

eDNA Lab Filtering Protocol

Protocol for filtering aqueous environmental samples in preparation for DNA extraction

MATERIALS

1. Glass vacuum filter set: funnel, clamp, vacuum base with sintered disc, and 1 liter flask
2. Silicone tubing
3. Lab supplied Vacuum tap or Vacuum hand pump (from auto parts store)
4. Filters; Whatman 934-AH, 47mm diameter, glass fiber, 1.5um pore size. (Fisher Cat number: 28496-886), or other project-specific filter
5. Latex or nitrile gloves (non-powdered)
6. Forceps (filter forceps if possible)
7. Laboratory/sharpie pen
8. Disinfectant: 50% bleach solution in a soaking tub, and 10% bleach solution in a spray bottle
9. Lab water, (RO or distilled) and scrub brush for decontaminating between samples
10. Paper towels
11. Sample list

PREPARATION

Avoid cross-contamination between samples! Contamination can result from a variety of factors at every step in the sample collection process. Contaminants are any biological material which could interfere with your results, including any related species. DNA from unrelated species, including humans or bacteria, should be minimized but won't ruin your results. Be careful to disinfect all equipment, but also avoid any contact between disinfectant and sample material.

1. Wear gloves throughout procedure. Change gloves between samples, or any time they may have come in contact with possible contamination.
2. Spray or wipe work surface with disinfectant, then line the surface with paper towels. Replace paper towels between samples, and re-wipe any areas where spills occur.
3. Decontaminate forceps and glass filter apparatus in 50% bleach for at least 5 minute between each sample. Rinse well with lab water.
4. Lab blanks to test for lab contamination; filter 1 liter of laboratory water before processing samples. If processing more than 10 samples, collect additional lab blanks every 10 samples.

FILTRATION

1. Pour sample slowly into filter funnel. Pause several times to swirl water in sample container before pouring remaining water into funnel.
2. Engage vacuum pump to begin filtration. During filtering, make sure vacuum pressure is sustained (monitor pump gauge if available, or watch water level to make sure water is flowing between the funnel and vacuum flask).
3. Continue to pour sample slowly as water level falls. If filter clogs (filter rate slows to less than a few drips per second), you will need to change the filter. Finish filtering the volume in the funnel, then turn off the vacuum, disassemble the funnel, and remove the filter as described below. Place a new filter and continue, repeating until full sample has been filtered. If flask fills, disassemble and empty flask.
4. Disassemble funnel and remove filter.
 - a. Remove funnel cup. Grasp funnel cup in one hand and the funnel base in the other. Gently twist and lift funnel cup to disconnect the funnel cup from the base, exposing filter membrane.
 - b. After removing funnel cup, wear clean glove (nitrile or other single-use gloves) on the hand that will touch filter membrane.
 - c. Using decontaminated forceps and gloved fingers, fold filter membrane in quarters by folding it in half and then in half again.
 - d. Place all of the filters from one sample in a small 'snack size' ziplock bag, or sterile centrifuge tube. Label with sample ID.
 - e. Freeze and store sample filters.