Extracting DNA Using Phenol-Chloroform

Reagents Needed

1. Phenol/chloroform/isoamyl alcohol (PCI) solution (25:24:1) DNase (RNase- and Protease-Free - Molecular Biology grade), pH 7.8-8.2 **Important**: acidic pH makes the DNA go into the phenolic layer while RNA goes into the aqueous layer
   Suppliers
   http://www.sigmaaldrich.com/catalog/product/sigma/77617?lang=en&region=US
   http://products.invitrogen.com/ivgn/product/15593031
   http://www.fishersci.com/ecomm/servlet/fsproductdetail_10652_657663_29104_-1_0

2. Chloroform/isoamyl alcohol, 24:1 (Molecular Biology grade)
   Suppliers
   http://www.sigmaaldrich.com/catalog/product/fluka/25666?lang=en&region=US
   https://new.fishersci.com/ecomm/servlet/fsproductdetail?aid=25168&&storeId=10652

3. Elution Buffer (10 mM Tris-HCl, pH 8.5)
4. NH_4OAc (Molecular Biology grade), concentrated solution (5M – 7.5M) or powder
   Supplier: http://www.sigmaaldrich.com/catalog/product/sigma/a2706?lang=en&region=US
5. Glycogen, 20 mg/ml (Molecular Biology grade), available from most biological reagent suppliers
6. 100% Ethanol
7. 80% Ethanol

Procedure

TUBE 1: Phenol/Chloroform/Isoamyl Alcohol Extraction

1. Start with 200 µL of material and a tube (label as **TUBE 1**). If necessary, bring the volume up to 200 µL using the Elution Buffer (“EB”) above.
2. Add an equal volume of the phenol/chloroform/isoamyl alcohol solution to **TUBE 1**.
3. Vortex **TUBE 1** vigorously for 1 minute.
4. Spin **TUBE 1** solution at high speed for 5 minutes.
5. Remove ~180 µL of the top aqueous solution and place into a new tube, **TUBE 2**. Avoid picking up any of the phenol/chloroform/isoamyl alcohol phase.
6. Add 200 µL of EB to **TUBE 1**.
7. Vortex **TUBE 1** vigorously for 1 minute.
8. Spin **TUBE 1** solution at high speed for 5 minutes.
9. Remove as much of the top aqueous solution as possible from **TUBE 1** without picking up any of the phenol/chloroform/isoamyl alcohol phase. Add the solution to **TUBE 2**.

TUBE 2: Chloroform Back Extraction (the following steps are to be performed in TUBE 2)

10. Add equal volumes of the chloroform/isoamyl alcohol solution to **TUBE 2**.
11. Vortex **TUBE 2** vigorously for 1 minute.
12. Spin **TUBE 2** solution at high speed for 5 minutes.
13. Remove as much of the top aqueous solution as possible and place into a new tube, **TUBE 3**. Avoid picking up any of the chloroform/isoamyl alcohol phase.

TUBE 3: Ethanol Precipitation (the following steps are to be performed in TUBE 3)

14. Add NH_4OAc to a final concentration of 0.75 M.
15. Add 1 µL of glycogen (20 µg).
16. Mix solution well.
17. Add 2.5X volume of 100% ethanol and mix well.
18. Incubate at 20 °C (optional).
19. Spin for 20 minutes in a 4 °C centrifuge at top speed.
20. Decant supernatant carefully without disturbing the pellet.
21. Wash by adding 300 µL of 80% EtOH and vortex 3 times.
22. Spin for 15 minutes in a 4 °C centrifuge at top speed.
23. Decant supernatant carefully without disturbing the pellet.
24. Repeat steps 21 – 23 for a second 80% EtOH wash.
25. Quick spin on table top centrifuge to draw residual EtOH to the bottom.
26. Remove residual EtOH with a P20 pipette. Be careful not to disturb the pellet.
27. Air dry for 1-2 minutes.
28. Re-suspend in appropriate volume EB (based on input amount).