MAY 14, 2021

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bfk8jkzw

Protocol Citation: Marine Vautier, Cécile Chardon, Isabelle Domaizon 2021. Fish eDNA: DNA extraction from water samples filtered through Sterivex filter unit and preserved in buffer . **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bfk8jkzw>

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's working

Created: Apr 27, 2020

Last Modified: May 14, 2021

PROTOCOL integer ID:
 36224

🌐 Fish eDNA: DNA extraction from water samples filtered through Sterivex filter unit and preserved in buffer

👤 In 1 collection

Marine Vautier¹, Cécile Chardon¹, Isabelle Domaizon¹

¹INRAE - UMR CARTEL - Pole R&D Ecla

CARTEL

Cecile Chardon

ABSTRACT

The objective of this protocol is DNA extraction from water samples filtered through 0.45 µm Sterivex™ filter units and preserved in 2 mL of buffer (dx.doi.org/10.17504/protocols.io.br5rm856).

Extraction is performed using NucleoSpin® Soil kit (MACHEREY-NAGEL) with specific modifications adapted to DNA extraction from Sterivex™ filter units.

This protocol is used upstream to molecular biology analysis (e.g. qPCR, metabarcoding, ddPCR) to specifically target fish eDNA.

GUIDELINES

- Sample preparation
- Sample lysis
- Contaminants elimination
- DNA fixation and washing
- DNA elution

Keywords: fish, extraction, eDNA, environmental DNA, water, buffer preservation, Sterivex

MATERIALS

■ Samples

- Sterivex cartridge filled with 2mL of buffer and stored at -20°C or 4°C ([dx.doi.org/10.17504/protocols.io.br5rm856](https://doi.org/10.17504/protocols.io.br5rm856))

■ Reagents

- NucleoSpin® Soil kit (MACHEREY-NAGEL)
- Ethanol (96 - 100%), molecular grade to prepare buffer SW2
- Lysozyme solution (add 2 mg lysozyme to 40 µL of Lysis Buffer): 40 µL per sample
- Proteinase K solution (20 mg/mL): 50 µL per sample
- 20% SDS: 100 µL per sample

■ Materials

- Specific DNA-work station (sterile area equipped with air filtration and UV systems)
- Microcentrifuge for 1.5 to 2 mL tubes (relative centrifugal force needed :11,000 x g)
- Incubator (37°C and 55°C)
- Tube Rotator with holder for 15mL tubes or Sterivex
- Pipettes : 1000 µL - 100 µL
- 2 trash cans : 1 for liquid and 1 for solid
- Vortex with holder for 15 mL tubes

■ Consumables

- Tips with filter : 1000µL - 100µL
- Sterile 10 mL or 5 mL syringe (1 per sample)
- 2 mL sterile microcentrifuge tubes (about 6 tubes are required per sample)
- 1.5 sterile microcentrifuge tube to collect DNA at the end of extraction (1 per sample)
- Gloves

SAFETY WARNINGS

- SDS 20%
CAS number : CAS 151-21-3
Signal word : Corrosive substance and Harmful
Hazard phrases : 315, 318, 335
Precaution phrases : 261, 280, 302+352, 304+340+312, 305+351+338+310

- Proteinase K solution
CAS number : CAS 56-81-5 + CAS 39450-01-6
Signal word : Health hazard
Hazard phrases : 334
Precaution phrases : 261, 284, 304+340, 342+311

- Absolute ethanol
CAS number : 64-17-5
Signal word : Harmful and Flammable
Hazard phrases : 225, 319
Precaution phrases : 210, 305+351+338

The manufacturer advise to wear gloves and goggles and to follow the safety instructions for 2 reagents :

- SB contains Guanidinium thiocyanate 45 - 60%,
CAS number : CAS 593-84-0
Signal word : Irritant
Hazard phrases : 302, 412
Precaution phrases : 264W, 273, 301+312, 330
- SW1 contains Guanidine hydrochloride 36 - 50% and 2 - propanol 20 - 35%
CAS number : CAS 50-01-1, 67-63-0
Signal word : Irritant and Flammable
Hazard phrases : 226, 302, 319, 336
Precaution phrases : 210, 260D, 264W, 280sh, 301+312, 330

BEFORE START INSTRUCTIONS

- The following precautions must be applied :
 - Wear gloves throughout the extraction process
 - Clean the bench with DNA-off solution
 - Use tips with filters to avoid contaminations
 - **All steps have to be performed under a specific DNA-work station (sterile area equipped with air filtration and UV systems).**
- Material preparation :
 - Clean a specific DNA work station and apply UV for 15min
 - Turn on the incubator at +37°C
- Solutions preparation :
 - Check buffer SW2 - before the first utilisation, you need to add the indicate volume of ethanol (96 - 100%) to buffer SW2 concentrate and mark the label of the bottle to indicate that ethanol was added. This solution is stable at room temperature (18 - 25°C) for at least one year
 - Check absence of precipitate in SDS 20%. Dissolve any precipitate by incubating the solution at 30-40°C for 5-10 min and shaking solution every 2 min.

Prepare the sample

30m

- 1 *Note : If the samples have been stored at RT or at 4°C, proceed directly to step 2. If the samples have been frozen, proceed to step 1.*

30m

- Take the Sterivex™ filter unit stored at -20 °C after sampling, filtration and preservation with Buffer.
- Defrost the sample at 4 °C (about 00:30:00)

Note : during this step heat the incubator at 37 °C

Sample Lysis

2h 50m

- 2
- Fix the Sterivex cartridges on the vortex and vortex for **00:05:00** to remove particles from the filter **2h 50m**
 - Remove the Inlet Cap (Figure 1) and with a pipette tip, add **40 μ L** of **lysozyme solution** into the Sterivex already filled with 2mL of buffer. Insert pipette completely into the inlet to see the pipette tip inside the unit just above the membrane. Re-cap the Sterivex.

Figure 1: Position of the two tips of the Sterivex™ filter unit

- Rotate for **00:45:00** at **37 °C** (during this step, place the SDS solution in the incubator for the next step)
- Remove the Inlet Cap and with a pipette tip, add **50 μ L** of **Proteinase K solution** and **100 μ L** of **SDS 20%**. Re-cap the Sterivex.

*Note : during this step heat the incubator at **55 °C***

- Incubate for **02:00:00** at **55 °C** with agitation (or shake manually every 15 min).
- Remove the Inlet Cap and fix a sterile syringe to the Sterivex.
- Aspirate the liquid contained in the Sterivex with the syringe by moving back and forth to collect all the liquid
- Transfer the lysate into two 2 mL tubes (about 1.1 mL per tube).

*Note : after this step, place the **Buffer SE** in the incubator heated at **55 °C** to anticipate the elution step*

Precipitate contaminants

6m

- 3
- In both 2mL tubes, add 200 μ L of **buffer SL3**.
 - Vortex for 00:00:02
 - Incubate for 00:05:00 at 4 °C in a fridge
 - Centrifuge at 11000 x g, 00:02:00

7m 2s

Filter lysate

5m

- 4
- Place a NucleoSpin® Inhibitor Removal Column (red ring) in a collection Tube (2 mL, lid).
 - Load up to 650 μ L of clear supernatant (obtained at the step 3) onto the filter.
 - Centrifuge at 11000 x g, 00:01:00
 - Remove the column and close the tube containing the flow through.
 - Repeat these steps as many time as there is still some supernatant from step 3 to be filtered.
For each centrifugation, collect the supernatant in a clean tube (about 4 tubes are required, but only 1 column for the filtration of all lysate from step 3).
 - Discard the NucleoSpin® Inhibitor Removal Column.

Note: If a pellet is visible after the centrifugation, transfer the clear supernatant to a new collection tube (not provided in the kit) to get rid of this pellet, and continue with the clear supernatant.

Adjust binding conditions

1m

- 5
- Add 250 μ L of **Buffer SB** per 2mL tube.
 - *Note : 0.38V of buffer SB for 1V of flow through from step 4.*
 - Close the lid.

- Vortex for 00:00:05 and centrifuge briefly.

Bind DNA

6m

- 6
 - Place a NucleoSpin® Soil Column (green ring) in a Collection tube (2 mL).
 - Load 650 µL of sample from step 5 onto the column.
 - Centrifuge at 11000 x g, 00:01:00
 - Discard the flow through and place the column back into the collection tube.
 - Repeat the three previous steps with all the tubes from step 5 and using the same column.
 - Discard the flow through and place the column back into the collection tube.

Wash silica membrane

5m

- 7

Note : the same collection tube is used throughout the entire washing procedure to reduce plastic waste.

7.1 1st wash :

- Add 500 µL of **Buffer SB** to the NucleoSpin® Soil Column.
- Centrifuge at 11000 x g, 00:00:30
- Discard the flow through and place the column back into the collection tube.

7.2 2nd Wash :

- Add 550 µL of **Buffer SW1** to the NucleoSpin® Soil Column.
- Centrifuge at 11000 x g, 00:00:30

- Discard the flow through and place the column back into the collection tube.

7.3 3rd Wash :

- Add 650 µL of **Buffer SW2** to the NucleoSpin® Soil Column.
- Vortex 00:00:02 and centrifuge at 11000 x g, 00:00:30 .
- Discard the flow through and place the column back into the collection tube.

7.4 4th Wash :

- Add 650 µL of **Buffer SW2** to the NucleoSpin® Soil Column.
- Vortex 00:00:02 and centrifuge at 11000 x g, 00:00:30 .
- Discard the flow through and place the column back into the collection tube.

Dry silica membrane

2m

- 8 ■ Centrifuge at 11000 x g, 00:02:00 .

Note: if for any reason, the liquid in the collection tube touched the NucleoSpin® Soil Column after the drying step, discard flow through and centrifuge again.

Elute DNA

- 9 ■ Place the NucleoSpin® Soil Column into a new 1.5mL microcentrifuge tube.
- Add 30 µL of **Buffer SE** previously heated to 55°C into the column.

Note: a larger volume of SE buffer can be used but the DNA concentration will be lower

- Do not close the lid and incubate for 00:01:30 at Room temperature (18-25°C).

- Close the lid and centrifuge at 11000 x g, 00:00:30

- Throw the column and keep the tube containing the DNA.

Note: A second elution can be performed with the same column to collect more DNA (the concentration will be lower for the second elution)

- We recommend storing DNA frozen at -20°C until the use of the DNA sample (or at -40°C to -80°C for longer storage).