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Preparing biological samples for metabarcoding V.2

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Protocol status: Working
We use this protocol and it works for us to generate metabarcoding libraries from biological samples (stored in e.g. Qiagen buffer ATL) for amplicon sequencing using MinION.

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Disclaimer

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.

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

MATERIALS

- Buffer AL **Catalog #**19075
- QIAgen DNeasy Blood and Tissue Kit, 50 rxn **Qiagen Catalog #**69504
- Buffer ATL **Qiagen Catalog #**19076
- Proteinase K, 100mg **Promega Catalog #**V3021
- PBS
- Ethanol 70%
- MetaPolyzyme **Sigma Aldrich Catalog #**MAC4L-5MG
- UltraPure™ DNase/RNase-Free Distilled Water **Thermo Fisher Catalog #**10977015
- Lysing Matrix A 2 mL tube **MP Biomedicals Catalog #**SKU 116910050-CF

STEP MATERIALS

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

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

Protocol materials

- Proteinase K, 100mg **Promega** **Catalog** #V3021
- Buffer AL **Catalog** #19075
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- Proteinase K, 100mg **Promega** **Catalog** #V3021
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- Ethanol 70%
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Safety warnings

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

Before start

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

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 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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**MAC4L-5MG

4 Add 20 µL of Proteinase K to each tube, vortex for 00:00:10 , then incubate at 16h

56 °C Overnight .

Proteinase K, 100mg **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 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:

- 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).