

RNA/DNA EXTRACTION (small scale)

keep all tubes on ice

make up all solutions with 0.1% DEPC treated dH₂O (except lysis buffer)

use filter tips if RNA/DNA is to be used for PCR

1. Grind tissue (250-350 mg) in liquid N₂ and transfer to 30 ml tube containing 700 μ l **Lysis Buffer** and 350 μ l phenol. Shake tubes well and put on ice.
2. Add 350 μ l 24:1 chloroform:isoamyl alcohol and shake well. Transfer the mix into 2 tubes of 1.5 ml Eppendorf tubes (so that it's easier to handle).
3. Spin 13K, 5' and transfer supernate to a new Eppendorf tube on ice. You now have two tubes of 350 μ l for each sample. To each add 350 μ l phenol:CHCl₃:IAA (25:24:1) and shake well.
4. Spin 13K, 10' and transfer aqueous layer to a new Eppendorf tube, staying away from interface. Collect two 350 μ ls from the same sample in one tube. Precipitate with 3.5 μ l acetic acid & 0.6 volumes isopropanol.
5. Freeze at -70°C for 30' or -20°C overnight.
6. Spin 13K, 10'. Pour off supernate and rinse pellet in 95% EtOH.
7. Dry and dissolve the pellet in 400 μ l DEPC treated dH₂O + 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 μ l 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 μ l DEPC dH₂O and add 40 μ l 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 μ l DEPC dH₂O.
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 μ l 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 μ l