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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 μ L 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 μ L 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: _____ μ L. It should be close to 800 μ L.



- 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; ______µI (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 μ L 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 4°C with gently mixing overnight to resuspend. Store at 4°C for use within one week, or store at -80°C for long-term storage.
- 33. Perform a 1X AMPure[®] Bead purification step and resuspend the DNA in PacBio EB immediately before shearing.

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