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DNA Barcoding Standard Operating Protocol, Plants and Lichens at RBGE, Lab methods: DNA extraction

Forked from DNA Barcoding Standard Operating Protocol, Plants and Lichens at RBGE, Sample Data

In 1 collection

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ABSTRACT

This is part of the collection DToL Taxon-specific Standard Operating Procedures for the Plant Working Group (protocols.io). The SOP collection contains guidance on how to process the various land plant taxa within the scope of the Darwin Tree of Life project. The guidance specifically refers to the tissue samples needed for DNA barcoding (which takes place at the Royal Botanic Garden (RBGE)) . Every specimen is submitted for DNA barcoding first before potentially being sent to the Wellcome Sanger institute.

This DNA barcoding SOP outlines DNA extractions from plant and lichen samples for the Darwin Tree of Life project (DToL) at the Royal Botanic Garden Edinburgh (RBGE).

DNA barcoding is used as part of the species identification process AND sample tracking (to check that the genome sequence corresponds to the material that was sent and that there have been no sample mix-ups).

Definition: Land plants (Embryophyta) and lichens
Including: Bryophyta, Marchantiophyta, Anthocerotophyta, Lycopodiophyta, Polypodiophyta, Pinophyta, Cycadophyta, Ginkgophyta, Gnetophyta, Magnoliophyta, lichenized fungi

Excluding: all non-lichenized fungi

Protocol status: Working
We use this protocol and it's working

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Keywords: DToL, RBGE, Plant Barcoding, Darwin Tree of Life, Lichen

Including: Marchantiophyta (liverworts), Bryophyta (mosses), Anthocerotophyta (hornworts), vascular plants, lichens

Note

Previous versions of the Plant Working Group SOPs can be found here:

RBGE DToL Sample collection Standard Operating Procedure Vascular Plants
RBGE DToL Sample collection Standard Operating Procedure Bryophytes
SOP RBGE Plant DNA Barcoding sample submission
Darwin Tree of Life DNA barcoding of vascular and non-vascular plants and of lichens - Standard Operating Procedure

	A	B	C	D
		Date	***Changes***	***Contributors***
	1.0	August 2020	First draft	Laura L Forrest, Michelle L Hart
	1.1	January 2021	Revisions	Laura L Forrest
	1.2	June 2021	Lichens added	Laura L Forrest

Previous Version History, RBGE DToL DNA Barcoding Standard Operating Procedure

Working SOP, checked by experts

MATERIALS

In the following list, general equipment and consumables that are typically available in molecular biology laboratories, such as benchtop centrifuges, water baths, heating blocks, orbital shakers, vortexers, thermocyclers, gel tanks and gel trays, agarose, UV or blue-light trans-illuminators, laminar flow hoods, fume hoods, water purification systems, autoclaves, micropipettes, tips, microcentrifuge tubes and tube racks, are generally omitted.

Plant and lichen DNA Extraction

- **Mixer Mill** (Retsch) or TissueLyser (QIAGEN), with 4 mm flattened “flying saucer” tungsten carbide or stainless steel beads, or 5 mm round tungsten carbide or stainless steel beads for single tube extractions.
- **Wide-based microtubes** (e.g. Axygen 2.0 mL microtubes, cat. no. MCT-200-C) (stops tissue and beads getting stuck in the end of the tube).
- **Mini-pestles** and **acid-washed sand** (for manual grinding of tissues that don't grind well in the mixer mill).
- **Narrow-based microtubes** (for use with mini-pestles)
- **DNeasy® Plant Kit** (QIAGEN) – mini-columns and plates.
- Other relevant plastics, including Rainen filter tips.

Note

- To avoid problems with tubes shattering, we do not use Mixer Mill frequencies over 20 Hz.
- Tungsten beads can be cleaned for reuse by rinsing them clean of plant debris in water, sonicating to remove small fragments, and washing for c. 1 min in either 0.4 M HCl or a bleach solution to remove any remaining DNA, rinsing well with water and (optionally) autoclaving.
- Mini pestles can be cleaned for reuse by rinsing them clean of plant debris in water, sonicating to remove small fragments, and washing for c. 1 min in either 0.4 M HCl or a bleach solution to remove any remaining DNA, rinsing well with water and autoclaving. We store these in sets of 8-16 in 50 ml Falcon tubes.

DNA extraction (Qiagen DNeasy plant mini kits)

1 **Sampling.**

Plant DNA barcoding for the Darwin Tree of Life (DToL) project involves extraction of DNA from silica gel dried tissue that has been provided, along with the required metadata, following the collecting and submission SOPS available in the collection **DToL Taxon-specific Standard Operating Procedures for the Plant Working Group (protocols.io)**

Note

All lab samples for DNA extraction are renumbered in consecutive order, with a short simple numbering scheme, rather than using the **DToL sample numbers**, as the numbers have to be transcribed onto several plastic tubes during the extraction process and this is least prone to error. Note the numbers both in a lab book and by annotating the individual silica gel packages on their top right hand corners.

Current **RBGE DToL DNA extraction numbering series** for barcoding are prefaced with **B** (mosses), **L** (liverworts), **F** (ferns and lycophytes), **A** (seed plants) and **X** (lichens).

These temporary tube numbers are entered into the EDNA_submission_sheet_211209.xlsx and recorded in the **EDNA database**. They are also tracked in the RBGE DToL barcoding google sheet.

In order to best troubleshoot contamination issues downstream, where possible avoid placing samples from the same genus, and particularly from the same species, into consecutive tubes.

2 **Homogenization**.

Plants.

1. On a vented bench, using clean forceps, put around 1-2 cm^2 silica-gel dried (or herbarium) plant material into a labelled open-based Axygen 2 mL microcentrifuge tube, along with a flattened tungsten bead.
2. Distribute the tubes or plates between two balanced adaptors.
3. Homogenize for 2 mins at 20 Hz, rotate the adaptor 180°, then homogenize for a further 2 mins at 20 Hz.
4. If the sample has ground poorly, add a second flattened bead and repeat.
5. If problems persist, transfer the sample or a new bit of plant tissue into a 1.7 ml eppendorf tube, add a pinch of acid-washed sand, and manually grind using a disposable plastic mini-pestle.

Note

The plant tissue should be slightly broken up prior to its homogenization in the tissue lyser. If it ends up lying vertically in the tube the bead may avoid hitting it and the sample will not consistently grind to a fine powder.

Lichens.

1. Place a small fragment of lichen into the bottom of a 1.7 ml eppendorf tube (if grinding with a mini-pestle) or a 2 ml eppendorf tube (if grinding with a mixer mill).
2. Soak the lichen tissue in c. 50 µl acetone for at least half an hour, ensuring that the tissue is completely covered by the acetone.
3. Pipette off the acetone into a labelled 500 µl tube.
4. Leave the lichen tubes and the acetone tubes open in a fume hood to air dry.
5. Once dry, grind the sample using either a disposable plastic mini-pestle and a little acid-washed sand, or a mixer mill and 1-2 flattened tungsten beads (following the plant protocol above).
6. Either pass the air-dried acetone sample on for thin layer chromatography (TLC), or store it with the lichen herbarium specimen in case TLC will be carried out in the future.

Note

Lichen barcoding samples may also be stored in eppendorfs at -20°C in TE buffer. Remove the samples from the freezer, allowing plenty of time for the buffer to defrost prior to homogenization.

The lichen thallus will have to be removed from the tube with forceps (do not use No. 5 forceps with a 2 ml tube, as these do not reach into the bottom of the tube); the forceps should be cleaned with alcohol between samples. Blot off excess TE buffer on a paper towel.

A small amount of lichen tissue can be transferred to a wide-based 2 ml tube with 2 flattened tungsten beads for homogenization using a tissue lyser (see above), or into a narrow-based 1.5 ml tube with a small quantity of acid-washed sand to be hand-ground using a disposable plastic mini-pestle.

If using a mini-pestle, adding the first 200 µL of pre-heated 65°C QIAGEN lysis buffer can help the sample grinding.

3 **DNA extraction** using DNeasy® Plant mini kits (QIAGEN):

Follow manufacturer's protocols, but:

- Extend the incubation period in lysis buffer to an hour at 65°C in a thermomixer block set to 800 RPM.
- Optionally, extend the on-ice incubation (this is a good point for a lunch break) - a longer incubation can help with polysaccharide-rich tissues.
- Avoid using too much starting material, as this can block the DNeasy column membrane. It is particularly important not to overdo starting material from mucous-rich plants.
- Always centrifuge for 5 minutes at full speed after the first incubation (this is an optional step in the DNeasy manufacturer's protocol) to pellet the homogenized material.
- Use filter tips whenever transferring or mixing liquids with DNA in them.

Note

Following the lysis step, and depending on sample numbers, the DNA extraction can either continue into individual QIAGEN plant DNeasy mini-columns, or be transferred into a QIAGEN DNeasy extraction plate for bulk processing. However due to the use of different marker sets and primers, samples from different lineages should never be mixed throughout a plate. If a plate is mixed at all, it should be in such a way that DNA from different lineages forms complete strips of 8 that can be rearranged into lineage specific sets prior to PCR.

- A double elution, with 75 µL pre-heated 65°C QIAGEN elution buffer at the first step and 50 µL pre-heated 65°C QIAGEN elution buffer at the second step, is recommended, giving a final extraction volume of c. 100 µL.

Note

We are also extracting plant barcoding samples using Qiagen MagAttract Plant kits, following the manufacturer's protocols, and lichen barcoding samples using quarter reactions with the extraction and dilution solutions from REDExtract-N-Amp™ Plant PCR kits.

4 Storage

Silica gel dried plant tissue – this should be stored out of direct light, in a temperature and humidity controlled environment.

Note

Short-term, we store the silica gel dried plant material in plastic bags in closed plastic DToL labelled boxes in the DNA extraction lab, in case extractions have to be repeated.

Once successful sequences have been obtained for each batch, the silica gel dried samples and the relevant sample manifest and EDNA accession numbers are passed on to Herbarium staff for long term curation in the RBGE silica-dried tissue store.

TE-preserved lichen material – if any lichen thallus is left, return the tubes to the freezer drawer, in case extractions have to be repeated.

DNA - as we elute in a buffer that contains EDTA, the DNA can be stored at 4°C for easy access and to avoid freeze-thaw cycles. Long term banking into a freezer is only necessary after all PCR from that extraction set is complete, including rePCRs of problem material.

Note

Short-term, we store barcoding DNA in labelled 1.7 mL elution tubes, at 4°C. DToL tubes are arranged taxonomically in 96-sample racks (i.e. by the temporary extraction numbers: A = seed plants; F = ferns and lycophytes; B = mosses; L = liverworts, X = lichens, each in numerical order in different racks).

Once good quality barcode DNA sequences have been obtained for all samples within a set of tubes, the DNA is transferred, in the same order, to the RBGE DNA bank, in barcoded fluidX tubes that have been scanned in their 96-tube racks. These are stored in a -80°C freezer. The tube and plate barcode details are entered into the relevant database (at RBGE, this is our in-house DNA database, EDNA).