

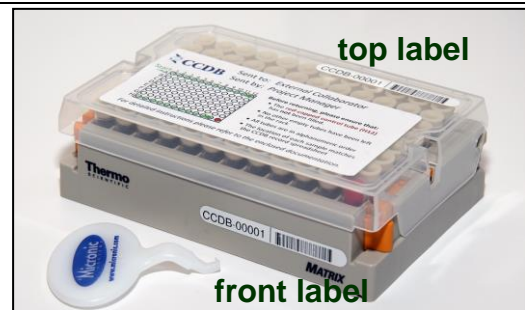
This Submission Package is aimed to facilitate the submission of **animal tissue samples** and collateral information for processing at the **Canadian Centre for DNA Barcoding (CCDB)**. The kit contains:

- **Tube racks (boxes containing coded tubes with silicone caps)**, for housing tissue samples;
- A digital CCDB Record file (MS Excel spreadsheet, attached to the automated notification email);
- These Sampling Instructions (enclosed in the package, copy sent by email);
- The Biological Material Analysis Agreement (BMAA) (enclosed in the package, copy sent by email).

Filled boxes together with the signed BMAA and filled CCDB Record files should be returned for analysis to the **Centre for Biodiversity Genomics** — the hosting institution of the CCDB. Specimen data should be submitted separately to the **Barcode of Life Data Systems (BOLD)**; a synopsis is provided below.

SAMPLE TUBE RACK (BOX)

Each tube rack (box) contains sample tubes with silicone stoppers that are arranged in a 12x8 rack corresponding to 96-well plates used in the CCDB sequencing lab. Tube A01 is the beginning of the sampling array. **One red-capped tube (H12)** corresponds to the negative control well in the sequencing plate and **should be left empty**, so each box will accommodate **95 samples**. Each tube has a marker at the bottom indicating its location within the rack. See below for details of the sampling procedure.



Each tube rack is individually numbered and will be shipped with **small labels** pre-affixed to the **front** side and **top** of the box lid. Each small label contains a unique **barcode** and human-readable **identifier (CCDB Number)**. This CCDB number should be entered in the corresponding *CCDB Record* spreadsheet (see page 4).

Note: Before adding samples into tubes, make sure the **front label is attached to the side corresponding to row H**. Always work with the front label facing towards you. The position of each tube within the rack (A01 through H12) should be consistent with the markers on the bottom of the tubes.

DIGITAL SPECIMEN DATA REQUIREMENTS

Prior to molecular analysis at the CCDB, accompanying data must be submitted in a compliant format via two different channels: the **CCDB** and the **Barcode of Life Data Systems (BOLD)**.

1. The **CCDB Plate Record** named **CCDB-00000_Record.xls**, is emailed to the recipient and used to record the location of samples in the corresponding sample container(s). Each sample must be assigned a **Sample ID**, which is a unique identifier connecting the sample with its source specimen. See section H for more details. Each container will have a corresponding plate record and up to 10 plate records can be included in the CCDB Plate Record file.

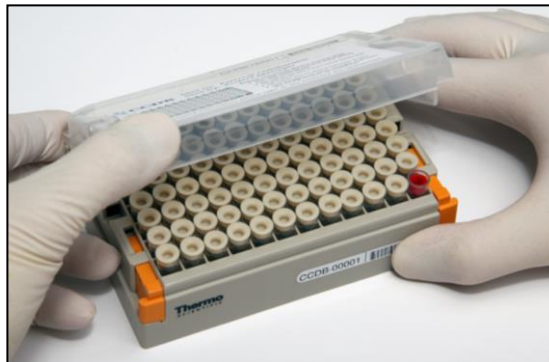
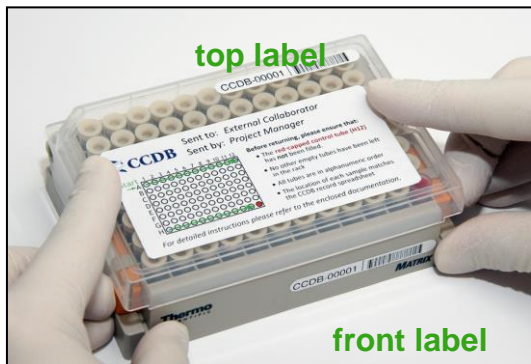
2a. A **BOLD Specimen Data Submission** is the first step in the process of creating records on BOLD. There will be one specimen data submission for each batch of containers. For more details on the specimen data submission protocol, please refer pg. 15 of the BOLD handbook accessible through this link: <https://boldsystems.org/resource-hub/documentation/>

Note: The 'Sample ID' field within this specimen data spreadsheet should be identical (including letter case) to the Sample IDs entered in the CCDB Plate Record and without any duplications.

2b. A **BOLD Specimen Image Submission** is an additional requirement for some analytical services (see <http://ccdb.ca/pricing/> for details) and should complement the specimen data submission. For details on the image submission protocol, please refer to pg. 26 of the BOLD handbook accessible through this link: <https://boldsystems.org/resource-hub/documentation/>

SAMPLING PROCESS: GENERAL INFORMATION

Before adding samples into the tubes, make sure that the **front label** is attached to the side **corresponding to row H**. Always work with the front label facing towards you. Make sure that the position each tube within the rack (A01 through H12) is consistent with the markers printed on the bottom of the tubes. Before closing the box, observe the orientation of the lid and make sure that the **CCDB Number** on the top label corresponds to that on the front label.



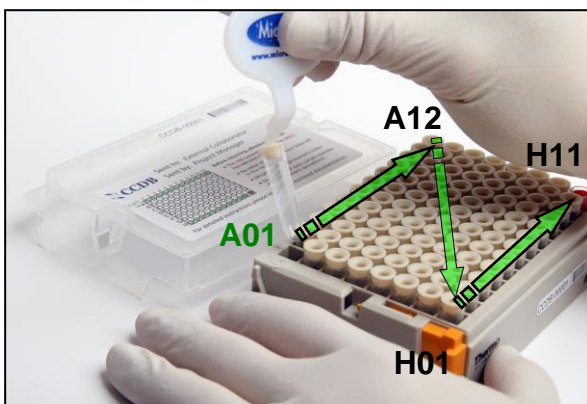
To begin the sampling process, position the box with the **front label** facing towards you, unlock and remove the box lid.



Note: To unlock the lid, slide the two orange handles towards the front. When the box is not in use, please keep the lid attached and locked, to prevent accidental spillage of sample tubes.



Use the supplied cap handling tool to fetch the tubes from the rack and remove caps from them. Use your fingers to fasten the caps back on and to place the tubes back in the rack. **Please do not discard the cap handling tool after use**; additional tools will be sent upon request only.



Start the sampling process with **tube A01** and proceed in alphanumerical order. **Do not remove tubes or leave empty tubes in the middle of the batch.** While sampling, remove only one tube at a time, to prevent mix-ups.

IMPORTANT: Do not fill the last red-capped tube H12! It corresponds to empty control well in the sequencing plate.

As you proceed with sampling, keep a full record of *Sample ID's* in the **Data Input** worksheet of the corresponding **CCDB Record** workbook. Refer to instructions on last page and in the CCDB Record Data Input Sheet for details.

SAMPLING PROCESS: THE PROCEDURE



Remove tube from rack, pop the cap off and leave it on the cap handling tool.



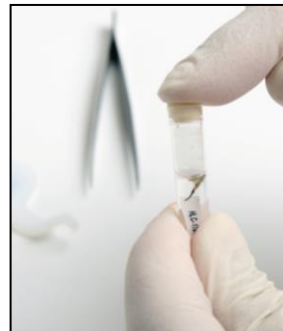
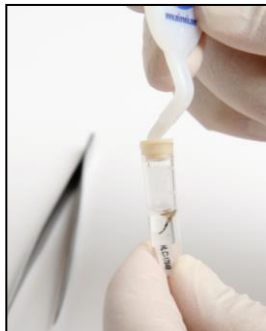
Place tissue inside sample tube. Prepare label with corresponding Sample ID and place it with sample.



Note: Labels should be laser-printed or written in pencil on archival/acid free paper.



Ensure that no residue from the tissue is left on the outside of the tube and that the label is not protruding beyond the lower surface of the cap. If fixative* is required, add it after placing the sample and label inside the tube. Do not overfill — leave ca. 0.5–1 cm from the top.



Place the cap back on the tube and press firmly with your finger to ensure proper seal. Pay special attention if fixative was added. Place the tube back in the rack in its original location (A04 in this example).

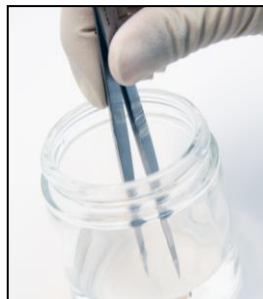


Note: Add the same fixative as originally used to preserve tissue. If no initial fixation was done (e.g., fresh or frozen samples), use 95% ethanol or Dimethyl Sulfoxide (DMSO). Dry tissues (e.g., insect legs or dry skin clips) do not require fixation. Tissues previously stored in 95% ethanol can be shipped with the fixative poured off, in order to comply with shipping regulations.

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.

When the work environment permits, use flame (e.g., dry insects) or bleach/specialized detergent such as Elimase (e.g., vertebrate tissue) to sterilize your sampling tools.

Note: If using bleach or detergent, make sure that all chemicals are completely removed from the tools by thoroughly rinsing them in distilled water before the next sampling round, to avoid DNA degradation.



After all the tubes have been filled with samples, ensure that all caps are pressed firmly into the wells, close the box lid firmly (**Note:** Observe proper lid orientation) and secure the side latches. Examine the tube rack from below to confirm that the markers on the bottom of the tubes (A01 through H12) match their coordinates inside the rack.

THE CCDB PLATE RECORD

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:

1. Select "tube rack" from the dropdown field under 'Type of sampling container'. Make sure that the image appearing below matches the container you plan to fill.

2. If intending to submit multiple containers within the same record file, mark the checkbox "Multiple containers..." located below the container dropdown field.

3. 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.

4. Confirm that the correct sampling order is followed: refer to the container map image and the tube coordinates indicated in the "Sample Locator" field.

5. 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 container, 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 tube(s)**.

6. Make sure that your data submission adheres to the requirements outlined in the 'DATA INPUT' worksheet. Watch for **error messages** appearing in red colour on yellow background in the field to the right of the corresponding CCDB numbers and Sample ID records and correct your entries accordingly.

NOTE: All coloured (non-white) cells in the CCDB Record workbook are write-protected to secure formulas and cross-links. Please type/paste your data only into white cells. Avoid moving (cutting and pasting) data between cells; use the copy-paste-delete procedure instead. When pasting data from another spreadsheet, make sure to paste 'values' or 'unicode text' using the 'paste special' function of MS Excel.

7. 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.

8. To visualize the correspondence between the data recorded and the position of samples in the tube rack, 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.

NOTE: If the CCDB record sheet is filled prior to sampling, the 'Array Map' sheet can be printed as a reference to use when sampling into the container.

DIGITAL SPECIMEN DATA SUBMISSION

1. The **CCDB Plate Record** file(s) and signed **BMAA** should be emailed to the CCDB via lims@ccdb.ca.
2. **Templates for BOLD data submissions can be found at the following link:**
<https://boldsystems.org/resource-hub/templates/>
 - a. The **Specimen Data** file can be submitted directly to BOLD using the specimen data submission protocol. Please refer to pg.15 of the BOLD handbook for instructions:
 - b. **Specimen Images** and the corresponding **Image Data** file can be submitted directly to BOLD using the online image submission protocol. Please refer to pg. 26 in the BOLD handbook for instructions:

For the BOLD handbook including detailed information on the BOLD data structure and submission procedures, please refer to the following link:

https://bench.boldsystems.org/libhtml_v3/static/BOLD4_Handbook_FinalVersion_Feb2023.pdf

Any questions concerning specimen data or image submissions should be directed to the BOLD Support Team support@boldsystems.org

SUBMITTING BIOLOGICAL MATERIALS

Fill all 95 samples in each container before proceeding to the next one in the batch. Do not ship back partly filled containers, unless specifically arranged with the CCDB. Whenever a container is transferred to another person for sampling, please notify the CCDB.

NOTICE: Unless explicitly negotiated otherwise, all biological materials submitted to the Canadian Centre for DNA Barcoding (CCDB) at the Centre for Biodiversity Genomics (CBG) fall under the standard provisions of their associated Biological Material Analysis Agreement (BMAA).

All data submitted to the Barcode of Life Database and generated by the CCDB will comply with the Data & Resource Sharing Policies. Full texts can be requested from the CCDB.

A synopsis of the conditions relevant to this transaction with the CCDB is contained in the BMAA included in this submission package. Please acknowledge that you have read and agreed to these conditions by signing the BMAA in electronic or hard copy form and returning it to the CCDB with the first batch of samples.

DISCLAIMER: It is the sender's responsibility to ensure that (1) biological materials are shipped to the CCDB in compliance with any applicable shipping regulations, (2) they have been obtained under appropriate collection, and (3) animal care permits in their country of origin and the necessary export/import documentation required by Canadian and International customs and conservation authorities has been provided, including, but not limited to:

- a) Export permit and/or zoosanitary certificate from the country of origin (if applicable)
- b) CITES export certificate from the country of origin (if applicable)
- c) Canadian Food Inspection Agency import permit (if applicable)

The CCDB cannot be held responsible in the event the provider fails to supply proper shipping documentation, causing the shipment to be held up or confiscated by customs, or any penalties resulting thereof.

After the sampling process has been completed, please return the samples by courier or registered mail to the following address. Please indicate a nil value on the shipping invoice.

**Sample Submission
Canadian Centre for DNA Barcoding
Centre for Biodiversity Genomics
University of Guelph
50 Stone Road East
Guelph, Ontario, Canada N1G 2W1
Phone: +1 (519) 824-4120 ext. 56393**

CCDB DISTRIBUTION OF DATA

Once CCDB processing is complete and all final sequences have been uploaded to BOLD, a **Sequence Upload Notification** will be sent to the external collaborator via the email address used during submission correspondence. The Sequence Upload Notification will contain the following information:

- The number of sequences uploaded
- Root plate name(s) associated with the data
- BOLD project name(s) associated with the data
- Contaminants, records flagged by BOLD, or other important information

The Sequence Upload Notification will contain the following attachments:

- Fasta file of uploaded sequences
- Taxon ID tree
- Spreadsheet of specimen information for sequences included in the taxon ID tree
- Specimen Images for sequences included in the taxon ID tree, if available
- BOLD Identification Engine results
- BOLD Sequence Submission Report
- Fasta file of sequences that contain a stop codon(s), indel(s), or identify as a contaminant, if any

In addition to the files attached to the Sequence Upload Notification, a copy of the CodonCode project, created by the CodonCode Aligner software used to perform sequence editing and assembly, as well as the fasta file of uploaded sequences will be attached to the associated BOLD Project for long term storage and review. All trace files will be uploaded to their corresponding specimen record on BOLD and can be reviewed or downloaded at any time.

Note: Sequences that contain greater than 1% ambiguous bases of the total recoverable sequence length (i.e. >5 N's in a 500bp sequence) do not meet CCDB sequence quality standards and will **not** be uploaded to BOLD.

Note: Final sequences that contain a stop codon(s), indel(s), or identify as a contaminant (e.g. *Homo sapiens*, *Mus musculus*, Proteobacteria (i.e. *Wolbachia*), fungal on a non-fungal plate, Nematoda on a non-Nematoda plate, etc.) will **not** be uploaded to BOLD. The sequence(s) will be provided as an attachment in the Sequence Upload Notification.

Note: Sequences that do not match the expected taxonomy at genus level or higher will be uploaded to BOLD and BOLD will flag the sequence to exclude it from use by the BOLD Identification Engine. The act of flagging maintains the integrity of the BOLD reference database used by the BOLD Identification Engine to determine closest matches for sequence identifications.

Validation of the final data is required from the external collaborator to address any mismatches between final sequence identifications and the expected taxonomy. To minimize mismatches, only known taxonomic information for specimens should be provided in the submitted **Specimen Data** file (i.e. specimens identified to Order if only Order level taxonomy is known). Flagged specimens require the review of specimen identifications. If the cause of the mismatch is a result of contamination from a non-target sequence, the sequence will remain flagged. If the cause of the mismatch is a result of specimen misidentification, the specimen data will be updated on BOLD by the external collaborator. Once taxonomic revisions of flagged sequences are complete, specimens with final sequence identifications matching the expected taxonomy can be unflagged upon request by the external collaborator to the BOLD Support Team at support@boldsystems.org.

All inquiries regarding CCDB processing or the information and data provided in the Sequence Upload Notification can be directed to CCDB Support Team at support@ccdb.ca.
