	<b>Crop: Yam</b>  <b>Function: Genotyping in the yam breeding program</b>	<b>SOP #</b>	IITA-YM-SOP04
		<b>Revision #</b>	IITA-YM-SOP04-01
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<b>SOP Owner</b>	Lead Breeder (Amele Asrat)	<b>Approval Date</b>	01-06-2022

## Standard Operating Procedures for Genotyping in Yam Breeding Program

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### ***1. Introduction***

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Yam (*Dioscorea* species) leaf samples contain high levels of polysaccharides and polyphenolic compounds. The extraction of DNA from leaves is usually problematic. Therefore, there is a need to extract good quality DNA to perform downstream DNA analysis; the cetyltrimethylammonium bromide (CTAB) DNA. Yam breeding optimizes DNA extraction from both leaves and tubers using a modified protocol for high-quality DNA.

### ***2. Purpose***

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To establish an appropriate DNA extraction procedure for routine genotyping activities in yam breeding.

### ***3. Scope***

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This standard operating procedure (SOP) covers the different activities related to yam genotyping.

### ***4. Definition of terms***

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DNA                      **Deoxyribo-Nucleic Acid**

IITA                      International Institute of Tropical Agriculture

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PCR	Polymorphic Chain Reaction
EDTA	Ethylenediamine Tetraacetic Acid
CTAB	Cetyltrimethyl Ammonium Bromide
QA	Quality Assurance
QC	Quality Control
QTL	Quantitative Trait Loci
PVP	Polyvinylpyrrolidone
WGRS	Whole Genome Referencing Sequence

before collecting each sample to avoid cross-contamination.

## ***5. Responsibilities***

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### **Scientist:**

- Identified trials to be genotyped with respective markers and the different sequencing platforms to be used. Provide protocol for sampling and high-quality DNA extraction

### **Research Associate/Manager**



- Assist in trial identification and provide trial layout for leave sampling

### **Supervisor/Technician**

- Assist in plot/clones' identification

### **Laboratory Technician:**

- Sampling data collection using coordinate App, DNA extraction, assessment of DNA quality, shipping of the sampling for sequencing

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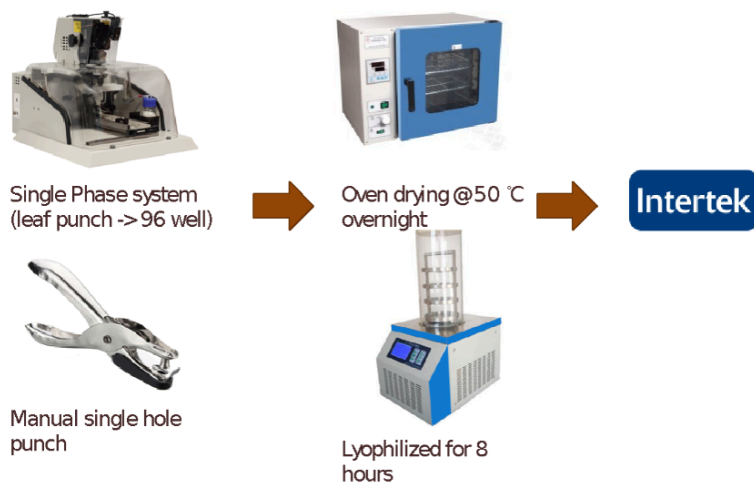


Figure 1: Different steps of leaf sampling and freeze-drying

## 6. Procedures



### Proper collection and storage of plant tissues

Fresh young, and healthy (two-month-old plant) plant leaves should be collected in an empty tea bag, or DNA extraction plate kept on ice and stored at -80° C. The leave should be contained in the middle part of the plant from the barcoded field plot (Figure 1). For this activity, the breeding program uses the coordinate app to track the sample collection in each PCR well (Figure 1).

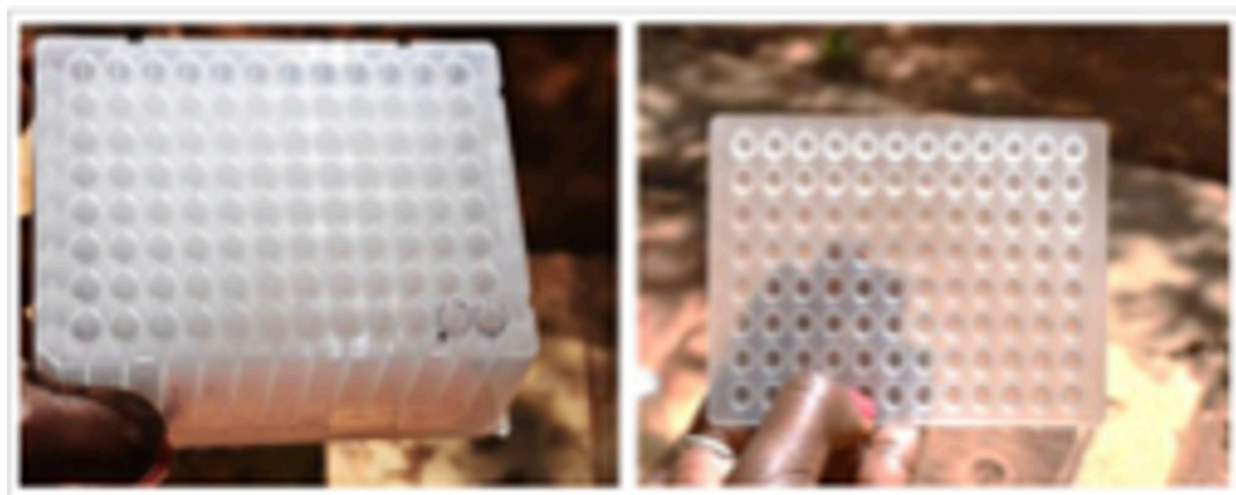
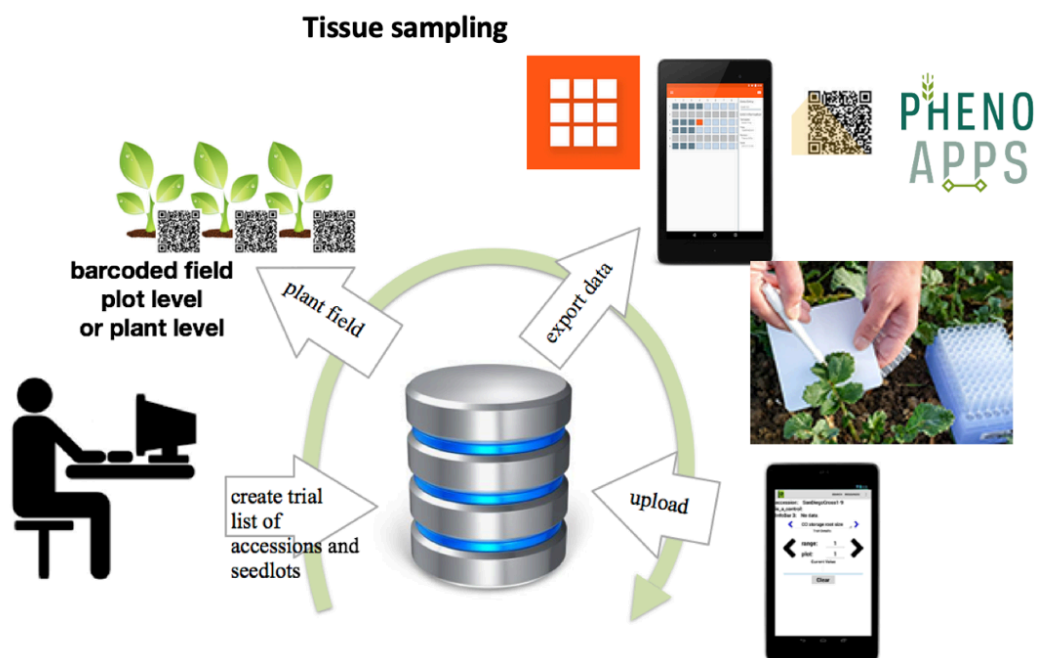
### SAMPLING REQUIREMENTS

#### Plates and Seals

- Samples are collected in 96-well format plates with round bottom wells (not conical) to sustain thorough tissue grinding (Figure 2).

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- We recommend below the 1,2ml AbGene 96-well plates (AB0564) and Silicone seals (AB0674).





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Figure 2: Yam leaves sampling and Summary of DNA extraction protocol and quality control in yam breeding

### **Precaution**

Sample collection must wear gloves and clean your working space with 50% -70% Ethanol.

The sampling device is sterilized by wiping a paper towel damped with 50%-70% ethanol or punching two or three punches of a clean, thick paper towel. This is generally done

### ***7. References***

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### ***8. Appendix***

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