**Plant DNA Extraction Procedure**

1. Freeze 100 mg plant material in liquid nitrogen
2. Grind to a powder in a mortar and pestle, keeping frozen at all times
3. Carefully tap into 400 ul DNA extraction buffer. Add a little at a time, being sure to **thaw in buffer** and **not in its own juices.**
4. Incubate at 65˚C for 5 mins
5. Extract with equal volume of 1:1 (v:v) Phenol:Chloroform (~450 µl) by shaking tubes vigorously, then placing on shaker for 10 mins
6. Centrifuge @ 14K RPM for 5 min.
7. Transfer as much of top phase as you can to fresh tube.
8. Add equal volume of isopropanol and mix gently but thoroughly.
9. Centrifuge @ 14K RPM for 5 min with hinges facing outwards.
10. Discard supernatant, then centrifuge for 5 seconds with hinges facing outwards.
11. Remove supernatant with pipetor, then “wash” pellet with 1ml 70% EtOH. Add 1 ml 70% EtOH, mix by inverting gently several times, then centrifuge @ 14K RPM for 1 min (if pellet is visible, 5 min if invisible) with hinges facing outwards.
12. Discard supernatant, then centrifuge for 5 seconds with hinges facing outwards.
13. Repeat steps 13 &14.
14. Remove as much fluid as possible, then air-dry until no fluid is left.
15. Dissolve DNA in 100 µl molecular grade H2O
16. Determine concentration using nanodrop.

DNA Extraction Buffer is 250 mM Nacl, 200 mM Tris-Cl, 25 mM EDTA, 0.5% SDS pH 7.5

For 100ml

20 ml 1M Tris-Cl (pH 7.5)

5 ml 5M NaCl

5 ml 0.5M EDTA

5ml 10% SDS

Fill to 100 ml with dH20