GENOMIC TOOLS FOR SWEETPOTATO IMPROVEMENT

Sweetpotato leaf preservation methods for high DNA quality, purity and yield

CENTRO INTERNACIONAL DE LA PAPA
CIP
A member of the CGIAR Consortium

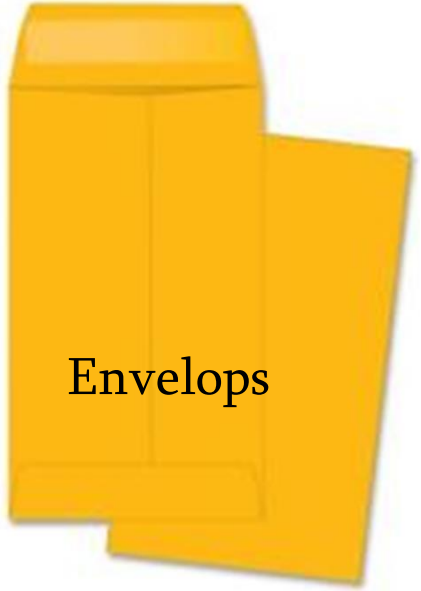
Leaf collection for high/medium-throughput DNA extraction

- Time saving leaf collection
  - Choose sample collection apparatus depending on the method you are going to use for mechanical cell wall breakage/grinding the leaf sample at the time of DNA extraction
- This prevents longer exposure of the leaf material to degradation especially when working with frozen material
- Avoid freeze and thawing

Materials

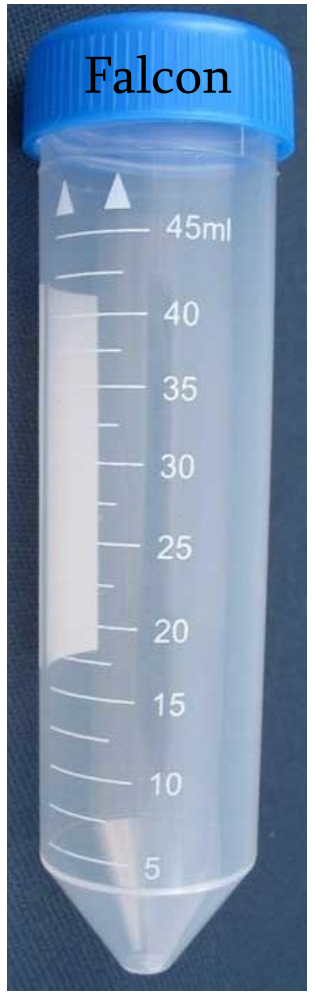
- Sterile Blades
- Ziplock bags/envelops/Falcon tubes/2M ml Eppendorf tubes
- Barcode Labels/blank labels
- Permanent marker pens/pencils
- 70% Ethanol
- Cotton wool/paper towels

Eppendorf



Envelops

Ziplock bags



Small (sandwich size) heavy gauge (freezer style) ziplock plastic bags are best because they do not tear

Leaf sampling

- Preferable to use young leaves (2-5) without necrotic areas or lesions
- Parts of/whole older leaves/parts, which are not senescent, may also be used
- Wipe leaf with with 70% cotton swab to surface sterilize
- Pack sampled leaves in an appropriate labelled bag/tube and preserve

Shorten the stems of the leaves if they are longer than 11/2 inches

Cryopreservation methods

- use of very low temperatures to preserve structurally intact living cells and tissues (typically -80 °C using solid carbon dioxide or -196 °C)

Pros and cons of Cryopreservation methods

Pros;

- Keeps DNA cells intact
- Long term preservation

- Remedy- collect leaves in extraction tubes for (high-medium throughput leaf grinding

  or

- Du/triplicate sample collection in labeled tubes/zip lock bags

Cons

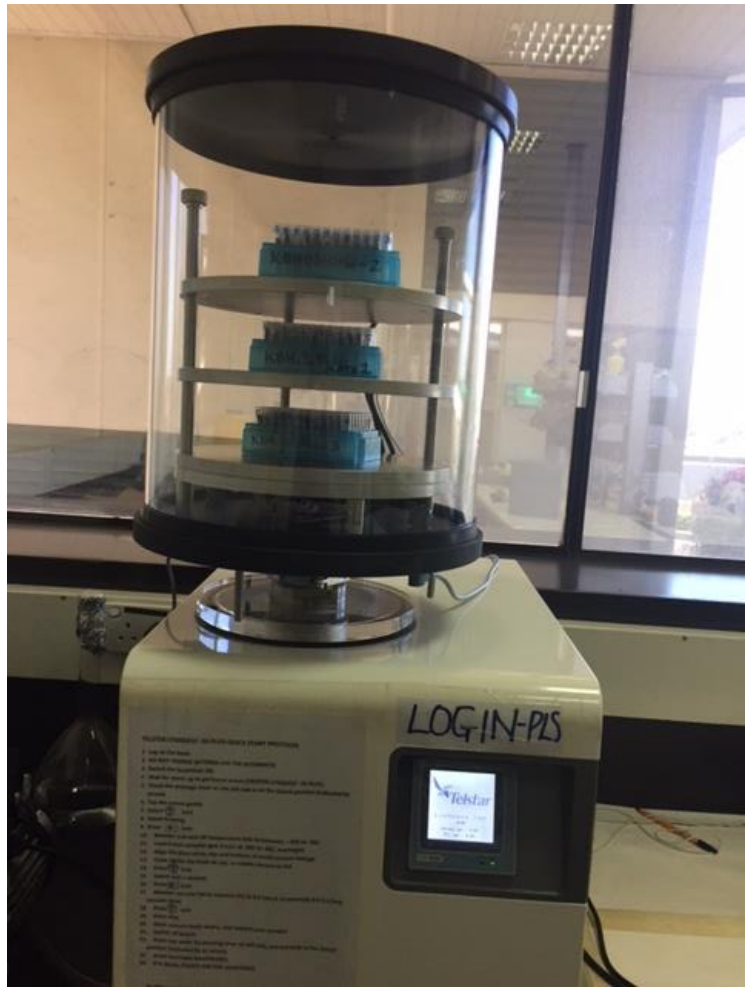
- Expensive
- May not be easily accessible to everyone
- Once preserved, exposure to external environment for several minutes will lead to DNA degradation
- Its tedious- especially if you have to pre-sample (for preservation) then sample into DNA extraction tubes/mortars during extraction

Cooling methods for temporary preservation

- Pre cool the ice packs in a -20°C to 80°C freezer
- Put the ice /cooler packs in a strofoam box to keep them cold for a longer time
- Do not over expose the ice or cooler packs to sun - if you are working in the field-keep the box under a shade
- Use waterproof sampling material (ziplock bags, Eppendorf or falcon tubes) and place samples into the ice or place in between the packs

- Pros; affordable and easy to use
- Cons; well frozen icepacks will best preserve the leaves for only 3 days therefore not long lasting

Lyophilizing (freeze drying) of sweetpotato leaves



- Leaves can be samples into Small (sandwich size) heavy gauge bags, falcon tubes or 2ml Eppendorf and Store fresh leaf samples at – 20°C to –80°C until ready to be lyophilized (overnight/24hours)
- Switch on the lyophilizer make sure that the temperature is down to ≤–55°C (the chamber is ≤ –60°C) and pulling a good vacuum (≤ 10 micronsHg) before loading samples
- Transfer frozen leaf samples to lyophilizer and avoid overload
- Align glass cover top and bottom to avoid vacuum leakage
- Close tightly the knob on the top to enable vacuum to fall (between o.5-0.4 is okay
- Samples should be dry in 72 hours.
- Lyophilized leaf samples can be stored at – 20°C for years

Leaf preservation by drying

Silica gel

Dry

Wet

- Silica gel may be doped with a moisture indicator that gradually changes its color when it transitions from the anhydrous (dry) state, to the hydrated (wet) state
- Common indicators are cobalt(II) chloride and methyl violet. Cobalt (II) chloride is deep blue when dry and pink when wet

- Place the leaves/branch between absorbed papers e.g newspaper
- place the papers with samples against a hard surface
- Keep the papers in an aerated place to prevent leaves from getting mold
- This can be done at room temperature

- Put the leaf samples in small envelops/tubes and leave open
- Place them inside the incubator at 37°C
- let the leaves stay for about 72 hours

Sweetpotato leaves preservation with Silica gel

- For particularly sensitive methods, such as AFLPs or Genotyping by sequencing (GBS), whole genome sequencing even microsatellites, or if you need higher quantities of intact DNA, a better method is necessary.

- That's where silica gel comes in.

- It's cheap, easy to handle, and most important can dry a leaf sample in 12-24 hours, fast enough to preserve enough high-quality DNA for most applications

- Silica gel is portable anywhere you go; Field, greenhouse, screen house, Laboratory …name it

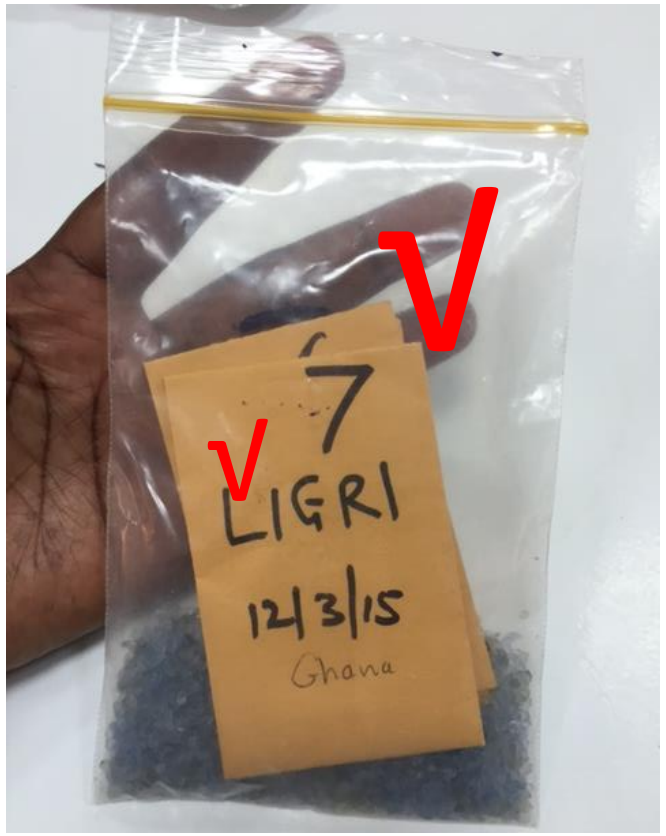
Place a bardoded/hand written label on to the envelop or Ziplock bag, place the leaf/leaves in and pour the silica gel

Dos` and Don'ts

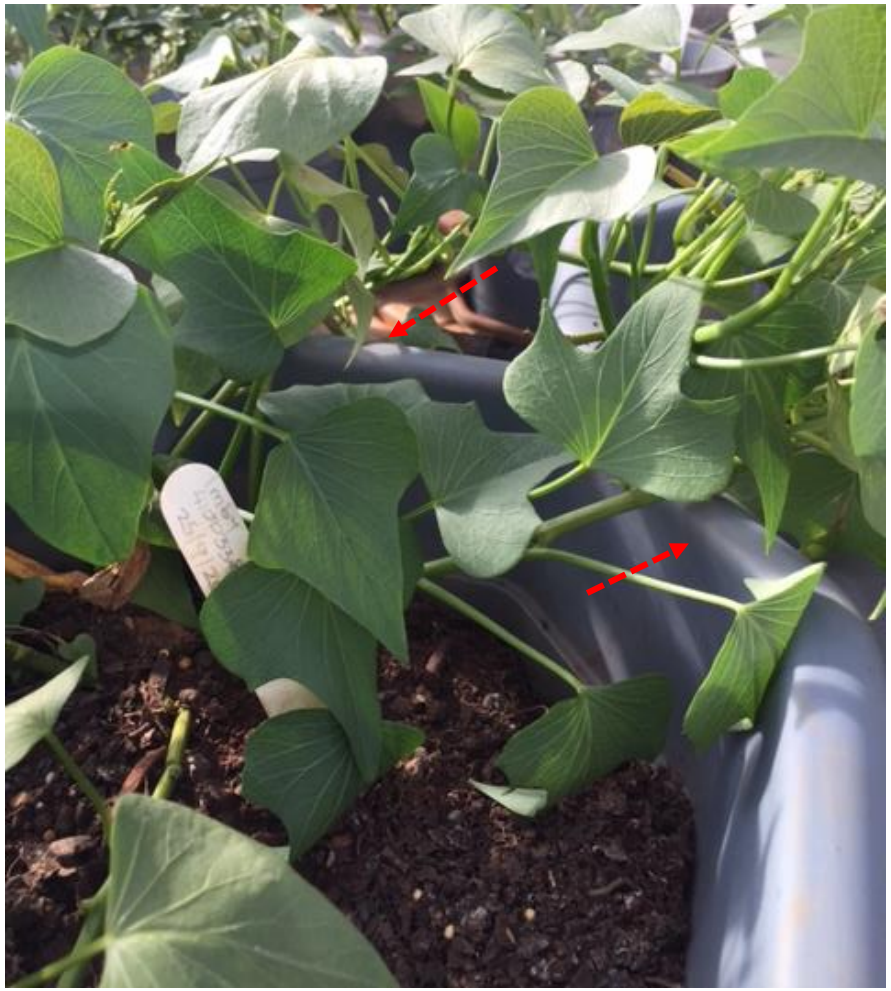
Large ziplock bag and a medium size envelop

- Ratio of plant material to silica gel 1 to 10 ratio is commonly used;
- Thus, to dehydrate 5g of tissue (wet weight), use 50 g of silica
- Change the silica gel if change in colour occurs

Right size of ziplock bag and envelop



Avoid biological contaminations while doing leaf sampling



Always follow a vine from the root bottom to make sure it belongs to the variety you are sampling

Important to note

- Put leaves of the same plant in one bag. DO NOT mix leaves of different plants even if they are clones
- The leaf material should be done dry within 24 hours.
- If it is not, the silica gel may not have been fully dehydrated or the weight ratio was incorrect.
- Grade 1or 2 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

- Poorly preserved sweetpotato leaves- either
  - in silica gel
  - or frequently frozen and thawed
- You end up with DNA looking like this!!!!