Standard Operating Procedure (SOP) FOR DNA EXTRACTION

Authors & Contributors

Abiodun Olutegbe, a.olutegbe@cgiar.org

Adaobi Nnadi, l.nnadi@cgiar.org

Mbanjo-Edwige Gaby-Nkouaya, e.mbanjo@cgiar.org

Ismail Rabbi, i.rabbi@cgiar.org

1. Introduction

Deoxyribonucleic acid (DNA) extraction is an important process in molecular plant breeding. DNA extraction involves isolating DNA from the nucleus of a cell. Once purified, scientists can study individual genes, sequence the entire genome, modify sections of DNA, among others.

2. Purpose

This document outlines the procedure for extracting high quality DNA from leaf samples.

3. Scope

This SOP highlights the steps involved in DNA extraction from plant leaf tissue as well as the materials needed. It also includes instructions for preparing the reagents needed for DNA extraction.

4. Definition of terms

Freeze Drying: This is a process in which water in the form of ice is removed from tissue or material under low pressure by sublimation

Gel electrophoresis: This laboratory technique is used to separate fragments of DNA, ribonucleic acid (RNA) and protein molecule according to their molecular size.

5. Roles and Responsibilities

Research Technician

• Prepares the leaf samples, reagents and materials needed for the DNA extraction

• Notifies the research supervisor of any chemicals or supplies not available in the laboratory and helps with DNA extraction process.

Research Supervisor

• Notifies the breeder of the materials not available in the laboratory,

•conducts the DNA extraction and shares the gel picture(s) and the concentration readings with the breeder.

Breeder

• The breeder orders the materials and chemicals needed for the DNA extraction.

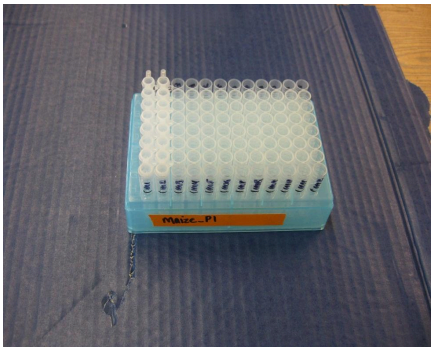
6. Procedure/Protocols

6.1 Materials

1.2ml extraction tube, water bath, centrifuge, Genogrinder, disposable gloves, micropipette, pipette tips, plastic trough, 3mm steel balls, reagent bottles

6.2 Sample collection and preparation for DNA extraction

1. Collect young cassava leaf tissues of about 2-3 leaflets weighing about 100mg into well labelled 1.2 ml extraction tubes on wet ice.



2. Transport samples to the lab and place in -80^{o}c freezer.
3. Transfer samples to a pre-running/chilled freeze-drier and dry for 24-48hrs. Alternatively, silica gel can be used for drying of leaves when a freeze drier is not available
4. Put two 3mm steel balls into each tube after drying using a multi-dispenser where possible to save time.
5. Grind dried leaves for 1 minutes at a rate of 1500 strokes/mins using a genogrinder. If the number of samples are few, the tissue can be ground with laboratory mortar and pestle containing warm extraction buffer or liquid nitrogen.
6. Remove steel balls immediately after grinding.

6.2.1 Preparation of reagents

Reagents required for the DNA extraction includes: Extraction buffer, 20% (w/v) SDS, 1% (w/v) PVP (40,000 MW), 5M potassium acetate, isopropanol, Chloroform:Isoamylalcohol mixture, 70% ethanol, low salt TE buffer, RNase A.

Note: *When working with β-mercaptoethanol and Chloroform : Isoamyalcohol, please use the fume cupboard.*

1. **Extraction buffer (1litre)**

100 mM Tris-HCl (100 ml of 1 M Tris-HCl, pH 8.0)
50 mM EDTA (100 ml of 0.5 M EDTA, pH 8.0)
500 mM NaCl (100 ml of 5 M NaCl)

Make up to 950 ml with de-ionized water and adjust to pH 8.0 using HCl. Autoclave for 15 min. Add 1% (w/v) PVP (40,000 MW), dissolve by mixing and adjust total volume to 1 litre with de-ionized water. Aliquot 100 ml of the stock Extraction Buffer and use it as your working buffer. To this working buffer, add 6.6 ml of 20% (w/v) SDS and 0.75% β-mercaptoethanol (75µl) just before use. Note: Extraction buffer can be stored for up to one month.

2. **Low salt TE plus 10mg/ml RNaseA (1 litre)**

10 mM Tris-HCl (10 ml of 1 M Tris-HCl, pH 8.0)
1 mM EDTA (2 ml of 0.5 M EDTA, pH 8.0)
Make up to 950 ml with de-ionized water and adjust to pH 8.0 using HCl and adjust total volume to 1 litre. Estimate amount of buffer you'll need and add 10 mg/ml RNAse-A to the volume of low salt TE needed.

3. **Chloroform:Isoamylalcohol (24:1) (100 ml)**

Chloroform (96 ml)
Isoamylalcohol (4 ml)

6.3 DNA extraction

6.3.1 Precipitation of Proteins and Polysaccharides

1. Add 400µl of extraction buffer into grinded leaf samples, homogenize mixture with a clean plastic pin and place in water bath at 65°C for 20 minutes with gentle rocking.
2. Remove the tubes from water bath and allow it to cool for 5 minutes. Add 200µl of ice-cold 5M Potassium acetate and mix by gentle inversion or with a vortex machine.
3. Place on ice for 20 minutes, remove from ice and add 350µl of Chloroform:Isoamylalcohol (24:1) into side of the wells or tubes. Mix with continuous rocking and centrifuge at 4000 g for 10 mins.

6.3.2 RNAse Treatment and Crude DNA Pellets Precipitation

1. Transfer upper layer to a new tube simply by pouring (as tissue debris would have locked down the Chloroform:Isoamylalcohol layer) and add 400µl of ice-cold Isopropanol and mix gently for about 1 minute. Put in -20ºC freezer for 30minutes for proper precipitation.
2. Remove tubes from freezer and allow temperature to drop to room temperature and then centrifuge at 4000 g for 20 minutes. Carefully discard the supernatant.

6.3.3 Washing and RNAse-A Treatment

1. Add 300µl of ethanol, flap tubes gently to let pellets float for easy washing and centrifuge at 4000g for 10mins (repeat the process twice for proper washing of DNA pellet).
2. Decant supernatant from each sample and air-dry pellet in a fume hood until ethanol evaporate completely. Do not over-dry pellet as it will make it difficult to re-suspend.
3. Re-suspend DNA in 90µl Low Salt TE buffer + 10µl RNAse-A and incubate at room temperature overnight or at 37 ºC for 1-2hrs.
4. Store the DNA at -20 ºC.

6.3.4 Visualization on UV spectrophotometer

1. Pipette 2ul of DNA sample and 3ul of loading dye in a 96-PCR plates.
2. Dissolve 1g of agarose in 100ml of TBE (Trisma base, Boric acid and EDTA) and microwave till you get a clear solution.
3. Cool under running water and add 0.5ul of ethiudium bromide or any dye-based DNA stains.
4. Swirl the flask and pour immediately in the gel tray containing the combs.
5. Carefully remove the comb after the gel solidifies.
6. Load the sample (DNA and loading buffer) in the gel and run the gel at a specified voltage and time. The loading buffer provides a visible dye which helps with loading and allows to assess how far the DNA has migrated.
7. Read result under the UV spectrophotometer.

6.3.5 Determination of concentration using Nanodrop Spectrophotometer

1. Blank the machine with 2ul low salt TE (Tris HCL and EDTA).
2. Add 2ul of the DNA samples on the machine, then run the analysis.
3. Export the concentration values at 260 – 280nm wavelength on excel sheets.

7. References

*Dellaporta SL, Wood J, Hicks JB. A plant DNA minipreparation Version II. Plant Mol Biol Rep. 1983; 1:19-21.

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