Phenol chloroform DNA purification

1. Adjust sample volume to 100ul with water. You could also add carrier at the same time. This will help to improve yield.

2. Add an equal volume (100ul) of TE-saturated phenol:chloroform:isoamil alcohol (25:24:1) to the DNA sample contained in a 1.5 ml microcentrifuge tube and vortex for 10 seconds. TE-saturated phenol:chloroform:isoamil alcohol (25:24:1) has TE upper phase. Make sure you are pipetting lower phase.

3. Centrifuge the sample for 15 minutes at room temperature to separate the phases.

4. Remove about 90% of the upper, aqueous layer to a clean tube, carefully avoiding proteins at the aqueous:phenol interface.

5. Add one-tenth volume of 3M NaOAc, pH 5.2 (10ul) to collected supernatant and mix.

6. Add 2.5 volumes (250ul) of cold 100% ethanol and mix.

7. Place at at -70°C for at least 30 minutes or -20°C for two hours to overnight (overnight will give you a better yield).

8. Centrifuge at 12,000 rpm in a microcentrifuge for 15 minutes at 4°C.

9. Remove supernatant without touching a pellet (even if pellet is invisible).

10. Add 1ml of cold 70% ethanol to the pellet, incubate at room temperature for 5-10 minutes and centrifuge again for 15 minutes at 4°C.

11. Remove supernatant without touching a pellet (even if pellet is invisible).

12. Centrifuge for a few seconds to get last drops down. Remove all liquid by careful pipetting.

13. Dry DNA pellet for 5-15 min.

14. Dissolve dried DNA in 10 mM Tris-HCl, pH 7.6-8.0 (buffer EB) or water.