Phenol Extraction and Ethanol Precipitation of DNA

1) Add an equal volume TE equilibrated phenol to the DNA. Generally 150 to 200µl is a good volume to work with. If the DNA is in a very small volume, is best to add $T_{10}E_{0.1}$ or ddH_2O to the sample prior to phenol extraction.

2) Vortex and spin ~ 2min (max RPM at RT) in a microfuge.

3) Transfer the aqueous layer (the upper layer) to a new tube. Avoid the interface. This is where much of the stuff we don't want is. If the volume of the aqueous phase transferred seemed too little, add some $T_{10}E_{0.1}$ or ddH_2O to the phenol layer and repeat the vortex and spin steps. Then simply combine the two aqueous phases that were removed from the organic phase (lower layer).

4) Add an equal volume of TE equilibrated phenol/Sevag (50:50); e.g., if your starting sample was 200µl, then add 100µl of TE equilibrated phenol and 100 µl of Sevag.

5) Vortex and spin ~ 2min (max RPM at RT) in a microfuge.

6) Transfer the aqueous layer to a new tube.

7) Add an equal volume of Sevag.

8) Vortex and spin (max RPM at RT) in a microfuge. This time only a brief spin is required since the Sevag and water is less miscible than phenol and water.

9) Transfer the aqueous layer to a new tube.

10) Add 1/10 volume 3M NaOAc (pH 5.2-6.0); e.g., if the original DNA volume was 200µl add 20µl 3M NaOAc.

11) Add two to 2.5 volumes of 95% EtOH.

12) Invert to mix well and spin for 1-2 min (max RPM at RT; 4^oC is okay too).

13) There should be a pellet of DNA visible at the bottom of the tube. This is what you want. The Ethanol is decanted off, usually into a waste container. Note however that if you are uncomfortable with pouring the ethanol off into the sink, pour it into another microfuge tube. That way if the pellet comes loose (or you forgot to add NaOAc, you still can go back and find your DNA. Note also that the dirtier the DNA, the easier it is to see the pellet; i.e., if the DNA is very clean and/or there is very little DNA, then it may be a bit harder to see.

14) Add about 1ml of 70% EtOH to the DNA pellet. This is a wash step. The 70% EtOH removes excess salts and other unwanted stuff.

15) Spin for about 2min (at max RPM and at RT) in a microfuge.

16) Decant the 70% off (into the sink or some other waste container).

17) Blot the opening of the microfuge tube witrh a kim-wipe to remove as much ethanol as possible.

18) Dry the DNA pellet. It usually takes about 10min in the speed vac. Alternatively just leave the tube open on your bench and the ethanol will evaporate in about 1h. (Residual EtOH will inhibit subsequent enzymatic manipulation of the DNA.)

19) Resuspend DNA in $T_{10}E_{0.1}$ pH 7.5. The volume is variable depending on what you next plan to do with the DNA.

20) Store the DNA at $-20^{o}C$.

Notes:

Phenol must be handled with care. It is a corrosive. It will cause severe burns if you get it on your skin. Use common sense and caution. Wear gloves, goggles and a lab coat and work in the fume hood when working with large quantities of phenol. If you spill it on yourself, wash the skin area well. If it has penetrated your clothing, remove your clothing immediately and wash your skin with water. If the burns are extensive, get help and go to the emergency room.

Phenol must be equilibrated with TE prior to use.

Never use old phenol; otherwise your DNA will be lost to the phenol. Store in the dark at $4^{o}C$. Generally the shelf life is about three months. If you prepare your own phenol, keep your stock at $-20^{o}C$ and remove aliquot every three months or so.

Sevag is Chloroform/Isoamyl alcohol (24:1) and is named for Sevag. Mix 240 ml of chloroform with 10 ml of Iso**amyl** alcohol and store at RT in the fume hood. This will stay good indefinitely as long as the bottle is sealed.

If the salt concentration in the sample being extracted is near saturation, the organic and aqueous phases will invert; i.e., the phenol will be on the top layer. If you are unsure, one way to check is to add a drop of $T_{10}E_{0.1}$ or H2O to the sample and note where it goes. If it passed through to the bottom layer, then the phases have inverted. Usually this only happens with high salt.

If the temperature is cold, the aqueous layer will become cloudy. Don't worry about this.

Phenol is what denatures the protein contaminants. The purpose of using the Sevag is that it helps to remove all traces of phenol.