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WildinSync - eDNA laboratory extraction V.1

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Martina Lüthi^{1,2}, Sarah Thurnheer^{1,2}, Flurin Leugger^{1,2}, Loïc Pellissier^{1,2}

¹Ecosystems and Landscape Evolution, Department of Environmental Systems Science, ETH Zürich, Zürich, Switzerland;

²Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland

WildinSync



Loïc Pellissier

ETH Zürich

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We use this protocol and it's working

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Abstract

This protocol is used in the context of the long-term global biodiversity monitoring initiative WildinSync, which applies environmental DNA (eDNA) metabarcoding to assess anthropogenic impacts and nature-positive actions on ecosystems. The filtration of eDNA from water bodies provides a non-invasive approach to assess biodiversity across marine and terrestrial systems. This protocol describes the processing of water filtrates conserved in a buffer followed by DNA extraction and purification. The extracted DNA is intended for downstream molecular analyses, including qPCR and metabarcoding, to enable biodiversity monitoring.

Guidelines

- FAIR terms are listed in the YAML frontmatter of this page.
- See <https://fair-edna.github.io/download.html> for the FAIR checklist and more information.
- See <https://fair-edna.github.io/guidelines.html#missing-values> for guidelines on missing values that can be used for missing FAIR or MIOP terms.

Materials

Durable equipment

- Horizontal reciprocating flask shaker (e.g., S50-type from Cat-ingenieurbüro) - Any validated supplier, 1, For liquid mixing should reach 800 rpm
- Microcentrifuge for 1.5-2 ml tubes (e.g., Accuspin micro 17R from Fisherbrand) - Any validated supplier, 1
- Refrigerated bench centrifuge for 50 mL tubes (e.g., Centrifuge GT2R Expert from Fisherbrand) - Any validated supplier, 1, Refrigerated and setup for 5,500 x g
- Freezer (-20 °C laboratory freezer) - Any validated supplier, 1, Reagent & sample storage, DNA precipitation
- Vortex mixer (e.g., Vortex Genie 1 from SI) - Any validated supplier, 1, Sample mixing
- Incubator or thermoblock (e.g., Touch Screen dry block heater from Thermo Scientific) - Any validated supplier, 1, For enzymatic incubation (lysis)
- Pipette controller (e.g., Pipette controller from ClearLine) - Any validated supplier, 1, For serological pipettes
- Micropipette (1000 µL) (e.g., Acura pipette from Socorex) - Any validated supplier, 1, Calibrated
- Micropipette (100 µL) (e.g., Acura pipette from Socorex) - Any validated supplier, 1, Calibrated

Consumable equipment

- Sterile pipette tips for 1000 µL (e.g., Sterile - with filter - low retention from ClearLine) - Any validated supplier, as required, Sterile, filtered, low-retention
- Sterile pipette tips for 100 µL (e.g., Sterile - with filter - low retention from Clearline) - Any validated supplier, as required, Sterile, filtered, low-retention
- Sterile serological pipette for 25 mL (e.g., Serological pipettes sterile from Sarstedt) - Any validated supplier, as required, Sterile, single-use
- 50 mL sterile tube (e.g., 50 mL sterile tube from Greiner) - Any validated supplier, as required, Sterile, rated for 5,500 x g
- 2 mL sterile microtubes (e.g., DNA LoBind 2 mL tube from Eppendorf) - Any validated supplier, as required, Sterile, low DNA binding
- 1.5 mL sterile microtubes (e.g., DNA LoBind 1.5 mL tube from Eppendorf) - Any validated supplier, as required, Sterile, low DNA binding
- Silica spin column & 6 2 mL collection tubes (Nucleospin Soil kit components) - Macherey-Nagel, 1 per sample, Included in extraction kit

Chemicals

- Absolute ethanol (e.g., Ethanol in bottle abs, zA ACS ISO 1L from Supelco) - Any validated supplier, 19.8 mL per sample
- 3M Sodium acetate pH 5.5 (e.g., 3M Sodium acetate pH5.5 from Invitrogen) - Any validated supplier, 0.9 mL per sample, Molecular grade
- Buffer ATL (Buffer ATL from Qiagen) - Qiagen, 0.72 mL per sample

- Proteinase K (Proteinase K from Qiagen) - Qiagen, 20 µL per sample, $A_{280} > 600$ mAU/ mL
- Binding buffer (SB) (SB from Nucleospin Soil kit) - Macherey-Nagel, 0.75 mL per sample, Included in extraction kit
- Wash buffer 1 (SW1) (SW1 from Nucleospin Soil kit) - Macherey-Nagel, 0.55 mL per sample, Included in extraction kit
- Wash Buffer 2 (SW2) (SW2 from Nucleospin Soil kit) - Macherey-Nagel, 1.2 mL per sample, Included in extraction kit
- Elution buffer (SE) (SE from Nucleospin Soil Kit) - Macherey-Nagel, 0.1 mL per sample, Included in extraction kit
- Sterile water (e.g., hyclone water-molecular grade from cytiva) - Any validated supplier, 30 mL per extraction, Extraction negative control

Safety warnings

- ! When working with DNA extraction kits containing guanidine and using bleach for decontamination, it is essential to avoid mixing these chemicals due to the potential formation of toxic fumes. Guanidine compounds, when combined with bleach, can produce harmful gases. Ethanol can also react dangerously with bleach; when ethanol and bleach come into contact, they can generate toxic chloroform vapors.

To mitigate these risks, always ensure that any surfaces or equipment that come into contact with guanidine-based buffers or ethanol are thoroughly rinsed with water before using bleach for decontamination. In the event of a spill or accidental release of guanidine-containing buffer or ethanol on the hood or work surface, immediately clean the area with water before proceeding with bleach disinfection. These precautions help prevent dangerous chemical reactions and ensure a safe working environment.

Personal protective equipment (PPE) must be worn at all times when handling bleach and chemical reagents. This includes laboratory gloves and safety goggles to prevent skin contact and eye exposure. Proper PPE use is essential to reduce the risk of chemical burns caused by bleach and other hazardous reagents.

Wear disposable gloves throughout the DNA extraction process. Gloves should be changed frequently and may be decontaminated with 10% bleach between steps when appropriate to minimize cross-contamination.

Clean all work surfaces before and after sample manipulation using DNA decontamination solution (e.g. DNA Exitus Plus for instruments) and 10% commercial bleach for surface and plastic racks.

Use sterile, filtered pipette tips for all liquid handling steps.



Before start

This protocol assumes that water samples have already been collected and filtered according to the Water eDNA Sampling Protocol. This protocol was adapted from Pont et al. (2018), with the following modifications:

- The centrifugation at $15,000 \times g$ for 15 min was replaced by $5,500 \times g$ for 35 min.
- The precipitation volume was adapted to the current filter setup.
- The elution volume was reduced to $2 \times 80 \mu\text{L}$ instead of $2 \times 100 \mu\text{L}$.

Safety

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Personal protective equipment (PPE) must be worn at all times when handling bleach and chemical reagents. This includes laboratory gloves and safety goggles to prevent skin contact and eye exposure. Proper PPE use is essential to reduce the risk of chemical burns caused by bleach and other hazardous reagents.

Training Requirements

- 2 The personnel must be trained to work in a clean laboratory environment, be experienced in working under a laminar flow hood, and be familiar with the molecular techniques used.

Time Needed to Execute the Procedure

- 3 The time required is approximately 3.5 hours on Day 1, followed by an overnight incubation. On Day 2, approximately 6 hours are required, including a 2-hour lysis incubation.

Standard Operating Procedure

- 4 Perform all extraction steps in a dedicated DNA work station or clean laboratory area designed for low-biomass or degraded DNA, equipped with appropriate air filtration and UV decontamination systems.
When possible, conduct extractions in a laboratory room specifically designated for rare or environmental DNA work (e.g., positive air pressure, UV treatment, frequent air renewal, restricted access).
DNA extraction must not be performed in rooms where PCR products are amplified or handled, to minimize the risk of cross-contamination.

Wear disposable gloves throughout the DNA extraction process. Gloves should be changed frequently and may be decontaminated with 10% commercial bleach between steps when appropriate to minimize cross-contamination.

Clean all work surfaces before and after sample manipulation using DNA decontamination solution (e.g. DNA Exitus Plus for instruments) and 10% commercial bleach for surface and plastic racks.

Use sterile, filtered pipette tips for all liquid handling steps.

Extraction

5 Sample preparation

- Agitate the filtration capsule containing the preservation buffer for 15 min at 800 rpm on an horizontal reciprocating flask shaker.
- Collect 30 mL of preservation buffer in a 50 mL sterile tube. Preserve the remaining buffer (about 5-10 mL) in another 15 mL sterile tube as a backup.
- Centrifuge at 5,500 x g for 35 min.
- Remove and discard (in non-halogenated solvents waste) 21 mL of the supernatant using a 25 mL sterile serological pipette. 9 mL should remain in the 50 mL sterile tube. Avoid disturbing the pellet.

DNA Precipitation

- Add 19.8 mL of absolute ethanol using a 25 mL sterile serological pipette.
- Add 0.9 mL of 3M sodium acetate pH 5.5 using a 1000 µL micropipette and its dedicated tips.
- Mix by inverting the tubes 5 times.
- Incubate in a freezer at -20 °C overnight.
- Centrifuge at 5,500 x g for 35 min.
- Discard (in non-halogenated solvents waste) the supernatant by pouring (do not pipette).

Sample lysis

- Add 0.72 mL of buffer ATL.
- Mix using the vortex mixer for 5 min at full speed.
- Transfer the mixture to a 2 mL sterile microtube containing 20 µL of Proteinase K.
- Mix by inverting the tubes 5 times.
- Incubate for 2 hours at 56 °C using a thermoblock.
- Centrifuge for 2 min at 11,000 x g using the microcentrifuge.
- Transfer the clear supernatant to a new 2 mL sterile microtube.

Adjusting binding conditions (step 6 from the NucleoSpin soil kit manual)

- Add 250 µL of binding buffer (SB).
- Close the lid and vortex 5 seconds using the vortex mixer.

DNA binding

- Place a silica spin column (green ring) into a 2 mL collection tube.
- Load 550 µL of sample into the column.
- Centrifuge for 1 min at 11,000 x g using the microcentrifuge.
- Discard the flow through (Guanidine salts waste) and place the column back into the 2 mL collection tube.
- Load the remaining sample onto the column.
- Centrifuge for 1 min at 11,000 x g using the microcentrifuge.
- Discard the flow through (Guanidine salts waste) and place the column back into the 2 mL collection tube.
- If a large pellet accumulates in the column and blocks the passage of the sample, resuspend the pellet in SB buffer and re-centrifuge the column.

Wash and dry silica membrane

1st wash

- Add 500 µL of binding buffer (SB) to the silica column.
- Centrifuge for 30 seconds at 11,000 x g using the microcentrifuge.
- Discard the flow through (Guanidine salts waste) and place the column back into the 2 mL collection tube.

2nd wash

- Add 550 µL of wash buffer 1 (SW1) to the silica column.
- Centrifuge for 30 seconds at 11,000 x g using the microcentrifuge.
- Discard the flow through (Guanidine salts waste) and place the column back into the 2 mL collection tube.

3rd wash

- Add 600 µL of wash buffer 2 (SW2) to the silica column.
- Close the lid and vortex for 2 seconds using the vortex mixer.
- Centrifuge for 30 seconds at 11,000 x g using the microcentrifuge.
- Discard the flow through (Guanidine salts waste) and place the column back into the 2 mL collection tube.

4th wash

- Add 600 µL of wash buffer 2 (SW2) to the silica column.
- Close the lid and vortex for 2 seconds using the vortex mixer.
- Centrifuge for 30 seconds at 11,000 x g using the microcentrifuge.
- Discard the flow through (Guanidine salts waste) and place the column back into the 2 mL collection tube.

Dry silica membrane

- Centrifuge for 2 min at 11,000 x g using the microcentrifuge.
- If for any reason, the liquid in the collection tube has touched the NucleoSpin® Soil Column after the drying step, discard flow through and centrifuge again.

DNA elution

- Place the silica column into a new 1.5 mL sterile microtube (not provided by the Nucleospin soil kit).
- Add 80 µL of elution buffer (SE) previously heated at 37 °C into the column.
- Do not close the lid and incubate for 1 min 30 seconds at room temperature (18-25 °C).
- Close the lid and centrifuge for 30 seconds at 11,000 x g using the microcentrifuge.
- Repeat the same steps with the same silica column and the same 1.5 mL sterile microtube to get a final extract of 160 µL: Add 80 µL of elution buffer (SE) previously heated at 37 °C into the column.
- Do not close the lid and incubate for 1 min 30 seconds at room temperature (18-25 °C).
- Close the lid and centrifuge for 30 seconds at 11,000 x g using the microcentrifuge.
- Throw away the column and keep the tube containing the 160 µL of DNA extract.
- Store in the freezer at -20 °C for short and middle term.
- For long term and biobanking, store your sample at -80 °C.

Quality Control

- 6 No DNA quantification or DNA quality assessment (e.g. spectrophotometric measurements such as NanoDrop 260/280 ratios, fluorometric quantification, or fragment analysis using systems such as TapeStation) is performed as part of this extraction protocol.

Assessment of amplifiable DNA and evaluation of PCR inhibition are conducted in a downstream workflow using the *qPCR Inhibition* protocol and are therefore not described here.

At the extraction stage, quality control is ensured through: - Strict contamination prevention measures (clean laboratory environment, filtered pipette tips, surface decontamination) - Inclusion of negative extraction control processed alongside samples.

Basic Troubleshooting Guide

- 7 Low or no DNA recovery: Verify that ethanol and sodium acetate volumes are added correctly during precipitation and that centrifugation speeds and durations are

respected. Ensure pellets are not lost during supernatant removal.

Clogged silica column during loading: If column flow is reduced or blocked, resuspend the pellet thoroughly by adding binding buffer (SB) prior to continuing sample loading onto the column.

Contamination risk: Confirm that work surfaces, equipment, and gloves are properly decontaminated. Use sterile, filtered pipette tips and process negative extraction controls alongside samples.

Kit-specific issues: For troubleshooting related to silica column performance, buffer handling, or unexpected extraction outcomes, refer to the Macherey-Nagel NucleoSpin® Soil Kit manufacturer's manual.

Protocol references

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