

# Environmental DNA (eDNA) extraction using Qiagen DNeasy blood and tissue kit

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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 are used by the University of South Florida (USF) for Florida Keys samples (FK) and the Monterey Bay Aquarium Research Institute (MBARI) for Monterey Bay, CA samples (MB). FK samples were extracted in the Breitbart Laboratory at the University of South Florida and all MB samples were extracted in the Chavez Laboratory at the Monterey Bay Aquarium Research Institute using the same extraction protocol.

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## Guidelines

Always observe proper laboratory safety warning and precautions.

## Protocol

### Preparation

#### Step 1.

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

### Preparation

#### Step 2.

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

### Preparation

#### Step 3.

0.25 g of each size was distributed into 2.0 ml extraction tubes, the tubes were subsequently autoclaved and UV treated for 30 min.

#### Extraction

##### Step 4.

Sample filters were transferred to bead tubes with subsequent bead beating steps as in Djurhuus et al., in press, and then [incubated] at 56 °C overnight with 720 µl Buffer ATL and 80 µl Proteinase K (Qiagen). Remaining steps followed the manufacturer's protocol for the DNeasy Blood and Tissue Kit

#### Extraction

##### Step 5.

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.

#### Extraction

##### Step 6.

Samples were subsequently diluted 1:10, split into three aliquots, and distributed to three separate research laboratories for marker-specific processing: 16S rRNA gene at the University of South Florida (secondary 16S amplification done at RTSF Genomics Core, MSU), 18S rRNA and COI gene at the Monterey Bay Aquarium Research Institute (secondary COI amplification at RTSF Genomics Core), and 12S rRNA gene at Stanford University. See "Environmental DNA (eDNA) metabarcoding Illumina MiSeq NGS PCR Protocol" for 12S, 18S, 16S, and COI.

#### Extraction

##### Step 7.

Additionally, each loci had a mock community that served as positive controls for library preparation steps and sequencing.

## 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](http://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
- 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