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Protocol for DNA Extraction from Cowpea

 Forked from [Protocol for DNA Extraction from Tepary Bean](#)

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol to extract high quality DNA from Cowpea leaves using modified CTAB method.



Materials

Buffer and Chemicals Required

1. CTAB Buffer pH.8

- CTAB- 20 g/L
- Tris- 12.11 g/L
- EDTA- 7.44 g/L
- NaCl - 81.89 g/L

2. RNase A (10mg/ml)- Dissolve in TNE buffer

- Tris-cl - 10mM
- Nacl - 15 mM
- EDTA - 1 mM
- Reconstitution as per Sigma Aldrich Roche Document (Product code: 10109142001)

RNase A can be dissolved at a concentration of 1 to 10 mg/ml in 10 mM Tris-HCl, pH 7.5, 15 mM NaCl, heated to

100 °C for 15 minutes to inactivate contaminating DNases and cooled slowly to room temperature and dispensed

into aliquots. Roche recommends subsequent storage at -15 to -25 °C.

3. Proteinase K (20mg/ml) : Dissolve in TE buffer

4. Phenol:Chloroform kit - Stored at 4 °C

5. Isopropanol (room temperature)

6. 3M Sodium Acetate

7. 70 % Ethanol (Ice-cold)

8. TE Buffer pH.8

- Tris- 10mM
- EDTA- 1mM

9. Agarose

10. Ethidium Bromide

11. Size Marker: 1 kb DNA ladder

Instrument

1. Centrifuge
2. Hot Water Bath
3. Spectrophotometer- Nanodrop
4. Gel Documentation system



Collection of leaf sample

- 1 Collect 1 fully expanded leaf from Cowpea plants in a 50ml centrifuge tube containing five sterile glass beads (5mm diameter) and freeze immediately in liquid nitrogen.
- 2 Grind tissue using vortex, ensuring tissue remains frozen. Transfer ~.5 mL of ground tissue to a new 2 mL eppendorf tube containing two 2mm glass beads.
- 3 Further beat the tissue by placing in the bead beater for 1 minute in one direction, then 1 minute in the opposite direction.

DNA Extraction

- 4 Before beginning:
Heat water bath to 65 °C and place CTAB buffer in 65 °C water bath for 20 minutes
Heat incubator to 37 °C
Place 70% ethanol in 4 °C
Make sure RNase (10mg/mL) is already made and treated
Make sure proteinase K (20mg/mL) is made

*Note on CTAB buffer: Because of high phenol content in the leaf tissue, it may be helpful to add 1% PVP to the CTAB buffer
- 5 Add 600 µl of pre-warmed CTAB buffer and 12 ul of proteinase K (20 mg/ml), invert the tubes gently to mix together and incubate the tubes in water bath at 65 °C for 30 min.

Invert the tubes once in every 10 min to homogenize the tissue and buffer.



CTAB buffer added to sample- clear green solution

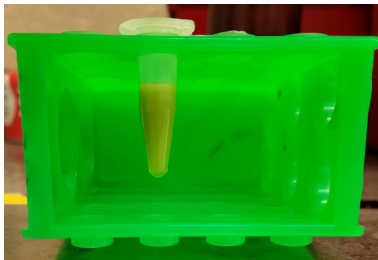
6

After 30 min, remove the tubes from water bath and centrifuge at 14000 rpm for 15 min to ensure lysate is cleared.

Transfer cleared lysate of 500 μ l to a new 1.5 ml tube. Add 10 μ l of RNase (10 mg/ml) and keep at 37 °C for 30 min.

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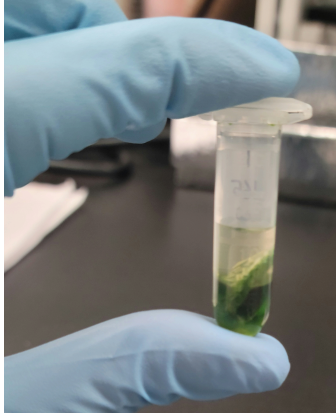
Add equal volume of Chloroform, and vortex well.



Tepary sample with Phenol:Chloroform mixed with sample

Note: for cowpea we use only chloroform, but it looks the same after vortexing

Centrifuge at 14000 rpm for 15 min. Transfer the upper aqueous layer to a new 1.5 ml Ep tube (~350-400 μ L)



Transfer upper aqueous layer to a new tube

Do not touch below layers while transferring the aqueous phase.

Repeat the Phenol: Chloroform alcohol step if the aqueous phase is not clear.

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Add equal volume of isopropanol, and mix very gently, keep for few minutes to precipitate nucleic acid.

Note: It may take up to 30 minutes of incubation at room temperature to observe precipitate nucleic acid



White strands noticed after adding isopropanol

Centrifuge at 14000 rpm for 10 min.

Pellet can be seen at the bottom.

Discard the supernatant.

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Add 300 μ l of 3M Sodium Acetate and 500 μ l 70 % ethanol and centrifuge at 14000 rpm for 5 min.

Discard the supernatant.

10

Add 200 μ l 70 % Ethanol and centrifuge at 14000 rpm for 5 min.

Discard the supernatant.

11

Air-dry the pellet, dissolve the pellet in 50 μ l TE Buffer.

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Place at 4°C overnight, then measure concentration and purity on a Nanodrop

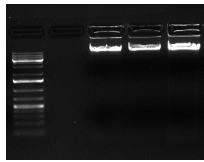
13

DNA is run on a 0.8% Agarose gel to check whether it is degraded or having RNA contamination.

If RNA is present, treat the samples with RNase A and precipitate again from phenol:chloroform step 4.

Nucleic acid concentration is measured in Nanodrop, and Ratio of A260/A230 with 1.8-2.0 indicates purity of DNA.

In case the values are <1.8, it indicates contamination such as carbohydrate or phenol.



0.8 % Agarose Gel picture

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Store DNA sample at -80 °C until use.