Guidelines for Using a Salt:Chloroform Wash to Clean Up gDNA

This protocol can be used to clean up high-molecular-weight genomic DNA (gDNA) prior to the SMRTbell™ library preparation. It describes how to use a high-salt low-ethanol percentage wash to remove polysaccharides before DNA is precipitated from the solution.

1. Prepare a buffer of 1 M NaCl and 2 mM EDTA. Label tube as **Buffer A** and set aside.

   Note that 500 µL of buffer will need to be prepared for each sample.

Buffer A	Volume (µL)
5 M NaCl	100
500 mM EDTA	2
PacBio® EB	398
TOTAL	**500**

2. Bring the volume of DNA up to 200 µL with Elution Buffer (EB) – label as **TUBE 1**.
3. Add the following reagents to **TUBE 1**.

Tube 1	Volume (µL)
gDNA in EB	**200**
5 M NaCl	**100**
500 mM EDTA	**2**
PacBio EB	**198**
TOTAL	**500**

4. Add 400 µL of Phenol:Chloroform:Isoamyl Alcohol (25:24:1) to **TUBE 1**.
5. Invert the tube 20 times to mix.
6. Spin the tube at maximum speed (at least 10 g) for 10 minutes.
7. Carefully remove the aqueous layer, do not disturb the interface. Place into a clean 2 mL microcentrifuge tube. Label the tube as **TUBE 2.**
8. Add 400 µL of **Buffer A** (from step 1) to **TUBE 1**.
9. Invert tube 20 times to mix.
10. Spin tube at maximum speed (at least 10 g) for 10 minutes.
11. Carefully remove the aqueous layer, do not disturb the interface. Place into **TUBE 2.**
12. Measure the volume in **TUBE 2**: _____ µL. It should be close to 800 µL.

13. Add an equal volume of Chloroform:Isoamyl Alcohol (24:1) to **TUBE 2.**
14. Invert tube 20 times to mix.
15. Spin tube at maximum speed (at least 10g) for 10 minutes.
16. Carefully remove the aqueous layer, do not disturb the interface. Place into a clean 2 mL microcentrifuge tube. Label the tube as **TUBE 3.**
17. Measure the volume in **TUBE 3**: _____ µL.
18. Add 0.3X volume of ethanol (99.99%) to **TUBE 3**. This high-salt, low-ethanol mixture precipitates the excess polysaccharides while gDNA remains in the solution. _____ µl (TUBE 3) x 0.3 = _____ µL of Ethanol
19. Invert tube 20 times to mix.
20. Spin tube at maximum speed (at least 10 g) for 15 minutes.
21. Carefully remove the supernatant without disturbing the polysaccharide pellet. (*Note that no visible pellet may be seen at this step)*. Place supernatant into a clean 2 mL microcentrifuge tube. Label the tube as **TUBE 4** and measure the volume**. Note: TUBE 4 contains the gDNA**.
22. Add 1.7X volume of ethanol (99.99%) to **TUBE 4**. The gDNA can be seen as falling out of the solution as long strands of gDNA; _____ µl (TUBE 4) x 1.7 = _____ µL of Ethanol.
23. Invert tube 20 times to mix.
24. Spin tube at maximum speed (at least 10 g) for 15 minutes.
25. Add 500 µL of 70% ethanol to remove the excess salt; do not disturb the pellet.
26. Spin the tube at maximum speed (at least 10 g) for 15 minutes.
27. Carefully remove supernatant, do not disturb the pellet. Save supernatant in a clean tube (label as **TUBE 5**).
28. Add 500 µL of 70% ethanol to remove the excess salt; do not disturb the pellet.
29. Spin the tube at maximum speed (at least 10 g) for 15 minutes.
30. Carefully remove the supernatant; do not disturb the pellet. Add supernatant to **TUBE 5**. Quick spin to gather the residual ethanol at the bottom of the tube and carefully remove with a P20 tip.
31. Let pellet air dry for 5 min at room temperature, taking care not to over dry.
32. Resuspend the pellet in 100 µL TE. Incubate at 4ºC with gently mixing overnight to resuspend. Store at 4ºC for use within one week, or store at -80ºC for long-term storage.
33. Perform a 1X AMPure® Bead purification step and resuspend the DNA in PacBio EB immediately before shearing.