C-TAB

Introduction

C-TAB Protocol for Isolating DNA from Plant Tissues

Materials

- Liquid nitrogen
- Ice
- Pestles
- Stirer
- 65^{0}C Bath
- Centrifuge
- CTAB buffer
  - 2% cetyl trimethylammonium bromide, 1% polyvinyl pyrrolidone,100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA
- 2-Mercaptoethanol
- Chloroform octanol (1:24)
- Sodium acetate (NaOAc)
- 100% Ethanol
- 70% Ethanol

Procedure

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1. Grind ≃200 mg of plant tissue to a fine paste.
2. Add 700ul CTAB buffer pre-heated to 65^{0}C + 7ul 2-Mercaptoethanol.
3. Vortex.
4. Incubation at 65^{0}C for 30minute (Vortex few times).
5. Chill on ice.
6. Add 700ul Chloroform octanol (1:24).
7. Vortex.

8. Centrifuge 5min 14,000 rpm.
9. Transfer the upper aqueous phase only (contains the DNA) to a clean tube.(550-600ul)
10. Add 400ul Chloroform octanol (1:24).
11. Centrifuge 5min 14,000 rpm.
12. Transfer the upper aqueous phase only (contains the DNA) to a clean tube. (350ul)
13. Add 35ul Sodium acetate (NaOAc). (X0.1 of sample volume)
14. Add 875 ul 100% Ethanol. (X2.5 sample volume).
15. Incubate at -80^{O}C for Hour/over night.
16. Centrifuge 10min 10,000 rpm.
17. Remove the supernatant
18. Add 1ml 70% Ethanol.
19. Centrifuge 10min 10,000 rpm.
20. Remove the supernatant.
21. Air dry the pellet, invert on paper towel.
22. Resuspend the DNA in sterile DNase free water (50ul)