OBJECTIVE: Recovery of DNA from agarose gels

Chemicals required: TE Buffer warmed to 67°C, buffer equilibrated phenol, chloroform, Phenol/chloroform (1:1), 100% ethanol (ice cold), 70% ethanol (room temperature), TE Buffer, pH 8.0, 10 M ammonium acetate

Plastic ware/glassware: autoclaved micro centrifuge tubes, 1.0 ml tips, 0.02ml-0.2 ml tips, enzyme tips, Scalpel or razor blade, glass or plastic stir rod

Equipments required: vortex mixer, microcentrifuge

PROTOCOL

1. Electrophorese DNA in an agarose gel prepared in 1X TAE Buffer.
2. Excise gel slice containing DNA and put it in a microcentrifuge tube.
3. Weigh the gel slice and calculate volume based on weight.
4. Mash the gel slice with the stir rod.
5. Add an equal volume of buffer-equilibrated phenol and vortex for 10 seconds.
6. Freeze at -70°C for 5 to 15 minutes.
7. Centrifuge for 15 minutes at room temperature and remove the supernatant which contains the DNA. Place the supernatant, in a clean microcentrifuge tube.
8. Phenol/chloroform extract the supernatant.
9. Follow with a chloroform extraction.
10. Chill the supernatant on ice for 15 minutes.
11. Centrifuge the sample for 15 minutes at maximum speed in a microcentrifuge.
12. Carefully collect the supernatant and place in a clean micro centrifuge tube.
13. Measure the volume of the sample and add 0.2 volumes of 10 M ammonium acetate to the sample. Add 2 volumes of 100% ice-cold ethanol.
14. Briefly vortex.
15. Store the mixture for 30 minutes to overnight at room temperature.
16. Centrifuge for 30 minutes at 12,000 rpm.
17. Decant the supernatant.
18. Wash the pellet with 70% ethanol.
19. Allow to air-dry at room temperature on the bench top.
20. Dissolve the DNA in TE Buffer.