

Collection Guide

I. Collect and Document Specimens

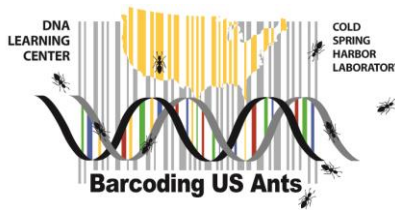
The steps below lay out the Collection and Documentation steps of the US Ants Barcoding Project. Detailed instructions for different ant collection techniques are summarized for reference, but not all collection techniques are necessary for all teams. Gear your collection to your geographical area and the ants that you are trying to collect.

Ants belong to a single family, the Formicidae, within the hyper-diverse insect order, Hymenoptera, and they exemplify the importance of insects as ecological indicators with their astounding biodiversity. Ants dominate the ecology of an area with their substantial biomass. They can manipulate species composition, influence trophic interactions, and shape both the abiotic (e.g., through soil shifting) and biotic (e.g., plant-insect interactions) factors affecting an ecosystem. They fill numerous ecological niches taking on the role of predators, mutualists and symbiotic partners, parasites, decomposers and often acting as “keystone species.” Additionally, ants possess a quasi-stable taxonomic and systematic status, creating a situation where species identification is assessable and widely understood. The extant Formicidae contains 21 subfamilies, 411 genera, and more than 15,600 species worldwide. In North America, there are 9 subfamilies, 70 genera, and nearly 800 species.

To help better document the diversity of ants in the United States and ask broader questions regarding natural history and ecology of ants in the US, the Barcoding US Ants Project is excited to have you play an important role as citizen scientists in collecting and identifying ants in your area through DNA barcoding. The hope is that you will collect at least a few ants that do not have or have only a few barcode records in a database, such as GenBank®, in order to enhance the resolving power of DNA barcoding as a broader taxonomic tool, and in the process, you will learn more about ants and their ecological roles! Additionally, your collection of even commonly collected species may support a census of life in a specific area or habitat and provide information about shifting range distributions.

Use common sense when collecting specimens. Respect private property; obtain permission and/or any necessary permits to collect in any location. Respect the environment; protect sensitive habitats, and collect only enough of a sample for barcoding. Do not collect specimens that may be threatened or endangered. Be wary of poisonous or venomous plants and animals.

Ants can be collected using one of the techniques laid out in the protocol below and euthanized using a kill jar by placing them in a freezer for several hours or placed directly into 95% ethanol until ready to extract DNA. Do not use more sample than you need. Only a small amount of tissue is needed for DNA extraction—for a large ant, only a leg might be necessary for extraction; for a smaller ant, a single whole specimen might be used. Try and collect 5-10 ant specimens, representative of a single species. Retaining



these replicates, as well as the remainder of the sample from which you extract DNA, will be important for later taxonomic identification and as voucher specimens that will be associated with your sequences. You will also need to take high quality photographs of your organisms using a phone camera or dissecting scope, especially in those cases where the entirety of a sample is used during extraction. Follow the instructions below for labeling and documenting environmental and behavioral information (i.e., metadata) associated with your sample.

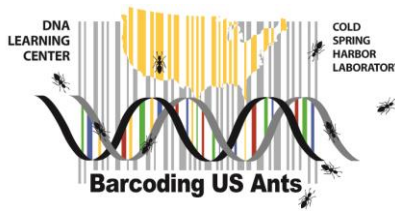
Ant collecting will be most successful after researching and preparing the field materials necessary to most productively collect target species. Remember that different ant species demonstrate different environmental preferences, habits and behaviors. Haphazard collecting techniques will not yield a diverse profile of ant species in your area.

Procedure

1. Before setting out to collect, having an idea of the ant species that occur in your area is important. This will help ensure a high species diversity in your collection and will help to keep your collection methodology focused. Prioritize the collection of ants that are missing DNA barcodes and on those species that occur locally, or are unique to your region.
 - a. Research the ants in your area and plan to target those species that are missing barcodes by consulting the **US Ants Regional Hit List(s)** in the US Ants Google Classroom.
 - b. Consult the **Ant Collecting Links** document in the US Ants Google Classroom for tips on ant collecting, collecting supply sources, species ranges, and details on where species have been collected before.
2. Determine a few suitable collection sites that might yield a good diversity of ant species in your area. For target species that are missing barcodes, consult the **Ant Collection Recommendations by Genus** document in the US Ants Google Classroom to determine the best collection methodologies for target species.
 - a. Ensure that there are no collection restrictions for those locations, and if there are, obtain appropriate permissions before going out to collect.
3. Prepare the equipment that you will need to collect ants.
 - a. Much of this equipment is available from local stores. Insect collecting equipment is available from a biological supply company, such as Bioquip.com or Carolina.com.
 - b. There are many different methodologies that can be used to collect ants, but regardless of the specialized techniques used, there are some essential items that you should always carry with you in the field.

Materials to bring to the field:

- Several pre-labelled collecting vials roughly half-filled with 90-95% ethanol (screw cap tubes, microfuge tubes, or some air-tight small container) – these will be provided to you by the DNALC and will have pre-assigned specimen numbers.
- Several dry collecting vials

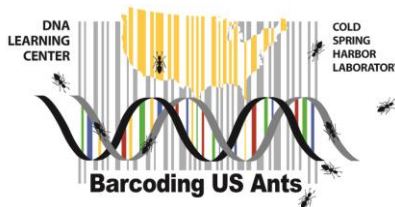


- Pre-written labels (on cardstock either hand written using a micron pen/pencil or printed), identifying the general location that you will be collecting from (see step 4).
 - Aspirator (invaluable for sucking up fast-moving, small insects, [i.e., ants] without damaging them) – cheap ones are available from Bioquip.com.
 - Forceps (softer forceps are better or dip the tip in ethanol to use them to stick to the ants – be careful not to crush specimens within the forceps!)
 - Mobile phone/camera for documenting specimens, habits, habitat, etc.
 - Permanent marker
 - Micron pen/Pencil for recording metadata and writing on labels
 - Field notebook (see step 5)
 - Field guide that identifies basic ants of your area
 - Water and first aid supplies
4. **Label Preparation** – In entomological collections, every specimen has a date/locality label associated with it that reflects the geographical information of the area from which a specimen was collected. These should be associated with your specimens, as well, *in addition to sample ID numbers* that will also be associated with your specimens. They can be prepared ahead of time to save time with blank spots for more specific information. Here is a sample of what sorts of information should be included:

Date/Locality Label

Print your labels on a computer (on a thicker paper like cardstock) for clarity. Use the following instructions to format your labels:

- a. Set margins at 0.5”.
- b. Create 11 columns.
- c. Use Times New Roman at 3.5 font.
- d. Type labels one after the other for the same locality and date; use a space to separate different localities and dates.
- e. Print labels on a heavy-duty paper, such as cardstock.
- f. Cut out labels. Specific information, like GPS or details about where the specimen was collected, can be left blank and added to the label at the collection site with a micron pen or pencil.
- g. These labels can be dropped into the collecting vials directly into the ethanol along with samples. Printer inks and micron/pencil marks should be unaffected by the ethanol.



Date/locality label template:

Example:

GPS Coordinates State: County Specific Site Coll. Date with month as roman numeral Collector (first initial. last name)	42.439286 -76.409754 NY: Tompkins County Cornell U: Ellis Hollow 13 – IX – 2007 J. Petracca
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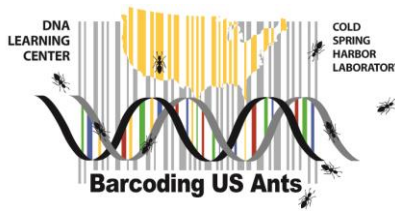
Actual size after formatting:

2.439286 -76.409754
 NY: Tompkins County
 Cornell U: Ellis Hollow
 13 – IX – 2007
 J. Petracca

5. **Field Notebook** – Prepare a field notebook for metadata, environmental and behavioral information pertaining to collected ant specimens.
 - a. Follow the model of the **US Ants Metadata Sheet** in the US Ants Google Classroom in terms of the types of metadata that you should collect. You can literally copy and paste these tables as needed, print them out, and place in your field notebook!
6. **Preparing for Specific Collection Techniques** – Based on your research, you should have a plan of what types of ants you can find in your area, which ants you may wish to target, and specific collection techniques might be used to obtain those targets.
 - a. Refer to the **US Ants Collection Clinic** recordings and presentations by Dr. Shawn Dash and Jeffry Petracca for more details on specific collection methodologies or consult the **Ant Collecting Links** document for more information. All recordings, presentations, and documents are in the US Ants Google Classroom.
 - b. Before going out to collect, be sure to prepare any additional materials for specific collection methodologies, as needed. Note that is not necessary to carry out each type of collection technique. Consult the **Ant Collection Recommendations by Genus** document in the US Ants Google Classroom to determine the best collection methodologies for target species. Choose one or more applicable collection techniques from the following list and gather or prepare the necessary materials:
 - i. **Directed Hand Collecting** – This is the simplest of the methodologies. Simply find ants in a habitat and collect them using forceps, your hands, or preferably, an aspirator. Be careful; many ants bite or sting, and forceps or other tools may crush specimens. You may wish to bring some tools, like a trowel or pry bar for digging up colonies, breaking into downed logs, etc.

Additional materials to bring to the field:

 - Optional: Trowel for digging up ants
 - Optional: Shears for trimming brush/accessing arboreal ants
 - Optional: Hand knife or pry bar (i.e., a tool for breaking into logs or flipping heavy rocks, etc.)
 - ii. **Leaf Litter Sifting** – For litter sifting, you can make your own leaf litter sifter easily. You want to collect a bunch of leaf litter, log or soil material and place it in the sifter, shake it back and forth, and collect the fine material that falls through the sifter. Ants and other small arthropods will be located in this fine material, which you can sort through later or scan for ants. You can purchase an expensive litter reducer, like a Winkler extractor, or produce your



own sifter with a repurposed cat litter box and a sifting pan from a local store. A sifter might also be produced by folding the edges up on a sheet of hardware cloth, and taping them for safety. Along similar lines, cut out the bottom of a plastic bin and replace it with hardware cloth. There are many options; consult the *Ant Collecting Links* document in the US Ants Google Classroom for some references on do-it-yourself options.

Additional materials to bring to the field:

- Sifter
- Collecting bags for sifted litter samples (i.e., siftate)
- Optional: Trowel for digging soil/leaf litter samples
- Optional: Small hand rake for gathering litter

iii. **Berlese Funnel** – An alternative to field sifting is to bring the litter back to the lab in a bag, and set it up in a Berlese funnel. Back in the lab, the litter can be suspended in a bag or dumped into a funnel over a collection vessel filled with ethanol and a light overhead. The idea here is that heat/illumination from the light will encourage ants and other critters to dig deeper into the litter and down into the funnel where they will eventually fall into the ethanol collection vessel.

Note: Keep these bags of litter cool and shaded while you continue collecting. If the specimens sit out in the sun, the ants within the sample will not survive, and the Berlese funnel will not work!

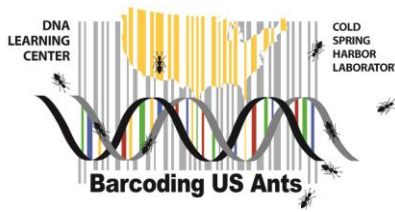
Additional materials to bring to the field:

- Collecting bags for leaf litter samples

iv. **Pitfall Traps** – This technique collects ants (and other organisms) that randomly wander into the trap, which is filled with a preservative until it can be collected. For a typical ground-level pitfall, it is a good idea to cover the trap with something to prevent water and other debris from falling in. It is also recommended that a second vessel is used to maintain the integrity of the hole. Pitfalls may be suspended from trees or dug below ground. For example, a subterranean pitfall trap might be produced by burning several small holes in a microfuge tube, attached to a string. For details of these pitfall variations, consult the *US Ants Collection Clinic* recordings in the US Ants Google Classroom.

Additional materials to bring to the field (for a typical ground-level pitfall):

- Trowel for digging holes
- Two cups (inner and outer, for placeholder and collection vessel, respectively. These cups should fit precisely within each other) – plastic Solo cups work well



- Preservative (ethanol is sufficient for short term periods, but will evaporate overnight. A 50/50 ethylene glycol and ethanol mixture with a pinch of denatonium benzoate to deter vertebrate drinking works better long-term)
- Lid and/or flashing material (i.e., thin weatherproof material) for preventing debris or water from collecting in your pitfall and reducing the volatilization of ethanol preservative
- Tape measure for laying out traps at uniform distances
- Optional: A “research” note (if you plan on leaving your trap for a few days, print a note to attach to your pitfall that a research project is being conducted, so someone doesn’t throw the materials away or mess with your trap!)
- Optional: Flags or brightly colored tape for locating traps

v. **Bait Traps** – Bait traps are effective for a wide range of ants, but beware: different ants are drawn to different types of baits! Pecan Sandies are classically an effective and commonly used bait. Other more proteinaceous baits can be used as well, such as Vienna sausage, hot dog, tuna, crushed crickets, etc., but they can get messy and contaminate samples. The idea is simple. Choose a bait, and place it out on a piece of paper or in a vial for a time (a few hours perhaps), and wait for ants to come to the bait. Try experimenting with new baits!

Additional materials to bring to the field:

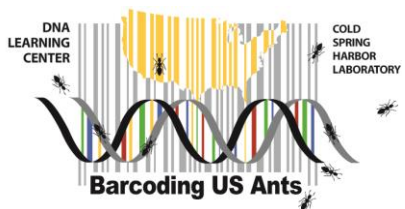
- Bait (e.g., Pecan Sandies)
- Dry collecting vials
- White paper (this makes it easier to collect ants, rather than trying to catch them amongst dirt and debris)
- Tape measure for laying out traps at uniform distances

vi. **Sweep Netting** – Sweep netting is an easy way to catch ants associated with plants, flowers, low-hanging branches, etc. A net is used to sweep back and forth across vegetation (literally hitting it), which dislodges specimens, causing them to fall into the net. An aspirator can then be used to collect ants amongst the debris or the debris can be bagged and brought back to the lab for specimen separation.

Additional materials to bring to the field:

- Sweep net (a lightweight, general insect collecting net is best – available from Bioquip.com)
- Optional: Collecting bags for sweep debris

vii. **Beat Sheeting** – Similar to sweep netting, this technique uses a white sheet laid out below plant material (usually low-hanging branches) and a stick (or the other end of a net) is used



to whack the vegetation, dislodging specimens onto the sheet below. An aspirator can be used to collect the ants from the sheet.

Additional materials to bring to the field:

- A white sheet (or a cut-open pillow case for smaller areas)

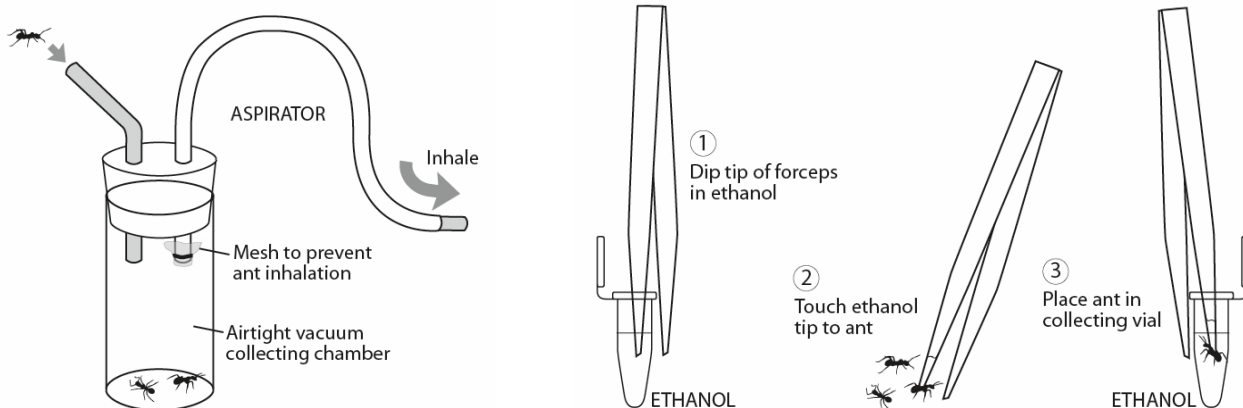
viii. Nocturnal Collecting AKA “Blacklighting” – Nocturnal collecting works well for certain types of reproductive, flying (alate) ants, and some other nocturnal species. However, the setup is more intense than other collecting methodologies on this list. A white cotton sheet can be suspended between two trees or branches and a Hg vapor light or black light can be set up in front of that sheet. Insects are then drawn to the diffuse light cast on the sheet. These lights require a generator, battery or a hookup to a vehicle. Power sources and the lights themselves can be costly. There are many variations to this methodology, but you can set up a quick and easy modified “black light” at your home simply by replacing normal incandescent exterior light fixtures with UV bulbs.

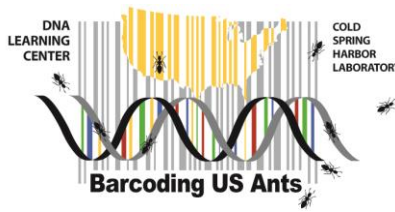
Materials to bring to the field:

- A white sheet
- Ropes or bungee cords for suspending the sheet
- Hg vapor light source or UV “black” light source
- Power source

7. Once you are prepped and ready to go into the field, it is time to collect! These steps apply to all collection methodologies, regardless of the one(s) that you use.

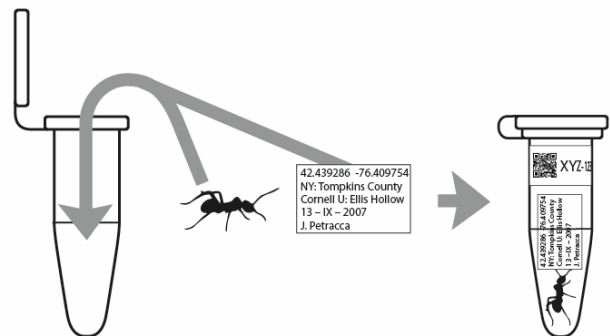
a. When collecting, remember to take caution not to damage the specimen. This is why an aspirator is so handy. It is difficult to catch ants as they scurry across dirt, leaf litter, bark, etc. The vacuum created in the aspirator will bring the ant into a collecting vial, so you can more easily slip it into the ethanol vial or a different tube. Forceps should be soft or should be moistened with a bit of ethanol to “stick” the ant to the forceps.





- b. Whenever possible, attempt to collect ants in series. It is preferred that you have 5-10 representatives of each specimen. At least one will be used for extraction and at least one will be used for taxonomic identification. *Remember that the best sequence data should have a voucher specimen associated with them for reference.* The other replicates are backup. If unsure whether all ants in a sample are the same species, you can separate these potential unknowns. You can either run them through DNA extraction as a separate sample if you strongly feel that it is a different species or you can just leave it off to the side, and a taxonomist will confirm its identity. Ant identification is hard, so use things like color, shape, size and habitat to distinguish among possibly different species. Use your field guide to help try and distinguish different species in the field. Store known replicates of the same series in the same tube.
- c. Once collected, ants can simply be placed directly into a pre-labelled collecting vial with ethanol so that they are totally submerged. The ethanol will dispatch the ants quickly. Otherwise, ants can be collected in dry tubes or containers that you have labelled with a permanent marker and placed in the freezer when returning to the lab. Leave them in the freezer for 24 hours to ensure that they are dispatched, then they can be placed in 90-95% ethanol tubes, or kept in the freezer until you are ready to extract DNA.

- d. Stay organized. Whether you do this in the field or back at the lab, your ant specimen(s) must ultimately wind up in a pre-labelled 90-95% ethanol collecting vial that you received from the DNALC along with a date/locality label. See step 4 for what information should be on this date/locality label. These labels should be placed directly in the ethanol with specimens. Simply fill in the pertinent specific information on a date/locality label (e.g., exact GPS coordinates) using the micron pen or pencil, and place the label in the ethanol with the specimen. The specimen ID number that is on the outside of the tube will correspond to the specimen and its sequence on the DNALC's Sample Database.



e. Procedures for Specific Collection Techniques

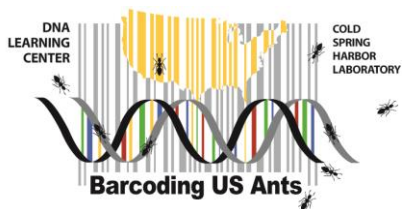
i. Directed Hand Collecting

- 1) Identify likely ant habitat in your collecting area.
- 2) Use your trowel to dig up ant colonies, or a pry bar to turn over heavy rocks and break apart rotting wood.
- 3) Collect ants. Refer to Step 7a.

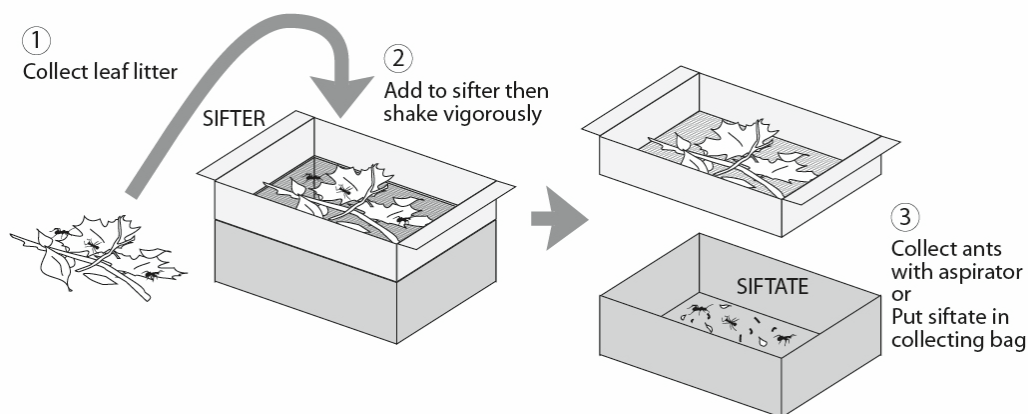
ii. Leaf Litter Sifting

In the field:

- 1) Collect leaf litter by hand, or use a trowel or small rake to gather litter/soil samples.

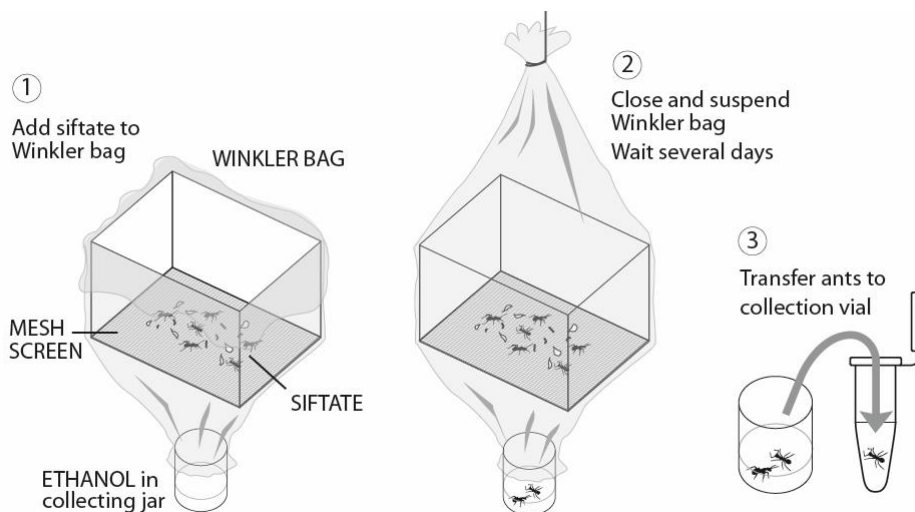


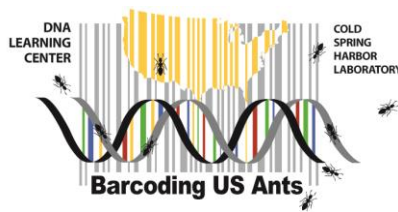
- 2) Place samples in your sifter.
- 3) Vigorously shake the sifter. Be sure that material is not falling out of the sifter.
- 4) Remove the sifter, and discard large leaf material (you can also save this litter for a more thorough Berlese funnel extraction – see step iii.).
- 5) Scan the fine, sifted material in the tray and use an aspirator to collect ants.
- 6) Optional: For a more thorough search, pour all fine material (siftate) into a collecting bag. Keep this bag cool and dry until you return to the lab, and don't forget to label it with the date and locality.



In the lab:

- 1) Set up a Winkler bag. Dump siftate into a breathable bag (i.e., a Winkler bag) that tapers at one end to an ethanol-containing collecting vessel covered with a piece of fine mesh.
- 2) Close bag, suspend vertically, and allow material to sit for up to three days. Check periodically to make sure ethanol does not evaporate. Refill as needed.
- 3) Scan the ethanol vial's contents under a dissecting scope for ant specimens.





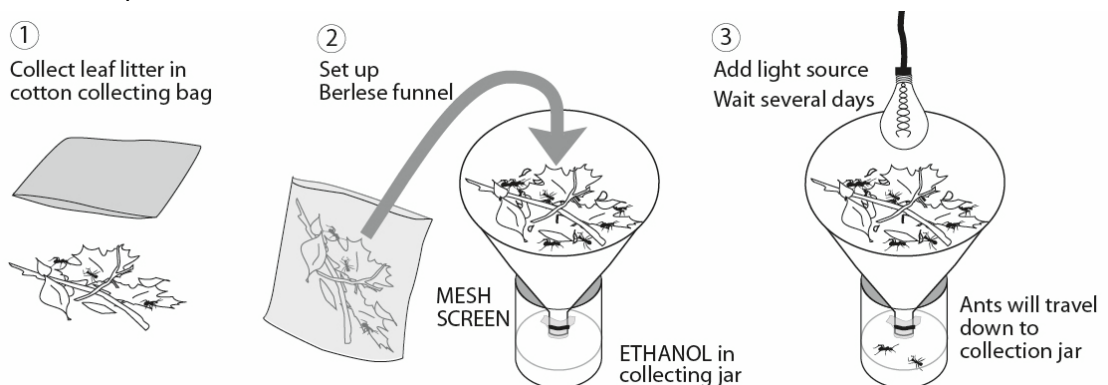
iii. Berlese funnel

In the field:

- 1) Collect leaf litter by hand, or use a trowel or small rake to gather litter/soil samples.
- 2) Place all litter/soil/wood samples in a collecting bag. Keep this bag cool and dry until you return to the lab, and don't forget to label it with the date and locality.

In the lab:

- 3) Set up a Berlese funnel. A simple way to do this is to dump the contents of the collecting bag into a large funnel with the small end fixed in a collecting vessel filled with ethanol.
- 4) Suspend a light source, such as a normal incandescent bulb above this funnel, and let sit for up to 2 days. Check periodically to make sure ethanol does not evaporate. Refill as needed.
- 5) Scan the collecting vessel for ants under a dissecting scope.
- 6) Litter samples can then be discarded.

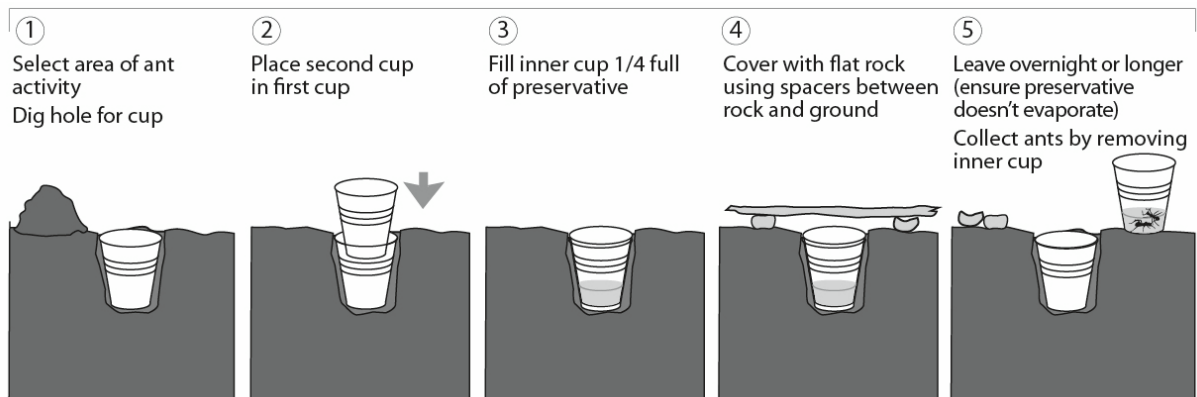


iv. Pitfall Trap (Ground-Level Pitfalls)

- 1) Select suitable locations for pitfalls (i.e., probable areas of ant activity).
- 2) Using your trowel, dig a hole to fit your first cup (i.e., the inner cup).
- 3) Ensure that the cup fits snugly in the hole and is approximately level with the ground. This will be the placeholder, so that the hole does not cave in.
- 4) Place the second cup (i.e., the outer cup) in the first one, and make sure that the lip of the cup is level with the ground. Note that if it is too high, ants may not enter the trap. This is the trapping cup.
- 5) Fill the trapping cup one quarter of the way with preservative, either ethanol or 50% ethylene glycol in ethanol mix.
- 6) Place a flat rock, piece of metal flashing, or oversized plastic lid over the top of your trap. Note do not obstruct the path around the trap; this step is meant simply to prevent unwanted debris from collecting in the trapping cup.
- 7) Using your tape measure, measure out other sites for traps, equidistant from the first. Place one trap every 3 ft. or so from another, and repeat steps 2-6.

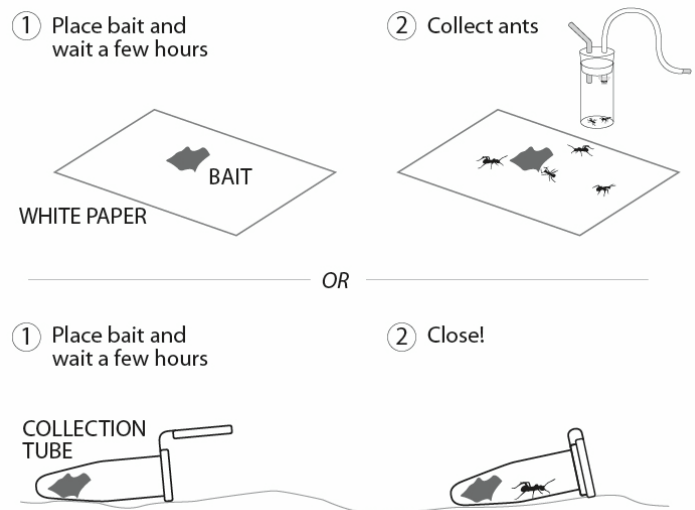


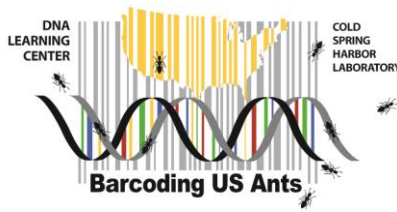
- 8) These traps may be left overnight or up to two days. Be sure to use an appropriate preservative for long term.
- 9) Optional: Place a “research” note near your trapping site so that rangers or other individuals do not discard your materials. This note should indicate that you are conducting an authorized research project, and that it should not be disturbed.
- 10) Optional: Place disposable flags or brightly colored tape at each trap location so that you can easily find them again. Depending on the size and type of pitfall, this can be very helpful – subterranean pitfall locations, for instance, can be hard to find after a few days!
- 11) After time has passed, return to your traps and remove organisms from preservative and place them in 90-95% ethanol collecting vials as soon as possible.
- 12) Traps may be reused again after preservative is replenished.
- 13) Consult the Ant Collecting Link document for references for different pitfall techniques.



v. Bait Traps

- 1) Select a bait specific to the ants that you are trying to lure. Pecan Sandies are a classic choice.
- 2) Select an area of probable high ant activity.
- 3) Crumble a small amount of bait and place it on a white sheet of paper or place within a dry collecting vial.
- 4) Place these baited traps at equal distances (perhaps every 1 foot) along your collecting site. Note that this idea can extend to ground or arboreal locations.
- 5) Let sit for an hour or so, and return to the collecting site.

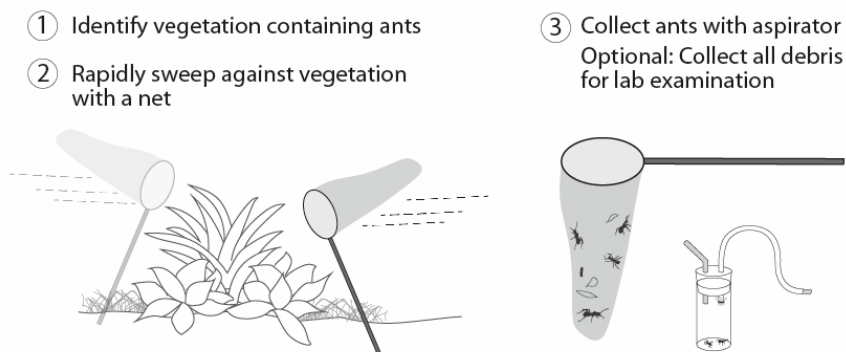




- 6) Ants can easily be spotted and collected from the white paper, and many can be collected at once by simply closing the dry collecting vial with bait.

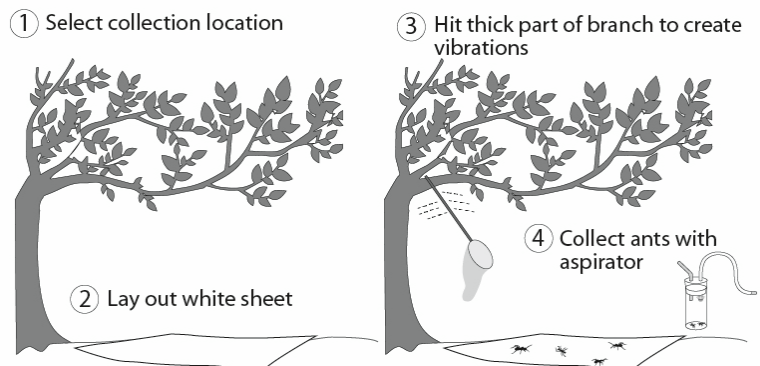
vi. Sweep Netting

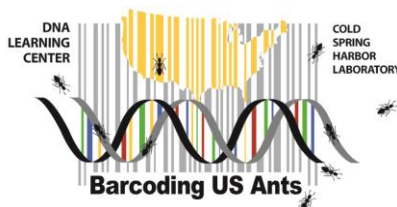
- 1) Identify an area of vegetation likely to harbor ants. Roadsides, forest edges, meadows, prairies, and grasslands are all excellent locations.
- 2) Using your collecting net, sweep the vegetation back and forth using rapid, figure-8 motions. If done correctly, small organisms, and some plant debris (flowers and leaves) will dislodge and fall into the net. If you are getting stems and branches, you are going too hard!
- 3) After a sweep, simply peer into the net and use your aspirator or dry collecting vials to collect ants.
- 4) Optional: Alternatively, sweep debris could be emptied into collection baggies for later examination in the lab.



vii. Beat Sheetting

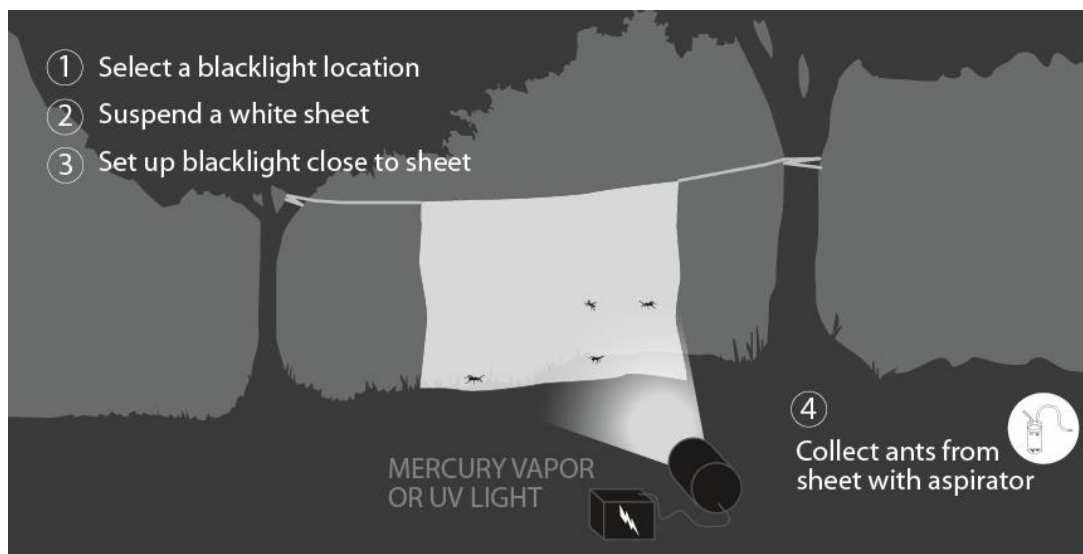
- 1) Select an area suitable under which a sheet can be placed. Low-hanging branches and shrubs are perfect places for this technique.
- 2) Lay out a white sheet as far as it will go beneath these branches. Do not leave many folds or it will be harder to see organisms.
- 3) Use the stick end of your collecting net or a firm branch, and whack the thicker branches above the sheet.
- 4) Insects will dislodge and fall onto the sheet. Against the white sheet, ants should be readily visible and easy to collect with an aspirator.



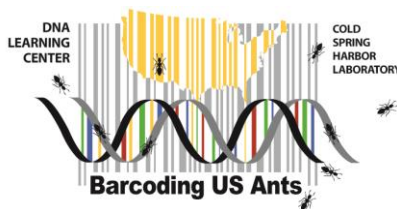


viii. Nocturnal Collecting AKA “Blacklighting”

- 1) Blacklighting is most productive after nightfall and up to 3 AM. Different organisms will become active at different times. Plan a blacklighting location that is distant from other artificial light sources. It is best to do this in a group for safety.
- 2) Suspend a white sheet from tree branch or against a rock face.
- 3) Hook up your power source to your mercury vapor bulb or UV light, and shine the light source on the white sheet.
- 4) Depending on the location, insects will cover the sheet after a short period of time. In terms of ants, you will draw nocturnally active reproductives or nocturnal workers that happen to be in the area. Please be responsible when using this technique. After collecting ants, turn off the light and allow the other organisms to go on their way.



8. Once your ant specimen(s) are safely in the collecting vial(s), record information about the area in your field notebook, following the guidelines of the ***US Ants Metadata Sheet***. When collecting litter or sweep samples for lab examination, do not forget to record metadata in the field.
 - a. Remember that you should record a profile of the locality in terms of geography, weather, climate, time of day, plant cover, leaf litter composition, and you should record a profile of the specimen itself in terms of behavior (what was it feeding on?; did you find it on a tree or in the ground?; what type of flower was it on?; etc.), nest structure, etc.
9. Use a smartphone or digital camera to photograph your specimen in its natural environment, or where it was obtained to clarify notes in your field notebook. Nests, food source, or interesting behaviors should be photographed when possible.
 - a. Take wide, medium, and close-up views, and always make sure to include a scale of some sort for reference.



10. GPS information must be recorded and associated with your specimen, which can be found with a smartphone.
 - a. In Apple iPhoto, click on "i" (image properties) to plot the photo on a map. Click on "Photo," then "Show extended photo info" to find GPS coordinates.
 - b. GeoSetter, photo metadata freeware for PCs, will plot your photo on a map.
 - c. In Google Picasa photo editor, click on "i" to find GPS coordinates.
 - d. Your smartphone's manual should explain how to use the GPS feature to obtain coordinates.
 - e. Many smartphones also have apps that make it easy to harvest GPS coordinates (e.g. Google maps).

11. Back in the lab, you must photograph your specimen. It is best to do this as soon as you are finished collecting or even while the specimen is alive. Ethanol can cause specimen color to change, which can make identification harder.
 - a. Photographs should be of high quality, using either a phone, a camera with a macro lens, or a dissecting scope fitted with a microscope.
 - b. Ideally, these photos will allow for an expert to identify your specimen to species when possible, and therefore should highlight the morphological features of the specimen from multiple angles. A series of photographs for each specimen is preferred. If you use the entirety of your specimen for DNA extraction, then this will be the *only* way to verify the species
 - c. These photos will ultimately be uploaded to the Sample Database with your metadata notes, and will help identify your sample. Be sure to save photos with the appropriate specimen ID number that corresponds to your sample on the pre-labelled collecting vials that you received from the DNALC.

12. You may wish to make an attempt to taxonomically identify your ant specimens prior to extraction. This is encouraged, but not required. You should first try to identify your ant to genus, and then make an attempt to identify it to species.
 - a. The book [Ants of North America](#), by Brian Fisher and Stefan Cover, is an excellent resource for genus-level identification.
 - b. Antwiki.com has numerous dichotomous keys available that can help identify specimens to species, depending on region or genus.
 - c. There are other references in the ***Ant Collecting Links*** document in the US Ants Google Classroom.

13. Specimens should then be stored with their date/locality labels in their appropriately labelled ethanol tubes in the freezer until you are ready to extract DNA. Repeated thawing and freezing, by shuffling them in and out of the freezer can be damaging to DNA.

Note: that when performing the extraction, your ant samples will have desiccated severely in the ethanol. They will be brittle! Take care when handling them to avoid breaking specimens.