Museum Sampling Instructions

To access the files referred to in this workflow and additional details, please visit: https://biodiversitygenomics.net/resources/sampling-protocols/

(!) NOTE: You will require CCDB sampling kits (i.e. labelled microplates), which require time to ship from Ontario, Canada. Please order these as early as possible if you have not received them in advance. To request CCDB sampling kits, visit: https://forms.gle/KSnH492YxkYGaWWAA

Before you begin:

- Determine harvesting strategy and generate specimen target list (Excel spreadsheet, names checked on BOLD or GGI analysis tool, Genbank etc.)
- Prepare museum harvesting tracking sheets (for recording taxonomy, cabinet/drawer location, important notes)
- Pre-fill microplates with ethanol (30 microlitres per well) and cap
- Make gridded 8X12 array box with foam base and lid (e.g. Schmidt box) to hold specimens during sampling
- Print placeholder labels (with plate number and well locator for the respective microplate) - See template available in link above
- Print DNA barcode labels to attach to the voucher specimens - See template available in link above
- Cut and pin placeholder labels in array box wells
- Review BOLD photography guidelines and determine imaging equipment requirements (https://v3.boldsystems.org/index.php/resources/handbook?chapter=3_submissions.html&section=image_submissions)

Step 1: Assemble Equipment Required to Process Specimens

Required Supplies:

- **Specimen Selection**
  - Gridded 8X12 array box with placeholder labels prepared
  - Microplate numbers (CCDB-XXXXX)
  - Target specimen list
  - Museum harvesting tracking sheet
  - Laptop
  - Cart (optional)

- **Specimen Labelling**
  - Printed specimen barcode labels
  - Foam tray for adding pinned labels

- **Specimen Imaging**
  - Digital camera/ photomicroscopy system capable of imaging each specimen so it fills the frame (suggestion below)
  - BOLD Image data sheet
  - Tripod

- DSLR camera
- Macro lens(es)
- Flashes/lights
- Pin holder/foam/needlepoint hoop with white or black fabric
- Computer/laptop

- **Specimen Digitization**
  - Computer
  - BOLD specimens data spreadsheet

- **Specimen Tissue Sampling**
  - CBG sampling kit (microplates and instructions)
  - Bunsen burner or equivalent
  - ~2oz jar
  - ~20ml etoh
  - Gloves
  - Forceps
  - Microscope (optional)

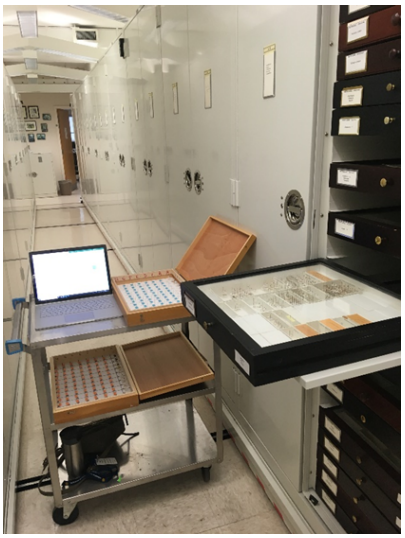
- **Specimen Return**
  - Gridded arrays of specimens
  - Museum harvesting tracking sheet with cabinet/drawer locations

Step 2: Specimen Selection/ Array specimens

Key Directive: Select 95 specimens and place into gridded array boxes. All specimens should belong to the same order.

Required Supplies:

- Gridded 8X12 array box
- Microplate number (CCDB-XXXXX)
- Specimen placeholder labels (A01 to H11)
- Target specimen list
- Museum harvesting tracking sheet
- Laptop
- Cart (optional)



How to Accomplish:

1. Label the exterior of your array with the first microplate number you will use.
2. **Work through the collection in a systematic way** according to your target list - beginning with the first unit tray in the order of interest. Once you encounter a species designated for analysis, select four specimens (if available). When possible, choose specimens from different sites and choose specimens that were collected most recently.
   - **NOTE: Be mindful of the following** during specimen selection:

    - **Curator specifications** (i.e. what material is appropriate/allowed to be sampled), how many representatives should remain per species/genera for a specific taxa, etc.
    - **Specimen collection date**: If selecting specimens for Sanger sequencing, do not include specimens collected before 1970. If selecting specimens for NGS, older specimens can be selected
    - **Any restricted material/types** (paratypes etc.) or material from restricted countries /without permits (if applicable)
    - **Collecting Method**: Some collecting methods lead to increased sequencing success (i.e. collecting into 95% ethanol), some inhibit
    - **Specimen size/condition**: Large enough that a leg can be removed and has a good probability of sequencing, legs are present, specimen not glued down to a point, etc.
    - **Data/Taxonomic (det.) label information:** Readability, language etc.
3. **When a specimen meets the requirements**, remove from the unit tray, and replace it with a specimen placeholder label. This ensures each specimen can be readily returned to the correct location in the collection after processing.
4. **Fill museum harvesting tracking sheet** to indicate the cabinet and drawer the sample came from and any information not readily available on the specimen label.
5. **Continue working through the collection** until you have filled the gridded box with 95 samples. Well 96 (H12) is left as a negative control.

Step 3: Labelling the Specimen

Key Directive: Add label with “DNA Barcode specimen” and sample ID to each specimen

Required Supplies:

- Printed specimen Barcode labels
- foam tray
- scissors

How to Accomplish:

1. Cut and place specimen Barcode labels on a foam tray (array order A01 to H11)
2. Checking that you have the correct label based on plate and well, attach a specimen Barcode label to each specimen (below specimen data labels, above any DET labels)

Step 4: Digitize Specimen Metadata and Submit to BOLD

Key Directive: Specimen Metadata must be digitized for each specimen. This step can be initiated as soon one array box is filled or multiple array boxes can be filled before beginning digitization.

Required Supplies:

- Computer
- BOLD specimen data sheet

How to Accomplish:

1. Use the plate number and well locator as the Sample ID for each voucher (e.g. CCDB-12345-A01). The list of Sample IDs is available in the label template file.
2. Reading directly from the specimen labels themselves, all details relating to the specimen should be extracted from the specimen's label and entered into the BOLD specimen data spreadsheet. Remember to also include any notes from your museum harvesting tracking sheet.
3. Enter information for each field in the databasing sheet according to the BOLD reference guide on data entry (https://v3.boldsystems.org/index.php/resources/handbook?chapter=3_submissions.html&section=data_submissions).
4. If the specimen label lacks GPS coordinates for its collection site, they should be obtained from a gazetteer.
5. After databasing is complete for one array box, upload the specimen data to BOLD following the submission guidelines.

Step 5: Photograph the Specimen

Key Directive: An image of each specimen must be uploaded to BOLD. This is essential for quality control.

Required Supplies (suggestion)

- Tripod
- DSLR camera
- Macro lens(es)
- Flashes/lights
- Pin holder/foam/needlepoint hoop with white or black fabric
- Computer/laptop

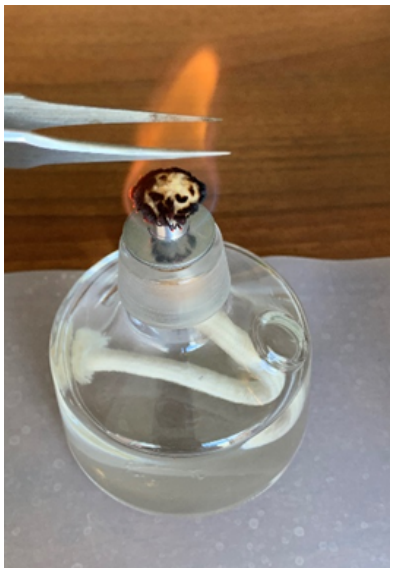
How to Accomplish:

1. Take an image of the array number as a placeholder before starting.
2. Take a picture of the whole array before imaging. This image is a reference for after the container is disassembled. Make sure the sticker with the container name is in the image.
3. Always start at A01 and go in order (A01, A02..H11)
4. Decide the best orientation for the specimen (dorsal or lateral). For specimens imaged dorsally, the anterior part of the specimen is placed on top of the image frame. Specimens imaged laterally, the anterior part of the specimen is placed on the left side of the image frame.
    - Recommended to take one image per specimen to avoid errors but BOLD allows up to 10 images per Sample ID.
5. The specimen should take up the majority of the frame and be in the center. No extra white space.
6. Image all 95 specimens then copy to the computer.

7. Rename each image by their Sample ID. Can use a batch rename program if only one image per specimen and in order. Verify specimens with image before it is disassembled.
    - Recommended to image only one array at a time to avoid errors
8. Edit any images that require cropping or rotating. Images should be cropped at a 4:3 ratio.
9. Upload images to BOLD using the ImageData file. Reference back to the BOLD reference guide for image upload.

Step 6: Specimen Tissue Sampling

Key Directive: A tissue sample (i.e. a leg) is harvested from each specimen in the array box, and placed into a corresponding labelled microplate.



Required Supplies

- CBG sampling kit (microplate)
- Ethanol burner or equivalent
- Gloves
- Forceps
- Microscope

How to Accomplish:

Refer to CCDB sampling kit instructions

1. Prepare your workstation with supplies
2. Light your bunsen burner (or equivalent)
3. Remove cap strip from row A01 to A12.
4. Dip your forceps in ethanol and sterilize in flame.
5. From your array, select specimen in cell A01 for tissue sampling
6. Remove leg from specimen (review size guide) and place into cell A01 of microplate
7. Repeat with each specimen (A01 to H11), sterilizing forceps between each and place into corresponding microplate well.
8. After tissue sampling is completed ensure caps are secured and turn off flame
9. Ensure CCDB plate records have been generated (see sampling kit instructions)

Step 7: Return the Specimens to the Collection

Key Directive: The specimens should be returned to their source location in the collection after step 6 is complete for a set of 95 specimens.

Required Supplies

- Arrayed boxes of specimens
- Museum harvesting tracking sheet with cabinet/drawer locations

How to Accomplish:

1. Use the tracking sheet to locate the proper cabinet/drawers, and then locate the placeholder label for each specimen
2. Replace the array placeholder label with the corresponding specimen
3. Discard placeholder label and repeat for all specimens in the array

Step 8: Ship microplates back to CBG

Key Directive: The completed microplates containing tissue should shipped back to CBG for analysis along with required documentation

Required Supplies

- Microplates with tissue samples
- BMAA
- CCDB plate records
- Shipping box and packing material

How to Accomplish:

Refer to CCDB sampling kit instructions