TOTAL NUCLEIC ACID (TNA) ISOLATION

This procedure is adapted from White and Kaper (1989).

1. Grind 0.15-0.25 g of plant tissue in a sterile mortar chilled on ice. Add 600 µl of freshly prepared extraction buffer and continue homogenization for 5 sec.
2. Transfer the homogenized material by pipetting to a chilled sterile microcentrifuge tube containing 600 µl of phenol. Mix immediately by vortexing for 10 sec. Centrifuge 5-10 min in a tabletop centrifuge at 16,000 X g, 4°C.
3. Transfer the upper phase to a fresh tube placed on ice containing 600 µl of 25:24:1 phenol/chloroform/isoamyl alcohol and repeat the extraction as described in step 2. Avoid precipitated material from the interface because it contains proteins. If a large precipitate forms after the first phenol/chloroform/isoamyl alcohol extraction, additional extraction may be required to remove all proteins including endogenous RNases.
4. Transfer 500 µl of upper phase to a new tube chilled on ice containing 500 µl of chloroform/isoamyl alcohol. Vortex 10 sec and centrifuge 5 min at 16,000 X g, 4°C.
5. Again, transfer 400 µl of upper phase to a sterile tube and precipitate the total nucleic acid by adding 1/20 volumes of 4 M sodium acetate, pH 5.2, and 2.5 volumes absolute ethanol. Mix by inversion and incubate 10-15 min on ice or store at -20°C overnight.
6. Recover the TNA by centrifuging for 10-15 min at 16,000 X g, 4°C. Remove the supernatant by aspiration.
7. To remove residual salts rinse the pellet with equal volumes of 70% ethanol used for precipitation and centrifuge immediately 5 min as described in step 2. Avoid resuspending the TNA pellet in 70% ethanol, because short RNAs are soluble to some extent in 70% ethanol in the absence of salt.
8. Remove the ethanol by aspiration without disturbing the pellet and collect the residual ethanol at the bottom of the tube by an additional 10 sec centrifugation. Remove the residual liquid completely using a small pipette tip and dry the tube at room temperature for 5 minutes.

9. Place the tube on ice, dissolve the pellet in 50 µl RNase-free water. Dilute 2 µl into 200 µl water to estimate the concentration of the total RNA by measuring the absorbance ($A_{260}$) in a quartz/plastic disposable cuvette. Store the remaining TNA extract at -70°C. Quality of RNA can be checked by denaturing 2-5 µl of sample with one volume of gel-loading solution at 65°C for 5 min and analyzing on a 1% agarose gel. The procedure can be scaled up if required. Grind the tissue in a mortar with liquid $N_2$ than add the powder to a 15 ml Falcon tube containing equal volume of phenol and extraction buffer. Mix immediately by vortexing for 15 sec. Centrifuge 5-10 min at 4,000 rpm, 4°C and proceed the protocol from step3.

LITERATURE CITED
MATERIALS

10X EB (see Reagents and Solutions)
Extraction buffer (see Reagents and Solutions)
Tris-buffered water-saturated phenol, pH 8.0
25:24:1 (v/v/v) phenol/chloroform/isoamyl alcohol
Chloroform
4 M sodium acetate pH 5.2
Absolute ethanol
Gel-loading solution (see Reagents and Solutions)
5X TBE (see Reagents and Solutions)
10 mg/ml ethidium bromide
Pestle and mortar
Liquid nitrogen
FALCON BLUE 15 ml Polypropylene conical tube
Refrigerated laboratory centrifuge with swing out rotors (Sigma 4K15C)
Spectrophotometer and 1-cm quartz cuvette/Eppendorf UVette
360-nm UV transilluminator
Table top centrifuge (Eppendorf 5415 D)

REAGENTS AND SOLUTIONS

Use deionized, distilled and autoclaved water in all recipes and protocol steps.

EB, 10X

1 M Glycine
100 mM EDTA
1 M NaCl
Adjust to pH 9.5, autoclave and store at room temperature. Avoid direct sunlight.
**Extraction buffer**
1X EB
2% SDS
Prepare freshly, keep at room temperature until use

**Gel-loading solution**
10 ml deionized formamide
200 μl 0,5 M EDTA, pH 8.0
1 mg xylene cyanol FF
1 mg bromophenol blue
Store up to 1 year at 4°C

**TBE, 5X**
54 g of Tris base
27.5 g of boric acid
20 ml of 0.5 M EDTA, pH 8.0
Adjust volume to 1 litre with water and sterilise by autoclaving, store at room temperature