SOP07:

Standard Operating Procedure (SOP) for Cassava Leaf Sampling for Genotyping

Single Phase system (leaf punch -> 96 well)

Manual single hole punch

Oven drying @ 50°C overnight

Lyophilized for 8 hours

Intertek

IITA Transforming African Agriculture CGIAR	**Crop:** Cassava **Function:** Leaf Sampling for genotyping	**SOP #**	IITA-CS-SOP07
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STANDARD OPERATING PROCEDURE (SOP) FOR LEAF SAMPLE COLLECTION FOR GENOTYPING

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1. Introduction

Application of deoxyribonucleic acid (DNA) technology in plant breeding offers an opportunity to enhance and accelerate genetic gain. Molecular markers, which are DNA or gene sequences with known locations on chromosomes are used as a tool for identification and genetic analysis. DNA extraction is the starting point and a critical step for genetic analysis. DNA can be extracted from diverse sources, including seeds and leaves of plants. Proper plant collection and storage conditions are essential for successfully extracting DNA of high quality and quantity from plants.

2. Purpose

The purpose of this document is to develop a reproducible workflow for leaf sampling, storage, freeze-drying, and shipment for genotyping in cassava breeding.

3. Scope

This document describes the step-by-step procedure used by the IITA cassava breeding program for tissue sampling, storage/freeze-drying, and shipment.

4. Definition of terms

Cassavabase: Open access repository developed to centralize information access to cassava research data and tools and support cassava breeding programs.

Freeze-drying: a dehydration technique based on the sublimation of water in a product.

5. Roles and Responsibilities

Molecular Breeder: Coordinates genotyping activities. Selects the population to genotype based on the breeding objectives and available resources.

Database manager: Uploads the plate layout into cassavabase; prints the barcode labels and plate maps.

Research Supervisor: Plans and coordinates all aspect of leaf sampling from the collection site to the laboratory, including sampling, collection, transport, processing, storage, and shipment.

Field Technician: Tags the genotypes to sample. Reconciles the field plot name with the printed plate layout to tally with the plate well positions.

Lab attendant: Collects the plates for storage in the -80^{o}C and supervise placement of samples into the lyophilizing machine.

6. Procedure/Protocols

6.1 Tissue (leaf) sampling

1. Ensure that all materials needed for sampling are ready a day prior to the collection date. Label each 96-well plate with their barcode labels or permanent marker to maintain identity.
2. Collect 3-4 leaf discs of 6mm in diameter from young leaves of each tagged clone, using an appropriate puncher and forceps. The use of young leaves is essential for good quality DNA.
3. Drop the leaf discs using the forceps into the appropriate well in the DNA plate on ice in a sampling bag. Each sample or well should come from a single plant.
4. Keep the last two wells H11 and H12 of the sampling plate empty for lab control, when using KASP low-density genotyping platform. When using the DaRTseq platform, the three wells G12, H11 and H12 are left empty.

6.2 Tissue storage and freeze-drying:

1. On getting to the lab, cover the plates with parafilm, and perforate on each plate well. Then keep the plates at -80^{0}C in a freezer prior to freeze-frying.
2. Dry the samples by lyophilizing them at -51°C and 5.0 pa for a minimum of 72 hrs (3 days) for complete freeze-drying. At the IITA Bioscience Centre, freeze-drying is done using LABCONCO FreeZone 18 Liter -50^{o}C freeze-dryer.
3. After freeze-drying, cap the DNA plate with a silicon sealing mat and store at room temperature until shipment.

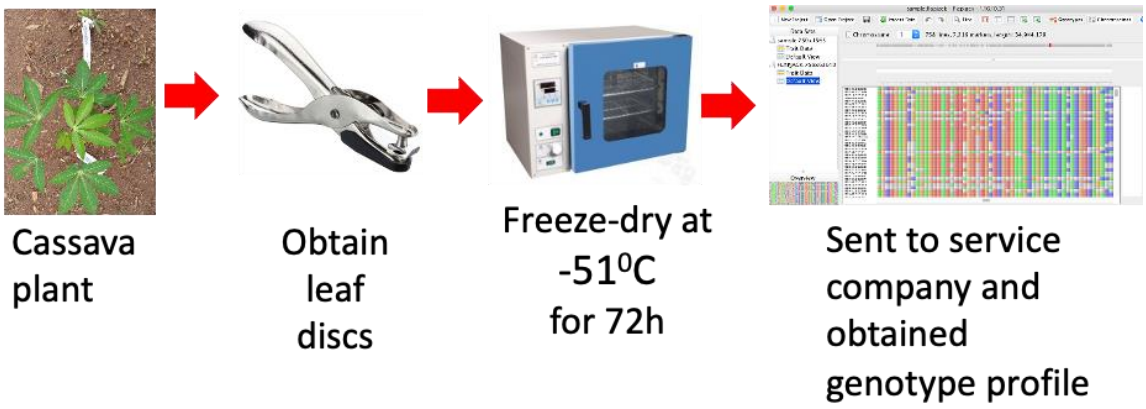
6.3 Sample shipment:

1. Complete all necessary documents/forms (depending on the lab, samples type and purpose of analysis) print and sign where necessary.
2. Place the DNA plates in an appropriate and logo-free carton and pad to avoid shaking during shipment **(because they are to be shipped via an air carrier)**
3. Take the carton along with necessary documents to the IITA mailroom for shipment (Note: A mail must be sent to the service provider prior to shipment).

7. References

Pascale, B. (2021). Molecular Plant Taxonomy: Methods, and Protocols. *Springer Nature*. doi: 10.1007/978-1-0716-0997-2

8. Annex: Forms/Templates to be used for monitoring and data collection



Workflow of tissue sample collection for genotyping at the IITA Cassava Breeding Program

Tools and Supplies

PCR Plate

Forceps

Puncher

Tape

Lyophilizer

Parafilm

Cooler bag

Cooler

Latex hand glove

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