

Short manual on how to collect plant material for DNA purposes

For each plant collected the following is compulsory:

a) **Herbarium voucher:** At least two specimens of the same plant must be prepared. One will be kept at the University of Johannesburg Herbarium, and the second deposited to a main herbarium in the region where the plant was collected.

b) **Few leaves stored in silica gel:** The silica gel is a hygroscopic material that is used to remove all moisture from plant material and conserve the DNA in the best condition as possible for further molecular work. Silica gel can be obtained from chemical companies, please contact us for more information. The product is not harmful, but contact to skin must be avoided.

c) **Collecting information:** The minimum information required is the a) Date of collection, b) the Botanical family of the plant collected, c) the GPS position/precise locality and d) collector information. More information is beneficial to each collection; please refer to the sample sheet for more information – Download.

All the information provided will be used to add your collection to the UJ-ACDB DNA bank. This is accessible online on the DNA bank page.

Silica dried leave material will be send to Canada for sequencing the two barcoding genes (*rbcLa* & *matK*). This data will be your property and you will be able to access them via BOLD. For more information on data release and protection you can refer to the BOLDSystems.

For creating your own project on BOLD you can follow the tutorial available on the system or contact us.

Herbarium voucher visual protocol:

It is recommended to collect plant specimens, which either have fruits, seeds or flowers to check field identification. Sterile material should be collected only if the collector can provide a 100% reliable identification. The herbarium specimen must be labelled with a unique vouchersing number. (i.e. collectors initial + collecting number from 1 to ∞).





Leaf material for Silica storage visual protocol:

It is compulsory that leaf material must be collected from a SINGLE plant specimen. Labelling must contain the same exact voucher name use for the herbarium specimens.

For each collection enough but not too much plant material must be collected to perform at least one DNA extraction (0.3 g of dry leaf material is required per extraction). Too much material in each envelope might result in leaves getting rotten and therefore be contaminated by fungi.

As examples:

A plant with the size of a 4-6 cm x 3x5 cm leave should have up to 6-7 leaves in silica.

In case of plants with very large leaves, a ¼ to 1/6 of the leave cut in smaller fragment would be enough.

In the case of succulent plants it is strongly recommended to peel and dry only the leaf surface. Removing the parenchyma will avoid extracting low quality DNA.



The most important protocol to follow when collecting material for DNA is getting the plant material to dry quickly and this is accomplished by increasing the surface area (cutting the leaves into smaller pieces). It is preferable to remove the midrib from leaf material before putting it into silica gel. The material is then placed in a Ziploc plastic bag with enough silica gel to ensure drying of the material in a couple of days. One or two days after collecting the leaves in silica it is necessary to mix the bag contents by turning it upside down.

Once the material is dry, most of the silica gel can be removed from the sample and dehydrated for future use. Indicator silica gel should be used to help determine the overall "dryness" of the sample. Check the

