

PROTOCOL 1

Soil Sampling Protocol: Overview

Sampling equipment

1. Items provided in the sampling kit for each park prepared by the Cornell Food Safety Lab

Note: all the items have been sanitized.

- Five 5 oz (150 mL) sterile scoops (1 for each site)
- Six 55 oz Whirl-Pak bags (5 for sample collection, 1 as back-up)
- Latex gloves
- Ethanol hand wipes
- Foam cooler
- Ice packs
- Six field datasheets printed on all-weather paper placed in a gallon freezer bag (5 for sample collection, 1 as back-up)
- Clipboard
- Pen
- Sampling protocol
- Map of trails and potential sites
- Permit
- Return shipping label

2. Items prepared by collectors

- A smartphone (to take photos, locate suggested sampling sites using Google Maps app, and obtain GPS coordinates of actual sites)
- A plastic shopping bag for trash when sampling
- Tape for sealing cooler and sampling kit when shipping

3. Optional

- Rain poncho(s)
- Sunscreen
- Bug spray

Sampling units

Sampling units	Number	Description
Sites	5 sites / park; sites within one park should be > 0.25 miles apart	Cornell will suggest sites within the given park to each sample collector, but sample collectors can provide input on alternative sites based on accessibility. If possible, please collect samples from locations with moist soils that are not covered in standing water (i.e., a vernal pool, pond).
Points	3 points / site; points within one site should be about 20 feet apart.	Please find, if possible, 3 points that look similar (e.g., similar type of soil, plant cover). Collect 1 subsample of topsoil (0-8 inches) at each point; these 3 subsamples will be pooled for one composite sample per site. The GPS coordinates of the central point (centroid) of the 3 points will be recorded. Figure 1 below shows the suggested distribution of the three sampling points around the centroid.

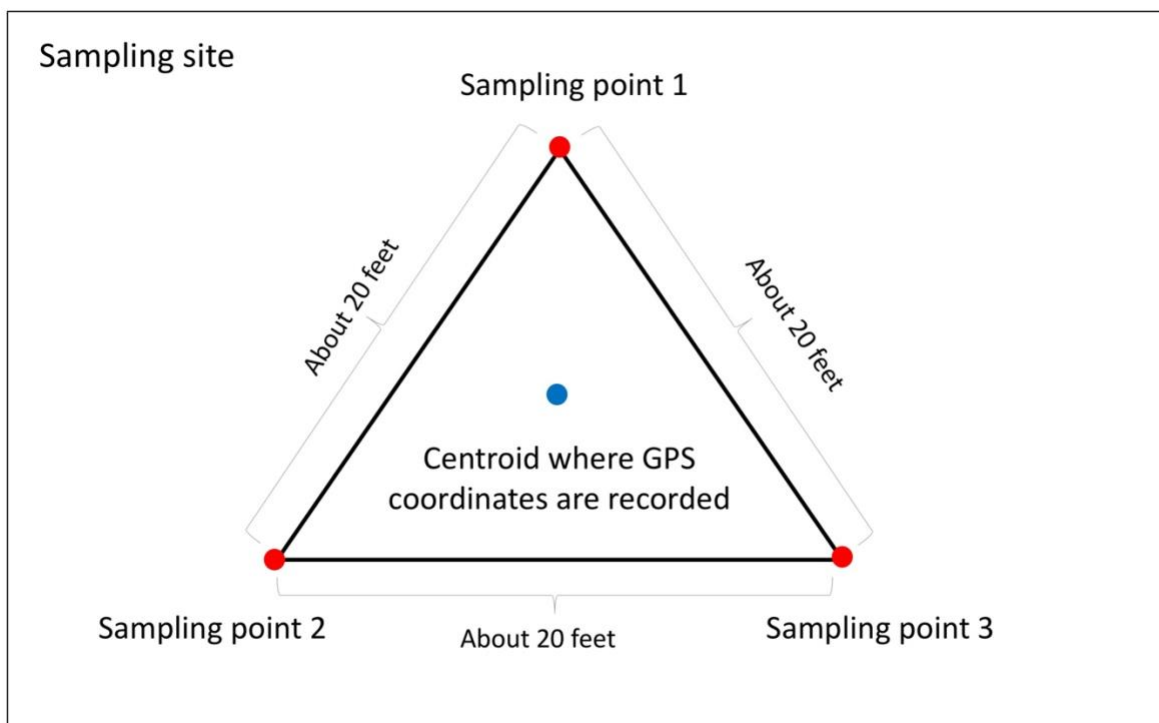


Figure 1 Diagram of sampling points

Soil Sampling Protocol: Sampling Methods

I. Background Information

* Soil samples will be collected at **5 sites** within the given park, **with each site being at least a quarter mile from the nearest site**. If possible, please collect samples from locations with moist soils that are not covered in standing water (i.e., a vernal pool, pond). At each sampling site, collect 3 subsamples and place all three subsamples in the same Whirl-Pak bag.

* Table 1 are the GPS coordinates for 5 suggested sampling sites along the trail/road. If the suggested sites are inaccessible or unsafe, find a location as close to the suggested site as possible. Sample sites should be between 30-150 feet from roads, or 20-150 feet from trails.

* Please call Jingqiu Liao (607-229-9983) if you have any questions during sampling.

Table 1 The GPS coordinates of 5 suggested sampling sites

No.	Latitude	Longitude	No.	Latitude	Longitude
1			4		
2			5		
3					

II. Preparation

1. Please download the Google Maps app on your phone, save the suggested sampling sites using the GPS provided coordinates (Table 1), and download the offline map from Google Maps prior to sampling following the steps shown in Figure 2, in case that there is no internet connection while sampling. *Note: please review the locations of sampling sites in Google Maps and decide the order to sample based on your hiking/driving route.*
2. **Place ice packs in the freezer >24 h before heading out to sample.** Immediately before heading out to sample, place ice packs in the cooler. When sampling the cooler can be left in the car, if hiking please take 3-4 ice packs with you, so the samples can be kept as cold as possible. Following sample collection, place samples on ice packs in the cooler as soon as possible.

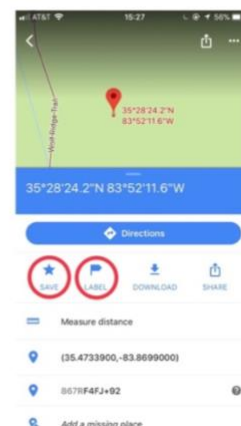
① Search the location of the site using the provided GPS coordinates in the search bar.



② Find the location and click on the pin.



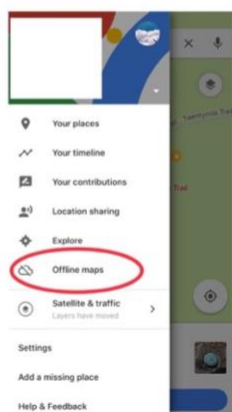
③ Save and Label dropped pin, e.g. Site 1. Repeat step 1-3 for the remaining suggested sites.



④ Search for trailhead/access point for hike/drive and click the menu button.



⑤ Click "Offline maps".



⑥ Click "Custom map".



⑦ Click "Download". Once completed, just use Maps normally, even when offline.



Figure 2 Instruction of locate sampling sites and create offline map using Google Maps

III. Soil collection

* **Watch out for loose rocks, cliffs, and other environmental and wildlife hazards** (e.g., bears, poison ivy, bees) while sampling. Photos of some potential poison plants are provided in Figure 3. Make sure you don't end up on the wrong trail/wrong direction; most trails are marked with a specific color blaze so please check your trail's color before starting the hike.

Poison ivy



Poison oak



Poison sumac



Figure 3 Photos of poison ivy, poison oak, and poison sumac

* Detailed steps:

1. Go to the first sampling site using the pre-saved location in Google Maps.
2. Take photos of the first sampling site and surrounding area. *Note: this step is optional.*
3. Fill in the field datasheet for the first sampling site.
4. Randomly pick a location within 20 feet of suggested location; this is the first sampling point.
5. Open the scoop package but do not remove scoop.
6. Put on the nitrile gloves.
7. Sterilize gloves by thoroughly wiping with ethanol wipes; following sterilization of the gloves do not touch your body or the cooler.
8. Remove the scoop from the package and using the scoop or your gloved hand remove as much leaf litter and vegetation as possible from the soil surface of the first sampling point.
9. Open the pre-labeled sterile Whirl-Pak bag following the instructions shown in Appendix A; make sure the ID on the Whirl-Pak bag is recorded and matches the label ID on the datasheet.
10. Push the sterile scoop into the ground (be careful not to break the scoop) and collect soil from 0-8 inches below the soil surface; transfer **2 full scoops** of soil into the Whirl-Pak bag. Make sure that you do not touch the inside of the Whirl-Pak bag with anything except the soil sample. Make sure not to touch the scoop to your body or clothes, and not to set the scoop on the cooler or the ground; if scoop is contaminated, please sterilize it using ethanol wipes. If a full scoop cannot be collected, collect enough soil to equal a full scoop.
11. Put the scoop back to the package (scoops will be sterilized in lab for future use, so please include all used scoops with package on in the sampling kit when shipping back). Please keep the soil hole as small as possible and after collection, please close the soil hole.
12. Identify a second location within 20 feet of the first sampling point as shown in Figure 1 that looks similar (e.g., similar type of soil, similar plant cover) to the first point; this will be the second sampling point.
13. Repeat steps 7-11 for the second sampling point; add the soil using the same scoop from the second sampling point to the Whirl-Pak bag containing the soil collected from the first point.
14. Identify a third location within 20 feet of the first and second sampling points as shown in Figure 1 that looks similar (e.g., similar type of soil, similar plant cover) to the first two points; this will be the third sampling point.
15. Repeat steps 7-11 for this third sampling point; add the soil using the same scoop from the third sampling point to the Whirl-Pak bag containing soil collected from the first and second points.
16. Close the Whirl-Pak bag containing all three subsamples following the instructions shown in Appendix A (squeeze the bag to remove air from the bag; roll five times).
17. Measure the GPS coordinates for the central point (centroid) of the 3 sampling points as shown in Figure 1 using Google Maps app following steps 1 to 3 in Figure 4 below. If offline (i.e. no

internet connection or service), continue following steps 4 to 6 in Figure 4 to get the GPS coordinates. **Record the GPS coordinates in the field datasheet.**

18. Place the bag containing the sample in the cooler that also contains the ice packs as soon as possible after sample collection. If hiking, please have a gallon bag with some ice packs in it so that the samples can be kept cool; once you get back to your car please put the samples in the cooler containing the ice packs.
19. Head to the next sampling site; repeat steps 1-18.
20. Repeat for the three remaining sampling sites.
21. Once you return home, store the soil samples on frozen ice packs in the foam cooler or 4 °C refrigerator until shipping. *Note: please do not freeze soil samples.* Please try to ship samples to Cornell within 24 and no more than 48 h after sample collection.

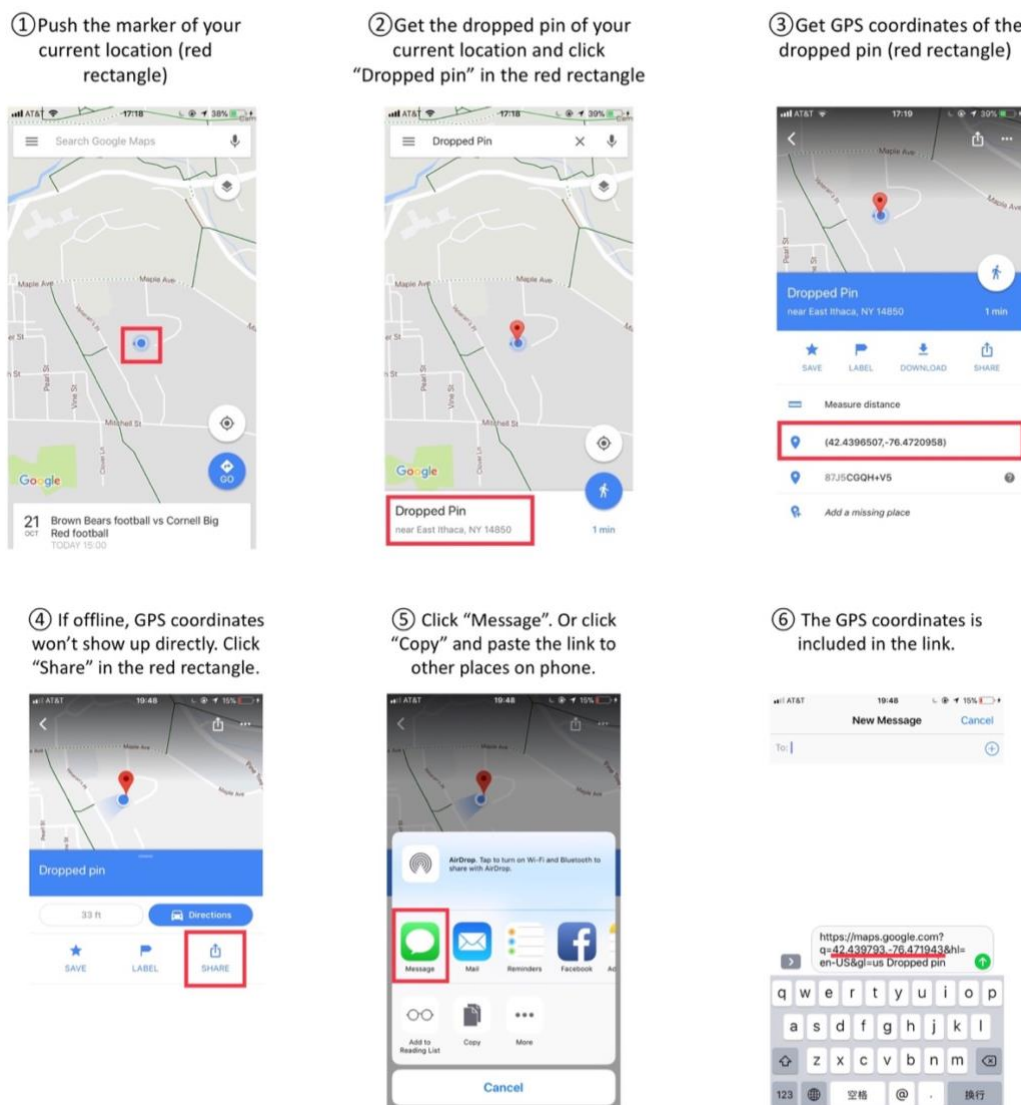
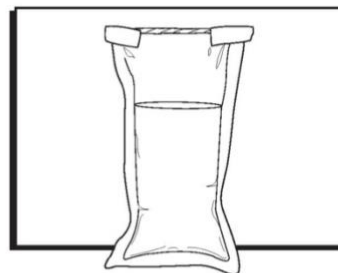
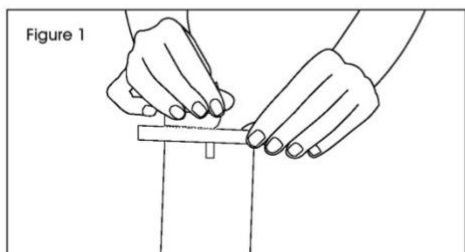


Figure 4 Instruction of getting GPS coordinates of current location using Google Maps

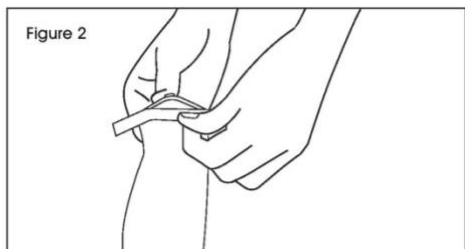
Appendix A

ULINE**WHIRL-PAK® BAGS**1-800-295-5510
uline.com**INSTRUCTIONS**

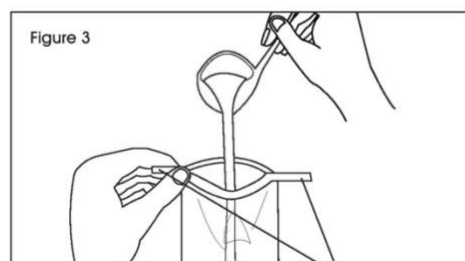
1. Tear off top of bag along perforation. (See Figure 1)



2. Use pull tabs on each side to open bag. (See Figure 2)



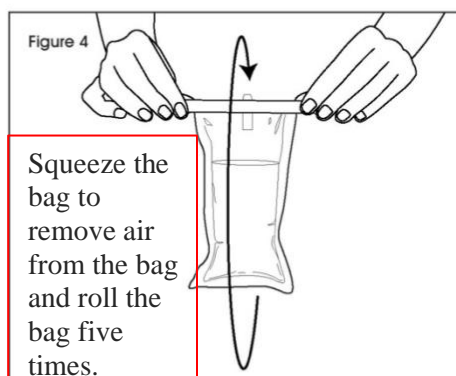
3. Put sample in bag. Leave enough space at top for closing and mixing. Pull the wire ends to close the bag. (See Figure 3)



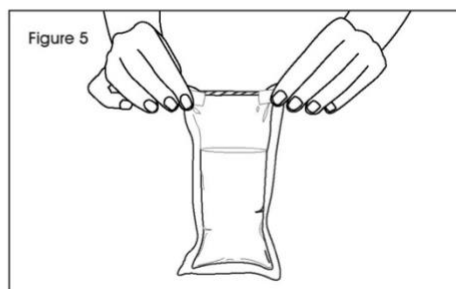
NOTE: If labeling bag with sample information, label before filling.

4. Holding the bag by the wire ends, whirl the bag three complete revolutions to form a leakproof seal. (See Figure 4)

NOTE: Whirling the bag will form the tightest seal. Larger bags can be closed by folding the tab over as tightly as possible.



5. Bend the wire ends over the bag to secure the seal. (See Figure 5)



Soil Sampling Protocol: Shipping Procedures

1. Within 48 h (ideally within 24 h) of sample collection, ship overnight the soil samples and sampling kit to Cornell using the procedures outlined below.
2. Make sure all Whirl-Pak bags are closed, and all of the air has been removed from the bag.
3. Place Whirl-Pak bags (i.e., soil samples) in the foam cooler with the frozen ice packs.
4. Place field datasheets in a gallon bag that is tightly sealed. Place the bag containing the datasheets in the cooler.
5. Place all used-scoops with package on and any unused Whirl-Pak bags in the cooler. Fill up the cooler with newspaper, plastic bags, additional ice or other fillers until the cooler is full.
6. Tape the cooler shut with packing tape.
7. Mail the cooler containing the soil samples, ice packs, field datasheets in the gallon freezer bag, and sampling materials to Jingqiu Liao, 358 Stocking Hall, 48 Judd Falls Rd, Cornell University, Ithaca, NY 14853 as soon as possible (within 48 hours; ideally within 24 hours). The return shipping label has been provided.
8. If photos are taken, please email them to Jingqiu Liao (jl3374@cornell.edu). Please include the corresponding sample ID numbers in the file names.

Mission Complete!
Well Done!
Thank you very much!

Field Datasheet 1 (please fill out one sheet per Whirl-Pak Bag)

Date: _____ Time: _____

Collector(s): _____

Sampling location (e.g. park name): _____

Sample ID: _____

Habitat type (please circle one): Forest Savanna Shrubland Grassland Other: _____

If close to a water body (e.g. river, stream, pond) (Y/N): _____

Latitude/Longitude of the centroid of the 3 sampling points at this site (obtained from Google Maps on smartphone)

Latitude: _____ Longitude: _____

Notes (e.g., presence of animal tracks or scat near the site, evidence of human disturbance, dominant plants, other weather and habitat information of note):

Field Datasheet 2 (please fill out one sheet per Whirl-Pak Bag)

Date: _____ Time: _____

Collector(s): _____

Sampling location (e.g. park name): _____

Sample ID: _____

Habitat type (please circle one): Forest Savanna Shrubland Grassland Other: _____

If close to a water body (e.g. river, stream, pond) (Y/N): _____

Latitude/Longitude of the centroid of the 3 sampling points at this site (obtained from Google Maps on smartphone)

Latitude: _____ Longitude: _____

Notes (e.g., presence of animal tracks or scat near the site, evidence of human disturbance, dominant plants, other weather and habitat information of note):

Field Datasheet 3 (please fill out one sheet per Whirl-Pak Bag)

Date: _____ Time: _____

Collector(s): _____

Sampling location (e.g. park name): _____

Sample ID: _____

Habitat type (please circle one): Forest Savanna Shrubland Grassland Other: _____

If close to a water body (e.g. river, stream, pond) (Y/N): _____

Latitude/Longitude of the centroid of the 3 sampling points at this site (obtained from Google Maps on smartphone)

Latitude: _____ Longitude: _____

Notes (e.g., presence of animal tracks or scat near the site, evidence of human disturbance, dominant plants, other weather and habitat information of note):

Field Datasheet 4 (please fill out one sheet per Whirl-Pak Bag)

Date: _____ Time: _____

Collector(s): _____

Sampling location (e.g. park name): _____

Sample ID: _____

Habitat type (please circle one): Forest Savanna Shrubland Grassland Other: _____

If close to a water body (e.g. river, stream, pond) (Y/N): _____

Latitude/Longitude of the centroid of the 3 sampling points at this site (obtained from Google Maps on smartphone)

Latitude: _____ Longitude: _____

Notes (e.g., presence of animal tracks or scat near the site, evidence of human disturbance, dominant plants, other weather and habitat information of note):

Field Datasheet 5 (please fill out one sheet per Whirl-Pak Bag)

Date: _____ Time: _____

Collector(s): _____

Sampling location (e.g. park name): _____

Sample ID: _____

Habitat type (please circle one): Forest Savanna Shrubland Grassland Other: _____

If close to a water body (e.g. river, stream, pond) (Y/N): _____

Latitude/Longitude of the centroid of the 3 sampling points at this site (obtained from Google Maps on smartphone)

Latitude: _____ Longitude: _____

Notes (e.g., presence of animal tracks or scat near the site, evidence of human disturbance, dominant plants, other weather and habitat information of note):

Field Datasheet (back-up)

Date: _____ Time: _____

Collector(s): _____

Sampling location (e.g. park name): _____

Sample ID: _____

Habitat type (please circle one): Forest Savanna Shrubland Grassland Other: _____

If close to a water body (e.g. river, stream, pond) (Y/N): _____

Latitude/Longitude of the centroid of the 3 sampling points at this site (obtained from Google Maps on smartphone)

Latitude: _____ Longitude: _____

Notes (e.g., presence of animal tracks or scat near the site, evidence of human disturbance, dominant plants, other weather and habitat information of note):
