Application note

Detection and Avoidance of Polysaccharides in Plant Nucleic Acid Extractions

NanoDrop Spectrophotometers

Introduction

Spectrophotometric measurement of nucleic acids is common, both to assess purity and to quantify DNA or RNA. By measuring the absorbance at 260 nm, it is possible to calculate nucleic acid concentration. Absorbance at other wavelengths, particularly 280 nm and 230 nm, may be used as a measure of the sample's purity by calculating the ratio of the absorbances at these wavelengths to the absorbance at 260 nm. Because proteins have an absorbance maximum at 280 nm, the A260/A280 ratio may be used to assess protein contamination: a ratio of less than 1.6 is commonly considered "poor", while ratios above 1.8 are considered "good". Similarly, because many polysaccharides absorb at or around 230 nm, the A260/A230 ratio may be used for their detection: ratios of 1.6 or less are considered "poor", while ratios above 1.9 are considered "good".

The A260/A230 ratio is of special importance for plant nucleic acid extractions because plants typically have high polysaccharide contents. In addition to cell wall material and sugar or starch content, plant cells contain a vast array of polysaccharides, which have been shown to inhibit the DNA polymerase in PCR and next-generation sequencing library preparation when co-precipitated with nucleic acids, preventing a successful reaction.[1,2]

Generally, plant nucleic acid extractions begin with tissue homogenization, either in buffer or using liquid nitrogen prior to adding buffer. The extraction buffer is a key component of the procedure, with each ingredient playing a specific role. The addition of sodium chloride to the buffer helps to prevent polysaccharides from precipitating following the addition of alcohol. Polysaccharides are therefore discarded in the supernatant following nucleic acid precipitation and centrifugation, resulting in DNA pellets with low polysaccharide contamination.[3,4]

In this study, the effect of salt concentration in extraction buffer on the polysaccharide content of the purified nucleic acids is demonstrated. By examining the A260/A230 purity ratio using a Thermo Scientific™ NanoDrop™ One/One^{C} Spectrophotometer, polysaccharide contamination was determined as being dependent on both the plant species used and the salt content of the extraction buffer. The NanoDrop One/One^{C} spectrophotometer uses a variable pathlength to determine concentration and purity using only 1-2 µL sample volumes.

Experimental Procedures

The DNA extraction method is a modification of the Invitrogen™ DNAzol™ Reagent protocol.[5] For isolation of RNA, 0.25 volumes of 10 M LiCl should be substituted for isopropanol in step 7. Nucleic acids were extracted from cucumber (Cucumis sativus) samples using the following procedure:

1. Two extraction buffers were formulated:
    - High salt buffer: DNAzol (Invitrogen, 10503027), 1% PVP-40, 1.5 M NaCl
    - Low salt buffer: DNAzol, 1% PVP-40, 0.5 M NaCl
2. Plant tissue (95 – 105 mg) was placed in a 1.5 mL microcentrifuge tube and mixed with 300 µL extraction buffer (High Salt and Low Salt buffer separately).
3. Samples were sonicated using a Branson Sonifier at 70% amplitude for 30 seconds to grind plant tissue and release cellular contents.
4. Chloroform:isoamyl alcohol solution (24:1, 300 µL) was added and samples were incubated at 25°C for 5 minutes.
5. Samples were vortexed at 12,000 rpm for 10 minutes and the top aqueous layers were transferred to fresh 1.5 mL microcentrifuge tubes.
6. Steps 4 and 5 were repeated.
7. Nucleic acids were precipitated using an equal volume of isopropanol and then incubated at room temperature for one hour.
8. Nucleic acids were pelleted by centrifugation at 12,000 rpm for 10 minutes.
9. Pellets were washed twice with 1.0 mL 70% ethanol and centrifuged at 12,000 rpm for 5 minutes.
10. Pellets were dried and resuspended in 30 µL tris-EDTA pH 8.0.
11. Nucleic acid concentrations and purity ratios were determined using 2.0 µL on the pedestal of a NanoDrop One^{C} spectrophotometer and the dsDNA application.

Results

There were no great differences in nucleic acid concentrations between the high salt and low salt buffers, but the A260/A230 purity ratios were markedly lower using the low salt buffer (Figure 1).

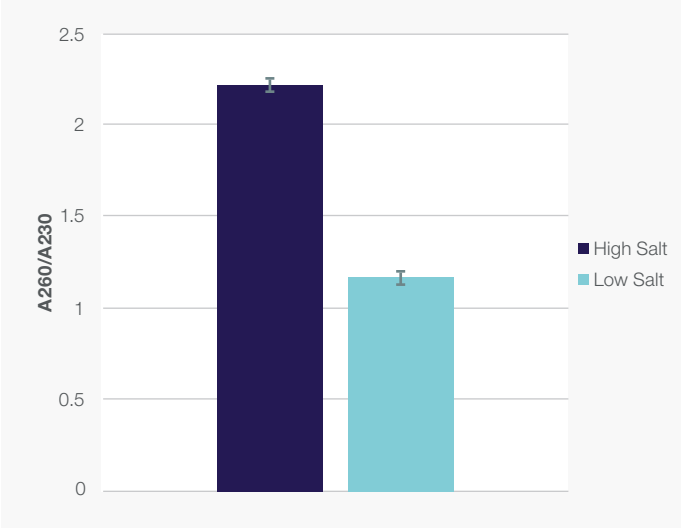

Figure 1. A260/A230 purity ratios of extracted nucleic acid samples from cucumber. Extractions were performed using both high salt buffer (1.5 M NaCl) and low salt buffer (0.5 M NaCl). For each bar, n=7; error bars represent ± standard deviation.

Examination of the absorbance spectra confirmed this observation (Figure 2). Although the absorbance peak for nucleic acids was consistently at 260 nm, the trough normally observed at 230 nm shifted to approximately 240 nm as a result of the increased sample absorbance at 230 nm using the low salt buffer for extraction.

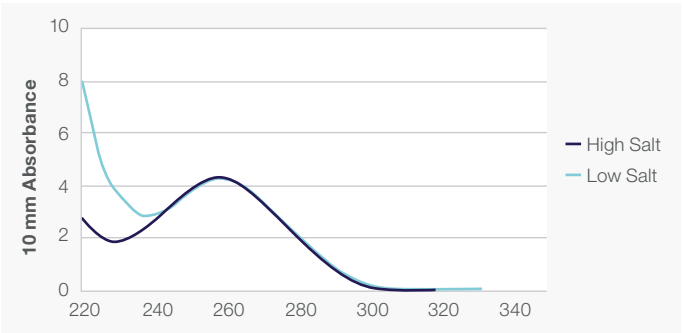

Figure 2. Absorbance spectra of cucumber nucleic acids extracted using high salt buffer (dark blue) and low salt buffer (light blue).

Conclusions

The presence of polysaccharides in extracted plant DNA is a common concern for plant molecular biologists as the polysaccharides inhibit reactions in PCR and NGS.[1,2] However, the presented data show that in many cases this can be averted with the use of increased salt concentrations in extraction buffer.[3,4]

The nucleic acid concentrations measured were approximately three times greater than what is measurable using a cuvette-based spectrophotometer. An advantage of microvolume quantification using NanoDrop spectrophotometers is the use of multiple pathlengths, automatically selecting the most appropriate. By doing so, the NanoDrop One/One^{C} spectrophotometer is capable of measuring dsDNA samples up to 27,500 ng/µL, where a cuvette-based spectrophotometer would require extensive sample dilution.

Although the automatic calculation of purity ratios by NanoDrop spectrophotometers is very convenient, it is important to view the sample spectra as other contaminants, such as phenol and guanidine, are also detectable. The large dynamic range and low volume requirements, coupled with the automatic calculation of purity ratios and display of spectra, make the NanoDrop One/One^{C} spectrophotometer an ideal instrument for the analysis of extracted nucleic acid samples.

References

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