Application Note 52645

Detection and Avoidance of Polysaccharides in Plant Nucleic Acid Extractions

Key Words

NanoDrop Spectrophotometers, Contamination, DNA, Nucleic Acids, Plants, Polysaccharides, Proteins, RNA

Introduction

Spectrophotometric measurement of nucleic acids is common, both in order to quantify the DNA or RNA, and also to assess purity. By measuring the absorbance at 260 nm, it is possible to calculate nucleic acid concentration. Absorbance at other wavelengths, particularly 280 and 230 nm, may be used as a measure of purity of the sample 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 A_{260}/A_{280} 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 230 nm, a A_{260}/A_{230} ratio may be used for their detection: ratios of 1.6 or less are generally considered poor, while ratios above 1.9 indicate very little polysaccharide contamination.

The A_{260}/A_{230} ratio is of special importance to plant molecular biologists because plant samples typically have relatively high polysaccharide contents. In addition to cell wall material and sugar or starch content, plant cells contain a vast (and often species specific) array of polysaccharides. Removal of these has led to many laboratories developing species-specific protocols. Generally, plant nucleic extractions begin with tissue homogenization, either in buffer, or using liquid nitrogen before the addition of buffer. The extraction buffer used 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.[1,2]

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 A_{260}/A_{230} ratio using a Thermo Scientific™ NanoDrop™ 2000 spectrophotometer, polysaccharide contamination was determined as being dependent on both the plant species used and the salt content of the extraction buffer. The NanoDrop 2000 spectrophotometer uses a variable pathlength to determine concentration and purity using only 1–2 µL samples.

Experimental Procedures

The extraction method used here is a modification of a previously published method, with volumes scaled down and both DNA and RNA extracted and measured simultaneously.[3] For isolation of pure DNA, a RNase step is necessary after extraction; for isolation of RNA, 0.25 volumes of 10 M LiCl should be substituted for the isopropanol in step 7.

Plant samples were obtained for both potato (*Solanum tuberosum* cv. Russett) and cucumber (*Cucumis sativus*), as these are commonly considered to have high and low carbohydrate contents, respectively. Nucleic acids were extracted using the following procedure:

1. Plant tissue (95–105 mg) was placed in a 1.5 mL microfuge tube and frozen in liquid nitrogen.
2. Tissue was ground to a fine powder using a micropestle (pre-chilled in liquid nitrogen). Care was taken to avoid samples thawing.

Thermo SCIENTIFIC

3. Prewarmed (60 °C) extraction buffer (2% SDS, 1% PVP-40, NaCl*, 25 mM EDTA, 0.2 M Tris pH 8.0, 2% β-mercaptoethanol) was added (500 µL) and the sample further ground to ensure thorough mixing.
4. Chloroform:IAA (24:1, 500 µL) was added and samples vortexed at maximum speed for 30 sec.
5. Samples were centrifuged at 13,000 × g for 5 min; supernatants were then transferred to fresh 1.5 mL tubes.
6. Steps 4 and 5 were repeated.
7. Nucleic acids were precipitated using an equal volume of ice-cold isopropanol and incubation at –20 °C overnight.
8. Nucleic acids were pelleted by centrifugation 13,000 × g for 15 min, pellets were then washed using 1 mL 70% ethanol.
9. Pellets were dried and resuspended in 50 µL dH_2O.
10. Nucleic acid concentration and purity ratios for the re-suspended samples were determined using a NanoDrop 2000 spectrophotometer.

** Low Salt Buffer = 0.5 M NaCl; High Salt Buffer = 1.5 M NaCl.*

Results

For each combination of plant species and extraction buffer, three nucleic acid samples were extracted and measured. Although there were no great differences in nucleic acid concentrations between samples, the A_{260}/A_{230} ratios were markedly lower in the potato nucleic acid samples extracted using the Low Salt Buffer than in any of the other extracted nucleic acid samples (Figure 1). Examination of the absorbance spectra confirmed this observation: although the peak of absorbance for nucleic acids was consistently at 260 nm, the trough normally observed at 230 nm shifted to approximately 240 nm (Figure 2) as a result of the increased sample absorbance at 230 nm.

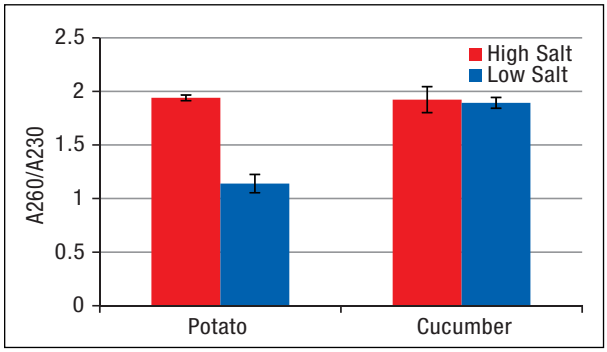

Figure 1: A_{260}/A_{230} ratios of extracted nucleic acid samples from potato and cucumber. For each species, extractions were performed using both High Salt Buffer (1.5 M NaCl) and Low Salt Buffer (0.5 M NaCl). For each bar, n = 3; error bars represent standard deviations.

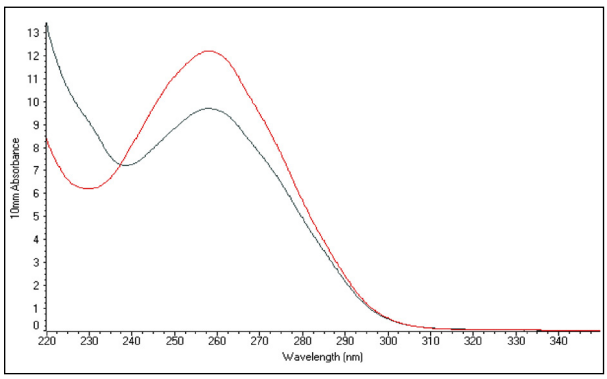

Figure 2: Examples of absorbance spectra of potato nucleic acids extracted using High Salt Buffer (red) and Low Salt Buffer (blue).

Conclusion

The presence of polysaccharides in extracted plant DNA is a common concern for plant molecular biologists, however the data presented here and elsewhere show that in many cases this can be averted with the use of increased salt concentrations in extraction buffer.[1,2] The fact that A_{260}/A_{230} ratios were unaffected by the salt concentration in the extraction buffer when using cucumber suggests that polysaccharides were at a low enough concentration in that plant tissue that they were effectively removed by both buffers tested. This was not so for the potato samples, where the use of a higher salt concentration in the extraction buffer was necessary in order to avoid polysaccharide carryover into nucleic acid samples.

It is worth noting that the nucleic acid concentrations measured in this study were approximately 10 times greater than what is measurable using a cuvette-based spectrophotometer. An advantage of microvolume quantification using NanoDrop spectrophotometers is that the instruments use multiple pathlengths, automatically selecting the best one. By doing so, the NanoDrop 2000 spectrophotometer is capable of measuring samples approximately 200 times more concentrated than those that can be measured using cuvette-based spectrophotometers. The use of a cuvette-based spectrophotometer in cases such as this study would require 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 or 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 2000 spectrophotometer an ideal instrument for the analysis of extracted nucleic acid samples.

References

1. Fang, G.; Hammar, S. and Grumet, R. (1992) *A Quick and Inexpensive Method for Removing Polysaccharides from Plant Genomic DNA. BioTechniques 13(1): 52–54.*
2. Arif, I.A.; Bakir, M.A.; Khan, H.A.; Ahamed, A.; Al Farhan, A.H.; Al Homaidan, A.A.; Al Sadoon, M.; Bahkali, A.H. and Shobrak, M. (2010) *A Simple Method for DNA Extraction from Mature Date Palm Leaves: Impact of Sand Grinding and Composition of Lysis Buffer.* International Journal of Molecular Science. 11(9): 3149–3157.
3. Page, A.F and Minocha S.C. (2005) *Analysis of Gene Expression in Transgenic Plants*. Methods in Molecular Biology 286: 291–312.

Africa +43 1 333 50 34 0
Australia +61 3 9757 4300
Austria +43 810 282 206
Belgium +32 53 73 42 41
Canada +1 800 530 8447
China +86 21 6865 4588
Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland/Norway/Sweden +46 8 556 468 00
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9494
Italy +39 02 950 591
Japan +81 45 453 9100
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Russia/CIS +43 1 333 50 34 0
Spain +34 914 845 965
Switzerland +41 61 716 77 00
UK +44 1442 233555
USA +1 800 532 4752

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand

AN52645_E 12/14M