

Short manual for collection and documentation of plant tissue samples for DNA analysis

How to select collection sites?

An individual with typical morphology from a regular habitat is to choose if only one individual per location and species shall be sampled. In general the sampling schema results from the analytical question to be answered. The selection of all **collection sites** of a taxon should represent its **morphological variability** and its entire **distribution range** if there is no other primary sampling objective.

To determine the genetic variability among populations it is statistically recommended to include 12 samples per collection site and population (Collect a minimum of 13 samples to compensate the loss of a sample during DNA analysis).

What and how to collect?



Figure 1: Herbarium specimen and tissue of *Corydalis cava*. Well documented DNA including a digital voucher image and detailed collection and DNA extraction data of that sample is available via the DNA Bank Network's webportal (www.dnabank-network.org)

Voucher specimen (herbarium specimen) – A least one full-grown flowering or fruiting individual including its roots has to be taken per sample site and prepared as voucher specimen for the herbarium. That individual must be labelled unmistakable by a collector's number (see below). Collection data should be recorded into a collection data list (see below) referring to that number.

Tissue samples – To extract high amounts of undegraded DNA young, fresh, and healthy leaf tissue should preferably be sampled. The tissue material should be taken using a tweezer. Samples can be stored and dried in paper or tea bags (see figure 2) one of each separately. Sample bags should at least be labelled by the collector's number and might have a unique tissue barcode and/or the species name too.

5 to 10 cm² leaf material per individual resulting in up to 0,1 g tissue after drying is sufficient for most molecular analyses. If too much material is sampled incomplete drying in the field will cause DNA degradation.

Please note that tissue material should as well be taken from the individual for the herbarium (herbarium specimen). That sample must be labelled to be unambiguously assignable to its source specimen.

About 10 to 20 sample bags, or e.g. all samples of a population, can be packed together with silicagel into air-tight zip-lock bags for drying and interim storage (see figure). Paper resp. tea bags should be folded carefully that the drying material cannot slip out of its bags or intermingle during transport.

Population samples - If multiple probes of a population are taken individuals of the sample set should represent the morphological variability of the population (if that is not against your collection goal) but they should precisely be assignable to a dedicated taxon. As a rule of thumb when higher plants are collected the sampled area should be at least 400 m². The population size should be estimated and noted. If seeds are sampled it should be kept in mind that they represent populations of related individuals (seed families). Thus, put seeds from different individuals into different bags and extract/analyse the DNA from single seeds or of seed families.

Hybrid samples - Samples from individuals which might be of hybrid origin should be labelled as such. Herbarium specimens of that samples should always be collected. Please note that often F1 hybrids only have intermediate characters, F2 and higher hybrids might have partly intermediate, extreme, and completely new features.

How to store the samples

The zip-lock bag resp. the silica gel should be checked for colour change after one or few days due to the water content of the sampled tissues. The silicagel has to be replaced if the colour indicator shows water saturation.

Dry samples can be stored in silicagel for years if low humidity can be guaranteed permanently.



Figure 2: Five tea bags containing tissue material, silica-gel to be put into a zip-lock bag for tissue drying and interim tissue storage.

What and how to document?

Sample bags should be labelled with the collector's number and unique tissue barcode to be identifiable unambiguously. It proved to be useful to label all samples by a systematic letter-figure combination. That might include the collector's initials, abbreviation/number of location, abbreviation of the species, serial number when several individuals per location (e.g. MR JAL 04-02; HZ KAB 23-06). The herbarium specimen and its associated tissue sample belonging to the same individual should be labelled that their relation can be traced back, in the case of population samples it could be labelled -01 (e.g. MR JAL 04-01).

Sample and Collection data

It becomes increasingly important to document all collected samples properly and to prepare data to be stored in primary data repositories for its long-term accessibility via data portals such as GBIF or the DNA Bank Network. To facilitate that process please fill in a suitable collection data form (e.g. available via <http://www.dnabank-network.org/downloads/collectiondataform.xls>) or contact the research collection/primary data repository you plan to store your specimens/tissue or DNA samples after project completion.

Summary: Important points to consider

- Distribution range, ecological preferences of the species/taxon
- Collection permissions
- Timing the collection trip, contact to collaborators/regional experts
- Collection tools and tissue fixation and storage materials
- Documentation form

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