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Collections Standard Operating Protocol, Plant group: Vascular Plants

Forked from Collections Standard Operating Protocol, Plant group: Bryophytes

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.

Sample collection for genome sequencing
Plant genome sequencing is carried out at the Sanger Institute; there is a cold chain from sample collection through to it arriving in the Sanger laboratories.

Sample collection for DNA barcoding
Plant DNA barcoding for DToL is carried out at the Royal Botanic Garden, Edinburgh (RBGE).

Sample collection for genome size estimation
Genome size estimation is carried out at RBG Kew.

Herbarium voucher collection
Where possible, the collection of voucher material is at the time of genome sampling in the field. Further preparation of the herbarium specimen can be carried out at a Genome Acquisition Lab.

Definition: Tracheophyta (vascular plants)

Including: Polypodiophyta, Pinophyta, Magnoliophyta

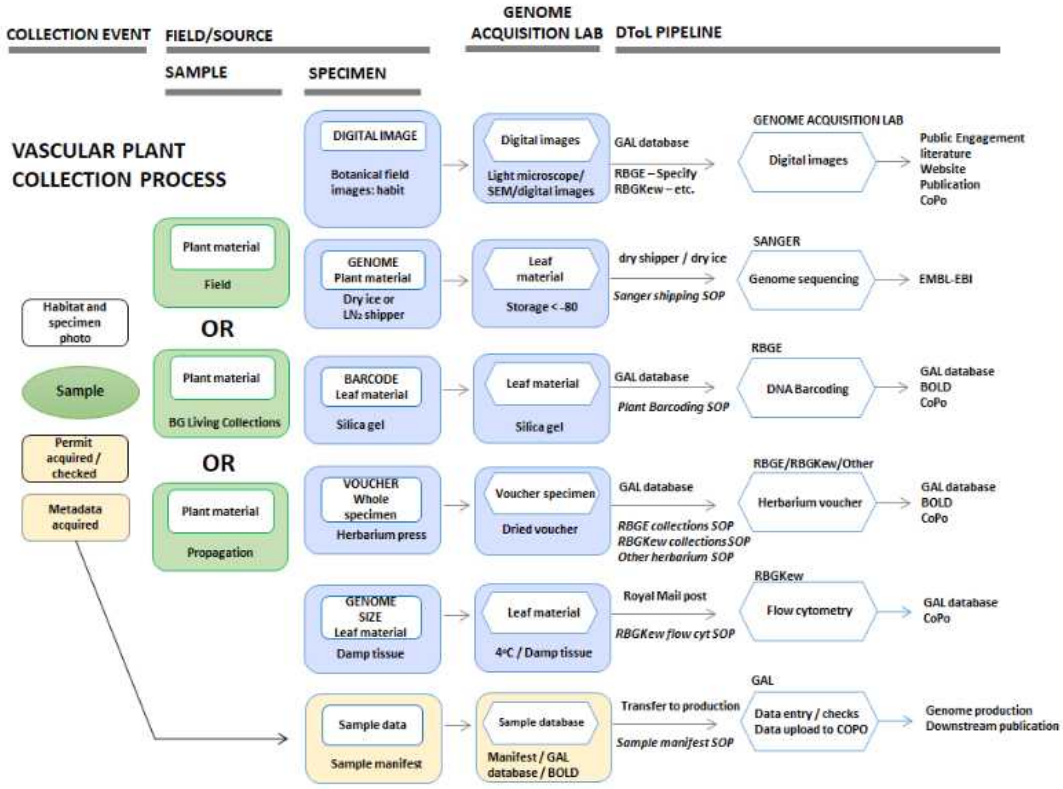
Excluding:

GUIDELINES

Including: Polypodiophyta (ferns), Pinophyta (conifers), Magnoliophyta (flowering plants)

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 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, vascular plants

Sampling

Field collection equipment

Samples for DToL 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.

Sampling equipment ready for field work

Equipment list

- Marker pen/pencil/pen
- Trowel
- Secateurs
- Sterilising wipes
- 5 inch forceps
- Fine watchmaker forceps
- 150 mm x 150 mm white dissecting tile
- 1.5 ml eppendorf tubes with attached lid
- GPS
- Cool box and ice packs/sheets with insulation (to prevent direct contact with leaf material)
- 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)
- Damp absorbent paper (kitchen roll)
- 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)
- Herbarium press

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
Only FluidX barcoded tubes are accepted at the Sanger Institute for DNA and RNA sample material; these can be provided by the Genome Acquisition Lab. Each single FluidX tube is barcoded and all barcodes must be scanned. Do not manually type tube barcode numbers into the manifest. A portable single tube scanner can be provided. For most plant collections, 7.6 ml FluidX tubes are used. For small specimens, 1.9 ml FluidX tubes are also available. FluidX tubes are supplied in a storage rack, already arranged consecutively by barcode number, for ease of use and tracking.

Sample collection for DNA barcoding
Plant DNA barcoding for DToL is carried out at the Royal Botanic Garden, Edinburgh (RBGE). Individual bags of silica gel desiccant can be provided for the preservation of plant material for DNA barcoding. The dried plant material should be posted to RBGE 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. Material should be stored in a refrigerator at 4°C prior to being posted to RBG Kew using Royal Mail first class delivery. The Genome Acquisition Lab can provide cold storage in the field - either cool packs or portable/chargeable cool boxes. Zip-lock bags can also be provided.

Herbarium voucher collection
Where possible, the collection of voucher material is made at the time of genome sampling in the field. Further preparation of the herbarium specimen is carried out at the Genome Acquisition Lab. A plant press can be provided.

Purpose: Correct collection 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.

No sampling should be undertaken that results in a threat to rare species.

Sampling should only commence after any necessary permit is in place.

Note

Here, "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.

Metadata: Please refer to the online version of the Recording Sample Metadata for DToL SOP for up-to-date information and instructions.

Note

Familiarity with the required data and format of the DToL sample manifest 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.

Sample metadata should include observations that might be useful for interpreting the genomic data. In particular, the sex of dioecious plants should be recorded, and the floral morph of heterostylous plants added to the Notes field.

Photographs: The Genome Acquisition Lab can provide a list of required/desired images, including:

Habitat photo with the entire plant and some of the surroundings.

Close-up pictures, including scale bar, of vegetative and fertile material (e.g. flowers, fruit, cones, fern sporangia).

Note

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_leaf_1.jpg, MR204_leaf_2.jpg; MR204_flower.jpg.

Collection of samples in the field

Tissue samples for genome sequencing

The exact method of collection will vary depending on the size and habit of the sample. It is critical that the sample is **healthy living material** when it is processed and placed in the FluidX tubes. 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 a dry shipper.

- Where possible, multiple specimens should be sampled from the same individual. For example, for large plants, all specimens (material for the genome sequencing, DNA barcoding, flow cytometry and voucher) should be from the same individual.
- Where this is not possible, due to the size of the sample, care should be taken to make specimens from the same part of a clump/population. For example, where individuals are not sufficiently large, all genome specimens should be from the same individual, but material for RNA, DNA barcoding, flow cytometry and the voucher specimen can be taken from other individuals within the same clump or population.
- Note that **if the plant has developing seeds, these cannot be assumed to be the same genetic individual as the parent plant** and should not be included in the material sampled for genome sequencing, although they can be included in the other samples.
- Where the plants are very small, each tissue collection for genome sequencing may be a different individual and specimens for DNA barcoding, flow cytometry and the voucher may be different individuals or a pool of multiple individuals.
- For dioecious plants, if flowers are present, note whether the sample is male (anther-bearing) or female (ovary-bearing); when both sexes are present in the population, collect samples from both for genome sequencing and for genome sizing, so that the decision about which to sequence may be made later. If fruits are present, note that the specimen is a female plant. Otherwise record sex as unknown.

Please ensure details are added to the sample manifest stating if the specimens are from a single individual (physically connected) or from multiple individuals. The SPECIMEN_ID must reflect the genetic identity of the individual. For example, ten different individual specimens each in their own tube would have 10 distinct SPECIMEN_IDs, even if they are all from the same species. However, a single specimen split across ten tubes would result in each of those ten tubes having the same SPECIMEN_ID.

Note

We currently collect up to 10 tubes per sample, with a minimum of 0.15g (Sanger Institute advice, May 2022), up to 1g of material per 7.6ml FluidX tube. Where possible, these tissues are sampled from the same individual plant. Where this is not possible, due to the size of the plant, care is taken to take material from plants growing as closely as possible together.

Details are added to the sample manifest stating whether the samples 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 samples (i.e. collections that are potentially from different genetic individuals) each in their own tube have 10 distinct SPECIMEN_IDs. However, a single plant with sampling split across ten tubes results in each of those ten tubes having the same SPECIMEN_ID.

Quantities of tissue required:
Genome sequencing: collect up to 10 tubes per plant specimen. DO NOT PACK TISSUE TIGHTLY INTO THE TUBE. Sanger requests at least 150 mg of plant tissue per 7.6 ml FluidX tube.

Relative amounts of tissue: c. 0.15 g 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.

Procedure
Plant material is sampled from a range of tissue types, depending on what is available. Where possible, make some of the collections from different tissue types, placing each different sample into a new FluidX tube. Prioritize samples from leaf buds/flower buds/leaves (unless the purpose is to collect specific tissue types for RNA extraction). Avoid collecting seeds for genome sequencing, as they are likely to be separate genetic individuals to the maternal plant.

Collecting plant material for genome sequencing: flash freezing tissue in liquid nitrogen, sampling from the RBGE living collection

Note

Example 1: LEAF MATERIAL from a tree species

1. Select a young, fully expanded leaf which appears free of pests or disease.
2. For ease of access, using sterilised secateurs, remove the branchlet holding your leaf of choice.
3. Again, check for signs of pest or disease.
4. Using a clean unused 1.5 ml eppendorf tube, ‘hole punch’ the leaf to produce a series of approx. 3mm diameter leaf discs. Avoid sampling the mid-rib if possible, as this prevents even grinding of the tissue in the DNA extraction process. Alternatively, tear the leaf tissue into approx. 5mm x 5mm pieces, again avoiding the mid-rib if possible.
5. Place the material into a single pre-chilled FluidX tube, noting the barcode number/rack position. Place immediately into the field cold storage - either dry ice/liquid nitrogen/dry shipper.
6. Do not jam material into the FluidX tubes - the leaf material should be able to be easily removed. Do not fill tubes up to the top as it is then not possible to get the tissue out without risk of it thawing.
7. On occasion, it may be necessary to collect samples from more than

one individual, to obtain sufficient material and/or for barcoding/voucher/flow cytometry. **Note: Read manifest SOP on the correct use of ID numbers for multiple specimens from the same individual sample vs bulk sample**.

8. Complete the mandatory fieldwork collection columns in the sample manifest and the data checklist provided.

Note

Example 2: LEAF BUD/FLOWER BUD

1. Select healthy leaf/flower buds.
2. For ease of access, using sterilised secateurs, remove the stem/branch holding your leaf/flower bud of choice.
3. Again, check for signs of pests or disease.
4. Using clean 5 inch forceps, remove the bud from the stem/branch.
5. Using clean watchmakers' forceps, and the dissecting tile if necessary, carefully remove the outer bud bracts. Again, check for signs of pests or disease.
6. Remove as much of the petiole/pedicel as possible, as these harder structures may prevent even grinding of the tissue in the DNA extraction process.
7. Place bud/buds into a single pre-chilled FluidX tube, noting the barcode number/rack position. Place immediately into the field cold storage - either dry ice/liquid nitrogen/dry shipper.
8. Do not jam material into the FluidX tubes - the material should be able to be easily removed. Do not fill tubes up to the top as it is then not possible to get the tissue out without risk of it thawing.
9. On occasion, it may be necessary to collect samples from more than one individual, to obtain sufficient material and/or for barcoding. **Note: Read manifest SOP on the correct use of ID numbers for multiple specimens from the same individual sample vs bulk sample**.
10. Complete the mandatory fieldwork collection columns in the sample manifest and the data checklist provided.

Note

Example 3: LEAF MATERIAL from small herb, e.g. willowherb

1. Prioritize samples from leaves (unless the purpose is to collect specific tissue types for RNA extraction).
2. Select a young, fully expanded leaf which appears free of pests or disease.
3. Using clean forceps, remove your leaf of choice.
4. Again check for signs of pests or disease.
5. Using a clean unused 1.5 ml eppendorf tube, 'hole punch' the leaf to produce a series of 3mm diameter leaf discs. Avoid the mid-rib if possible, as this prevents even grinding of the tissue in the DNA extraction process. Alternatively, tear the leaf tissue into approx. 5mm x 5mm pieces, avoiding the mid-rib. If the leaves are small, using the watchmakers forceps, remove the leaves from the stem, removing any mid-rib if possible.
6. Place the material into a single pre-chilled FluidX tube, noting the barcode number/rack position. Place immediately into the field cold storage - either dry ice/liquid nitrogen/dry shipper.
7. Do not jam material into the FluidX tubes - the leaf material should be able to be easily removed. Do not fill tubes up to the top as it is then not possible to get the tissue out without risk of it thawing.
8. It may be necessary to collect samples from more than one individual, to obtain sufficient material and/or for barcoding/voucher/flow cytometry. **Note: Read manifest SOP on the correct use of ID numbers for multiple specimens from the same individual sample vs bulk sample**.
9. Complete the mandatory fieldwork collection columns in the sample manifest and the data checklist provided.

Note

Example 4: WHOLE STEM including leaves, e.g. tiny eyebright

1. Select a young growing stem with fully expanded leaves which appears free of pests or disease.
2. Using clean forceps, remove the stem or for very small specimens, the whole plant.
3. Again, check for signs of pests or disease.
4. Remove all root material.
5. Place the plant material into a single pre-chilled FluidX tube, noting the barcode number/rack position. Place immediately into the field cold storage - either dry ice/liquid nitrogen/dry shipper.

6. Do not jam material into the FluidX tubes - the plant material should be able to be easily removed. Do not fill tubes up to the top as it is then not possible to get the tissue out without risk of it thawing.
7. It is likely that multiple samples will be required to obtain sufficient material and/or for barcoding/voucher/flow cytometry. **Note: Read manifest SOP on the correct use of ID numbers for multiple specimens from the same individual sample vs bulk sample**.
8. Complete the mandatory fieldwork collection columns in the sample manifest and the data checklist provided.

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

Shipping samples to the Sanger Institute.

Information taken from the Sanger DToL sample Submission SOP (July 2020); refer to the online document for up to date information and instructions.

Note

Prior to shipping any samples, the sample manifest is completed and sent to treeoflifesamples@sanger.ac.uk whereupon the manifest is 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, as well as for 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. Where this is not possible due to the size of the sample, care should be taken to sample from the same clump/patch of plants.

> **Note**
>
> 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 DNA barcoding: DNA barcoding: minimum tissue amount c. 5 mm^2; where individual plants are smaller than this, pool tissue from multiple individuals. [Link to RBGE Barcoding SOP]

Procedure

1. Prepare and label 50 mm zip-lock bags with 10 g of silica gel OR prepare and label a teabag. Label with the COLLECTOR_ID number.
2. Processing procedure is the same as sampling for genome sequencing - use a clean 1.5 ml eppendorf to prepare leaf discs; use sterilised fine forceps to remove small leaves; use sterilised fine forceps to break any stems up into 5 mm fragments.
3. Place the tissue to be barcoded in the labeled bag of silica gel/tea bag.
4. All tissue must be dried as quickly as possible. Please ensure there is either sufficient silica gel in the zip-lock specimen bag to totally submerge the plant tissue or sufficient DRY silica gel in the storage tub to cover tea bag specimens.
5. Record the COLLECTOR_ID and register the sample in the DToL Manifest. Email the completed manifest to edtolnumbers@rbge.org.uk, cc lforrest@rbge.org.uk.
6. 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, is carried out at RBGKew for all DToL plant samples. Flow cytometry requires living material; this can briefly be stored at 4°C to preserve condition.

> **Note**
>
> Please contact Ilia Leitch (i.leitch@kew.org) and Sahr Mian (s.mian@kew.org) 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: up to 5 cm^2 of young, actively growing material, avoiding any pigmented areas if possible [link to RBGKew flow cytometry SOP]. While most analyses are conducted on leaf material, other tissues such as buds, petioles and stems can also be collected if available as, in some cases, they may provide better results especially if leaves are heavily pigmented. In addition to the material from the plant that has been sampled for DToL, collect material from 3 or 4 additional individuals from the site, where possible, placing these into a separate zip-lock bag within the main bag.

Procedure

1. Prepare and label zip-lock bags with water-dampened kitchen roll/white roll. Label with the COLLECTOR_ID number.
2. Remove intact leaves/whole stems.
3. Place the specimen in a labelled grip seal bag and store in the cool bag - avoid direct contact between the leaf material in the specimen bag and ice pack (wrap ice pack in tissue).
4. Record the COLLECTOR_ID in a Sample Processing Sheet and register the sample in the DToL Manifest.
5. Store the specimens at 4°C (refrigerator) prior to shipping to Kew (N.B. Do **not** freeze samples at any point as this will degrade the nuclei and reduce the quality of the genome size estimate).
6. 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 that are contained in the envelope.

DToL ℅ Ilia Leitch/Sahr Mian
Jodrell Laboratory
Royal Botanic Gardens, Kew
Richmond
Surrey TW9 3DS
UK.

Voucher specimens

A voucher specimen should be made for all collections. Ideally the voucher will be the same genetic individual sent for genome sequencing. Where this is not possible due to the size of the sample, take care to make the voucher from the same clump/patch of plants. In addition to a voucher of the genome sequence specimen, it may be prudent to voucher other representative material (e.g. proxy voucher), for example to capture fruit or floral structure. Ensure details are added to the sample manifest stating if the voucher specimen has the same genetic identity as the genome sequence specimen. The voucher may also be made from more than one individual from a clump if the plant is particularly small, but make a note where this is the case.

Procedure

1. Selecting material

- Select material from the plant.
- For larger plants take all material from a single individual – do not mix individuals.
- For smaller plants, where multiple individuals are collected, any material from the individual used for genome sequencing must be processed separately.
- Include fertile material (e.g. sporangia, cones, flowers, fruit) if possible.
- Include extra material (e.g. leaf, flower, fruit) for later dissection
- Include below-ground parts (e.g. roots, bulbs, corms) if possible.

2. Preparing Material for Pressing

- Remove soil and any obvious invertebrates.
- Ensure the material will fit a standard herbarium sheet (42 x 26.4 cm). Either trim excess or divide into two or more sheets, clearly marked as such (e.g. sheet 1 of 2, sheet 2 of 2...)
- Remove any excess material to reduce bulkiness and ensure important features are visible.
- Make all cuts obvious, e.g. keep leaf base or section of petiole on specimen.

- For fertile angiosperms, prepare a flower to show internal structure.
- Cut bulky tissues (e.g. fruits and seeds, fleshy roots/bulbs/corms) in half.
- Any bulky fruits or seeds need a separate label.

3. Pressing Plant Material

- Lay out on a folded sheet of newspaper, leaving space for the label and a capsule (envelope) to contain loose material.
- Leave loose – do not attach to the newspaper with tape.
- Mark newspaper with COLLECTOR_ID number on outside bottom left hand corner – clearly mark any specimens which need more than one sheet.
- Ensure that both sides of the leaf can be seen by turning at least one leaf the other way up. If there is only one leaf, fold it to show both sides.
- Fold any long stems or leaves into 'N', 'V' or 'Z' shapes, as appropriate.
- Place newspaper between blotting paper in a plant press.

4. Drying

- Dry specimens using a drying frame over a source of heat, ensuring the heat source is not too hot, to prevent the specimens becoming brittle.
- Alternatively place the plant press in a warm, dry place (e.g. a drying room).
- Keep the number of specimens in the press to a minimum to aid drying.
- Change the blotting paper daily until the specimens are dry.
- Corrugated metal sheets can be used at intervals in the press, to increase heat and air flow.

The dried herbarium specimens are posted to a Herbarium where they will be databased and mounted.

Mounted specimen ready to be laid away in the RBGE herbarium

	A	B	C	D

	A	B	C	D
		Date	***Changes***	***Contributors***
	1.0	July 2020	First draft	Michelle L Hart, Laura L Forrest
	1.1	8th July 2020	changes to Collecting for Barcoding procedure and Genome sequencing procedure	Pete Hollingsworth, Alex Twyford
	1.2	9th June 2020	Changes to Specimens for Genome sequencing procedure	Michelle L Hart
	1.3	26th March 2021	notes on recording plant sex	Alex Twyford
	1.4	16th May 2022	Note on collecting both male and female individuals for dioecious species; notes on naming plant image files	Laura L Forrest

Previous Version History, RBGE DToL Sample collection Standard Operating Procedure_Vascular

Working SOP, checked by experts