Phenol Chloroform extraction

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WEAR GLOVES

DISPOSE OF ALL PHENOL CHLOROFORM WASTE IN THE FUME HOOD.

If Phenol does spill on the skin wash off with PEG, use water if no PEG available.

Use Tris buffered Phenol:Chloroform:isoamylalchol 25:24:1

1. Add and equal volume of phenol chloroform – remember to put pipette tip below the buffer in the reagent bottle.
    a. The liquid will drip from a pipette tip so be prepared and work efficiently.
2. Vortex for a few seconds to mix well.
3. Centrifuge for 10min RT 13,000rpm.
4. Pipette off and keep the top (aqueous layer) avoiding taking and precipitated protein or phenol.
5. Add1/10 volume sodium acetate pH 5.5.
6. Add 2 volumes of 100% ethanol.
7. (If possible spool the DNA out of the ethanol and into 70% ethanol).
8. If the DNA does not spool
    a. Vortex or invert to mix well for a few seconds.
    b. Centrifuge for 15min RT 13,000rpm.
    c. Carefully remove the ethanol
        i. You can tip this off but take care the pellet does not slip.
9. It is advisable to keep this ethanol if you cannot see a pellet – the DNA may not have fully precipitated – see note below.
10. Add 70% ethanol – typically 500µl in 1.5ml microtube.
11. Centrifuge 2min max speed to re-pellet the DNA
12. Remove ALL the ethanol (re pellet again if necessary).
    a. The pellet may now be slippery so it is advisable to pipette off the liquid.
13. Air dry for 5-10 minutes to ensure ALL ethanol has gone BUT do not over dry.
14. Resuspend in dH2O or TE as appropriate

NOTE if your expected DNA yield is small you may need to put the ethanol precipitation stage at -80C for a few hours/overnight.