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MBARI Environmental DNA (eDNA) extraction using Qiagen DNeasy Blood and Tissue Kit

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MBON eDNA

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We use this protocol and it's working

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Abstract

Nucleic acids extraction from the filters using the Qiagen DNeasy Blood and Tissue Kit with some modifications to the manufacturer's protocol. These extraction protocols, adapted from Thomsen et al (2012), are used by the Monterey Bay Aquarium Research Institute (MBARI) for Monterey Bay, CA samples (MB). All MB samples were extracted in the Chavez Laboratory at the Monterey Bay Aquarium Research Institute, Moss Landing California.

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Thomsen, P. F., Kielgast, J., Iversen, L. L. N., Møller, P. R., Rasmussen, M. & Willerslev, E., 2012, Detection of a Diverse Marine Fish Fauna Using Environmental DNA from Seawater Samples. *Plos One,* 7(8)**,**e41732.

Qiagen Inc., July 2006 DNeasy Blood and Tissue Handbook; available online from Qiagen Inc§ .

Qiagen, Inc., June 2012 Qiamp DNA Mini and Blood Mini Handbook, available online from Qiagen, Inc.

Guidelines

Always observe proper laboratory safety warning and precautions.

Safety warnings

This section is taken directly from the Qiagen DNeasy Blood and Tissue Handbook.

- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/ts/msds.asp where you can find, view, and print the MSDS for each QIAGEN kit and kit component.
- CAUTION: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.
- Buffer AL and Buffer AW1 contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. If liquid containing this buffer is spilt, clean with suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.
- The following risk and safety phrases apply to components of DNeasy Blood & Tissue Kits and DNeasy 96 Blood & Tissue Kits.
- Buffer AL and Buffer AW1 (concentrate)Contains guanidine hydrochloride: harmful, irritant. Risk and safety phrases:* R22-36/38, S13-26-36-46
- 1 Proteinase K Contains proteinase K: sensitizer, irritant. Risk and safety phrases:* R36/37/38-42/43, S23-24-26-36/37 13.10 24-hour emergency information
- Emergency medical information in English, French, and German can be obtained 24 hours a day from: Poison Information Center Mainz, Germany Tel: +49-6131-19240; or Poison Control USA Tel: (800) 222-1222.
- R22: Harmful if swallowed;
- R36/37/38: Irritating to eyes, respiratory system and skin;
- R36/38: Irritating to eyes and skin;
- R42/43: May cause sensitization by inhalation and skin contact;
- S13: Keep away from food, drink, and animal feeding stuffs;
- S23: Do not breathe spray;
- S24: Avoid contact with skin;
- S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice;
- S36: Wear suitable protective clothing;
- S36/37: Wear suitable protective clothing and gloves;
- S46: If swallowed, seek medical advice immediately, and show container or label

Preparation

1 Nucleic acids were extracted from the filters using the Qiagen DNeasy Blood and Tissue Kit with some modifications to the manufacturer's protocol.

2 Prior to extraction, 0.5 mm and 0.1 mm glass beads (BioSpec Products) were ashed at 500 °C for 5 hours.

3 Bead tubes: 0.25 g of each size glass bead was distributed into sterile 2.0-ml conical microcentrifuge tubes (with screw cap and o-ring). The tubes were subsequently autoclaved for 30 min.

Extraction

4 Sample filters were transferred to bead tubes with sterile forceps, 720 µl Buffer ATL (Qiagen) was added, and two bead-beating steps were done: tubes were shaken in a MiniBeadbeater (BioSpec Products) at maximum speed for 45 sec, followed by incubation at 56 °C for 30 min and a second round of bead-beating (45 sec) and incubation (30 min). After the second incubation, 80 µl Proteinase K (Qiagen) was added. Tubes were then placed in a 56 °C shaking incubator for overnight incubation.

5 After incubation, tubes were vortexed for 15 sec then centrifuged for 1 min at 4,000 x g. 650 µl of supernatant was transferred to new 1.5-ml tubes then spun at 13,000 x g for 1 min. After the final spin, 600 µl of supernatant (avoiding any remaining glass beads) was transferred to new 2-ml tubes for next steps.

6 Remaining steps followed the manufacturer's protocol for the Qiagen DNeasy Blood and Tissue Kit with the following modifications: 600 µl of Buffer AL and 600 µl of 100% ethanol were used; 500 µl of lysate was pipetted to spin column then pulled through with aQiagen vacuum manifold each time until the entire volume of lysate (1.8 mL) was pulled through the spin column; two 500-µl washes of Buffer AW1 and two 500-µl washes of Buffer AW2 were done; elutions were done in two 50-µl steps for a total of 100 µl extracted DNA.

7 To serve as a control in each set of extractions, an additional empty tube with beads was carried through the process as an extraction blank.