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Nov 22, 2021

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

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

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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.io**https://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 Luca Mirimin

protocol

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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Nov 22, 2021

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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**https://dx.doi.org/10.17504/protocols.io.by7rpzm6

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)
QIAgen DNeasy Blood and Tissue Kit, 50 rxn **Qiagen Catalog #69504** In 2 steps
Oakton™ 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

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

PowerMax® Soil DNA Isolation 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)

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PowerMax® Soil DNA Isolation

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:

Environmental DNA sampling protocols for the surveillance of marine non-indigenous species

by Luca Mirimin

PREVIEW RUN

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:

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

A	B
Protocol title	**Description/purpose**
PROTOCOL 1 - eDNA extraction from water samples (low volume water)	Extracting eDNA from filter membranes following filtration of low voume marine water (e.g. 1L)
PROTOCOL 2 - eDNA extraction from water samples (high volume water)	Extracting eDNA from High Volume filter capsules (e.g. 1 µm polyethersulfone filter membrane with an Effective Filtration Area of 1,300 cm2)
PROTOCOL 3 - eDNA/eRNA co-extraction from water samples (high volume water)	Co-extracting eDNA and eRNA from filter membranes following filtration of marine water
PROTOCOL 4 - eDNA extraction from water samples (high volume tow net)	Extracting eDNA from marine samples collected with a fine mesh (e.g. 50um) plankton net
PROTOCOL 5 - eDNA extraction from sediment samples	Extracting eDNA from marine 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 ☒Oakton™ 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 **720 µL** of ☒Buffer ATL (tissue lysis buffer) **Qiagen Catalog #19076** , **950 µL** of

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distilled water and 100 µL of Proteinase K
Proteinase K,
2mL **Qiagen Catalog #19131** (2 mg/L final concentration).

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

Mixer Mill MM 400
Retsch **MM400**

(or equivalent)

2.4 Follow subsequent steps as per manufacturer's recommendations

2.5 During the final elution phase, add **100 µL** of
Buffer
AE **Qiagen Catalog #19077** and spin through the column.
Repeat the step above for a final total volume of **200 µL** .
Store extract at **-20 °C** .

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

3 PowerMax® Soil DNA Isolation
Extract eDNA using the Kit **Mobio Catalog #12988-10** following manufacturer's instructions.

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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Mark II InDepth eDNA sampler
Semi-automated water sampler
Applied Genomics **Mark II**

Further details are available at Biodiversity and eDNA Survey, Analysis and Monitoring | Applied Genomics

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

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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™ 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 proccess, 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.

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4.2 Reverse Transcribe eRNA into cDNA using ☒SuperScript II reverse transcription kit **Life Technologies** or equivalent kit.

cDNA can be stored at **-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** sub-sample 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 **1350 µL** 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 **100 µL** of ☒Buffer AE **Qiagen Catalog #19077** and spin through the column. Repeat the step above for a final total volume of **200 µL** .

Store extract at **-20 °C** .

PROTOCOL 5 - eDNA extraction from sediment samples

6 Extract eDNA from a **5 g** sub-sample using ☒ PowerMax® Soil DNA Isolation Kit **Mobio Catalog #12988-10** following manufacturer's recommendations.

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