**DNA isolation from plant materials**

**Materials**

CTAB buffer (Hexadecyl trimethy l-ammonium bromide)

Microfuge tubes

Mortar and Pestle

Microfuge

Absolute Ethanol (ice cold)

70 % Ethanol (ice cold)

7.5 M Ammonium Acetate

55o C water bath

Chloroform: Iso Amyl Alcohol (24:1)

Water (sterile)

Agarose

6x Loading Buffer

1x TBE solution (Tris base, boric acid, EDTA (pH 8.0)).

Agarose gel electrophoresis system

Ethidium Bromide solution

**Procedure**

1. Grind 200 mg of plant tissue to a fine paste in approximately 500 μl of CTAB buffer.
2. Transfer CTAB/plant extract mixture to a microfuge tube.
3. Incubate the CTAB/plant extract mixture for about 15 min at 55o C in a recirculating water bath.
4. After incubation, spin the CTAB/plant extract mixture at 12000 g for 5 min to spin down cell debris. Transfer the supernatant to clean microfuge tubes.
5. To each tube add 250 μl of Chloroform : Iso Amyl Alcohol (24:1) and mix the solution by inversion. After mixing, spin the tubes at 13000 rpm for 1 min.
6. Transfer the upper aqueous phase only (contains the DNA) to a clean microfuge tube.
7. To each tube add 50 μl of 7.5 M Ammonium Acetate followed by 500 μl of ice cold absolute ethanol.
8. Invert the tubes slowly several times to precipitate the DNA. Generally the DNA can be seen to precipitate out of solution. Alternatively the tubes can be placed for 1 hr at -20 o C after the addition of ethanol to precipitate the DNA.
9. Following precipitation, the DNA can be pipetted off by slowly rotating/spinning a tip in the cold solution. The precipitated DNA sticks to the pipette and is visible as a clear thick precipitate. To wash the DNA, transfer the precipitate into a microfuge tube containing 500 μl of ice cold 70 % ethanol and slowly invert the tube. Repeat.
10. (alternatively the precipitate can be isolated by spinning the tube at 13000 rpm for a minute to form a pellet. Remove the supernatant and wash the DNA pellet by adding two changes of ice cold 70 % ethanol )).
11. After the wash, spin the DNA into a pellet by centrifuging at 13000 rpm fo 1 min.
12. Remove all the supernatant and allow the DNA pellet to dry (approximately 15 min).
13. Do not allow the DNA to over dry or it will be hard to re-dissolve.
14. Resuspend the DNA in sterile DNase free water (approximately 50-400 μl H2O; the amount of water needed to dissolve the DNA can vary, depending on how much is isolated). RNaseA (10 μg/ml) can be added to the water prior to dissolving the DNA to remove any RNA in the preparation (10 μl RNaseA in 10ml H2O).
15. After resuspension, the DNA is incubated at 65o C for 20 min to destroy any DNases that may be present and store at 4o C.
16. Agarose gel electrophoresis of the DNA will show the integrity of the DNA, while spectrophotometry will give an indication of the concentration and cleanliness.