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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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KEYWORDS

Metabarcoding, metagenomics, DNA extraction

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GUIDELINES

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** Step 9

Buffer

ATL **Qiagen Catalog #19076**

Proteinase K,

100mg **Promega Catalog #V3021** Step 4

PBS **Contributed by users**

Ethanol 70% **Contributed by users**

MetaPolyzyme **Sigma**

Aldrich Catalog #MAC4L-5MG Step 3

UltraPure™ DNase/RNase-Free Distilled Water **Thermo**

Fisher Catalog #10977015

Lysing Matrix A 2 mL tube **MP**

Biomedicals Catalog #SKU 116910050-CF Step 1

Centrifuge.
Bead beater.
Incubator (for 37C and 56C).
Pipettes and tips.

SAFETY WARNINGS

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

DISCLAIMER:

ABSTRACT

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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. 30m

Buffer ATL

Qiagen Catalog #19076

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Lysing Matrix A 2 mL tube **MP Biomedicals Catalog #SKU 116910050-CF**

2 Perform bead beating in a disruptor at at 5.0 M/s (speed) for **00:00:40** x2 (ensure tube looks homogenous). 15m

Enzymatic digestion 2h 15m

3 Add **5 µl** of Metapolyzyme to each tube and vortex briefly. 2h 15m

Incubate samples at **37 °C** for **02:00:00** .

MetaPolyzyme **Sigma Aldrich Catalog #MAC4L-5MG**

4 Add **20 µl** of Proteinase K to each tube, vortex for **00:00:10** , then incubate at **56 °C** **Overnight** . 16h

Proteinase K, 100mg **Promega Catalog #V3021**

DNA extraction

5 Vortex samples for **00:00:15** and centrifuged **13000 x g** for **00:01:00** .

6 Transfer the supernatant from each tube (up to **900 µl**) into a new tube and centrifuged at **13000 x g, 00:01:00** .

7 Transfer up to **600 µl** of bead-free supernatant to a new **2 mL** tube.

8 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.

Buffer AL, Lysis buffer **Qiagen Catalog #19076**

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

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- Load < **600 µl** of lysate mixture (ATL, AL and EtOH) at a time into the column
- Spin at **6000 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 **100 µl** 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 ~ **50 ng** of DNA in a **20 µl** per sequencing library PCR reaction (see amplicon library PCR protocol).

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