

Phenol/ Chloroform Extraction and Ethanol Precipitation
V.1 12.29.03
NDJ

Required Solutions:

Buffer Saturated Phenol pH for DNA

Chloroform.

Chloroform should be mixed 24:1 with IAA (IsoAmyl Alcohol) to prevent foaming (CIAA)

Sodium Acetate 3M (NaAc) pH 5.5

99% Ethanol

70% Ethanol

Phenol/Chloroform Extraction

1. Prepare waste bag for phenol/chloroform waste
2. Make 1:1 mixture of phenol:CIAA. Prepare an amount equal to volume of samples
3. Aliquot out an amount of CIAA equal to the volume of samples
4. Add 1:1 of Phenol/CIAA to microfuge tubes with samples
5. Vortex briefly and spin for 30 seconds
6. Remove top layer (aqueous) and place in new microfuge tube.
7. Add 1X volume of CIAA to sample.
8. Vortex briefly and spin for 30 seconds
9. Remove top layer (aqueous) and place in new microfuge tube

Ethanol Precipitation

1. Add 1/10 volume of NaAc 3M pH 5.5 to sample
2. Invert tube once
3. Add to 2 volumes 99% ethanol.
4. Place in -80°C freezer for 15 minutes
5. Centrifuge at 4°C for 10 minutes
6. Decant supernatant
7. Add 1ml 70% ethanol.
8. Invert twice
9. Centrifuge at 4°C for 10 minutes
10. Decant supernatant
11. Lay diagonally upside down on paper towel for 30 minutes
12. Resuspend in 20ul H₂O. Pipette up and down.