

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:

- **Colourless** empty **microplates**, 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).

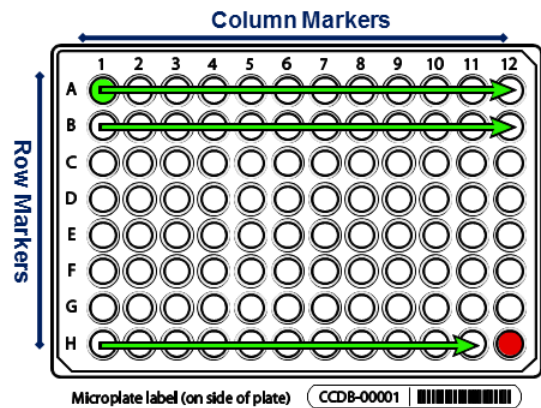
Filled plates, together with the signed BMAA and filled CCDB Record files, should be returned for analysis to the **Biodiversity Institute of Ontario** — the hosting institution of CCDB. Specimen data should be submitted separately to the **Barcode of Life Data Systems (BOLD)**; a synopsis is provided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

MICROPLATE

Each microplate contains sampling wells that are arranged in a 12x8 format. The sampling array starts with well **A01**. Well **H12** should be **left empty** for control, so each plate will accommodate 95 tissue samples. See below for details of the sampling procedure.

Each plate will be individually numbered, and will be shipped with the label pre-affixed to the plate. Each label contains a unique **barcode** and human-readable **identifier (CCDB Number)**. This CCDB number should be entered into the corresponding *CCDB Record* (see page 4).

Note: Before adding samples into a plate, make sure the **label is attached to the side corresponding to row H**. Always work with the plate label facing towards you. Pay special attention to the position of **row** (A through H) and **column** (1 through 12) markers: they should be on the **left** and **top** margins of the plate, respectively.



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.

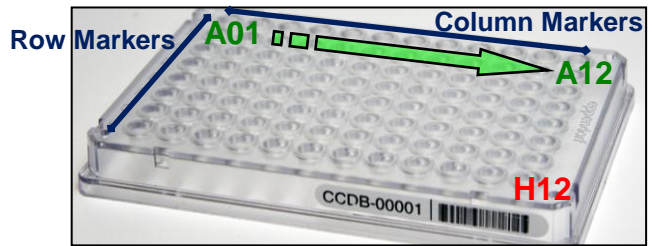
Specimen Images are an additional requirement for some projects (refer to the Biological Material Analysis Agreement – BMAA, for details) and should be uploaded to BOLD to complement the specimen data records. Please refer to the following link for details on the BOLD image submission procedure: http://www.boldsystems.org/index.php/resources/handbook?chapter=3_submissions.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.

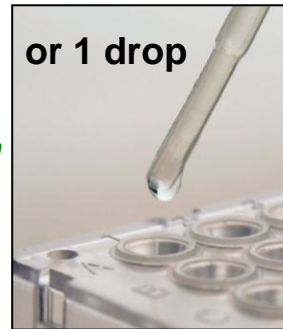
SAMPLING PROCESS: PLATE PREPARATION

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.



IMPORTANT!
Add Ethanol
to avoid static
cross-contamination

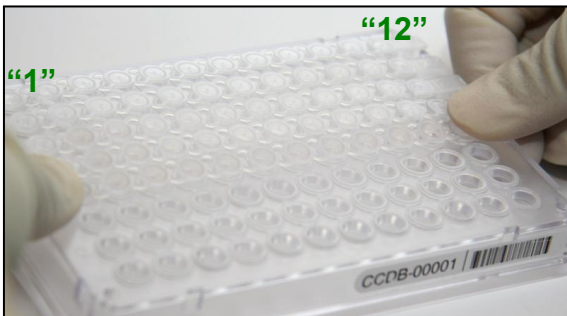


If samples are prone to spontaneous **displacement because of static electricity** (e.g., **arthropod legs**), sampling wells should be pre-filled with **30 µl** (microlitres) of 95-100% **Ethanol**, e.g., using a multi channel pipettor. If a pipettor is not available, add **one drop** of **Ethanol** to each well using an eyedropper, just prior to sampling.

Note: Do not add excess ethanol - 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.

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

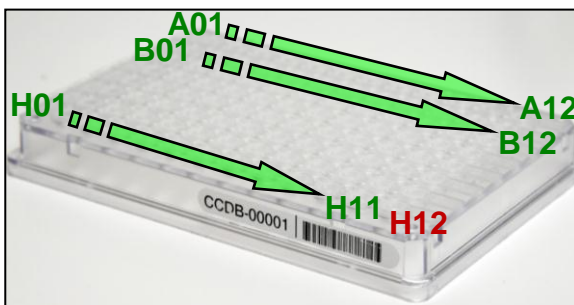
IMPORTANT! Never use Ethanol if tissue was fixed or stored in Dimethyl sulfoxide (DMSO)!



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.

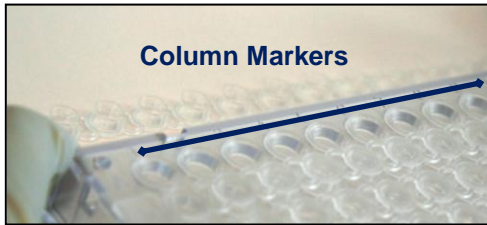


Start the sampling process with **A01** (row 1) and proceed in alphanumeric order to **A12** (left to right). When done with the first row, proceed to the second row (**B01**) and repeat the process until all 12 rows are filled. **Do not leave empty wells in the middle of the plate.** While sampling, remove only one cap strip at a time to prevent cross-contamination.

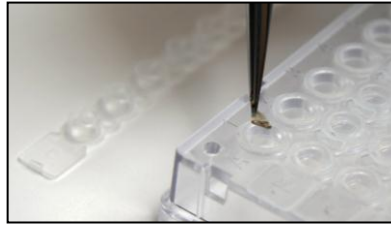
IMPORTANT: Do not fill the last well, H12! It should be left empty as a negative control.

As you proceed with sampling, keep a full record of Sample ID's in the Data Input worksheet of the corresponding CCDB RECORD workbook. For details, refer to instructions on page 4 of this manual and in the CCDB Record Data Input Sheet.

SAMPLING PROCESS: THE PROCEDURE



Before beginning the sampling procedure, remove the cap strip from the first row. If required, make sure that wells are pre-filled with fixative (see previous page).

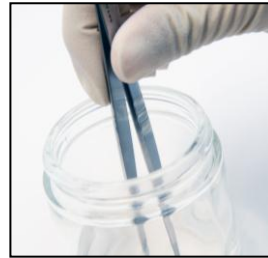


Sample or subsample the right amount of tissue with fine forceps (as shown in the image) and place it into the sampling well. Confirm that the tissue remains inside the well. Once done, enter the corresponding sample ID into the CCDB Record (see below).

Note: DO NOT place any foreign objects (e.g. labels) into sampling wells.

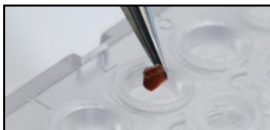
Before proceeding to the next sample, ensure that no residual tissue is present on the forceps by rinsing them in 95% Ethanol and wiping them with a clean napkin or paper towel.

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

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 mm³ 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.

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

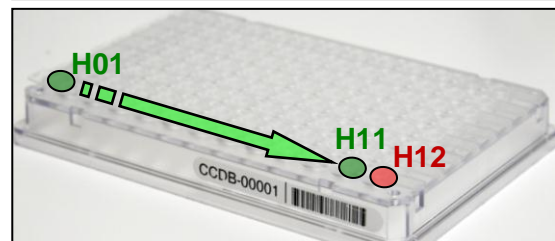
Avoid sampling from digestive tracts or from areas which may have been in contact with digestive tract contents or other contaminants.

To visualise well contents (e.g., to evaluate the correct amount of fixative or tissue sampled) examine the plate from below.

After samples have been added to all wells in a row, replace the cap strip and seal it firmly before proceeding with next row.

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.

Once the plate is filled with samples, ensure that all cap strips are pressed firmly into the wells.



Note: All samples sent in microplates will be completely used up for molecular analysis; no residual tissue will remain.

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:

Sample Locator	BOLD Sample IDs	Container CCDB Number(s)
4	CCDB-01234 A01 MUS_CLL-0001	CCDB-01234
5	CCDB-01234 A02 MUS_CLL-0002	CCDB-01235
6	CCDB-01234 A03 MUS_CLL-0003	CCDB-01236
7	CCDB-01234 A04 MUS_CLL-0004	
8	CCDB-01234 A05 MUS_CLL-0005	
9	CCDB-01234 A06 MUS_CLL-0006	
10	CCDB-01234 A07 MUS_CLL-0007	
11	CCDB-01234 A08 MUS_CLL-0008	
12	CCDB-01234 A09 MUS_CLL-0009	
13	CCDB-01234 A10 MUS_CLL-0010	
14	CCDB-01234 A11 MUS_CLL-0011	
15	CCDB-01234 A12 MUS_CLL-0012	
16	CCDB-01234 B01 MUS_CLL-0013	
17	CCDB-01234 B02 MUS_CLL-0014	
18	CCDB-01234 B03 MUS_CLL-0015	
19	CCDB-01234 B04 MUS_CLL-0016	
20	CCDB-01234 B05 MUS_CLL-0017	
21	CCDB-01234 B06 MUS_CLL-0018	
22	CCDB-01234 B07 MUS_CLL-0019	
23	CCDB-01234 B08 MUS_CLL-0020	
24	CCDB-01234 B09 MUS_CLL-0021	
25	CCDB-01234 B10 MUS_CLL-0022	
26	CCDB-01234 B11 MUS_CLL-0023	
27	CCDB-01234 B12 MUS_CLL-0024	
28	CCDB-01234 C01 MUS_CLL-0025	
29	CCDB-01234 C02 MUS_CLL-0026	
30	CCDB-01234 C03 MUS_CLL-0027	
31	CCDB-01234 C04 MUS_CLL-0028	
32	CCDB-01234 C05 MUS_CLL-0029	
33	CCDB-01234 C06 MUS_CLL-0030	
34	CCDB-01234 C07 MUS_CLL-0031	
35	CCDB-01234 C08 MUS_CLL-0032	
36	CCDB-01234 C09 MUS_CLL-0033	
37	CCDB-01234 C10 MUS_CLL-0034	
38	CCDB-01234 C11 MUS_CLL-0035	
39	CCDB-01234 C12 MUS_CLL-0036	
40	CCDB-01234 D01 MUS_CLL-0037	
41	CCDB-01234 D02 MUS_CLL-0038	

1. 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.

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 well 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 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).

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 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.

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