

Best practise manual

# DNA barcoding at NHM/Canada

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## **Introduction**

DNA barcoding manager at NHM is Gunnhild Marthinsen, Room 323, Robert Collett's house, [gunnhilm@nhm.uio.no](mailto:gunnhilm@nhm.uio.no), phone 22851874, 99535482.

Contact Gunnhild regarding all DNA barcoding activity, at least to inform her about ongoing projects.

DNA barcoding at NHM is performed through two channels; NorBOL and in-house.

NorBOL processes up to five specimens of any species sampled in Norway. These samples are shipped to Canadian Centre for DNA Barcoding (CCDB) for analyses, and NorBOL pays the costs. All requests goes through Gunnhild or Arild Johnsen.

In addition there is a DNA barcoding pipeline at NHM. In 2015 mainly Diptera and lichens are processed through this line. The plan is to expand this in the coming years.

## **Data- and specimen-flow for samples to be barcoded**

1. Specimen into relevant NHM-collection and database
  - Vertebrates: [Corema](#)
  - Vascular plants, fungi and insects: MUSIT
  - Lichens: Einar Tindal's database/MUSIT
2. If more tissue is left after barcoding-analysis, this sample goes into the DNA-bank
3. Data regarding (potentially all spent) tissue sample and (in future existing) DNA-extract goes into Corema (sample-information is exported from MUSIT)
4. Data is exported from Corema to be submitted to BOLD; the DNA barcoding database.
5. Photos are to be submitted to BOLD
6. Samples are sent to CCDB, or is entered into the NHM pipeline

## General tips on data

UTM-soner i Norge: <http://www.entomologi.no/hobby/utmsoner.jpg>

## Sample IDs

All samples shall have a number from the start. This can be a MUSIT reg.number, an UUID or a number designed appropriately in each case (e.g. Field number, collection number).

## How to get data into MUSIT

Punch manually or use an import-file.

Contact the relevant collection manager.

## How to get data from MUSIT to Corema

1. Find the relevant entries in MUSIT
2. Set up the "Resultatoppsett" needed, ask Gunnhild or Lars Erik
3. Export all entries as an excel-file
4. Add columns at the end corresponding to the ones listed in N:\barcoding\Div\corema-extra-fields.xlsx
  - Look at Corema import file found at [https://wiki.uio.no/nhm/skf/best-practices/index.php/DNA\\_Bank](https://wiki.uio.no/nhm/skf/best-practices/index.php/DNA_Bank)
5. Split the species-column (Accepted taxon name for fungi) in Genus, epithet and uncertainties (e.g. cf., aff., coll.)
6. Dates without day or month should be on the format 00.06.2011 or 00.00.2011
7. Send the excel-file to Lars Erik Johannessen (DNA-bank technical curator).
8. Fungus/Lichens: New samples from previously sampled specimens (resampling) shall be registered as **new** accessions in Corema

## How to get non-MUSIT data to Corema

(in case of samples that do not belong to NHM)

1. Use Corema import file found at [https://wiki.uio.no/nhm/skf/best-practices/index.php/DNA\\_Bank](https://wiki.uio.no/nhm/skf/best-practices/index.php/DNA_Bank)
2. If there are more than 500 samples, copy the last row as far down as necessary *before* filling in data, since there are formulas in several columns that need to be there.

## How to get data from Corema in a BOLD-friendly format

(To get access to Corema, ask Lars Erik)

1. Go into relevant collection (File --> Change collection)
2. Check that the newly imported data have correct coordinates by plotting them:
  - Choose Reports\Maps
  - Search for accessions, e.g. by event
  - Button: View...
3. Make a report (search for accessions and make a report in the same operation):
  - Go to Reports (panel to the left)

- Under “02 Accession items”; select the “Item02 report” (gives you a report of BOLD-relevant data)
- Search for the relevant entries. Example: search for accessions that have consecutive numbers (see below for dealing with not-consecutive numbers):
  - o Under “Accession”; tick off for Acc. no
  - o Choose “between”, and enter your numbers (only numbers, no prefixes should be included)
- Click button “View...” in top right corner, and get an excel file that you can save where you like
- (Find first and last acc no:
  - search for the first individual
  - button: prepare for search or new entry (blank page)
  - enter f.ex. species name under “Taxon name” at the top, then Ctrl F
  - note the acc no for the specimen you want
  - do the same for the last individual)
- to search for a batch of accessions that do not have consecutive numbers, make a task and search for this task under Event when making the report. How to make a task:
  - o Go to “Tasks” under “Events” and get a blank form
  - o Enter a Name for the task, e.g. GM\_asco\_160520
  - o Choose Type --> Temporary
  - o Choose Entries kind --> Accession
  - o Click “click here to add a new row”
  - o Write an acc-no (without prefix) in the field below Code/Name and Enter, and it adds to the list that is the task
  - o For several at once, paste a column with acc-no from excel in the same field
  - o For searching, click the ...-button and click advanced search (binoculars with yellow +)
    - enter search criteria
    - Ctrl + F
    - choose entries in list with Ctrl+A (all) or clicking lines with Ctrl to choose several
    - Click Select and close, and these are added to the task-list
  - o

### How to fill in BOLD’s SpecimenData sheet

1. Download sheet (Template\_Version\_3.0) from [http://www.boldsystems.org/index.php/resources/handbook?chapter=3\\_submissions.html&section=data\\_submissions](http://www.boldsystems.org/index.php/resources/handbook?chapter=3_submissions.html&section=data_submissions)
2. For fungus: fill in the sheet based on the report from Corema using the reformatting-file [N:\barcoding\Div\templat\\_corema\\_to\\_bold\\_sheet\\_fungus.xlsx](#) (work in progress, use with care!)  
For insects: use the reformatting-file N:\barcoding\Div\templat\_corema\_to\_bold\_sheet\_insects.xlsx
3. See same link as above regarding how to fill out the fields, but pay attention to the following:
  - o Sample ID is hard to change later, **be careful to get it right**
    - Used to be full Acc no from Corema

- birds: NHMO-BI-xxxxx
      - fungi and lichens: O-DFL-xxx (??)
      - insects: NHMO-DAR-xxxx
    - **new** for fungi and lichens: MUSIT-no
  - Field ID
    - Optional. Whatever is suitable.
  - Museum ID
    - birds: same as Sample ID
    - MUSIT-entries: MUSIT reg.number
      - This also applies to insects. UUID shall be under “Notes”
    - **new** fungi and lichens: Acc.no
  - Taxonomy:
    - uncertain species identification, e.g. cf. or aff. for fungi, is indicated in “Taxonomy notes”. Do not put it in the name.
    - do not use family as interim genus-name, create a new one
    - put initials and numbers after sp. example: Bos sp. 1KHR
  - Collection Code
    - birds: BI
    - fungus: F
    - lichens: L
    - insects: NHMO
  - Institution storing
    - **Format shall be (if NHM):** University of Oslo, Natural History Museum
  - Identifier: only one person. First Name Last Name.
4. Send SpecimenData file to Gunnhild for control check, and submission to BOLD, or submit yourself to BOLD
- Create a new project if there are none

### How to take photos and submit to BOLD

- BOLD Photo guide:
  - [http://boldsystems.org/index.php/resources/handbook?chapter=3\\_submissions.html&section=image\\_submissions](http://boldsystems.org/index.php/resources/handbook?chapter=3_submissions.html&section=image_submissions)
- Download the file **imagedata.xls** from
  - [http://www.boldsystems.org/index.php/resources/handbook?chapter=3\\_submissions.html&section=image\\_submissions](http://www.boldsystems.org/index.php/resources/handbook?chapter=3_submissions.html&section=image_submissions) and fill out
    - To create the list of the image files in a folder, open a terminal window (Start > Run > cmd in Windows), navigate to the folder containing the image files, and then run the command  
dir /b \*.jpg>list.txt
    - copyright license: if no specific request, put **by-nc-sa** in Licence (Creative Commons - Attribution Non-Commercial Share-Alike), the present year in Licence year, **University of Oslo, Natural History Museum** in Licence Institution, [arild.johnsen@nhm.uio.no](mailto:arild.johnsen@nhm.uio.no) in contact, and yourself as Photographer. Licence holder is not important if you do it like this.
- Renaming of photos:

- Not necessary for BOLD, but could be convenient, and is necessary for MUSIT (?)
  - Make a bat-script and put it in the same folder as the photos (example: N:\barcoding\Div\rename\_photos\_script\_example.bat), e.g. by using the excel-file-templat “mal for rename-script til bilder.xlsx” in the same folder as the example-script
  - run the script by double clicking it - but keep backup copies of the photos!
- maximum allowed size of zipped folder is 190 MB
  - Reduce size of photos in FastStone (free download from internet):
    - copy photos to new folder
    - mark all
    - Tools --> convert chosen photos (Verktøy --> konverter valgte bilder)
    - remove tick for “målmappe”
    - tick off for advanced choices (?) (Avanserte valg)
    - click on “avanserte valg”
    - long side = e.g. 2400 pkt
    - ok
    - “utfør”

## Submission of samples

### NorBOL-samples:

Put samples in barcoding-plate that you get from NorBOL manager/Gunnhild. (If you have a plate from elsewhere, inform Gunnhild and give her the plate-number)

### **How to fill plate**

See instructions from CCDB:

- *insects*: <N:\barcoding\BOLD protocols and sheets\BOLD protocols and sheets 2013\ Sample Submission Package-microplate 2010-07.pdf>
- *plants*: <N:\barcoding\BOLD protocols and sheets\Sample submission package plants and fungi\Sample Submission Package Plant.pdf>

Insect legs (or whole insects if small) should be put in 96% ethanol.

Be sure to get the direction right; one row at the time. Keep the caps on the rows you are not working with.

Keep track of the samples; fill out the Record-sheet at the same time

- *plants*: <N:\barcoding\BOLD protocols and sheets\Sample submission package plants and fungi\plants: CCDB-00000 PLANT Record.xls>
- *insects*: [N:\barcoding\BOLD protocols and sheets\BOLD protocols and sheets 2013\CCDB-00000\\_Record \(2\).xls](N:\barcoding\BOLD protocols and sheets\BOLD protocols and sheets 2013\CCDB-00000_Record (2).xls)

## **Packing for shipment**

### *Dry material:*

Put in cardboard-box big enough to fit address-note and shipping papers on same side.

Use cushioning material (e.g. bubbled plastic left-over from the DNA-lab, or from packing desk in zoomus or geomus).

### *Material on ethanol:*

OBS! You must be trained to pack ethanol ([https://wiki.uio.no/nhm/skf/best-practices/index.php/Import\\_og\\_eksport](https://wiki.uio.no/nhm/skf/best-practices/index.php/Import_og_eksport))

Gunnhild interprets the terms and do the packing like this:

- **Inner packing: specimens are placed in rigid containers with no more than 30 mL of alcohol**
  - ➔ Each plate is one rigid container. Each well contains maximum 250µl ethanol = 24mL in plate (or more often 95\*30µl = max 3mL). (I don't interpret each well as single rigid containers, because they are connected, and if one break several brake, and also, you cannot have more than 33 single units in an outer packing - I think.)
- **1<sup>st</sup> re-packing: specimens are placed in a plastic bag that is heat sealed**
  - ➔ all plates together are heat-sealed in plastic (or divide if many)
- **2<sup>nd</sup> re-packing: bagged specimens are places inside another plastic bag with absorbent material and then heat sealed**
  - ➔ wrap bag in paper-towels enough to absorb 24 mL\*no of plates and heat-seal in plastic

Put in cardboard-box big enough to fit address-note on one side, and pad with cushioning material (e.g. bubbled plastic left-over from the DNA-lab, or from packing desk in zoomus or geomus).

### Shipping documents

Make sure values correspond in all documents!

[https://wiki.uio.no/nhm/skf/best-practices/index.php/Import\\_og\\_eksport](https://wiki.uio.no/nhm/skf/best-practices/index.php/Import_og_eksport)

Put one copy of each document inside package.

Put two copies of each document on the outside.

DHL: give them the two (**three!?**) copies of each document, together with what you print from the ordering of the shipment (which should be printed one-sided). Also bring the Shipment receipt for them to sign and you to keep.

### Shipping to CCDB

Gunnhild can ship the plates.

Address:

University of Guelph  
Biodiversity Institute of Ontario  
50 Stone Road East  
Guelph, Ontario, N1G2W1  
Canada

Phone#:+1 (519) 824-4120 ext 56393

Shipping with regular mail (dry samples, i.e. fungi and lichens):

- <http://www.uio.no/for-ansatte/drift/post/pakke/>
- (get an address-note from Blindern in intern-mail, same day if you're early)
- custom invoice from Posten: <http://www.posten.no/produkter-og-tjenester/pakker/carryon-cash-pakker-til-utlandet>
- The wrapping should be big enough to fit the address card and the shipping documents on the same page?

Shipping with DHL (if on ethanol):

We are obliged to use DHL

[http://www.uio.no/for-ansatte/arbeidsstotte/anskaffelser/innkjop/regler/rutiner/rutine\\_dhl\\_v3.0.pdf](http://www.uio.no/for-ansatte/arbeidsstotte/anskaffelser/innkjop/regler/rutiner/rutine_dhl_v3.0.pdf)

Get an account with DHL

<https://webshipping2.dhl.com/wsi/WSIServlet?moduleKey=Login&countryCode=NO>

kundenr 240044873

organisasjonsnr 971035854

Check price online (know size and weight and address of package)

Order shipment from Maria, give her account information and receiver institution and country

Order shipment online, include E-number from Maria

Make custom invoice (automatic through dhl web page) and shipping document (more detailed about content, dangerous, e.g. ethanol, packing etc.), see above

Order pickup online

Make room for address-note on package.