

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

- **Red-coloured microplates** filled with dry storage preservation medium, for housing PCR products;
- 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).

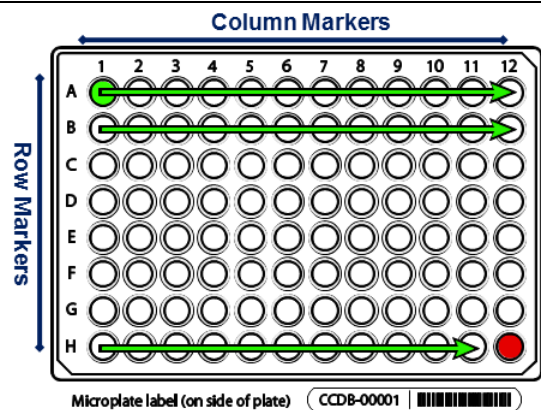
Filled plates, together with the signed BMAA and filled CCDB Record files, should be returned for analysis to the **Biodiversity Institute of Ontario** — the hosting institution of CCDB. Specimen data should be submitted separately to the **Barcode of Life Data Systems (BOLD)**; a synopsis is provided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

MICROPLATE

Each microplate contains wells pre-filled with dry storage media for aliquoting PCR products 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 PCR aliquots. See below for details on the aliquoting 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 PCR products 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.



IMPORTANT: Only designated red-coloured PCR plates can be used for sending PCR products to the CCDB. Do not store or ship PCR plates together with tissue or whole genomic DNA extracts.

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

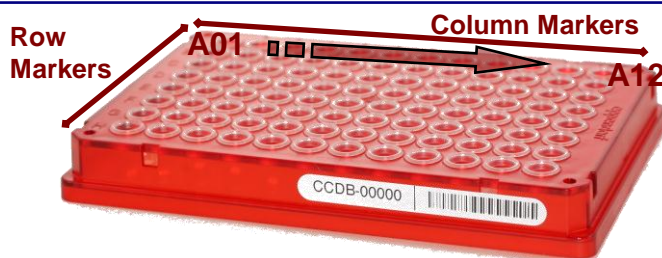
NOTE: Although data uploads to BOLD are independent from the submission of samples and CCDB record files to the lab, they are a critical prerequisite for running molecular analyses at the CCDB.

SAMPLING PROCESS: PLATE PREPARATION

IMPORTANT: The plates will be shipped to you sealed with clear film or aluminum foil. Before beginning the sampling process, remove the seal completely and ensure that no film or foil residue remains around plate wells.

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

NOTE: Column markers (1–12) should be at the top and the row markers (A–H) should be on the left side of the plate.



SAMPLING PROCESS: PCR PRODUCT PREPARATION

Basic recipe for Polymerase Chain Reaction (PCR)
PCR reagents per 12.5 µl reaction:

# of reactions	1	100
10% trehalose	6.25 µl	625 µl
ddH ₂ O	2 µl	200 µl
10X buffer	1.25 µl	125 µl
50 mM MgCl ₂	0.625 µl	62.5 µl
10 µM primer A	0.125 µl	12.5 µl
10 µM primer B	0.125 µl	12.5 µl
10 mM dNTPs	0.0625 µl	6.25 µl
Polymerase (5 U/µl)	0.06 µl	6 µl
Total	10.5 µl	1050 µl
DNA template	2 µl per well	

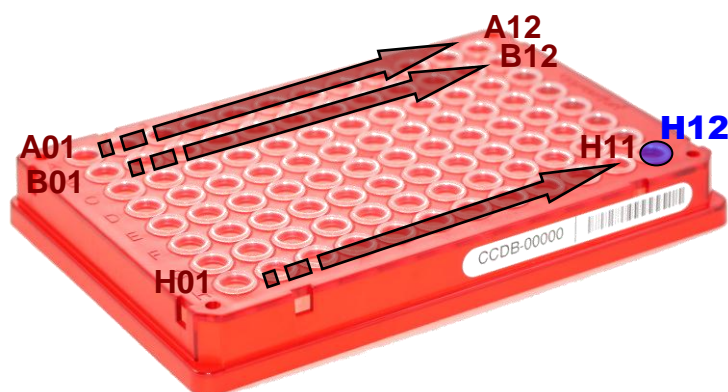
Prepare PCR products according to CCDB PCR protocol: http://www.dnabarcoding.ca/CCDB_DOCS/CCDB_Amplification.pdf.

Do not cleanup PCR products. The optimal concentration of PCR product is 100-150 ng/µl. If it is within recommended range, then add 8-10 µl of PCR product to each well.

IMPORTANT: Normalize PCR product concentration if necessary. If concentration exceeds recommended range, add less PCR product. For example, if PCR product concentration is 300-400 ng/µl, apply 5 µl of PCR product. Do not dilute PCR product prior to transfer.

Upon arrival of the PCR plates in the CCDB, PCR products will be resuspended in molecular grade water and incubated for 30 min at room temperature prior to gel check and sequencing.

SAMPLING PROCESS: THE PROCEDURE



Start aliquoting PCR products with well **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.**

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

As you proceed with sampling PCR products into wells, 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



IMPORTANT: Replace tips between each PCR product sample transfer. Filter tips are strongly preferred.

When transferring PCR products from individual tubes, it is recommended to organize tubes in a 12x8 rack in the same order as they will be transferred into the plate. Each tube should be repositioned, e.g., to another rack, as you retrieve sample from it. This will help minimize human error during the transfer process.



To visualize well contents (e.g., to ensure that PCR products have been added to all wells) examine the plate from below. When sampling into the last row (Row H), remember to leave the last well (**H12**) empty. If PCR transfer takes more than 1 day, the plate can be sealed with aluminum foil (see below) and kept at 4°C for several days. Make sure to centrifuge the plate at 1000xg for 1 min prior to opening and subsequent transfers. If your facility does not have a plate centrifuge, dry the samples completely (as described below) before sealing the plate.



Before sealing, leave the plate to dry either overnight at room temperature or incubate for several hours at 37-56°C.

NOTE: Dry PCR product will concentrate at the bottom of the plate forming a film or sticky residue.

IMPORTANT: Do not seal the plate until ALL wells are completely dry. Make sure that no liquid or condensate is present in the wells.



When the drying is complete, cover and seal the plate with aluminum foil (provided). Carefully seal the edges with a back side of aluminum foil.

When stacking multiple plates, use spacers to avoid puncturing foil with the wells of the plate above.



Please provide information on PCR product concentration range and the volume of diluted PCR product added to each plate. This can be written in marker on the foil seal or supplied separately (e.g., by e-mail).

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 shows the 'DATA INPUT' worksheet of the CCDB record template. The spreadsheet is organized into columns: 'Sample Locator' (A1-A12), 'BOLD Sample IDs' (B1-B12), and 'Container CCDB Numbers' (C1-C12). The 'Type of sample container' is set to 'PCR plate'. A checkbox for 'Multiple containers (up to 10)' is checked. A well locator grid is visible, showing a 96-well plate layout with columns 1-12 and rows A-H. A red PCR plate image is shown below the grid. Instructions in green italics guide the user on how to enter data, including a note to 'Proceed to type or paste Sample ID's for your samples into the white cells of the 'Well Locator' field (column B)'. A warning message at the bottom states: 'Please ensure that your data submission meets the following criteria: Sample ID's are unique identifiers unambiguously linking each tissue sample with the corresponding BOLD record and collection voucher specimen. The order of Sample ID's in this CCDB record should match the position of the corresponding tubes, wells or blotting circles (refer to sample layout map on next sheet)'.

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

NOTE: Selecting the container “DNA plate” allows you to choose the sampling order: either A01-H12, control H12 (preferred standard) or H01-A11, control A12 (transposed, used in some molecular labs). To change the sort order, select the default control well in the top right corner of the DATA INPUT worksheet.

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 <lims@ccdb.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