

This Submission Package is aimed to facilitate the exchange of **PCR products** 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 PCR products aliquots 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)**, <http://www.barcodinglife.org>.

MICROPLATE

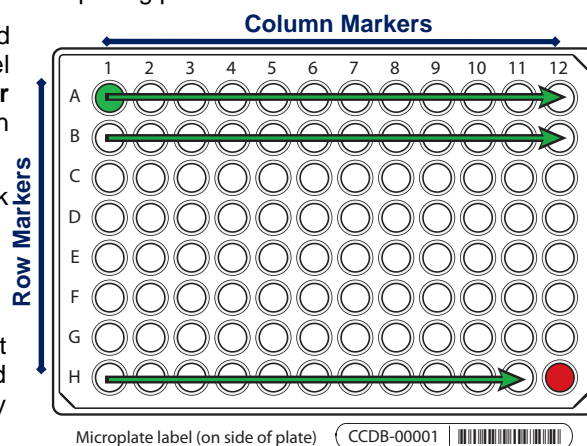
Each microplate contains wells pre-filled with dry storage media for aliquoting PCR products that are arranged in a 12x8 format. The sampling array starts with well **A01**. Well **H12** should be **left empty** for control, so each plate will accommodate 95 PCR aliquots. See below for details of the aliquoting 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)**. This CCDB number should be entered in the corresponding *CCDB Record* spreadsheet (see page 4).

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 (sample 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 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 filled PCR plates together with tissue or whole genomic DNA extracts.

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 DNA aliquot 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: 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 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>

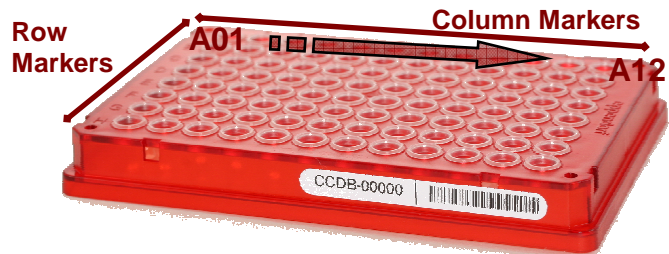
NOTE: Submission of specimen data and images is independent from sample submission. Submission of the specimen data and images to BOLD is a **critical prerequisite** before PCR products can be analyzed in the lab. To facilitate effective processing of samples, their accompanying data must be submitted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

SAMPLING PROCESS: PLATE PREPARATION

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	

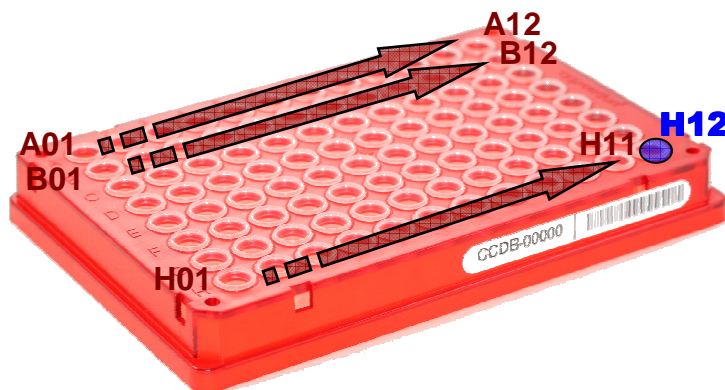
Prepare PCR products according to CCDB PCR protocol: http://www.dnabarcoding.ca/CCDB_DOCS/CCDB_Amplification.pdf.

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 alphanumerical 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

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, select 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 “PCR plate”.

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 PCR products. 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 PCR products 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 PCR PRODUCTS 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 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). The above requirements do not apply to laboratory-generated artifacts, such as PCR products; however, they may apply to residual genomic DNA, if present within mix.

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

IMPORTANT: Make sure to indicate on the package that these are PCR products; this will ensure that they are treated with respective precautions upon arrival at the CCDB!

SUBMITTING 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.

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>.