# Aim 52

# Isolation of Genomic DNA from Eukaryotic Cells

## Introduction

It is the simplest and fast method for the isolation of DNA from plants. Homogenize the plant tissue in the medium containing SDS (denatures proteins), Urea and NaCl (Dissolve nucleoprotein complex). Chloroform-isoamyl alcohol is used to remove proteins and alcohol finally precipitate DNA.

**Requirements**

1. Fresh leaves of lemon, chilli, curry, pudina, tomato, potato, hibiscus, mango etc. in dry form.
2. Chloroform: isoamyl alcohol (24 : 1).
3. RNase A solution.
4. DNA extraction buffer:

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|---|---|
| a. Urea | 42 g |
| b. Redistilled phenol | 5 ml |
| c. 1 M Tris –EDTA-HCl (pH 8.0) | 5 ml |
| d. 20 % SDS | 0.75 ml |
| e. 5 M NaCl | 7.0 ml |
| f. 0.25 M EDTA | 8.0 ml |
| g. Final volume | 100 ml |

5. Tris- EDTA (TE) buffer (1 mM EDTA in 10 mM Tris – HCl, pH 8.0)
6. Isopropanol
7. Centrifuge

**Procedure**

1. 1 g of dried powder of leaves is taken and suspended in 10 ml of extraction buffer at 65°C.
2. The mixture is mixed well and incubated at 65°C for 30 min.
3. 10 ml of chloroform : isoamyl alcohol mixture is added and mixed properly with immediate shaking.
4. Centrifuge at 5000 rpm for 10 min.
5. The formation of three layers appears.
6. The aqueous upper layer is taken out into another centrifuge tube.
7. Equal volume of cold isopropanol is added and mixed softly.
8. After 15 min the mixture is centrifuged at 10,000 rpm for 10 min.
9. Supernatant is discarded. The pellet is dried at room temperature by inverting the tube.
10. The pellet is dissolved in 1 ml of TE buffer.
11. 10 µL of RNase A (10 µg/µL) is added and incubated at room temperature for 10 min.
12. Test the quantity and quality of DNA preparation by spectrophotometric method after proper dilution.
13. The DNA is stored at 4°C for use.