

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 **tube racks** for housing **plant or fungal** 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)**. These tube racks should not be used for sampling/shipping animal tissues.

SAMPLE TUBE RACK (BOX)

Each box contains strips of sample tubes that are arranged in a 8x12 rack corresponding to 96-well plates used in the CCDB sequencing lab. Tube H01 is the beginning of the sampling array. **Tube A12** corresponds to the negative control well in the sequencing plate and **should be left empty**, so each box will accommodate **95 samples**. See below for details of the sampling procedure.

Each tube rack is individually numbered and will be shipped to you with small labels pre-affixed to the side of the box and to the top of the box lid. 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).

ATTENTION: The sampling order in the plant tube rack is different from that of the sampling media used to accommodate animal tissue.



Before adding samples into tubes, make sure the **side label is attached to the side corresponding to row 12**. Always work with the side label facing towards you. Make sure to use the only **box record specifically designated for plant tube racks (CCDB-00000_PLANT_Record.xls)**.

Note: Plant tube racks are sterilized and recycled after processing samples. Please avoid affixing mailing labels to the racks or marking them with alcohol-resistant marker.

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_PLANT_Record.xls** is intended to record critical information on the locations of each sample in its corresponding tube rack. 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).

IMPORTANT: Only the file template **CCDB-00000_PLANT_Record.xls** is valid for plant/fungal samples.

Note: A single CCDB Record can contain data for up to 10 tube racks. 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>

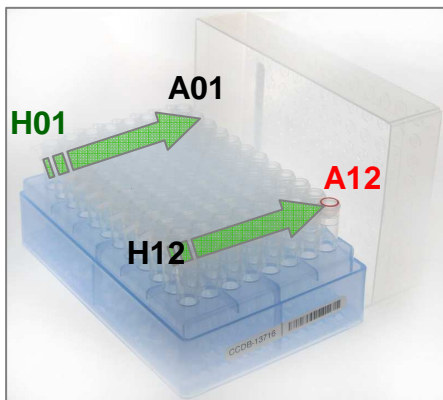
3. The file **ImageData.xls** in the folder **/ImageSubmission/** should house data on the digital images of the voucher specimens that provided the tissue samples. Refer to the link below for image submission details:

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

IMPORTANT: 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: GENERAL INFORMATION

Before adding samples into the tubes, make sure that the **side label is attached to the side corresponding to row 12 (H12–A12)**. Always work with the side label facing towards you. **Sampling tube strips are unmarked**, so caution should be taken to track the correct position of tissue samples.

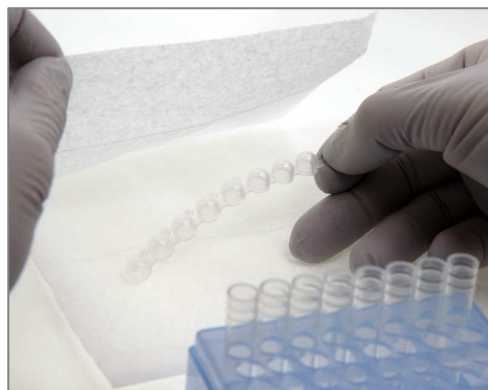
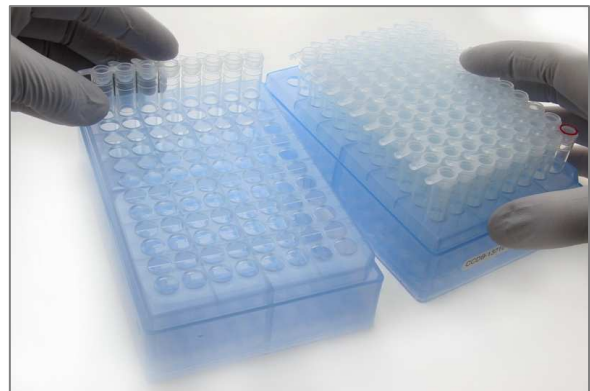
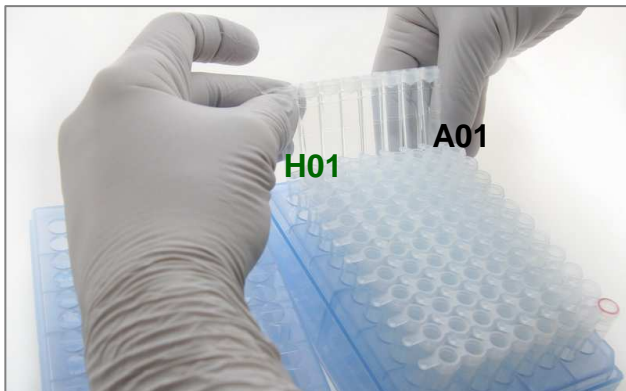


Sampling **tube strips will arrive pre-capped** with cap strips; in addition, an empty tube rack and spare tube/cap strips are provided. Use the empty rack to house the tube strips while they are being filled with samples; keep the main tube rack closed and away from the area where tissue samples are being handled. This will minimize the risk of cross-contamination during the sampling process.

Prior to sampling, position the empty rack in a location convenient for sampling. Position the tube rack with the **side label facing towards you** and remove strips of lab tape securing the box lid.

Begin sampling with tube strip H01–A01. **Start** the sampling process with tube **H01** and proceed in reverse order **to A01**. Fill tube strips in numerical order (**1–12**). **Do not** detach tubes from the strips; **do not** leave empty tubes in the middle of the batch.

IMPORTANT: Do not fill the colour-marked last tube in row 12 (**A12**)! It corresponds to the **empty control** well in the sequencing plate.

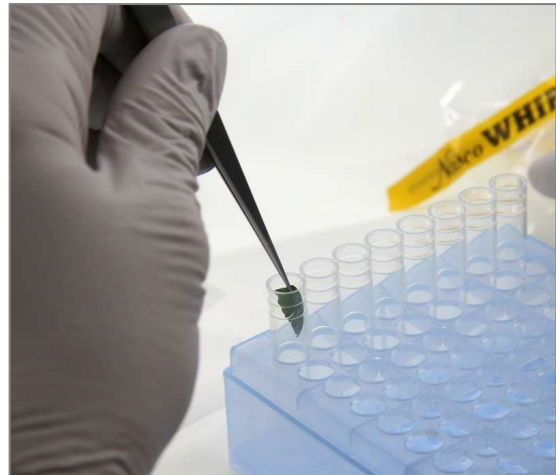
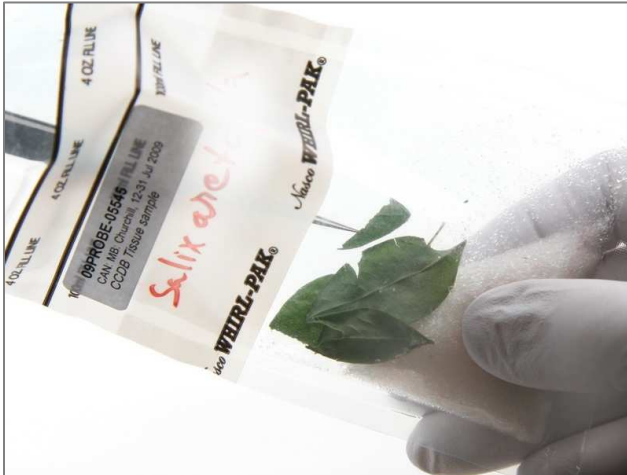


To begin sampling, open the tube rack, remove the first strip of tubes (**H01–A01**) and place into the empty rack.

Remove cap strip from the row of tubes, place it on a clean paper tissue (KimWipe or analog), and cover it to prevent accidental contamination.

Important: Maintain original orientation of tube strip!

SAMPLING PROCESS: THE PROCEDURE



Subsample a tissue piece ca. 1×0.5 cm in area with clean forceps and place it deep inside sampling tube. Ensure that no tissue residue remains on the outside of the tube after the procedure.

IMPORTANT: Only thoroughly dried plant or fungal tissue can be used for sampling, e.g., taken from a herbarium specimen or preserved in a bag with desiccant, as shown above.

Note: Dry samples are fragile and prone to static displacement—use caution when sampling.



Note: Tissue source should be fragile enough to facilitate mechanical sample disruption with grinding beads in a tissue homogenizer. **Do not** use hard or lignified parts (e.g. bark or seeds). In plants, **give preference** to the youngest and greenest parts, rich in plastids and meristematic cells (e.g., **tip of the leaf or shoot, buds, or petals of the flower**).

Use the picture on the left as a guideline to the optimal amount of tissue to sample:

h) correct: subdivided grass leaf or long conifer needle;

g) correct: piece of leaf/frond/thallome ~0.5 cm² in area;

f) incorrect: tissue sample is too long;

e) incorrect: tissue oversampled.

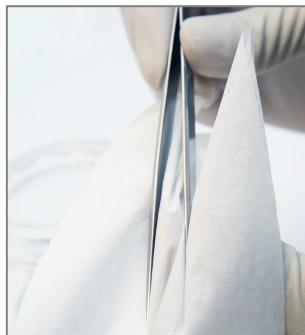
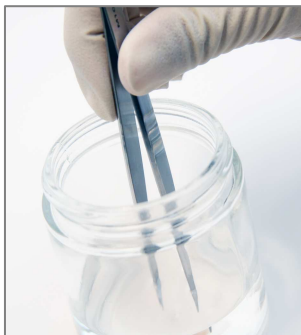


DO NOT sample from moist tissue, e.g., freshly collected or fluid-preserved.

DO NOT add fixatives, desiccant or any other objects or chemicals into the tube.

DO NOT place labels in tubes together with samples or on the outside of the tubes.

DO NOT oversample; **DO NOT** add tissue pieces that exceed **1/3** of the height of the tube.

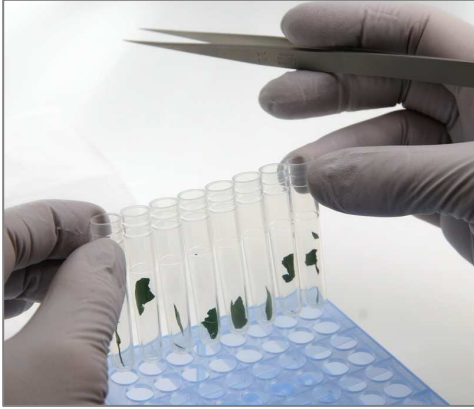


Before proceeding to next sample, **ensure that no residual tissue remains on the forceps or on the gloves** by rinsing them in 95% Ethanol and wiping them with a clean absorbent paper (e.g., KimWipe).

When the working environment permits, use flame to sterilize your sampling tools.

Change gloves as necessary and maintain a contaminant-free working area.

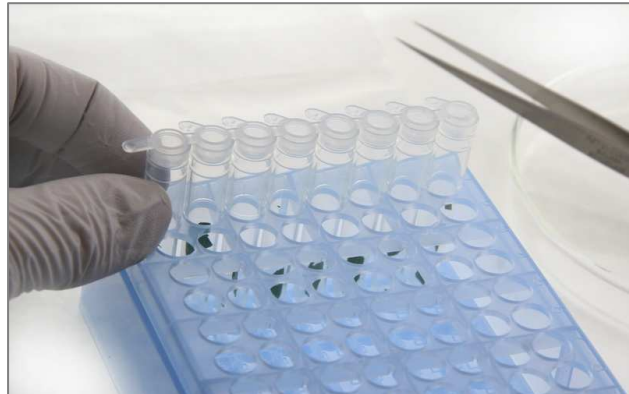
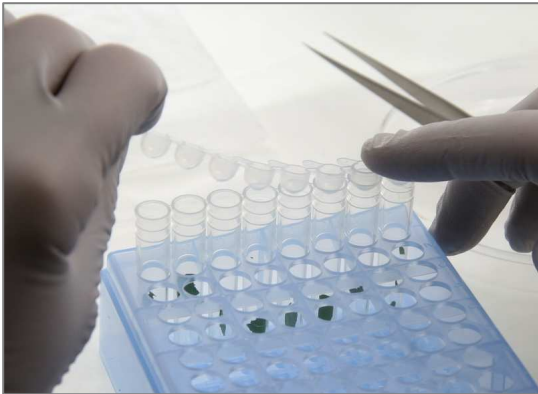
SAMPLING PROCESS: THE PROCEDURE



Proceed sampling in reverse alphanumeric order (G01–A01) until the first row is filled.

After confirming that the correct amount of tissue has been sampled, place the cap strip over the row of tubes and fasten caps securely. Observe proper orientation of the cap strip: the tabs on the strip should be directed towards column markers (H–A) as shown.

Note: 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.



When finished with the tube strip, place it back into the main tube rack and proceed with the next tube strip in the same manner. Remember to work in the empty tube rack and keep the box closed during sampling.

IMPORTANT: Maintain the correct position and orientation of all tube strips. If necessary, use marker on tubes or cap strips, but do not add labels.

Remember to leave the last tube in the last row (**A12**) empty as a control. After all the tubes have been filled with samples, ensure that all caps are pressed firmly into the tubes, and close the box lid

IMPORTANT: Prior to shipping, secure box lid with pieces of lab tape (provided), to prevent it from opening while in transit.



SAMPLING PROCESS: KEEPING A RECORD

Open the MS Excel file titled **CCDB-00000_PLANT_Record.xls** (**ATTENTION: Standard ('animal') templates are not valid for plant/fungal data**). By default, the table will open 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:

1. The type of sample medium in the first drop-down menu is defaulted to "plant tube rack".

2. Select the appropriate iBOL workgroup from the second dropdown menu (defaulted to "WG1.2 Land Plants").

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 tube rack labels 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 tube, 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 tube rack, 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 tube (A12).**

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 tube rack, 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 tubes in each rack before proceeding to the next one. Do not ship back partly filled racks, unless specifically arranged with your BOLD Project Manager. Whenever a tube rack 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 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 the tube rack 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 <ccdbcol@uoguelph.ca>, with a copy to your CCDB Contact.

The **Specimen Data** file should be e-mailed directly to the BOLD Data Management Team at <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>.