RAPID RNA EXTRACTION

make up all solutions with 0.1% DEPC treated dH20
use filter tips if RNA/DNA is to be used for PCR

1. Harvest tissue (preferably 5+ small Arabidopsis leaves, but more tissue=more RNA) and flash freeze in liquid N_2.

2. Grind tissue in liquid N_2 in a pestle and mortar and transfer a spatula full to an eppendorf containing 200µl **TLES buffer**.

3. Add 200 µl phenol/chloroform/IAA (25:24:1) and shake by hand, or vortex briefly.

4. Spin at 13K for 10'. Remove upper layer to fresh eppendorf and dispose of remaining debris.

5. Repeat phenol/chloroform extraction (steps 4-5).

6. Add 2.5 x volume ethanol EtOH and 0.1 x volume 3M NaOAc pH 4.5 and leave at –20°C for 30'.

7. Spin at 13K for 10'. Remove and dispose of supernate and air-dry pellet.

8. Dissolve pellet in 75µl DEPC treated H_2O, add 25µl 8 M LiCl and leave
on ice for 30'.

9. Spin at 13K for 10' and wash pellet in 70% ethanol (5'spin).

10. Air dry pellet for 5-10'.

11. Resuspend in DEPC treated H_2O (25-40µl).

TLES buffer (10ml)

50mM Tris-HCl pH 9.0	250 µl 2 M
150mM LiCl	188 µl 8M
5mM EDTA	100 µl 0.5 M
5% SDS	5 ml 10%