytical sensitivity and versatility for GM element screening, circumventing many trade-offs inherent to conventional platforms.

Based on the above analysis, this review summarizes the current application status of representative molecular detection technologies for GM crops and focuses on the application potential of CRISPR-based nucleic acid detection systems in common GM element screening, parallel multi-target detection, and rapid on-site testing. This discussion provides a systematic reference for research and practical application of GM detection technologies and promotes their development toward greater precision, portability, and intelligence.

2. Research, Development, and Application of GM Detection Technologies Support Safety Regulation

GM detection technologies are important for increasing crop yield, safeguarding food security, and reducing environmental pollution [20]. A variety of existing methods can directly detect exogenous expression products and support rapid on-site screening [21,22], thereby providing technical support for improving global biosafety regulation of genetically modified organisms [1,4].

2.1. Development of GM Crops and the Current Research of Global Regulation

Large-scale cultivation of GM crops plays an important role in reducing the threat of pests and diseases to grain yield, addressing pressure on arable land resources, and mitigating greenhouse gas emissions. It has generated significant benefits for socioeconomic development and sustainable use of resources and the environment [23,24]. Since the first GM tobacco and petunia plants were produced in the United States in 1983, 41 years of industrialization have led to rapid expansion of GM crop cultivation. The global area has increased from 1.7 million hectares at the initial stage to 209.8 million hectares, with annual growth rates exceeding double digits. Crop types have also expanded from early cotton, tobacco, and maize to wheat, rice, and soybean [1,25]. Breeding objectives have shifted from simply increasing yield to designing traits that meet diverse needs in cultivation, processing, and consumption, including herbicide tolerance, insect and disease resistance, drought resistance, and water-saving properties [26–28]. Target traits in GM crops currently under development have become even more diverse [29], including tolerance to glyphosate and glufosinate [30,31], resistance to rice blast and stripe rust [32–35], resistance to brown planthopper and Colorado potato beetle [36–38], and tolerance to saline-alkali and water stress [39,40]. Quality-improvement traits are also becoming more varied, such as increasing folic acid and vitamins A and E, enhancing photosynthetic efficiency, and inhibiting browning [41]. In addition, GM

technology has promising applications in reducing pesticide use, lowering carbon emissions, enhancing soil carbon sequestration, and improving the efficiency of water and soil resource utilization [42–45]. More GM products are expected to enter commercialization within the next two to three years.

At present, global biotechnology innovation is accelerating, GM technology continues to advance, and new genes, traits, and products continue to emerge. However, uncertainties regarding the biosafety of genetically modified products remain, and coupled with the expansion of their cultivation scale and the introduction of new varieties, there is an urgent need to strengthen the corresponding regulatory framework. Countries and regions differ substantially in GM legislation, labeling management, threshold standards, and regulatory models, creating obstacles for import-export inspection and quarantine, international mutual recognition, and cross-border trade involving GM products. Therefore, research and application of rapid and precise GM detection technologies can help establish a unified safety regulatory mechanism, enable source control of GM crop cultivation risks, effectively balance risk prevention with agricultural industry development, and respond to public safety concerns while reducing cognitive differences and public controversy regarding GM products.

2.2. Upgrading and Challenges of Molecular Detection Technologies for GM Crops

With the rapid commercialization and increasing genetic complexity of GM crops, existing surveillance frameworks predominantly established on exogenous nucleic acid and protein detection face profound translational bottlenecks (**Table 1**) [46,47]. While these platforms offer distinct analytical capabilities, practical GM regulation in non-standardized field or port environments exposes two fundamental trade-offs:

First, a trade-off between analytical fidelity and on-site deployability governs nucleic acid-based assays. Although PCR-derived frameworks (e.g., qPCR and digital PCR) remain the gold standard for reliable quantification, their strict reliance on precision thermal cyclers and multi-step workflows restricts their utility to centralized laboratories [48–50]. Isothermal amplification strategies (e.g., loop-mediated isothermal amplification) resolve the hardware hurdle by operating under constant temperatures; however, their practical implementation is severely bottlenecked by assay engineering complexity and vulnerability to false results. For example, in 2013, Randhawa and colleagues used GM cotton as a research model and showed that loop-mediated isothermal amplification (LAMP) requires four to six primers targeting six specific regions of the target gene, making primer design difficult [51].

Second, a trade-off between operational simplicity and target specificity limits protein-based screening [17,52]. Immunological methods like enzyme-linked immunosorbent assay (ELISA) bypass laborious nucleic acid purification, facilitating high-throughput screening [17,47]. Nevertheless, the conformational susceptibility of antibodies frequently induces false positives triggered by cross-reactivity with structural homologs or denatured variants in processed agricultural

matrices [52,53].

Table 1. Comparison of Advantages and Disadvantages of Detection Methods.

Target	Method	Technical Principle	Core Target	Advantages	Limitations
Protein	ELISA	Specific antigen-antibody binding with enzyme-catalyzed color development	Exogenous proteins (CP4-EPSPS, Cry1Ab)	Quantitative analysis; simultaneous detection of multiple samples; widely recognized and accepted	Requires specific antibodies; insufficient antibody specificity may cause cross-reactions and false-positive results
Protein	Western blot	Protein separation by electrophoresis followed by membrane transfer and antibody hybridization/color development	Exogenous proteins	Provides molecular weight and presence/absence information; detects modified proteins; has confirmatory value	More complex than traditional ELISA; long analysis time
Nucleic acid	Real-time PCR	quantitative Fluorescent probes or dyes monitor nucleic acid amplification in real time and quantify Ct values	Exogenous DNA (CaMV35S, FMV35S, NOS)	Excellent specificity and sensitivity; compatible with multiplex qPCR; eliminates post-processing, saving time and reagents	Each sequence requires a dedicated probe; background may be high; quantification can be affected by reagent and enzyme-activity fluctuations
Nucleic acid	Digital PCR	Sample is partitioned into tens of thousands of micro reactions; endpoint counting enables absolute quantification	Exogenous DNA and endogenous reference genes	Absolute quantification; higher sensitivity and specificity; detects low-copy samples	Expensive instruments and reagents; complex operation; long assay time; relatively narrow detection range
Nucleic acid	LAMP	Efficient amplification at 65°C using four to six primers	Exogenous DNA	High amplification efficiency; short reaction time; strong specificity; no special equipment required	Aerosol contamination can cause false positives; products are not suitable for cloning/sequencing; stringent primer requirements
Nucleic acid	High-throughput sequencing	Massively parallel sequencing of total sample DNA followed by bioinformatic comparison	Whole-genome DNA	High sequencing efficiency; higher accuracy than traditional Sanger sequencing; significantly reduced sequencing cost	Low assembly accuracy; complex data analysis
Metabolite	Chromatography-mass spectrometry	Chromatographic separation and MS-based qualitative/quantitative detection of characteristic metabolites	Characteristic metabolites [glyphosate and aminomethylphosphonic acid (AMPA)]	Very low sample requirement; directly reflects functional activity; high throughput	Limited resolution of isomers; extra desalting or dilution may be required when interfaces are incompatible
Physicochemical	Near-infrared spectroscopy	Measures absorption and scattering spectra of organic molecular bonds in the near-infrared region	Overall composition (protein, oil, fiber content)	Non-destructive analysis; high analytical efficiency; no sample pretreatment	Low sensitivity; model-dependent
Physicochemical	Laser-induced breakdown spectroscopy	Laser ablation generates plasma; atomic/ionic emission spectra are analyzed	Elemental composition and content	Enables <i>in situ</i> and remote analysis; simultaneous multi-element analysis; insensitive to sample form	Limited spatial resolution and depth analysis; poor repeatability

Existing GM detection technologies commonly face limitations such as strong dependence on instruments, complex operation, long assay time, high cost, and difficulty in achieving rapid on-site testing. To overcome these inherent limitations and achieve truly portable analysis, there is an urgent need for innovative detection solutions. In recent years, CRISPR-based detection technologies have attracted broad attention because of their simple operation, rapid reaction, and high sensitivity. They have shown disruptive and revolutionary characteristics and provide key core technical support for GM molecular diagnostics.

2.3. Emergence and Innovation Potential of CRISPR Detection Technology

Clustered regularly interspaced short palindromic repeats (CRISPR) rapidly became a research focus in life sciences after the publication of the first related research papers and was recognized by the 2020 Nobel Prize in Chemistry [54]. The

system formed by CRISPR and CRISPR-associated proteins (Cas proteins) has reshaped molecular biology as a revolutionary tool. This mechanism originates from an adaptive immune defense system where archaea and bacteria have evolved over billions of years: foreign DNA fragments are captured and integrated into CRISPR arrays as spacer sequences, thereby establishing an immune memory library (**Figure 1**) [55–57]. Based on the ability of CRISPR systems to precisely recognize and cut genomes, gene-editing technology has gradually been established. It functions as both a “molecular scalpel” and a “suture needle”, enabling targeted deletion of specific DNA fragments as well as introduction of gene sequences associated with desired traits (**Figure 2**). The CRISPR/Cas system can modify multiple loci simultaneously and shows unique advantages due to its strong affinity and specificity for target DNA. Its short operation cycle and high editing efficiency further support its strong development prospects in life sciences [58].

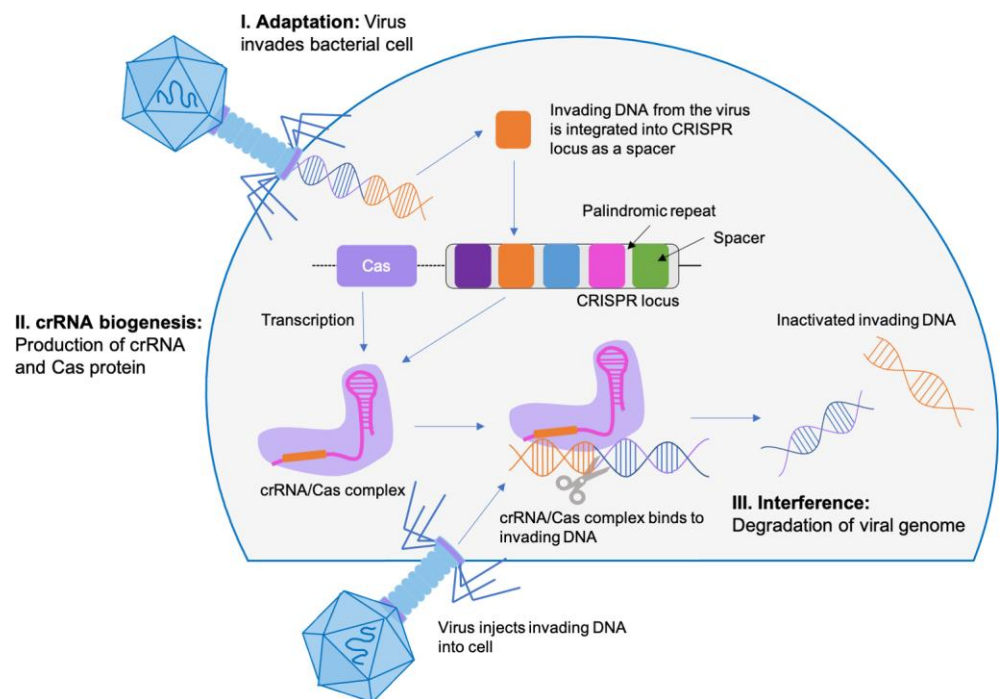


Figure 1. Schematic diagram of the CRISPR editing principle. (I) Adaptation: Upon initial viral invasion, a fragment of the invading foreign DNA is captured and integrated into the host's CRISPR locus as a novel spacer flanked by palindromic repeats. (II) crRNA biogenesis: The CRISPR array and adjacent *Cas* genes undergo transcription and processing, resulting in the production of mature CRISPR RNA (crRNA) and Cas proteins, which assemble to form the functional crRNA/Cas ribonucleoprotein complex. (III) Interference: During subsequent infections, the crRNA guides the Cas nuclease complex to specifically recognize and bind to the complementary sequence of the newly injected invading DNA.

Beyond their classical utility in genome modification, the programmable sequence-recognition capacity of CRISPR/Cas systems has catalyzed their repurposing as powerful *in vitro* biosensing platforms [59]. In agricultural surveillance, for instance, these platforms circumvent the need for complex laboratory workflows, enabling the rapid and sensitive on-site identification of

genetically modified (GM) traits and crop pathogens. Mechanistically, specific recognition and binding of a target nucleic acid by guide RNA activate the conformational rearrangements of Class 2 Cas nucleases (notably Cas12 and Cas13), triggering their non-specific collateral (*trans*-cleavage) activity to indiscriminately shred surrounding single-stranded nucleic acids. By coupling this catalytic turnover with quenched-fluorescent or colorimetric reporter probes, Cas enzymes translate single-molecule recognition events into orders-of-magnitude signal amplification, establishing a versatile molecular diagnostic paradigm that underpins next-generation point-of-care testing (POCT) devices.

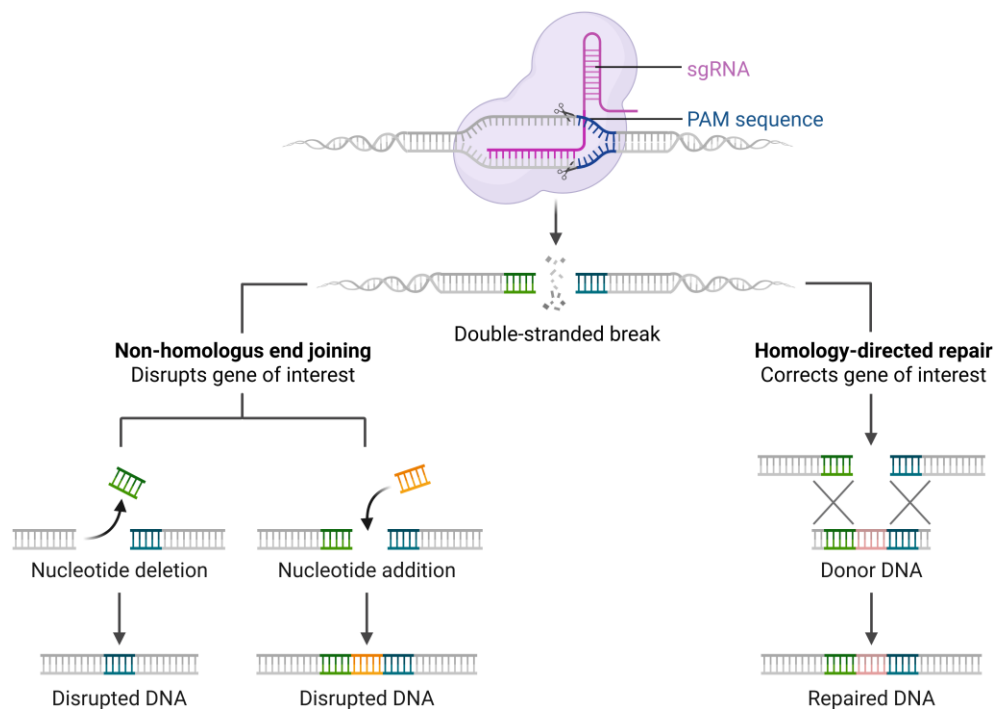


Figure 2. Schematic diagram of the CRISPR base-cutting principle. The CRISPR-Cas9 system achieves targeted genome modification via RNA-programmable DNA cleavage. The Cas9 endonuclease is directed to a specific genomic locus by a single guide RNA (sgRNA), which hybridizes with the complementary target DNA strand. Stable target binding requires the presence of a protospacer adjacent motif (PAM) sequence immediately downstream of the complementary target region. Upon sequence-specific recognition, Cas9 catalyzes a site-specific double-stranded DNA break (DSB) at the target locus.

3. Working Principles of CRISPR Molecular Detection Technology

3.1. Principles of CRISPR Molecular Diagnostics

In 2011, the teams of Makarova and Koonin, and in 2015 the team of Feng Zhang, classified Cas proteins into two major classes and six types (I–VI) according to composition and function [60–62]. Class 1 includes types I, III, and IV, in which target-sequence cleavage requires the cooperation of multiple Cas proteins. In contrast, the Class 2 systems type II Cas9, type V Cas12, and type VI Cas13 require only a single Cas protein to target the sequence of interest, making their structures

more streamlined and their operation more unified (**Figure 3**).

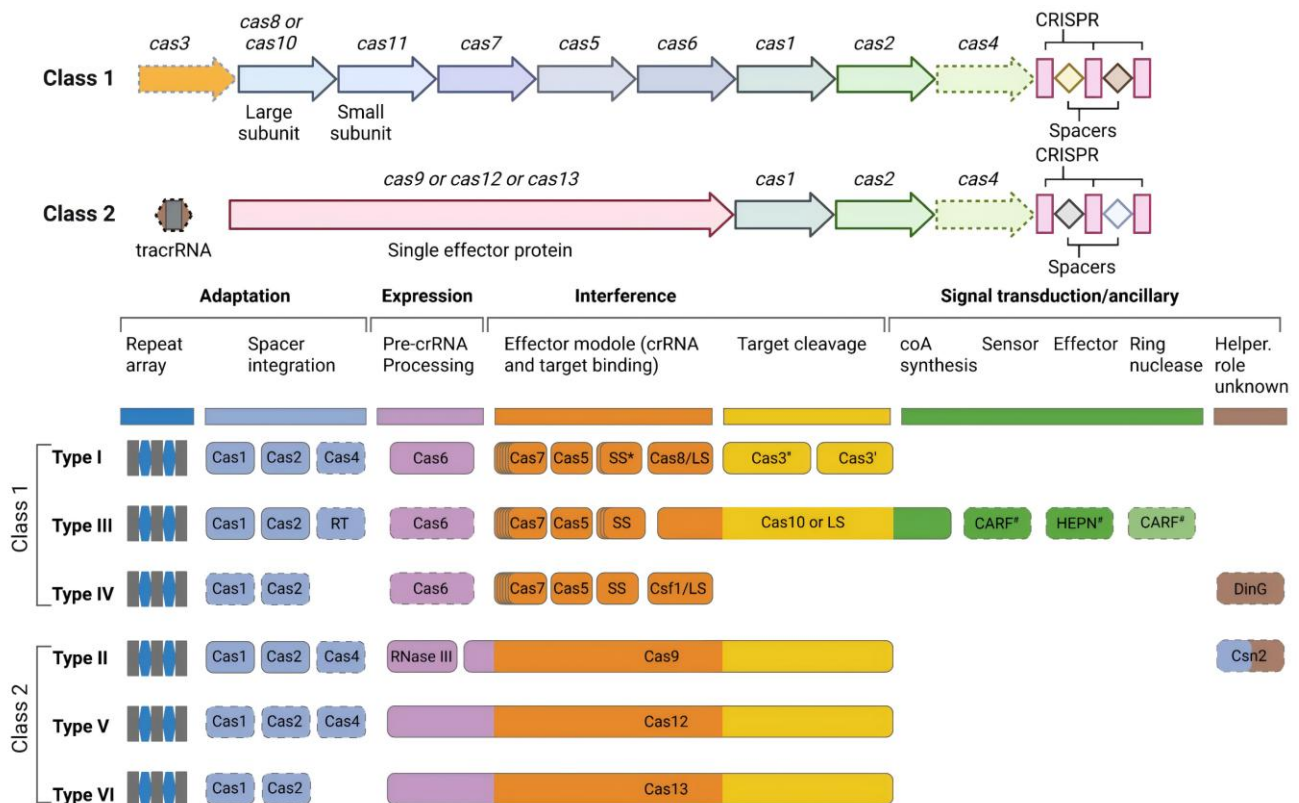


Figure 3. Schematic diagram of Cas protein classification. Class 1 systems (comprising Types I, III, and IV) employ multi-subunit ribonucleoprotein complexes for target interference. In contrast, Class 2 systems (comprising Types II, V, and VI) utilize a single, large multidomain effector protein, such as Cas9, Cas12, or Cas13. The functional cascade is categorized into distinct physiological stages: adaptation (spacer integration, predominantly mediated by the conserved Cas1-Cas2 complex), expression (pre-crRNA processing), interference (crRNA-guided target binding and subsequent nucleic acid cleavage), and ancillary signal transduction. The diversification of effector modules and stage-specific proteins forms the basis for categorizing the systems into six major types.

Although Class 2 accounts for only about 20% of CRISPR/Cas systems, it has consistently been central to scientific research and practical applications [63]. The CRISPR/Cas9 system belongs to Class 2 type II Cas systems and was the first CRISPR/Cas family system applied to gene editing [64]. The Cas9 protein contains two nuclease domains and one recognition domain. Cas9 nuclease requires both *trans*-activating (tracrRNA) and crRNA to cleave target DNA. Its cleavage activity is directed by sgRNA: once bound, Cas9 can precisely locate and cleave target double-stranded DNA (dsDNA), and this process is strictly dependent on the presence of a PAM sequence. In addition, nuclease-deactivated dead Cas9 (dCas9) retains DNA-binding ability, which makes it a core component for constructing various biosensors [65]. Because of its excellent precise cleavage of dsDNA, the Cas9 system is highly favored in genetic diagnosis and editing, particularly in genotyping. In 2023, Zhai et al. developed a CRISPR/dCas9-mediated ELISA detection method that can detect GM rice seeds within 1.5 h [66], with a detection limit of 12.5 copies/μL.

CRISPR/Cas12a (Cpf1) also belongs to Class 2 and is one of the systems most similar to Cas9. It recognizes the PAM sequence 5'-TTTV-3'. Guided only by

crRNA, this system can cleave dsDNA using the RuvC-like domain and is completely independent of tracrRNA. Cpf1 produces sticky-end cleavage products, a feature that significantly improves the success rate of targeted gene integration [67,68]. Cas12a is smaller than proteins in many other CRISPR-Cas systems and requires only a 42-nt crRNA, giving it high application flexibility [69]. In 2018, Li et al. found that after the Cas12a/ crRNA/ target DNA ternary complex forms, Cas12a carries out collateral cleavage (also referred to as *trans*-cleavage) of non-target single-stranded DNA (ssDNA). On this basis, they developed one-Hour Low-cost Multipurpose highly Efficient System (HOLMES), which uses quenched fluorescent ssDNA probes for rapid detection of target DNA and target RNA (**Figure 4**) [70].

CRISPR/Cas13a is an RNA-guided RNA editor that can specifically target and cleave viral RNA and endogenous RNA in eukaryotic cells [71–73]. This technology has shown excellent application potential in early screening and drug-resistance analysis, and its application boundary may be extended to rapid pesticide monitoring in the future. Following Cas12a, Doudna’s team identified a new Cas protein, Cas14 [74–76]. Cas14a occurs almost entirely in ultra-small archaea known as Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota, and Nanohaloarchaeota (DPANN) and can specifically cleave ssDNA. After target recognition, it activates “*trans*-cleavage” activity and indiscriminately degrades nearby ssDNA reporter molecules. Compared with Cas9 and Cas12, Cas14 has a smaller protein size, and its high-fidelity characteristics can markedly reduce off-target risk. In multiple crops, editing efficiency exceeds 60%, which facilitates the construction of multi-gene editing vectors. It has broad development prospects in infectious-disease detection and genetic mutation screening [77].

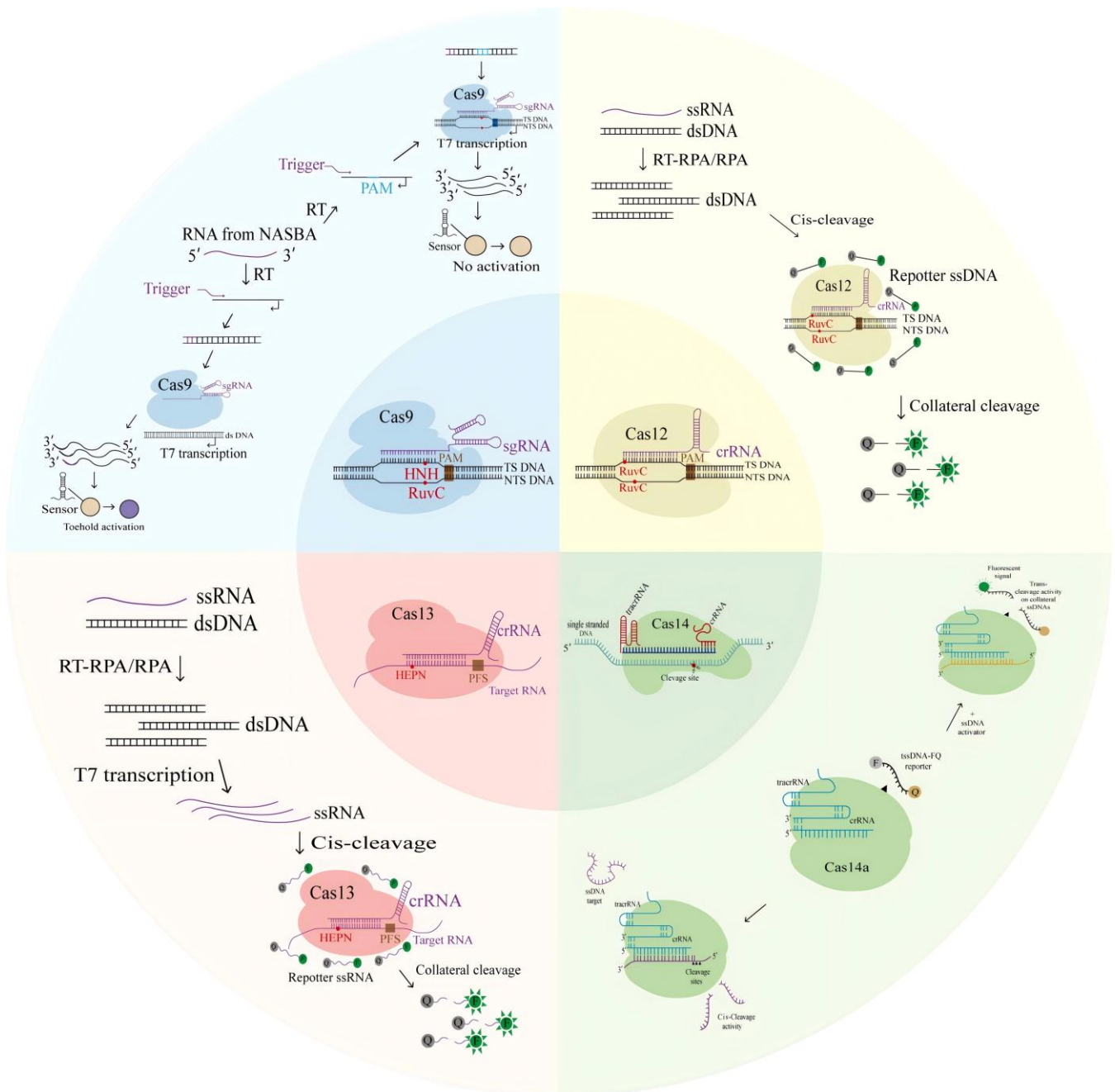


Figure 4. Nucleic acid detection technologies developed based on different Cas proteins [78]. Top left (Cas9): The diagnostic mechanism relies on PAM-dependent dsDNA recognition by the Cas9-sgRNA complex, which can be coupled with upstream amplification strategies (e.g., NASBA) and T7 transcription to trigger a downstream toehold sensor for signal readout. Top right (Cas12): Upon specific binding to a target dsDNA sequence via PAM and crRNA recognition, the Cas12 nuclease undergoes conformational activation. These triggers localized *cis*-cleavage of the target followed by robust, non-specific collateral (*trans*-) cleavage of bystander single-stranded DNA (ssDNA) reporters, generating a quantifiable fluorescent signal. Bottom left (Cas13): Activated by the specific binding to a target single-stranded RNA (ssRNA) containing a Protospacer Flanking Sequence (PFS), the HEPN domains of Cas13 initiate indiscriminate collateral cleavage of dual-labeled reporter ssRNA. Upstream isothermal amplification (e.g., RT-RPA) and T7 transcription are typically employed to enhance target availability. Bottom right (Cas14): Distinctively, the Cas14 effector recognizes single-stranded DNA (ssDNA) targets independent of a PAM requirement, subsequently inducing collateral cleavage of ssDNA fluorophore-quencher (FQ) reporters for signal amplification.

3.2. Signal Readout Methods

By coupling Cas effector proteins (Cas12, Cas13, Cas14, and others) with signal amplification and conversion approaches, including fluorescence, potentiometry, colorimetry, and lateral flow, a series of diagnostic platforms with high sensitivity, strong specificity, and low cost have emerged, opening new pathways for diversified target screening [79].

For fluorescence-based readouts, the *trans*-cleavage activity of Cas effectors (e.g., Cas12a and Cas13a) is commonly harnessed to design fluorophore-quencher (FQ) reporter systems. Upon target recognition and subsequent activation, the non-specific degradation of fluorophore- and quencher-labeled single-stranded reporters relieves quenching, generating an amplified fluorescent signal that correlates quantitatively with target abundance.

Lateral flow assay (LFA), also known as immunochromatography, is a portable device designed for on-site testing of liquid samples. In a Cas9-based LFA strategy, isothermal amplification products are introduced through biotin-labeled primers and subsequently bind specifically to avidin immobilized on the test line (T line) [80–84].

Electrochemical biosensors convert biochemical reactions into electrical signals to enable precise detection of specific nucleic acid sequences. CRISPR/Cas-based electrical-signal detection platforms often use catalytically inactive dCas9, which retains DNA-binding ability but does not cleave target nucleic acids. This makes amplification-free direct detection possible and provides high sensitivity [85]. In 2021, Ge and colleagues designed a CRISPR/Cas12a-mediated dual-mode electrochemical biosensor that enables sensitive and reliable detection of the GM soybean SHZD32-1 without PCR amplification [86].

4. Specific Applications of CRISPR Detection Technology in GM Crop Analysis

4.1. Screening and Detection of Common GM Elements

For nucleic acid detection of GM crops, common promoter elements such as CaMV35S and FMV35S are often selected according to target differences [68]. In addition to promoters, the detection scope also includes terminators such as NOS, E9'3, and T-CaMV35S; selectable marker genes such as *NptII* and *HPT*; and target genes such as *Bt*, *Bar*, *Pat*, and *EPSPS*. Moreover, specific verification of vector construction and transformation-feature analysis of insertion-site boundary sequences have become key screening steps [69,87–90]. Yan et al. established a multi-RPA technology combined with a CRISPR/Cas12a-dependent visual detection system (multi-RPA-CRISPR/Cas12a) that can simultaneously detect three GM elements: CaMV35S, *NptII*, and *Hyg*. Wang et al. developed a PCR-CRISPR/Cas12a method that can detect as few as 60 copies of the NOS terminator element in the reaction system, showing high sensitivity [91,92].

4.2. Realization of Multiplex Detection Capability

High-throughput screening for genetically modified (GM) elements increasingly demands multiplex detection capabilities to enhance efficiency, reduce turnaround

times, and lower regulatory costs [93]. However, homogeneous multiplex detection in CRISPR-based diagnostics is fundamentally constrained by the inherent non-specific collateral (*trans*-) cleavage mechanisms of Cas12 and Cas13 effector proteins. Once activated by a specific target-crRNA duplex complex, these nucleases indiscriminately cleave surrounding single-stranded reporter probes, thereby erasing target-specific information and causing severe signal crosstalk within the same reaction system.

To overcome this biophysical limitation, existing methods primarily rely on two strategies: orthogonal Cas enzymology and spatial/physical compartmentalization [94,95]. Orthogonal strategies utilize Cas proteins with distinct, non-overlapping cleavage preferences (e.g., combining ssDNA-cleaving Cas12 with ssRNA-cleaving Cas13, each coupled to a different fluorophore) to enable multi-channel readout in a single-tube reaction. In contrast, spatial compartmentalization strategies isolate individual reactions within independent microenvironments using microfluidic droplets, multi-channel lateral flow assays, or encoded solid-phase supports. In 2025, Wang et al. successfully circumvented crosstalk between reporter probes by integrating a CRISPR/dCas9 system with polymer-coated magnetic beads (MB@polymer) that were dual-encoded via physical size and Raman fingerprints [96]. By confining each target recognition event to the surface of a uniquely encoded magnetic bead, they achieved the simultaneous identification of six GM maize lines (including MON810, BT11, DBN9501, and MON89034) without cross-contamination, offering a robust solution for high-throughput regulatory testing.

4.3. Rapid On-Site Detection and Commercialization Potential

As related technologies are applied more deeply, the standardization and commercialization of CRISPR/Cas for rapid GM detection are expected to accelerate further [79]. To improve nucleic acid extraction efficiency, enhance operational safety, and meet on-site testing needs by non-professionals in non-laboratory environments, rapid nucleic acid testing technologies suitable for point-of-care testing are receiving extensive attention. Their standard workflow comprises rapid nucleic acid extraction and purification, isothermal amplification enrichment, and CRISPR-specific detection [97–101].

Notably, front-end sample preparation remains the rate-limiting bottleneck for in-field agricultural POCT. Unlike clinical biofluids, complex agricultural matrices particularly recalcitrant crop seeds and fibrous leaves are laden with polysaccharides, polyphenols, and secondary metabolites [102]. These interferences frequently co-extract with nucleic acids, quenching the enzymatic activity of isothermal pre-amplification polymerases (e.g., RPA, LAMP) and severely compromising Cas ribonucleoprotein (RNP) collateral *trans*-cleavage kinetics. To bypass laborious, equipment-dependent protocols such as cetyltrimethylammonium bromide (CTAB) extraction and high-speed centrifugation, matrix-tailored isolation strategies have emerged: (i) direct lysis formulations using alkaline, chelating, or detergent-based buffers that rapidly disrupt rigid plant cell walls while chemically passivating polyphenolic inhibitors; (ii) nanomaterial- and solid-phase-assisted platforms, including surface-functionalized magnetic nanoparticles (MNPs) and

cellulose/Flinders Technology Associates (FTA)-membrane capture strips, which enable rapid matrix clearance and nucleic acid enrichment with reduced dependence on conventional laboratory equipment [103]; and (iii) integrated microfluidic “sample-to-answer” cartridges that monolithically integrate tissue disruption, passive filtration, and cascade isothermal-CRISPR reactions within closed fluidic networks, effectively preventing aerosol contamination [104].

Coupled with addressing upstream matrix interferences, further optimization of lyophilized crRNA formulations and long-term reagent stability will be essential for field deployment [105]. With continuous engineering innovations in both reaction systems and sample-to-answer integration, CRISPR rapid on-site detection will solidify its role as a core technical solution for the field regulation of GM crops.

5. Advantages, Challenges, and Countermeasures for CRISPR-Based GM Detection Technology

5.1. Advantages of CRISPR/Cas-Based GM Detection Technology

Overall, CRISPR/Cas detection technology has significant advantages for rapid on-site testing of GM products [106–108]: (1) the workflow is simple and requires relatively little equipment and operator skill; (2) it does not depend on complex instruments, and results can be visualized; (3) the entire process can be completed within 30 min; (4) the cost is low, reducing the expense of each test; (5) sensitivity is high, with detection limits reaching the attomolar level, comparable to or even better than qPCR; and (6) the approach is highly flexible, because for different target sequences, redesigning RPA or LAMP primers or crRNA sequences can effectively reduce false-positive risk. Together, these advantages explain why CRISPR/Cas systems have become prominent in GM crop detection.

5.2. Current Challenges and Limitations

Although CRISPR/Cas detection technology is highly active in molecular diagnostics, its practical application still faces many inherent limitations [109]: (1) off-target effects may occur, because Cas proteins may cleave non-target DNA or RNA sequences with high similarity, leading to false-positive results; (2) reliance on pre-amplification steps can introduce variation; (3) CRISPR/Cas systems require complex optimization to enable multi-target detection in a single reaction, which greatly limits efficiency when screening GM organisms containing multiple transgenic elements; (4) standardized protocols and quantitative benchmarks are lacking; and (5) large-scale application remains constrained in resource-limited regions. Therefore, it is essential to develop CRISPR/Cas systems into trusted tools for routine GMO detection.

5.3. Future Development Directions

As the technology matures, CRISPR/Cas systems not only provide sensitive and rapid detection but have also achieved major breakthroughs in automation, portability, and multiplex detection [110]. Future development should focus on: (1) developing highly specific new Cas effectors and constructing high-fidelity variants

through protein engineering to improve detection accuracy; (2) optimizing stable and inhibitor-resistant isothermal amplification methods to reduce false-negative results; (3) combining multiple crRNAs with microfluidic technology to further optimize reaction conditions and signal detection and achieve higher-order multiplex detection; (4) establishing broadly recognized reference materials, validation guidelines, and quantitative models to meet requirements for reproducible, standardized experiments and safety regulation; (5) developing stable lyophilizable reagents and improving portability; (6) integrating advanced technologies with smartphone imaging, cloud computing, and artificial intelligence to achieve fully automated, user-friendly, high-throughput on-site rapid detection through data analysis; and (7) conducting cost-benefit analysis and technology transfer to transform experimental achievements into commercialized and routine agricultural biosafety tools.

6. Conclusions and Prospects

CRISPR technology continues to advance, and its application in GM crop detection is becoming increasingly recognized. Relying on advantages such as speed, convenience, high efficiency, accuracy, and low cost, point-of-care testing platforms have made considerable progress [59,111]. Detection specificity and efficiency have been further improved, PAM sequence recognition sites have expanded, the editing capacity of homologous recombination repair pathways has been enhanced, and plant tissue-culture techniques have been optimized. Nevertheless, this detection model still faces many challenges [112,113]: (1) standardized experimental procedures are lacking; (2) aerosol particles may interfere with CRISPR/Cas detection and trigger non-specific positive responses; (3) strengthening target-response intensity generally requires isothermal amplification, which indirectly increases operational burden; and (4) the technology still has difficulty achieving efficient high-throughput parallel detection of multiple components and multiple samples. By taking advantage of the extensibility of CRISPR components, integrating multiple signal acquisition schemes, and maintaining compatibility with existing commercial platforms, this technology is expected to move toward automation and standardization. Future research should examine the feasibility of applying this technology to molecular-level detection in plant production, food quality, environmental monitoring, and safety control [114,115]. At the same time, attention should focus on solving current technical limitations through technological innovation, introducing smartphone- and artificial-intelligence-assisted approaches to achieve more convenient and precise point-of-care testing, and using policy and regulatory support to promote the dissemination of related detection technologies. We believe that with continued scientific research and process iteration, the application scenarios and applicable fields of CRISPR/Cas systems will further expand, providing stronger support for crop safety regulation and the industrialization of biological breeding.

Author contributions: Conceptualization, C.Z. and R.Q.; methodology, K.H. and Y.D.; software, F.H.; validation, H.N., F.H., T.D. and C.Y.; formal analysis, K.H. and J.Z.; investigation, K.H., Y.D. and F.H.; resources, C.Z. and R.Q.; data curation, K.H.;

writing—original draft preparation, K.H.; writing—review and editing, C.Z. and R.Q.; visualization, K.H. and Y.D.; supervision, C.Z. and R.Q.; project administration, C.Z. and R.Q.; funding acquisition, C.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China, grant number 32572933.

Acknowledgments: The authors gratefully thank Prof. Pengcheng Wei from Anhui Agricultural University for his valuable guidance and constructive discussions on the molecular detection of genetically modified crops and CRISPR-based diagnostic technologies.

Conflict of interest: The authors declare no conflict of interest.

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