Protocols (DNA extraction): Conventional extraction

Short Method

- Put 100mg of leaf sample in 2.0ml epindorff tube, grind using liquid nitrogen and wash 2-3 times with1000µl Hepes solution, spin down at 13000rpm for 5mins and decant supernatant to remove secondary metabolites
- Add 800µl of SDS buffer and vortex briefly, incubate in water bath at 65^0C for 30mins
- Add 600µl of Phenol, Chloroform and Iso-amyl alcohol(25:24:1), vortex and centrifuge at 13000rpm for 20mins
- Remove 600µl of the supernatant into new sterile 1.5ml tube
- Add 400µl (2/3 of supernatant) of cold isopropanol and invert mix gently
- Incubate at -80^0C for 30mins
- Centrifuge at 13000rpm for 15mins to sediment the nucleic acid
- Decant the supernatant gently and ensure the pellets are not disturbed
- Add 500µl of 70% ethanol to the pellets and centrifuge at 10000rpm for 10mins(use this to wash twice)
- Decant the ethanol and dry the Nucleic acid(pellets) at 37^0C for 30mins
- Pellets are suspended in 50µl TE buffer/sterile distilled water and store at 4^0C for dissolution.
- Add 3µl of 10mg/ml RNAse to degrade the RNA in the nucleic acid leaving only DNA
- Incubate at 37^0C for 1hr and store at -20^0C

NOTE:

For the preparation of Herpes solution (4-(2-hydroxyethyl)-1-piperazoneethanesulfonic acid) the following constituents are required:

0.1M of Hepes

PVP (polyvinylpyrrolidone)

L-ascorbic acid

2-mercaptoethanol

sterile distilled water

For the preparation of SDS-extraction buffer the following constituents are required:

1M Tris Hcl pH 8

0.5M EDTA pH 8

5M Nacl pH 8

1% Mercapethanol

2% Sodium dodecyl sulphate(SDS) and sterile distilled water

Long method (using SDS)

1. Disrupt the lyophilized yam leaf tissue with genogrinder at 1400 - 1500 RPM for 2 minutes.
2. Wash the ground leaf samples with 1m Hepes buffer 2 times

3. Add 800 µL of isolation buffer, mix well, and vortex (SDS buffer).

4. Mix contents of the tubes vigorously and incubate for 30 min at 65°C.

5. Centrifuge at 13,000 rpm for 20 min.

6. Transfer the supernatant into a clean 2ml microcentrifuge tube and add 600uL 24:1 Chloroform –Isoamyl. Mix well by inverting the tubes. Centrifuge for 20 minutes at 4000 rpm.

7. Transfer the supernatant to a clean microcentrifuge tube, and add 500 µL of cold isopropanol.

Mix gently to precipitate the DNA.

8. Incubate for 30 minutes in -80°C or 1 hour in -20°C

9. Centrifuge at 10,000 rpm for 20 min to precipitate the DNA.

10. Carefully decant the supernatant.

11. Rinse the DNA pellet with 500 µL of cold 70% ethanol twice.

12. Drain the ethanol completely, and dry the pellet for 1 hour.

13. Suspend the DNA in 100 µL of TE and 3 uL RNase. Store at -80ºC.

PURIFICATION

1. Resuspend the DNA samples in 200ul of 25:24:1 Phenol:Chloroform:Isaamyl Alcohol and mix very well by inverting the tubes
2. Centrifuge at 13,000 RPM for 20 mins
3 Carefully Collect the Supernatant without disturbing the interface to a new 1.5ml tubes
4 Add 300ul of cold Absolute Ethanol and 15ul Sodium Acetate and invert for proper mixing.
5 Incubate in -20C for 20 mins
6 Centrifuge at 13,000rpm for 10 mins
7 Remove the supernatant and wash twice with 70% ethanol.
8 Centrifuge at 10,000rpm for 10 mins
9 Decant the ethanol and dry the DNA pellets for 1 hour
10 Resuspend the DNA in 50ul TE buffer and store in -20C for subsequent use

NOTE:

For the preparation of Herpes solution (4-(2-hydroxyethyl)-1-piperazoneethanesulfonic acid) the following constituents are required:

0.1M of Hepes

PVP (polyvinylpyrrolidone)

L-ascorbic acid

2-mercaptoethanol

Sterile distilled water

For the preparation of SDS-extraction buffer the following constituents are required:

1M Tris Hcl pH 8

0.5M EDTA pH 8

5M Nacl pH 8

1% Mercapethanol

2% Sodium dodecyl sulphate(SDS)

Sterile distilled water