

# STANDARD OPERATING PROCEDURE

## CMNH SPECIMEN PREPARATION FOR DNA BARCODING

### 1. PURPOSE

- 1.1 To provide a procedure to define the general guidelines to ensure proper handling and sampling of specimens for DNA barcoding applications.

### 2. SCOPE

- 2.1 This procedure applies to all CMNH personnel involved in the handling of Carabid beetle specimens for the National Ecological Observatory Network (NEON) program at CMNH.

### 3. RESPONSIBILITIES

- 3.1 The NEON Carabid beetle taxonomic identifier is responsible for: 1) identifying specimens to be included in the DNA barcoding sample set, 2) pulling specimens from NEON drawers and organizing and placing them in order by their sample barcode number (relative to the Excel list generated by NEON), 3) supply the organized specimens to the person sampling tissues and 4) then return sampled specimens to their proper place in the NEON drawers.
- 3.2 The tissue sampling person shall: 1) select specimens in the order provided by the taxonomic identifier, 2) will check their sample ID number against the master list of DNA specimens (any sample ID number that is incorrect will be flagged and the proper specimen will be retrieved and sampled), 3) pull a leg and place it into the 96-well plate in the proper position and 4) return the specimen to its original position. Once sampling is complete, plates will be submitted to the Canadian Center for DNA Barcoding (CCDB) for analysis and NEON personnel will be notified that the samples have been shipped.

### 4. BACKGROUND, DEFINITIONS & ABBREVIATIONS

- 4.1 **ID:** identification.
- 4.2 **COI:** cytochrome c oxidase subunit I, the standard gene used in DNA barcoding.
- 4.3 NOTE: the “**Microplate and Data Submission Package Sampling Instructions**” PDF document (filename: “CCDB\_Microplate\_Instructions.pdf”) from the Canadian Centre for DNA Barcoding (CCDB) should also be consulted as it gives step-by-step instructions and other information. Subsets of this document are included below, where appropriate.

## 5. PROCEDURE

- 5.1 Taxonomic expert identifies Carabid beetle specimens to lowest taxonomic level possible (usually to species level).
- 5.2 Once identified, the expert will supply the specimen taxonomic IDs to NEON along with an indication of which specimens s/he thinks will make good DNA vouchers (selections usually based on specimen quality and/or taxonomic uniqueness).
- 5.3 NEON personnel will supply a final list of specimen ID numbers (taking into account the taxonomic expert's suggestions) in Excel format for each NEON domain for which CMNH has committed to examine.
- 5.4 Once received, the Excel file will be sorted by Domain and individual ID number. Two additional columns should be added to the spreadsheet including: "plateID" and "wellCoordinate". The plateID column will record the verbatim 96-well plate barcode number (e.g., CCDB-33917; supplied by the CCDB) and the wellCoordinate column will record the specific well (i.e., A1) on the 96-well plate to which the specimen will be assigned. Once sorted and assigned, the spreadsheet will be printed out and given to the person taking the tissue samples so that every specimen can be checked off the list as it is being processed.
- 5.5 The taxonomic expert will take the generated list and will pull specimens from the NEON Domain drawers and place the specimens in order based on the Individual IDs so they match the order on the generated list.
- 5.6 The person processing tissues will then take the ordered specimens and double check the individual ID label on the specimen against the number on the list to make sure they match. If they do match, a leg will be pulled from the specimen and placed into the appropriate well on the plate. If the specimen IDs do not match, then that will be recorded, and the specimen will be returned to the NEON Domain drawer where it came from and then the correct specimen will be found and sampled.
- 5.7 To begin the sampling process, position the plate on a flat surface with the plate label facing towards you. The column markers (1–12) should be at the top and the row markers (A–H) should be on the left side.
- 5.8 If samples are prone to spontaneous displacement because of static electricity (e.g., arthropod legs), sampling wells should be pre-filled with 30 µl (microliters) of 95-100% Ethanol (e.g., using a multi-channel pipettor). If a pipettor is not available, add one drop of Ethanol to each well using an eyedropper, just prior to sampling. Do not add excess ethanol as this may cause well caps to pop off during shipping. If the samples are compact and were previously fixed in ethanol (e.g. moist vertebrate muscle tissue), then adding fixative to the plate is optional.

- 5.9 Before proceeding with sampling, place the cap strips (supplied with the sampling kit) over all well rows to avoid cross-contamination during sampling. Observe the orientation of cap strips: terminal markers 1 (wide) and 12 (narrow); these should match the corresponding columns of the plate. Do not fasten caps tightly, as you will need to remove the strips just prior to sampling. When sampling, remove corresponding cap strips one at a time and fasten them back when paused or after finishing each row.
- 5.10 Start the sampling process with A01 (row 1) and proceed in alphanumeric order to A12 (left to right). When done with the first row, proceed to the second row (B01) and repeat the process until all 12 rows are filled. Do not leave empty wells in the middle of the plate. While sampling, remove only one cap strip at a time to prevent cross-contamination.  
*IMPORTANT: Do not fill the last well, H12! It should be left empty as a negative control.*
- 5.11 Sample or subsample the right amount of tissue with fine forceps (as shown in the image) and place it into the sampling well. Confirm that the tissue remains inside the well. Once done, enter the corresponding sample ID into the CCDB Record (see below). Below are some examples of recommended tissue sizes. *NOTE: Samples should be shipped within 48 hours of removal from the voucher specimens.*
- **Small insect:** whole leg, antenna — ~ 5 mm length
  - **Large insect:** tibia or femur only — ~ 2 mm length
  - **Vertebrate/invertebrate:** muscle — ca. 1 mm<sup>3</sup> volume or 1 mm diameter
  - **2-dimensional tissue:** skin/body wall — ca. 2–4 mm diameter
  - **Minute invertebrate:** whole specimen — ca. <3 mm length
- NOTE: Do not place excessive tissue into the sampling wells - this may inhibit DNA extraction. If the sample exceeds the recommended dimensions, subdivide it into fragments to obtain the right amount.*
- 5.12 Before proceeding to the next sample, ensure that no residual tissue is present on the forceps by rinsing them in 95% Ethanol and wiping them with a clean napkin or paper towel.
- 5.13 To visualize well contents (e.g., to evaluate the correct amount of fixative or tissue sampled) examine the plate from below.
- 5.14 After samples have been added to all wells in a row, replace the cap strip and seal it firmly before proceeding with next row.
- 5.15 When sampling into the last row (Row H), remember to leave the last well (H12) empty. It is OK to add fixative to this well if dispensing with a multi-channel pipettor.
- 5.16 Once the plate is filled with samples, ensure that all cap strips are pressed firmly into the wells.
- 5.17 **Specimen Data Submission.** — To facilitate the effective processing of samples, their accompanying data must be submitted in a timely manner and in a compliant format to CCDB. These data should be submitted via two independent channels (i.e., specimen data sheets via e-mail and specimen images, if available, via website upload).

- 5.18 **The CCDB Record.** The MS Excel file CCDB-00000\_Record.xls, which has been emailed to the recipient of this sampling kit is intended to record locations of samples in the corresponding sample container(s). This information is required by the CCDB Core Analytical Facility in order to begin the molecular analyses. Therefore, it is a critical component of the analytical chain. Each sample must be assigned a Sample ID — a unique individual identifier unambiguously linking the sample (tissue or DNA) with its source specimen. Ideally, it should be a permanent collection catalogue number prefixed by the museum acronym or, if unavailable, a field collection number prefixed by the collector’s initials.
- 5.19 Open the MS Excel file titled “CCDB-00000\_Record.xls”. By default, it will start with the tab (worksheet) called “DATA INPUT”. Please follow the worksheet filling instructions typed in green italics and ensure that no warning messages remain in the header of the worksheet indicating missing information.
- 5.20 Select “microplate” from the dropdown field under “Type of sampling container”. Make sure that the image appearing below matches the container you plan to fill.
- 5.21 If intending to submit multiple containers within the same record file, mark the checkbox “Multiple containers...” located below the container dropdown field.
- 5.22 Enter the CCDB container number(s) into the white cell(s) under “Container CCDB Number(s)” (type digits only, do not add prefixes). This will unhide the fields for entering Sample ID numbers.
- 5.23 Confirm that the correct sampling order is followed: refer to the container map image and the well coordinates indicated in the “Sample Locator” field.
- 5.24 As you place the samples into each container, enter their Sample ID numbers into the corresponding white cells of the column “BOLD Sample IDs”. Each CCDB Record should contain 95 entries per plate, corresponding to 95 samples. If preferred, the entire spreadsheet could be populated all at once, e.g., by pasting a column of data. In this case, please ensure that all measures are taken to ensure complete correspondence between the actual position of samples and the CCDB Record. *NOTE: Do not attempt to paste more than one column of data and do not enter data for the control well(s).*
- 5.25 Make sure that your data submission adheres to the requirements outlined in the “DATA INPUT” worksheet. Watch for error messages appearing in red color on yellow background in the field to the right of the corresponding CCDB numbers and Sample ID records and correct your entries accordingly.
- 5.26 When data entry is completed, rename the file to incorporate the CCDB numbers included in it. For example, rename it to CCDB-01234\_Record.xls for a single container or to CCDB-01234-01236\_Record.xls for a set of several containers. This numbering format simplifies the archival and retrieval of these files after when they are submitted to the CCDB.

- 5.27 To visualize the correspondence between the data recorded and the position of samples in the microplate, refer to the next tab (worksheet) titled “Array Map”. If errors were detected when entering Sample ID information, an additional map will be displayed below the general layout map to help localize problematic sample entries. Please ensure that all error messages disappear before submitting the CCDB Record.
- 5.28 **BOLD Specimen Data/Image Submission.** — Data submissions are the first step in the process of creating records on BOLD. Refer to the link below for details on the data submission procedure:  
[http://www.boldsystems.org/index.php/resources/handbook?chapter=3\\_submissions.html&section=data\\_submissions](http://www.boldsystems.org/index.php/resources/handbook?chapter=3_submissions.html&section=data_submissions)  
*NOTE: The 'Sample ID' field within the spreadsheet submission template, which can be found at the above link, should contain numbers identical to those entered in the CCDB Record.*
- 5.29 **Specimen Images** are an additional requirement for some projects (refer to the Biological Material Analysis Agreement – BMAA, for details) and should be uploaded to BOLD to complement the specimen data records. Please refer to the following link for details on the BOLD image submission procedure:  
[http://www.boldsystems.org/index.php/resources/handbook?chapter=3\\_submissions.html&section=image\\_submissions](http://www.boldsystems.org/index.php/resources/handbook?chapter=3_submissions.html&section=image_submissions)  
*NOTE: Although data uploads to BOLD are independent from the submission of samples and CCDB record files to the lab, they are a critical prerequisite for running molecular analyses at the CCDB.*
- 5.30 Once all specimens have been sampled and the plates have been capped, they should be packaged and shipped to the CCDB via FedEx. A hard copy of the NEON manifest should be included with the shipment. *NOTE: This shipment crosses international borders and should include a filled-out Customs declaration form!*
- 5.31 **NEON Barcoding Data Ingest.**— In addition to the files that need to be submitted to CCDB, a “Barcoding file” in csv format should also be uploaded to the NEON Data Ingest website (<https://data.neonscience.org/web/external-lab-ingest/>). This file will be used to link the submitted specimens to the CCDB sequence data, once generated.