



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

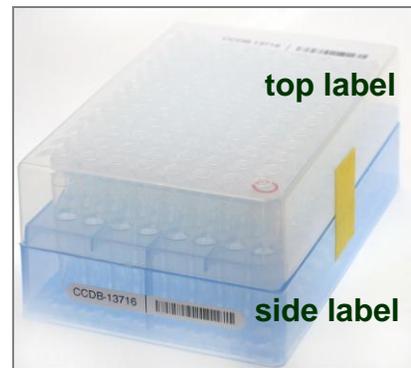
- **Tube racks (boxes containing capped tube strips)**, for housing plant/fungal tissue samples;
- A digital CCDB Record file (MS Excel spreadsheet, attached to the automated notification email);
- These Sampling Instructions (enclosed in the package, copy sent by email);
- The Biological Material Analysis Agreement (BMAA) (enclosed in the package, copy sent by email).

Filled boxes 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 the CCDB. Specimen data should be submitted separately to the **Barcode of Life Data Systems (BOLD)**; a synopsis is provided below.

SAMPLE TUBE RACK (BOX)

Each box contains strips of sample tubes that are arranged in a 8×12 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 5).



Note 1: The sampling order for the plant tube rack is transposed, relative to that for animal containers, but the same CCDB Record file is used as of January 2014. 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.

Note 2: 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.

REQUIRED DATA

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

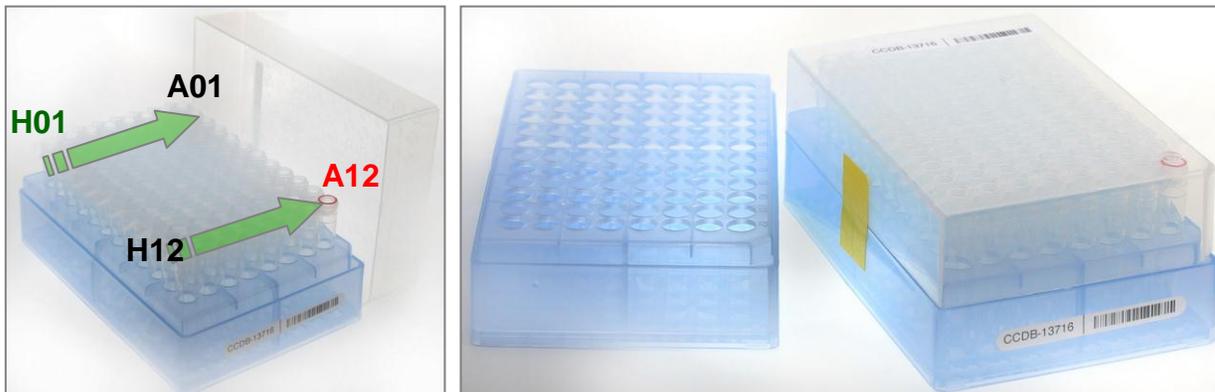
NOTE: The 'Sample ID' field within the spreadsheet submission template, which can be found at the above link, should contain numbers identical to those entered in the CCDB Record.

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: 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.

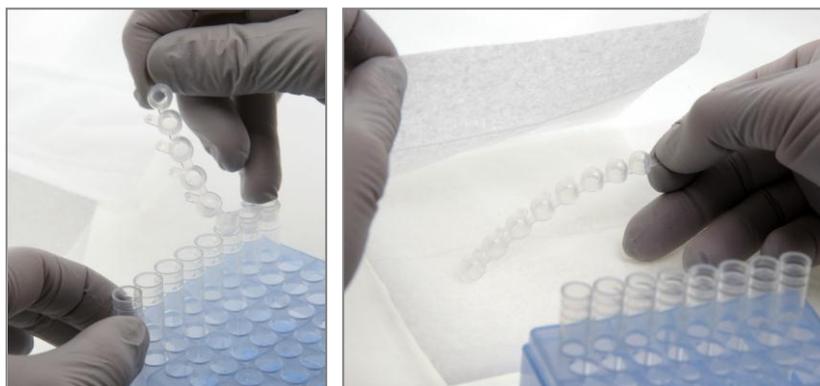
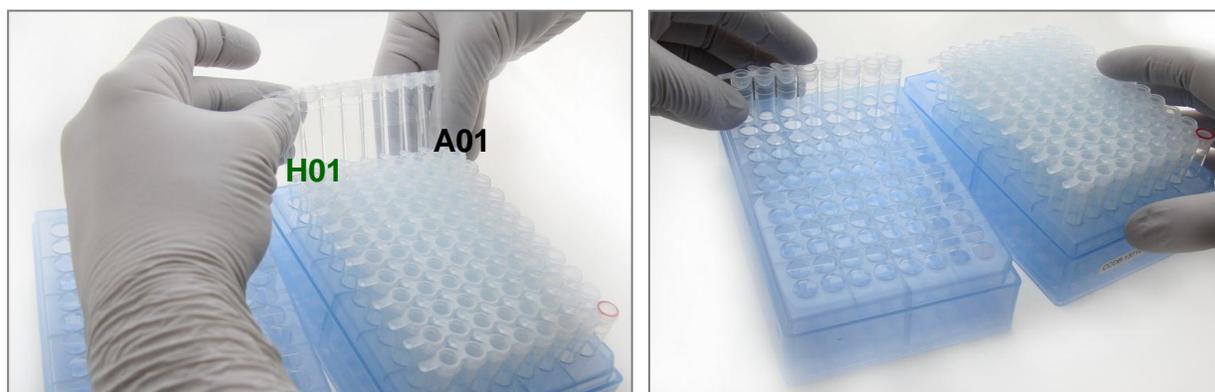


Sampling **tube strips will arrive pre-capped** with cap strips; in addition, an empty tube rack and spare tube/cap strips are provided. Use the empty rack to house the tube strips while they are being filled with samples; keep the main tube rack closed and away from the area where tissue samples are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

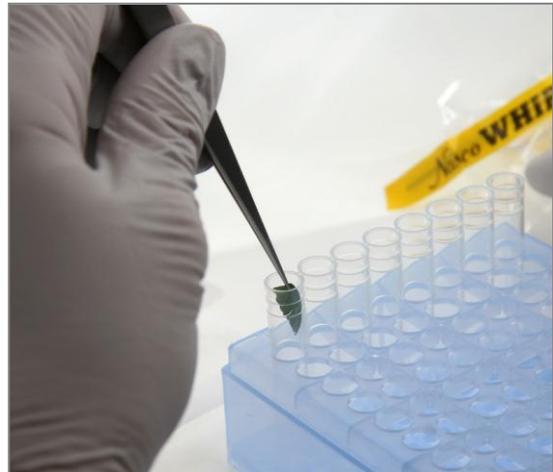


To begin sampling, open the tube rack, remove the first strip of tubes (**H01–A01**) and place into the empty rack.

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.

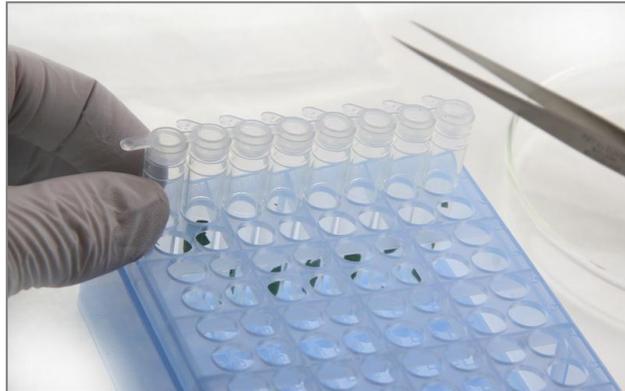
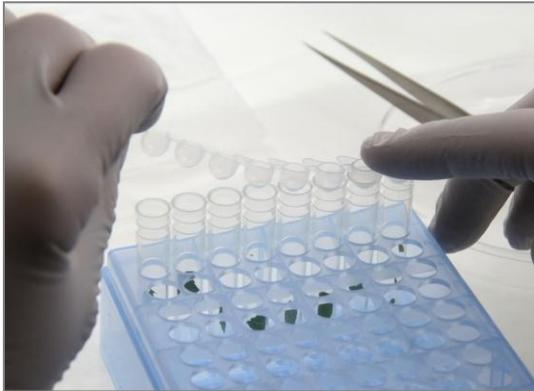
SAMPLING PROCESS: THE PROCEDURE



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

Attention: New CCDB record template introduced in January 2014 – please read carefully!

NOTE: The sampling order for the plant tube rack is transposed, relative to that for animal containers, but the same CCDB Record file is used as of January 2014.

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. It features a table with columns for 'Sample Locator', 'BOLD Sample IDs', and 'Container CCDB Numbers'. The 'Sample Locator' column lists 95 sample IDs (e.g., CCDB-01234 H01, CCDB-01234 G01). The 'BOLD Sample IDs' column contains corresponding IDs (e.g., MUS_CLL-0001, MUS_CLL-0002). The 'Container CCDB Numbers' column is currently empty. A dropdown menu for 'Type of sample container' is set to 'plant tube rack'. A checkbox for 'Multiple containers (up to 10)' is checked. A 'Well Locator' grid is visible, showing a 12x5 grid of wells labeled H, G, F, E, D, C, B, A across the top and 1-12 down the right side. A 'Plant Tube Rack' image is shown with a green path indicating the sampling order starting at H01 and ending at A12. A 'SAMPLING ORDER' note states: 'Begin sampling with position H01 and finish at A12 DO NOT fill and DO NOT ENTER DATA for CONTROL WELL - A12'. A 'Please ensure that your data submission meets the following criteria:' box contains instructions: 'Sample IDs are unique identifiers unambiguously linking each tissue sample with the corresponding BOLD record and collection voucher specimen. The order of Sample IDs in this CCDB record should match the position of the corresponding tubes, wells or blotting circles (refer to sample layout map on next sheet)'. The spreadsheet footer includes 'DATA INPUT', 'Array Map', and 'For lab use only'.

1. Select “plant tube rack” 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 tube 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 container, 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 tube(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 tube rack, 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