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.

<table>
<thead>
<tr>
<th>Buffer A</th>
<th>Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 M NaCl</td>
<td>100</td>
</tr>
<tr>
<td>500 mM EDTA</td>
<td>2</td>
</tr>
<tr>
<td>PacBio® EB</td>
<td>398</td>
</tr>
<tr>
<td>TOTAL</td>
<td>500</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Tube 1</th>
<th>Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gDNA in EB</td>
<td>200</td>
</tr>
<tr>
<td>5 M NaCl</td>
<td>100</td>
</tr>
<tr>
<td>500 mM EDTA</td>
<td>2</td>
</tr>
<tr>
<td>PacBio EB</td>
<td>198</td>
</tr>
<tr>
<td>TOTAL</td>
<td>500</td>
</tr>
</tbody>
</table>


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.
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 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.