**CTAB DNA Extraction Protocol**

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**BEFORE STARTING:**  
1. Make sure isopropanol/100% ethanol and chloroforme/isopropanol is in freezer (needs to be cold when mixed with aqueous solution).

Note: using isopropanol ensure a higher yield of DNA as DNA is less soluble in isopropanol than 100% ethanol. However, salts will also precipitate. Using 100% cold ethanol result in higher quality DNA but lower yield of DNA.

2. Make sure 80% ethanol remains cold.

3. Samples should always be in fridge (or freezer for long term storage) before or after DNA extraction.

**DAY 1: Sample homogenization and extraction**

1. Place 40 - 60 mg of tissue in a mortar. Add liquid nitrogen until it covers the leaf tissue. While the liquid nitrogen is evaporating, break the tissue into pieces and grind when liquid nitrogen is almost gone. Repeat if samples are not grinded enough. With a spatula pick 25-30ng of grinded tissue in a fresh tube, save the remaining tissue.

2. Add 600 µl of pre-warmed CTAB buffer + B-me to each sample. Use the pipette tip to break the leaf powder plug at the bottom of the tupbe

3. Vortex tubes to ensure homogenate is dissolved in CTAB buffer. If necessary, grind samples in homogenizer for an additional cycle.

4. Incubate the tubes at 65ºC for 1 hour. After incubation, cool samples at room temperature for 15 minutes.

5. In a fume hood, add 5 µl of Proteinase K to each tube, Incubate at 50º C for 30 minutes. After incubation, cool samples at room temperature for 15 minutes.

6. In a fume hood, add 6 µl of RNAse A to each tube. Incubate at 37º C for 30 minutes.

7. In a fume hood, add 600 µl of (24:1) chloroform:isoamyl alcohol. Rock or shake vigorously for 5 minutes to form an emulsion.

7. Centrifuge for 20 minutes at 14,000 rpm. You will see 3 layers:

* Top - clear solution with nucleic acids (this is what we want)
* Middle - cellular debris and proteins (avoid sucking this up, you will get streaky DNA)
* Bottom - chloroform (avoid at all costs as chloroform will inhibit downstream restriction digest/PCR)

8. In a fume hood, use a P-1000 pipette (100 - 1000 µl) to remove the aqueous (top) phase to a new, flip-top tube. You will yield ~ 400 - 500 µl. Do not give into the temptation to try and suck all of the aqueous phase out. Using the P-1000 prevents mechanical shearing of DNA.

9. Dispose of the tubes with middle and bottom phase follow the hazardous waste disposal guidelines mandated by your institution.

10. Add an equal volume of ice-cold, molecular-grade isopropanol or 100% ethanol to the aqueous phase. You should see a white precipitate form.

11. Mix the isopropanol-aqueous phase solution by hand. The white precipitate should dissolve into solution.

12. Incubate at -20ºC overnight (or 2 hours if chosen to use Ethanol).

**DAY 2: Clean up**

13. Centrifuge for 20 minutes at 14,000 rpm.

14. Pour off isopropanol from each tube. Drag the tube across a clean paper towel to remove any excess.

15. Wash pellets by adding 500µl of 80% cold ethanol and vortexing gently. Make sure that the pellet releases from the bottom of the tube.

16. Centrifuge for 10 minutes at 14,000 rpm.

17. Pour off the ethanol as in step 14.

18. Repeat Steps 15 - 17.

18. After the 2nd 80% EtOH wash, keep the lids off of the tubes and let the pellet dry overnight in a fume hood (this step can be reduced to few hours if chosen).

**Day 3: Elution**

19. Resuspend the pellet in 30 µl of AE (Qiagen Buffer). Recap the tubes and incubate the solution for 1-2 hours at 37ºC to assist with re-suspension (flick the tubes from time to time to assist in resuspension).

For 20 ml of CTAB buffer: (enough for ~30 samples + excess)

**6 ml CTAB 10% in H2O or 0.2g of CTAB**

**5.6 ml 5 M NaCL**

**0.8 ml 0.5 M EDTA (pH 8.0)**

**2 ml 1 M Tris-Cl (pH 8.0)**

**4.96 ml molecular grade H20**

**40 µl ß-Mercaptoethanol (add right before use)**

**0.5 g**  **PVP (polyvinylpyrrolidone)**

**Total: ~20 ml**