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Extracting high molecular weight DNA from *Halichondria panicea* (Phylum: Porifera)

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This protocol was used to extract high molecular weight DNA from the sponge (porifera) *Halichondria panicea.* This protocol contains slight modifications from that presented in Ausubel et al (1995).

References:
Ausubel, F.; Brent, R.; Kingston, R.; Moore, D.; Seidman, J.G.; Smith, J.;Struhl, K. Short Protocols in Molecular Biology(1995), 3rd ed., Unit 2.1: page 2-3

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protocol

DNA extraction, High Molecular Weight DNA, Sponge, Porifera

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MATERIALS

☒ Buffer P3 **Contributed by users Catalog #19053**

☒ RNase A **Qiagen Catalog #19101**

☒ Isopropanol **Contributed by users**

☒ Buffered Phenol Chloroform Isoamyl alcohol (P:C:I) ((25:24:1, saturated with 10 mM Tris, pH 8.0 and 1 mM EDTA **Sigma Catalog #Sigma P2069**

☒ 70% Ethanol **Contributed by users**

☒ Proteinase K (2 ml) **Qiagen Catalog #19131**

☒ Buffer AP1 **Qiagen Catalog #1014630**

☒ Molecular biology grade 3M sodium-acetate pH 5.2±0.1 **Sigma Catalog #S7899**

☒ Buffer AE **Qiagen Catalog #19077**

Note: one could also make up a solution of 800 mM guanidine HCl, 20 mM Tris-Cl pH 8.0, 30 mM EDTA, 5 % Tween-20, and 0.5 % Triton-X 100 to replace Buffer AP1.

Similarly, buffer P3 can be replaced with 3 M potassium acetate buffer at pH 5.5.

AE buffer can be make up with 10 mM Tris-HCl pH 8.0, 1 mM EDTA, and 0.1 % Tween 20.

1 Rinse sample in filter sterilized seawater three times. Use a mortar and pestle to finely grind approximately 1 gram of tissue in liquid nitrogen.

2 Transfer homogenized sample into a 15 mL tube.

3 Add 5 mL of buffer AP1 (QIAGEN, Germany) and mix with 50 µL of 100 mg/mL RNase A. Invert tube a couple of times to mix. Incubate at 65°C for 30-60 minutes, inverting the tube approximately every 5-10 minutes (as often as possible). During this step, cells are lysed in presence of SDS. RNase will remove RNA.

4 Allow the tube to "cool" to approximately 50°C. Add 150 µL 20 mg/mL Proteinase K. Mix by inverting the tube a couple of times and then incubate at 50°C for a minimum of 2 hours. As often as possible, invert the tube to mix. Proteinase K will digest intracellular proteins such as histones and other DNA-binding proteins. It will also digest membrane-bound proteins which will further facilitate cell-lysis.

5 Cool the sample on ice for 5-10 minutes (depending on the volume) and add 1,700 µL of P3 (QIAGEN, Germany) buffer. Mix gently by inverting the tube 5-10 times. This step will precipitate SDS and polysaccharides (if present).

6 Spin down the sample in centrifuge at 7,200 RCF for 30 minutes.

7 Transfer supernatant with a wide-bore, i.e. cut with sterile blade, P1000/P5000 pipette-tip to a 15 mL tube. Subsequently, add Chloroform:Phenol:Isoamyl in a 1:1 mixture by volume. For initial protocol evaluation, vortex the sample until emulsions have formed. Prolonged incubation is encouraged. If downstream DNA yields turn out high, revert to a non-vortexing approach to minimize DNA shearing (gentle, prolonged mixing) Prolonged incubation is encouraged/essential.

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8 Spin down the sample at 7,200 RCF for 20 minutes and remove the DNA-containing supernatant (slowly and wide a wide-bore tip).

9 Repeat step 7-8 one to two times and subsequently transfer the supernatant to a clean 15 mL tube.

10 Add Molecular biology grade 3M sodium-acetate pH 5.2±0.1 (S7899 Sigma) to a final concentration of 0.3 M. This step will lower pH and neutralize the phosphodiester backbone, thus rendering the DNA ready for precipitation.

11 Add 1 volume of room-temperature isopropanol if HMW DNA is the intended target or use 1 volume of ice-cold isopropanol if both LMW and HMW is the target (with the option of including an over-night incubation at -20°C). For HMW DNA, invert tube a few times to mix until DNA precipitates as nice jelly-fish threads (within a few min). Then centrifuge for 10 min at 7,200 RCF (at room-temperature if HMW DNA is the target).The short incubation with isopropanol will preferentially precipitate HMW DNA species and by keeping the sample at room-temperature, salt carry-over is further minimized.

12 Remove the supernatant and add as much ice-cold 70 % ethanol as possible and gently invert tube to mix. Gently dislodge (if possible) the pellet. Centrifuge for 10 min at 7,200 RCF and replace as much liquid with fresh ice-cold 70 % ethanol. Spin again and remove as much liquid as possible. Finally aspirate residual ethanol with a P10 pipette (after a short spin).This will remove salts and replace isopropanol with the more volatile ethanol.

13 Air-dry the pellet for 5-10 minutes. Do not over-dry!

14 Add 100-200 µL AE buffer. Incubate the sample at room-temperature or fridge for a minimum of two days. Longer incubations are encouraged (1-2 weeks if time allows and no DNase activity is assumed present). If the pellet is not readily dissolving, then try to pull out "DNA threads" with a P10 pipette.

15 Measure quality and concentration with NanoDrop (or similar) and dsDNA (Qubit) and evaluate DNA size-distribution.

16 If A260/A230 is < 2.0, perform a clean-up step with the Qiagen PowerPro CleanUp kit.

17 Measure quality and concentration with NanoDrop (or similar) and dsDNA (Qubit) and evaluate DNA size-distribution.

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