

LeDNA: Global measure of biodiversity by sampling environmental DNA from lakes

Environmental DNA sampling protocol

Overview:

Sampling environmental DNA from one lake entails the filtration of up to 30 liters of surface water collected from multiple sampling sites along the shoreline. With a maximum of 10 sites distributed around the lake, focus on sampling water from locations in the lake but near the main rivers or streams flowing into the lake and if possible, in the lake near the lake's outflow.

The sampling of the lake water will all be done through a single filter, resulting in one sample per lake, but have water from many locations. The total volume of water to sample should be split across the sites, thus it will be important to have an idea of where you plan to sample before you go. In cases where the lake has less than 10 rivers or streams or accessibility to these points is limited or not allowed (e.g., in a protected area and you do not have a permit to sample), it is perfectly acceptable to sample fewer sites. For example, if the lake has only 2 main rivers flowing into it, the sampling strategy would involve filtering 10 liters of water in proximity to each river and 10 liters near the outflow. If accessibility to the outflow is restricted, sample as close as feasible.

Our sampling kits are equipped with a custom filter cartridge (Figure 1) designed for high-volume filtration, using a 5 μm filter. This filtration system has demonstrated efficacy in handling volumes over 40 liters of water. While the recommended sampling volume is 30 liters, it is essential to note that this is an estimate. Instances such as algae blooms or high levels of suspended particles in the water may necessitate a lower filtration volume that must be distributed across all sites rather than focusing solely on one site. In the event of filter clogging, which is experienced when the water flow through the pump is low and it becomes extremely hard to pump the water, simply record the filtered volume obtained to that point. The bucket you are provide with is 3 L so if filled to half each time this is 1.5 L per location. As this is not an exact measurement device, its okay to make an estimate plus or minus a few liters is not going to make a huge difference. The value of the sample will come from sampling multiple locations near rivers.

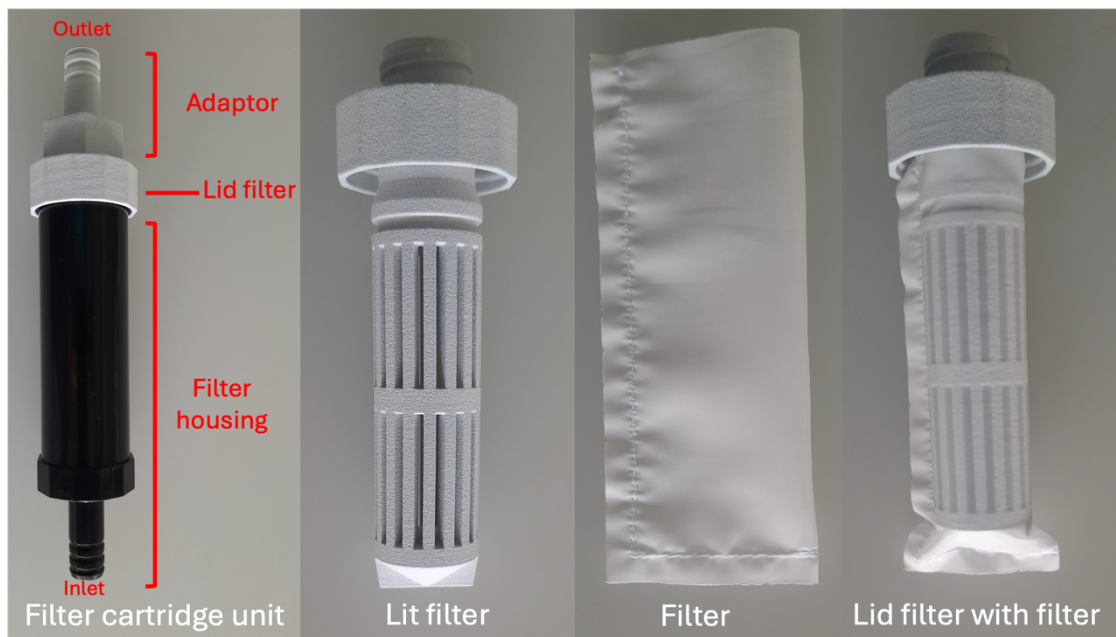


Figure 1. Components of the filter cartridge unit.

Important:

1. Read the sampling protocol carefully.
2. If you have questions, refer to our training materials prior to reaching out:
 - [Sampling video and Webinars](#)
 - [FAQs](#)
3. Read the [sampling sheet](#) designed for data collection during sampling. Print the sampling sheet if you will not have access to internet and a phone to enter the data on site.
4. All equipment has undergone bleaching and UV treatment, and the filter cartridge is assembled and ready for sampling. Sampling kits cannot be used for practice.
5. Measuring environmental variables (e.g., water temperature, pH, dissolved oxygen, conductivity, etc.) is not required. If you are able to, please record the measurements after water collection for eDNA sampling and provide the respective units in which they were measured.
6. Paper towels are not provided but would be good if you can bring some with you to wipe or dry material if needed.

Sampling kit for one lake:

When you first receive the sampling kit, check if you have all equipment you will need (Figure 2):

- 1x 3L plastic bucket
- 1x Hand suction pump for liquids
- 1x Big plastic bag:
 - 1x Filter cartridge unit with nylon filter (Figure 1)
 - 2x Hose (25 cm)
 - 2x pairs of nitrile gloves
- 1x Small plastic bag:
 - 1x pair of nitrile gloves
 - 1x 50 ml plastic tube with 25 ml of preservation buffer
 - 1x 15ml plastic tube lid (red cap)



Figure 2. Sampling kit.

When you arrive to a sampling site:

1. Use the QR code or the link provided in the sampling sheet to record information during sampling.
Note: You can register information in the sampling sheet and complete the form later.
2. Record the Hylak_id specific to your lake (point 2 in sampling sheet).
Note: You can find the Hylak_id number in the table provided to you earlier.
3. Record the sample code found on the barcode label (e.g., LeDNA240001) located inside the bucket lid (point 3 in sampling sheet).



4. Record the coordinates of sampling site (point 5 in sampling sheet).

Sampling eDNA:

5. Open the big plastic bag, put on a pair of gloves to prevent contamination of the water sample with human DNA.
6. Thoroughly rinse (3x) the bucket and hose with lake water.
7. Using the plastic bucket, collect surface water (3L).
Note: Avoid contact with the water (e.g., standing, falling in, etc.) to minimize the risk of sample contamination. In case the water sample contains large debris (e.g., leaves or twigs), discard the collected water and obtain a fresh sample. If the lake exhibits signs of an algae bloom or elevated levels of suspended particles, reduce the volume of water collected from all sampling sites.
8. Assemble the filter cartridge and hand pump by inserting the adapter into the pump inlet (near the pump handle).



9. Attach the hose to the outlet of the filter cartridge (black end).



10. Insert the other end of the hose into the water in the bucket.

Note: Once the filtration system is assembled, the use of gloves is not mandatory. However, avoid touching the material in contact with the water without gloves.

11. Pull and push the pump handle to initiate suction and pull water through the filter.

Note: Be careful to prevent filtered water from re-entering the bucket through the pump outlet. You can adjust the orientation of the hand pump outlet to control its direction.

To facilitate, you can tilt the bucket, or a team member can help by holding the hose and bucket.

12. When finished, disassemble filter cartridge from the pump, and remove the PVC hose.

13. Carefully, shake the filter cartridge to remove excess water.

14. Store filter cartridge and hose in plastic bag 1, put everything in the bucket and move to next sampling site.

15. Repeat steps 4 to 14 in all sampling sites around the lake.

Preserve eDNA sample:

16. In your last sampling site, once all water is filtered, remove the hose, and disassemble filter cartridge from hand pump.

17. Open the small plastic bag and put on a fresh pair of gloves.

18. Unscrew the adaptor unit from the lid filter unit.



19. Screw the red cap, from plastic bag 2, onto the lid filter unit.



20. Unscrew the lid filter unit from the filter housing unit.



21. Move and screw lid filter unit into the 50 ml tube with preservation buffer.

Note: Some buffer may spill, avoid contact with skin. Add a note in sampling sheet if you spill a large volume of buffer.



22. Carefully, shake the 50 ml tube to be sure the filter is covered in the buffer.

23. Place the 50 ml tube with the now preserved filter back in the small bag.

Note: The sample is room temperature stable for 6 months. Keep it away of direct sunlight and below 45 °C.

24. Place everything inside the bucket. If you do not have means to dispose the gloves, put them in the big bag.

25. Mail everything back to:

Cátia Lúcio Pereira
ETH Zürich
Department of Environmental Systems Science
Institute of Biogeochemistry and Pollutant Dynamics (IBP)
Universitaetstrasse 16, G50.4
8092 Zurich, Switzerland
(+41) 44 632 34 37