RNA Isolation for cDNA Library Construction
(Modified Rutger van der Hoeven Protocol)

Procedure:

Harvest 2-3 grams of plant tissue using clean technique. (We typically put tissue into 15 or 50mL tubes that are sitting in liquid nitrogen as it is collected.)

Store tissue at -80^{o}C until RNA extraction.

Use liquid nitrogen to precool a mortar and pestle.

Grind the plant material into a very fine powder, supplying more liquid nitrogen when necessary to ensure powder remains frozen. It is easier to obtain very fine powder if the LN is allowed to evaporate completely at the end of the grind – DON'T let the tissue warm up – this requires speed & care!)

Collect powder in a precooled 15 or 50mL tube and place at -80°C. The powder can be stored here indefinitely. (Keep tubes in liquid nitrogen when working at the bench in the subsequent steps.)

Heat water bath to 85°C.

To a sterile 40ml, polypropylene Oak Ridge tube, add a 1:1 mixture of RNA extraction buffer (10ml) and phenol (10ml).

Determine the weight of the tube with the 1:1 RNA extraction buffer/ phenol mixture.

Heat the 1:1 RNA extraction buffer/ phenol mixture to 85°C in the water bath. This mixture is ready when the phases no longer separate quickly when mixed.

Transfer ~2.5-3.5g (5-6 spatula 'spoonfuls') of frozen, powdered tissue into the 20mls of 1:1 RNA extraction buffer/ phenol.

Quickly add the frozen powder, vortex briefly & reweigh tubes. **If using freshly ground tissue be sure that no liquid nitrogen remains in the powder.** Subtract original weight from the final weight to get an accurate weight of the amount of tissue used.

Vortex sample (high speed) for at least 30 seconds (or shake to mix).

Add 10ml chloroform.

Vortex again vigorously and then centrifuge for 20 minutes at 12,000 x g in JA-25-50 rotor (or equivalent) at 4^{o}C.

Using a 25ml glass pipette, transfer the resultant aqueous phase (upper layer) to a new polypropylene Oak Ridge tube.

Repeat chloroform extraction (2-3x) until no interphase can be detected (use a volume equal to the aqueous phase).

Transfer resultant aqueous phase to a new polypropylene Oak Ridge tube. (This can be stored frozen for 24 hours.)

Add an equal volume of 8M LiCl and precipitate overnight sitting in a container of ice in a 4°C refrigerator (or at least 3 hours).

Centrifuge at 12,000 x g in JA-25-50 rotor at 4^{o}C to precipitate the RNA.

Remove supernatant, taking care not to disturb whitish pellet.

Resuspend pellet in 400ul sterile DEPC-treated ddH_2O.

Vortex to dissolve pellet & transfer to a 1.5ml microfuge tube.

Add 400ul phenol and vortex for 30 seconds. Add 400ul chloroform & vortex 30 seconds more.

Centrifuge at 4^{o}C for 10-15 min. Transfer aqueous phase to a clean tube.

Extract aqueous phase 3x more with equal volumes of chloroform.

Add 10% (v/v) of 2M NaOAc, pH 5.0 (DEPC treated).

Add 2 volumes ice-cold 100% ethanol & precipitate O/N at -20^{o}C.

Centrifuge at 4^{o}C for 10-15 min. There should be a fairly large and visible whitish pellet. Aspirate off the supernatant and dissolve pellets in 500ul DEPC-treated ddH_2O.

Check purity & concentration with the spectrophotometer (1:50 & 1:100 dilutions usually give a good measurement range on the Spec.). Calculate concentration.

Run a 5ug aliquot on a formaldehyde gel to check the quality of the RNA. The gel can be northern blotted if desired. Freeze the remaining sample at -80^{o}C.

Solutions:

RNA Extraction Buffer		100ml	250ml
100.0 mM	Tris-HCl (pH 8.0)	10ml of 1M Stock	25ml of 1M Stock
100.0 mM	LiCl	0.424g	1.06g
10.0 mM	EDTA	2ml of 0.5M Stock	5ml of 0.5M Stock
1.0 %	LiDS or SDS	10ml of 10% Stock	25 ml of 10% stock

Buffered Phenol

Invitrogen cat no. 15513021

8M LiCl	100ml	250ml
LiCl	33.9g	84.78g
DiH2O	to volume	to volume
DEPC (O/N)	100ul	250ul
Autoclave		

2M NaOAc pH 5.0	50ml
NaOAc	13.61g
Glacial acetic acid	to pH 5.0
ddH_2O	to 50ml
DEPC	50uL
Incubate at RT	1-2hrs.
Autoclave	

Notes:

We use microfuge tubes straight out of the bag from Sarstedt (cat no. 72.690) and consider these to be RNase free.

Mortars and pestles are cleaned and dried and then stored at -20^{o}C until use but are not DEPC treated or baked.

Pipettes are baked at 220^{o}C overnight.

Spatulas are not baked but are wiped with EtOH just before using.