Jan 13, 2023

Collections Standard Operating Protocol, Plant group: Bryophytes

Forked from Taxon group: Non-larval Arthropods (TSS1)

In 1 collection

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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 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)) 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 plant samples for the Darwin Tree of Life project. DToL aims to generate high quality genome sequences from these samples. To achieve this goal the DToL Genome Acquisition Labs (GALs) must access 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, flow cytometry and herbarium vouchers. In some instances, material for RNA extraction is also required. It is the responsibility of the GALs to also link accurate and information-rich metadata to all collections.

Definition: Bryophytes.

Including: Bryophyta, Marchantiophyta, Anthocerotophyta.

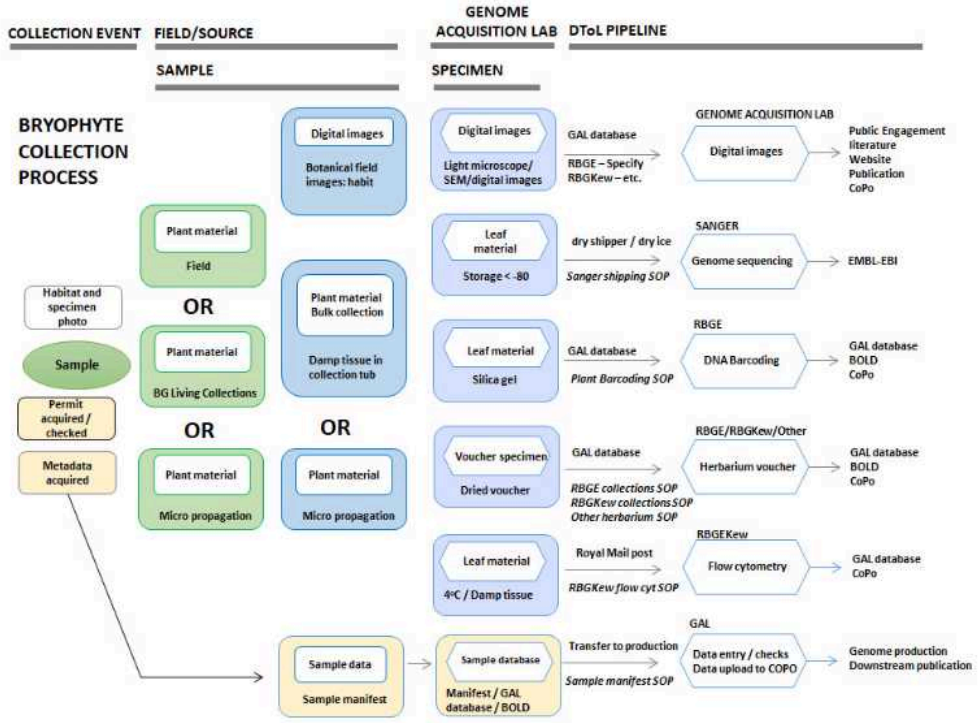
Excluding:

Guidelines

Including: Marchantiophyta (liverworts), Bryophyta (mosses), Anthocerotophyta (hornworts)

Pre-fieldwork preparation

- Ensure collecting permits are in place and you are familiar with the 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 silica gel 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 pathway for Bryophyte specimens, with processing of material in the lab only

Field sampling

Field collection equipment

Specimens are either a) processed in the field, or b) sent to a Genome Acquisition Lab for processing. The Genome Acquisition Lab can provide collectors with FluidX tubes or sample containers, and with printed and electronic datasheets to associate specimens with collection vessels. The Genome Acquisition Lab can also

provide equipment and specimen containers for DNA barcoding, genome size estimation and herbarium vouchering.

Equipment list
Sample containers - sealable plastic containers
Damp absorbent paper (kitchen roll)
Marker pen
Trowel
Knife
GPS
Cool box and ice packs/sheets
Field version of sample manifest
Packets of silica gel - for DNA barcode sample (if collecting sample in field)
Ziplock 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 for genome sequencing
Bulk collection into sample containers: We encourage bulk sampling of a clump of bryophyte material in the field, for subsequent processing in the lab at the Genome Acquisition Lab. Sample containers are sealable plastic tubs. Each container should be directly labelled. We recommend the inside bottom of these collection tubs be lined with kitchen roll dampened with water, to prevent the plants drying out, and that the tubs be quickly shipped/returned to the Genome Acquisition Lab in a cool box or on ice packs where possible (or at ambient temperature if that is similar to their natural growth conditions).

Sample collection for DNA barcoding
Plant DNA barcoding for the Darwin Tree of Life project is carried out at the Royal Botanic Garden, Edinburgh (RBGE). The majority of the sample sorting of bryophyte material for DNA barcoding will be undertaken in the labs at RBGE, not in the field. Thus the default position is collection and transport of fresh plant material in plastic tubs with dampened kitchen roll. However, individual bags of silica gel desiccant can be provided for the preservation of plant material for DNA barcoding by other institutes or for use in the field where required. Material that cannot be delivered in-person to RBGE should be sorted into ziplock bags and posted using Royal Mail first class delivery.

Sample collection for genome size estimation
Genome size estimation is carried out at RBG Kew. Plant material should be stored between sheets of damp, cool tissue-paper in a zip-lock bag in the field, or retained in the tubs with the bulk material and sorted into a zip-lock bag at the Genome Acquisition Lab. Material should be stored in a refrigerator at 4°C prior to being posted to RBG Kew.

Herbarium voucher collection

For the vast majority of bryophytes samples, voucher material will be processed at the Genome Acquisition Lab at RBGE, from the bulk material collected. If appropriate, however, the collection of voucher material can be made at the time of genome sampling in the field by separating some of the collection into a labelled envelope or folded paper packet, and leaving it to air dry.

Tupperware or picnic boxes with field-collected bulk bryophyte specimens for lab processing

Field-based sampling

Purpose: Correct collection methods of healthy living plant material for genome sequencing, DNA barcoding, flow cytometry and herbarium vouchers and the collation of information-rich metadata describing the specimens.

Note

Sample refers to the material collected in the field. This may be an individual plant or a bulk sample, comprising multiple individuals. Specimen refers to a discrete physical unit of material that is a single genetic individual. Specimen ID numbers are unique for each genetic individual. No sampling should be undertaken that results in a threat to rare species. Sampling should only commence after any necessary permit is in place.

Metadata

Please refer to the online version of the Recording Sample Metadata for DToL SOP 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 Sample Manifest (electronic and hard copy) containing only the 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.

Photographs

Habitat photos. Please save 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.

Plant habit photos. Please save 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_thallus_1.jpg, MR204_thallus_2.jpg; MR204_seta.jpg.

Collection of bulk samples in the field

It is critical that the sample is healthy living material when it is processed into specimens and placed in the FluidX tubes. We recommend the collection of bulk samples of bryophyte material in the field, followed by processing in a lab, with use of a dissecting microscope for sample cleaning and close-up photography.

If the species is known to be dioicous and sexual organs are present in the field, where possible a note should be taken of whether the specimen being sampled is male (with antheridia) or female (with archegonia, or with sporophytes attached). If both sexes are present at a site, they should both be sampled, so that a decision about which is most suitable for genome sequencing can be made after the collections have been genome sized - and allowing for potential additional sequencing or resequencing of the other sex.

Procedure

1. Line the sample container with H2O dampened kitchen roll.
2. Using a clean trowel or knife if required, sample a clump of bryophyte material, taking (if necessary) only a shallow layer of substrate.
3. Remove any unconnected plant material and insects.
4. Place the sample in the assigned sample container, ensuring the lid can fully seal.
5. On occasion, it may be necessary to collect multiple unconnected clumps of material to obtain sufficient material and/or for barcoding/voucher/flow cytometry. Collect any unconnected material into a different labelled sample container.
6. Place the sample container(s) in the cool box or on the ice packs.
7. Complete the mandatory fieldwork collections columns in the sample manifest and the data checklist provided.

Lab-based processing

Purpose: Correct processing methods of specimens to ensure the delivery of sufficient quantities of high quality preserved plant material for genome sequencing; correct processing methods of specimens for DNA barcoding, flow cytometry and herbarium vouchers; collation of rich metadata describing the specimens and catalogue of images for taxonomic description and public engagement literature.

Specimens for genome sequencing

It is critical that the plant material is healthy and living when it is processed into specimens and placed in the FluidX tubes. Samples must be processed in a clean and preferably cool environment, therefore we recommend processing the samples, using a binocular microscope, in a petri dish that sits on wet ice. Only FluidX barcoded tubes will be accepted at Sanger, either 1.9ml or 7.6ml. To ensure maximum DNA recovery, the specimens must be frozen as quickly as possible in the FluidX tubes, using either a FluidX cooling rack, dry ice or Liquid nitrogen.

Note

We have been advised to collect up to 10 tubes per specimen/sample, with up to 1g of material per 7.6ml FluidX tube. Where possible, these multiple specimens should be sampled from the same individual. Where this is not possible, due to the size of the sample, care should be taken to take specimens from the same part of a clump or from patches of the plant growing as closely as possible together. Please ensure details are added to the sample manifest stating if the specimens are believed to be from a single individual (physically connected) or may have come from multiple individuals. The SPECIMEN_ID must reflect the genetic identity of the individual. For example, ten different individual specimens (i.e. collections that are potentially from different genetic individuals) each in their own tube would have 10 distinct SPECIMEN_IDs, even if they are all from the same clump. However, a single specimen split across ten tubes would result in each of those ten tubes having the same SPECIMEN_ID.

Quantities of tissue required:

Genome sequencing: please collect up to 10 tubes per specimen. DO NOT PACK TISSUE TIGHTLY INTO THE TUBE. Sanger requests at least 150mg of plant tissue per 7.6ml FluidX tube (May 2022). **This will not be possible for many bryophytes - in these cases, collect as much as possible.**

c. 0.15g of fresh plant tissue from the angiosperm Agrimony (centre), the fern *Equisetum* (left) and the moss *Polytrichum* (right), with a lab Sharpie pen and a 7.6ml Fluidx tube for comparison. Note that for the moss, this includes several stems that we would usually treat as potentially separate genetic individuals for genome sequencing

Procedure

1. Precool the FluidX cooling rack and/or FluidX tubes on dry ice. We recommend the use of a FluidX cooling rack so that tubes are stable and at the right temperature to receive tissue.
2. Gently remove any substrate material (soil/plant debris) from a clump of the sample as follows: wash thoroughly with several changes of water, gently rubbing at any clumps of dirt with a mounted needle or no. 5 forceps to loosen it. Blot dry between rinses with tissue paper or filter paper (which can also help remove some of the dirt). Remove rhizomes with either forceps or a scalpel, particularly if they're dense, as they can contain a lot of dirt and fungi. Place all waste material into an autoclave bag for safe disposal.
3. Place the cleaned material into a 100mm petri dish with a little water.
4. Working with the petri dish on wet ice, gently tease the clump of tissue apart, taking care to keep connected strands intact.
5. Using clean tweezers, carefully remove a strand or segment of tissue no wider than 10mm or longer than 70mm (for use of 7.6ml tubes). Place the specimen into a single pre-chilled FluidX tube, noting the barcode number/rack position. Please do not fill tubes to the top as it is then not possible to get the tissue out without risk of thawing. **Note: Read manifest SOP on the correct use of ID numbers for multiple specimens from the same individual sample vs bulk sample**.
6. With the FluidX tubes still on dry ice, transfer the samples to the -80oC freezers and register the sample in the DToL Manifest.

All tissue must be kept as cold as possible as fast as possible, so whenever possible place the tubes containing specimens immediately into the -80oC freezer for storage until shipping to the Sanger Institute, Dry ice and liquid nitrogen are suitable for material for genome sequencing. Wet ice and -20oC freezers are not suitable at any point for the storage of FluidX tubes containing these samples, and freeze/thaw cycles must be avoided.

Note

Dwarf males can be found growing on the females of some mosses - in species where these are known to occur, extra care must be taken in separating out genetic individuals.

Shipping samples to the 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 Sample RT tracking ID for that batch of samples. Once your manifest is approved you will receive back a validated version 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.

Samples for DNA barcoding

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). 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 most bryophytes, 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. Thus for bryophytes, DNA barcoding may either involve:

(a) DNA barcoding the same individual that is genome sequenced (only for species with very large individuals),
(b) DNA barcoding an equivalent discrete genetic individual collected in immediate proximity and with identical morphology to the genome sequenced individual (where the individual is large enough for Sanger sequencing), or
(c) DNA barcoding a 'clump' of tissue, with identical morphology to the genome sequenced individual but consisting of multiple individuals to give enough DNA to obtain a DNA barcode sequence.

Please ensure details are added to the sample manifest stating if the barcode specimen has the same genetic identity as the genome sequenced specimen, or if it is a proxy barcode from one or more adjacent conspecific individuals.

Quantities of tissue required for barcoding: DNA barcoding: minimum tissue amount c. 5mm2, where smaller than this, pool tissue from multiple individuals. [Link to RBGE Barcoding SOP]

Procedure

1. Prepare and label a 100mm zip-lock bag containing approx. 10g of silica gel. Label with the COLLECTOR_ID number.
2. Processing procedure is the same as sampling for genome sequencing - Gently remove any substrate material (soil/plant debris) from a clump of the sample (up to 50 × 50mm). Place all waste material into an autoclave bag for safe disposal.
3. Place the cleaned material into a 100mm petri dish with a little water.
4. Working with the petri dish on wet ice, gently tease the clump of tissue apart, taking care to keep connected strands intact.
5. Using clean tweezers, carefully remove individual strands or clumps of connected strands.
6. Remove as much water as possible by gently blotting the material on a tissue.
7. Place the specimen in the labelled bag of silica gel.
8. All tissue must be dried as soon as possible. Please ensure there is sufficient silica gel in the specimen bag to totally submerge the plant tissue.
9. Record the COLLECTOR_ID and register the sample in the DToL Manifest. Email the completed manifest to xxxx@rbge.org.uk, cc lforrest@rbge.org.uk.
10. Post the sample to the address given below, or bring it to the DToL silica gel collection at RBGE.

DToL Barcoding Submission % Laura L Forrest
Balfour 1.01
Royal Botanic Garden, Edinburgh
20A Inverleith Row
Edinburgh, Scotland
EH3 5LR

Samples for Genome size estimation (flow cytometry)

Genome size estimation is used to inform sequencing effort and genome assembly. Genome size estimation, by flow cytometry, will be carried out at RBGKew for all plant samples. Flow cytometry requires living material; this can briefly be stored at 4°C to preserve condition.

Note

Please contact Ilia Leitch at Kew, prior to fieldwork, to inform of your intention to ship samples to Kew for flow cytometry. Shipping without prior agreement may mean that Kew is unable to appropriately deal with your samples on arrival, and the material will not remain suitable for flow cytometry for longer than a few days.

Quantities of tissue required for genome size estimation: 4 to 6 20mm long strands of actively growing material, preferably from several individuals [link to RBGKew flow cytometry SOP]

Procedure

1. Prepare and label zip-lock bags with water-dampened kitchen roll/white roll. Label with the COLLECTOR_ID number.
2. Processing procedure is the same as sampling for genome sequencing - Gently remove any substrate material (soil/plant debris) from a clump of the sample (approx 50 × 50mm). Place all waste material into an autoclave bag for safe disposal. Place the cleaned material into a 100mm petri dish with a little water.
3. Working with the petri dish on wet ice, gently tease the clump of tissue apart, taking care to keep connected strands intact.
4. Using clean tweezers, carefully remove individual strands or clumps of connected strands.Rhizoids can be included for these samples.
5. Place the specimen in a labelled zip-lock bag.
6. Record the COLLECTOR_ID in a Sample Processing Sheet and register the sample in the DToL Manifest.
7. Store the specimens at 4°C prior to shipping to Kew.
8. Ship the sample, in a padded envelope, to the address given below. Use Royal Mail first class post; also include a print-out of the manifest details for the samples in the envelope.

DToL % Ilia Leitch
Royal Botanic Gardens, Kew

Richmond, Surrey,
TW9 3DS, UK.

Voucher specimens

After removing as much substrate as possible without washing or damaging the bryophyte, put a small clump of the plant into a labelled paper packet and dry at room temperature with the packet open to facilitate air flow. Label with the COLLECTOR_ID number.

Put delicate fertile material or material that has been washed into separate small sub-packets within the main packet. Label these with the COLLECTOR_ID number, specifics of the separated tissue (e.g. "mature sporophytes"), and any treatment given.

If parts of any of the individual plants that were sampled for genome sequencing, barcoding and/or flow cytometry remain, put these into individual labelled sub-packets within the main packet. This sub-packet should be labelled with the COLLECTOR_ID number and the SPECIMEN_ID number to identify the material as the same genetic individual used for genome sequencing.

Air dry specimens at ambient temperature as quickly as possible to prevent mould. Ideally open the packets and use a fan to provide air circulation. Avoid direct heat.

After drying, transfer specimens to archival paper storage packets. Ensure all material, including any dirt at the bottom, is transferred, as this may include small structures that have become detached.

Annotate the packet to mark whether the material is the same as that used for genome sequencing or if it is a proxy voucher (morphologically identical material, collected adjacent to the sequenced individual).

	A	B	C	D
		Date	***Changes***	***Contributors***
	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

	A	B	C	D
	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

Previous Version History, RBGE DToL Sample collection Standard Operating Procedure_Bryophytes

Working SOP, checked by experts

Troubleshooting