Isolation of marine meiofauna from sandy sediments: from decanting to DNA extraction.

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Method Article

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Abstract

This protocol describes the separation of marine meiofauna from sediment and subsequent environmental DNA extraction. In this study meiofauna samples were taken with a 45 mm core from the upper 5 to 10 cm of sediment layer. Separation from sediment was achieved using a decantation process followed by isolation from fine silt using repetitive centrifugation steps with a 1.16 specific gravity \(sg) LUDOX-TM solution. Meiofauna were deliberately separated from macrofauna by using a 1 mm sieve on top of a bottle-top sterile 45 µM sieve. High quality DNA was subsequently obtained using the QIAamp DNA Blood Maxi Kit \(Qiagen) with minor adjustments to the manufacturer's protocol. This procedure allowed efficient isolation of meiofaunal representatives from marine sediments and also extraction of high quality environmental DNA that can be used for downstream metagenetic analysis.

Reagents

• DESS: 20% DMSO, 0.25 M disodium EDTA, saturated with NaCl, pH 8.0 \(1). • LUDOX-TM concentrate: 800 ml of conc. LUDOX-TM, 1200 ml of dH2O, shake vigorously until solution is homogenous, rectify specific gravity \(sg) until it is 1.16. • DNA lysis buffer: 100 mM Tris-HCl, pH7.5; 100 mM NaCl; 100 mM EDTA; 1% SDS, 500 µg/ ml proteinase K • QIAamp DNA Blood Maxi Kit \(Qiagen) • 100% Ethanol • 1.5 ml Eppendorf tubes, 15 ml falcon tubes, 50 ml falcon tubes

Equipment

1 mm and 45 µM analytical grade stainless steel wire mesh sieves • Bottle-top 47 mm 45 µM membrane filters and disposable filter holder \(Millipore, NY4104700 and MVHAWG124) • UV transilluminator chamber • Bench-top centrifuge with swing-arm rotor and adapters for 15ml and 50 ml rotor adapters • -20 C and -80 C freezers

Procedure

CRITICAL Where possible, all equipment should be autoclaved between samples. If not possible, the use of a UV transilluminator/ cross linker \(according to manufacturer's instructions) and/or immersion in sodium metabisulfite solution will reduce cross contamination between samples. **Meiofauna extraction** _Separation from sediment – decantation_ 1. Collect benthic samples \(ca. 150 ml) into storage pots containing 300 ml of DESS solution. **TIP:** Maintain at least 1:3 ratio of wet sediment sample to DESS ratio for efficient DNA preservation. 2. Dispose the aqueous DESS-phase of each benthic sample through a 45 µM wire mesh sieve into a chemical waste container and rinse with 45 µM filtered water. DMSO should not be introduced into freshwater ecosystems. 3. Pour all the sediment-phase of each benthic sample into a 2 L measuring cylinder and rinse the storage pot as well as the 45 µM wire mesh sieve with 45 µM filtered tap water into the cylinder. 4. Fill the 2 L measuring cylinder with 45 µM filtered tap water, seal with lid, invert the cylinder vigorously 10 times and let it rest for no more than 30 seconds. 5. Gently pour the decanted samples onto a 45 µM wire mesh sieve and repeat steps 4 and 5 six

times. **TIP:** Do not lean the cylinder more than a 40 degree angle; the less sediment in suspension decanted with your community, the better. 6. Localise your biotic sample into the corner of the sieve and rinse into a sample pot using DESS and store the pots at 4 C until use. _Separation from fine silt – centrifugation_ 1. Use a 1 mm sieve on top of a bottle-top sterile 47 mm 45 µM membrane filter and pour in the DESS decanted sample, keep a DESS container under the last sieve \(e.g. petri dish). 2. Wash the sample retained on the bottle-top 47 mm 45 µM membrane filter with distilled water. 3. Again, wash the sample retained on the bottle-top 47 mm 45 µM membrane filter but this time with 1.16sg LUDOX-TM and pour it into a 50ml falcon tube and top up if necessary with 1.16sg LUDOX-TM up to 2 cm from the top. **TIP:** Do not use too much 1.16sg LUDOX-TM since each sample should fit into a 50 ml falcon tube. 4. Shake the 50 ml falcon tube vigorously, centrifuge for 5 min at 4000rpm and carefully pour the centrifuged sample into the bottle-top membrane filter. **TIP:** If samples are contaminated with fine sediments, use a vacuum filter system to help with the process. 5. Re-use the retained 1.16sg LUDOX-TM for each sample and repeat step 3 five times. 6. Rinse the sample retained on the bottle-top 47 mm 45 µM membrane filter with distilled water. 7. Carefully detach the bottle-top sterile 47 mm 45 µM membrane filter and with the help of sterile forceps and blade, carefully fold and slice the 45 µM filter into three pieces. 8. Place the sliced 45 µM membrane filter inside a 15 ml falcon tube and store at -80 C until subsequent DNA extraction. **DNA extraction** C overnight in 3ml of DNA lysis buffer assisted by spinning wheel mixing.1. Incubate each sample retained in the 15 ml falcon tubes at 56 2. Transfer the 3 ml digested solution into a sterile 50 ml falcon tube. 3. Add 6 ml of Buffer AL \(QIAamp DNA Blood Maxi Kit, Qiagen) and mix thoroughly by inversion. 4. Add 5 ml of 100% ethanol and mix thoroughly by inversion. 5. Follow QIAamp DNA Blood Maxi Kit \(Qiagen) manufacturer's protocol. **TIP:** Centrifugation and incubation times were increased by up to 15-30 minutes as this will ensure complete removal of Buffers AW1 and AW2. 6. Incubate each sample in 700 µl of AE Buffer \(Qiagen) for at least 4h-8h, prior to elution via centrifugation for 20 minutes at 5000rpm. **TIP:** A second elution with the same amount of AE Buffer can be performed overnight at room temperature as this results in higher DNA yields. 7. Keep samples in 1.5 – 2 ml tubes at -20C until subsequent PCR amplification.

Timing

About 2 days Meiofauna extraction, 1h and 20 minutes per sample; sample digestion, 16 hours; DNA extraction, 2 hours; DNA elution, 4-6 hours

References

1 Yoder, M. et al. DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. Nematology 8, 367-376 \(2006)