**STANDARD OPERATING PROCEDURE (SOPs) FOR GENOMIC SELECTION**

**SAMPLE COLLECTION:**

**Materials needed:**

Extraction tubes and caps

Plate maps

Ice bowls

Marker pen

**Procedures:**

* Ensure plates are well labelled with a tape and marker pen.
* Write the clone name on the appropriate well location in the plate map.
* Collect the apical leaf of the cassava plant and place in appropriate well in the extraction box.

PS: If apical leaf is small, 2-3 can be collected but if it is big, a leaf should be collected.

* Suspend the plate on ice while on the field to avoid degradation of leaf tissue.
* After concluding sample collection, keep the plate in -20°C freezer in the laboratory.
* Freeze dry the leaves tissue using a lyophilizer.
* Extract DNA from leaf tissue using a standardized DNA method.

**DNA EXTRACTION**

 (Ensure all reagents are available by preparing them with distilled water. Check the box for every extraction step!)

**Reagents required:**

100mM Tris-HCL (50ml of 1M Tris-HCL, pH 8.0)

50mM EDTA (50ml of 0.5 EDTA, pH 8.0)

500mM NaCl (50ml of 5M NaCl)

 20% (w/v) SDS

5M Potassium Acetate,

Isopropanol

Chlorofoam

Isoamyalcohol

70% Ethanol

Low salt TE

RNase A.

**Materials and equipments:**

Water bath

Centrifuge

Pipette

Tips

Trough

Steel balls

Magnet

-20ºC freezer

**Procedures:**

* Put two steel balls in each well of the extraction box containing the lyophilized samples.
* Grind tissue (about 100mg) in 1.2ml extraction strip tubes using a geno-grinder, after grinding, remove the steel balls using a magnet.
* Add 400ul of extraction buffer, homogenize mixture with a clean plastic pin and place in water bath @65ºC for 20 minutes with gentle rocking.
* Remove tubes from water bath and allow it to cool for 5 minutes. Add 200ul of ice-cold 5M Potassium acetate and mix by gentle inversion or with a vortex machine.
* Place on ice for 20 minutes, remove from ice and add 350ul of Chlorofoam:Isoamyalcohol (24:1) into the tubes. Mix with continuous rocking and centrifuge at 4000g for 10 minutes.
* Transfer upper layer to a new tube and add 400ul of ice-cold Isopropanol and mix gently for about 1 minute. Put in -20ºC blast freezer for 30 minutes for proper precipitation.
* Remove tubes from freezer and allow temperature to drop to room temperature and then centrifuge at 4000ng for 10 minutes. Carefully discard the supernatant.
* Add 300ul of Ethanol, flap tubes gently to let pellets float for easy washing and centrifuge at 4000ng for 10 minutes (repeat the process twice for proper washing of DNA pellet).
* Decant supernatant from each sample and air-dry pellet in a fume hood until ethanol evaporate completely.
* Re-suspend DNA in 100ul Low salt TE buffer +10ul RNase A and incubate at room temperature overnight or at 37ºC for 1-2hrs.
* Store at -20ºC for a long time storage.

**QUALITY CONTROL USING NANO-SPECTROPHOTOMETER AND GEL ELECTROPHORESIS**

**Materials and Equipments for nano-drop spectrophotometer:**

Computer system

Nano spectrophotometer

Tips

Pipette

**Procedures:**

* Turn on the computer.
* Click on the nanodrop software in the desktop.
* Click on the Nucleic acid icon.
* Save result in desired location.
* Clean the pedestal and load 2ul of water on the pedestal to blank.
* Lower the sampling arm and click OK.
* Open the sampling arm and wipe the water then load 2ul of TE buffer and then click on BLANK.
* After blanking wipe the pedestal and load 2ul of sample on the pedestal and click MEASURE.

PS: Ensure samples are tapped gently before loading on pedestal.

* Wipe the pedestal after loading each sample.
* Continue this process until all the sample have loaded.
* After completion of the work, exit the software.

**AGAROSE GEL ELECTROPHORESIS**

**Materials and Equipments:**

Gel tray

Combs

Stoppers

Gel Tank

Power tank

Microwave

Gel doc/imager

Staining solution (ethidium bromide/safe view)

Bromophenol blue

(Ensure every instrument is washed and dried before using).

**Procedures:**

* Place the combs in the tray containing stoppers at both ends and ensure it is balanced.
* Place the agarose tablets in a conical flask and add the appropriate volume of 0.5% TBE (Tris-HCl, Boric acid and Ethylenediaminetetraacetic acid) buffer.
* Allow the tablet to dissolve completely by gentling rocking the flask.
* Place the flask in the microwave for 3min at 650 watts.
* Allow the solution to cool down for about 5mins.
* Add 7ul of safe view or ethidium bromide to the agarose solution and stir.
* Pour the agarose solution into the already balanced tray.
* Allow to solidify.
* Place the solidified gel into the electrophoresis chamber containing TBE buffer.

PS: Ensure the TBE buffer is covering the gel to a depth of 1mm.

* Remove the combs gently to avoid breaking the gel.
* Load your DNA samples containing bromophenol blue.

PS: Add 2ul of bromophenol blue to 3ul of DNA sample and centrifuge.

* Connect the gel tank to the power tank and turn on the power tank.
* Allow sample to run at 100V for 45mins.
* Gently remove the gel from the tray and place in the gel imager.
* Click on the Ultra-violet light indicator on the monitor.
* Capture the image.
* Save inside a desired folder.