VERSION 2

SEP 28, 2020

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bms8k6hw

Protocol Citation: Tim Regan 2020. Preparing biological samples for metabarcoding. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bms8k6hw> Version created by Tim Regan

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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.

Created: Sep 28, 2020

Last Modified: Sep 28, 2020

🌐 Preparing biological samples for metabarcoding V.2

Tim Regan¹

¹The Roslin Institute, University of Edinburgh

Tim Regan

DISCLAIMER

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to [protocols.io](#) is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with [protocols.io](#), can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

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.

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

 QIAgen DNeasy Blood and Tissue Kit, 50 rxn **Qiagen Catalog #69504** Buffer ATL Qiagen Catalog #19076

PBS Contributed by users

Ethanol 70% **Contributed by users**

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

 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.

SAFETY WARNINGS

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

BEFORE START INSTRUCTIONS

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

30m

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 15m somewhat homogenous).

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 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).