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Preparing biological samples for metabarcoding V.1

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ABSTRACT

This protocol describes the preparation of biological samples (specifically from a marine environment e.g. hatchery or RAS unit) for amplicon sequencing. Starting with a biological sample stored in Qiagen buffer ATL, or similar, it begins with a bead beating process to homogenise the sample. Enzymatic lysis using Metapolyzyme and Proteinase K are emplyed to ensure efficient DNA release. The Qiagen DNeasy kit is used to column extract DNA from lysates. Following concentration estimates of DNA elutions, samples are diluted >1:10 to avoid PCR inhibition during amplicon library preparation.

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PROTOCOL CITATION

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KEYWORDS

Metabarcoding, metagenomics, DNA extraction

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In every step following enzymatic digestion of samples (and in general), ensure samples are kept at 4C to maximise sample stability.

Freeze DNA samples if not being used for >1 week following extraction.

Otherwise, storing DNA at 4C in fridge is preferable.

MATERIALS TEXT

MATERIALS

⊠ Buffer AL **Contributed by**

users Catalog #19075

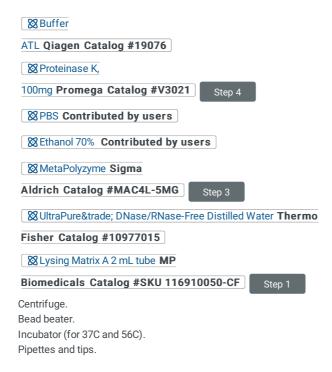
⊠ QIAgen DNeasy Blood and Tissue Kit, 50

rxn Qiagen Catalog #69504

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SAFETY WARNINGS

Refer to manufacturer's MSDS information for each reagent used to ensure appropriate and safe use.

DISCLAIMER:

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ABSTRACT

This protocol describes the preparation of biological samples (specifically from a marine environment e.g. hatchery or RAS unit) for amplicon sequencing. Starting with a biological sample stored in Qiagen buffer ATL, or similar, it begins with a bead beating process to homogenise the sample. Enzymatic lysis using Metapolyzyme and Proteinase K are emplyed to ensure efficient DNA release. The Qiagen DNeasy kit is used to column extract DNA from lysates. Following concentration estimates of DNA elutions, samples are diluted >1:10 to avoid PCR inhibition during amplicon library preparation.

BEFORE STARTING

Ensure leaving time for samples to thaw if frozen. Avoid leaving samples thaw for too long as this may lead to degradation.

Bead beating 45m

1 Starting with biological sample (filter, swab, water, biofilm, tissue etc.) stored in Qiagen Buffer ATL (or similar), transfer up to 1 mL to Matrix A bead tube.

⊠ Buffer ATL

Qiagen Catalog #19076

 ■ Lysing Matrix A 2 mL tube MP Biomedicals Catalog #SKU 116910050-CF 15m Perform bead beating in a disruptor at at 5.0 M/s (speed) for © 00:00:40 x2 (ensure tube looks homogenous). Enzymatic digestion 2h 15m 2h 15m Add $\Box 5 \mu I$ of Metapolyzyme to each tube and vortex briefly. Incubate samples at § 37 °C for © 02:00:00. Aldrich Catalog #MAC4L-5MG Add **20** µl of Proteinase K to each tube, vortex for **00:00:10**, then incubate at **56°C 00vernight ⊠** Proteinase K, 100mg Promega Catalog #V3021 DNA extraction Vortex samples for \bigcirc 00:00:15 and centrifuged 313000 x g for \bigcirc 00:01:00. Transfer the supernatant from each tube (up to 900 µl) into a new tube and centrifuged at **313000 x g, 00:01:00**. Transfer up to $\Box 600 \mu I$ of bead-free supernatant to a new $\Box 2 \mu L$ tube. Premix 70% ethanol and Qiagen lysis buffer AL 1:1 to add to sample at a ratio of 1:1:1 e.g. for 10 samples of ⊒500 μl each, premix ⊒550 μl of buffer AL and ⊒550 μl of 70% ethanol and add ■1 mL of ethanol/buffer AL mixture to each sample.

9 Hereafter, the manufacturer's protocol for the Qiagen DNeasy Blood and Tissue kit is followed with some modifications:

⊠ Buffer AL, Lysis

buffer Qiagen Catalog #19076

- Load < □600 µl of lysate mixture (ATL, AL and EtOH) at a time into the column
- Spin at **36000** x g, 00:01:00 and discard flow-through.
- Repeat as necessary until all lysate is loaded on column e.g. mixture of □1500 μl may take x3 initial spins and flow through discarding to complete column binding.

⊠QIAgen DNeasy Blood and Tissue Kit, 50

rxn Qiagen Catalog #69504

- 10 Place the DNeasy Mini spin column in a new 2 mL collection tube (provided), add 500 μl Buffer AW1, and centrifuge a 6000 x g, 00:01:00 (8000 rpm).
 - Discard flow-through and collection tube.
- 11 Place the DNeasy Mini spin column in a new **2 mL** collection tube (provided), add **500 μl** Buffer AW2, and centrifuge for at **20000 x g**, **00:03:00** to dry the DNeasy membrane.
 - Discard flow-through and collection tube.
- 12 Perform final elution in $\boxed{100}$ µI of AE buffer.

Preparing concentration for library preparation

- 13 Check approximate concentration of extracted DNA using a Nanodrop.
- 14 Prepare 1:10 dilution of each extraction for PCR (to avoid PCR inhibition).

 Perform further dilution of sample to a maximum final concentration of ~ [M]1 ng/μl [M]10 ng/μl
- 15 Use ~ 30 ng of DNA in a 30 μ l per sequencing library PCR reaction (see amplicon library PCR protocol).