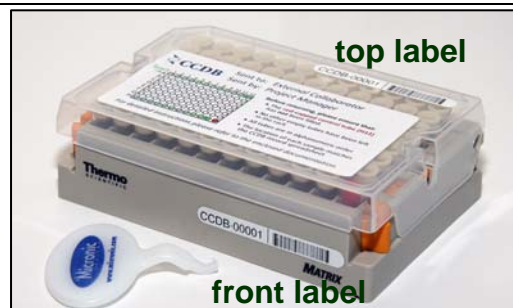


This Submission Package is aimed to facilitate the exchange of tissue samples and specimen data between **Research Collaborators** and the **Canadian Centre for DNA Barcoding (CCDB)**, one of the central analytical nodes for the **International Barcode of Life Project (iBOL)**. It contains **tube racks** for housing tissue samples that should be returned to the **Biodiversity Institute of Ontario** (the hosting institution of the CCDB) for analysis and spreadsheets for entering specimen data for submission to the **Barcode of Life Data Systems (BOLD)**.

## SAMPLE TUBE RACK (BOX)

Each 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 to you 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).



There is a large label affixed to the box lid which contains the following additional information:

**Sent to:** *Collaborator* responsible for filling sample tubes and providing specimen data (tissue provider).

**Sent by:** CCDB contact responsible for the shipment (recipient). This person will usually oversee analyses and facilitate the submission of specimen data to BOLD; may coincide with *BOLD Project Manager*.

**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. 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.

## DATA SUBMISSION SPREADSHEETS

The CD included in this Sample Submission Package contains three blank spreadsheets corresponding to the three blocks of data needed for a complete specimen record (**plate record**, **specimen data** and **image data**).

1. The file **CCDB-00000\_Record.xls** is intended to record locations of samples in the corresponding tube rack (or array of racks) for the lab staff running 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 tissue sample with its source specimen* (ideally, a permanent collection catalogue number prefixed by the museum acronym or, if unavailable, a field collection number prefixed by the collector's initials).

**Note: Note: A single CCDB Record can contain data for up to 10 tube racks.** See last page for details.

2. The file **SpecimenData.xls** is intended for entering geographic, taxonomic and other collection data for the specimens to be analyzed. The '*Sample ID*' field should contain numbers identical to those entered in the CCDB Record. Please provide as many details for each entry as possible. Please refer to the following link for further information on filling in this sheet:

<http://www.boldsystems.org/docs/handbook.php?page=datasubprotocol>

3. The file **ImageData.xls** in the folder **/ImageSubmission/** should house data on the digital images of the voucher specimens that provided the tissue samples. Please refer to the following link for details on the image submission procedure:

<http://www.boldsystems.org/docs/handbook.php?page=imagesubprotocol>

**NOTE: Submission of specimen data and images is independent from sample submission.** Submission of the specimen data and images to BOLD is a **critical prerequisite** before tissue samples can be analyzed in the lab. To facilitate effective processing of samples, their accompanying data must be submitted in a BOLD compliant format.

## 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.

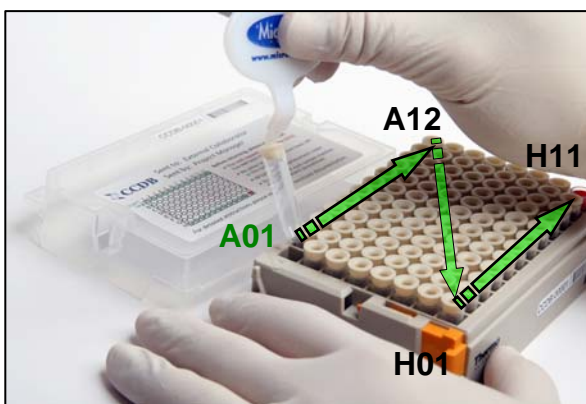


To begin the sampling process, position the box with the **front label** facing towards you, unlock and remove the box lid.



**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 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

Open the MS Excel file titled **CCDB-00000\_Record.xls**. By default, it will start with the page outlining the iBOL Biological Material Transfer and Data Policy. To proceed with data entry, you must agree to the provisions of this document by checking the appropriate tickbox.\* This will take you to the “DATA INPUT” worksheet of the table. If macros are disabled or if you are using a program other than MS Excel for Windows, go to the tab “DATA INPUT” at the bottom of the table and follow instructions typed in green:

1. Select the type of sample medium from the first dropdown menu. It should be “**tube rack**”.

2. Select the appropriate iBOL workgroup from the second dropdown menu.

3. If intending to fill a multiple plate array, mark the checkbox “**Multiple array...**” located below the dropdown menus.

4. Enter the proper CCDB number(s) from tube rack labels in the designated field(s) (type in digits only, do not add prefixes). This will unhide the fields for entering Sample ID numbers.

5. After filling each sampling well, enter the Sample ID number into the corresponding cell of the CCDB Record DATA INPUT sheet. Ultimately, each CCDB Record should contain 95 entries per tube rack, corresponding to 95 samples. If preferred, the entire spreadsheet could be populated at once (e.g., by pasting a column of data), provided that all measures are taken to ensure complete correspondence between samples and CCDB Record.

Sample ID's should be entered in columnar format in the white cells of the DATA INPUT spreadsheet. Please ensure that the **Locator** next to each record matches the position of the corresponding sampling well. **Do not enter data for the control tube (H12).**

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 change your entries accordingly.

To visualize correspondence between the data recorded and the position of samples in the tube rack, refer to the next worksheet titled ‘**Submission Results**’. 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.

When data entry is completed, rename the file to incorporate the CCDB numbers included in it, for example, rename it to **CCDB-00001\_Record.xls** for a single plate or to **CCDB-00001-00007\_Record.xls** for a multiple plate array.

**NOTE: All coloured 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.

\* If your samples are not destined for an iBOL-related project, please ask your CCDB contact for further instructions.

## SUBMITTING TISSUE SAMPLES AND DATA

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***Fill all 95 sampling tubes in each rack before proceeding to the next one. Do not ship back partly filled racks, unless specifically arranged with your BOLD Project Manager. Whenever a tube rack is transferred to another person for tissue sampling, please notify your BOLD Project Manager.***

**NOTICE:** Unless explicitly negotiated otherwise, all biological materials shipped to the Biodiversity Institute of Ontario fall under the standard provisions of the iBOL Biological Material Transfer Agreement (v. 2009-07), and all data submitted to BOLD and generated by the Canadian Centre for DNA Barcoding (CCDB) will comply with the iBOL Data & Resource Sharing Policies (v. 2009-07). 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 printed synopsis of these documents should have been provided as part of the sampling kit. Please acknowledge that you have read and agreed to these conditions by filling out and signing the Implementing Letter either in electronic or hard copy form. Please sign and return it with the first batch of samples. If your research project is not part of iBOL, ensure that all relevant administrative questions are resolved with the CCDB before proceeding with sampling.

**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 in 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 (registry certificate CA022).

*After you have completed the sampling procedure, please return the tube rack 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. 56393

## SUBMITTING DATA

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**CCDB Record** files should be e-mailed to the Canadian Centre for DNA Barcoding Collections Unit <[ccdcol@uoguelph.ca](mailto:ccdcol@uoguelph.ca)>, with a copy to your CCDB Contact.

The **Specimen Data** file should be e-mailed to the BOLD Data Management Team <[submissions@boldsystems.org](mailto:submissions@boldsystems.org)> with a copy to your CCDB Contact and the BOLD Project Manager. Be sure to indicate the name and code of the BOLD project to which your data are being submitted.

**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/docs/handbook.php?page=imagesubprotocol>. Questions concerning image submission should be directed to the BOLD Support Team <[support@boldsystems.org](mailto: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/docs/handbook.php>.