Version 3

Sep 20, 2022

CTAB genomic DNA extraction from Arabidopsis leaf material V.3

Diep R Ganguly[1], Pip Wilson[1], Gonzalo Estavillo[1], Xin Hou[1], Barry Pogson[1]

[1]Australian National University

dx.doi.org/10.17504/protocols.io.8epv5y2dl1bz/v3

Pogson Group EBL_ANU

Diep R Ganguly
The Australian National University

ABSTRACT

CTAB-based extraction of genomic DNA from Arabidopsis leaf tissue.

DOI

dx.doi.org/10.17504/protocols.io.8epv5y2dl1bz/v3

PROTOCOL CITATION

Diep R Ganguly, Pip Wilson, Gonzalo Estavillo, Xin Hou, Barry Pogson 2022. CTAB genomic DNA extraction from Arabidopsis leaf material. **protocols.io** https://protocols.io/view/ctab-genomic-dna-extraction-from-arabidopsis-leaf-buebntan
Version created by Diep R Ganguly

KEYWORDS

genomic DNA, Arabidopsis

CREATED

Apr 21, 2021

LAST MODIFIED

Sep 20, 2022

PROTOCOL INTEGER ID

49315

GUIDELINES

Gives reasonable quality and yield of gDNA. Ideal for DNA in PCRs applications, Dye-terminator Sanger sequencing reactions, and cloning. Can be used for next-generation sequencing applications, however, ensure you perform additional cleaning steps and longer centrifuge times. Run on a 1% agarose gel to ensure you have good quality and clean DNA preparation (and Nanodrop), often these preps yield a substantial portion of sheared nucleotides (likely RNA, so make sure you add RNase A during extraction).

MATERIALS TEXT

MATERIALS

RNase

A **Qiagen Catalog #19101**

EDTA **Contributed by users**

1.5 mL Eppendorf tubes **Contributed by users**

water **Contributed by users**

Ethanol **Contributed by users**

NaCl **Sigma**

Aldrich Catalog #53014

Hexadecyltrimethylammonium bromide **Sigma**

Aldrich Catalog #H6269

Tris-HCl (Tris-Hydrochloride),

100gm **Promega Catalog #H5121**

2-Propanol (IsoPropanol) **Bio Basic**

Inc. Catalog #PC8601.SIZE.4L

Tris-EDTA, pH

8.0 **Ambion Catalog #AM9849**

Chloroform **Sigma Catalog #366919-1L**

Centrifuge **Contributed by users**

Water bath set to 65°C **Contributed by users**

Tissue lyser
1/8" steel ball bearings
Vortex
Centrifuge
RNase A (e.g. Promega #A7973 or Sigma #R6148)

SAFETY WARNINGS

Citation: Diep R Ganguly, Pip Wilson, Gonzalo Estavillo, Xin Hou, Barry Pogson CTAB genomic DNA extraction from Arabidopsis leaf material https://dx.doi.org/10.17504/protocols.io.8epv5y2dl1bz/v3

Perform chloroform steps in fume hood.

BEFORE STARTING

Ensure you grind your leaf tissue into a fine powder using mortar and pestle or Qiagen tissue lyser (place 1/8" steel ball bearing into tube with tissue sample).

Make sure leaf tissue remains frozen until the addition of CTAB buffer.

Cell lysis 15m

1 Prepare 2% CTAB buffer. 15m

A	B
Reagent	**[Cf]**
hexadecyltrimethylammonium bromide	2% (w/v)
NaCl	1.4 M
EDTA (pH 8)	20 mM
Tris-Cl (pH 8)	100 mM

2% CTAB buffer recipe

2 Aliquot required volume of CTAB buffer and heat in water bath at 60 ºC for 5-10 minutes immediately before use. 10m

3 Add 300 µL / 100 mg leaf tissue of CTAB buffer. 2m

4 2m

Add RNase A solution to a concentration 50-100 µg/mL.

5 Mix well with a vortex. Invert samples by hand to ensure that all ground tissue is in solution. 5m

6 Incubate in water bath at 60 ºC for 30 - 60 minutes. Mix tubes periodically by inversion. 1h

7 Cool samples to room temperature . 10m

Citation: Diep R Ganguly, Pip Wilson, Gonzalo Estavillo, Xin Hou, Barry Pogson CTAB genomic DNA extraction from Arabidopsis leaf material https://dx.doi.org/10.17504/protocols.io.8epv5y2dl1bz/v3

Phase separation 15m

8 Add 300 µL chloroform and mix thoroughly with a vortex or vigorous shaking for 15 seconds. 5m

9 Centrifuge samples for 10 minutes at 20,000 rcf. 10m

10 Transfer upper aqueous (approx. 200 µL) phase to clean tube. 5m

11 20m

Repeat steps 9-10 for a cleaner extract.

Precipitation 15m

12 Add equal volume of ice-cold 2-propanol and mix by inversion. 2m

13 Incubate for 30-60 min @ -20 °C 1h

14 Centrifuge for 15 min @ 20,000 rcf. 15m

15 Discard supernatant using pipette. 2m

Resuspend DNA 17m

16 Wash pellet with 1 mL of 70 % ethanol (mix by inversion). 2m

17 Centrifuge samples @ 9,200 rcf for 5 min. 5m

Citation: Diep R Ganguly, Pip Wilson, Gonzalo Estavillo, Xin Hou, Barry Pogson CTAB genomic DNA extraction from Arabidopsis leaf material https://dx.doi.org/10.17504/protocols.io.8epv5y2dl1bz/v3

18 Remove as much ethanol as possible using a pipette, then allow pellet to air dry for 5 minutes. 10m

19 Resuspend gDNA in nuclease-free H_2O or low EDTA TE buffer (10 mM Tris-Cl pH 8, 0.1 mM EDTA).

20 Test yield and purity of samples using a Nanodrop and running samples on a 1 % agarose gel.

Citation: Diep R Ganguly, Pip Wilson, Gonzalo Estavillo, Xin Hou, Barry Pogson CTAB genomic DNA extraction from Arabidopsis leaf material https://dx.doi.org/10.17504/protocols.io.8epv5y2dl1bz/v3