Please note: the shared protocols described herein may not have been validated by Pacific Biosciences and are provided as-is and without any warranty. Use of these protocols is offered to those customers who understand and accept the associated terms and conditions and wish to take advantage of their potential to help prepare samples for analysis using the PacBio® RS system. If any of these protocols are to be used in a production environment, it is the responsibility of the end user to perform the required validation.

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://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

3. Elution Buffer (10 mM Tris-HCl, pH 8.5)
4. NH₄OAc (Molecular Biology grade), concentrated solution (5M – 7.5M) or powder

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/Isomyl 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 NH₄OAc 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).