

eDNA

Sampling and filtration with peristaltic pump or syringe

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Generally

Analyses using eDNA (environmental DNA) are DNA traces that organisms leave behind in the environment. The traces come from, among other things, skin cells, faces, urine, sweat, germ cells and saliva. Detection of these traces can be used to inventory species in different environments. DNA traces are found in soil, air and water. At the Centre for Genetic Identification (CGI) at the National Museum of Natural History, we carry out eDNA analyses on behalf of Swedish authorities and environmental organisations.

We have extensive experience working with different types of material for DNA analysis and can both analyze entire species groups with so-called barcode analysis and look for specific species which is often called single-species analysis. In environmental monitoring today, it is most common to work with water samples and the simplest and most effective method for collection is to collect and filter water in the field. This manual describes how water samples for eDNA can be taken and also contains information about what equipment is required.

Equipment list

Most of the equipment needed for sampling and filtration is standard water sampling equipment. Special materials other than the pump and tubing listed in the table below are available from CGI (cgi@nrm.se).

A good and efficient pump for use in the field is the "Eijkelkamp peristaltic pump 12 VDC standard" which is available for purchase or rental from Unoson, among others. Note that both municipalities and county administrative boards sometimes own such a pump which is often used for groundwater sampling, so it may be worth checking around among colleagues.

If filtering in the field is not possible, water samples can be filtered at CGI's laboratory. It is then important that the samples are transported there as quickly as possible. DNA breaks down faster at high temperatures and bright light, so keep the samples cool and dark during transport. The samples can also be frozen if they are not filtered within 30 hours of sampling. However, we recommend filtering in the field as in our experience this gives both better and more stable results.

Equipment	Available where
Peristaltic pump (brand Unoson) – bookable	Unoson
Battery charger – included in booking of pump	Unoson (included in booking)
Sterile plastic bottles (0,5 - 1,0 liter)	CGI
Large test tubes with stoppers for sediment samples	CGI
Thermometer	
Sterile gloves	CGI
Hose, hard	Unoson or the person who owns the pump
Hose, soft	Unoson or the person who owns the pump
Syringe sleeves, i.e. syringes “without a needle”	CGI
Scissors	
Filter (Photo 1)	CGI
Filter caps (Photo 2)	CGI
Buffer	CGI
Cable ties	
Nippers	
Marker pen	
Labels	
Chlorine (mix 1 + 9)	
Bucket with lid	
Garbage bags	



Photo 1. Filter.



Photo 2. Orange-colored filter caps.

Bring into the field

- Field forms
- Pump with charged battery
- Sterile plastic bottles
- Thermometer
- Hoses (hard and soft) pre-cut. new ones or cleaned with chlorine 10% + tap water
- Scissors
- Filters and filter caps
- Cable ties and nippers
- Syringe sleeve
- Marker pen, labels and garbage bags
- GPS or mobile with the ability to take coordinates and pictures
- Possibly chlorine solution and bucket, if multiple samples require purification in the field
- Possibly test tube for sediment sample

Sampling with peristaltic pump

1. Take coordinates, water temperature + photo at the location. Fill out the field protocol.

2. Put on disposable gloves and rinse the clean sampling bottle + any sampling equipment such as a net or similar downstream of the sampling point, or alternatively 10 m away. If the sample is taken from a boat, rinse on the windward side. Also rinse the hose. Note that all equipment that has been in contact with other water or equivalent should be cleaned with a 10% chlorine solution before rinsing off in the water to be sampled.



3. Take the sample upstream or, when sampling from a boat, on the windward side, avoiding the bottom. Also, remember not to step down with “dirty” shoes near the sample collection point.

- a. If you want to take a pooled sample from an area, you can take 0.5 litre from several different points and then mix these before filtering.
- b. If the water is cloudy, it is a good idea to let the bottle sit and settle for a while before filtering.



4. Cut 30 cm from both soft and hard tubing, or install cleaned pre-cut tubing. Preferably use gloves when handling below, so that the amount of human DNA is minimized.

- i. Soft hose to the filter.
- ii. Be careful with the hard tubing so that it does not get contaminated, as it will be immersed in the sample.
- iii. Put on filter + cable tie to prevent leaks between soft hose and filter (where metal buckle is located in photo 5).



Photo 5.

5. Turn on the pump. Check that it is running in the correct direction. Turn off.

6. Insert the hard hose into the sample, not at the bottom of the bottle (Photo 6). Turn on the pump and let it filter at least 0.5 litre of water, or as much as possible. Turn the pump off.



Photo 6.

7. Remove filter, cut off cable ties with wire cutters.

8. Screw on the spray nozzle and squeeze out as much water as possible (normally 2 - 3 times), so that the filter becomes "dry".

9. Take out the syringe with buffer, remove the cap and screw the syringe onto the filter.

10. Fill the filter with buffer, approximately 1 ml of buffer.

11. Put the caps on (two caps for each filter, reuse the cap from the small syringe and another cap that comes with it). Label the filter with the date and location. Place in a plastic bag and label this as well.

12. Filter a control sample with municipal tap water. This is so that the operator/company analysing the filters can see that any results or contaminations are not due to incorrect handling during filtration.

13. Send to operator/company for analysis as soon as possible (same day) if refrigerated. Alternatively, refrigerate/freeze filter and send later.

14. If the sample water is to be saved for the future; label and freeze this. Otherwise, pour out the remaining sample water and rinse the sample bottle and hoses in the chlorine solution (bucket).

15. Send filters for analyses.

16. Compile the field protocols (Appendix 1), images, type of analysis, etc. and send to the actor responsible for the analysis.

Sampling with syringe

NB! Do not reuse syringes, bottles or other sampling equipment between samples.

1. Take coordinates, water temperature and photo of the location. Fill out the field protocol (Appendix 1).
2. Put on disposable gloves and rinse the clean sampling bottle and any sampling equipment such as a net or similar downstream of the sampling point, or 10 m away. If the sample is taken from a boat, rinse on the windward side. Note that all equipment that has been in contact with other water or the like must be cleaned with a 10% chlorine solution before rinsing in the water to be sampled.
3. Take the sample upstream or, when sampling from a boat, on the windward side, avoid the bottom. Also, remember not to step down with "dirty" shoes near the sample collection point.
 - a. If you want to take a pooled sample from an area, you can take 0.5 litre from several different points and then mix these before filtering.
 - b. If the water is cloudy, it is a good idea to let the bottle sit and settle for a while before filtering.
4. Take out the 50 ml syringe and sylphuim/sterivex filter. Pull the syringe plunger out of the syringe sleeve. Screw on the filter. Fill the syringe with sample water and press the syringe plunger. Filter the entire sample volume. Unscrew the filter and refill the syringe with sample water. Screw the filter back on and filter the entire sample volume. Repeat until the water runs out or no more water can be filtered. Note the volume filtered.
5. Fill the syringe with air, screw on the filter and squeeze out as much water as possible from the filter.
6. Take out the 3 ml syringe filled with 1 ml of buffer. Unscrew the orange cap and screw the syringe onto the filter. Fill the filter with approximately 1 ml of buffer. Unscrew the syringe and screw the two orange caps onto the filter.
7. Place the buffer-filled filter in a zip-lock bag and label the bag with the sample name. The filter can also be labelled.
8. The sample can be stored in the refrigerator, freezer or at room temperature, but refrigeration is preferred.
9. Send the sample.

Tip for good results

Some species are easier to work with than others. For mussels, the chances of detection are increased if samples are taken when they release gametes, as otherwise there may be too small amounts of DNA traces from these in the water. For frogs, toads and salamanders, the greatest chance of detection is during the periods when the animals are active.

As with all types of research, one should be careful not to draw conclusions from only one time point and/or collection occasion. This is perhaps even more important with eDNA as it is an indirect detection, and the origin of the DNA detected is not known. For example, organisms from the catchment area will often be detected in a body of water because DNA residues can be transported in running water.

Questions that have arisen

How do you clean equipment?

Equipment such as hoses and bottles are cleaned of DNA traces with a solution consisting of 1 part chlorine and 9 parts water. It is important to rinse thoroughly afterwards, otherwise the chlorine will remove DNA the next time you take a sample. It is advisable to put the equipment in tap water overnight to remove the chlorine.

Is tap water sterile?

When cleaning equipment, such as a hose, in a chlorine solution (1 part chlorine to 9 parts water), the chlorine must be rinsed away. Can we then use tap water?

Answer: Yes.

Tap water in Sweden is usually purified with UV light, so there is no major danger in rinsing with that water and directly filtering a water sample in the office. However, you should filter the tap water and send the filter (blank sample) for analysis.

If you do filtration in the field, you can rinse both the hose and bottles thoroughly about 10 meters from the sampling point/downstream or with sampling water - this will also remove any traces of tap water.

Short of hose?

It is convenient to bring as many hoses as needed when sampling in the field and then put them in chlorine solution for a few minutes. Then you can put them in tap water overnight.

Delivery of samples

Samples can be dropped off or sent to:

Naturhistoriska riksmuseet
Centrum för Genetisk Identifiering
Svante Arrhenius väg 3
114 18 STOCKHOLM

It is important to contact cgi@nrm.se before sending the samples to ensure that someone is on site to receive the samples.

Appendix 1.

Field protocol eDNA

Local:

Date:

Water temperature and weather report:

Sampler:

Sample number	Name of sampling point	Coordinate N (SWEREF 99)	Coordinate E (SWEREF 99)	Type of analysis, possibly several

Other comments: