

Purification of RNA from Recalcitrant Plant Tissues

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Introduction

The isolation of high-quality RNA from recalcitrant plant tissues is complicated by varying levels of polyphenolics, polysaccharides and other compounds that, when oxidized, avidly bind nucleic acids upon cell lysis.^{1,2} Once bound, these substances coprecipitate with RNA and render it unusable for enzymatic reactions such as reverse transcription. In addition, these impurities can interfere with UV absorbance readings for quantitation.³ Many approaches to RNA purification from plant tissues use organic solvents that are toxic and require special treatment for disposal. Other methods involve spin columns that can clog with tissue debris if homogenization is not complete or if too much starting material is used. Columns also have yield limitations and some size bias, so that small RNAs are not always recovered. In this report, we introduce the MasterPure™ Plant RNA Purification Kit, a salt-based extraction method with a lysis solution and procedure designed specifically to address the challenges associated with recalcitrant plant tissues, without the use of toxic reagents.

The MasterPure Plant RNA Purification Kit contains a Plant Tissue and Cell Lysis Solution designed to inactivate and remove polyphenols and polysaccharides, while releasing nucleic acids and inactivating ribonucleases. The contaminants are simply pelleted with the insoluble cellular debris, and the RNA is further purified by a simple protein precipitation step and nucleic acid precipitation step. There is no size selection with the MasterPure procedure, so small-RNA types are not lost. Unlike other plant RNA extraction kits, a DNase I treatment step is included as well as reagents to remove the DNase I and remaining contaminants, so the resulting RNA is highly purified and ready for reverse transcription, RNA amplification, or hybridization.

Methods and Results

Overview

We tested the MasterPure Plant RNA Purification Kit with a variety of plant tissues that were harvested fresh and either used immediately or quick-frozen in liquid nitrogen. The tissues were weighed and 100 mg of each was ground to a fine powder with mortar and pestle in liquid nitrogen. Without allowing the homogenate to thaw, lysis solution was added, the tissue was mixed by vortexing, and incubated at 56°C. The debris and contaminants were pelleted by centrifugation, the supernatant was chilled, proteins were precipitated, and the nucleic acid was recovered by isopropanol precipitation. A 10-minute DNase I treatment was sufficient to remove the remaining DNA. The DNase I was then removed by protein precipitation and the total cellular RNA was precipitated, resuspended, and was ready for use. RNA was purified from similar samples using three different vendors' plant purification products, following manufacturers' instructions.

Evaluation of purity

We tested many sources of plant tissues during the development of the MasterPure Plant RNA Purification Kit. Purified RNA from maize seedlings, grape leaves, alfalfa sprouts, strawberry leaves, raspberry leaves, pine needles, and maize roots was separated by formaldehyde gel electrophoresis using standard methods (Fig. 1). The intact high-molecular-weight rRNA bands, and lack of

Table 1. Assessment of purity and yield of RNA purified using different methods.

Vendor	Source	Yield (µg)	A ₂₆₀ /A ₂₈₀	A ₂₆₀ /A ₂₃₀
EPICENTRE	Maize leaves	17.67	2.01	2.017
Vendor 1		8.23	2.10	0.566
Vendor 2		13.39	2.21	1.37
Vendor 3		2.83	1.98	0.153
EPICENTRE	Grape leaves	8.98	1.49	0.524
Vendor 1		0	ND	ND
Vendor 2		0.336	1.48	0.124
Vendor 3		0.264	1.54	0.201

ND, not determined.

larger genomic DNA bands indicates the integrity and purity of the RNA. Smaller RNA is also clearly visible, and is not lost during MasterPure purification.

Using spectrophotometry, we assessed the purity of RNA isolated from 100 mg of maize leaf and grape leaf. The leaves were weighed, quick-frozen, homogenized with mortar and pestle and then RNA was purified using one of four different methods, in duplicate. Absorbance readings at 230, 260, and 280 nm were averaged and used to evaluate the purified total cellular RNA (Table 1).

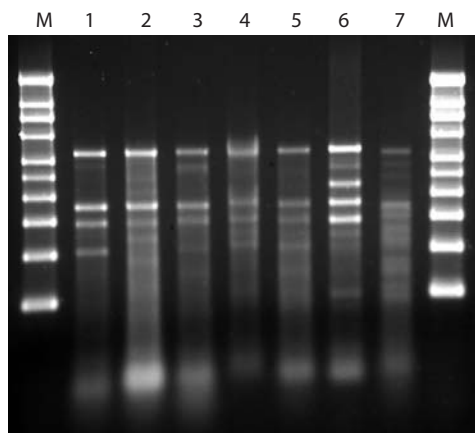


Figure 1. Purification of intact plant RNA from a variety of plant tissues. Plant tissue RNA was purified using the MasterPure™ Plant RNA Purification Kit, and 500 ng of each type of RNA was separated on a 1% formaldehyde gel and visualized with SYBR® Gold I. Lanes M, RNA ladder; lane 1, maize seedling RNA; lane 2, grape leaf RNA; lane 3, alfalfa sprout RNA; lane 4, strawberry leaf RNA; lane 5, raspberry leaf RNA; lane 6, pine needle RNA; lane 7, maize root RNA.

Higher yields of both types of RNA were obtained using the MasterPure Plant RNA Kit. The grape-leaf RNA purification proved to be recalcitrant to the other methods, yielding very little, if any, usable RNA. Absorption at 230 nm reflects impurities of carbohydrates, peptides, chaotropic salts, or aromatic compounds.⁴ Samples with a low A_{260}/A_{230} ratio still contain a significant presence of contaminants that may interfere with other downstream processes like RT-PCR or the *in vitro* transcription steps in RNA amplification. RNA purified using the MasterPure Plant RNA Kit had the highest A_{260}/A_{230} ratios obtained (Table 1).

cDNA synthesis and PCR amplification

Using the RNA samples obtained from maize leaf as described above, we performed reverse transcription with 500 ng of each RNA sample, with random primers and EPICENTRE's MMLV Reverse Transcriptase 1st-Strand cDNA Synthesis Kit according to the protocol. We used 1 μ l of each cDNA for end-point PCR in a 25- μ l reaction, using the FailSafe™ PCR System (EPICENTRE) with PreMix D and 10 pmol of each primer. PCR primers were designed to span an intron in the genomic DNA sequence of maize GAPDH.

All of the cDNA samples produced the expected 584-bp product (Fig. 2), but the cDNA produced from RNA isolated by other methods also produced larger bands. These amplicons are likely from regions of GAPDH genomic DNA

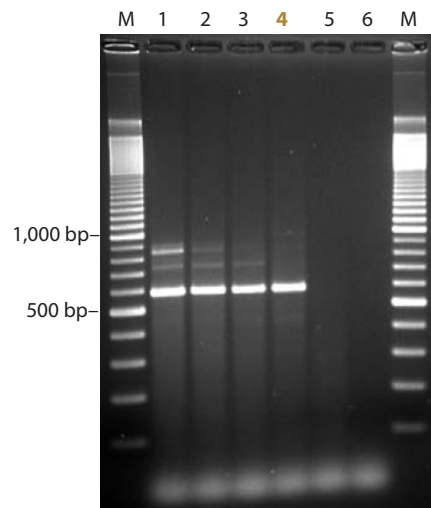


Figure 2. End-point RT-PCR of RNA purified using the MasterPure™ Plant RNA Kit and other kits. RNA was reverse-transcribed using the MMLV Reverse Transcriptase 1st-Strand cDNA Synthesis Kit and random primers. FailSafe™ PCR was performed with PreMix D and 10 pmol of each primer, using 1 μ l of each cDNA. The RT-PCR products from each type of cDNA were separated by electrophoresis and visualized with SYBR® Gold I. Lanes M, 100-bp ladder; lane 1, vendor 1; lane 2, vendor 2; lane 3, vendor 3; lane 4, MasterPure Plant RNA Kit; lane 5, MasterPure no-RT control; lane 6, no-template control.

contaminating the RNA preparations. Only the MasterPure Plant RNA Purification Kit includes specific DNA removal reagents, thereby reducing interference from genomic DNA.

We also used the random-primed cDNA in real-time PCR with 10 pmol of each primer and the FailSafe™ PCR System (EPICENTRE). We used a series of primer pairs that amplify messages of varying abundance in mature maize leaf.

The traces in Fig. 3 depict the specific amplification of each of four targets from MasterPure-purified maize leaf RNA. MatK is a highly abundant chloroplast RNA, GAPDH and actin 1 are of high to medium abundance, and TubG1 is expressed at lower levels in these leaves. The specificity of these amplifications was verified by melt-curve analysis and gel electrophoresis (data not shown).

Conclusions

The MasterPure Plant RNA Purification Kit produces highly purified RNA from a variety of plant sources and tissue types. The isolated plant RNA is suitable for reverse transcription in end-point and real-time PCR as well as RNA amplification methods such as EPICENTRE's MessageBOOSTER™ cDNA Synthesis Kit for qPCR (data not shown). The optimized lysis solution removes common inhibitors and provides the highest possible A_{260}/A_{230} ratios, assuring the user of RNA purity and fewer difficulties with downstream applications. The

lysis solution allows isolation of RNA from some of the most recalcitrant plant tissues such as grape leaves, pine needles, and citrus leaves, and produces better RNA yields from these tissues than other kits tested.

References

1. Salzman, R. A. *et al.* (1999) *Plant Mol. Biol. Reporter* **17**, 11.
2. Dimick, P. S. *et al.* (1999) *Can. Inst. Food Sci. Technol. J.* **14**, 269.
3. Newbury, H. J. *et al.* (1977) *Plant Physiol.* **60**, 543.
4. Cheng, S.-H. and Seeman, J. R. (1998) *Meth. Mol. Biol.* **86**, 27.

Cat. #	Quantity
MasterPure™ Plant RNA Purification Kit	
MPR09010	10 Purifications
MPR09100	100 Purifications

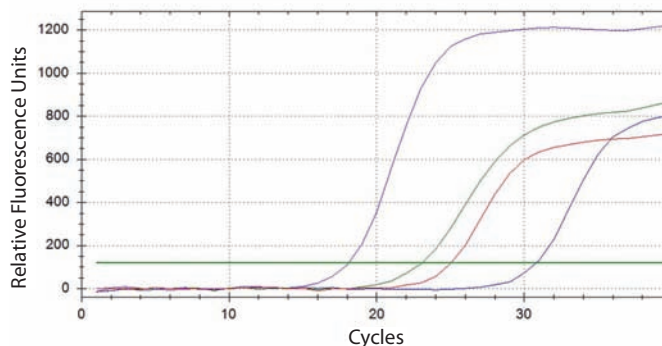


Figure 3. Real-Time PCR detection of plant messages of varying abundance in RNA purified with the MasterPure™ Plant RNA Purification Kit. Maize leaf cDNA samples from Fig. 2 were used in real-time PCR with the FailSafe™ PCR System and 10 pmol of each primer. Messages detected were MatK (purple), GAPDH (green), actin 1 (red), and TubG1 (blue).



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