Technical Brief

Comparison of Methods for Isolating High-Quality RNA from Leaves of Grapevine

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Abstract: High concentrations of polyphenols and polysaccharides make it challenging to extract high-quality RNA from grape organs. To determine an optimal protocol for grape leaves, 15 different methods of RNA extraction were evaluated based on cost, time to complete the extraction, and quality of the RNA isolated. The addition of specific compounds to the extraction buffer to remove polyphenols and polysaccharides is often critical for downstream applications such as polymerase chain reaction (PCR) and microarray hybridization. RNA quality was assessed using spectrophotometric methods, formaldehyde-agarose gel electrophoresis, reverse transcription (RT)-PCR reactions, and Agilent 2100 Bioanalyzer. Large differences in RNA yield and quality among protocols were found. Some protocols that are commonly used for other species did not yield usable RNA from grapevine leaves. The optimum methods were Tris-lithium chloride, which, while relatively time-consuming, gave consistently high yields of quality RNA at very low cost and was suitable for PCR and microarray hybridization, and RNeasy Midi + polyethylene glycol, which rapidly provided high-quality RNA, but with lower yields.

Key words: RNA isolation, leaf, grape, Vitis vinifera

Extraction of high-quality total RNA is essential for the successful application of many molecular techniques, such as reverse transcription polymerase chain reaction (RT-PCR), cDNA library construction, and gene expression profiling studies using microarrays. RNA is degraded rapidly by ribonucleases (RNases) and, therefore, must be extracted quickly and efficiently (Sambrook et al. 1989). Denaturing reagents such as phenol (Davies and Robinson 1996) or guanidine thiocyanate (John 1992, Salzman et al. 1999) and inhibitors of RNases such as aurintricarboxylic acid (ATA) (Franke et al. 1995, Lewinsohn et al. 1994) are often added to extraction buffers.

Extraction of high-quality RNA from the leaves of woody plants, such as grapevine, is particularly challenging because of high concentrations of polysaccharides, polyphenols, and other secondary metabolites (Loulakakis et al. 1996, Salzman et al. 1999). When applied to grape leaves, some RNA extraction methods yield pellets that are poorly soluble, indicating the presence of unknown contaminants, whereas others are gelatinous, indicating the presence of polysaccharides (Tesniere and Vayda 1991). Other procedures yield reddish pellets indicative of phenolic contamination (Bahloul and Burkard 1993, Katterman and Shattuck 1983). RNA can become complexed with polysaccharides and phenolic compounds (Bahloul and Burkard 1993, Lewinsohn et al. 1994, Salzman et al. 1999), particularly if chaotropic agents (Newbury and Possingham 1977) or vinyl-pyrrolidone polymers and antioxidants (Salzman et al. 1999) are not present in the extraction buffer. Polysaccharide and phenolic complexes render the RNA unusable for applications such as reverse transcription and cDNA library construction (Salzman et al. 1999).

Whereas some standard procedures developed for model plant species, such as Arabidopsis and rice (for example, TRIzol, RNAwiz, RNeasy Plant), are very reliable, these methods are often inadequate when applied to leaves of Vitis vinifera. While several reports using recalcitrant plant tissues compared a few methods of RNA isolation (Logemann et al. 1987, Loulakakis et al. 1996, Tesniere and Vayda 1991), the scope of these comparisons was relatively limited. Logemann et al. (1987) compared six methods using various combinations of cetyltrimethyl-
ammonium bromide (CTAB), cesium chloride (CsCl), and guanidine for extraction of RNA from potato tubers. Tesniere and Vayda (1991) applied six methods to grape
berry, five of which included CsCl density centrifugation. Loulakakis et al. (1996) compared four methods, three including CsCl density centrifugation, on grapevine callus
culture, berry, and leaf. Although many procedures exist, a more substantial comparison was needed. Therefore, we compared RNA extraction methods that had been reported
to be successfully used with grapes (Davies and Robinson 1996, Loulakakis et al. 1996, Nassuth et al. 2000, Saltz-
man et al. 1999), as well as other plant species from which
RNA extraction is known to be a challenge (Bahloul and
isolation methods were evaluated and ranked according to
time, cost, quality, and quantity of RNA obtained. Two
methods were found to be the most suitable for a wide
range of molecular biology applications: Tris-lithium clor-
ride (Tris-LiCl) and RNeasy Midi + polyethylene glycol
(PEG).

Materials and Methods

Plant material. Vitis vinifera L. cv. Chardonnay and
Cabernet Sauvignon plants were grown in the field at
Reno, NV (lat. 39°33′N; long. 119°47′W), in pots in green-
houses, in growth chambers (model AR-75L, Percival Scien-
tific, Perry, IA), or hydroponically in greenhouses. Growth
conditions were 16-hr days at 25 to 28°C and 8-hr nights
at 20°C, regardless of location. Plants were grown from
cuttings or rooted cuttings and were one- to two-years
old. Potted plants were grown in Scott’s Metro-Mix 200
(Sun Gro Horticulture, Vancouver, BC). The composition of
the hydroponic solution was according to Gibeaut et al.
(1997). Plants used for RNA isolations were subjected to
a variety of cold (4°C), water deficit, flooding, or heat
(42°C) stresses or were unstressed. Young leaves, young
shoots with leaves, or berries were harvested, immediately
frozen in liquid nitrogen, then stored at -80°C until use
(up to two years). Roots were rinsed to remove soil, blot-
dry, and then harvested as above. All RNA methods
were tested on young unstressed leaves. For all methods
except CTAB and CTAB + RNeasy, young stressed leaves
were also used.

RNA protocols. All glassware for RNA extraction was
baked at 180°C for at least 4 hr, whereas plasticware was
either new disposable or treated with 0.1% (v/v) diethyl-
pyrocarbonate (DEPC) water and autoclaved before use.
All solutions used in the RNA extractions, except those
containing Tris, were treated with 0.1% (v/v) DEPC to de-
stroy potentially damaging RNases. Five of the methods
used for RNA isolation were guanidine thiocyanate (Salz-
man et al. 1999), hot sodium acetate (NaOAc) (Bahloul
and Burkard 1993), TRIzol-PEG (Gehrig et al. 2000), modified
RNeasy method (Nassuth et al. 2000), and Plant RNA Iso-
lation Reagent (Invitrogen, Carlsbad, CA).

Several unpublished modified methods were also used,
including modified hot borate (David Shintani 2001, per-
sonal communication) based on Hall et al. (1978). The ex-
traction buffer contained 200 mM sodium borate, 30 mM
[ethylenbis(oxyethylenenitritro)]-tetraacetic acid, 1% so-
dium dodecyl sulfate (SDS), 1% (w/v) deoxycholate, 2%
(w/v) polyvinylpyrrolidone (PVP). The solution was auto-
claved, and just before use, 10 mM dithiothreitol (DTT)
was added and the extraction buffer heated to 80°C. One
gram of tissue was ground in a liquid-nitrogen-cooled
mortar and placed in a chilled 15-mL tube, then 3.5 mL ex-
traction buffer was added after which 3.5 mL of fresh 1:1
phenol:chloroform was added, and the mixture was vor-
texed until thoroughly mixed. The liquid phases were
separated by centrifugation at room temperature for 20 min
at 10,000 x g. The aqueous phase was extracted with one
volume of chloroform, adjusted to 2 M lithium chloride
(LiCl) and incubated overnight on ice at 4°C. The precipi-
tate was collected by centrifugation at room temperature
for 20 min at 10,000 x g. The pellet was resuspended in
500 µL DEPC-treated water, after which 50 µL of 2 M po-
tassium acetate pH 5.5 and 1.1 mL of 100% ethanol were
added. The precipitate was pelleted by centrifugation at
10,000 x g for 20 min. The supernatant was completely
removed, and the pellet allowed to air dry for 10 min, and
then resuspended in 500 µL DEPC-treated water.

A Tris-LiCl method (Wang et al. 2000) was modified by
adding a phenol:chloroform extraction step. In short, the
extraction buffer consisted of 200 mM Tris-HCl pH 8.5,
1.5% (w/v) lithium dodecyl sulfate, 300 mM Na-EDTA
(ethylenebridiaminetetraacetic acid), 1% (w/v) sodium deoxy-
cholate, and 1% (v/v) tergitol NP-40. Just before use, 2
mM ATA, 20 mM DTT, 10 mM thiourea, and 2% (w/v)
PVP (polyvinylpolypyrrolidone) were added. Approxima-
tely 1.5 g leaf or 3 g berry or root tissue was ground in a
liquid-nitrogen-cooled mortar and combined with 25 mL
extraction buffer. The mixture was frozen at -80°C for at
least 2 hr. The homogenate was thawed in a 37°C water
bath, and centrifuged for 20 min at 4°C and 5,000 x g. The
supernatant was transferred to fresh tubes, and 0.106 M
NaOAc and 10% (v/v) ethanol added. After 10 min on ice,
this was centrifuged 4°C for 20 min at 5,000 x g. The super-
natant was retained and 0.33 M NaOAc and 33% (v/v) iso-
propanol was added. The mixture was incubated at least 2
hr at -20°C. The tubes were then centrifuged for 30 min at
4°C and 5,000 x g. The pellet was resuspended in 3 mL TE
(10 mM Tris pH 7.5, 1 mM EDTA), and incubated on ice 30
min, then centrifuged for 30 min at 4°C and 5,000 x g. The
supernatant was transferred to fresh tubes, LiCl added to
2.5 M, and incubated on ice at 4°C overnight. The tubes
were centrifuged for 30 min at 4°C and 10,000 x g. The pel-
let was resuspended in 1.5 mL TE and potassium acetate
was added to 3 M. The mixture was incubated on ice for 3
hr, and centrifuged for 30 min at 4°C and 10,000 x g. The
pellet was resuspended in 1 mL TE, and a single phenol:
chloroform:isoamyl alcohol (25:24:1) extraction performed,
followed by a single chloroform:isoamyl alcohol (24:1) extraction. Ten percent 3.3 M NaOAc and 2 volumes ethanol were added and incubated at -20°C for at least 2 hr. Tubes were centrifuged at 4°C and 10,000 x g for 30 min. The pellets were washed with 500 µL absolute ethanol, dried on ice, and resuspended in 500 µL DEPC-treated water.

A Tris-LiCl ultracentrifugation method was also performed as described (Loulakakis et al. 1996), except that CsCl was not added to the RNA solution before ultracentrifugation because it would not dissolve in the solution, even at a reduced concentration of 0.2 g/mL.

Two modified sodium perchlorate (NaClO₄) methods were tested that were based on the method originally developed by Davies and Robinson (1996). In the first modification, the extraction buffer was changed to contain 0.2 M Tris pH 8.3, and the PEG was omitted. A single TRIzol extraction was substituted for the phenol:chloroform:isoamyl alcohol extractions. The second modified method was from Steve van der Merwe and Johan Burger (2002, personal communication). The extraction buffer was modified to contain 3 M NaClO₄, 0.2 M Tris-HCl pH 8.3, 5% (w/v) SDS, 8.5% (w/v) PVPP, 2% PEG (w/v), and 1% (v/v) β-mercaptoethanol. One mL of extraction buffer was used for each 100 mg tissue, and autoclaved Miracloth (Calbiochem, La Jolla, CA) was used to filter the eluate instead of a glasswool filled syringe. The phenol:chloroform:isoamyl alcohol extractions were performed repeatedly until the interphase was negligible. At least six phenol:chloroform:isoamyl alcohol extractions were needed. The RNA was precipitated using 2 M LiCl. The data from these two variations of NaClO₄ method were combined in the analyses, as they have significant similarities.

A CTAB method based on the methods of Jaakola et al. (2001) and Chang et al. (1993) was modified by Alberto Iandolino (2003, personal communication). The ground tissue was incubated with extraction buffer for 30 min with frequent agitation. Two chloroform:isoamyl alcohol extractions were performed. The RNA pellet was resuspended in DEPC-treated water and a final purification of the RNA was done using the RNeasy (Qiagen, Valencia, CA) cleanup method.

The RNeasy Midi (Qiagen) + PEG method was performed according to the manufacturer’s instructions for isolation of RNA from plants, with a few modifications: 3.3% (w/v) PEG (MW 15,000 to 20,000) was added per mL of buffer RLT before addition of plant material (Gehrig et al. 2000). Following initial centrifugation, the supernatant was filtered through Miracloth. Final elution was done using 150 µL RNase-free water, and the eluate was run back through the column for the second elution. RNeasy Mini cleanup was performed as described by the manufacturer, except that no more than 70 µg RNA was applied to the column.

Reverse transcription PCR. RT-PCR was performed in a PTC-200 thermocycler (MJ Research, Waltham, MA). Primers were designed to grape malate dehydrogenase (GenBank accession # U67426). The sequence of the forward primer was 5’-TCAGCCTGCTTGCCAGTTAC-3’ and the reverse primer was 5’-CCAAATCTTTTGTGCGGT-3’. Reaction conditions were as follows: 1x Invitrogen PCR buffer (20 mM Tris-HCl [pH 8.4], 50 mM KCl), 3 mM MgCl₂, 10 mM DTT, 0.5 mM dNTPs, 0.5 µM each primer, 12 units RNaseOUT (Invitrogen), 30 units Superscript II (Invitrogen), 0.5 units Taq polymerase (Invitrogen), 100 ng total RNA. The thermocycling conditions were reverse transcription at 50°C for 30 min, denaturation at 94°C for 2 min, denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 94°C for 15 sec, annealing at 50°C for 30 sec, and extension at 72°C for 45 sec, and a final extension at 72°C for 5 min.

RNA analysis. RNA concentration and purity were determined using either a BioWave S2100 (Biochrom, Cambridge, UK) or a Beckman DU530 (Beckman Coulter, Fullerton, CA) spectrophotometer. For purity assessment, the absorbance for the A260/280 and A260/230 ratios was taken in water, unless noted otherwise. Formaldehyde-agarose gel electrophoresis was performed according to the protocol in the Qiagen RNeasy instruction manual. In brief, 1.2% agarose gels containing ethidium bromide were prepared. The running buffer consisted of 1 mM EDTA, 5 mM NaOAc, 20 mM 3-(N-morpholino)propanesulfonic acid (MOPS), adjusted to pH 7.0 with NaOH, and 246 mM formaldehyde was added. The 5x RNA loading dye consisted of 80 mM MOPS, 20 mM NaOAc, 8 mM EDTA, 886 mM formaldehyde, 20% (v/v) glycerol, 31% (v/v) formamide, 0.16% (v/v) saturated bromphenol blue. One microgram of total RNA was loaded, except as indicated otherwise. Before loading, RNA with loading dye was heated at 65°C for 4 min, then chilled on ice. The gel was run at 7 V/cm. Gels were imaged using the GelDoc 2000 gel documentation system and QuantityOne image analysis software version 4 (Bio-Rad, Hercules, CA). Agilent 2100 Bioanalyzer RNA LabChip assays were performed following the manufacturer’s instructions (Agilent Technologies, Palo Alto, CA). All statistical analyses were performed using JMP (version 5, SAS Institute, Cary, NC).

Results and Discussion

RNA yield. There were large differences in the amount of RNA extracted per gram of tissue depending on the protocol used (Table 1). For example, the CTAB and TRIzol-PEG methods gave the highest mean raw yields in micrograms RNA per gram fresh weight (846 and 821 µg/g FW, respectively). In contrast, the modified RNeasy method gave the lowest mean yield (13 µg/g FW). The Tris-LiCl method gave a moderate yield of over 300 µg/g FW. Yields were significantly reduced by the addition of an RNeasy cleanup step (Tables 1 and 2). Mean yield from all methods for stressed tissue was 399 ± 34 µg/g FW and was not significantly different from unstressed tissue (392 ± 39 µg/g FW, p = 0.89).

RNA quality. RNA quality was assessed by five methods: A260/280, A260/230, appearance on a denaturing agarose gel, ability to produce RT-PCR products, and Agilent
Isolating High-Quality RNA from Grapevine Leaves – 403

2100 Bioanalyzer RNA LabChip assay. A260/280 ratios indicate the level of protein contamination, based on the principle that nucleic acids display an absorbance optimum at 260 nm, whereas proteins (primarily aromatic amino acids) display an absorbance optimum at 280 nm (Winfrey et al. 1997). After running on a denaturing gel, high-quality RNA shows two or more sharp rRNA bands with little smearing, depending on whether the gel is run long enough to separate the 16S from the 18S and the 23S from the 25S rRNA. The modified RNeasy, RNeasy Midi + PEG, CTAB + RNeasy, and Tris-LiCl ultracentrifugation methods gave RNA with very low amounts of protein contamination (Table 1). In contrast, RNA isolated by the hot borate and CTAB methods showed significantly more protein contamination, as indicated by the lower A260/280 ratios.

A260/230 ratios are used to assess the level of contamination by polysaccharides and polyphenols (Loulakakis et al. 1996, Schultz et al. 1994). A ratio of 2.5 is considered free of contamination. The modified RNeasy and hot borate methods yielded RNA that was significantly contaminated with polysaccharides (Table 1). In contrast, the RNeasy Midi + PEG, Tris-LiCl ultracentrifugation, Tris-LiCl, and Tris-LiCl + RNeasy methods yielded RNA with very little polysaccharide.

The yield and quality of RNA from the Tris-LiCl method was improved significantly by the addition of a phenol:chloroform step as compared to the original Tris-LiCl-Wang (2000) protocol (Table 1). The Tris-LiCl ultracentrifugation method (Loulakakis et al. 1996) yielded RNA that had excellent purity and amount based on spectrophotometric measurements, but 6 of 10 samples showed significant to severe degradation upon denaturing agarose gel electrophoresis.

Most of the methods for which RNA could be seen on a gel had a clear band pattern, although many had some smearing (Figure 1A), indicative of RNA degradation. These patterns were reproducible and are seen more clearly in the gel image and electropherograms from the Agilent 2100 Bioanalyzer (Figure 1B, C).

RNA preparations were further tested for quality using RT-PCR reactions, performed in triplicate. The RNA from NaClO₄, TRIzol-PEG, RNeasy Midi + PEG, Tris-LiCl, and Tris-LiCl + RNeasy methods consistently resulted in amplification (Table 3, Figure 2), while the CTAB, guanidine thiocyanate, hot NaOAc, and Tris-LiCl ultracentrifugation methods consistently gave no amplification.

**Cost.** Approximate hands-on time required for each method and cost per µg RNA were analyzed. Hands-on time included incubations or centrifugations of less than

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### Table 1: Comparison of yield and quality from various methods of RNA isolation from grapevine young leaves as assessed using a spectrophotometer.

<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>µg/g FW</th>
<th>A260/280</th>
<th>A260/230</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTAB</td>
<td>2</td>
<td>846 ± 68</td>
<td>1.45 ± 0.35</td>
<td>1.80 ± 0.06</td>
</tr>
<tr>
<td>TRIzol-PEG</td>
<td>9</td>
<td>821 ± 24</td>
<td>1.61 ± 0.18</td>
<td>1.70 ± 0.05</td>
</tr>
<tr>
<td>Hot borate</td>
<td>10</td>
<td>665 ± 30</td>
<td>1.49 ± 0.06</td>
<td>&lt;0.95 ± 0.21</td>
</tr>
<tr>
<td>Plant RNA Isolation Reagent</td>
<td>46</td>
<td>624 ± 77</td>
<td>1.74 ± 0.02</td>
<td>1.22 ± 0.02</td>
</tr>
<tr>
<td>Tris-LiCl</td>
<td>75</td>
<td>387 ± 29</td>
<td>1.80 ± 0.01</td>
<td>2.49 ± 0.03</td>
</tr>
<tr>
<td>Tris-LiCl + RNeasy</td>
<td>42</td>
<td>302 ± 15</td>
<td>1.70 ± 0.01</td>
<td>2.55 ± 0.05</td>
</tr>
<tr>
<td>Plant RNA Isolation Reagent</td>
<td>23</td>
<td>299 ± 20</td>
<td>1.75 ± 0.04</td>
<td>1.73 ± 0.07</td>
</tr>
<tr>
<td>Tris-LiCl ultracentrifugation</td>
<td>10</td>
<td>228 ± 87</td>
<td>2.13 ± 0.11</td>
<td>2.40 ± 0.08</td>
</tr>
<tr>
<td>Tris-LiCl-Wang</td>
<td>8</td>
<td>159 ± 72</td>
<td>1.54 ± 0.05</td>
<td>1.28 ± 0.10</td>
</tr>
<tr>
<td>Guanidine thiocyanate</td>
<td>9</td>
<td>97 ± 63</td>
<td>1.79 ± 0.39</td>
<td>1.47 ± 0.28</td>
</tr>
<tr>
<td>CTAB + RNeasy</td>
<td>6</td>
<td>85 ± 10</td>
<td>2.00 ± 0.06</td>
<td>1.91 ± 0.11</td>
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<tr>
<td>RNeasy Midi + PEG</td>
<td>4</td>
<td>54 ± 3.0</td>
<td>2.76 ± 0.06</td>
<td>4.21 ± 0.49</td>
</tr>
<tr>
<td>NaClO₄</td>
<td>11</td>
<td>40 ± 8.0</td>
<td>1.87 ± 0.13</td>
<td>2.23 ± 0.23</td>
</tr>
<tr>
<td>Hot NaOAc</td>
<td>6</td>
<td>26 ± 7.0</td>
<td>1.61 ± 0.08</td>
<td>1.27 ± 0.03</td>
</tr>
<tr>
<td>Modified RNeasy</td>
<td>9</td>
<td>13 ± 3.0</td>
<td>2.41 ± 0.45</td>
<td>&lt;0.21 ± 0.04</td>
</tr>
</tbody>
</table>

### Notes:

- n: number of extractions.
- Means ± SE; superscript uppercase letters indicate p ≤ 0.05 significance level based on least square means student’s t test.
- Yield is given in µg RNA per gram fresh weight.

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### Table 2: Comparison of leaf RNA before and after RNeasy cleanup isolated by Plant RNA Isolation Reagent and Tris-LiCl methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>µg/g FW</th>
<th>A260/280</th>
<th>A260/230</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before cleanup</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A260/280</td>
<td>1.72 ± 0.03</td>
<td>1.82 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>A260/280 µg/g FW</td>
<td>1.80 ± 0.01</td>
<td>2.05 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>A260/230</td>
<td>1.30 ± 0.04</td>
<td>2.65 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>After RNeasy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A260/280</td>
<td>1.68 ± 0.02</td>
<td>1.70 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>A260/280 µg/g FW</td>
<td>2.13 ± 0.01</td>
<td>2.18 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>A260/230</td>
<td>1.81 ± 0.11</td>
<td>2.66 ± 0.07</td>
<td></td>
</tr>
</tbody>
</table>

### Notes:

- Data based on a subset of what is listed in Table 1 for which measures were taken in both water and 10 mM tris pH 7.5.
- 24 extractions.
- 12 extractions.
one hour; longer periods were not included, as researchers may perform other tasks. The labor involved in preparing solutions was not included in the cost estimates. Total cost per µg RNA isolated was based on mean yields, reagent costs, labor at $8.00/hr, and the cost of single-use tubes, filters, or columns. Generally, the higher the RNA yield per gram of tissue, the lower the cost per µg, regardless of the cost of reagents or the labor involved. Some methods can be scaled up, which gives a savings in labor costs per µg, while scaling down can increase the cost per µg RNA. The Tris-LiCl ultracentrifugation, CTAB, Plant RNA Isolation Reagent, and Tris-LiCl methods were the most economical (Table 3), and the guanidine thiocyanate and modified RNeasy methods were the most costly because of low yields and/or small scale.

Application of RNA methods to roots and berries. We have used the Tris-LiCl + RNeasy method extensively, and it was used to isolate RNA for the leaf, root, and berry cDNA libraries that our group has prepared for EST sequencing. When run on a gel, four rRNA (16S, 18S, 23S, and 25S) bands can be seen for Tris-LiCl + RNeasy isolated RNA. Although this method is relatively time-consuming, it consistently yields a good quantity of quality RNA, is economical, and does not require specialized equipment. We have stored Tris-LiCl + RNeasy isolated RNA at -80°C for more than six months without noticeable degradation in quality, and even after four freeze-thaw cycles it is still competent for RT-PCR. Furthermore, we have performed high-quality global transcript analysis with Tris-LiCl + RNeasy isolated RNA using the Vitis Affymetrix...
GeneChip (www.affymetrix.com). The average coefficient of variation for six technical replicates from young shoots of unstressed plants was low (16%), indicating that the quality of the RNA was good.

In a few cases, we have had some degraded RNA from berry extractions using the Tris-LiCl method. The RNeasy Midi + PEG method was tested for berry RNA isolation, and it gave consistently good quality and adequate yield in a much shorter time than the Tris-LiCl + RNeasy method. This method was also tested on leaves and roots (Tables 1 and 3, Figure 2). It seems to be very effective at removing polysaccharides and phenolics from leaves and berries; the absorbance at 230 nm was nearly undetectable for many samples, giving unusually high A260/230 ratios (Table 1). RNeasy Midi + PEG RNA from leaves or berries was RT-PCR competent (3/3 reactions), but the RNA from roots failed to amplify by RT-PCR.

The Tris-LiCl method was also applied successfully both to roots and to berries at various developmental stages (from preveraison to over-ripeness). The mean yield for roots was 20 mg/g FW and for berries was 40 µg/g FW (Table 4). With a Tris-borate method, Franke et al. (1995) reported that good-quality RNA was obtained from berries at stages up to the beginning of veraison, but not from postveraison berries. The average yield for five preveraison stages of berries was 26 µg/g FW. Using an extraction protocol similar to the Tris-LiCl ultracentrifugation and Tris-LiCl methods, Tesniere and Vayda (1991) reported yields from berries of 18 µg/g FW, based on at least three replicates. These yields are half of what we obtained using the Tris-LiCl method (40 µg/g FW) and their method requires an ultracentrifugation step. A yield of 30 µg/g FW was obtained from berries using the RNeasy Midi + PEG method.

**Conclusions**

The choice of method depends upon the application in which the RNA will be used. Yield, A260/280, and A260/230 ratios are not necessarily good indicators of RT-PCR–competent RNA. RNA should be checked for degradation. For RT-PCR, only four extraction protocols gave consistent product: NaClO₄, TRIzol-PEG, RNeasy Midi + PEG, and Tris-LiCl (with or without RNeasy). The TRIzol-PEG extraction had significant RNA degradation, making quantification of RT-PCR questionable. Sodium perchlorate extractions had slight RNA degradation, low extraction efficiency, and cost substantially more than the Tris-LiCl method. The modified Tris-LiCl + RNeasy method was a good choice for large quantities of RNA because it provided a good yield and excellent quality for low cost; it
also provided high-quality and consistent results for shoots and leaves for both RT-PCR and transcript profiling using oligonucleotide-based microarrays. The RNeasy Midi + PEG method gave lower yields and was significantly more expensive than Tris-LiCl + RNeasy, but it was faster and provided high-quality undegraded RNA. It was also less prone to error but was unsuitable for obtaining RT-PCR–competent RNA from roots. For small quantities of RNA from leaves or berries, such as for RT-PCR or microarrays, the RNeasy Midi + PEG method provided ease of use and excellent quality.

**Literature Cited**


