Aim 57 Isolation of RNA from Plant Material by SDS Phenol Method

Introduction

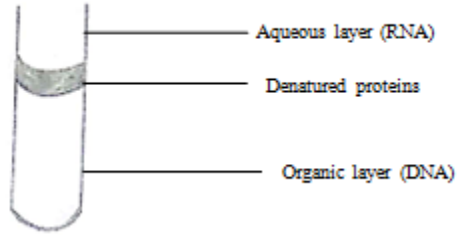
It is very simple and fast procedure for the isolation from plant material. SDS and phenol are used to denature the proteins and to dissociate the nucleoprotein complexes. Phenol : Choloroform : isoamyl alcohol are used to remove the denatured protein. During this DNA goes into the phenol phase and RNA can be obtained by precipitation of aqueous with ethanol.

Requirement

1. Plant material
2. *SDS solution*
   a. SDS 1 %
   b. Tris HCl (pH 8.0) 50 mM
   c. EDTA 5 mM
   d. Sodium acetate 0.15 M
3. *Phenol reagent*
   a. 8-hydroxy quinoline 0.5 g
   b. Redistilled phenol 500 g
   c. Water 150 ml
4. *Tris EDTA buffer (TE)*
   10 mM Tris- HCl (pH 8.0) containing 1 mM EDTA
5. *Deproteinizing solution*
   Phenol reagent : chloroform : isoamyl alcohol
   24 : 24 : 2
6. Ethanol
7. Centrifuge

Procedure

1. 1 g of plant material is homogenize in pestle and mortar with 10 ml of SDS solution.
2. Homogenate mixture is transferred to polypropylene centrifuge tube.
3. Equal volume of deproteinizing solution is added.
4. Mix it well.
5. Leave it for 10 min at room temperature with intermittent shaking.
6. Centrifuge for 10 min at 10,000 rpm at 4°C.
7. Three layers will be formed:
   - RNA (aqueous layer)
   - Denatured proteins
   - DNA (organic layer)



8. Carefully take out the aqueous layer then transfer it to another centrifuge tube.
9. Two volumes of chilled ethanol are added and mixed well. Leave it to stand in the freezer overnight.
10. Again centrifuge the tube as above.
11. Dry the pellet.
12. The precipitate is dissolved in TE buffer and by using spectrophotometer estimate the amount of RNA.
13. At 4°C store the RNA for further use.