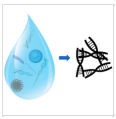

Nov 26, 2019

Environmental DNA (eDNA) extraction with modified Qiagen DNeasy Blood & Tissue kit

Collin Closek[1], Hilary Starks[1], Elizabeth Andruszkiewicz[1], Alexandria Boehm[1]

[1]Stanford University

1 Works for me dx.doi.org/10.17504/protocols.io.yrqfv5w

MBON eDNA

Collin Closek

ABSTRACT

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

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Place holder for publication

GUIDELINES

Workspace and equipment were cleaned prior:
Workspace was wiped down with 10% bleach, followed by 70% ethanol.
Pipettes and centrifuges were sprayed and wiped down with RNase Away.
Pipettes, molecular grade water, and racks were UV sterilized for 20 min (10 min each side).

SAFETY WARNINGS

Always observe proper laboratory safety warning and precautions.

Below 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
- 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) transferred to glass beaker, covered with tin foil, and ashed at 500 °C for 5 hours.

3 0.75 g of each bead size was distributed into sterile 5 ml extraction tubes, the tubes with caps loosened were subsequently autoclaved and UV treated for 30 min.

Extraction

4 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.

5 Ashed beads were added to sample filters tubes with subsequent bead beating steps as in Djurhuus et al., 2017, and then incubated at 56 °C overnight with 900 µl Buffer ATL and 100 µl Proteinase K (Qiagen).

6 Bead free supernatant was transferred to 2 ml tube, 650 µl Buffer AL was added, and then briefly vortexed. An additional 650 µl Ethanol (96-100%, molecular grade) was added, then vortexed briefly.

7 Mixture was added into mini spin column (650 µl, 3x). Remaining steps followed the manufacturer's protocol for the DNeasy Blood and Tissue Kit.

8 Samples were subsequently diluted 1:10 for 12S rRNA gene amplicon and metabarcoding preparation.