Leaf tissue collection for DNA extraction

As methods for extraction, purification and amplification of DNA improve, we actually need very little plant material in order to adequately supply molecular labs. In general, we recommend collecting about 3-5 square inches of fresh, young plant material, normally 3-5 leaves or cut parts of larger leaves. There are several methods that can be used to preserve plant material for DNA substances. Traditional approaches use liquid nitrogen or dry ice for tissue collection and transportation. Fresh tissues are stored usually in -70°C and freeze dried for grinding.

Labeling and Shipment.

Label information is important, so please provide the following:

- Taxon name
- Collection number
- Date of Collection
- Specific Locality (latitude and longitude encouraged)

The label should be placed inside the ziplock bag with the specimen. For shipping - simply use the most reasonable postal rate available (no need for overnight or express service). Also, no special permits are required for dry material. Mark outside of package "For Scientific Study - No Commercial Value".

Silica Gel

Collecting on silica is very straightforward. A 1 to 10 ratio of plant material to silica gel is commonly used. Thus, to dehydrate 5g of tissue (wet weight), use 50 g of silica. Simply place the silica gel in a ziplock style plastic bag, add the plant tissue, and seal. Small (sandwich size) heavy gauge (freezer style) ziplock plastic bags are best because they do not tear. The material should be bone dry within 24 hours. If it is not, the silica gel may not have been fully dehydrated or the weight ratio was incorrect. Grade 12 silica with a bit of indicator silica mixed in is usually used. The indicator silica "reports" when the silica is dehydrated (blue) or hydrated (pink). Some labs reuse silica gel (after baking it in an oven), but cross contamination may occur, so always use new silica.

Lyophilization

1. Harvest leaves from greenhouse or field grown plants. It is preferable to use young leaves without necrotic areas or lesions, although older leaves, which are not senescent, may be used.
2. If the midrib is thick and tough, remove it. Cut or fold leaves into 10-15 cm sections and place in a fiberglass screen mesh bag along with the bag identifying the sample (aluminum foil or paper bags may be substituted if holds are punched to allow good air flow). Place bags in an ice chest or other container with ice to keep samples cool (but do not allow to freeze).
3. Place leaf samples in a Styrofoam container or some type of container able to hold liquid nitrogen. Add liquid nitrogen to quick-freeze samples. *Once frozen, do not allow samples to thaw until freeze-dried.*

   NOTE: Leaf samples may be stored at –80°C until ready to be lyophilized.
4. Transfer frozen leaf samples to lyophilizer. Make sure that the lyophilizer is down to temperature (the chamber is ≤ –60°C) and pulling a good vacuum (≤ 10 micronsHg) before loading samples. Do not overload lyophilizer make sure vacuum is always ≤100 microns and condenser temperature is ≤ –60°C. Samples should be dry in 72 hours. Typically, fresh weight = 10X dry weight.
5. Dried leaf samples may be stored in sealed plastic bags at room temperature for a few day or, preferably, at 20 °C for several years.
6. Fill out a harvesting record sheet.

Grinding

1. Grind to a fine powder with a mechanical mill, into a plastic scintillation vial or any other appropriate plastic container that can be closed airtight.

   NOTE: If the plant material weighed less than 4 g fresh weight, grind to a powder with a mortar and pestle in the presence of a pinch of acid washed sand. The finer the grind, the greater the yield of DNA from a given amount of material.
2. Store ground samples tightly capped at -20°C. Samples are stable for several years.

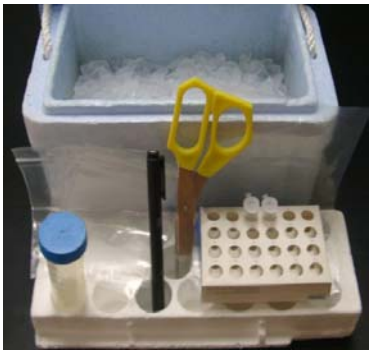
Reference

Hoisingto, D, Khairallah, M and Gonzalez-de-Leon, D. 1994. Laboratory Protocols: CIMMYT Applied Molecular Genetics Laboratory. 2nd Ed. Mexico, D.F.:CIMMYT.

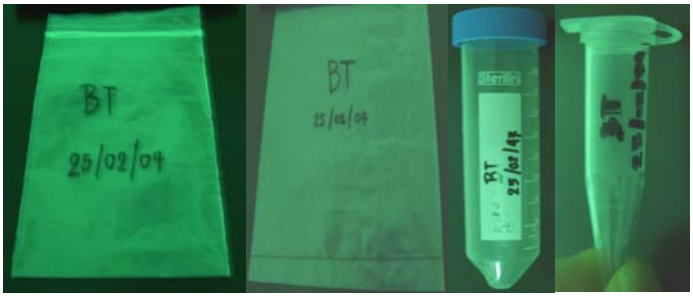
http://www.science.siu.edu./plant-biology/faculty/nickrent/Plant.DNA.html

http://www.gramene.org/newsletters/rice_genetics/rgn14/v14p164.html

http://www.mobot.org/MOBOT/research/applied_research/methods.shtml

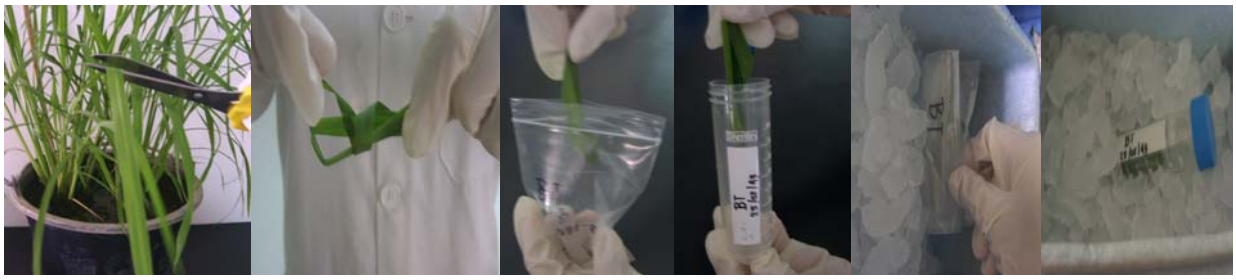


Prepare materials for collecting fresh leaf



Labelling sample name and date

Seedling and tillering stage



Cutting leaf, folding, put in the tube or plastic bag or zip bag and the put in the icebox