

Progress in On-the-Go Genetically Modified Product Identification: Transitioning from Defense Biosensing to On-Site Sequencing.

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Abstract

Transgenic technology has ushered in a new era, revolutionising life as we know it. As genetically modified products emerge and gain widespread adoption, the imperative to ensure their safety and authenticity has grown exponentially. This demand for assurance has driven the quest for faster, more precise, and easily deployable on-site detection systems. This perspective delves into the current landscape of technology, exploring the realm of portable immune biosensing, innovative portable ultrafast PCR detection devices, and cutting-edge on-site biosensors leveraging functional nucleic acids. It also sheds light on superior field detection instruments. Notably, the landscape briefly mentions on-site genetic sequencing for genetically modified organisms, an exciting frontier. This comprehensive overview showcases the current state of detection technology and provides a compass pointing towards the future of genetically modified product detection. It is an invaluable reference for researchers and developers navigating this dynamic domain.

Keywords: *genetically modified products, ultra-fast PCR, portable immune biosensors, functional nucleic acids, GMO sequencing*

Introduction

By introducing target genes, transgenic technology has proven to be a potent tool for enhancing the characteristics of host organisms and creating new, improved iterations of the source creatures. Concerns over the safety of genetically engineered products have grown in recent decades, leading to increased debate. The demand for mutagenesis testing is driven by people's growing concern over detecting whether a product has been genetically modified. This growing focus emphasises the importance of detecting Transgenic material, where protein components, nucleic acids, and metabolite concentrations may be critical markers.

The setting for testing G.M. products has significantly grown in response to this rising demand. The public's right to transparency has improved thanks to the development of more straightforward, quicker, and portable field-testing methods. The ability of antibodies to recognise and bind to proteins made from foreign genes, effectively converting chemical signals into visible forms like optical signals, has altered the identification of G.M. products. Additionally, the signal's detection, enhancement, and output are all critical for determining D.N.A. levels.[4]

The polymerase chain reaction (PCR), which requires significant equipment and time, has historically been the most reliable approach for identifying genetically altered organisms. Ultra-fast PCR, on the other hand, enables effective and speedy on-site diagnosis without compromising tolerance or specificity because of recent advances in theoretical PCR research.

Transgenic on-site screening methods have gained broader accessibility with advancements in technology and research. Notably, ultra-fast PCR improvements have played a vital role in this accessibility. Among the isothermal amplification methods, recombinase polymerase propagation and loop-mediated isothermal amplification (LAMP) have emerged as rapid detection tools.

LAMP, developed by Notomi et al. in 2000, relies on precisely designed primers binding to multiple sites on a template gene, enabling nucleic acid synthesis at consistent temperatures within 30 to 60 minutes. In contrast, recombinase polymerase amplification (R.P.A.) employs recombinase, single-stranded D.N.A. binding proteins, and strand-shifting D.N.A. polymerase, enabling exponential nucleic acid synthesis at a constant temperature in approximately 30 minutes, making it particularly valuable for swift viral genome detection.

This perspective distinguishes on-site G.M. product identification procedures from traditional laboratory processes. These streamlined techniques encompass simple sample preparation, automated data management, and straightforward result interpretation, focusing on generating rapid results in field settings. The approach involves portable immunological biological detection, transportable ultra-fast PCR detection equipment, improved field detection machinery, and surveillance sensor technology leveraging functional amino acids. It also encompasses ultra-fast amplification methods for on-site detection. The concept of on-site G.M.O. sequencing is briefly touched upon, followed by insights into challenges and potential future directions in this evolving landscape.

Portable Immune Biosensors

A practical method for improving the properties of native species is the detection of proteins produced by foreign genes in genetically altered organisms. Due to their speed, simplicity, and affordability, immunoassays are often used for the quantitative and qualitative evaluation of these proteins, making them suited for the field evaluation of fresh food.

The protein strip analysis (P.S.A.), which requires little equipment and specialised knowledge, is very straightforward. The mutually advantageous impacts of P.S.A. and PCR methods in G.M. detection were discovered by Van et al. Mutoni et al. used protein crossbands to characterise G.M. components in maise-based food products, which PCR confirmed to aid in food labelling and consumer decision-making.[5]

The development of gold colloidal immunochromatographic strips (I.C.S.) by Zeng et al., which allow for the simultaneous identification of several transgenic proteins in crops such as maise, soybeans, beetroot sugar, and cotton, is one innovation in the detection of G.M. proteins. To quantify the Cry1Ac protein in maise, Lv et al. developed a double-antibody combination immunosorbent assay with enzymes (ELISA). This invention made it possible to check and isolate transgenic products trustworthy and sensitively [6].

These developments highlight the importance of protein-based G.M. detection, which provides excellent productivity, straightforwardness, and visible evidence with exceptional accuracy. This point of view emphasises the importance of protein-based detection methods for guaranteeing the effectiveness and transparency of G.M. products.

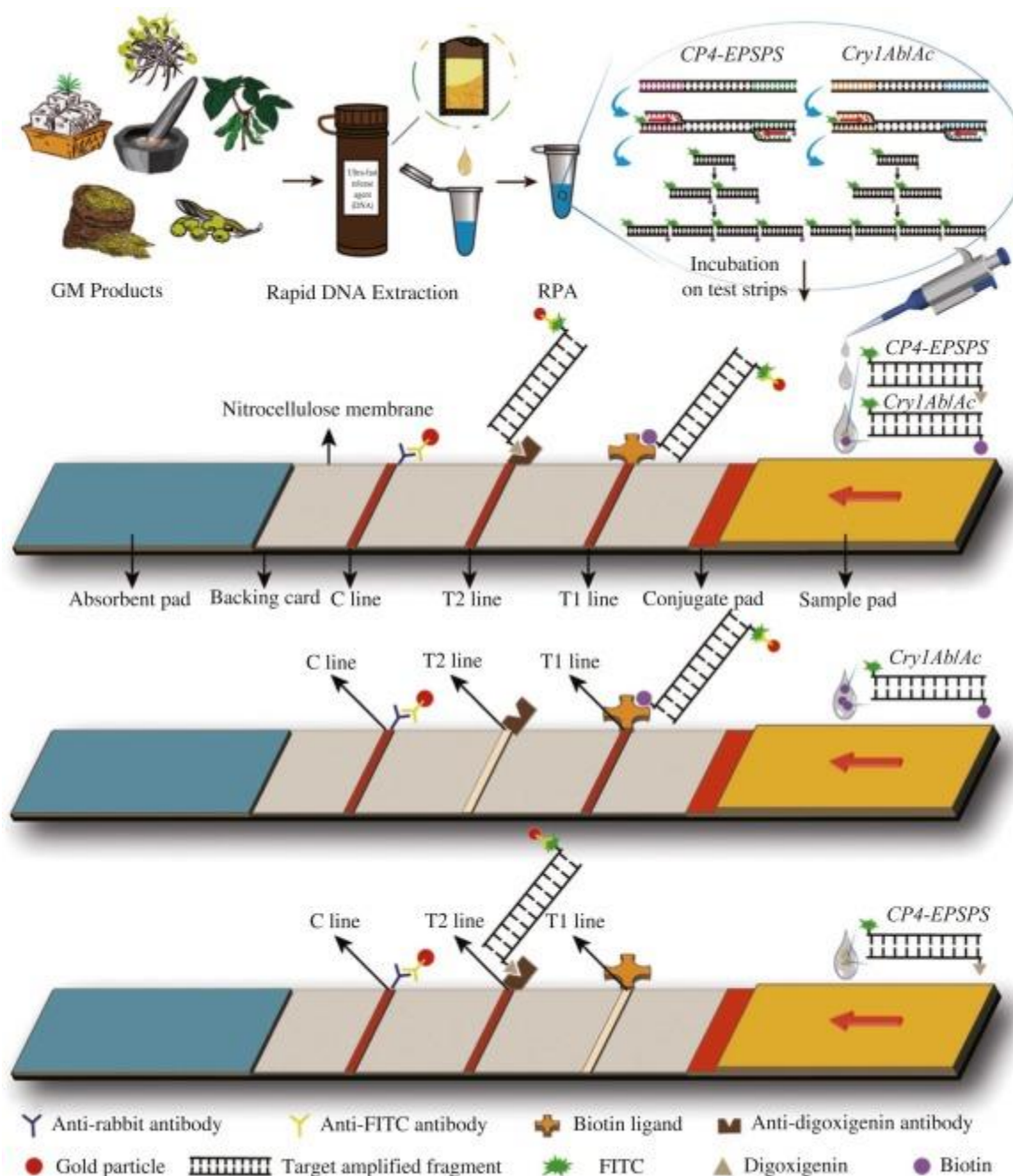


Figure 1 illustrates an immunochromatographic strip containing colloidal gold designed for concurrently detecting multiple transgenic proteins.[1]

Handheld PCR devices for rapid and sensitive pathogen identification

With continuous advancements, the PCR technique has gained renown for its capacity to detect D.N.A. quantities. The advent of ultra-fast Polymerase enzymes has notably decreased turnaround times while streamlining traditional PCR processes' once intricate and labour-intensive equipment requirements. It now allows for rapid on-site diagnostics by integrating microfluidic chips, longitudinal flow biologic sensors, and other cutting-edge technologies with ultra-fast PCR.

Gao and colleagues achieved the replication of target D.N.A. segments in under 25 seconds by combining dual hyperpolymerase store chain interaction with a comprehensive longitudinal flow bioassay (LFB) for swift visual screening of two genetically modified components. Furthermore, the LFB achieved an astonishingly low identification limit, as low as 0.05% of modified wheat, and delivered dual amplification results in less than ten minutes without expensive machinery.[7]

To swiftly identify the maize transgene MON810, Li and collaborators devised a magnetic screening strip that is both quick and highly accurate. This strip is based on the blocked stimulated PCR (BS-PCR) technique, enabling the detection and amplification of a target gene's sequence in under five minutes, significantly reducing the overall processing time. Signal translation and output, utilising ferromagnetic technology, require an additional five minutes.

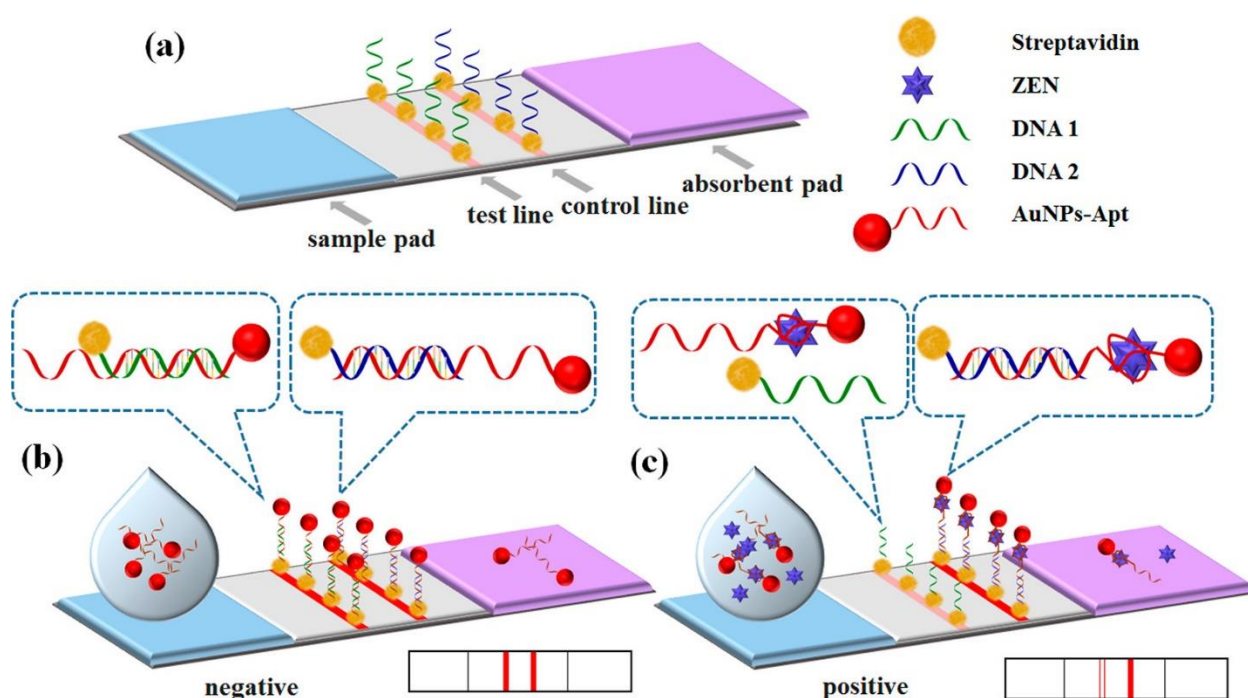


Figure 2. depicts a highly stable and rapid magnetic test strip built upon the BS-PCR technique[2].

Innovations in On-Site G.M.O. Identification

Functional nucleic acids represent diverse molecules with unique structures and specialised metabolic roles, making them viable substitutes for conventional antibodies and proteases. A typical biosensor comprises three essential components: a recognition element, a signal amplification segment, and an information presentation element. These biosensors can discern various biological components and convey diagnostic signals corresponding to varying concentrations. Functional nucleic acids, including the CRISPR/Cas system, DNAzymes, specific primers, hairpin platforms, and nucleotide aptamers, are commonly harnessed for detection applications. When applied to verify altered elements directly, these functional D.N.A. molecules can significantly enhance detection efficiency while reducing associated costs.[8]

For instance, the CRISPR/Cas system frequently interfaces with nucleic acid replication procedures. Zhang and collaborators devised a nucleotide identification method based on CRISPR/Cas12, using the *Magnaporthe oryzae* gene and the *Cry1C* gene found in the human genome, assessing its effectiveness in identifying rice diseases and G.M.O.s. They also introduced a technique for D.N.A. extraction using filter paper strips, which combined a lateral flow assay (LFA) with RPA-Cas12a. This modification renders the test suitable for on-site monitoring, as the entire test can be conducted at room temperature, eliminating the need to remove the paper filters or additional components from the LFA strip.[9]

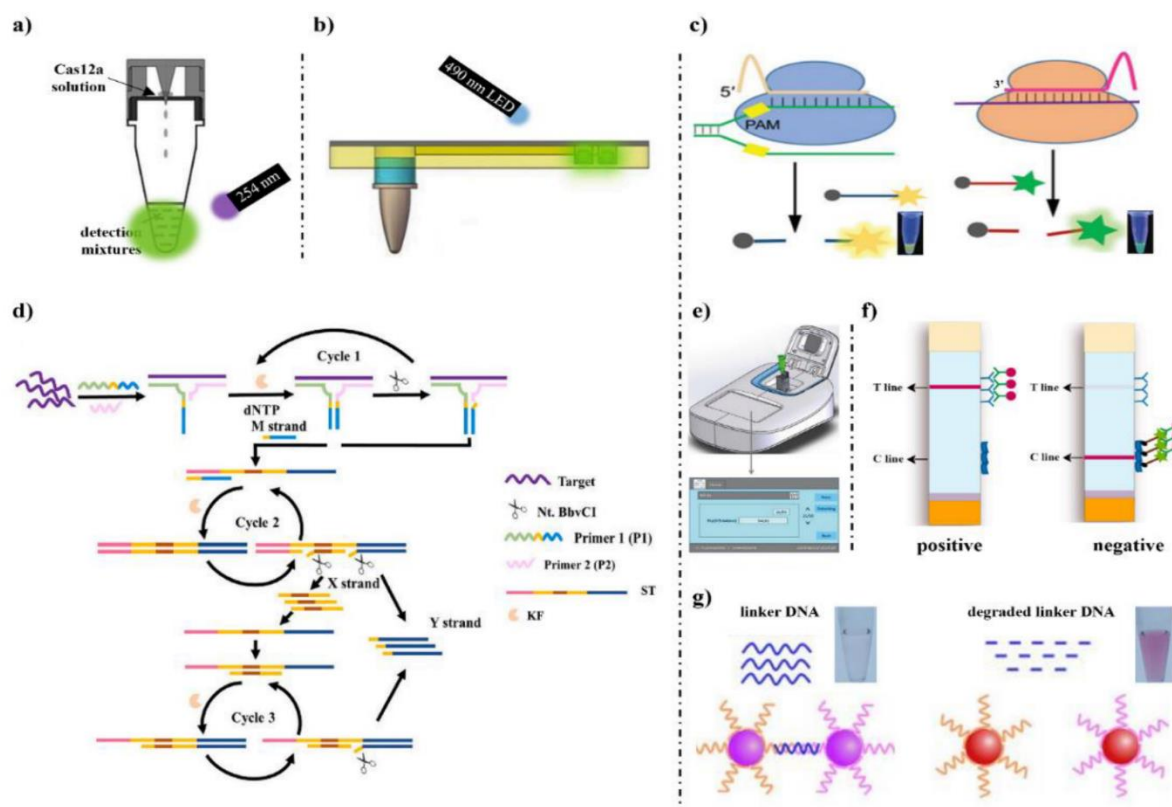


Figure 3: Nucleic acid detection platform based on CRISPR/Cas12[3]

Conclusion

The Post-Stamping Technique (PST) has played a pivotal role in G.M. product identification, much like the PCR technique's significance in nucleic acid detection. Recent advancements in nanomaterials research have revitalised conventional PST methods, harnessing their unique catalytic properties and plasmonic attributes. The integration of nanomaterials and the growing adoption of immunoassay strips have significantly elevated on-site G.M. product testing, yielding remarkable results.

Furthermore, ultra-fast PCR technology has reinvigorated the conventional "gold standard" for transgene detection. Its rapid response times allow versatile utilisation with various signal output devices to meet diverse sensing needs. Incorporating functional molecules and nucleic acid isothermal amplification technology has notably enhanced signal amplification and output methods, enabling the integration of micro platforms, microfluidics, semiconductors, and printed devices for on-site G.M. product testing.

Moreover, the development of quick on-site sequencing techniques has dramatically improved on-site genetically modified organism detection methodology. These advancements underscore the ongoing importance and promising future of on-site G.M. product detection, driven by technological progress and enhanced efficiency.

Reference

1. Wang J, Wang Y, Hu X, et al. A dual-RPA based lateral flow strip for sensitive, on-site detection of *CP4-EPSPS* and *CryIAb/Ac* genes in genetically modified crops. *Food Science and Human Wellness*, 2024, 13(1): 183-190. <https://doi.org/10.26599/FSHW.2022.9250015>
2. Aptamer-Based Lateral Flow Test Strip for Rapid Detection of Zearalenone in Corn Samples Shijia Wu, Lihong Liu, Nuo Duan, Qian Li, You Zhou, and Zhouping Wang *Journal of Agricultural and Food Chemistry* 2018 66 (8), 1949-1954 DOI: 10.1021/acs.jafc.7b05326
3. Wang, M.; Wang, H.; Li, K.; Li, X.; Wang, X.; Wang, Z. Review of CRISPR/Cas Systems on Detection of Nucleotide Sequences. *Foods* 2023, 12, 477. <https://doi.org/10.3390/foods12030477>
4. VanGuilder, H.D.V.K. Twenty-five years of quantitative PCR for gene expression analysis. *BioTechniques* 2008, 44, 619–626
5. Mutoni, C.K.; Magiri, E.; Boga, I.H.; Mugo, S.; Gichuki, S.T. Inadvertent presence of genetically modified elements in maize food products in Kenyan markets. *Afr. J. Biotechnol.* 2013, 12, 4881–4890.
6. Lv, X.; Zhou, X.; Man, Y.; Zhang, J.; Wang, P.; Liu, Y. Double-antibody Sandwich ELISA for the Quantitative Detection of CryI Ac Protein in Transgenic Plants. *Mod. Food Sci. Technol.* 2014, 30, 6.
7. Wu, H.; Zhang, X.; Wu, B.; Qian, C.; Zhang, F.; Wang, L.; Ye, Z.; Wu, J. Rapid on-site detection of genetically modified soybean products by real-time loop-mediated isothermal amplification coupled with a designed portable amplifier. *Food Chem.* 2020, 323, 126819.
8. Xu, W. A Developed Accurate Digital PCR Detection Technology in Food Safety. In *Functional Nucleic Acids Detection in Food Safety*; Springer: Singapore, 2016; Chapter 7; pp. 123–141.
9. Cheng, N.; Shang, Y.; Xu, Y.; Zhang, L.; Luo, Y.; Huang, K.; Xu, W. On-site detection of stacked genetically modified soybean based on event-specific TM-LAMP and a DNAzyme-lateral flow biosensor. *Biosens. Bioelectron.* 2017, 91, 408–416.