

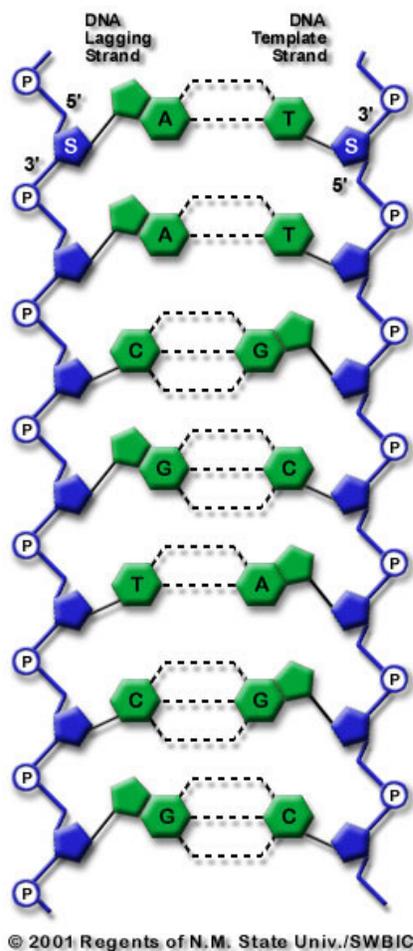
## Development of Primary Methods for Determining the Mass of Nucleic Acids

The accuracy of measurements done on DNA and other nucleic acids is dependent on the amount and quality of the DNA used in the measurements. This is the case for determinations of the amounts of biotech crop material in a grain shipment or food, forensic identification, medical diagnosis, and disease monitoring. Since current methods have serious limitations, NIST has developed a new primary method of measuring the amounts of nucleic acids, using phosphorus as the measurand.

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The most common methodology to determine the amounts of nucleic acids in solution is to measure the absorbance at 260 nm. Isolation of nucleic acids from biological material frequently leaves impurities that interfere with accurate absorbance measurements of the amount of DNA. Dyes that fluoresce upon binding single- and double-stranded DNA are more sensitive than absorbance of UV light, but the accuracy is also limited by contaminants. Thus, our project goal is the development of a new primary method of measuring the amounts of nucleic acids. We have selected phosphorus as the measurand because the measurement of the amount of phosphorus can be used to quantitate any type of nucleic acid, e.g., from individual nucleotide monophosphates, oligo-nucleotides, RNA, and DNA. Also, this approach yields results that are directly traceable to the SI.

The methods selected are Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) and ion chromatography (IC). ICP-OES is a highly accurate and precise tool for the measurement of phosphorus. The NIST-developed high-performance ICP-OES method employs an internal standard and an innovative drift correction technique.



The NIST-developed primary method based on phosphorus measurements will improve the quality of the measurements that impact on the trade of biotech commodity crop materials.

The major advantage of the IC method is that, unlike ICP-OES, one can work with small amounts of solution. We have compared ICP-OES and IC methodologies traceable to a SRM for phosphorus and found good agreement between these two quite different methods. Analysis has been done on a variety of nucleic acids. We have also entered into collaboration with the Korean Research Institute of Standards and Science on measurements of nucleotide monophosphates.

Since the ICP-OES method is very accurate and precise, we continue to work on reducing the amount of sample required for ICP-OES, the current major disadvantage of the technique. Biological materials contain many phosphorylated compounds. Each type of nucleic acid, isolated from biological material represents a challenge to provide pure nucleic acid material for analysis so that the measured phosphorus mass is representative of only the nucleic acids. Work has gone into preparation and monitoring of the purity of the nucleic acid preparations. We have also determined appropriate digestion procedures to be implemented prior to ICP-OES and IC. In some cases these measurements can be complemented by nucleotide analysis. But with some nucleic acid types, it is difficult to achieve adequate digestion without altering the individual nucleotide components.



The primary methods validated at NIST will be used to certify purified nucleic acid SRMs. We plan to quantitate genomic DNA from various plant sources and then document the exact nature of their differing responses to spectroscopic methods.