



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:

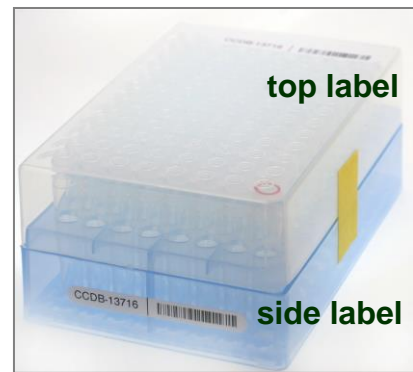
- **Tube racks (boxes containing capped tube strips)**, for housing plant/fungal tissue samples;
- A digital CCDB Record file (MS Excel spreadsheet, attached to the automated notification email);
- These Sampling Instructions (enclosed in the package, copy sent by email);
- The Biological Material Analysis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

DIGITAL SPECIMEN DATA REQUIREMENTS

Prior to molecular analysis at the CCDB, accompanying data must be submitted in a compliant format via two different channels: the **CCDB** and the **Barcode of Life Data Systems (BOLD)**.

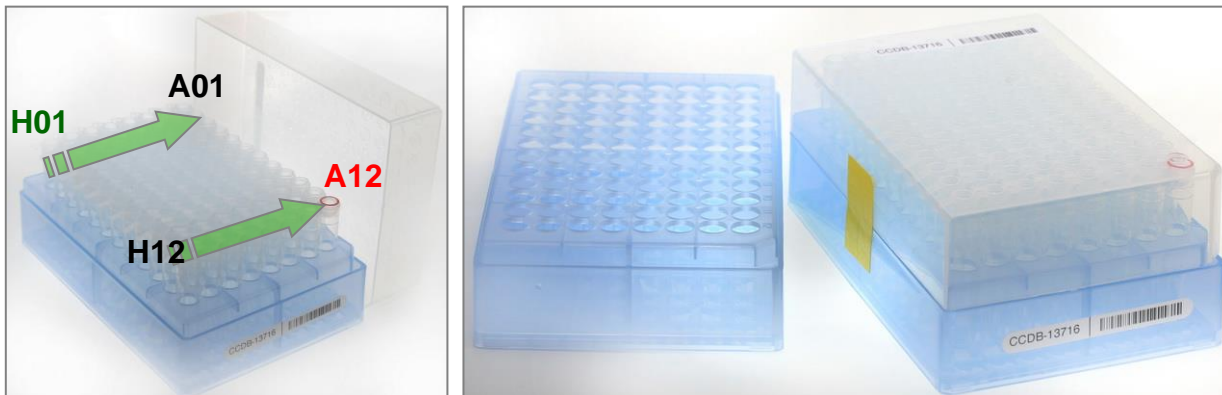
1. The **CCDB Plate Record** named **CCDB-00000_Record.xls**, is emailed to the recipient and used to record the location of samples in the corresponding sample container(s). Each sample must be assigned a **Sample ID**, which is a unique identifier connecting the sample with its source specimen. See section H for more details. Each container will have a corresponding plate record and up to 10 plate records can be included in the CCDB Plate Record file.
- 2a. A **BOLD Specimen Data Submission** is the first step in the process of creating records on BOLD. There will be one specimen data submission for each batch of containers. For more details on the specimen data submission protocol, please refer pg. 15 of the BOLD handbook accessible through this link: <https://boldsystems.org/resource-hub/documentation/>

Note: The 'Sample ID' field within this specimen data spreadsheet should be identical (including letter case) to the Sample IDs entered in the CCDB Plate Record and without any duplications.

- 2b. A **BOLD Specimen Image Submission** is an additional requirement for some analytical services (see <http://ccdb.ca/pricing/> for details) and should complement the specimen data submission. For details on the image submission protocol, please refer to pg. 26 of the BOLD handbook accessible through this link: <https://boldsystems.org/resource-hub/documentation/>

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.

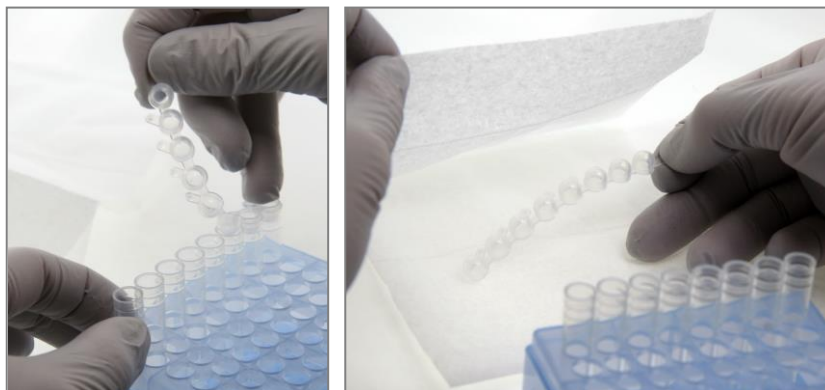
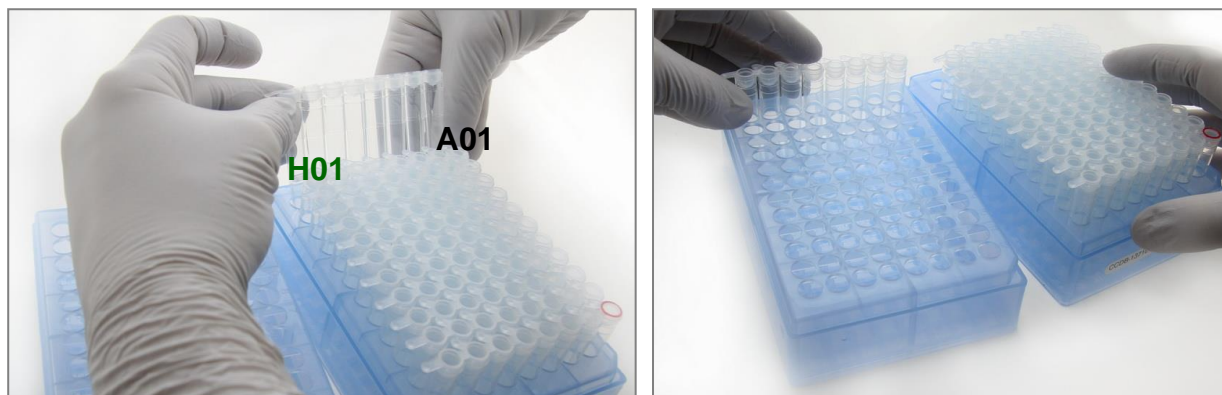


Sampling **tube strips will arrive pre-capped** with cap strips; in addition, an empty tube rack and spare tube/cap strips are provided. Use the empty rack to house the tube strips while they are being filled with samples; keep the main tube rack closed and away from the area where tissue samples are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

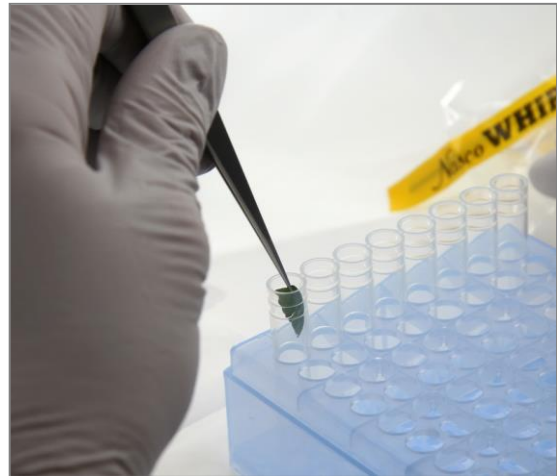
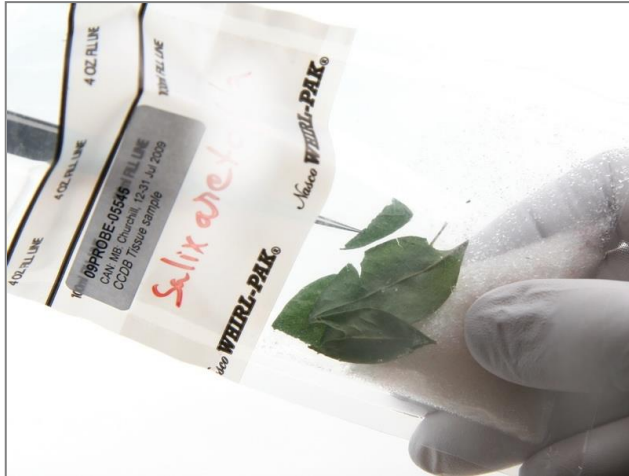


To begin sampling, open the tube rack, remove the first strip of tubes (**H01–A01**) and place into the empty rack.

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.

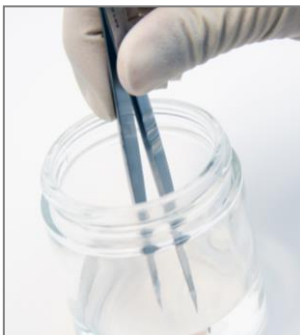


DO NOT sample from moist tissue, e.g., freshly collected or fluid-preserved.

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.

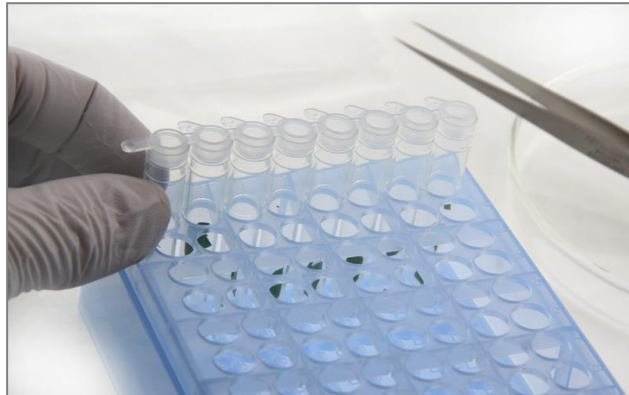
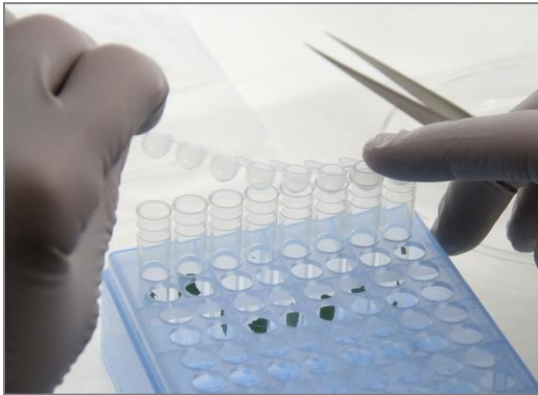
SAMPLING PROCESS: THE PROCEDURE



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 in 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:

Sample Locator	BOLD Sample IDs	Container CCDB Numbers
CCDB-01234 H01	MUS_CLL-0001	CCDB-01234
CCDB-01234 G01	MUS_CLL-0002	CCDB-01235
CCDB-01234 F01	MUS_CLL-0003	CCDB-01236
CCDB-01234 E01	MUS_CLL-0004	
CCDB-01234 D01	MUS_CLL-0005	
CCDB-01234 C01	MUS_CLL-0006	
CCDB-01234 B01	MUS_CLL-0007	
CCDB-01234 A01	MUS_CLL-0008	
CCDB-01234 H02	MUS_CLL-0009	
CCDB-01234 G02	MUS_CLL-0010	
CCDB-01234 F02	MUS_CLL-0011	
CCDB-01234 E02	MUS_CLL-0012	
CCDB-01234 D02	MUS_CLL-0013	
CCDB-01234 C02	MUS_CLL-0014	
CCDB-01234 B02	MUS_CLL-0015	
CCDB-01234 A02	MUS_CLL-0016	
CCDB-01234 H03	MUS_CLL-0017	
CCDB-01234 G03	MUS_CLL-0018	
CCDB-01234 F03	MUS_CLL-0019	
CCDB-01234 E03	MUS_CLL-0020	
CCDB-01234 D03	MUS_CLL-0021	
CCDB-01234 C03	MUS_CLL-0022	
CCDB-01234 B03	MUS_CLL-0023	
CCDB-01234 A03	MUS_CLL-0024	
CCDB-01234 H04	MUS_CLL-0025	
CCDB-01234 G04	MUS_CLL-0026	
CCDB-01234 F04	MUS_CLL-0027	
CCDB-01234 E04	MUS_CLL-0028	
CCDB-01234 D04	MUS_CLL-0029	
CCDB-01234 C04	MUS_CLL-0030	
CCDB-01234 B04	MUS_CLL-0031	
CCDB-01234 A04	MUS_CLL-0032	
CCDB-01234 H05	MUS_CLL-0033	
CCDB-01234 G05	MUS_CLL-0034	
CCDB-01234 F05	MUS_CLL-0035	
CCDB-01234 E05	MUS_CLL-0036	
CCDB-01234 D05	MUS_CLL-0037	
CCDB-01234 C05	MUS_CLL-0038	

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.

DIGITAL SPECIMEN DATA SUBMISSION

1. The **CCDB Plate Record** file(s) and signed **BMAA** should be emailed to the CCDB via lims@ccdb.ca.
2. **Templates for BOLD data submissions can be found at the following link:**
<https://boldsystems.org/resource-hub/templates/>
 - a. The **Specimen Data** file can be submitted directly to BOLD using the specimen data submission protocol. Please refer to pg.15 of the BOLD handbook for instructions:
 - b. **Specimen Images** and the corresponding **Image Data** file can be submitted directly to BOLD using the online image submission protocol. Please refer to pg. 26 in the BOLD handbook for instructions:

For the BOLD handbook including detailed information on the BOLD data structure and submission procedures, please refer to the following link:

https://bench.boldsystems.org/libhtml_v3/static/BOLD4_Handbook_FinalVersion_Feb2023.pdf

Any questions concerning specimen data or image submissions should be directed to the BOLD Support Team support@boldsystems.org.

SUBMISSION OF BIOLOGICAL MATERIALS

Fill all 95 samples in each container before proceeding to the next one in the batch. Do not ship back partly filled containers, unless specifically arranged with the CCDB. Whenever a container is transferred to another person for sampling, please notify the CCDB.

NOTICE: Unless explicitly negotiated otherwise, all biological materials submitted to the Canadian Centre for DNA Barcoding (CCDB) at the Centre for Biodiversity Genomics (CBG) fall under the standard provisions of their associated Biological Material Analysis Agreement (BMAA).

All data submitted to the Barcode of Life Database and generated by the CCDB will comply with the Data & Resource Sharing Policies. Full texts can be requested from the CCDB.

A synopsis of the conditions relevant to this transaction with the CCDB is contained in the BMAA included in this submission package. Please acknowledge that you have read and agreed to these conditions by signing the BMAA in electronic or hard copy form and returning it to the CCDB with the first batch of samples.

DISCLAIMER: It is the sender's responsibility to ensure that (1) biological materials are shipped to the CCDB in compliance with any applicable shipping regulations, (2) they have been obtained under appropriate collection, and (3) animal care permits in their country of origin and 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 export certificate from the country of origin (if applicable)
- c) Canadian Food Inspection Agency import permit (if applicable)

The CCDB 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.

After the sampling process has been completed, please return the samples by courier or registered mail to the following address. Please indicate a nil value on the shipping invoice.

Sample Submission
Canadian Centre for DNA Barcoding
Centre for Biodiversity Genomics
University of Guelph
50 Stone Road East
Guelph, Ontario, Canada N1G 2W1
Phone: +1 (519) 824-4120 ext. 56393

CCDB DISTRIBUTION OF DATA

Once CCDB processing is complete and all final sequences have been uploaded to BOLD, a **Sequence Upload Notification** will be sent to the external collaborator via the email address used during submission correspondence. The Sequence Upload Notification will contain the following information:

- The number of sequences uploaded
- Root plate name(s) associated with the data
- BOLD project name(s) associated with the data
- Contaminants, records flagged by BOLD, or other important information

The Sequence Upload Notification will contain the following attachments:

- Fasta file of uploaded sequences
- Taxon ID tree
- Spreadsheet of specimen information for sequences included in the taxon ID tree
- Specimen Images for sequences included in the taxon ID tree, if available
- BOLD Identification Engine results
- BOLD Sequence Submission Report
- Fasta file of sequences that contain a stop codon(s), indel(s), or identify as a contaminant, if any

In addition to the files attached to the Sequence Upload Notification, a copy of the CodonCode project, created by the CodonCode Aligner software used to perform sequence editing and assembly, as well as the fasta file of uploaded sequences will be attached to the associated BOLD Project for long term storage and review. All trace files will be uploaded to their corresponding specimen record on BOLD and can be reviewed or downloaded at any time.

Note: Sequences that contain greater than 1% ambiguous bases of the total recoverable sequence length (i.e. >5 N's in a 500bp sequence) do not meet CCDB sequence quality standards and will **not** be uploaded to BOLD.

Note: Final sequences that contain a stop codon(s), indel(s), or identify as a contaminant (e.g. *Homo sapiens*, *Mus musculus*, Proteobacteria (i.e. *Wolbachia*), fungal on a non-fungal plate, Nematoda on a non-Nematoda plate, etc.) will **not** be uploaded to BOLD. The sequence(s) will be provided as an attachment in the Sequence Upload Notification.

Note: Sequences that do not match the expected taxonomy at genus level or higher will be uploaded to BOLD and BOLD will flag the sequence to exclude it from use by the BOLD Identification Engine. The act of flagging maintains the integrity of the BOLD reference database used by the BOLD Identification Engine to determine closest matches for sequence identifications.

Validation of the final data is required from the external collaborator to address any mismatches between final sequence identifications and the expected taxonomy. To minimize mismatches, only known taxonomic information for specimens should be provided in the submitted **Specimen Data** file (i.e. specimens identified to Order if only Order level taxonomy is known). Flagged specimens require the review of specimen identifications. If the cause of the mismatch is a result of contamination from a non-target sequence, the sequence will remain flagged. If the cause of the mismatch is a result of specimen misidentification, the specimen data will be updated on BOLD by the external collaborator. Once taxonomic revisions of flagged sequences are complete, specimens with final sequence identifications matching the expected taxonomy can be unflagged upon request by the external collaborator to the BOLD Support Team at support@boldsystems.org.

All inquiries regarding CCDB processing or the information and data provided in the Sequence Upload Notification can be directed to CCDB Support Team at support@ccdb.ca.
