

This sampling kit is aimed to facilitate the submission of **animal tissue samples** and collateral information for processing at the **Canadian Centre for DNA Barcoding (CCDB)**. It consists of two elements:

**Physical items enclosed with the shipment:**

- Microplate(s): 8×12 well plastic, colourless container(s) for housing tissue samples;
- Cap strips to cover wells containing tissue samples;
- Biological Material Analysis Agreement (BMAA) – hard copy;
- Sampling Kit Instructions – hard copy.

**Digital items attached to the confirmation email:**

- CCDB Plate Record file (an MS Excel spreadsheet named CCDB-00000.xls);
- Digital copies of the BMAA and Sampling Kit Instructions.

The following sections (A-J) are designed to help you prepare the material being sent to the CCDB. Please read all instructions before proceeding with tissue sampling.

To avoid delays in processing, please ensure that all required information and digital specimen data pertaining to the material is submitted prior to the arrival of samples at the CCDB.

**IMPORTANT: For submission of DNA extracts or PCR products, a different sampling kit is required.**

## **A – CONTAINER DESCRIPTION - MICROPLATE**

Each **microplate** contains 96 sampling wells that are arranged in an 8×12 format. The sampling array starts with well **A01**. Well **H12** should be **left empty** as a negative control, so each plate accommodates 95 tissue samples. The sampling procedure is described in detail in section G.

Each plate is individually numbered with a unique barcode (**CCDB Number**), which should be entered into the corresponding CCDB Plate Record (see section H).

## **B – 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/>

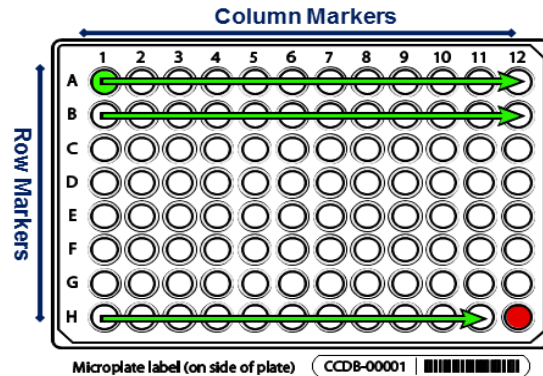
**Note:** The 'Sample ID' field within this specimen data spreadsheet should be identical (including letter case) to the Sample IDs entered in the CCDB Plate Record and without any duplications.

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/>

## C – MICROPLATE ORIENTATION AND ADDITION OF ETHANOL

Position the microplate on a flat surface with the CCDB plate label facing towards you and with well **A01** positioned at the **TOP LEFT** corner.

**IMPORTANT: Add ethanol**



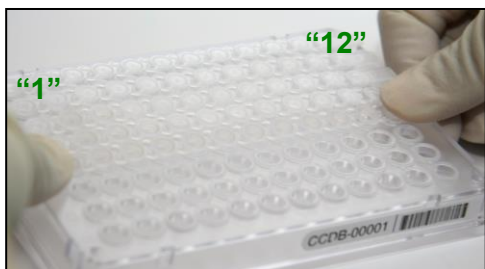
Sampling wells should be pre-filled with **30 µl** of 95-100% **ethanol (not provided with the kit)** using a multi-channel pipettor. If a pipettor is not available, add **one drop** of ethanol to each well using an eyedropper just prior to sampling. Always wear gloves when handling the microplate.

**Note:** Do not add excess ethanol. If the samples are compact and were previously fixed in ethanol (e.g. moist vertebrate muscle tissue), then the addition of ethanol is optional.

**Note:** Tissue that has not been dried or preserved should not be sampled into a microplate.

**IMPORTANT: Never use ethanol if tissue was fixed or stored in dimethyl sulfoxide (DMSO)!**

## D – STRIP CAP PLACEMENT



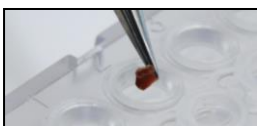
Before proceeding with tissue sampling, place the cap strips (supplied with the submission package) over all rows of wells to avoid contamination. Observe the orientation of cap strips: markers "1" and "12" should match the corresponding columns of the plate.

**Note:** If sampling immediately, do not fasten caps tightly. If not sampling immediately, seal properly.

When sampling, remove cap strips one at a time and fasten them back when paused, or after finishing each row.

## E – RECOMMENDED TISSUE SIZE

Below are some examples of recommended tissue sizes for sampling into microplates:



- **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:** one whole specimen — ca. <3 mm length



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 for the well.

**Avoid** sampling from body parts containing scales, hairs or bristles.

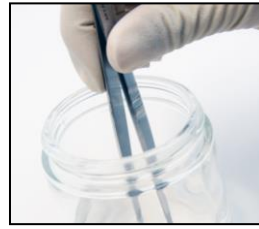
**Avoid** sampling from digestive tracts or from areas which may have been in contact with digestive tract contents or other contaminants.

## F – FORCEP STERILIZATION BETWEEN SAMPLES

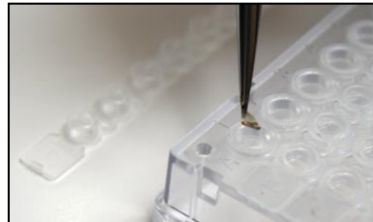
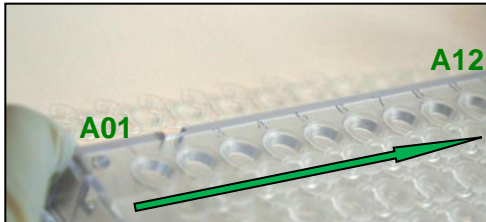
Before proceeding to the next sample, ensure that no residual tissue remains on the forceps. Rinse in 95% ethanol then wipe with a clean kimwipe or tissue.

When the work environment permits, use flame (e.g. for dry insects) or bleach/specialized detergent such as Eliminate (e.g. for 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 in distilled water before the next sampling round, to avoid DNA degradation.



## G – THE TISSUE SAMPLING PROCESS



Start the tissue sampling process with **A01** (row 1) and proceed to **A12** (left to right). Sample or subsample the recommended amount of tissue from only one specimen with fine forceps (as shown above) and place it into the current sampling well. Confirm that the tissue remains inside the well.

Once complete, enter the corresponding **Sample ID** into the **CCDB Plate Record** (section H). Next, proceed with sampling into **A02**, progressively moving towards **A12**.

When row A is complete, replace the cap strip and seal firmly. Proceed to row B filling from left to right (B01-B12). Repeat this process until all 8 (A-H) rows are filled.

**Note:** Do not leave empty wells in the middle of the plate. NEVER remove and replace samples.

**Note:** Do not place any foreign objects (e.g. labels) into sampling wells.

**IMPORTANT: Do not fill the last well, H12. It should be left empty as a negative control.**

Once the plate is filled with samples, ensure that all cap strips are pressed firmly into the wells. Ensure the correct amount of fixative and/or tissue has been sampled from only one specimen into each well by examining the microplate from underneath.



**Note:** Tissue samples sent in the container(s) may be destroyed unless voucher recovery is requested. Please notify the CCDB if voucher recovery is necessary.

# H – THE CCDB PLATE RECORD

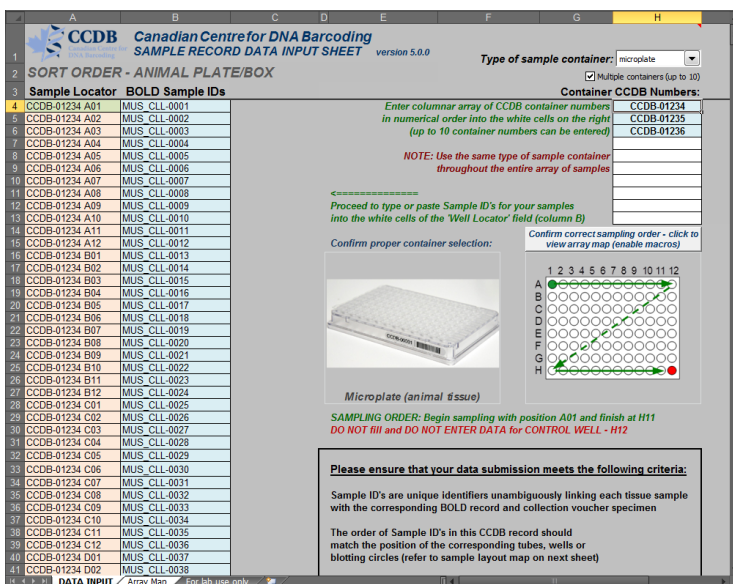
Download and open the MS Excel file titled **CCDB-00000\_Record.xls**. By default, it will start with the tab (worksheet) called “DATA INPUT”. Please follow the worksheet filling instructions typed in **green italics** and ensure that no **error messages** remain in the header of the worksheet indicating missing information.

1. Select “**microplate**” from the dropdown field under ‘Type of sample container’. Make sure that the image appearing below matches the container you plan to fill.

2. If submitting multiple containers within the same plate 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)**”. This will unhide the fields for entering Sample ID numbers.

4. Confirm that the correct sampling order is followed by referring to the container map image and the well coordinates indicated in the “**Sample Locator**”.



5. As you place the samples into each well, enter their Sample ID numbers into the corresponding white cells of the column “**BOLD Sample IDs**”. *Ideally, the Sample IDs 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. The corresponding Sample Locator value (i.e. CCDB-00000-A01) is also commonly used as a default.* Each CCDB Plate Record should contain 95 entries per plate, corresponding to 95 samples in the particular container. If preferred, the entire spreadsheet could be populated at once by pasting a column of data, however, do not attempt to paste more than one column of data at a time.

**Note:** All coloured (non-white) cells in the CCDB Plate Record spreadsheet are write-protected to secure formulas and cross-links. Please type/paste your data only into white cells. Avoid moving (i.e. cutting and pasting) data between cells in the spreadsheet. When pasting data from another spreadsheet, make sure to paste ‘values’ or ‘unicode text’ using the ‘paste special’ function of MS Excel.

**IMPORTANT:** Please ensure that all measures are taken to guarantee correspondence between the actual position of samples and the CCDB Plate Record and do not enter data for the control well(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 BOLD Sample ID and correct these entries accordingly.

7. To visualize the correspondence between the position of samples in the microplate and the data entered in the CCDB Plate Record, 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 saving and submitting the CCDB Plate Record.

8. When data entry is complete, rename the file to incorporate the unique CCDB number assigned to it (e.g. **CCDB-01234\_Record.xls** for a single container or **CCDB-01234-01236\_Record.xls** for multiple containers). This numbering format simplifies the archival and retrieval of these files after they are submitted to the CCDB.

**Note:** If the CCDB Plate Record file is filled prior to sampling, the ‘Array Map’ sheet can be printed and used as a reference when sampling into the container(s).

## I – DIGITAL SPECIMEN DATA SUBMISSION

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1. The **CCDB Plate Record** file(s) and signed **BMAA** should be emailed to the CCDB via [lims@ccdb.ca](mailto: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](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](mailto:support@boldsystems.org).

## J – SUBMISSION OF BIOLOGICAL MATERIALS

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***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**

## K – CCDB DISTRIBUTION OF DATA

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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](mailto: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](mailto:support@ccdb.ca).

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