PHENOL EXTRACTION AND ETHANOL PRECIPITATION

1. Add an equal volume of TE saturated phenol to the DNA solution to be purified. Phenol is nasty stuff. It denatures proteins so that is what it will do to yours on your hand and arms, etc. Wear gloves, labcoat and safety glasses--NO EXCEPTIONS. Refer to the MSDS for more information.
2. Vortex 10 sec. and spin in ufuge for 30 sec.
3. Suck off the organic phase (yellow). The organic phase (phenol solution) is usually on the bottom however, high salt concentrations occasionally result in the inversion of the layers. Either way, you want to get rid of the yellow one.
4. Add 1/10th volume of ammonium acetate (5M), finger vortex and add 2.5 vols of ethanol or 0.6 vols isopropanol. Isopropanol is good for relativiely large pieces of DNA (the size of a plasmid) as less RNA and free nucleotides are precipitated. Since isopropanol is more deleterious to enzymes etc. always wash with ethanol. For this reason I usually use ethanol for precipitating unless isopropanol is specified (ie plasmid preps). Contrary to popular misconception there is no advantage to cooling (or freezing) after adding ethanol. Room temp. Is fine and may even be better. Ethanol and isopropanol are flammable and should be stored in the flammable liquids cupboard when not in use. Read the msds's for these compounds and don't be careless.
5. Spin 10 min in microfuge, suck off sup carefully, wash pellet by addition of 70% ethanol (volume equal to the original total) and respin 5 minutes, suck dry carefully with a pulled pipette, air dry at 37 C for a few minutes or with speed vac and dissolve in TE (volume depends on the next step). After the wash the DNA pellet does not stick very well to the side of the tube so be careful. If the DNA is small (less than 200 bases) wash with 95% ethanol.

BUFFERING PHENOL

When you are using more than a few ul of phenol you must work in the fume hood. This is a perfect example of using too much to be safe anywhere else.

Start with an unopened 1 L bottle of liquified phenol form stores (88% phenol). Add 1 gram of 8-hydroxyquinoline to a 2 litre flask and pour in the phenol. Hydroxyquinoline is nasty too. It can irritate the skin and eyes and is a possible mutagen. Wear gloves, safety glasses and lab coat. Read the MSDS. Luckily it comes as fairly large crystals and has a high vapour pressure which means you can weigh it out CAREFULLY but without a mask. The phenol will turn yellow when the hydroxyquinoline dissolves--it is added as an anti-oxidant and scavages free radicals.

Add 500mLs of 50mM Tris base. Stir up the two phases either by hand or witha magnetic stirrer (remember to take all precautions when you retrieve the stirring bar later).

Allow the phases to seperat and aspirate off the aqueous phase. Make sure you don't get any phenol in the process as fish don't like it. It doesn't matter if you can't get all the aqueous phase off each time. Repeat twice with 50mM Tris-CL pH8.

Dispense into foil wrapped 50mL falcon tubes-- label them properly and store at -20 C. Store the aliquot that is in use in a brown bottle at 4 C See the current protocols book for more information.