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Preparing biological samples for metabarcoding V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bms8k6hw

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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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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

NAME	CATALOG #	VENDOR
Buffer AL	19075	
QIAgen DNeasy Blood and Tissue Kit, 50 rxn	69504	Qiagen
Buffer ATL	19076	Qiagen
Proteinase K, 100mg	V3021	Promega

NAME	CATALOG #	VENDOR
PBS		
Ethanol 70%		
MetaPolyzyme	MAC4L-5MG	Sigma Aldrich
UltraPure™ DNase/RNase-Free Distilled Water	10977015	Thermo Fisher
Lysing Matrix A 2 mL tube	SKU 116910050-CF	MP Biomedicals

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Buffer ATL	19076	Qiagen
Lysing Matrix A 2 mL tube	SKU 116910050-CF	MP Biomedicals
MetaPolyzyme	MAC4L-5MG	Sigma Aldrich
Proteinase K, 100mg	V3021	Promega
Buffer AL, Lysis buffer	19076	Qiagen
QIAgen DNeasy Blood and Tissue Kit, 50 rxn	69504	Qiagen

MATERIALS TEXT

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:

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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 30m up to **1 mL** to Matrix A bead tube.

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Buffer ATL
by Qiagen
Catalog #: 19076

Lysing Matrix A 2 mL tube
by 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 somewhat 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
by 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
by Promega
Catalog #: V3021

DNA extraction

5 Vortex samples for **00:00:15** and centrifuge a **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

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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
by Qiagen
Catalog #: 19076

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

- 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
by 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.

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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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