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ALGAE DNA COLLECTION PROTOCOL

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details the collection, filtering, and preservation of algae samples for DNA sequencing. Using 47mm Whatman/Swinnex filter holders or a filter funnel with 0.45µm cellulose nitrate filters, algae are captured from a homogenized bioassessment sample. The filtered algae are placed in screw cap tubes with a preservation solution, labeled, and stored at -20°C or -80°C for up to six months. Field blanks are collected by filtering deionized water. Sterilization of equipment is essential to prevent cross-contamination. This protocol ensures reliable algae sample collection and preservation for DNA analysis.

Guidelines

If your filter is clogging:

- **Option A:** Take replicate filters of small volume to ensure there is adequate biomass being preserved (remember to record volumes filtered).
- **Option B:** If necessary, you can ship your unfiltered sample back to SCCWRP. Please contact Susie Theroux (**susannat@sccwrp.org)** before doing so. This may exclude your sample from the statewide algae DNA study.

Materials

- 47mm Whatman/Swinnex filter holders* -OR- Filter funnel*
- 47mm 0.45um cellulose nitrate filters (Millipore HAWP04700)
- 2ml screw cap tube pre-loaded with preservation solution (bead solution, Qiagen # 12955-4-BS) **labelled with sample site code, date, and replicate number**
- 60ml Syringe with luer lock* (for syringe filtering only)
- 25mm Swinnex filter holder with luer lock* (for syringe filtering only)
- 500ml or 1L bottle*
- 100ml deionized water (DI H2O)
- Latex gloves
- Tweezers/forceps*
- Whirlpaks **labeled with sample site code, date, and replicate number**

Sample collection

1 The sample for DNA sequencing will be sourced from the bioassessment composite bucket. To separate sample, mix bioassessment composite bucket to re-suspend any settled particles, and allow sand and other large particles to briefly settle. Pour entire supernatant into a 1L bottle.

2 Homogenize sample in 1L bottle by vigorously shaking. If necessary, remove large biomass to individually cut-up and place back in 1L bottle.

Filtering

3 Using forceps to avoid contamination, place 0.45µm cellulose nitrate filter on to 47mm Swinnex (Figure 1B) or filter funnel (Figure 1C). Ensure that the filter is secured evenly and held in place by rubber gaskets. If filter is slipping, you can use sterile DI H2O to help secure the filter in place.

4 Vigorously shake 1L bottle with sample to homogenize. Filter between 10-25 ml of homogenized sample. If the filter becomes clogged, make note of total volume of sample filtered. Record this volume on worksheet.

After Filtering

5 To remove filter from filter-holder, gently fold filter using forceps, optimally folding three times to create triangle-shaped filter with filtrate protected on the inside of the triangle. Place filter in pre-loaded screw cap tubes and cap. Label tube. **Filter must be submerged in preservation solution,** shake vigorously if needed. Place tube in labeled Whirlpak bag.

6 Keep tubes with filters on ice until being transferred to a -20°C or -80°C freezer. Samples at -20°C are stable for six months.

7 If there is adequate material, taking **three replicate filters from each composite sample is preferred**. Simply repeat Steps #1-6 for the two additional filters. When possible, freeze (-20°C) any remaining composite algal sample in the event of low biomass DNA extractions.

Collecting a Field Blank

8 Please collect a field blank at each sampling site. Filter 50ml of DI H2O onto a 0.45µm filter and place in tube with preservation solution.

Sample Metadata

9 Please record all sample metadata in the CoC form (example **HERE**). A sample label template can be found **HERE**.