

## Soil/Mulch Collection Protocol:

*This method is intended for the eDNA sampling of various substrates utilizing self-desiccating Smith-Root filters.*

### Materials:

- 2x Peristaltic pumps w/ tubing pieces
- Pump Charger
- Filters (Smith-Root, PES, 5 $\mu$ m)
- DI Sprayer
- Carboy filled with DI
- Boxes of Gloves
- Field Clipboard
- Data sheet + Pen/Paper
- Sharpies
- Graduated cylinder
- Sterile Bags
- Bags for garbage

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### Sampling Prep

1. A 10% bleach solution is used to clean the exteriors of all sampling materials. Materials are subsequently wiped down with deionized (DI) water to remove any excess bleach residue.

### Sampling Protocol

1. Before sampling, set up the peristaltic pump and tubing. Place one end of the tubing into a graduated cylinder while keeping the other end clean as it will come into contact with your filter housing.
2. Generate a negative control by following steps 3 – 7, without collecting any substrate. This can be done before and after sampling efforts or at each sampling site.
3. To begin sampling, begin by opening your sterile bag and turning it inside out over a gloves hand.
  - a. **Note: Contamination can happen easily here. As the inside of the bag is now exposed, anything that comes into contact with it can deposit unwanted, exogenous DNA.**
4. With the bag over your hand, grab a fistful of substrate and invert the bag so your sample sits safely inside. Fill with enough DI water to sufficiently cover your substrate.
5. Seal your bag and begin shaking/massaging for approximately 30 seconds to fully homogenize your sample.
6. With clean gloves, assemble your Smith-Root filter and snorkel and begin filtering the sample in your collection bag. When filtering is complete, invert the filter housing for ~30 seconds and continue pumping to allow the filter inside to dry.
  - a. **Note: Your filter may not filter through your entire sample as it may clog and form a collection of the substrate. For this reason, make sure your sample has been properly homogenized before filtering to obtain an accurate representative sample.**

7. Detach your filter housing from the pump tubing and place it back into its original bag. Be sure to properly label your bag with the **site name**, **date**, and **sample number**.

*\*\*This process can all be done using general plastic filter holder assemblages, as well. If using these, be sure to pull and preserve your filters ASAP to avoid DNA degradation. Smith-Root Filters are self-desiccating and therefore can be preserved at room temperature for prolonged periods of time.\*\**