Optimizing Bead Homogenization of Plant Tissues for DNA and RNA Isolation

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INTRODUCTION

The isolation and purification of DNA and RNA from plant tissues is a common practice in both research and agriculture fields. The purified nucleic acids can be used with a variety of tools and techniques such as PCR, Southern blotting and sequencing, and can be used with the goal of DNA fingerprinting, detecting plant pathogens, genotyping or detecting genetically modified organisms. It is essential that the purified nucleic acids be of high quality, with as few contaminants, such as polysaccharides and starches, which inhibit PCR, as possible. It is also essential to minimize the amount of shearing that occurs to the sample's nucleic acid, while maximizing the quantity of nucleic acid that can be detected and used.

DNA and RNA isolation from plant tissues requires the homogenization of the sample. This is often achieved by mortar and pestle tissue homogenization or glass bead homogenization. The first of these methods involves grinding the sample into a powder with a mortar and pestle following the addition of liquid nitrogen. Samples can also be homogenized by adding them to tubes containing glass beads and rapidly shaking or vibrating the tube to break up the tissue. Both methods risk shearing the nucleic acid, and in particular destroying the largest fragments. Though it yields more nucleic acid, the amount of shearing caused by the bead homogenization method renders much of the DNA and RNA unusable for most applications and so using a mortar and pestle is considered the industry standard for plant tissue homogenization.

Plant tissues such as leaves, flowers, roots and stems homogenize differently due to the structural differences between them and the relative quantities of vascular, epidermal, ground and meristematic tissues. Some tissue types, for example, are more prone to shearing, while others may yield more or less nucleic acid than others. Different plants species may also yield varying amounts of DNA and RNA.

Norgen Biotek's Plant/Fungi DNA Isolation Kit and Plant/Fungi RNA Purification Kit have already been proven to isolate high quality DNA and RNA from many plant tissue types, such as roots, leaves, stems and fruit skin for a variety of plant species. This application note illustrates that it is possible to decrease the amount of shearing that occurs during glass bead homogenization while still yielding significant quantities of high quality DNA and RNA by adding lysis buffer to the bead tube prior to homogenization. This method was successful for all tissue types tested (dandelion leaf, flower, stem and root), increasing the efficiency of nucleic acid isolation for these samples using Norgen's DNA and RNA isolation kits.

MATERIALS AND METHODS

Plant DNA Isolation

DNA was isolated from 100 mg of dandelion tissue (equivalent to ~5 x 10^6 plant cells) using Norgen's Plant/Fungi DNA Isolation Kit (Cat. 26200) and Norgen Bead Tubes containing glass beads for homogenization (Cat. 26230; can be purchased separately). Before homogenization, varying amounts of the Lysis Buffer L provided with the kit (0 µL, 250 µL or 500 µL) was added to the Bead Tubes containing the plant tissue. The tissue (leaf, flower, stem and root) was then homogenized using an Omni Bead Ruptor (Omni International) with S = 6.0 for 30 seconds and Norgen's glass AP beads to grind the tissue into a fine powder. Additional Lysis Buffer L (for a total of 500 µL) was added to the homogenized samples, which were then incubated at 65°C for 10 minutes with intermittent mixing. The protocol was then followed as written to complete the DNA isolation.

Plant RNA Isolation

RNA was isolated from 50 mg of dandelion leaves using Norgen's Plant/Fungi Total RNA Purification Kit (Cat. 52800) and Norgen Bead Tubes containing glass beads for homogenization (Cat. 26230; can be purchased separately). Before homogenization, varying amounts of the Lysis Buffer C provided with the kit (0 µL, 300 µL or 600 µL) was added to the bead tubes containing the plant leaves. The leaves

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were then homogenized as with the DNA samples. Additional Lysis Buffer C (for a total of 600 µL) was added to the homogenized samples, which were then incubated at 55°C for 5 minutes. The protocol was then followed as written to complete the RNA isolation.

Gel Electrophoresis

For visual inspection of genomic DNA, 10 µL of the elution was loaded onto a 1.2% agarose TAE gel and run for 22 minutes at 170 V alongside Norgen's HighRanger 1 kb DNA ladder (Cat. 11900). For visualization of RNA, 7.5 µL of the elution was denatured at 70˚C for 10 minutes with 2x RNA loading dye and run on a 1X MOPS, 1.4% formaldehyde-agarose gel for 22 minutes at 170 V. Gel photos were taken using an AlphaImagerTM IS-2200 (Alpha Innotech).

PCR Amplification

The purified DNA from the different plant tissues was used as a template in end-point SYBR Green PCR reactions. To amplify plant genomic DNA, 2 µL of isolated DNA was added to 18 µL of reaction mixture containing 0.5 µM 18S rRNA primers. Reactions were run using the program; 95°C for 3 minutes for an initial denaturation, 45 cycles of 95°C for 15 seconds for denaturation, 60°C for 30 seconds for annealing and 72°C for 45 seconds for extension followed by melting curve analysis. The reaction was run on an iCycler iQ thermocycler (Bio-Rad).

RT-PCR Assay

The purified RNA from leaves was used as a template for one step SYBR Green RT-qPCR with plant 18s rRNA primers. To amplify plant RNA, 2 µL of purified RNA was added to 18 µL of RT-PCR reaction mixture. Reactions were run using the program; 50°C for 30 minutes followed by 95°C for 3 minutes for an initial denaturation, 40 cycles of 95°C for 15 seconds for denaturation, 60°C for 30 seconds for annealing and 72°C for 45 seconds for extension followed by melting curve analysis. The reaction was run on an iCycler iQ thermocycler (Bio-Rad).

RESULTS AND DISCUSSION

DNA concentrations of samples were determined spectrophotometrically using a Nanodrop 2000. Figure 1 demonstrates the effectiveness of adding differing concentrations of Lysis Buffer L (none, 250 µL or 500 µL) before the bead homogenization process and the resulting DNA yield from various plant tissues (leaf, flower, stem and root).

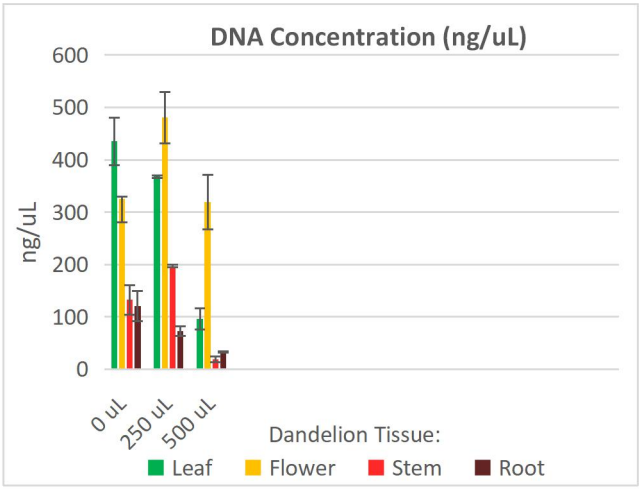

Figure 1. DNA Concentration of Samples Isolated from Dandelion Tissues Using Different Quantities of Lysis Buffer Before Bead Homogenization. The DNA yield from leaves and flowers was higher than from stems and roots. The maximum amount of DNA was yielded from leaves and roots when no Lysis Buffer was added before homogenizing. Maximum DNA for flowers and stems was yielded when half of the lysis buffer is added before homogenizing.

Gel electrophoresis was conducted to visualize the samples and to analyze any shearing that may have occurred. **Figure 2** shows the DNA yielded by the different plant tissues tested with varying lysis buffer additions.

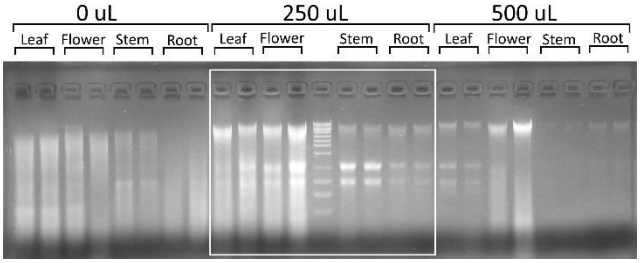

Figure 2. Isolation of DNA from Various Dandelion Tissues and Homogenization Conditions. DNA was isolated from 100 mg of dandelion leaves, flowers, stems and roots. Samples were homogenized using an Omni Bead Ruptor with 0 µL, 250 µL or 500 µL of lysis buffer added to the bead tube prior to homogenization. Samples homogenized when half of the lysis buffer (250 µL) was added before homogenization and half after provided high DNA yields with minimal shearing of large DNA fragments (white box).

PCR was used to determine the relative quantities of high-quality DNA yielded by the different types of dandelion tissues. Figure 3 shows the amplification of the DNA isolated by Norgen's Plant/Fungi DNA Isolation Kit and a bead homogenization method for leaf, flower, stem and root tissues. The samples shown in this figure were prepared with 250 µL of the lysis buffer added before

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homogenization. This demonstrates that the bead homogenization method was able to yield DNA from all the different plant tissues, and that the DNA can be successfully amplified using PCR applications.

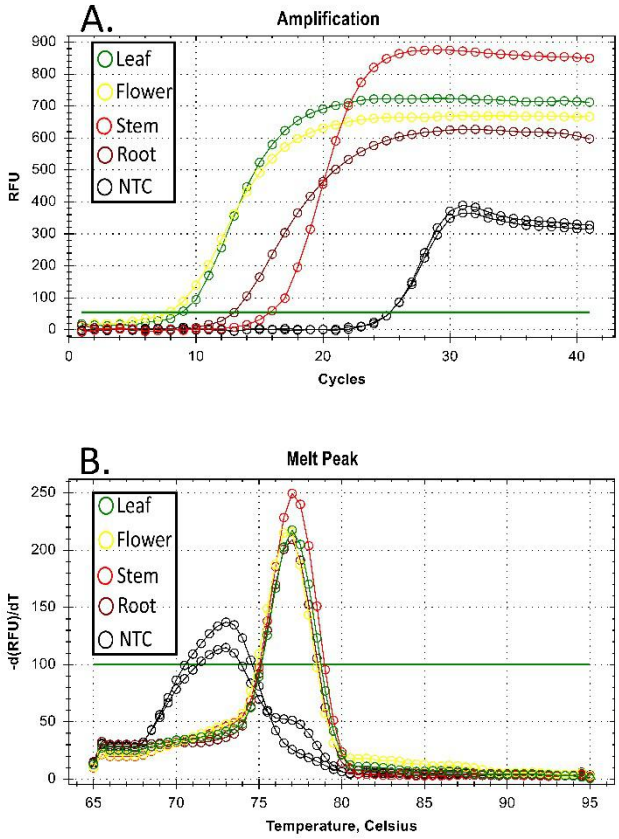

Figure 3. Detection of 18s rRNA in the DNA from Various Tissues and Homogenization Conditions by SYBR Green PCR. (A) Leaves and flowers yield superior results to roots and stems. NTC = No Template Control. (B) Melting curve of plant tissue types validate the 18s rRNA by qRT-PCR. Melting curves coincide for all DNA samples (Tm=77˚C).

Different tissue types in plants yield different quantities of DNA, with leaves and flowers yielding the most and stems and roots yielding the least. Leaves and roots yielded more DNA when no lysis buffer was added before homogenizing, but the DNA experienced significant shearing, rendering it unusable for most applications.

RNA concentrations of samples were determined spectrophotometrically using a Nanodrop 2000. Figure 4 demonstrates the effectiveness of adding differing amounts of Lysis Buffer L (none, 300 µL or 600 µL) before the homogenization process and the resulting RNA yield from dandelion leaves.

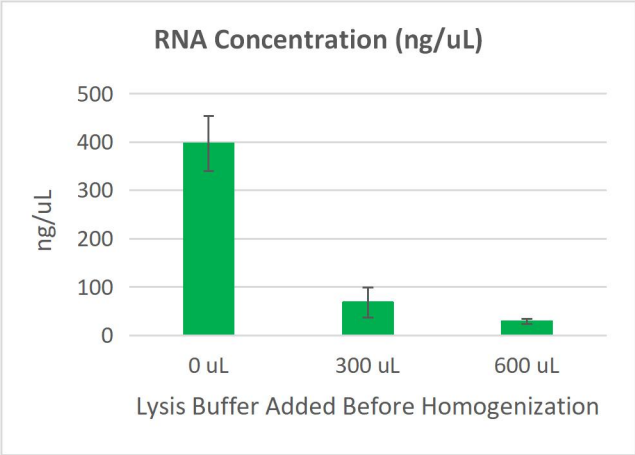

Figure 4. RNA Concentration of Samples Isolated from Dandelion Leaf Using Different Quantities of Lysis Buffer Before Bead Homogenization. RNA yield was significantly higher when no lysis buffer was added before homogenization and decreased as more lysis buffer was added.

Figure 5 shows the RNA quality and quantity from dandelion leaves with varying amounts of lysis buffer added before homogenization.

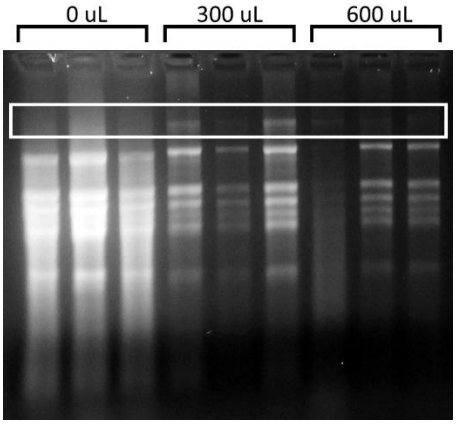

Figure 5. Isolation of RNA from Dandelion Leaf with Varying Lysis Buffer Additions Before Bead Homogenization. RNA was isolated from 50 mg of dandelion leaves with samples homogenized using an Omni Bead Ruptor with 0 µL, 300 µL or 600 µL of lysis buffer added to the bead tube prior to homogenization. Samples homogenized with no lysis buffer provided high RNA yields, though they experienced shearing of the largest RNA fragments (white box).

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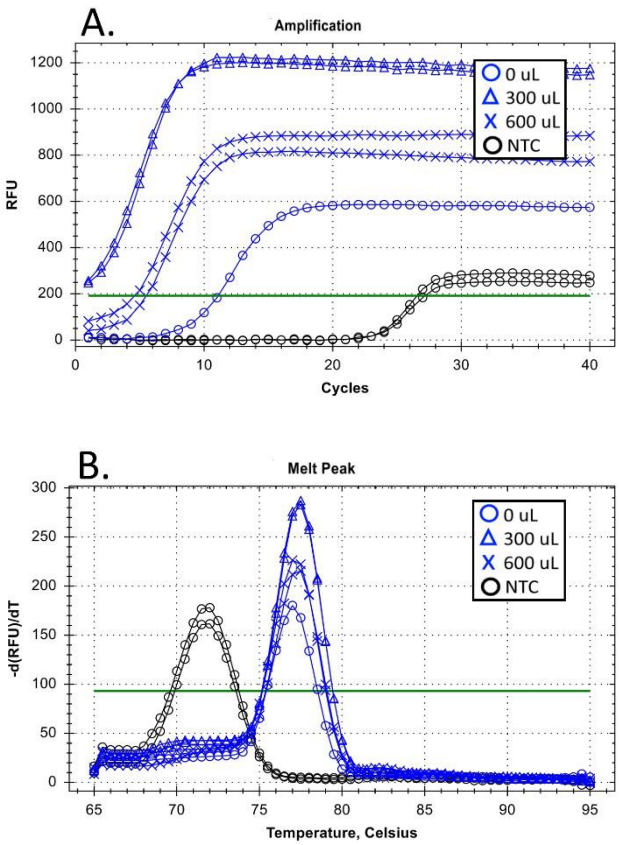

Figure 6. Detection of 18s rRNA in the RNA from the different homogenization conditions by qRT-PCR (Tri = 300 µL, X = 600 µL, O = No lysis buffer added). Samples prepared with 300 µL of the lysis buffer added before homogenization produced the best results with the optimized bead homogenization protocol.

Adding 250 µL of the Lysis Buffer L required by Norgen's Plant/Fungi DNA Isolation Kit or 300 µL of the Lysis Buffer C required by Norgen's Plant/Fungi Total RNA Purification Kit to the bead tube prior to homogenization increases the yield of high quality DNA and RNA while minimizing shearing of large DNA or RNA fragments.

CONCLUSIONS

1. **Efficient and rapid sample preparation with bead homogenization** – As opposed to the traditional liquid nitrogen grinding method, the bead homogenization method rwas convenient and fast to process samples designed for a high through put analysis.

2. **Lysis Buffer Addition for Optimization of Bead Homogenization**- Bead homogenization of plant samples using Norgen's Plant/Fungi DNA Isolation Kit or Norgen's Plant/Fungi Total RNA Purification Kit can be improved by adding half of the required lysis buffer (250 µL for plant DNA or 300 µL for plant RNA respectively) prior to homogenization to minimize shearing while maintaining a high DNA and RNA yield.

3. **Optimization for Various Plant Tissues** –Flowers and leaves yield the most DNA, while stems and roots yield less. Softer samples such as flowers and stems yield the most DNA when homogenized with 250 µL of the lysis buffer added prior to homogenization. Tougher samples such as leaves and roots yielded less DNA with the same condition (250 µL of the lysis buffer added prior to homogenization) but no difference was observed in the DNA quality, and the DNA was suitable for any downstream analysis such as qPCR.

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