Tips for Phenol Chloroform Extraction

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One of the most common molecular biology lab procedures, phenol extraction removes proteins from nucleic acid samples during isolation, and purifies nucleic acids after enzymatic reactions; e.g., removal of restriction enzymes during preparation of transcription template.

Do
Add an equal volume of a 1:1 phenol: chloroform solution to the aqueous sample and vortex for 1-2 minutes (longer for larger volumes) creating an emulsion. Please note that isolating genomic DNA requires gentle mixing because the DNA can be sheared by vortexing in phenol solutions. Centrifuge at high speed for 2 minutes (10 minutes for larger volumes). Carefully remove the aqueous or top layer to a new tube, avoiding any flocculent material at the interface. Even though it is sometimes present in the aqueous layer, this flocculent material contains proteins and other debris which should be avoided.

For optimum recovery, the spent phenol can be back extracted to obtain residual aqueous material left at the interface. Back extraction involves adding an equal volume of aqueous buffer to the spent phenol solution. The solution is vortexed, centrifuged and the aqueous layer removed again as described above. The two aqueous solutions are then pooled and precipitated to concentrate the nucleic acids.

Don't
Don't simply invert the extraction a few times, because inadequate mixing results in insufficient removal of contaminating proteins. (Inversion is only an efficient extraction means if it is done over a period of several hours, as with genomic DNA.)

Don't be tempted to completely remove the aqueous layer. The purpose of the extraction is to remove the contaminating proteins, lipids and carbohydrates some of which can be in the aqueous layer near the interface.

If working with genomic DNA samples, don't vortex. The samples should be gently and constantly inverted for several hours to ensure good mixing while preserving the integrity of the DNA strands.

Tip
To avoid interface material, set your pipettor to only remove 80-90% of the aqueous phase in the initial extraction. Use a back extraction to dilute and recover the remaining 10-20% of the aqueous phase.

When performing phenol extractions on large volumes (10 ml or greater), a short incubation on ice (5 minutes) can help compact the residue at the interface.

Ethanol Wash

Ethanol washes are performed after salt/EtOH precipitations to remove any residual salt from the nucleic acid pellet. The wash employs 70-80% EtOH which will solubilise salts but not nucleic acids.

Do
Add 70 - 80% EtOH to the nucleic acid pellet. The volume should be sufficient to at least cover the pellet and wet the sides of the tube when vortexed (there is no volume too large). Vortex the sample for 1 minute; the pellet should come loose from the tube and be broken up in the EtOH. Centrifuge the sample 10 - 30 minutes, to recollect the pellet. Aspirate off the EtOH.

Don't
Don't just add the EtOH and immediately decant. The pellet should be vortexed so that the EtOH can penetrate the sample and solubilise salt.

Don't forget to respin! The pellet must be firmly reaffixed to the tube so that it is not lost during aspiration.

Double Aspiration

Double aspiration is useful for removing the last traces of EtOH supernatant after precipitations. It involves a second quick spin and aspiration to ensure removal of any precipitation supernatant e.g. on the walls of the tube, that might interfere with downstream steps of the protocol.

Do
After pelleting the precipitation, aspirate the precipitation supernatant off the nucleic acid pellet. Follow immediately with a quick 1-2 second centrifugation and aspirate again.

Aspiration can be done with a syringe needle or a drawn-out Pasteur pipette connected to a vacuum source with a trap. Alternatively, a drawn-out Pasteur pipette can be used with a pipette bulb. To make drawn-out Pasteur pipettes, soften the pipette tip with a flame and draw the tip out with forceps, break the tip at the narrowest point and flame polish if needed.

Slant the tube, pellet facing up, and aspirate from beneath.

Don't
Don't let the tubes sit very long between aspirations as the pellet may come loose.

Resolubilizing RNA Pellets

During RNA isolations and purifications, it is often necessary to precipitate the sample. Resolubilizing the RNA pellet after precipitation can be time-consuming and the presence of proteins or other contaminants can make it difficult.

Do
After precipitation, perform double aspiration as above and air dry for 5-10 minutes.

Use the largest volume of solute possible to increase solubility. The less dry the pellet, the easier it is to solubilise.

Don't
Don't dry by vacuum centrifuge (speed vac).

Tip
To aid resolubilization of over dried RNA, store the RNA pellet and solute together overnight at -80°C. The freeze-thaw process helps in subsequent solubilization