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We use this protocol and it's working

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CTAB genomic DNA extraction from Arabidopsis leaf material V.2

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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 **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**

SAFETY WARNINGS

Perform chloroform steps in fume hood.

BEFORE START INSTRUCTIONS

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 (vorte...

- 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...

- 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..

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

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

- 5
 - Perform in fume hood.

Centrifuge 5 - 15 min at 20,000g and transfer upper aqueou..

- 6
- Transfer ~100 - 200 μ l (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 m..

- 7
- Can incubate longer or at -80 °C depending on yield required.

Centrifuge @ 20,000g for 5 - 15 min to form a pellet and dis..

8

Add 500 μ l 70 - 80 % Ethanol to sample and centrifuge @ 9,..

9

Resuspend gDNA in desired volume of water or Tris-EDTA b..

10