

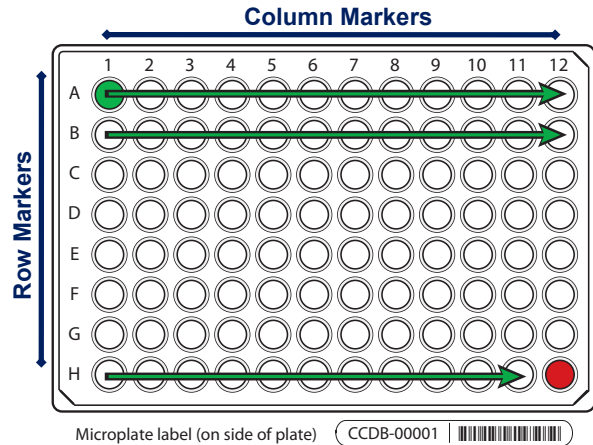
This Submission Package is aimed to facilitate the exchange of tissue samples and specimen data between **Research Collaborators** and the **Canadian Centre for DNA Barcoding (CCDB)**, one of the central analytical nodes for the **International Barcode of Life Project (iBOL)**. It contains **microplates** 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)**.

MICROPLATE

Each microplate contains sampling wells that are arranged in a 12×8 format. The sampling array starts with well **A01**. Well **H12** should be **left empty** for control, so each plate will accommodate 95 samples. See below for details of the sampling procedure.

Each plate will be individually numbered, and will be shipped to you with the label pre-affixed to the plate. Each label contains a unique **barcode** and human-readable **identifier (CCDB Number)**. The CCDB number should be provided in the corresponding *CCDB Record* spreadsheet (see last page).

A separate large label will be affixed to the top of the zip-lock bag containing the plates, with the following information:



Sent to: *Collaborator* responsible for filling sample wells 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 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.

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 microplate (or array of plates) 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 plates.** 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 Plate 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:

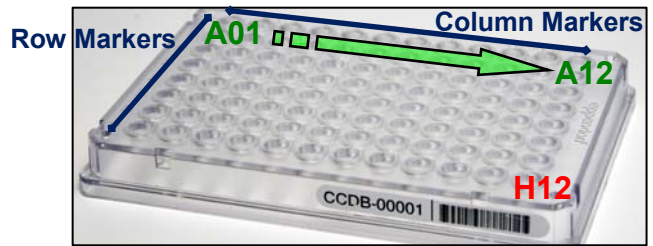
<http://www.boldsystems.org/docs/handbook.php?page=imagesubprotocol>

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: PLATE PREPARATION

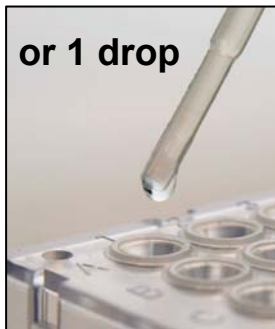
To begin the sampling process, position the plate on a flat surface with the plate label facing towards you.

The **column markers** (1–12) should be at the top and the **row markers** (A–H) should be on the left side.



30 µl

IMPORTANT!
Add Ethanol
to avoid static
cross-contamination



or 1 drop

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.

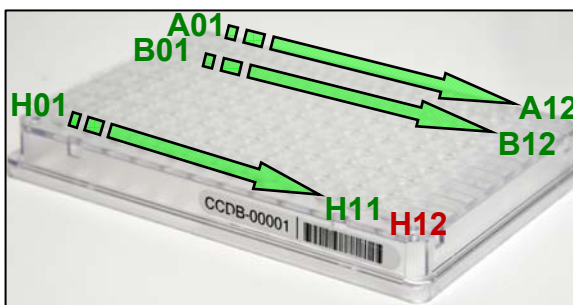
Tissue that has not been dried or preserved should not be sampled into a microplate.

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.

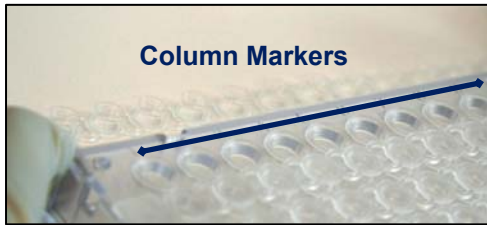


Start the sampling process with **A01** (row 1) and proceed in alphanumeric order to **A12** (left to right). When done with the first row, proceed to the second row (**B01**) and repeat the process until all 12 rows are filled. **Do not leave empty wells in the middle of the plate.** While sampling,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

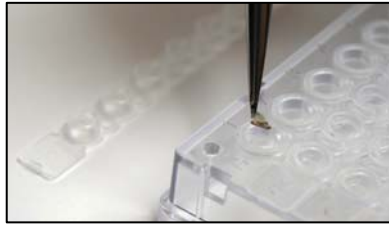
IMPORTANT: Do not fill the last well, H12! It should be left empty as a negative control.

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 5 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 — ca. 5–6 mm length
- **Large insect:** tibia or femur only — ca. 2–4 mm length
- **Vertebrate/invertebrate:** muscle — ca. 8 mm³ volume or 2 mm diameter
- **2-dimensional tissue:** skin/body wall — ca. 3–4 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.

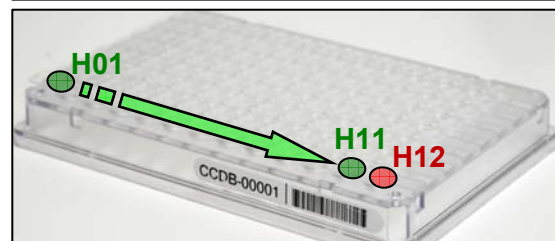
Avoid sampling from body parts containing scales, hairs or bristles, when possible.
Avoid sampling from digestive tracts or from areas which may have been in contact with digestive tract contents.

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

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:

Sample Locator	Sample ID	CCDB Number
CCDB-00001 A01	SAMPLE-0001	CCDB-00001
CCDB-00001 A02	SAMPLE-0002	CCDB-00002
CCDB-00001 A03	SAMPLE-0003	CCDB-00003
CCDB-00001 A04	SAMPLE-0004	CCDB-00004
CCDB-00001 A05		CCDB-00005
CCDB-00001 A06		CCDB-00006
CCDB-00001 A07		CCDB-00007
CCDB-00001 A08		
CCDB-00001 A09		
CCDB-00001 A10		
CCDB-00001 A11		
CCDB-00001 A12		
CCDB-00001 B01		
CCDB-00001 B02		
CCDB-00001 B03		
CCDB-00001 B04		
CCDB-00001 B05		
CCDB-00001 B06		
CCDB-00001 B07		
CCDB-00001 B08		
CCDB-00001 B09		
CCDB-00001 B10		
CCDB-00001 B11		
CCDB-00001 B12		
CCDB-00001 C01		
CCDB-00001 C02		
CCDB-00001 C03		
CCDB-00001 C04		
CCDB-00001 C05		
CCDB-00001 C06		
CCDB-00001 C07		

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

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 the microplates 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 plate, 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 well (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 microplate, 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

Fill all 95 sampling wells in each plate before proceeding to the next plate. Do not ship back partly filled plates, unless specifically arranged with your BOLD Project Manager. Whenever a plate 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 your plates 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

CCDB Record files should be e-mailed to the Canadian Centre for DNA Barcoding Collections Unit <lims@ccdb.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> 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>.

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>.