Phenol-Chloroform Extraction of DNA

1. Add 0.5 ml phenol:chloroform:isoamylalcohol pH 8.0 (25:24:1) to DNA extracts in Phase-lok gel tubes (Eppendorf 2.0 ml Heavy). Shake tubes gently by hand.
2. Tubes are centrifuged at 12000 x g for 5 min.
3. The aqueous layer is removed and combined with an equal volume of chloroform:isoamylalcohol (24:1) in another phase-lok gel tube.
4. Tubes are mixed well and centrifuged at 12000 x g for 5 min at room temperature. The aqueous layer is removed into a sterile 1.5 ml microcentrifuge tube.

   You can try this extract in PCR (should be clean enough now) or alternatively put this extract through a spin column clean up like a PCR product clean up kit. You can also follow with an ethanol precipitation to further concentrate your DNA.

Ethanol Precipitation of DNA

5. Add 0.25ul glycogen (20mg/ml – Roche), 1/10 volume 3M sodium acetate (pH 5.2) and mixing gently. 2.5 volumes ice cold ethanol (95%) are then added and tubes well-mixed.
6. Centrifuge at 16,000 x g for 15 minutes at 4C. Position the tube so the hinge is facing outward, as the resulting pellet might not be visible.
7. Being careful not to dislodge the pellet, remove the supernatant.
8. Carefully rinse the pellet twice with 200 ul of ice-cold 70% ethanol while centrifuging at 16,000 x g for 2 minutes at 4C between each wash.
9. Dry the pellet for ~5 minutes in a vacuum desiccator.
10. Resuspend the pellet in 10ul water or 10 mM Tris buffer.