Isolation of DNA

Dr. Mahesha H B, Yuvaraja's College, Mysore.

Aim: To isolate DNA from plant source.

Principle: Extraction of DNA is accomplished by the rupturing of cell wall, cell and nuclear membrane followed by deproteinization and precipitation of the nucleic acid using ethanol.

Materials Required:

1. Extraction buffer: 100mM Tris-Cl buffer containing 0.15 M NaCl, 0.1MEDTA, 2.5% SDS pH 7.8.
2. Chloroform and isoamyl alcohol mixture 24:1.
3. 3M sodium acetate
4. Tris-EDTA buffer: 10 mM Tris-Cl pH 7.5, 1mM EDTA.
5. 70% ethanol, Sample, Glass wares, centrifuge, water bath etc.,

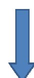
Procedure:

1. Grind 0.5gm of the sample (mulberry leaf) material in 0.5 – 0.75 ml of extraction buffer in a mortar and pestle.
2. Transfer the homogenized sample in to a micro centrifuge tube and incubate at 65 °C for 30 min.
3. After incubation centrifuge at 3000 rpm for 10 min at room temperature.
4. Collect the supernatant in to an another micro centrifuge tube. To this, add an equal amount of chloroform – isoamyl alcohol mixture and shake the contents.
5. Centrifuge the contents at 8000 rpm for 10 min at room temperature.
6. After centrifugation, carefully pipette out the upper clear aqueous phase from the coagulated protein emulsion at the interface in to a fresh tube.
7. To this, add $1/20^{th}$ volume of 3 M sodium acetate and double volume chilled ethanol and swirl gently to precipitate DNA. White fibrous DNA appears in the tube. For further precipitation incubate the tube at -20°C for 30 – 60 min.
8. Centrifuge the contents for 10 min at 8000 rpm, discard the supernatant and air dry the DNA pellet. Wash the DNA with 70 % ethanol twice.
9. Suspend the pellet in 100 µl TE buffer and store at -20° for future use.

Result: DNA is isolated from plant source, which is observed as white fibrous material.

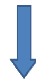
Flow chart:

0.5 g Mulberry Leaf /any plant tissue



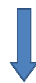
0.5 – 0.75 ml of extraction buffer

Homogenize and incubate at 65 °C for 30 min

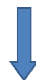


Centrifuge the homogenate at 3000 rpm for 10 min

Transfer the supernatant in to a fresh tube

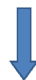


Add equal volume of Chloroform-isoamyl alcohol mixture, mix and centrifuge at 8000 rpm for 10 min

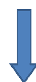


Collect the upper clear supernatant in to fresh tube and add $1/20^{th}$ volume of sodium acetate and double volume of chilled ethanol and swirl gently

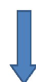
Fibrous White DNA appears



Keep the tube at -20°C for 30-60 min for further precipitation



Centrifuge 8000 rpm for 5 min; discard the supernatant and air dry the pellet



Wash in 70% ethanol (1-2 times) and suspend in 100 µl TE buffer and preserve for future use