

Sequencing Request – US Ants

To request sequencing for your ant samples, you will need to provide a gel image of the amplified PCR products through the *Barcode Sample Database* (*SD*). Please enter all sample metadata and upload sample pictures into the *SD* before requesting sequencing. The *SD* can be accessed at:

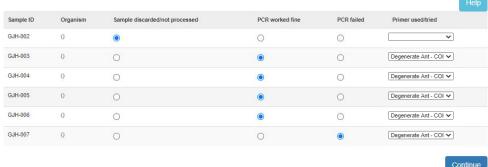
http://sampledb.dnalc.org/barcoding/

- 1. Log in to the SD with your username and password. Once logged in, click on you US Ants project.
- 2. On the bar above the project info, next to the "Samples" tab, a "Sequencing" option will become available once a minimum of three samples have been added to the project. *Gels cannot be uploaded without satisfying this minimum*.



3. Select the "Sequencing" option. Here, you will indicate if any of the samples you added to the database were not processed, whether or not the PCR worked for samples that were processed, and the primer used for amplification. Be sure to enter this information accurately – samples that are considered "not processed" or "PCR failed" cannot be selected for sequencing later. Participants should select "Degenerate Ant – COI" as their primer. You must complete this process for each sample, otherwise you will receive a "missing data" error. Click "Continue" when finished.



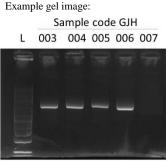








- 4. To upload the gel image for your samples, click "Choose File."
 - a. Gels must be uploaded as a .jpeg. Only one image can be uploaded at a time, though multiple gel images can be uploaded per project.
 - b. Gels should be labeled with the team's sample code, sample numbers above the appropriate lanes, and primer type. Ensure gel images are completely labeled before uploading. Text can be added to gel images in programs such as Microsoft Paint, PowerPoint, Photoshop, or other photo-editing software.
 - c. In the text box below the upload gel image section, indicate which samples should be sent for sequencing. Example text: "We feel samples GJH-003, 004, 005, and 006 are sufficiently amplified for sequencing."



Projects » US Ants Project » Sequencing request

Seq Request status: new

Please upload a get image:

Choose File US Ants Get Image. pg

Please indicate which samples you consider to be good for sequencing.

We feel samples GJH-003, 004, 005, and 006 are sufficiently amplified for sequencing.

- d. Click "Upload gel image." You will be given the option to "Add another gel" below the image that was just uploaded. If you have additional gel images to add, you will need to upload the new images and indicate samples to be sent for sequencing in the same manner as the previous gel. Please ensure that all gel images are uploaded before submitting your request. Once a request is submitted, you will not be able to upload additional gels for the project unless project staff reject the request.

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- 5. Once all gel images are uploaded, use the drop-down menu to the right of the image to link each sample to its corresponding well, and select the primer used to amplify the sample. When finished, click "link sample" and another set of drop-down options will appear for your next sample. You will not have the option to include a lane for your ladder or positive control. If, for example, your ladder is

Sample code GJH

Loo3 004 005 006 007

Primer: Ant COI

We feel samples GJH-003, 004, 005, and 006 are sufficiently amplified for sequencing, added on 10056020 ff. 32 a.m. by apspending added on 100

loaded in well 1, then link your first sample to "well 2."







- a. You may notice one or more samples in the drop-down box that are marked with * and cannot be linked to the gel image. These are samples that you designated as "not processed" or "PCR failed" OR are missing important metadata such as geolocation or at least one sample image in your project. If you need to link one of these unavailable samples to your gel, select "Delete and start over" and update the sample in the SD with the missing information, or correctly indicate the sample as "PCR worked fine;" then, repeat the gel image upload procedure. Please do not request sequencing for a sample that is not linked to your gel image; this is how we link DNA barcoding sequence data to your samples in the SD.
- 6. When all samples are linked to the gel image, click "Submit Request." You will receive the message "Your request was received" and US Ants staff will be notified of your submission. Once you submit, you will not be able to edit your gels or upload additional gels. The US Ants staff will review your gel images within a few days of your submission and will approve or reject your request. If approved, you will be able to package your samples for shipment to GENEWIZ (see Sample Return protocol for additional details). If rejected, you will receive an explanation and follow-up from a US Ants staff member.

