**Large-Scale DNA Extraction** (modified from Doyle and Doyle, 1987)

1. Use a 10% Bleach solution to wash 5g of fresh leaf tissue (preferably young), and then rinse immediately in water.
2. Quickly pat the leaves dry and grind in liquid nitrogen with a mortar and pestle (do not let the leaves thaw). This usually requires at least 3 rounds of grinding.
3. After the tissue has been ground into a fine pounder, add 20ml of 2% CTAB buffer w/BME that had been preheated to 65C.
4. Grind the tissue in the buffer to mix well and then transfer to a 50mL Falcon tube.
5. Incubate in a 65C water bath for 1 hour, inverting to mix every 15 minutes.
6. Remove the samples from the water bath and allow them to cool to RT.
7. Add 20mL of phenol/chloroform/isoamyl alcohol (25:24:1).
8. Mix well by inverting the tube until the phases are no longer separate.
9. Spin at 4,000g for 30 minutes at 4C.
10. Transfer the aqueous phase to a clean 50mL Falcon tube on ice.
11. Add an equal volume of chloroform/isoamyl alcohol (24:1) and repeat steps 8-10.
12. Add an equal volume of Isopropanol (prechilled to 4C).
13. Mix by gently inverting until phases are no longer separate and DNA has precipitated.
14. Use a glass Pasteur pipette to spool the DNA and transfer it to a clean 50mL Falcon tube containing 25mL of 76% EtOH, 10mM ammonium acetate.
15. Mix by gently inverting to wash the pellet.
16. Use a glass Pasteur pipette to hook the DNA pellet and transfer it to the side of a clean 15mL Falcon tube.
17. Allow the pellet to air dry (sometimes overnight).
18. Re-suspend the pellet in 1xTE buffer (I prefer to start with 1mL and continue to add TE until the pellet is solubilized). This can be done overnight using a platform shaker set to a low speed at 4C.
19. Add 3-5ul of 10ug/ul RNase A for every 1mL of TE buffer.
20. Incubate at 37C with gentle shaking (50 rpm) for 1 hour.
21. Add an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1).
22. Mix well by inverting the tube until the phases are no longer separate.
23. Spin at 4,000g for 30 minutes at 4C.
24. Transfer the aqueous phase to a clean 15mL Falcon tube on ice.
25. Add an equal volume of Isopropanol (prechilled to 4C).
26. Mix by gently inverting until phases are no longer separate and DNA has precipitated.
27. Spin at 4,000g for 15 minutes at 4C.
28. Pour off the supernatant and wash with 10mL of 70% EtOH (prechilled to 4C).
29. Spin at 4,000g for 15 minutes at 4C.
30. Repeat 28-29 once.
31. Allow the pellet to air dry.
32. Re-suspend the pellet in 1xTE buffer (I prefer to start with 0.5mL and continue to add TE until the pellet is solubilized). This can be done overnight using a platform shaker set to a low speed at 4C.
33. Quantify the DNA using a Qubit, and check the quality of the DNA on a 0.8% agarose gel with Lambda DNA standards.

**2% CTAB Buffer**

(2% CTAB, 100mM tris, 1.4M NaCl, 20mM EDTA)

1M tris (pH 8) 5mL

5% CTAB 20mL

5M NaCl 14mL

05M EDTA (pH 8) 2mL

dH2O 8.5mL

BME 0.5mL

50mL