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Adventitious Presence (AP) Testing and its Benefits to Corn Seed Production

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Adventitious presence (AP) is defined as the unintended presence of unwanted biotech traits in a seed lot. It is vital in any breeding or production program to conduct AP testing at the proper stages, with quality samples, statistically significant sample sizes and for appropriate targets to ensure the highest quality product. This paper outlines the basic steps required to design a robust and informative AP testing program.

Sample Collection

Proper sample collection is critical to producing quality test results. Care should be taken to ensure that a seed lot to be tested is not accidently contaminated with seed (partial or whole) from other sources (e.g. bin carryover), and that the sample collected is representative of the entire seed lot. Refer to the AASCO *Handbook on Seed Sampling*[1] for proper sampling procedures.

If leaf tissue is being harvested for testing, contact BioDiagnostics (BDI) for specific instructions on proper sampling.

Sample Size

The number of seeds to be tested is determined by the size of the seed lot and the acceptable threshold for contamination. For production lots where seed is not limited, refer to Table 1 to determine sample size. For breeder seed or very small lots, no more than 10% of the seed should be tested.

The whole sample may be randomly sampled into smaller seed pools at BDI. Each pool will be extracted and tested individually. The number of pools to be tested is determined by the acceptable contamination threshold and desired confidence level. Confidence level is the percent likelihood that contamination in a lot is below the acceptable threshold listed. A higher confidence level requires testing a greater number of seeds. 95% confidence is standard unless otherwise specified by the customer.

Target Selection of Biotech Contaminants

The selection of targets to test for is determined by three factors:

1. Is the seed lot conventional or trait-containing?
2. If trait-containing, which trait provider's technology has been used in the production or breeding program?
3. Is it important to know the specific identity of the contaminant, or just that a contaminant is present?

For conventional seed, testing for the Cauliflower Mosaic Virus 35s promoter (CaMV 35s) and *A. tumafaciens* NOS terminator (NOS) will detect every commercially available trait combination as of the 2012 growing season.

Hybrid or inbred seed containing traits should be tested for every event from each trait provider whose technology is being licensed (Table 2). The number of available traits is expanding each year; contact BDI for a current list or visit our website at www.biodiagnostics.net.

Testing Methodologies

Semi-Quantitative PCR (sqPCR)

In most cases, semi-quantitative PCR is the method of choice for AP detection and quantification in seed and leaf tissue. sqPCR provides a qualitative (positive or negative) result on a number of individual pools for each sample. A pool is a subsample of the seed submitted for testing and pool size is usually determined by specific program requirements and capabilities. The statistical software program SeedCalc is then used to obtain an estimate of the contamination level and an upper contamination range limit for the specified confidence level based on the number of positive and negative pools. SeedCalc is available free of charge from the International Seed Testing Association (ISTA) on their website at www.seedtest.org/en/content---1--1143.html.

Quantitative Real-Time PCR (qPCR)

qPCR is also performed at BDI. qPCR uses known standards to plot a curve and quantify unknowns based on the number of DNA copies present for the target.

Strengths and Limitations of each Method

Each PCR method has advantages and disadvantages. sqPCR can give a better estimate of contamination by directly detecting the number of pools with the contaminant(s) without regard to zygosity or number of copies per genome. sqPCR does require at least one negative pool in order to estimate AP level. In the event that all pools test positive, an estimate of the contamination and an upper limit are not possible using sqPCR.

qPCR does not require a negative pool in order to estimate AP level, but testing can be complicated by zygosity or number of copies per genome. Homozygous contaminants or seeds with multiple copies of a target can lead to a higher estimate of the contamination than an estimate from sqPCR. qPCR results may also be affected by the efficiency of the reaction which can result in more ambiguous or intermediate results than would testing by sqPCR.

Conclusion

A strong AP testing program should be an integral component of any breeding or production program. AP testing can save time and money by identifying the presence of unintended biotech traits early in the breeding or production program. AP testing can also be utilized as a powerful marketing tool. An AP testing program will provide customers with tangible evidence of a quality product and can be a significant competitive advantage over programs that do not verify product integrity through AP testing.

The experts at BioDiagnostics will help design a testing program that will meet the specific needs of your business including when to test, at what level to test and for which targets to test. Please contact the DNA QA Laboratory Manager at 715-426-0246 for further information or assistance.

Table 1. Determination of sample size requirements

Acceptable Contamination Threshold (Customer-Defined)	Sample Size Required (95% Confidence)	Sample Size Required (99% Confidence)
0.75%	400	600
0.50%	600	1000
0.30%	1000	1600
0.20%	1500	2400
0.10%	3000	4400

Table 2: Assay Selection by trait provider

Monsanto	Syngenta	Dow	Bayer
MON 810	Bt11	DAS 59122-7	T25
MON 863	GA21	TC1507	
MON 88017	MIR604		
MON 89034	MIR162		
NK603			

[1]Association of American Seed Control Officials, *Handbook on Seed Sampling*, 2006