

## **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 transgenic events or 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 accidentally 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*<sup>1</sup> 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 divided 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. tumefaciens* NOS terminator (NOS) will detect every genetic event released as of the 2009 growing season except MON89034.

Hybrid or inbred seed containing traits should be tested for each event or gene available from the 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](http://www.biodiagnostics.net).

### **Testing Methodology: Semi-Quantitative PCR (SQ-PCR) vs. Quantitative (Real-Time) PCR**

In most cases, semi-quantitative PCR is the method of choice for AP detection and quantification in seed and leaf tissue. SQ-PCR 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 specified by the technology provider. 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](http://www.seedtest.org/en/content---1--1143.html).

Quantitative (Real-Time) PCR testing is also performed at BDI. Q-PCR uses known standards to plot a curve and quantify unknowns based on the number of DNA copies present for the target.

Each PCR method has its advantages and disadvantages. Semi-quantitative PCR 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. SQ-PCR 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 SQ-PCR.

Quantitative PCR 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 SQ-PCR. Q-PCR results may also be affected by the efficiency of the reaction which can result in more ambiguous or intermediate results than would testing by SQ-PCR.

## 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 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 Laboratory Manager or Supervisor at 715-426-0246 for further information or assistance.

**Table 1. Determination of sample size requirements**

Acceptable Contamination Threshold—Customer Defined	Sample Size Required for 95% Confidence Level	Sample Size Required for 99% Confidence Level
0.0–1.0%	400	600
0.0–0.5%	600	1000
0.0–0.3%	1000	1600
0.0–0.2%	1600	2400
0.0–0.1%	3000	4600

**Table 2. Assay selection by trait provider. These tests are only available to licensees of each trait provider.**

Monsanto*	Syngenta	Dow
Cry 1Ab or MON810	MIR604	Herculex™ (TC1507)
Cry 2Ab	Bt11	Herculex RW™ (DAS 59122-7)
Cry 3Bb or MON863	GA21	
CP4 or NK603		
GA21		
Pat		
Bar		

\*This combination of targets will detect all commercially available Monsanto traits including 88017 and 89034 but may not specifically identify them by event.

<sup>1</sup>Association of American Seed Control Officials, *Handbook on Seed Sampling*, 2006.

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