Leaf sampling and submission for DNA Extraction Procedure

Purpose

To guide the client in sample collection and submission to Segolip Unit for the purpose of DNA extraction and other analysis services offered by the unit per client specification.

Procedure

Segolip Unit accepts variable sample types for automated DNA extraction. These ranges from animal tissues, whole blood, plant leaves, seeds, just to name a few. The focus in this protocol is mainly on sampling and submission of leaf samples.

Please also see appended in Annex1 the sample specification details for the other Category of the DNA extraction samples.

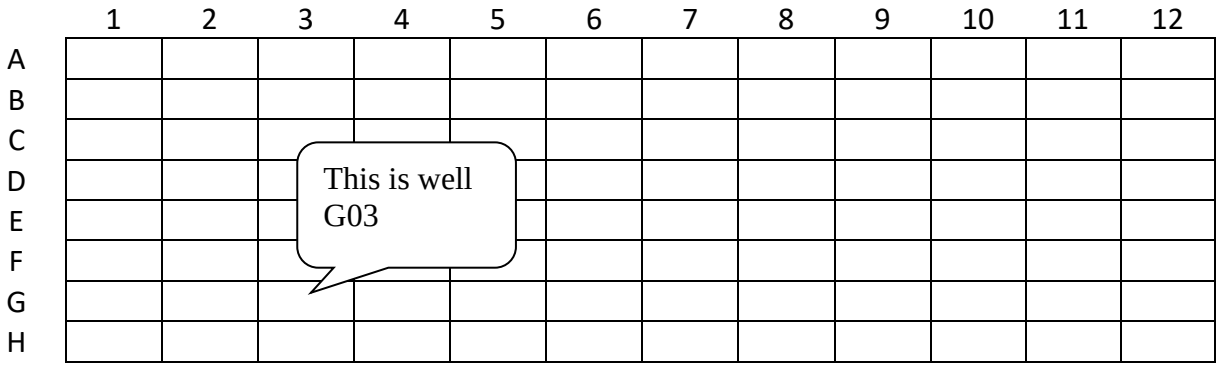
Materials and Equipment

1. Sample collection sheet
2. Marker pens
3. 96-well plate holder and cover (holds 12 x 8-strip tubes), LGC Kit where applicable. 96 Deep welled plates of superior quality can also be used for sampling.
4. 8-well strip tubes and caps (Perforated)
5. 11X7.5cm khaki envelope containing 50gm of silica gel, sealed.
6. Masking Tape
7. Plant material (Leaf)
8. Latex gloves (optional)
9. Forceps (optional)
10. 70% Ethanol.

96 well plate layout and labelling.

- 96-well plate columns are labelled 1-12 and rows A-H (Fig.1). The sample ID in the plate and the sampling sheet layout must match to circumvent inappropriate sample information. I.e. Sample information in your collection sheet and sample orientation in plate layout must correspond. It's imperative to constantly validate during the sampling process. Do not remove strips from plate during collection**.**

Fig.1. Plate layout



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NB; Label Clearly to avoid confusion

Example of collection sheet, recording the sample ID in each well of the plate.

Plate1

	1	2	3	4	5	6	7	8	9	10	11	12
A	1	9										
B	2	10										
C	3	11										
D	4	12										
E	5	13										
F	6	14										
G	7	15										
H	8	16										96

A. Leaf sample collection procedure

The LGC plant sample collection kit is recommended, (Fig1) because it is very convenient for sample collections. It has the following constituents:

- 1 x 96-well tube storage rack with lid, containing 12 x 8-strip tubes
- 12 x perforated 8-strip caps
- 1 x 50 g desiccant sachet. Please note that this sachet is enclosed in plastic bag. Do not remove it from this bag until plates are being prepared for shipping as this will dramatically reduce its ability to desiccate leaf samples.
- 1 x large labelled sealable bag
- Elastic band
- 1 x leaf cutting tool (where applicable)
- 1 x leaf cutting mat (where applicable)

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Fig.1

1.Label the plates according to the plate ID assigned on your collection sheet. Put strips tubes in the rack. You can label each strip 1-12 at row H to help you keep orientation correct and not turn the plate around! (Fig 2)

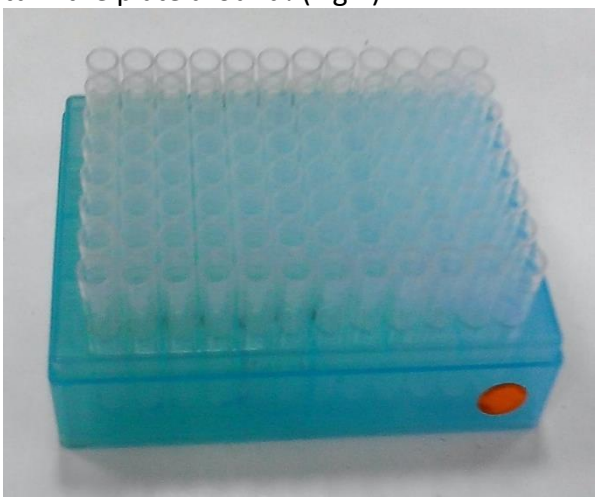

Fig 2

(However, sampling kits/plates from other suppliers may also be used, ensure superior quality plates are used to prevent loss of material due to breakage by steal balls during geno-griding step).

2.Do not remove strips from the rack. With strips remaining in plate, place the Leaf on cutting mat and hold the leaf cutting punch perpendicular to your leaf. (Fig 3)

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Fig 3

3. Rotate the punch back and forth until the sample is collected at the tip of the punch. To remove the sample from the punch, simply press the plunger on top of the punch. Collect about **Three to four leaf discs from the same leaf. This will ensure enough sample material to generate good amount of DNA.** Always sterilize the leaf punch device with 70% alcohol before punching the next leaf to prevent cross contamination. Fill wells 1A to 1H, then 2A to 2H etc.
If another service is required after DNA extraction, **Leave wells 12G and 12H empty** for in controls. Complete the sample sheet as you collect the materials (Fig 4)

Fig. 4

4. Where the cutting mat and leaf cutting punch are not available for sampling, you can cut a bout 3cm of the leaf material and push it down the strip tube/deep welled plate using a forceps. Similarly, ensure correct sample orientation is maintained and sterilize the forceps with 70% ethanol before use on the next sample to prevent cross contamination.

5. Once collection is complete, dry the fresh leaf disks overnight in a **drying oven at 35°c**. Put the plate in the oven, without caps. Ensure the leaf disks are completely dry to prevent any fungal

growth and DNA degradation. Cover the strips using strip caps once dry. No silica gel is necessary if samples are oven dried.

6. When oven drying is not feasible, cover the samples with perforated caps. Remove the sealed khaki envelope (11X7.5 cm) containing 5gm of silica gel from the plastic bag. Place the silica gel desiccant satchet on top of the strips capsealed tubes. Place the plastic lid cover on top of the 96 well storage plate ensuring the desiccant sachet is situated beneath the plastic lid.

7.Secure the lid in place using the elastic band provided and place the sealed rack into the large labelled sealable bag provided. Force excess air out of the bag and seal the bag tightly.(Fig 5-8)

8.Place the sealed bag into the shipment kit box.

Fig 5

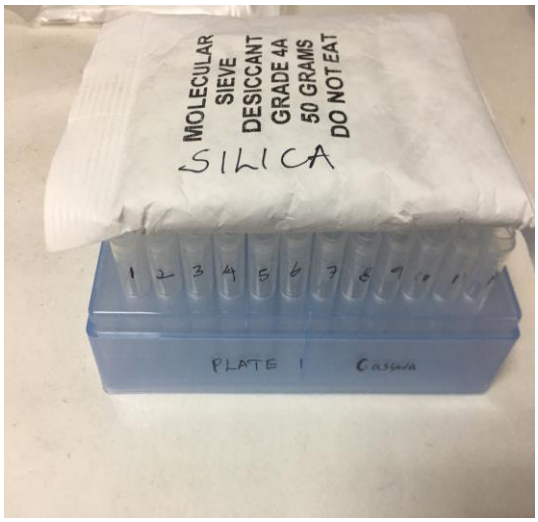

Fig 6

9. Where samples are harvested within ILRI campus, there will be no need of oven drying or use of desiccant satchet. The freshly sampled leaf material can be put in -20 freezer for 4 hours, then freeeze dried overnight and submitted to Segolip Unit for DNA extraction.

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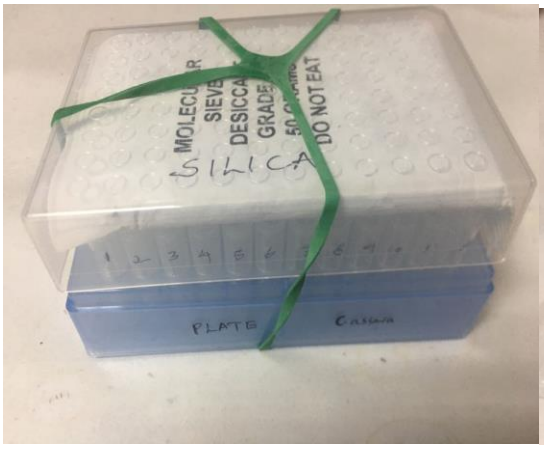

Fig 7 Fig 8

Send a DNA extraction request to segolilb@cgiar.org, attach soft copies of sample collection sheets with the following information: Plate ID and sample orientaion.

10.Shipping of collection plates

Place all the labelled collection plates, import permit and phytosanitary certificate, print out of sample specification sheets in a container and tape. Ship the material using DHL or any other courier service of choice to the following address. Ensure you indicate door to door delivery for speedy delivery.

NB/ Physical delivery of Samples collected inhouse is permittable.

Segolip Unit

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Leaf sampling and submission for DNA Extraction Procedure

Annex 1

DNA Extraction Specifications.

Blood DNA	
Features	**Specification**
Sample Type	Whole blood/ Frozen/ Buffy coat
Purified Nucleic Acid	Genomic DNA
Sample Amount	300 µl whole blood/Frozen blood/ Buffy coat
Typical yield	2 - 5 µg

Blood RNA	
Features	**Specification**
Sample Type	Whole blood/ Frozen/ Buffy coat
Purified Nucleic Acid	Genomic RNA
Sample Amount	50 - 300 µl whole blood/Frozen blood/ Buffy coat
Typical yield	50- 300 ng

Stool cell DNA	
Features	**Specification**
Sample Type	Stool
Purified Nucleic Acid	Genomic DNA
Sample Amount	50mg Stool
Typical yield	2 - 16 µg

Tissue DNA	
Features	**Specification**
Sample Type	Animal tissue
Purified Nucleic Acid	Genomic DNA
Sample Amount	50 - 100mg tissue
Typical yield	5 -40 µg

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Viral DNA/ RNA	
Features	**Specification**
Sample Type	Serum, PBS suspension, VTM swabs
Purified Nucleic Acid	Viral DNA and RNA
Sample Amount	200 µl / 300 µl serum, PBS suspension
Typical yield	1 -20 µg

Plant DNA	
Features	**Specification**
Sample Type	Plant tissue
Purified Nucleic Acid	Genomic DNA
Sample Amount	50-100 mg plant tissue
Typical yield	2 - 5 µg

Plant RNA	
Features	**Specification**
Sample Type	Plant tissue
Purified Nucleic Acid	Genomic RNA
Sample Amount	50-100 mg plant tissue
Typical yield	2 - 5 µg

Bacteria DNA	
Features	**Specification**
Sample Type	Cultured Bacteria
Purified Nucleic Acid	Genomic DNA
Sample Amount	<3 X 10^5 cells
Typical yield	1 - 20 µg

Fungal DNA	
Features	**Specification**
Sample Type	Fungi
Purified Nucleic Acid	Genomic DNA
Sample Amount	50-100 mg
Typical yield	2 - 10 µg

Forensic DNA

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Features	**Specification**
Sample Type	Forensic specimen (swab/ Butts/ Stain/ Hair/ Chewing gum/ Fingerprints)
Purified Nucleic Acid	Total DNA
Sample Amount	Variable
Typical yield	2 - 5 µg