Development of ICP-OES Determination of Phosphorus as a Primary Measurement Tool for Quantitation of Plant Deoxyribonucleic Acid (DNA)

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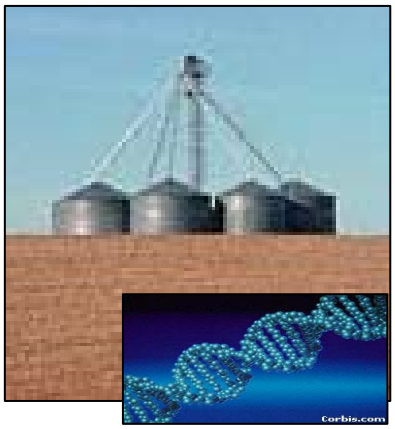
The successful detection and quantitation of biotech crop material in grain or food is highly dependent on the acquisition of pure and non-degraded DNA in a quantity that is appropriate for the limits of detection and quantitation of the measurement methods. The most important measurements, related to US export of biotech commodity crops and prepared foodstuffs, are the ones relevant to the detection of trace amounts of biotech material. Thus the amount of DNA that is used for the detection becomes critical. Laboratories currently use spectroscopic methodologies to quantitate DNA preparations, for example, DNA absorbance at 260 nm or fluorescent-dye binding. The values obtained can be seriously compromised by impurities in the DNA preparations or the state of the DNA itself.

CSTL researchers develop primary methods that provide accurate and traceable measurement of total plant DNA.

The primary methods developed will support the development of Standard Reference Materials for the calibration and validation of plant DNA measurements using other methods that are appropriate for testing and research laboratories. In this project the primary measurands are phosphorus and the four nucleotide bases that comprise DNA. A substantial effort has been directed during FY04 toward the development of a high-performance inductively-coupled plasma optical emission spectrometric (HP-ICP-OES) method for determining the total mass of phosphorus present within a given sample of DNA. HP-ICP-OES employs a clever experimental design, a well-chosen internal standard, and an innovative drift correction technique to enable expanded uncertainties on the order of a few parts per thousand. A methodology for measuring phosphorus has been developed, and several determinations using phosphorus spectrometric solution standards as 'mock' DNA samples have been demonstrated. As a more realistic test, a sample of corn DNA has been successfully digested, and suitable phosphorus spectra have been acquired. Quantitation of real DNA samples using the HP-ICP-OES approach is forthcoming.



DNA quantitation plays an important role in commerce, for example it provides a basis for the detection of biotech crop material commingled with conventional crops.

A remaining challenge concerns instabilities within the microconcentric nebulizer that is required for the combination of high sensitivity and small sample volumes. High Performance Liquid Chromatography (HPLC) is a second unrelated technique for the quantitation of phosphorus. The team has developed a suitable digestion protocol to release phosphorus and measured the phosphorus mass in DNA preparations. This methodology will provide an independent validation of the phosphorus content. Nucleotide analysis is the other critical component. This analysis is accomplished using both gas chromatography and liquid chromatography coupled with mass spectroscopy. These investigations have shown that DNA from corn kernels and soybeans responds differently to the digestion and analysis protocols that work with human DNA. Work is continuing to find the best protocol suitable for use with plant DNA.

The importance of this work has been highlighted recently in experiments we conducted to compare dye binding properties of plant DNA with that of animals and microbes. Significant differences in response were observed with two commonly used fluorescent dyes which highlight the inappropriate use of mammalian and microbial DNA as calibrants for plant DNA measurements and the necessity of new plant DNA Standard Reference Materials.