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🌐 Guidelines for Using a Salt:Chloroform Wash to Clean Up HMW DNA for PacBio

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We use this protocol and it's working

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Abstract

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

Materials

PacBio EB buffer **or** 10 mM Tris-HCL, pH 8.5 (Rockland, MB-027-1000).

Phenol:Chloroform:Isoamyl Alcohol (25:24:1), pH 8 (Sigma, P2069-100ML).

Chloroform:Isoamyl Alcohol (24:1) (Sigma, C0549-1PT).

Pure Ethanol (Sigma, E7023).

10 mM Tris-HCl, pH 8 (Corning, 46031CM)

Pure water (Invitrogen, 10977-015).

500 mM EDTA, pH 8 (Homemade, solution must be filtered with 0.22 µm filter).



- 1 Prepare **Buffer A** and set aside:

5 M NaCl	100 μL
500 mM EDTA, pH 8	2 μL
PacBio EB (10 mM Tris-HCl, pH 8.5)	398 μL
TOTAL	500 μL

Note

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

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

DNA in EB	200 μL
5 M NaCl	100 μL
500 mM EDTA, pH 8	2 μL



	PacBio EB (10 mM Tris-HCl, pH 8.5)	198 μL
	TOTAL	500 μL

- 4 Add 400 μL of Phenol:Chloroform:Isoamyl Alcohol (25:24:1), pH 8 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 at Room Temperature (RT).
- 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**) $\times$ 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**) $\times$ 1.7 = _____ μL of Ethanol.

**Note**

If the final volume is larger than 2 mL, I recommend splitting the volume in two tubes (two tech reps).

- 23 Invert tube 20 times to mix.
- 24 Spin tube at maximum speed (at least 10 g) for 15 minutes. Discard supernatant with pipette, do not disturb the DNA pellet.
- 25 Add 500 μ L of 70% ethanol to DNA pellet to remove the excess salt; do not disturb the DNA pellet.
- 26 Spin the tube at maximum speed (at least 10 g) for 15 minutes. Discard supernatant with pipette, do not disturb the DNA pellet.
- 27 Add 500 μ L of 70% ethanol to DNA pellet to remove the excess salt; do not disturb the pellet.
- 28 Spin the tube at maximum speed (at least 10 g) for 15 minutes. Carefully remove the supernatant; do not disturb the DNA pellet.
- 29 Quick spin to gather the residual ethanol at the bottom of the tube and carefully remove with a P20 tip.
- 30 Let DNA pellet air dry for 5 min at room temperature, taking care not to over dry.
- 31 Resuspend the DNA pellet in 100 μ L 10 mM Tris-HCl, pH 8. 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.

Note

If you have two technical reps, resuspend both DNA pellets with one round of 100 μ L 10 mM Tris-HCl, pH 8.