

POLYMERASE CHAIN REACTION TECHNOLOGY AS AN ANALYTICAL TOOL IN AGRICULTURAL BIOTECHNOLOGY

Summary of Key Findings and Industry Perspective

Preface

A group of 7 authors from Biotechnology companies, grain traders and private testing laboratories completed a manuscript describing in detail the needs and requirements for a successful application of the most widely used DNA-based detection method, the Polymerase Chain Reaction (PCR). Regulatory authorities that operate mandatory labelling schemes for food and feed heavily rely on this method. PCR is a very challenging technique, and this manuscript provides a detailed description on the issues that need to be considered for sampling and testing for the presence of GMOs in grain. The information in this manuscript can be used to educate regulators and private laboratories in order to achieve higher levels of reliability and global compatibility and comparability of testing for the presence and amounts of GMO.

Background

This manuscript addresses the application of polymerase chain reaction (PCR) technology to food biotechnology in the soybean and corn (maize) industries only, though the principles are applicable to other crops as well. PCR is one of several techniques that are employed for the detection of GM material in a product, but it is the technique that receives most attention by regulatory agencies outside North America.

A number of countries have adopted or are in the process of developing legislation related to the approval of GM products. Authorities in many countries require that DNA sequence information be provided as part of the registration package. In addition, a PCR detection method that is specific to the event may also be requested. The term "event" is used to describe a plant, and its offspring, that contain a specific insertion of DNA. Such an event is distinguishable from other events by its unique site of integration of the introduced DNA. A PCR method that can distinguish such an event from all other events is described as being "event-specific" and generally is based on the detection of a junction fragment between the original plant DNA and the introduced DNA.

Rationale and Scope of the Manuscript

The agricultural biotechnology industry applies polymerase chain reaction (PCR) technology at numerous points in product development. Commodity and food companies, as well as third-party diagnostic testing companies also rely on PCR technology for a number of purposes. The primary use of the technology is to verify the presence or absence of genetically modified (GM) material in a product, or to quantify the amount of GM material present in a product. The document highlights the many areas to which attention must be paid in order to produce reliable test results. These include sample preparation, method validation, choice of appropriate reference materials, and biological and instrumental sources of error. The article also discusses issues related to the analysis of different matrices and the effect they may have on the accuracy of the PCR analytical results. This is the first manuscript that tries to exhaustively address the many issues, starting from sampling to the interpretation of measurement results that need to be considered when applying PCR.

Conclusions

Polymerase Chain Reaction technology is often used for the detection of products of agricultural biotechnology. It is critical that such methods are reliable and give the same results in laboratories across the world. This can only be achieved by proper validation of the methods. The choice of the appropriate reference material will impact the reliability and accuracy of the analytical results. It is important that analysts pay proper attention to the effect of specific matrices on the methods. In addition, there are numerous biological and analytical factors that need to be taken into account when reporting results. This is particularly important when interpreting quantitative data.

Key Findings

- **PCR is an important, but not the only, detection method that is extensively used by all parties involved**

The agricultural biotechnology industry applies many analytical techniques at numerous steps throughout product development. These include PCR, immunoassays, and a multiplicity of other methods. The major uses of PCR technology during product development include gene discovery and cloning, vector construction, transformant identification, screening and characterization, and seed quality control. Commodity and food companies, as well as third-party diagnostic testing companies, rely on PCR technology to verify the presence or absence of GM material in a product, or to quantify the amount of GM material present in a product. Quantitative PCR technology also has been used to estimate GM copy number and zygosity in seeds and plants.

- **Careful consideration needs to go in the selection of the correct sampling procedure**

If applying PCR to test for GM material, one must carefully conduct the sampling in a manner that avoids erroneous results. When PCR-based diagnostic assays are used to test for the presence of GM material in seed or grain, a number of sampling steps occur:

1. Sampling the consignment of seed or grain to obtain the bulk sample.
2. Sampling of the bulk sample to obtain the laboratory sample.
3. Subsampling the laboratory sample to obtain the test sample.
4. Sampling the meal that results from grinding the test sample to obtain the analytical sample.
5. Sampling the DNA solution that results from extraction of the meal sample to obtain the test portion.

Each of the sampling steps has the potential to introduce error that may impact the detection of GM material at the desired threshold. As such, designing a sampling strategy that will be suitably representative requires knowledge of the particle size characteristics of the test sample and the meal, the genome size of the species in question and the Limit of Detection (LOD) or Range of Quantification (ROQ) of the analytical technique.

While it is often overlooked, the overall sampling method must be carefully designed and characterized. Particular attention should be given to understanding

the limitations of the analytical technique as it relates to the sampling plan when testing for GM material at low thresholds of detection.

- **Matrix effects determine the selection of an appropriate DNA extraction procedure**

The performance of an analytical method will vary with the nature of the sample under study. Typically, a method will be developed and validated for only one sample type or a very restricted set of different matrices. Modifications to the method may be required to accommodate other matrices, thereby creating a different method /procedure.

A prudent approach to this challenge is not to rely solely on method validation for a particular matrix or to assume all samples that are considered the same matrix will behave in exactly the same way. The detection limit will remain unknown for the any given matrix unless established by appropriate validation.

- **The applicability of PCR methods on mixed products with DNA from multiple sources needs to be demonstrated by appropriate validation**

The specificity of a chosen method with respect to the event and the crop under investigation needs to be carefully assessed and documented. Targeting genetic elements that are common to more than one event can easily lead to overestimation, especially in mixed products with multiple plant species, e.g., the 35S promoter can be found in canola, soy and corn plants and thus easily lead to overestimation if used for the determination of the amount of GMOs with respect to a specific crop only.

- **Application of qualitative PCR**

Qualitative PCR assays are used in two main ways. The first way is a simple test to determine whether the sequence in question is present in a bulk sample (usually flour or other processed material). The second way is semi-quantitative, where analysis of multiple samples from the same lot of seeds or grain can be used to estimate the number of kernels that contain the target analyte. An advantage of the semi-quantitative approach is that the method can be applied in a range that is well above the LOD (limit of detection); thus, the likelihood of false positive or false negative results can be significantly reduced. Nevertheless, care has to be taken that contamination with fragments of seed, grain, or dust does not cause false positive results.

- **Application of quantitative PCR**

Various approaches to quantification of GM material in a sample using PCR are used in different laboratories. In all cases, quantification by PCR determines the amount of GM DNA versus a reference DNA target (e.g., maize or soy DNA). This is not a direct weight-to-weight (of seed) measurement. The manuscript discusses in detail the various Real-Time PCR chemistries, as well as different approaches for standard curve generation and data analysis with their respective benefits and drawbacks. It is the responsibility of the analyst to understand the limitation of each method and select the most appropriate analytical approach.

- **Choice of reference materials**

The analyst conducting PCR to detect the presence of a GM material in seed or processed materials must make a number of decisions. A key decision is the type of reference material to use. This decision will be influenced by the availability of

reference materials and any consideration of matrix effects. In any case, each method should be validated in the laboratory using a reference material of the highest metrological standard available (SRM™ or CRM if possible). The laboratory may then use a reference material or working standard that has been calibrated back to the CRM/SRM™.

- **Method validation**

A method must be validated using the protocols and reaction conditions under which it will be performed. Changes, as well as the application to a different matrix, are likely to affect method characteristics such as the specificity or sensitivity. PCR may have a tendency to produce artefacts if operated outside the scope of the method as it was validated.

- **Biological Sources of Errors**

In determining the percent GM value for an unknown sample, the laboratory must convert the analytical result (copies of the GM gene/copies of the endogenous gene) into a percent GM value (weight to weight if required by the customer). This conversion assumes there is a direct 1:1 relationship between the endogenous control gene and the GM gene. However, there are many biological factors that can affect this 1:1 relationship and, as such, this basic assumption is not valid in many circumstances. Of most significance is the effect of biological factors on the 1:1 relationship. This effect is most pronounced in maize and wheat grains and grain products, but soybeans and cotton are not exempt from the basic physiological issues discussed below. The manuscript discusses the major factors that impact the 1:1 ratio assumptions: hybrid status, copy number, DNA degradation, DNA endoreduplication, outcrossing vs. inbreeding, effects of grain processing and variability in the genome.

- **Analytical/Instrumental Sources of Errors**

Total analytical error (or measurement error) refers to assay errors from all sources derived from a data collection experiment. The accuracy and precision of a PCR method for GM detection or quantification are subject to influences of total analytical error. Total analytical error is of paramount importance in judging the acceptability of PCR- based GM detection or quantification methods. Errors in PCR assays can be classified and are discussed as random (indeterminate) error and systematic (determinate) error.