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

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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 TEXT

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

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**) 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.

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.

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 µL 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 µL 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 µL 10 mM Tris-HCl, pH 8. 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.

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