Rapid RNA Preparation from Plant Tissues Enriched in Secondary Metabolites

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Abstract

RNA preparation is an important step in functional genomics that employs technologies such as RT-PCR, microarray, and RNAi. In many plant tissues, however, secondary metabolites such as polyphenolic compounds, tannins, and polysaccharides often interfere with RNA isolation and its use in downstream applications. As a consequence, laborious procedures, as well as extraction with hazardous organic solvents, are often required to prepare RNA from such plant tissues. Sigma-Aldrich has developed a novel RNA purification chemistry that enables rapid RNA preparation from difficult plant tissues that contain high levels of secondary metabolites without employing organic extraction or salt precipitation procedures. This method employs unique lysing and binding conditions to allow preparation of RNA free from secondary metabolites. Agilent Bioanalyzer and QRT-PCR analyses reveal that purified RNA is of high quality. This novel purification method has been verified with various difficult plant tissues that yield no or very little RNA with TRI Reagent or RNeasy Plant Mini Kit (such as needles of spruce and pine, and leaves of Red Maple, grape, and cotton), as well as common research plant tissues.

Introduction

Plants are well known for their diversity in secondary metabolites. Some plant tissues, such as spruce and pine needles and grape and cotton leaves, are enriched in polyphenolic compounds and/or tannins; others, such as potato tuber and corn seed, are enriched in polysaccharides. These secondary metabolites often hinder RNA preparation or compromise RNA sample quality. Current protocols for RNA isolation from conifer needles (1, 2), grape and cotton leaves (3), or other difficult plant tissues (4) may take hours or even days to carry out and require hazardous organic extractions. These protocols typically involve precipitation of crude RNA from extracts with alcohol, differential precipitation by lengthy centrifugation in high salt to remove polysaccharides, phenol and chloroform extractions, and final precipitation of purified RNA with alcohol again. Many commercial RNA purification kits are totally ineffective for such difficult plant tissues. Here we present Sigma's Spectrum™ Plant Total RNA Kit that employs a newly developed purification chemistry to overcome the interfering materials from difficult plant tissues in a simple and streamlined silica bind-wash-elute format, without using hazardous organic solvents such as phenol and chloroform. High quality total RNA can be obtained in 30 minutes after the tissue has been ground in liquid nitrogen.

Materials and Methods

All materials were supplied by Sigma-Adrich Corporation (St. Louis, MO) unless otherwise stated.

Plant Tissue Preparation
Plant tissues were ground to a fine powder in liquid nitrogen with a mortar and pestle and stored at –70 °C before RNA purification.

RNA Purification
Ground plant materials were weighed into 100 mg aliquots. Each aliquot was extracted and purified with one of the following RNA preparation methods: 1) Spectrum Plant Total Kit, 2) TRI® Reagent, and 3) RNeasy® Plant Mini Kit, purchased from Qiagen. RNA preparation with TRI Reagent or Qiagen's RNeasy Kit was carried out according to the manufacturer's instructions. The procedure for Spectrum Plant Total RNA kit is outlined in Figure 1. Briefly, ground tissue is extracted in a lysis solution that releases RNA and at the same time inactivates ribonucleases and interfering secondary metabolites such as polyphenolic compounds. After the removal of cellular debris, RNA is captured onto a binding column using a unique binding solution, which effectively prevents polysaccharides, as well as genomic DNA from clogging the column. Residual impurities and most residual genomic DNA are removed by wash solutions, and purified RNA is eluted in RNase-free water.

Spectrophotometer Analysis
RNA samples were diluted in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and analyzed at 230, 260, 280, and
320 nm on a Spectramax® microplate spectrophotometer (Molecular Devices).

Agarose Gel Electrophoresis
RNA samples were analyzed on a non-denaturing 1% agarose gel prepared in TBE buffer.

Agilent Bioanalyzer
Pine RNA samples were analyzed with an RNA 6000 Nano LabChip® Kit on an Agilent 2100 Bioanalyzer.

RT-PCR
Pine RNA samples were analyzed with a SYBR® Green Quantitative RT-PCR Kit (Sigma Product Code QR0100)
in one-step RT-PCR on an Mx3000P™ Real-Time PCR System (Stratagene). A pine alpha-pinene sythase mRNA (AF543527) was targeted with forward primer 5'-GAGTATGGTTGGCACACGTA-3' and reverse primer 5'-TAGTACTCCACGTGACGATG-3', which generated a 242-bp amplicon.

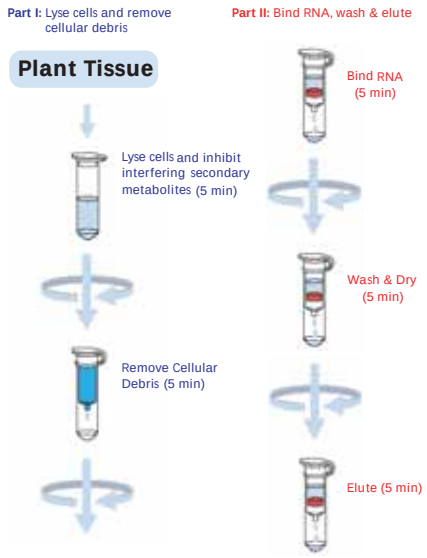

Figure 1. Spectrum Plant Total RNA Kit protocol overview.

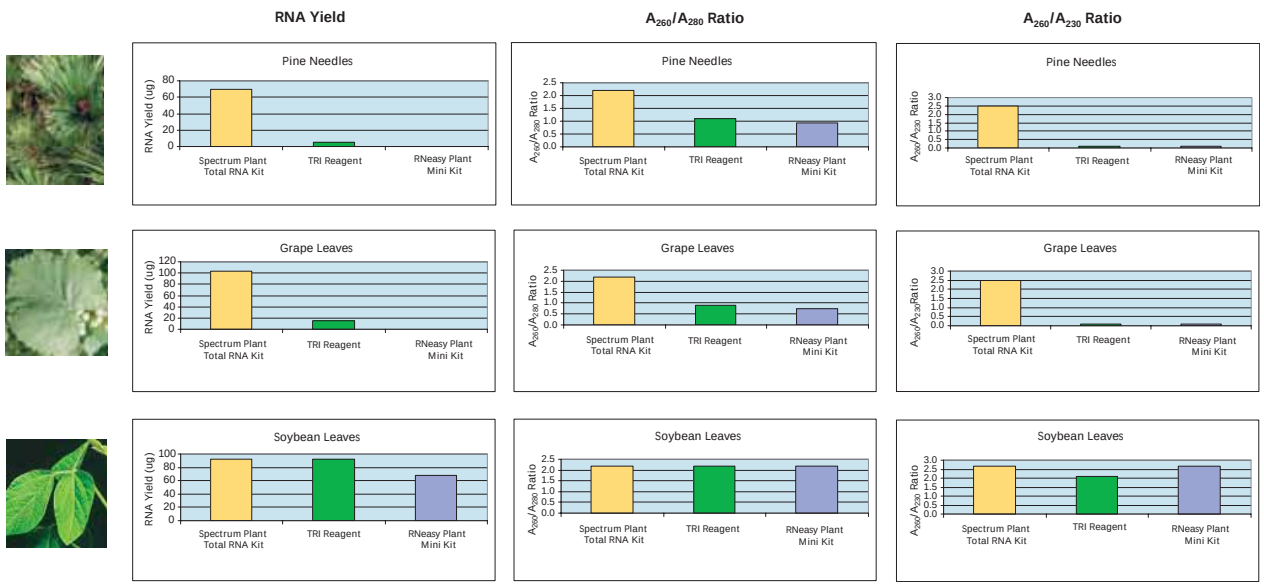

Figure 2. Spectrophotometer analysis of RNA samples purified by Sigma's Spectrum Plant Total RNA Kit, TRI Reagent, and Qiagen's RNeasy Plant Mini Kit. Samples that exhibited very low A_{260}/A_{280} and A_{260}/A_{230} ratios contained no RNA, as was confirmed in agarose gel electrophoresis.

Figure 3. Agarose gel analysis of RNA samples prepared by Sigma's Spectrum Plant Total RNA Kit, TRI Reagent, and Qiagen's RNeasy Plant Mini Kit.
Pine samples: Each lane was loaded with 2% of total recovery (S samples) or 10% of total recovery (T and Q samples).
Grape samples: Each lane was loaded with 1.5% of total recovery (S samples) or 10% of total recovery (T and Q samples).
Soybean samples: Each lane was loaded with 1.5% of total recovery.
Molecular markers: 1 kb DNA ladder.

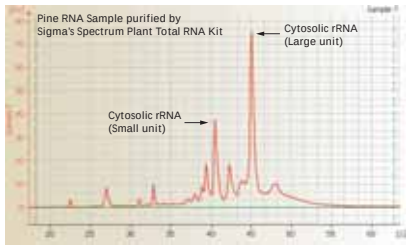

Figure 4. Agilent Bioanalyzer analysis of pine total RNA sample purified by Sigma's Spectrum Plant Total RNA Kit.

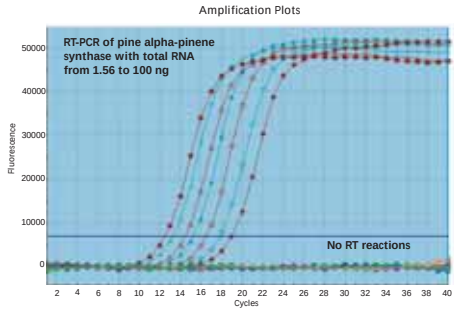

Figure 5. SYBR Green quantitative RT-PCR of pine RNA samples purified by Sigma's Spectrum Plant Total RNA Kit on an Mx3000P Real-Time PCR System (Stratagene).
The amplification curves sequentially correspond to 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 ng of input pine total RNA. No amplicon was detected within 40 cycles without RT.

Plant Species	Tissue Type
Norway Spruce	Needle
Pine	Needle
Red Maple	Leaf
Apple	Leaf, branch
Citrus	Leaf, orange peel
Grapevine	Leaf, root, flower, berry
Grape	Leaf
Cotton	Leaf, fibers
Plectranthus barbatus	Leaf
Cistus creticus	Leaf
Salvia officinalis	Leaf
Watermelon	Leaf, root
Tobacco	Leaf
Tomato	Leaf, stressed leaf, fruit
Soybean	Leaf, root
Canola	Seed
Potato	Tuber
Arabidopsis thaliana	Leaf, flower, silique
Corn	Leaf, root, seed, embryo
Rice	Leaf

Table 1. Plant species and tissue types validated with the Spectrum Plant Total RNA Kit.

Results

Both Sigma's Spectrum Plant Total RNA Kit and Qiagen's RNeasy Plant Mini Kit required approximately 30 minutes in procedure time, while TRI Reagent required a longer procedure time (>45 minutes). However, as shown in Figures 2 and 3, the Qiagen kit and TRI Reagent both failed to isolate any detectable RNA from pine needles and grape leaves, although they were effective with soybean leaves. These two common RNA purification methods also were totally ineffective with other difficult plant tissues, such as spruce needles, Red Maple leaves, and cotton leaves (data not shown). In contrast, the Spectrum Plant Total RNA Kit was able to purify RNA in high yields and high quality from all the plant tissues; and as shown in Figures 4 and 5, RNA purified from the difficult tissue of pine needles exhibited excellent integrity and was highly suitable for QRT-PCR. The Spectrum kit also proved to be effective with other difficult plant tissues, such as spruce needles, Red Maple leaves, cotton leaves, and *Plectranthus barbatus* leaves (data not shown). To date, the Spectrum Plant Total Kit has been validated in 20 plant species, representing a wide taxonomy spectrum (Table 1).

Conclusion

Sigma's Spectrum Plant Total RNA Kit provides a convenient method for purifying high quality total RNA from demanding plant tissues as well as common research plant tissues. The procedure is simple and rapid, requiring no hazardous organic extractions. Purified RNA samples are suitable for QRT-PCR, Northern blots, and other applications.

References

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