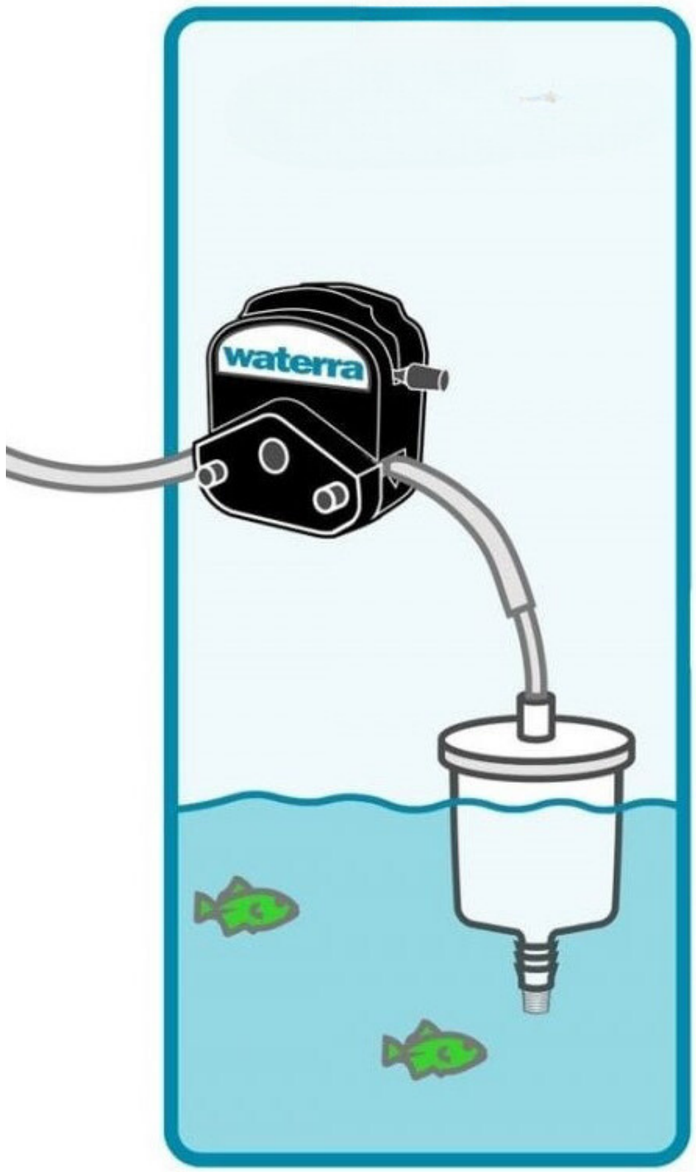
waterra

eDNA Filters

Sample collection and preservation protocols



Intro

Waterra makes high-quality eDNA filters for field collection of eDNA.

Water is pulled through eDNA filters using a suction device, be it a peristaltic pump or a diaphragm pump. Since the filter is positioned at the intake end of the system, tubing and other materials upstream of the filter do not need to be replaced.

This represents a significant time and cost savings reducing the risks of cross-contamination.

Overview

An efficient protocol has been developed whereby a non-lysis initiating buffer is injected into the filter after sample collection.

Lysis is the rupture of cell membranes which in this protocol is not encouraged as the released DNA is subject to degradation.

The buffer - eDNA mixture is pushed from the filter and into a 50 ml sample vial in the field. These small vials can be placed in coolers for transport for eDNA processing at a laboratory.

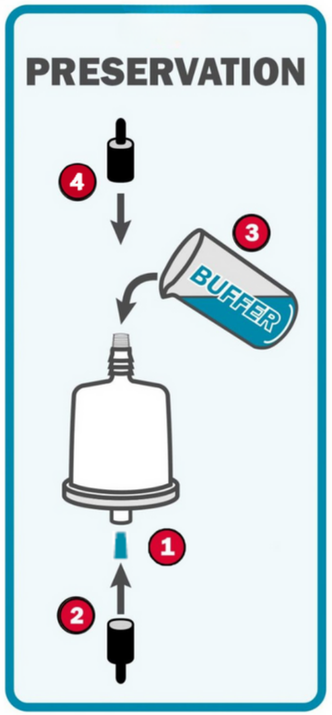
Samples treated in this manner can be stored for days or even weeks.

Here are the steps:

1. Don fresh “Nitrile” gloves.

2. Insert tubing (VOC/nano tubing) from the pump into the outlet (cylindrical end, not the barbed end) of the filter. Submerge the filter to sample depth.

3. Once the requisite volume has been “pulled” through the filter, the filter is lifted out of the water body holding the filter with the inlet (barbed end) pointing up. This limits the spilling of eDNA because eDNA is trapped on the up-flow side of the filter which is correspondingly pointing up. Continue to run the pump until there is no more discharge.



Waterra eDNA filters include 1 orangey-red plug and 2 blue caps for sample retention.

4 Remove the tubing from the outlet of the filter.

5 Insert the orange conical plug into the outlet end (cylindrical protrusion on flat end) perhaps pushing down on a hard surface to make sure it is properly inserted. For added security, place a blue cap over top of the orange plug and onto the cylindrical outlet.

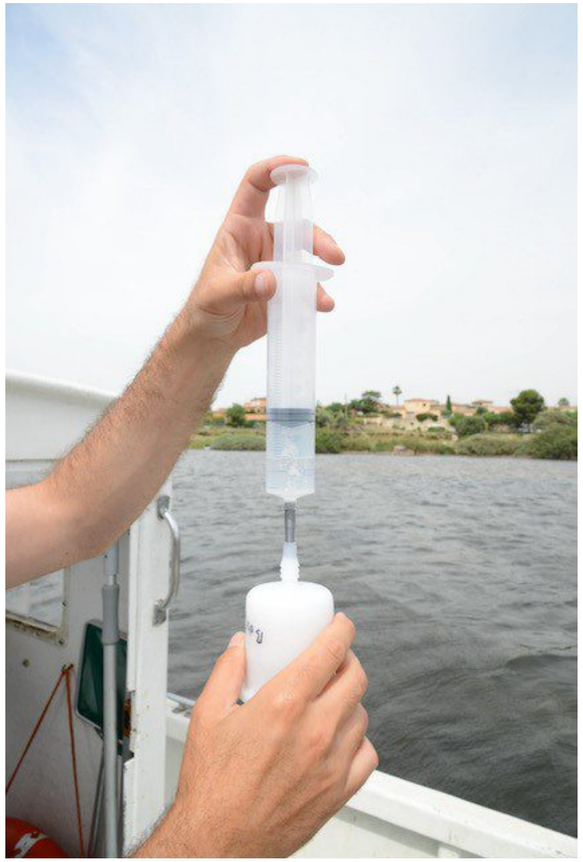
A NOTE ABOUT BUFFERS - THERE ARE TWO STRATEGIES:

Select a buffer that preserves the sample and does not encourage lysis – **Waterra recommended strategy.**

Select a buffer that does encourage lysis but appreciate that the sample should be processed forthwith to avoid DNA degradation.

6 While keeping the outlet pointed down, use the 100 ml syringe with a Waterra eDNA Syringe Adapter installed and draw up 50 ml of the buffer solution into the syringe.

7 Insert the syringe/ adapter into the inlet of the filter (pointed up). Make sure the small hole on the side of the adapter is not covered by your gloved hand. This is an air release feature so that the buffer can be injected into the filter and the air has an escape way.



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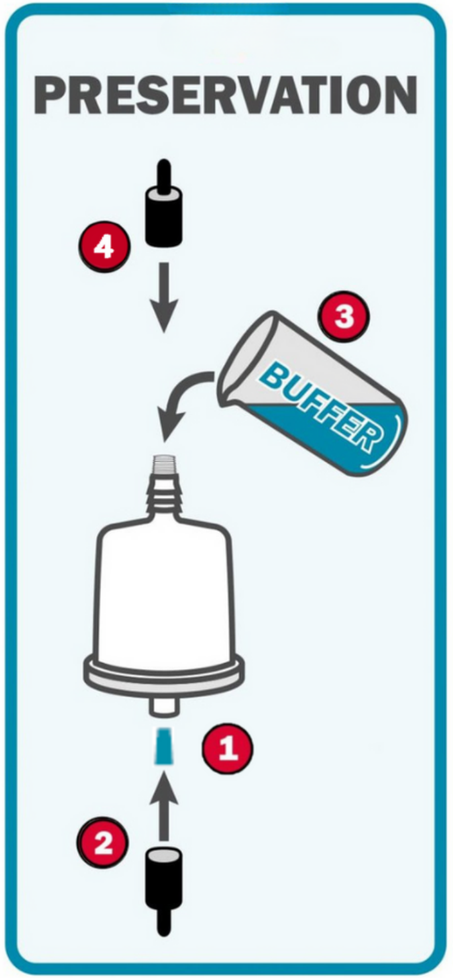
After injection of the buffer, place the second blue cap over the inlet of the filter which traps the buffer solution in the filter itself.



NOW THERE ARE 2 PROTOCOL OPTIONS:

Place the filter in a cooler for storage at 1 – 20° C for transport to the laboratory - DO NOT FREEZE unless lysis is encouraged.

OR

RECOMMENDED - Place the capped filter in a Waterra eDNA shaker with the cylindrical outlet pointing up. Note that the gussets under the filter ring must align with gaps in the shaker ring.

Filter Shaking

9 **Hold the shaker away from your body, where it will not hit anything and depress the trigger and shake the filter for 15 seconds.**

10 **Stop shaking, push the filter up relative to the ring and rotate the filter 90 degrees.**

11 **Push the filter back down again aligning the gussets and shake for a further 15 seconds.**

12 **Stop and remove filter from shaker.**

13 Have a labelled 50 ml sample vial ready.
Hold the filter with the outlet up (barbed pointing down).
Remove the blue cap and orange plug from the outlet of the filter.

14 Using the same syringe and adapter draw in 100 ml of air.
Insert the adapter into the outlet with the outlet still pointing up.
Remove the vial cap.

15 With the filter positioned over the sample vial (outlet still pointing up), remove the blue cap from the inlet of the filter and direct any discharge into the vial.

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Depress the syringe pushing air into the outlet while covering the small hole on the side of the adapter with a gloved finger. This forces air into the filter so that the buffer and particulate containing eDNA from the upstream side of the filter flows into the sample vial.

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Cap the vial and place the sample vial in a cooler to maintain 1 - 25 ° C temperature. DO NOT FREEZE unless you want to encourage lysis. The sample should be stable for days or weeks with the use of a non-lysis buffer.

Send your samples to the laboratory.

Recommended Laboratory Processing

The laboratory process for samples collected in Waterra eDNA filters and field processed as described above begins with a centrifuge process. The vials are centrifuged generating a particulate concentrate at the bottom of each sample vial. The supernatant fluid does not contain appreciable amounts of eDNA and can be disposed of.

From here the concentrate can go straight into initiating of lysis and further processing OR the sample can be dried and stored indefinitely.

If you have any questions, Waterra is here to help!

Contact us

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Thank you!

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