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CTAB genomic DNA extraction from Arabidopsis leaf material V.2

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We use this protocol and it's working

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Abstract

Extraction of genomic DNA from Arabidopsis leaf material.

Guidelines

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

Materials

MATERIALS

- RNase A **Qiagen Catalog #**19101
- EDTA
- 1.5 mL Eppendorf tubes
- water
- Ethanol
- NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**53014
- Hexadecyltrimethylammonium bromide **Merck MilliporeSigma (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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**366919-1L
- Centrifuge
- Water bath set to 65°C

Safety warnings

Perform chloroform steps in fume hood.

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).

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

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

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

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.

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.

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

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

5 - Perform in fume hood.

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

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

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.

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.

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.

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