This protocol was generously contributed by the Lowe Lab at Hopkins Marine Station, Pacific Grove, California

Preparing Genomic DNA from Sperm

This protocol can be used to prepare purified genomic DNA from sperm for sequencing on PacBio Systems. It is recommended to start with ~250 µl of whole sperm, which can generate >60 µg of purified genomic DNA.

Supplies, Materials, and Instrumentation

Item	Vendor
1.5 ml microcentrifuge tubes	Sigma-Aldrich cat. no. T9661
50 ml conical tubes	Fisher Scientific cat. no. 14-432-22
Microcentrifuge	Fisher Scientific cat. no. 05-403-90
2 ml 5PRIME Phase Lock Gel tubes	VWR cat. no. 2302830
Spectrophotometer	Fisher Scientific cat. no. ND-2000
RNase cocktail	Ambion cat. no. AM2286
Sodium acetate	Ambion cat. no. AM9740
Proteinase K	New England Biolabs cat. no. P8107S
DNA extraction buffer: 1M Tris-HCL (pH 8.0) 10% (wt/vol) SDS dH_2O	
Phenol-chloroform-isoamyl alcohol (25:24:1): Phenol (pH 8.0) Chloroform Isoamyl alcohol	
Tris-EDTA (TE) buffer: 1M Tris-HCL (pH 8.0) 1M EDTA (pH 8.0) dH_2O	
DEPC-treated H_2O: DEPC dH_2O	

Generation of Solutions

DNA extraction buffer:

Mix 1ml of 1M Tris-HCL (pH 8.0) with 5ml of 10% (wt/vol) SDS and add to 90ml of dH_2O. After mixing, bring volume to 100ml with dH2O. Filter and autoclave the solution. Stable at room temp (~21°C) for up to 1 year.

Phenol-chloroform-isoamyl alcohol (25:24:1)

Combine 50ml of phenol (pH 8.0), 48 ml of chloroform, and 2 ml of isoamyl alcohol in a brown glass bottle (or a clear bottle covered with foil). Store the solution at 4 °C for up to 1 month or freeze it at − 20 °C for long-term storage. For convenience, and to reduce the pipetting of hazardous reagents, a premixed solution of phenol- chloroform-isoamyl alcohol may also be used (e.g., Sigma-Aldrich, cat. no. P2069).

Tris-EDTA (TE) buffer

Add 1 ml of 1 M Tris-HCl (pH 8.0) and 0.2 ml of 1 M EDTA (pH 8.0) to 98.8 ml of dH_2O. Filter and autoclave the solution. Store the solution in a tightly sealed glass bottle at room temperature for up to 1 year.

DEPC-treated H_2O

Add 1 ml of DEPC to 1 liter of dH_2O in a loosely capped bottle. Mix the solution on a stir plate in a fume hood overnight at room temperature. Autoclave the solution for 30 min to inactivate DEPC. Store the solution in a tightly sealed glass bottle at room temperature for up to 1 year.

Procedure

Step	Instructions
1: Tissue Digestion	1. Add 500 µl of DNA extraction buffer supplemented with 50 µl of RNase cocktail. 2. Gently swirl with pipette tip to disperse the tissue in the buffer. 3. Incubate the sample at 37°C for 1 hour with gentle rocking. 4. Add 50 µl of 20 µg µl-1 proteinase K. 5. Incubate the tissue at 50°C for 2 hours with gentle rocking. 6. Centrifuge the digestion solution at 500 g for 2 minutes at room temperature to pellet any remaining undigested tissue. 7. Split the aqueous layer into two 5PRIME Phase Lock Gel tubes and proceed with extraction in parallel with both tubes. Note: Overdigestion with proteinase K can lead to DNA degradation. Monitor the viscosity of the digestion solution with the aim of a viscosity close to that of water.
2: DNA Isolation	1. Add one volume of phenol-chloroform-isoamyl alcohol (25:24:1) to the isolated aqueous layer. 2. Blend the organic and aqueous layers by gently inverting the tube several times. 3. Centrifuge the tube at 5,000 g for 15 minutes at room temperature. 4. Carefully transfer the aqueous phase to a new tube. 5. Repeat steps 2-4 twice more for a total of three times. 6. Transfer clear aqueous phase to a clean sterile tube. Note: If using Phase Lock Gel tubes, the aqueous layer can simply be poured to a fresh tube. However, if using a different type of tube, the aqueous layer can be transferred to a fresh tube with a wide bore pipette (trimmed tip off of a 1 ml pipette tip) carefully avoiding the flocculent white material at the interface between organic and aqueous phases as this contains proteins and membranes.
3. DNA Precipitation	1. Add a one-ninth volume of ammonium acetate. 2. Add 2.5 volumes of 100% ethanol. 3. Gently mix solution by gentle inversion. DNA should visibly precipitate in the form of white cotton-like threads. 4. Dissolve DNA in 200 µl of TE buffer overnight at 4°C. Note: High molecular weight DNA dissolves quite slowly, especially at 4°C. Therefore, if after dissolving overnight you do not measure DNA even though DNA strands were seen, consider dissolving for longer.

Example Results

PFGE DNA QC

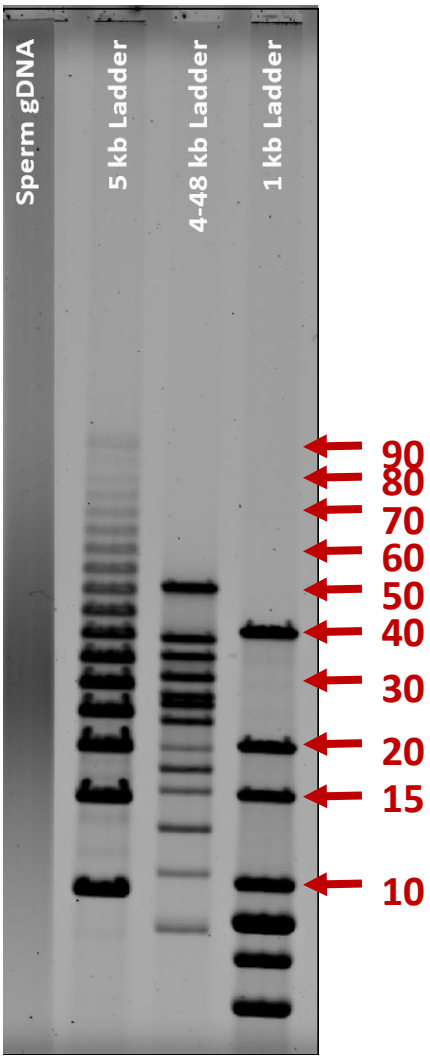

Figure 1. Pippin Pulse gel of DNA purified from marine invertebrate sperm. Lane 1, sperm gDNA; lanes 2-4, ladders.