

## GENOMIC DNA ISOLATION- CTAB BASED

1. Grind 2-3 gm of (5-6 leaves) leaves or any other tissue to a fine powder. Use liquid nitrogen if grinding with mortar and pestle and dry ice if using an electric coffee grinder. For lyophilized ground tissue use up to 5 mL mark of 50 mL tube (This is about 500-600 mg).
2. Transfer the powder to a 50 mL tube and add about 20mL of CTAB extraction buffer. Mix with a spatula breaking all the clots to make fine slurry. Incubate in a preheated water bath at 60°C for 2 to 3 hours and mix after every 15-20 min.
3. Let the tubes cool down to room temperature and add 20mL (or equal volume) of Chloroform:Octanol (24:1). Mix thoroughly but gently, or until top layer is milky green in color (Use shaker for mixing for 15-20 min).
4. Centrifuge at room temperature at 4000 rpm for 15 minutes.
5. Transfer the supernatant (using inverted 10mL pipette or 5 mL manual pipette) to a new tube and add 2/3 volume of isopropanol (room temp). Mix gently to get a DNA clot. Do not leave for long at this stage.
6. Drain the solution by retaining the DNA clot (using a pipette tip) in the tube. Wash DNA with 20mL of 76% ethanol + 0.2M Sodium acetate. Leave at room temperature for 30 minutes, mix occasionally. Drain the ethanol and repeat the wash with 10 mL of 76% ethanol + 10mM Ammonium acetate. Keep at RT for 10 min. (can be left over night at 4°C).
7. After draining ethanol, transfer the DNA pellet to 2 mL eppendorf tube and air dry the DNA pellet, and add 1000  $\mu$ L 1mM Tris and 1  $\mu$ L of RNAase (10mg/mL). Incubate at 37°C for about 10 minutes. Leave at 4°C overnight. If not dissolved, incubate in 65°C water bath until dissolved, with inversion and gentle tapping every 30 to 60 min or until dissolved (often several hours).
8. Add an equal volume (1000 $\mu$ L) of phenol:CI (1:1), (CI is a mixture of chloroform and isoamyl alcohol (24:1). Mix thoroughly but gently for 5-10 minutes manually or on the shaker. Spin at 14000 rpm at room temperature in a microcentrifuge for 15 minutes.
9. Carefully transfer the supernatant to a new 2 mL eppendorf tube using a wide bore pipette tip.
10. Add 1/10th volume of 3M NaAc, pH 5.5 and 1 volumes of cold isopropanol and mix well.
11. Drain the solution by retaining the pellet in the tube (use a pipet tip to hold the pellet). Fill the tube with 70% ethanol and leave for at least 30 min. The pellets may also be left in 70% ethanol overnight at 4°C.
12. Drain the ethanol and dry the pellet in the speed vac (5-20 minutes) (air drying at room temperature as well as in laminar flow works too). Resuspend the pellet in 1000 $\mu$ L or 1mL of 1mM Tris. Leave it overnight for dissolving.

13. Determine the DNA concentration by running a 1:40 dilution {DNA:(3 TE:1 loading buffer)} on an agarose gel along with a conc. marker (uncut lambda DNA at conc. of, 10, 25, and 50 ng). For details, See DNA Quantification protocol. Visually compare the intensity of the DNA bands with the markers to determine the concentration.

### CTAB EXTRACTION BUFFER

[For DNA extraction from lyophilized tissue use **1X** CTAB extraction buffer. If extracting DNA from fresh leaves ground with liquid Nitrogen or dry ice use **2X** CTAB extraction buffer].

#### 1X CTAB extraction buffer

| <b>Stock</b>        | <b>10 mL</b> | <b>50 mL</b> | <b>150 mL</b> |
|---------------------|--------------|--------------|---------------|
| dd H <sub>2</sub> O | 7.3 mL       | 36.5         | 109.5         |
| 1M Tris pH 7.5      | 1.0 mL       | 5.0          | 15.0          |
| 5M NaCl             | 1.4 mL       | 7.0          | 21.0          |
| 0.5 M EDTA pH 8.0   | 0.2 mL       | 1.0          | 3.0           |
| CTAB                | 0.1 g        | 0.5          | 1.5           |
| *14M BME            | 0.1 mL       | 0.5          | 1.5           |

#### 2X CTAB extraction buffer

| <b>Stock</b>        | <b>10 mL</b> | <b>50 mL</b> | <b>150 mL</b> |
|---------------------|--------------|--------------|---------------|
| dd H <sub>2</sub> O | 4.6 mL       | 23.0         | 69.0          |
| 1M Tris pH 7.5      | 2.0 mL       | 10.0         | 30.0          |
| 5M NaCl             | 2.8 mL       | 14.0         | 42.0          |
| 0.5 M EDTA pH 8.0   | 0.4 mL       | 2.0          | 6.0           |
| CTAB                | 0.2 g        | 1.0          | 3.0           |
| *14M BME            | 0.2 mL       | 1.0          | 3.0           |

**CTAB** = Cetyl Trimethyl Ammonium Bromide

**\*BME** = 2-β-mercaptoethanol: Add to warmed buffer just prior to use in the hood.