RNA EXTRACTION (HOT PHENOL)

keep all tubes on ice
make up all solutions with 0.1% DEPC treated dH20
use filter tips if RNA/DNA is to be used for PCR
use phenol and phenol chloroform equilibrated to pH 9.0-9.4

1. Prewarm 5.0 ml phenol and 5.0 ml **RNA Extraction Buffer** at 60ºC. Grind tissue (0.5 to 2.0g) in liquid N_2 and transfer to 30 ml tube. Shake tubes well and put on ice.

2. Spin 8K 10' and transfer supernate to new 30 ml tube on ice. Add 5.0 ml chloroform:isoamyl alcohol and shake well.

3. Spin 8K 10' and transfer aqueous layer to new 30 ml tube. Add 5ml isopropanol to precipitate nucleic acids.

4. Spin 8K 10'. Pour off supernate and rinse pellet in 70% EtOH.

5. Resuspend pellet in 750 µl DEPC-treated dH20. Add 250 µl 8M LiCl and place at 4°C overnight.

6. Spin 13k 30'. Pipette off supernate (precipitate DNA from supernate with = volume isopropanol, wash in 70%, dry and resuspend in TE). Wash pellet with 70% EtOH and dissolve in DEPC treated dH20 on ice.

7. Run 5µl on a 0.8% agarose gel to check integrity of RNA. Take OD A260/A280 to quantitate.

RNA Extraction Buffer (100 ml)

100 mM Tris pH 9.0	5 ml 2M Tris pH 9.0
1% SDS	1 g SDS
100 mM LiCl	1.25 ml 8M LiCl
10 mM EDTA	2.0 ml 0.5M EDTA