



# Preparing biological samples for metabarcoding V.1

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In Development

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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 employed 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.

## DOI

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## PROTOCOL CITATION

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## KEYWORDS

Metabarcoding, metagenomics, DNA extraction

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Sep 07, 2020

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## PROTOCOL INTEGER ID

41713

## 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<sup>®</sup> 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.

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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.

[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 homogenous). <sup>15m</sup>

#### 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**. <sup>16h</sup>


 **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:

- 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 at **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 ~ **1 ng/µl** - **10 ng/µl**
- 15 Use ~ **50 ng** of DNA in a **20 µl** per sequencing library PCR reaction (see amplicon library PCR protocol).