DNA ISOLATION USING CTAB

1. Freeze plant tissue in liquid nitrogen and grind to fine powder.

2. Place a small spatula-full amount of ground tissue in 2 ml conical bottom eppendorf. Add 1 ml of **extraction buffer** (very viscous) and mix with yellow tip or cocktail stick.

3. Incubate at 65°C for 15 minutes. Mix by inversion after 5 minutes.

4. Add 714 µl of chloroform:isoamyl alcohol (24:1). Centrifuge at 8000 rpm for 5 minutes at room temperature

5. Transfer aqueous (top) layer to a 1.5 ml eppendorf and add 70 µl of 3M ammonium acetate. Mix by inversion.

6. Add 726 µl of isopropanol and mix by inversion.

7. Centrifuge at 7500 rpm for 5 minutes at room temperature, discard supernate and wash pellet in 600 µl of 70% ethanol.

8. Centrifuge at maximum speed for 15 minutes, discard ethanol and vacuum (or air) dry pellet.

9. Resuspend pellet in appropriate volume of 1 x TE buffer.

EXTRACTION BUFFER (100 ml)

10% PEG 8000	10 ml
1 M Tris-HCl, pH 7.0	10 ml
5 M NaCl	28 ml
10% CTAB (heat to dissolve)	20 ml
500 mM EDTA	4 ml
10% SLS (sodium lauryl sarcosine)	3 ml
dH_2O	25 ml