Highly Accurate Measurement of Plant Genomic DNA to Improve Genetic Identity Testing of U.S. Export Grains

NIST has developed a High Performance Inductively Coupled Plasma – Optical Emission Spectroscopy (HP-ICP-OES) method for the measurement of phosphorus content of acid-digested DNA. This is a more accurate method for quantitating the amount of DNA that goes into PCR-based genetic identity assays. This work in expected to improve on inter-laboratory variability, making it easier to assure compliance with export regulations for commodity grain trade.

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Commodity grains, developed using techniques of modern biotechnology, are prominent in the current U.S. agricultural sector. Export of raw and processed foods, especially those containing corn and soy, often need to be tested for compliance with regulations of importing countries. Accurate testing for identification significantly impacts U.S. trade in agricultural products. While international harmonization of sampling and testing procedures is as yet unrealized, improvement in the analysis of commodity grains is a goal that can be moved forward.

NIST has developed a new and more accurate method for determining the amount of plant DNA tested for genetic purity. This work is expected help assure the genetic integrity of U.S. agricultural products through more accurate identity testing.

DNA measurements are the methods of choice for identification and analysis of the biotech crop components. These methods, particularly quantitative real-time PCR (Q-PCR), are highly sensitive, and it is now possible to achieve the regulated requirements for limits of quantitation which are often quite low (<1% w/w). While Q-PCR is a powerful technique, it is imperative that an appropriate amount of template DNA is added to the assay so that trace detection is possible. It is also important not to overload the template in the reaction as impurities co-isolated with the DNA often interfere with the amplification reaction.

FILE-Mature corn awaits harvest in a field near Eureka, Walla Walla County, Wash. (Washington State University Photo/Terence L. Day 1998.)

Therefore, quantitation of total genomic plant DNA is important, prior to conducting quantitative PCR studies to determine that presence and the amount of transgenic material. However, quantitation of genomic DNA isolated from plants is also problematic as there is often a discrepancy between data obtained using common spectroscopic methods for detection. Therefore, we have been exploring other methods for quantitation of total DNA in order to certify genomic DNA reference materials and investigate spectroscopic inconsistencies. This year, we have investigated the use of High Performance Inductively Coupled Plasma – Optical Emission Spectroscopy (HP-ICP-OES) for the measurement of phosphorus content of acid-digested DNA. It was determined that measurement of DNA can be achieved with excellent accuracy and very high reproducibility using this method.

This protocol is now being applied to the analysis of maize and soy genomic DNA isolated from seeds. The methodology requires that impurities be removed from the DNA prior to analysis as biological systems contain many phosphorus compounds, some of which might co-purify with DNA. Prior to HP-ICP-OES analysis, DNA is cleaned using a series of enzymatic treatments and extraction steps to remove residual proteins and carbohydrates, and then washed to remove small molecules. It is important to note that cleaning the DNA can damage the integrity of the

molecule leading to small fragments or even single stranded DNA which can have an effect on the spectroscopic measurements. Therefore, it is necessary to analyze the integrity of the DNA following these steps.

With the development of these procedures, we have produced a validated independent measure of the total DNA content making it possible to certify pure genomic DNA reference materials. We are currently investigating the accuracy of commonly used spectroscopic methodologies for quantitating plant genomic and other DNAs.

Publications:

"Traceable Phosphorus Measurements by ICP-OES and HPLC for the Quantitation of DNA" MJ Holden, SA Rabb, YB Tewari, MR Winchester; *Anal. Chem*, Web release 1/10/07.