# DNA Isolations and Purification

## Small Scale DNA Isolation

1. Grind freeze dried tissue in 2mL tube.
2. Add 700uL CTAB to tissue, vortex.
3. Incubate at 65°C for 60-90 minutes, invert every 15 min.
4. In fume hood, add 400uL Chloroform:IA (24:1), vortex.
5. Spin 5 minutes.
6. Transfer top phase to a new 1.6mL tube.
7. Add 400uL Chloroform:IA, vortex.
8. Spin 5 minutes.
9. Transfer top phase to new 1.6mL tube.
10. Add 300uL Isopropanol, mix by inversion.
11. Spin 5 minutes, pour off top phase.
12. Add 500uL 70% EtOH, vortex.
13. Spin 5 minutes.
14. Pour off top phase, air dry.
15. Resuspend in 250uL water or low Tris (pH8)

## Large Scale DNA Isolation

1. *Perform in a fume hood with protective goggles.*
2. Add 300-400 mg dry tissue to a 15mL Falcon Tube (screw cap).
3. Add 7 mL CTAB stock solution (with freshly  added β-mercaptoethanol). Vortex to mix well.
4. Incubate 90 min. at 65°C. Invert every 15 minutes.
5. Remove tubes from the bath and cool 10 min.
6. Add 8mL 24:1 Chloroform: IsoAmyl alcohol.
7. Invert for 10 min. on the rocker.
8. Centrifuge for 10 min. at 3,700 rpm.
9. Pour off the supernatant into a new snap-top tube.
10. Add 5uL of 20 mg/mL Rnase A. Incubate 30 min. at 37°C.
11. Add 4 ml C/I and shake 10 min.
12. Centrifuge for 10 min. at 3,700 rpm.
13. Remove the supernatant with transfer pipette into a new snap-top tube.
14. Add 5 ml isopropanol to precipitate DNA.
15. Remove DNA with glass hook or spin down to pellet the precipitated DNA
16. Re-suspend DNA in 300-500ul nuclease-free water.
17. Transfer to a 1.5mL tube.
18. Quantify DNA using a nanodrop.

## DNA Cleanup

1. Add equal volume of phenol:chloroform to DNA sample and mix well/vortex gently.
2. Spin at 8,000rpm for 3 minutes.
3. Transfer the aqueous layer to a new 1.5mL tube and add equal volume chloroform.
4. Spin at 8,000rpm for 3 minutes.
5. Transfer the aqueous layer to a new 1.5mL tube.
6. Add 0.1 volume cold 3M Na-Acetate (pH 5.5) and 2 volumes cold 100% ethanol.  Incubate for 30 minutes to 1 hour at -20°C.
7. Spin at 10,000rpm for 10 minutes, 4°C.
8. Remove supernatant and wash with 400uL cold 70% ethanol.
9. Spin at 10,000rpm for 5 minutes and remove ethanol.
10. Air dry for 5-10 minutes.
11. Resuspend DNA in 200uL nuclease-free water.
12. Quantify DNA using a nanodrop.