

Sampling guide for collection of eDNA samples with Sterivex filtration and sediment

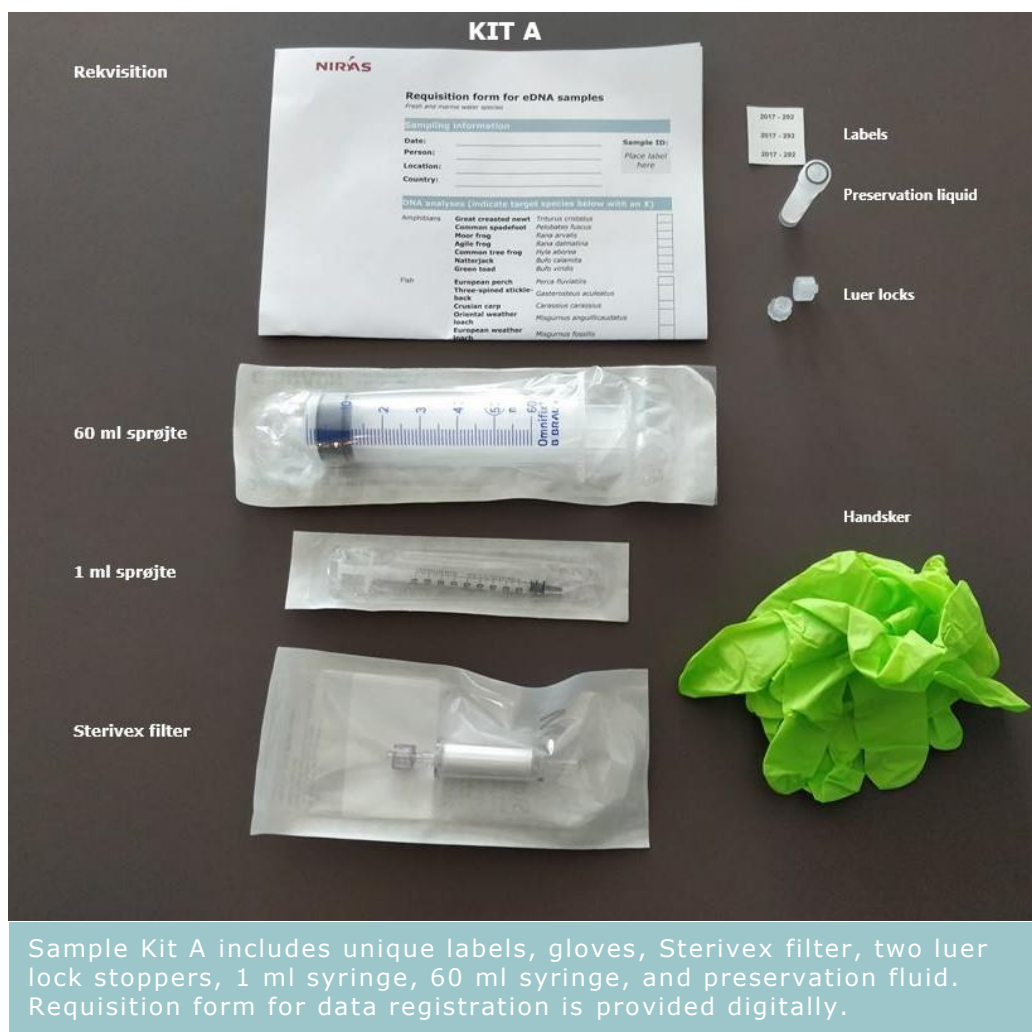
This sampling guide has been customized for the project:

“Tykskallet malermusling i Suså - 2021”

Collection of eDNA samples using Sterivex filters is performed using sample kit A (image below). Individual kits are provided for each sample to be collected. Sediment samples will be collected using equipment provided by the sampler, a short guide to best practices is included at the bottom of this document.

The requisition form is customizable, and has been provided digitally. Please fill out a requisition form for each collected sample.

For this specific project, six units of sampling kit A have been provided, as well as blank labels for marking containers and filters. Please mark each sample clearly, and indicate the sample name clearly on the requisition form.



Procedure for Sterivex filtration

Step 1 - Filling the 60 ml. syringe

Fill the provided 60 ml syringe with water from the site of investigation. Fill the syringe in 30 cm depth and try to avoid larger particles in the water sample (e.g. insects, leaves and dirt).

Step 2 - Attach the syringe to the Sterivex filter and filtration

Attach the syringe to the Sterivex filter unit and add pressure to the syringe to filtrate the water. This step is to be repeated with step one as long as it is still possible to filtrate water through the Sterivex filter unit. Try to avoid that the filter is fully clogged, since this will make step 3 more difficult.

Step 3 - Removing water from the filter

Note down the volume of filtrated water on the requisition form for later estimation of DNA concentration in the sample.

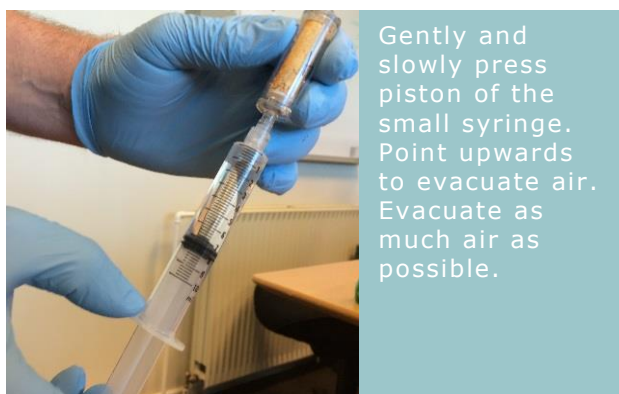
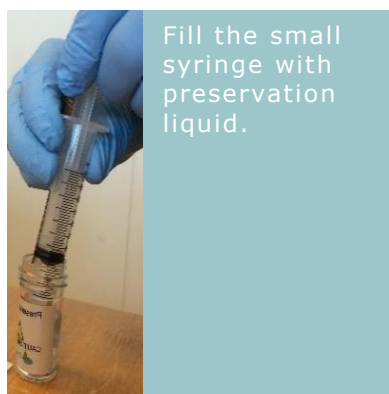
Remove all water from Sterivex filter with the empty 60 ml syringe. Fill the syringe with air and connect to the filter as done in step two. Use the air to press out remaining water in the filter. Repeat until filter is completely empty. This step is performed to preserve DNA and to not dilute the preservation liquid solution, if used.

4 Step 4 - preparing the samples:

Choose either Step 4A or 4B. Note: Samples are sensitive to heat.

- Choose 4A when preservation liquid is to be used.
- Choose 4B when preservation liquid is not to be used.

4A Step 4A: Preservation liquid is used.



Add preservation liquid to the Sterivex filter using the 1mL syringe and close the ends of the filter with stoppers.

Store sample at low temperature as soon as possible (-20 °C) within 2-3 days. Ship samples to laboratory cooled as much as possible (e.g. cooling elements, crushed ice or dry ice)



Close the filter with the unisex stoppers/plugs.

4B **Step 4B:** Preservation liquid is not used.

Remove water from Sterivex filter by pressing surplus water through the Sterivex filter with a syringe. Store the Sterivex filter on ice (or at -20°C) as soon as possible. (The sooner - the better). Ship samples to laboratory on dry ice or as cool as possible (e.g. cooling elements or crushed ice).

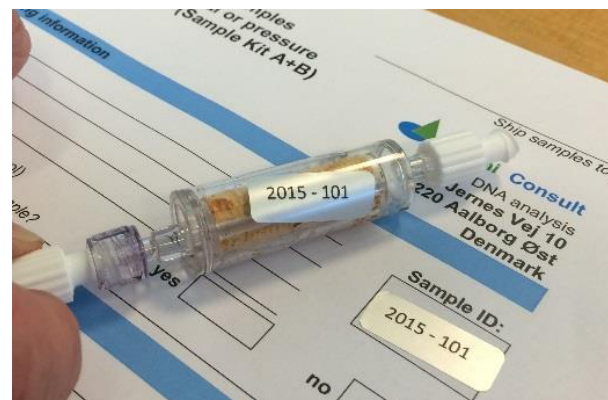


Store the filter on ice (or at -20°C) as soon as possible

Step 5 - Marking the samples:

Mark the filter clearly using the provided labels. Use an identical label on the requisition form. Fill out the requisition form with information, and ship requisition form and filter unit to us for analysis.

Shipping conditions must be according to 4A or 4B.



Put identical labels on filter and requisition form. Fill out empty fields on requisition form.

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Sediment sampling

Equipment:

- Sediment sampler
- Collection containers (1L/2.5L bucket w. lid)
- 70-100% ethanol
- Gloves
- Requisition forms + labels
- Waterproof marker

Step 1: Document the site.

Document all relevant information about the sampling location on the requisition form, including stream substrate and number of samples taken. Make sure the sample name is clearly visible on the requisition form.

Step 2: Prepare the equipment.

Saturate the sampling tube and container by flushing them 3 times with water from the stream.

Step 3: Collect representative samples.

Use the sampling tube to bore 3-5 sediment cores of the upper 5 cm of the stream bed across the width of the stream, depending on stream width. Should the sampling location be on a bend in the stream, a diagonal sampling may be appropriate.

Step 4: Transfer samples to container.

Deposit the sampled sediment cores into the container, and cover with 1 cm of water from the stream. Close the container, and mark the sample name clearly on the side. Keep the container cool until storage in the fridge (4°C).

Step 5: Clean up.

Wash the sampling equipment thoroughly with water from the stream to remove all remaining sediment. Clean the sampling tube on the inside with ethanol and air dry. Discard protection items such as gloves between locations.