CALeDNA x NPS Protocols
Guide to eDNA Water Sampling

Current as of April 13th, 2022

Please follow the steps below to sample eDNA for CALeDNA

Metadata collection: We provide a webform for sample collection, that we often load onto our cell phones and 'save to home screen' for easy access. The form will load, reload, and save data in the field without cell service. We even recommend to work in airplane mode because the GPS is more precise for most cell phones in that mode. You may prefer to record similar information in this form on paper and then email it to us later. That's fine, too!

Minimally, we need the kit code that's on the three tubes, the GPS location, and the date and time. Our webform collects additional non-essential metadata, including a site photo and a place to add notes for anything you think might influence biodiversity.

Link to KOBO webform for recording sample metadata: http://tinyurl.com/CALeDNA-KoBo

Labels: Sediment collection tubes are labeled K1XXX-XX. The last digits are A1-T9. Each Whirlpak bag should contain three cryotubes with the same kit label. There are other symbols on the tubes that we internally use to track individual tubes – you don't need to worry about those.

Your water samples are NOT prelabeled. You'll need to assign them a kit label. We recommend you either come up with your own unique sample names should you repeat sampling in the same area next season, or that you use the K1XXX-XX code provided, but add a W before the K. This would be useful should you want to pair your water and sediment collections, because then you only need to record field metadata once.

For labeling water samples, be prepared with a thick Sharpie and clear tape to write the chosen kit label directly on to the filter column plastic. Cover the Sharpie label with clear tape so it does not rub off.

Field Collection: Please follow the steps below to obtain water samples for environmental DNA. Consult the associated video protocol for a demonstration: http://tinyurl.com/CALeDNA-videos

Step 1: Check your sampling kit to ensure you have the required components, including: 1) one pair of gloves; 2) one 50 mL syringe used for water sampling; 3) one 3 mL syringe used to add Zymo DNA storage solution to the filter; 4) one vial of Zymo solution; 5) one Luer lock Sterivex filter unit; 6) two Luer lock caps for sealing the Sterivex filter; 7) one Whirl-Pac bag to hold the Sterivex filter after sampling; and 8) one Sharpie for writing the sample ID on the bag and filter.

Step 2: Remove the Sterivex filter from its sealed package, and being careful not to touch the sterile ends, write the sample ID on the filter with a Sharpie marker. Return to its package until needed for Step 5.

Step 3: Remove the 50 mL syringe from its sealed package, and draw up 50 mL of water to rinse completely with water from the sampling location. The syringe is now ready for sampling eDNA.

Step 4: Obtain a 50 mL water sample from the target area using the 50 mL syringe.

Step 5: Screw the Sterivex filter onto the end of the 50 mL syringe until firmly in place. Then, gently flush your water sample through the syringe, discarding the water. Remove the Sterivex filter from the 50 mL syringe.

Step 6: Repeat **Steps 4 and 5** above until filtering water becomes difficult and the filter has reached capacity. Record the total amount of water filtered directly on the syringe as well as in your metadata sheet.

Step 7: Discard any remaining water in the 50 mL syringe, and draw 50 mL of air. Screw the Sterivex filter onto the 50 mL syringe to flush the filter of any remaining water. It is ok if a small amount of water remains in the filter after this step.

Step 8: Using the 3 mL syringe, draw up 2.5 – 3 mL of Zymo solution. Screw the 3 mL syringe with Zymo solution onto the inlet end of the Sterivex filter and fill completely with Zymo solution. It may be helpful to hold the filter outlet upward to remove the air and add Zymo solution.

Step 9: Add a Luer lock cap to the outlet end of the Sterivex filter containing Zymo solution. Remove the 3 mL syringe and add another Luer lock cap to the inlet end of the Sterivex filter.

Step 10: Place the sealed Sterivex filter into the provided Whirl-Pac bag, and write the sample ID on the bag with a Sharpie marker. Sampling of water eDNA is now complete! Store the filters at room temperature or in the fridge.

For inquiries about this protocol or related work, please contact:

Van Wishingrad, Ph.D.
vwishingrad@ucsc.edu
UC Santa Cruz, CALeDNA Program
Ecology and Evolutionary Biology
Coastal Science Campus, CBB 143
130 McAllister Way
Santa Cruz, CA 95060

Got an urgent question? Contact Van at: (831) 221-0033 or Rachel at: (206) 351-7997.