

Sample Return

If you are NOT doing PCR amplification and gel electrophoresis at your facility, please refer to section I below for DNA and specimen return instructions. If you ARE doing PCR and gel electrophoresis at your facility, please refer to section II below for DNA, ant specimen, and positive PCR amplicon return instructions. All participants should refer to section III for shipping information.

I. DNA and specimen return to the DNALC

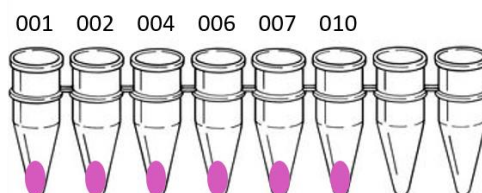
1. Return several of each species for taxonomic identification - use a separate tube of ethanol for each different species you intend to send. Ensure the ethanol and purified DNA/Chelex microfuge tubes are labeled with the corresponding sample ID (ex: ABC-001). Use a permanent marker to label the top and sides of the tubes with the sample ID. Include locality labels with the samples in ethanol, when appropriate (see *US Ants Collection Guide* for locality label details).
2. Place the labeled screw-cap tubes containing ethanol and your specimens and the labeled Parafilm tubes containing Chelex/tissue/DNA into the microcentrifuge tube storage box for mailing. Label the box with your last name, team name, the date, and "US Ants." Place the pestles and cap locks into a zip top bag for return mailing. Label the bag with your last name, team name, the date, and "US Ants."

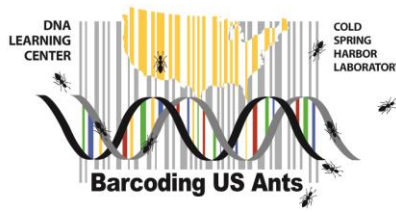
II. DNA, specimen, and PCR amplicon return to the DNALC

Please complete the following steps ONLY if your sample PCR amplifications have been confirmed by a DNALC staff member. If you have not yet uploaded a gel image to request sequencing for your samples, please refer to the *Sequencing Request* protocol before proceeding.

1. US Ants team leaders will be notified via email once your team's sequencing request has been accepted by a DNALC staff member. This email will inform you of the samples that were approved to be packaged and sent for sequencing. *If your sequencing request was not approved, you will receive an explanation and follow-up from a DNALC staff member. Important* – not all samples may be approved by DNALC staff. Be sure to ONLY send the samples that were approved – discard PCR samples that were not. Please contact us if it is unclear why a sample was not approved.
2. Transfer the entirety of each approved PCR amplicon into the long connected strip tubes that you received. Samples must be transferred to strip tubes in increasing numerical order, as indicated in the approved sample email. DO NOT cut apart strip tubes, even if you do not fill all 8 tubes per strip.

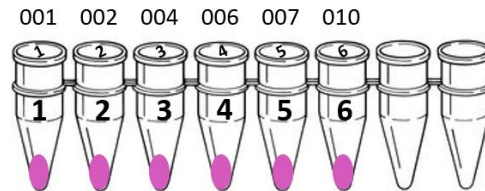
Example:





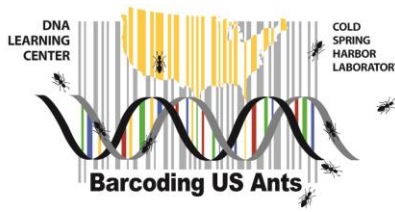
- The sequencing facility GENEWIZ requires that sample tubes be numbered consecutively, as opposed to labeled with our sample IDs. For example, if team samples ABC-001, 002, 004, 006, 007 and 010 are approved for sequencing, the tube containing sample 001 will be labeled with "1," 002 with "2," 004 with "3," 006 with "4," 007 with "5" and 010 with "6." These numbers must be written on the tops and sides of the strip tubes. Do not number empty tubes.

Example:



Note: Your sequence data will be assigned the original sample IDs when returned from GENEWIZ. The tube relabeling is for internal GENEWIZ purposes only.

- Ensure caps are closed tightly to avoid sample loss during shipping, and place the strip tube(s) inside the provided petri dish. Tape the petri-dish closed and write your last name, team name, the date, and your team's 3 letter code on the tape. Keep the PCR amplicons and purified DNA/Chelex microfuge tubes refrigerated until they are ready to be shipped to the DNALC.
- Return several of each species for taxonomic identification - use a separate tube of ethanol for each different species you intend to send. Ensure the ethanol and purified DNA/Chelex microfuge tubes are labeled with the corresponding sample ID (ex: ABC-001). Use a permanent marker to label the top and sides of the tubes with the sample ID. Include locality labels with the samples in ethanol, when appropriate (see *US Ants Collection Guide* for locality label details).
- Place the labeled screw-cap tubes containing ethanol and your ant specimens and the labeled Parafilmed tubes containing Chelex/tissue/DNA into the microcentrifuge tube storage box for mailing. Label the box with your last name, team name, the date, and "US Ants." Place the pestles and cap locks into a zip top bag for return mailing. Label the bag with your last name, team name, the date, and "US Ants." Return the box of specimens/DNA, bag with materials, and petri dish with PCR amplicons to the DNALC, along with any other borrowed equipment.



III. Shipping

1. Send purified DNA/Chelex tubes, specimens, PCR products (if applicable), and borrowed equipment (if applicable) by **three day** shipping method to:

Sharon Pepenella
DNA Learning Center – US Ants
Cold Spring Harbor Laboratory
1 Bungtown Road
Cold Spring Harbor, NY 11724
516-367-5167

Samples will be stable at ambient temperature for the duration of the shipment, so there is no need to send samples on ice.

Participants are welcome to use any three day shipping method to return samples and materials/equipment. **However, if participants would like the DNALC to cover return shipping costs, please return via FedEx; if FedEx shipping is not an option for you and you require coverage of the shipping costs, please contact us.** Please use the pre-filled FedEx form included with your kit for the return shipping. **If you did not receive a pre-filled form, please contact the DNALC for shipping instructions.**

2. Please notify DNALC staff when you return your materials, so we can be on the lookout for your kit. You can email Justin Burke (burke@cshl.edu) or Sharon Pepenella (spepenel@cshl.edu) with this information.