Sprayer Aggregation Protocol:

This method is intended for foliage surface eDNA sampling utilizing self-desiccating Smith-Root filters.

Materials:

- 2x Peristaltic pumps w/ tubing pieces
- Pump Charger
- Filters (Smith-Root, PCTE, 10 µm)
- DI Sprayer
- Carboy filled with DI
- Boxes of Gloves
- Field Clipboard
- Data sheet + Pen/Paper
- Sharpies
- Graduated cylinder
- Buckets / Sterile Bags
- Bags for garbage

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. Pressurize your sprayer and ensure the surface area of the spray does not exceed the opening of your collection bucket/bag.

    a. **Note: Contamination can happen easily here. Be sure to only adjust the spray nozzle with *extremely clean gloves* as the spray it generates becomes your sample.**

3. Generate a negative control by spraying DI water into a clean receptacle and following steps 5 – 6. This can be done before and after your sampling efforts OR at each sampling site.

4. To begin sampling, grab a collection receptacle (sterile bucket or bag), being sure to only handle the exteriors, and place it below your sample. Begin spraying the surface of your sample from approximately 30 cm away, keeping your collection bucket/bag directly underneath to collect as much run-off as possible.

    a. **Note: It's only necessary to spray the surface of your sample for 1-3 seconds – just enough to collect the DNA on the surface. Prolonged spray may only collect an excess of organic compounds that could inhibit downstream molecular assays.**

    b. **The amount of vegetation you should collect run-off from will vary depending on the biology of your species and its abundance.**

5. With clean gloves, assemble your Smith-Root filter and snorkel and begin filtering your suspended sample in your collection receptacle. When filtering is complete, invert the filter housing for ~30 seconds and continue pumping to allow the filter inside to dry.

6. 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.

Environmental and Natural Resource Building | 14 College Farm Road New Brunswick, NJ 08901 | julie.lockwood@rutgers.edu | (p.): 848 - 932 - 9336