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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 Tepary Bean 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

- **T**ris-cl - 10mM
- **N**acl - 15 mM
- **E**DTA - 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

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

Troubleshooting

Collection of leaf sample

1

Collect 100 mg of young leaf from Tepary Bean plants in a 2ml eppendorf tubes containing two sterile glass beads and freeze immediately in liquid nitrogen.

DNA Extraction

2

Add 600 μ l of pre-warmed CTAB buffer and 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

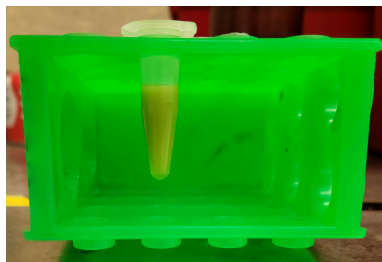
3

After 30 min, remove the tubes from water bath and centrifuge at 14000 rpm for 15 min.

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

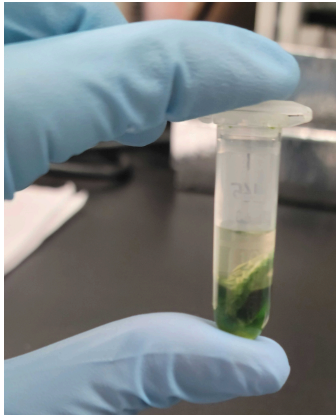
4

Add equal volume of Phenol: Chloroform (phenol:chloroform kit), and vortex well.



Phenol:Chloroform mixed with sample

Centrifuge at 14000 rpm for 15 min. Transfer the upper aqueous layer to a new 1.5 ml Ep tube.



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.

5

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

6

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.

7

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

Discard the supernatant.

8

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

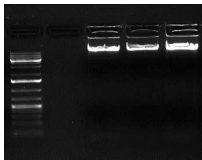
9

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

10

Store DNA sample at -80 °C until use.