

Roller Aggregation Protocol:

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

Materials:

- 2x Peristaltic pumps w/ tubing pieces
- Pump Charger
- Filters (Smith-Root, PCTE, 10 μ m)
- Carboy filled with DI
- DI Sprayer
- Bleach Cleaned Rollers in Sterile Bags
- Bleach Cleaned Roller Handles
- Cleaned Pole
- Boxes of Gloves
- Spare Sterile Bags
- Field Clipboard
- Data sheet + Pen/Paper
- Sharpies
- Graduated cylinder
- Stopwatch for Visual Surveys
- Bucket
- Bags for Used Rollers and Handle
- Bags for garbage

Sampling Prep

1. A 10% bleach solution is used to clean the exteriors of all sampling materials. For methods, reference our sampling prep protocols.
2. To set up sampling kits, wipe down your counter with a 10% bleach solution. Let this air dry and wipe down again with deionized (DI) water.
3. Using your clean surface, individually pack clean, damp paint rollers into small, sterile bags. You can pack a number of these into larger, sterile 2-gallon bags along with your filter bags however is necessary and most efficient. ***We recommend generating sampling kits for each unique site (i.e. 1 roller and filter per sample + 1 filter for a negative)***

Sampling Protocol

1. Before sampling, set up the peristaltic pump and tubing. Place one end of the tubing into a graduated cylinder and keep the other end clean as it will come into contact with your filter housing.
2. To begin sampling, place a damp, **clean roller** and **clean handle** on your sampling pole. Be sure to avoid touching the roller and only handle the handle with clean gloves.
 - a. You can use this first roller to generate a field negative, by following steps 4-7. Place it back onto the roller handle after step 5 to begin taking your field samples.
3. Upon arriving at the target tree, roll the trunk of the tree by starting at the base and moving as far up as possible (~3m) around the entire circumference of the tree and roll tops of any branches in that range near the trunk.

- a. **Note: Where you roll your tree should be determined by the biology of your species. If you're sampling for a species that drops lots of excrement, you may want to focus your sampling efforts on the tops of branches/bottom of trunk/where their excrement will likely land. Moreover, if your species burrows, you may want to focus your efforts on potential holes/burrow sites on the trunk.**
4. Remove the roller from the handle using a sterile bag. With the roller inside the bag, fill it with ~150ml of DI water using a sprayer or carboy, taking care not to contaminate DI water source.
5. Close the bag and massage the roller through the bag for ~30 seconds to suspend your sample in DI, then discard the roller in a designated "used roller" bag.
6. With clean gloves, assemble your Smith-Root filter and begin filtering your now suspended sample. When filtering is complete, invert the filter housing for ~30 seconds and continue pumping to allow the filter inside to dry.
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**.

Collect One negative and Two roller samples (~3-6 trees per roller) per site.

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.