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Protocol for DNA Extraction from Tepary Bean

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ABSTRACT

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

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PROTOCOL CITATION

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Semagn K. Leaf tissue sampling and DNA extraction protocols. Methods Mol Biol. 2014;1115:53-67. doi: 10.1007/978-1-62703-767-9_3. PMID: 24415469.

KEYWORDS

DNA Extraction, CTAB, Tepary bean

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MATERIALS TEXT

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 dispend
  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

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1. Centrifuge

2. Hot Water Bath

3. Spectrophotometer- Nanodrop

4. Gel Documentation system

Collection of leaf sample

1

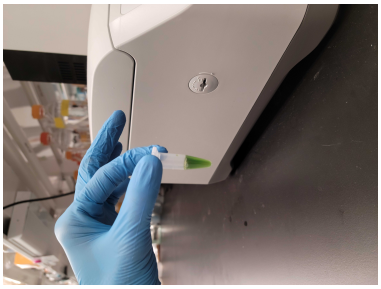
Collect 100 mg of young leaf from Tepary Bean plants in a 2ml eppendorf tubes containing two sterile glass beads and freeze immediately in liquid nitrogen.

DNA Extraction

2

Add 600 µl 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 µl 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.

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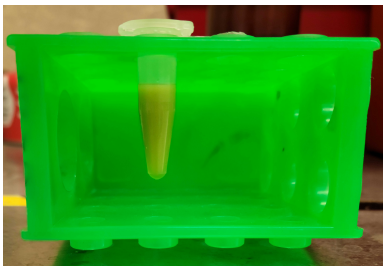
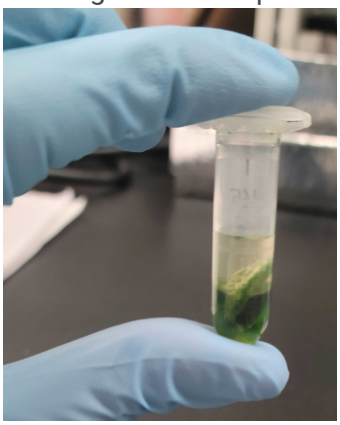


Phenol:Chloroform mixed with sample

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.

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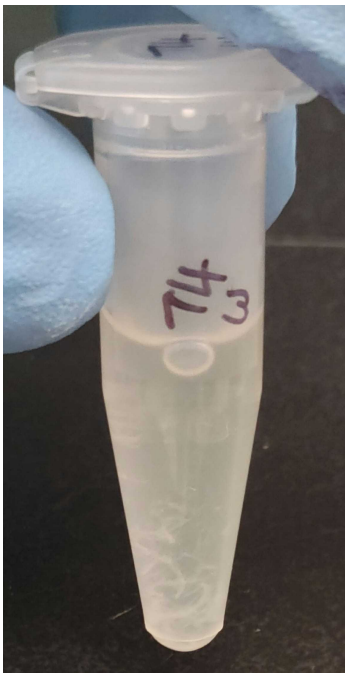

White strands noticed after adding isopropanol

Centrifuge at 14000 rpm for 10 min.

Pellet can be seen at the bottom.

Discard the supernatant.

6

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

Discard the supernatant.

7

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

Discard the supernatant.

8

Air-dry the pellet, dissolve the pellet in 50 µl 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

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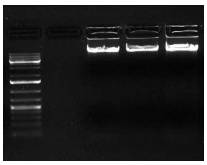
phenol:chloroform step 4.

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.

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