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# Protocols for eDNA/eRNA extraction from marine samples V.2

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This document provides a series of protocols used to extract eDNA or eRNA from marine environmental samples such as small and large volume (filtered) water, sediment or (fine mesh net) plankton. Specifically, these protocols are recommended for use following sampling as described in Mirmin et al. (2021; Environmental DNA sampling protocols for the surveillance of marine non-indigenous species.protocols.iohttps://dx.doi.org/10.17504/protocols.io.by7rpzm6).

DOI

dx.doi.org/10.17504/protocols.io.bz95p986

Luca Mirimin, Dulaney Miller, Sara Fernandez 2021. Protocols for eDNA/eRNA extraction from marine samples. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bz95p986
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protocol

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Sara Fernandez, Dulaney L. Miller, Luke E. Holman, Arjan Gittenberger, Alba Ardura, Marc Rius, Luca Mirimin, Environmental DNA sampling protocols for the surveillance of marine non-indigenous species in Irish coastal waters, Marine Pollution Bulletin, Volume 172, 2021, 112893, ISSN 0025-326X, https://doi.org/10.1016/j.marpolbul.2021.112893

eDNA, eRNA, extraction, environmental samples, protocol

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While these protocols can be adapted and used for a wide range of sources and sample types, these protocols are recommended for samples collected using the methods described in:

Luca Mirimin, Dulaney Miller, Sara Fernandez2021. Environmental DNA sampling protocols for the surveillance of marine non-indigenous species. **protocols.io** <a href="https://dx.doi.org/10.17504/protocols.io.by7rpzm6">https://dx.doi.org/10.17504/protocols.io.by7rpzm6</a>

# PROTOCOL 1 - eDNA extraction from water samples (low volume water)

Micropipette set (1-1000ul) and relevant filter tips

Microcentrifuge to accommodate 1.5-2.0mL tubes

Bead beater

Incubator (at last up to 60C)

Vortexer for 1.5-2.0mL tubes

Ethanol (96-100%) (not denaturated alcohol)

rxn Qiagen Catalog #69504 In 2 steps

**⊠** Oakton<sup>™</sup> Glass beads for Mills **Fisher Scientific** Step 2.1

**⊠** DNA-

ExitusPlus Applychem Catalog #A7089 Step 2.1

Buffer ATL (tissue lysis

buffer) Qiagen Catalog #19076 In 2 steps

⊗ Proteinase K,

2mL Qiagen Catalog #19131 In 2 steps

10mL tubes suitable to beat beater

1.5mL microtubes

## PROTOCOL 2 - eDNA extraction from water samples (high volume water)

Micropipette set (1-1000ul) and relevant filter tips

Pipettes 1-10mL

Centrifuge to accommodate 1.5-2.0mL microtubes

Centrifuge capable of spinning 50 ml tubes at 2500 x g using swing-out rotor

Rocker/shaker for HV filter capsules

Incubator (at last up to 60C)

Vortexer for 1.5-2.0mL tubes with adapter for 50mL



### 

Kit Mobio Catalog #12988-10 In 2 steps

### PROTOCOL 3 - eDNA/eRNA co-extraction from water samples (high volume water)

Micropipette set (1-1000ul) and relevant filter tips

Pipettes 1-10mL (not needed if extracting from small volume samples)

Centrifuge to accommodate 1.5-2.0mL microtubes

Centrifuge capable of spinning 50 ml tubes at 2500 x g using swing-out rotor (not needed if extracting from small volume samples)

Rocker/shaker for HV filter capsules (not needed if extracting from small volume samples) Incubator (at last up to 60C)

Vortexer for 1.5-2.0mL tubes with adapter for 50mL (adapter not needed if extracting from small volume samples)

#### ZR-Duet™ DNA/RNA MiniPrep Plus **Zymo**

Research Catalog #D7003 Step 4.1

SuperScript II reverse transcription kit Life Technologies Step 4.2

#### PROTOCOL 4 - eDNA extraction from water samples (high volume tow net)

Micropipette set (1-1000ul) and relevant filter tips

Microcentrifuge to accommodate 1.5-2.0mL tubes

Incubator (at last up to 60C)

Vortexer for 1.5-2.0mL tubes

◯ ♥ QIAgen DNeasy Blood and Tissue Kit, 50

rxn Qiagen Catalog #69504 In 2 steps

🛭 Buffer ATL (tissue lysis

buffer) Qiagen Catalog #19076 In 2 steps

**⊠** Proteinase K,

2mL Qiagen Catalog #19131 In 2 steps

1.5mL microtubes

### PROTOCOL 5 - eDNA extraction from sediment samples

Micropipette set (1-1000ul) and relevant filter tips

Microcentrifuge to accommodate 1.5-2.0mL tubes

Incubator (at last up to 60C)

Vortexer for 1.5-2.0mL tubes

Weighing scale (up to 10g)



3

Kit Mobio Catalog #12988-10 In 2 steps

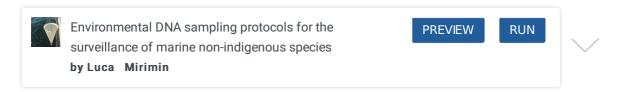
Make sure to establish a Risk Assessment to mitigate any adverse effect on users while carrying out any of these protocols. Useful information (e.g. Safety Data Sheets) should be sourced from the relevant suppliers who procured the materials.

To minimize risk of sample contamination, note that all protocols should be carried out in dedicated eDNA extraction laboratories/rooms.

Ensure that each protocol and list of materials is checked before starting any of the procedures. Specifically, make sure that all key materials(e.g. kits) have not been modified or discontinued by the relevant supplier.

## INTRODUCTION

1 Note that these nucleic acid extraction protocols have been adapted and tested in conjunction with sampling protocols as detailed in:



See also the relevant peer-reviewed publication here:

Fernandez S, Miller DL, Holman LE, Gittenberger A, Ardura A, Rius M, Mirimin L (2021). Environmental DNA sampling protocols for the surveillance of marine non-indigenous species in Irish coastal waters.. Marine pollution bulletin.

https://doi.org/10.1016/j.marpolbul.2021.112893

1.1 Overview of protocols included in this document:

Α	В
Protocol title	Description/purpose
PROTOCOL 1 - eDNA extraction from	Extracting eDNA from filter
water samples (low volume water)	membranes following filtration of
	low voume marine water (e.g. 1L)
PROTOCOL 2 - eDNA extraction from	Extracting eDNA from High Volume
water samples (high volume water)	filter capsules (e.g. 1 µm
	polyethersulfone filter membrane
	with an Effective Filtration Area of
	1,300 cm2)
PROTOCOL 3 - eDNA/eRNA co-	Co-extracting eDNA and eRNA from
extraction from water samples (high	filter membranes following filtration
volume water)	of marine water
PROTOCOL 4 - eDNA extraction from	Extracting eDNA from marine
water samples (high volume tow net)	samples collected with a fine mesh
	(e.g. 50um) plankton net
PROTOCOL 5 - eDNA extraction from	Extracting eDNA from marine
sediment samples	sediment

PROTOCOL 1 - eDNA extraction from water samples (low volume water)

15m

2 Extract eDNA using the

**⊠QIAgen DNeasy Blood and Tissue Kit, 50** 

rxn Qiagen Catalog #69504

following

manufacturer's instructions on "Purification of Total DNA from Animal Tissues (Spin-Column Protocol)", with the following modifications:

2.1 Following sample collection as per "Protocol for collection of water samples (low volume water)", cut each filter membrane in half with scissors and place it in a ■10 mL tube containing 0.25 g of 0.1 mm glass beads and 0.25 g of 0.5 mm glass beads ②Oakton™ Glass beads for Mills Fisher Scientific or equivalent. Both scissors and tweezers used for cutting and handling the membranes should be decontaminated with

**⊠**DNA-

ExitusPlus Applychem Catalog #A7089 (or equivalent) prior to use.

2.2 Add  $\blacksquare$ 720  $\mu$ L of

■ Buffer ATL (tissue lysis)

buffer) Qiagen Catalog #19076

, **⊒950** μL of



distilled water and 100 µL of Proteinase K

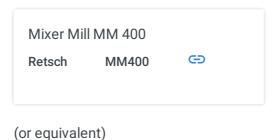
<a href="mailto:RProteinase">RProteinase K</a>,

2mL Qiagen Catalog #19131

(2 mg/L final concentration).

15m

2.3 Bead beat the mixture for © 00:15:00 at half speed using a



- 2.4 Follow subsequent steps as per manufacturer's recommendations
- 2.5 During the final elution phase, add  $\Box 100 \, \mu L$  of

3 × Powe

■ PowerMax® Soil DNA Isolation

Extract eDNA using the Kit **Mobio Catalog #12988-10** following manufacturer's instructions.

PROTOCOL 2 - eDNA extraction from water samples (high volume water)

3.1

Note that some initial modifications should be incorporated when extracting from samples collected using a semi-automated eDNA sampler.

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6

Mark II InDepth eDNA sampler Semi-automated water sampler

Applied Genomics Mark II

Further details are available at <u>Biodiversity and eDNA Survey</u>, <u>Analysis and Monitoring | Applied Genomics</u>

PROTOCOL 3 - eDNA/eRNA co-extraction from water samples (high volume water)

4

The following protocol was adapted from Pochon et al. (2017)

Pochon X, Zaiko A, Fletcher LM, Laroche O, Wood SA (2017). Wanted dead or alive? Using metabarcoding of environmental DNA and RNA to distinguish living assemblages for biosecurity applications.. PloS one.

https://doi.org/10.1371/journal.pone.0187636

4.1 Co-extract eDNA and eRNA using

**⊠**ZR-Duet<sup>™</sup> DNA/RNA MiniPrep Plus **Zymo** 

Research Catalog #D7003

following manufacturer's recommendations.

Store eDNA at & -20 °C and eRNA at & -80 °C.

To monitor posible crosscontamination in the extraction process, include a negative control in the DNA isolation step, consisting of the same components but no starting material, to be processed alongside the eDNA/eRNA samples throughout all subsequent steps. In case there is a contamination, it can be detected after sequencing.



4.2 Reverse Transcribe eRNA into cDNA using

SuperScript II reverse transcription kit **Life Technologies** or equivalent kit.

cDNA can be stored at 8 -20 °C .

PROTOCOL 4 - eDNA extraction from water samples (high volume tow net)

5 Extract eDNA using the

**⊠QIAgen DNeasy Blood and Tissue Kit, 50** 

rxn Qiagen Catalog #69504

following

manufacturer's instructions on "Purification of Total DNA from Animal Tissues (Spin-Column Protocol)", with the following modifications:

- 5.1 Following sample collection as per "Protocol for collection of water samples (high volume tow net)", mix by briefly vortexing and place a 15 mL subsample into a new clean 50 mL falcon tube (conical bottom). Centrifuge at max speed to form a pellet. Remove supernatant by pipetting.
- 5.2 Add  $\blacksquare$ 1350  $\mu$ L of

**⊠** Buffer ATL (tissue lysis

buffer) Qiagen Catalog #19076

, **□**100 μL

⊗ Proteinase K,

2mL Qiagen Catalog #19131

and follow manufacturer's

recommendations for all subsequent steps. Including an overnight incubation step.

5.3 During the final elution phase, add  $\Box 100 \, \mu L$  of

**⊠** Buffer

AE Qiagen Catalog #19077

and spin through the column.

Repeat the step above for a final total volume of  $200 \, \mu L$ .

Store extract at 8 -20 °C.

PROTOCOL 5 - eDNA extraction from sediment samples

6 Extract eDNA from a  $\Box$ 5 g sub-sample using

○ PowerMax® Soil DNA Isolation

Kit Mobio Catalog #12988-10

following

manufacturer's recommendations.

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8

**Citation**: Luca Mirimin, Dulaney Miller, Sara Fernandez Protocols for eDNA/eRNA extraction from marine samples <a href="https://dx.doi.org/10.17504/protocols.io.bz95p986">https://dx.doi.org/10.17504/protocols.io.bz95p986</a>

