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Collections Standard Operating Protocol, Organism group: Lichens

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We use this protocol and it's working

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Abstract

This is part of the collection "DToL Taxon-specific Standard Operating Procedure (SOP) for the Plant Working Group". The SOP collection contains guidance on how to process the various land plant taxa and lichens within the scope of the Darwin Tree of Life (DToL) project. The guidance specifically refers to the collection and processing of tissue samples needed for DNA barcoding (which takes place at the Royal Botanic Garden Edinburgh (RBGE)) and outlines the flash frozen tissues required for whole genome sequencing (WGS), which takes place at the Wellcome Sanger Institute. Every specimen is submitted for DNA barcoding first, before potentially being sent to the Wellcome Sanger institute.

This Sample Collection SOP outlines the collection of lichen samples for the DToL project, which aims to generate high quality genome sequences from these samples. To achieve this goal, the DToL Genome Acquisition Labs (GALs) must collect a sufficient quantity of healthy living material, preserve it in a manner that conserves its DNA quality, and supply it to the appointed sequencing facility. Material must also be available and appropriately preserved for DNA barcoding and the provision of herbarium vouchers. It is the responsibility of the GALs to link accurate and information-rich metadata to all collections.

Definition: Lichens

Including: Lichenized Ascomycota and Basidiomycota (Agaricomycetes)

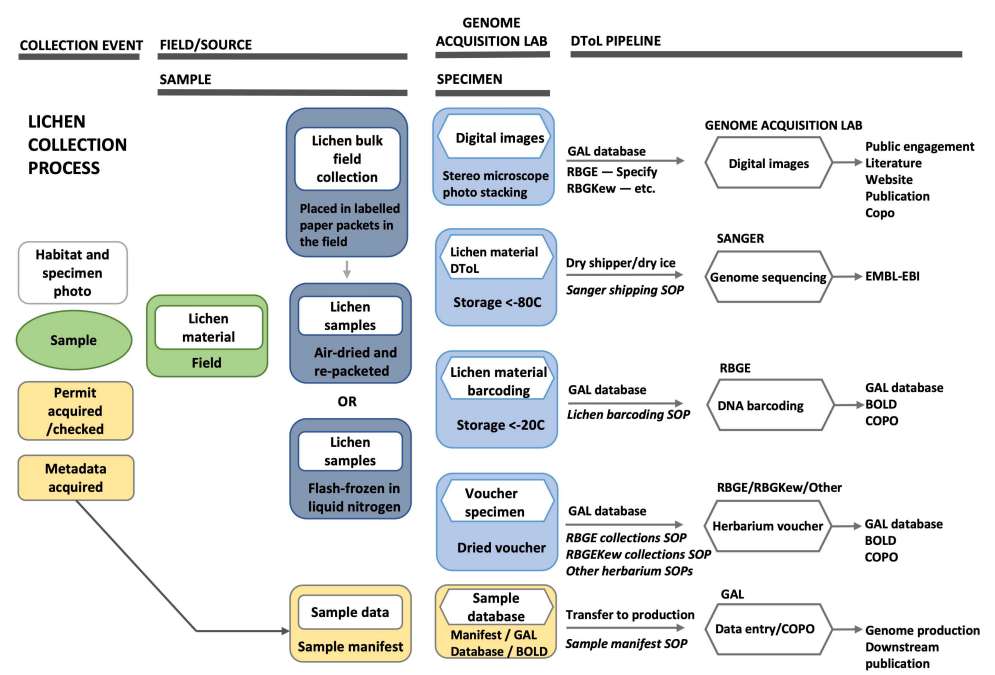
Excluding: Non-lichenized fungi

Guidelines

Including: Lichenized fungi (Ascomycota and Agaricomycetes of Basidiomycota)

1. Pre-fieldwork preparation

- Ensure collecting permits are in place and you are familiar with the Darwin Tree of Life (DToL) Code of Conduct document.
- Ensure all H&S documentation including institutional risk assessment documents have been completed and approved.
- Ensure the risk assessments for the use of liquid nitrogen has been completed.
- Ensure the risk assessment for Fieldwork Plant Health work has been completed and you are familiar with methods of footwear and tool decontamination.



Genome sequencing and DNA barcoding pathway for lichen specimens, with processing of samples in the lab only

2. Field sampling materials/considerations

Field collection equipment

Lichen samples are either a) processed in the field, or b) more often sent to a Genome Acquisition Lab (GAL) for processing. The GAL can provide collectors with FluidX tubes or sample containers/packets, and with printed and electronic datasheets to associate specimens with their collection vessels, for both genome sequencing and DNA barcoding.

Equipment

Sample collection carrier bag
Sample collection paper packets or bags
Marker pen
Knife/razor blades
EtOH for flaming
Sterilizing wipes
Lighter
Gloves
Forceps
Chisel
Safety glasses
GPS
Lichen spot test chemical kit
UV torch
Hand lens
Ruler
Field version of sample manifest
Seal-able bags - for genome size sample (if collecting sample in field)
FluidX tubes (if collecting straight to sample tube)
Barcode reader (if collecting straight to sample tubes)
Liquid Nitrogen shipper (if collecting straight to sample tubes)

Biosafety kit

Biosafety kits contain materials for cleaning footwear and field tools, and should be readily accessible at all times.

Sample collection vessels and storage

Lichen sample material can be collected directly into FluidX tubes if processing in the field (flash freezing with liquid N) and sent to Wellcome Sanger Institute (WSI) for genome sequencing. Alternatively, and more often, lichen samples are collected into labelled paper packets and taken back to RBGE (the GAL) for sample cleaning and picking, air dried and then frozen at -20°C. In the lab, samples are processed into FluidX tubes for whole genome sequencing. These are kept at -80°C until they are sent to WSI. A sub-sample is then processed for the associated DNA barcoding carried out at RBGE, and a corresponding herbarium voucher collection is made into a labelled envelope or packet.



Lichen collection packets air drying in the lab

3. Field sampling

Purpose: Correct collection methods for lichen material for genome sequencing, DNA barcoding, herbarium vouchers; the collection and collation of information-rich metadata describing the samples/specimens.

Note

Sample refers to the material collected in the field. In the case of lichens, this may reflect an individual, or a sample comprising multiple individuals. Specimen refers to a discrete physical unit of material that likely represents a single genetic individual (e.g. from a single thallus). Specimen ID numbers generated in DToL processing are unique for each genetic individual. Notes should be made to detail any information that might help interpret the status of specimens with regard to likely genetic homogeneity.

No sampling should be undertaken that results in a threat to rare species. Sampling should only commence after any necessary permits are in place.

Metadata

Please refer to the online version of the [DNA Barcoding Standard Operating Protocol, Plants and Lichens at RBGE, Sample Data](https://dx.doi.org/10.17504/protocols.io.dm6gp3y7jvzp/v1) for up-to-date information and instructions.

Note

Ensure you are familiar with the required data and format of the DToL sample manifest as this will facilitate the successful update of data. The Genome Acquisition Lab uses field versions of the manifest (electronic and hard copy) containing only the necessary columns for completion in the field. This includes instructions, e.g. Lat/Long decimal degree to a minimum of 3 decimal places, and a checklist of mandatory field columns.

Habitat and substrate photos

In situ photos should be taken in the field to provide information on lichen habitat/substrate and growth forms, as well as the possibility of mixed samples and nearby associated lichens or other organisms. Save photos as .JPG for upload to BOLD. Images should be named in a standard manner, preferably by the collector number as it appears in the manifest, a one-word description, and a number if there are several images in a series, e.g. MR204_habitat_1.jpg, MR204_habitat_2.jpg.



Lichen sample *in situ*



Lichen sample *in situ* showing wider substrate/habit

Collection of bulk samples in the field

Healthy living lichen material should be collected in the field, followed by processing in a lab with the use of a stereo microscope for sample cleaning and close-up photography.

Procedure:

1. Label a paper collecting packet with a collector number (or a temporary field collection number, e.g. site name and a number in a series). Wear gloves when handling samples (except when flame sterilizing).
2. *Corticolous/lignicolous crustose lichens*: use a flame-sterilized knife/blade to remove a shallow layer of wood/bark substrate and the lichen sample. *Saxicolous lichens*: use a chisel to cleave a portion of rock containing the lichen sample (wear safety glasses). If the rock surface should not be damaged for some reason, it is possible to slide a razor blade between the adhering layer of tissue and the rock surface. While slicing with one hand, apply pressure with the other thumb to the flat surface of the blade to achieve a thin slice (see image below). This technique does not work well when wearing gloves, instead, a sterilizing wipe can be used on hands. The resulting shaving of lichen will be thin, and can be carefully folded into tissue. In this case, it is imperative to have a good macro photo showing the specimen in detail. *Terricolous or foliose/fruticose lichens*: detach the lichen from its substrate, maintaining all diagnostic features intact (e.g. holdfasts), but avoiding excess unconnected material, soil, etc. *Basidiomycete lichens*: smaller fruiting bodies can be divided into parts, with portions sampled directly into FluidX tubes and flash frozen and another portion or fruitbody retained as a voucher, or vegetative thalli can be collected as above.
3. Place the sample in the collection packet and into a dry collecting bag.
4. On occasion, it may be necessary to collect multiple samples of material to obtain sufficient material for genome sequencing, DNA barcoding and a herbarium voucher. If there is a risk that the multiple specimens

representing a sample are from different genetic individuals, these should be collected, packaged and numbered separately.

5. Complete the mandatory fieldwork collections columns in the sample manifest and the data checklist provided.
6. Lichen samples should be air dried at the earliest opportunity, then re-packeted with provisional field IDs and collection numbers and stored at -20°C until processed for genome sequencing and DNA barcoding.



Slicing a thin layer of lichen tissue from the surface of a rock, in cases where the rock substrate cannot be damaged or collected

4. Lab-based processing

Purpose: Correct processing methods for lichen specimens to ensure the delivery of sufficient quantities of high quality preserved lichen material for genome sequencing and DNA barcoding.

Materials

1.9 ml FluidX tubes and 1.7ml Eppendorfs

Micro-forceps, needles, other micro dissection tools

Razor blades (double-edged)/scalpel and blades

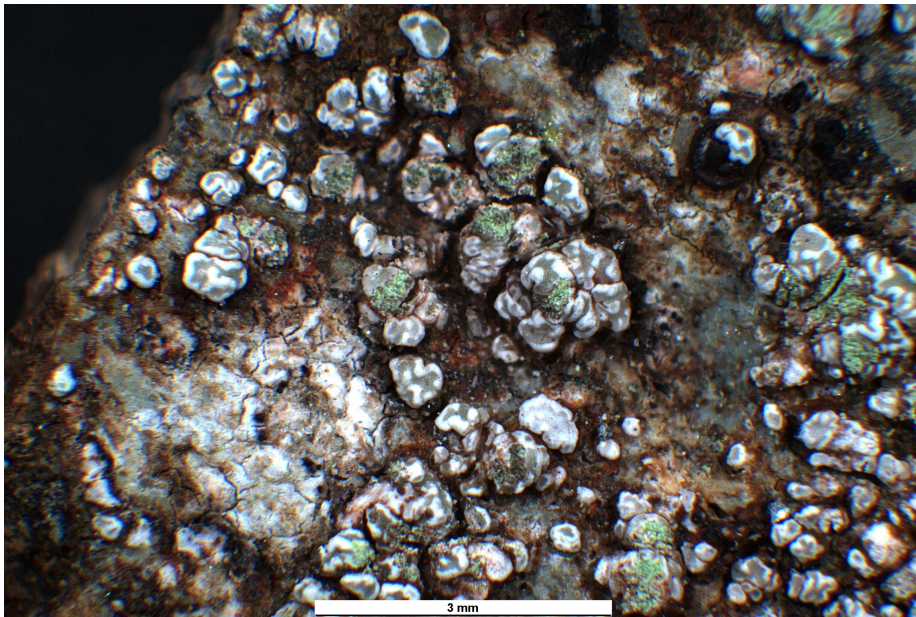
EtOH and a lighter for flaming

Clean white paper for amassing lichen tissue and funneling into tubes

Sample and specimen photos

Photos should first be taken to record the position of the lichen specimen in the context of the wider sample, substrate and other lichens in a mixed sample (this can be done with any high resolution phone camera, as the field of view of a stereo microscope is normally too narrow). Photos can be marked up on phone cameras or simple hand-drawn maps showing the specimen and specific area sampled can be kept with the photographs or specimens, respectively.

Under the stereo microscope, photograph the specific area and tissues/features comprising the specimen. Use a standard scale bar 3 or 5 mm (calibrated with the magnification used). Ideally, use the focus-stacking function to get better detail/depth of field to represent sampled areas. Ensure photographs are saved to a network drive with a naming convention following this format: collector number followed by tissue sampled (e.g. thallus/apothecium). If uploading images to BOLD, include sample ID in the name (the EDNA number).jpg.



Lichen specimen photo showing areas of tissue to be sampled

Sampling specimens for genome sequencing

Procedure:

1. Use 70% industrial denatured alcohol (IDA) to clean all work surfaces and use 100% ethyl alcohol (EtOH) to flame sterilize a blade/scalpel and forceps.
2. Working under a stereo microscope (generally 6X-20X magnification), locate an area of clean healthy lichen tissue near, but not at the growing edge of the lichen.
3. Using flame-sterilized forceps/blades, collect sterile tissue/thallus, placing the material into FluidX tubes. In cases where no thallus is present, sample soredia/soralia/isidia or apothecia, with preference for asexual material.

- Gently scrape asexual propagules onto a clean white piece of paper, amassing the material and funneling into the tube; or thinly slice apothecia before adding to a FluidX tube.
4. Continue sampling until c. 100–500 mg of material is collected; ideally collect 2 tubes per sample. A minimum of 50mg per tube is recommended.
 5. Store labelled sample tubes at -80°C until shipping.

Note

Ensuring collection of clean lichen material requires removing dirt/debris from specimens, including removal of sections of tissue containing rhizines or other structures which are likely to collect dirt, e.g. the lower surfaces of many Parmeliaceae lichens. Additionally, asexual propagules often contain contaminants and should be carefully examined before sampling.

Care should be taken to sample one individual, where possible. Note that apothecia from the same sample area may represent more than one genetic individual and attempts should be made to sample tissues originating from a single branch in the case of branching lichens, e.g. *Ephebe* and *Usnea*. Due to the small size of some lichens, collection of more than one individual may be necessary to provide sufficient material for genome sequencing.

Specimen documentation

In a notebook, document the size of the tissue area sampled, type of tissue sampled (e.g. apothecia or thallus), and the likelihood of the sample representing more than one individual. Draw a map of the sample area in relation to the wider sample and possible other individuals, other species, or contaminants. Fill in the DToL sample manifest, including noting whether the specimens are believed to be from a single individual, or that those specimens may represent more than one organism.



Lichen sample from a mixed species specimen



Rough map documenting the position of the lichen sampled from a rock with mixed lichen species

Shipping samples to the Wellcome Sanger Institute.

Information taken from the Sanger DTOL sample Submission SOP July 2020. Please refer to the online document for up to date information and instructions.

Prior to shipping any samples, the sample manifest needs to be completed and sent to treeoflifesamples@sanger.ac.uk whereupon the manifest will be checked and validated. You will be provided with a unique Sanger TOL (Tree of Life) Sample tracking ID for that batch of samples. Once your manifest is approved you will receive a validated version of the manifest and the Sanger TOL Samples team will agree with you when to ship your samples. Do not ship any samples until your manifest has been approved.

Sampling specimens for DNA barcoding

DNA barcoding is used as part of the DTOL 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). It is therefore crucial that wherever possible, the material used for DNA barcoding relates to the same genetic individual sent for genome sequencing. However, due to the amount of tissue required for genome sequencing, and the small size of many lichens, it will often be necessary to DNA barcode representative material (i.e. proxy barcoding) of the specimen that is genome sequenced, rather than sequencing the same genetic individual.

Sampling specimens for DNA barcoding follows the same basic procedures as above for genome sequencing. However, only 1-3mm² of clean, healthy lichen tissue is required--sampling into labelled 1.7 ml Eppendorfs and storing at -20°C until use in DNA barcoding.

Herbarium vouchers

After sampling for genome sequencing and DNA barcoding, sub-packet the remaining sample material within the larger specimen packet (which will be vouchered in the RBGE herbarium). Add a label/slip within the packet to indicate that the specimen has been sampled for DNA barcoding, including the sampler's name and a DNA number.

	A	B	C	D
		<i>Date</i>	<i>Changes</i>	<i>Contributors</i>
	1.0	April 2020	First draft	Laura L Forrest, Michelle L Hart
	1.1	6th June 2020	Updated flow chart and reference to Manifest and shipping SOPs	Michelle L Hart
	1.2	8th June 2020	Changes to Collecting for Barcoding procedure	Laura L Forrest
	1.3	16th May 2022	Updated protocol for dioicous species, and revised information for photographs	Laura L Forrest
	1.4	12th October 2023	New protocol for lichen collections	Amanda L Jones

Previous Version History, RBGE DTOL Sample collection Standard Operating Procedure_Bryophytes

Working SOP, checked by experts

