# DNA sampling best practices (SOP 1c)

#### **INTRODUCTION**

Strict procedures need to be employed at every stage of DNA sampling to avoid contamination. Contamination can result from a variety of factors at every step in the sample collection process. This document will help alert you to the common causes of contamination and offers guidance on best-practices for avoiding these situations.

1. **Contamination that originates from equipment**
   1. Any item that comes into contact with your sample should be DNA-free, preferably single-use, and should be opened just before use. If equipment is re-used between sites or samples, then stringent decontamination protocols (see #3 below) must be followed.
2. **Contamination that originates from the sampler**
   1. To avoid introducing exogenous DNA (which can decrease the sensitivity of downstream analyses), ensure that collected water never comes in contact with skin or non-sterile objects. Avoid entering the water wherever possible so that you do not introduce your own DNA, DNA originating from other environments you have visited, or resuspend settled DNA. In wetlands or small pools, this can be done by collecting from the margin of the water body by assuring that the person sampling works from the shore and does not enter the water. In these situations, modified samplers with long extensions can be used if necessary to sample greater distances from shore. In running water, this risk can be mitigated if it is necessary to enter the water, by ensuring that the water is collected upstream from the sampler. For beach/coastal sampling, ensure that samples are collected during an incoming wave. For sampling at depths, ensure Niskin samplers are rinsed thoroughly (with on-site water for 15-20 minutes) between deployments.
   2. Single-use laboratory gloves must be worn during contact with collection or filtration equipment and samples.
   3. When necessary, a new pair of gloves may be used to collect each replicate sample. If field conditions make it difficult to change gloves, gloves can be layered and peeled off between samples.
   4. If an open filter is used, a new pair of gloves should be put on after filtration is complete for transfer of the filter membrane into its storage container. New gloves should also be used during filtering.
   5. Surgical masks can be useful to avoid contamination when filtering samples in the field, and are recommended to be worn during filtration if open filters are used.
3. **Equipment sterilization and DNA-removal**
   1. When possible, avoid the need for any in-field decontamination by using single-use materials or using materials/equipment that was decontaminated before arrival at the field site and is dedicated to sampling from a single site. Where in-field decontamination is unavoidable, the following guidelines should be adhered to:
      1. Items should be fully covered with bleach for a minimum of 1 minute, then rinsed with DNA-free water to remove all traces of bleach.
      2. Bleach is extremely harmful to the environment so all waste from bleach used in the field must be collected, removed from the field and disposed of appropriately.
      3. Other solutions that have been demonstrated to be equally effective in destroying DNA and may be less harmful for the environment could be used (e.g. DNA-Away, Eliminase).
      4. Many solutions labelled as ‘decontaminants’ kill microorganisms but do not remove DNA (e.g. ETOH). These are not regarded as viable decontaminants for the purposes of environmental DNA sampling.
      5. For guidance on bleach preparation and use, please see [Appendix I](https://drive.google.com/open?id=1fq9juJUKNPLQA2xJmO34l5uG-JSftDoz).
4. **General guidelines**
   1. Be careful with gloves and other supplies. Do not leave them unprotected and do not toss them in a backpack or places they can get wet. It is critical to use a sterile, secondary containment system whenever possible to minimize potential contact with other items and keep items sterile until just before use. Gloves, Whirl-Paks, and other small supplies can be kept in newly opened (i.e., not reused) plastic ziplock bags. Larger items, like sampling bottles or nets, can be kept in larger ziplock bags or clean garbage bags.
   2. Guidelines for wearing gloves:
      1. Sterile (non-powdered) nitrile or latex gloves should be worn at all times, including when collecting a water sample and filtering the sample.
      2. Change gloves before removing filters and placing them in storage tubes. Do not touch anything other than the filter or decontaminated tips of the forceps before you handle the filter. If your gloves touch anything that might not be clean, replace them with a clean pair.
   3. Guidelines for working with open filters:
      1. When filtering samples, be careful not to touch the top or inside of the filter cup.
      2. If reusing forceps, decontaminate them in bleach for at least 1 minute between each sample. Rinse well with distilled or deionized molecular grade water. Other methods, including autoclaving/ETOH, do not remove DNA and will cross-contaminate your samples!
      3. If using disposable forceps, use new forceps for each sample, discarding after use. Remove disposable forceps from plastic wrappers by the hinged end, being careful not to touch the tips.
   4. Clean boots, waders, and other bulk equipment that could touch the water body being sampled thoroughly between sites. Remove all dirt, pebbles, etc. from soles and sides of boots. Decontaminate in 10% bleach if they came in contact with water or mud during sampling. Rinse well in tap water (not water from the site).
   5. Bleach vacuum flask and stopper in 10% bleach between sites to prevent disease transport. If the vacuum pump and tubing got wet during sampling or filtering, bleach them as well. Submerge equipment in 10% bleach for at least 1 minute, then rinse thoroughly with tap water.
   6. To re‐use Nalgene grab bottles, bottles must be decontaminated prior to collecting new samples. Submerge bottles in bleach solution for at least 1 minute. Rinse thoroughly with clean molecular grade water (fill, cap, shake, and rinse; repeat at least 3 times). At the sampling site, rinse again with water from the water body 3 times (shaking with cap on each time) before collecting samples to make sure there is no bleach residue in the bottle. Discard rinse water on the shore where it won’t run back into the water body. If the water body you’re sampling isn’t deep enough for the second round of rinsing, consider using single-use bottles.
5. **Field blanks**
   1. To test for contamination in the field by user or equipment, collect field blanks. The number of field negatives should be dictated by research questions and analytical capacity.
      1. The field negative is molecular grade water that is filtered and preserved using the same equipment and procedures as the water samples. Fill a collection receptacle (Whirl-Pak or bottle, whichever is being used for the samples) with distilled water. Using methods for filtering samples, filter the distilled water. Remove and preserve filters using the same method used to process eDNA samples.
      2. Decision criteria:
         1. What is your research question? What is the management risk?
         2. Cost/resolution/risk
         3. How much data might I need to throw away if contamination is discovered?
         4. Can you pool negative libraries together?
         5. Sampling timeline x days in field x replicates x cost x ?
         6. qPCR versus metabarcoding
      3. Potential minimum: 1 field blank per sample outing, with a minimum of 10-30% of samples should have field blank.

**REFERENCES**

Modified from Goldberg Lab, 2017 & Working draft of CEN Standard