CTAB genomic DNA extraction from Arabidopsis leaf material

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Abstract

Extraction of genomic DNA from Arabidopsis leaf material.

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Guidelines

Gives reasonable quality and yield of gDNA, typically would use for PCRs, sequencing, and cloning but not for next-generation sequencing.

Before start

Make CTAB buffer:

- 2% (w/v) CTAB

- 1.4 M NaCl

- 0.1 M Tris-HLC pH 8

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

Materials

RNase A 19101 by Qiagen
EDTA by Contributed by users
1.5 mL Eppendorf tubes by Contributed by users
water by Contributed by users

- ✓ Ethanol by Contributed by users
  NaCl 53014 by Sigma Aldrich
  Hexadecyltrimethylammonium bromide H6269 by Sigma Aldrich
  Tris-HCl (Tris-Hydrochloride), 100gm H5121 by Promega
  2-Propanol (IsoPropanol) PC8601.SIZE.4L by Bio Basic Inc.
  Tris-EDTA, pH 8.0 AM9849 by Ambion
  Chloroform 366919-1L by Sigma
- ✓ Centrifuge by Contributed by users
- ✓ Water bath set to 65°C by Contributed by users

Protocol

Pre-heat 2% CTAB buffer at 65 °C in water bath
Step 1.

Heat only desired volume for use (300 µl/100mg leaf tissue)

Add 300 µl CTAB buffer to each sample and mix well (vortex)
Step 2.

- Adjust CTAB volume = 300 µl/100mg tissue

- Can add 3 µl RNase A solution (100 µg/µl) to each sample if desired.

Incubate samples at 65°C (water bath) for at least 30 mins with occasional mixing by inversion of tubes.
Step 3.

Can be up to several hours or as needed based on input tissue and desired yields.

Remove samples from water bath and allow to cool to room temp.
Step 4.

- Can place on ice/in fridge to speed up.

Add 300 µl chloroform to each sample. Mix well (vortex or shaking).
Step 5.

- Perform in fume hood.

Centrifuge 5 - 15 min at 20,000g and transfer upper aqueous layer to new tube.
Step 6.

- Transfer 100 - 200 µl (lower volume = less chance of contamination from organic layer).

- Depending on gDNA use, yield/cleanliness required, can repeat steps 5-6.

Add equal volume ice cold 2-propanol and incubate for 30 min @ -20 °C

Step 7.

- Can incubate longer or at -80 °C depending on yield required.

Centrifuge @ 20,000g for 5 - 15 min to form a pellet and discard supernatant using pipette.

Step 8.

Add 500 µl 70 - 80 % Ethanol to sample and centrifuge @ 9,000 - 10,000g for 5 min. Pipette off ethanol (as much as possible) and air dry for 1-2 min.

Step 9.

Resuspend gDNA in desired volume of water or Tris-EDTA buffer. Determine concentration by Nanodrop or Qubit or run on agarose gel to check quality.

Step 10.

Warnings

Perform chloroform steps in fume hood.