Phenol extraction and Ethanol Precipitation of DNA

This protocol describes the most commonly used method of purifying and concentrating DNA preparations using phenol extraction and ethanol precipitation; it is appropriate for the purification of DNA from small volumes (<0.4ml) at concentrations ≤1mg/ml.

Materials

-DNA to be purified (≤1 mg/ml) in .1 to .4 ml volume
-25:24:1(v/v/v) phenol/chloroform/isoamyl alcohol (made with buffered phenol: Support Protocol 1)
-3M sodium acetate, ph 5.2
-100% ethanol,ice cold
-70% ethanol, room temperature
-Ultra pure water

1. Add an equal volume of phenol/chloroform/isoamyl alcohol to the DNA solution to be purified in a 1.5-ml microcentrifuge tube.
2. Vortex vigorously 10 sec and microcentrifuge 15 sec at room temperature
3. Carefully remove the top (aqueous) phase containing the DNA using a 200 microliter pipettor and transfer to a new tube. If a white precipitate is present at the aqueous/organic interface, reextract the organic phase and pool the aqueous phases.
4. Add 1/10 volume of 3M sodium acetate, pH 5.2, to the solution of DNA. Mix by vortexing briefly or by flicking the tube several times with a finger
5. Add 2 to 2.5 vol (calculated *after* salt addition) of ice-cold 100% ethanol. Mix by vortexing and place in crushed dry ice for 5 min or longer.
6. Spin 5 min in a fixed-angle microcentrifuge at high speed and remove the supernatant.
7. Add 1ml of room temperature 70% ethanol. Invert the tube several times and microcentrifuge as in step 6.
8. Remove the supernatant. Allow to air dry for 15 minutes
9. Resuspend DNA pellet in 100 microliters of Ultra pure water

Protocol adapted from:
Ausubel, F.; Brent, R.; Kingston, R.; Moore, D.; Seidman, J.G.; Smith, J.;Struhl, K. Short Protocols in Molecular Biology(1995), 3rd ed., Unit 2.1: page 2-3.