

Phenol/Chloroform Extraction & Ethanol Precipitation

- Add equal volume of Phenol/Chloroform to DNA or digest
- Vortex for 10-15 seconds
- Spin 5', 14,000rpm @ RT
- Transfer aqueous layer (top) to a clean tube
- Add equal volume of Phenol/Chloroform and repeat above steps
- Optional: Add equal volume 100% Chloroform then vortex and spin as above

- Add 1/10th volume 3M NaOAc (ie. 10ul to 100ul of DNA)
- Add 2.5 volumes 100% EtOH (ie. 250ul to 100ul reaction)
- Spin 30' at 4°C, 15,000rpm
 - (Alternatively put reaction at -20°C for at least 30' to o/n then spin 10' at 4°C, 15,000rpm)
- Remove EtOH solution, wash pellet in 2 volumes 70% EtOH
- Spin 5' at 4°C, 15,000rpm
- Remove EtOH, air dry pellet
- Resuspend DNA in appropriate volume of H₂O or DEPC H₂O