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The Use of DNA and Protein-Based Detection Methods in Agricultural Biotechnology

Elina Jones*

Department of Genetic Engineering, University of Sao Paulo, Sao Paulo, Brazil

***Corresponding author:** Elina Jones, Department of Genetic Engineering, University of Sao Paulo, Sao Paulo, Brazil, E-mail: chemtech@alliedresearch.org

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Description

Detection methods based on DNA and proteins are commonly used instruments for monitoring biotechnology-derived crops and their products around the world. For product development, seed production, compliance, and contractual demands, agricultural biotechnology firms, food/feed providers and supply chains, diagnostic testing organizations, and regulatory authorities rely extensively on these two technologies. DNA and protein-based detection methods are primarily used to validate the presence or absence of Genetically Engineered (GE) materials, as well as to quantify the amount of GE material contained in a product.

Several countries have passed legislation prohibiting the sale of Genetically Modified (GE) foods in national markets. Genetically Modified (GM) products are also known as Bioengineered (BE) or biotechnology-derived products. The availability of test methods capable of identifying the presence and/or concentration of GE components in food or bulk consignments of agricultural commodities such as seed and grain is critical to ensuring compliance with GE food labeling legislation. As a result, the agricultural biotechnology business uses DNA and protein-based testing methods to detect biotechnology-derived features on a regular basis. These assays detect either the novel protein or the old protein. While DNA-based technologies, such as the widely-used Polymerase Chain Reaction (PCR), are considered the “gold standard” in testing, the technology does have limitations.

For example, PCR analysis can have substantial instrumentation and consumables costs, and it requires highly trained personnel and suitable laboratory conditions. Protein-based (immunoassay) technologies encompass methods such as Enzyme-Linked Immunosorbent Assays (ELISA) and Lateral Flow Strips (LFS). Immunoassays provide a flexible format for diverse applications, offering simple, specific, and cost-effective protein detection methods. While DNA-based methods, such as the commonly used Polymerase Chain Reaction (PCR), are regarded the "gold standard" in testing, they are not without flaws.

PCR analysis, for example, can be expensive in terms of instrumentation and consumables, and it necessitates highly trained

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workers and ideal laboratory conditions. Enzyme-Linked Immunosorbent Assays (ELISA) and Lateral Flow Strips are examples of protein-based (immunoassay) technologies (LFS). Immunoassays are a versatile format that offers easy, specific, and cost-effective protein detection methods for a variety of applications.

They meet a wide range of applications; however they have drawbacks such cross-reactivity and sample processing effects that can denature the protein of interest. Both types of detection procedures are well-known and provide qualitative, semi-quantitative, and quantitative results. Quantitative detection platforms are widely employed in local and foreign testing labs. PCR is a technique for determining whether a given DNA sequence is present or absent in a biotechnology-derived plant product. PCR methods can be used subjectively to determine the presence or absence of a biotechnology-derived trait in a sample, or quantitatively to determine the amount of biotechnology-derived DNA present. Specific sequences that are unique to a biotechnology-derived trait may be detected using PCR techniques. This method is sometimes referred to as "event-specific" PCR. Multiple unique targets, such as the transgenic itself, its promoter, and/or border area sequences, may be available using DNA-based approaches. Another advantage of testing at the DNA level is the toughness of the DNA molecules themselves.

They can withstand harsh environmental conditions and retain the features that allow for long-term testing after being removed from or decaying from the organism. When testing processed food, this is especially crucial because, even if all proteins have been denatured and degraded, DNA molecules may still be present as a target for testing. The quantitative test, also known as real-time, quantitative PCR (qPCR, or rtqPCR), is performed using either absolute quantification against a standard curve or a relative calculation by comparing the transgenic DNA relative frequency to an endpoint. The semi quantitative, or sub-sampling method, which combines qualitative end-point assays with a statistical model for quantification, is another way to acquire absolute quantitative results. In general, GE testing can be classified into two categories based on the matrices that are being tested. Seed, grain, food, and feed all have their own set of market demands and technical obstacles. The sections that follow will address this variance by addressing the benefits and drawbacks of employing PCR for GE testing.

PCR and the general structure of the molecule it investigates both have a high level of selectivity. The sequence and length of the oligonucleotide primers employed determine the specificity of a PCR reaction; the more unique the sequences that the primers anneal to, the more selective and specific the assay will be. PCR allows testing at distinct and increasing degrees of specificity by constructing primers with varied stringencies. Screening approaches, which target genetic components that are common to numerous transgenic events, are at a lower level. This addresses the broad question of whether (and how much) GE DNA is present in a sample without being able to pinpoint which event(s) caused the positive result. The 35S promoter and the NOS terminator are the most prevalent targets for screening approaches. Trait-specific assays, which target the transgenic gene's DNA sequence, are the next tiers on the specificity scale. Without the analyst being able to identify the precise contributing event(s), all events carrying the same gene will result in a positive result and contribute to the quantity collected. Construct-specific assays, which target the DNA backbone of a vector that is shared by multiple events, achieve a similar level of specificity.

By probing junctions that will be different combinations of the above, various intermediate levels of specificity can be obtained. The intersection of a regulatory element and the construct backbone, or the intersection of a transgenic gene and a regulatory element or construct sequence, are examples. The event-specific assays are at the top of the specificity range; these tests target the chimeric DNA sequence at the genomic insertion site and usually amplifies a DNA segment made partially

from the transgenic sequence and partially from the flanking, native, genomic sequence, a one-of-a-kind and highly specific combination.