RNA/DNA EXTRACTION

keep all tubes on ice
make up all solutions with 0.1% DEPC treated dH_2O (except lysis buffer)
use filter tips if RNA/DNA is to be used for PCR

1. Grind tissue (1 - 5g) in liquid N_2 and transfer to 30 ml tube containing 10 ml **Lysis Buffer** and 5 ml phenol. Shake tubes well and put on ice.

2. Add 5 ml 24:1 chloroform:isoamyl alcohol and shake well.

3. Spin 8K, 5 ' and transfer supernate to a new 30 ml tube on ice. Add 10 ml phenol:$CHCl_3$:IAA (25:24:1) and shake well.

4. Spin 8K, 10' and transfer aqueous layer to new 30 ml tube, staying away from interface. Precipitate with 50 µl acetic acid & 0.6 volumes isopropanol.

5. Freeze at –70°C for 30' or –20°C overnight.

6. Spin 8K, 10'. Pour off supernate and rinse pellet in 95% EtOH.

7. Dry and dissolve the pellet in 400 µl DEPC treated dH_2O + 0.1% SDS. Pipette up and down to dissolve. Place at 37°C for 10' and then on ice for 15'. At this time there may still be a lot of stuff not dissolved but most of it is not nucleic acid so spin briefly in the microfuge and remove the supernate to a new tube.

8. Add 100 µl 8M LiCl and place at 4°C overnight.

9. Spin 10'. Pipette off supernate to eppendorf tube and precipitate with 2 x volume EtOH **(this is the DNA).**

10. Dissolve pellet in 400 µl DEPC dH_2O and add 40 µl 3M NaOAc pH 4.5.

11. Add 1 ml 95% EtOH and freeze at –70°C for 10'.

12. Spin 10' and resuspend pellet in 40 μl DEPC dH_2O.

13. Place at 60°C for 10' and then on ice for 30'. After this time, all RNA should be dissolved. Spin in microfuge for 10' and then transfer liquid to a fresh tube, leaving behind opaque viscous pellet.

14. Run 1 μl on 0.8% agarose gel to check integrity of RNA.

RNA Lysis Buffer (100ml)

100 mM Tris-HCl pH 8.6	5 ml 2M
2 % sarkosyl	2 g
4 M Guanidine thiocyanate	50 g
25 mM EDTA	5 ml 0.5 M
25 mM EGTA	5 ml 0.5 M
100 mM β-mercaptoethanol	694 μl
20 mM diethylcarbamic acid**	343 mg

** Only need to use DECA if tissue has a high concentration of polyphenolics