

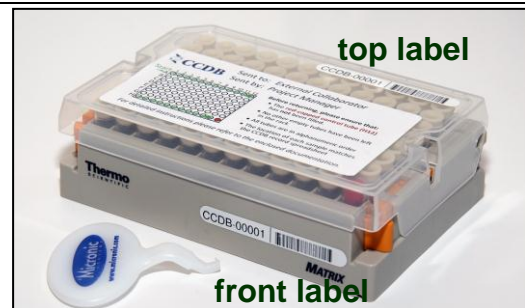
This Submission Package is aimed to facilitate the submission of **animal tissue samples** and collateral information for processing at the **Canadian Centre for DNA Barcoding (CCDB)**. The kit contains:

- **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 **Biodiversity Institute of Ontario** — 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 12×8 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.

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. These data should be submitted via two independent channels:

1. **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.*
2. **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§ion=data_submissions

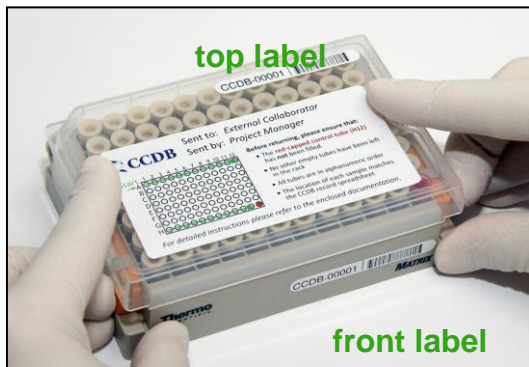
NOTE: The 'Sample ID' field within the spreadsheet submission template, which can be found at the above link, should contain numbers identical to those entered in the CCDB Record.

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§ion=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.

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.



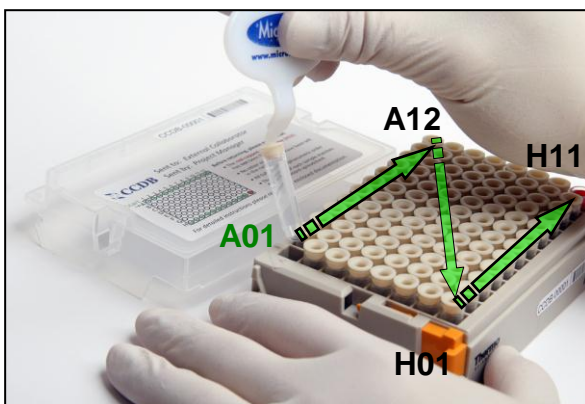
To begin the sampling process, position the box with the **front label** facing towards you, unlock and remove the box lid.



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 alphanumeric 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.

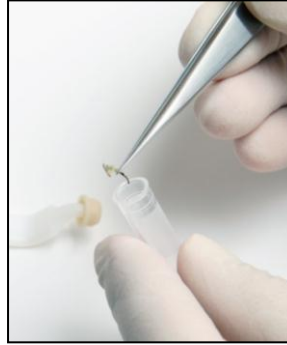
IMPORTANT: Do not fill the last red-capped tube H12! It corresponds to empty control well in the sequencing plate.

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.



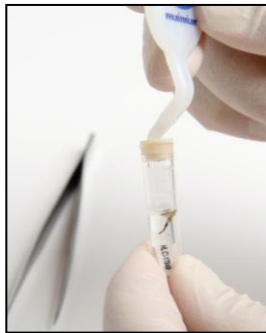
Place tissue inside sample tube. Prepare label with corresponding Sample ID and place it with sample.



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).

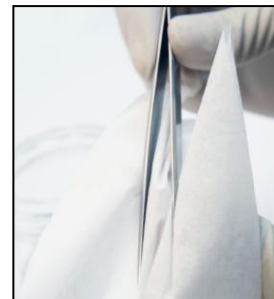


Note: Add the same fixative as originally used to preserve tissue. If no initial fixation was done (e.g., fresh or frozen samples), use 95% ethanol or Dimethyl Sulfoxide (DMSO). Dry tissues (e.g., insect legs or dry skin clips) do not require fixation. Tissues previously stored in 95% ethanol can be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

SAMPLING PROCESS: KEEPING A RECORD

Attention: New CCDB record template introduced in January 2014 – please read carefully!

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:

The screenshot displays the 'DATA INPUT' worksheet of the CCDB record template. It features a grid for entering sample data, with columns for 'Sample Locator', 'BOLD Sample IDs', and 'Container CCDB Numbers'. A 'Type of sample container' dropdown is set to 'tube rack', and a 'Multiple containers (up to 10)' checkbox is checked. A 'Tube Rack (animal tissue)' image is shown. A 'SAMPLING ORDER' section includes instructions and a grid for marking sample positions. A 'Please ensure that your data submission meets the following criteria:' box is also visible.

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.

SUBMITTING BIOLOGICAL MATERIALS

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

NOTICE: Unless explicitly negotiated otherwise, all biological materials shipped to the Canadian Centre for DNA Barcoding and the Biodiversity Institute of Ontario fall under the standard provisions of the iBOL Biological Material Transfer Agreement (v. 2011-11).

All data submitted to BOLD and generated by the Canadian Centre for DNA Barcoding under the iBOL framework will comply with the iBOL Data & Resource Sharing Policies (v. 2012-01). Full texts can be downloaded from the iBOL website at <http://www.ibolproject.org> or requested from your contact person at the CCDB or iBOL Theme Coordinator.

A synopsis of the conditions relevant to this transaction is contained in the Biological Material Analysis Agreement (BMAA) attached with this sampling kit. Please acknowledge that you have read and agreed to these conditions by signing BMAA in electronic or hard copy form and returning it together with the first batch of samples.

DISCLAIMER: It is the sender's responsibility to ensure that biological materials are shipped to the Biodiversity Institute of Ontario in compliance with any applicable shipping regulations, that they have been obtained under appropriate collection and animal care permits in their country of origin and that 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 registry certificate for the provider institution (if applicable);
- c) Canadian Food Inspection Agency import permit (if applicable).

The Biodiversity Institute of Ontario 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. Upon request, BIO staff will advise on Canadian import requirements and assist in obtaining relevant import permits. The Biodiversity Institute of Ontario is a CITES-registered institution (COSE registry certificate CA022).

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

Sample Submission
University of Guelph
Biodiversity Institute of Ontario
50 Stone Road East
Guelph, Ontario, Canada N1G 2W1
Phone: +1 (519) 824-4120 ext. 58259

SUBMITTING COLLATERAL DATA

CCDB Record files should be e-mailed to the Canadian Centre for DNA Barcoding Collections Unit <ccdbcol@uoguelph.ca>, with a copy to your CCDB contact person.

The **Specimen Data** file can be submitted directly to BOLD using the online batch specimen data submission procedure. Please refer to the following link for instructions:

http://www.boldsystems.org/index.php/resources/handbook?chapter=3_submissions.html§ion=data_submissions

Questions concerning specimen data submission should be directed to the BOLD Data Management Team <submissions@boldsystems.org>.

Digital images of specimens and the corresponding **Image Data** file can be submitted directly to BOLD using the online image submission procedure. Please refer to the following link for instructions:

http://www.boldsystems.org/index.php/resources/handbook?chapter=3_submissions.html§ion=image_submissions

Questions concerning image submission should be directed to the BOLD Support Team <support@boldsystems.org>.

For detailed information on the BOLD data structure and submission procedures, please refer to the BOLD online manual: http://www.boldsystems.org/index.php/resources/handbook?chapter=1_gettingstarted.html