### Modified Phenol Extraction of DNAFrom Low Melting Point Agarose

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1. Surface stain gel briefly (about 30 minutes) with ethidium bromide, or use an indirect visualization method. Excise bands into a plastic tube in foil.

2. Weigh band. Transfer to a silanized Corex tube.

3. Add one volume of 2 buffer:

|  |  |
| --- | --- |
|  | for 100 ml |
| 400 mM NaCl | 8.0 ml 5 M NaCl |
| 20 mM Tris-Cl pH 7.4 | 2.0 ml 1 M Tris |
| 2 mM EDTA | 0.4 ml 0.5 M EDTA |

 Melt at 68° until all signs of agarose disappear (keep dark)

4. Add Tris-saturated phenol (*no* chloroform) in the ratio of 400 l phenol to one ml original buffer volume in the following manner:

a. While agarose is warm, add ~1/3 of the phenol and immediately vortex briefly to mix. It should be fairly clear.

b. Add second 1/3, vortex. It should be cloudy.

c. Add remaining phenol. A flocculant precipitate appears.

5. Immediately spin tubes 10k rpm 10 minutes at 4°C. (Cold rotor, room temp adapter)

6. Quickly pipette supernatant into new corex, avoiding agarose-phenol pellet. Add about 1 volume chloroform:octanol (24:1). Vortex until homogenized to a milky white. Allow to sit on ice until layers separate (about 1 minute). Remove lower (cloudy) phase and discard.

7. Butanol reduce volume about 1/2 (not more). Chloroform-octanol extract 1 time more. Ethanol precipitate aquaous phase. Wash pellet with 70% ethanol-acetate, before drying and resuspending. (Spin 5 minutes at 5k rpm, for butanol reduction and chloroform extraction)

### FREEZE SQUEEZE PURIFICATION OF DNA RESTRICTION FRAGMENTS

1. Run the DNA on a gel

2. Stain and visualize bands with UV light

3. Cut at bands and place in 15 ml tubes

4. Place in freezer for 5-10 min

5. Mash fragments with a glass rod (do not use round ended rod)

6. Add 100 l phenol per gel slice

7. Vortex for 10 seconds

8. Freeze at -20°C for 30 min (5-15 min at -70°C)

9. Centrifuge for 15 min at room temperature

10. Remove upper (agareous) layer and transfer to a new tube

11. Extract with phenol:

Add an equal volume of phenol
Vortex for 10 seconds
Centrifuge for 5 min
Collect the upper phase

12. Extract once with phenol-chloroform

13. Add 10 l of 3 M Sodium acetate per 100 l of supernatant

14. Add 2 volumes of cold 100% Ethanol

15. Place at -20°C for >2 h

16. Centrifuge for 5 minutes and pour off supernatant

17. Dry pellet

18. Resuspend the DNA in 10 l of TE