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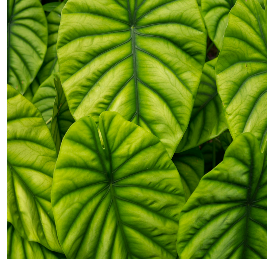
96 well spin column TNA extraction from plants - CTAB method



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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is designed for extracting total nucleic acids (TNA) from plant material. In reality the drying step probably means that you won't isolate plant mRNA but viral RNA (and probably plant ribosomal RNA) are recovered. This protocol is based on patent US 20110092687 A1 to allow the use of CTAB with spin columns instead of the more usual chloroform/Isoamyl alcohol method.

This protocol is significantly improved through the use of high volume multichannel electronic repeaters (e.g. a **Gilson PIPETMAN M P8X1200M**) or a 96 well pipette (e.g. the **Gilson Platemaster**) but can be performed using manual multichannel pipettes.

Attachments

Plant_protocol_CTAB_...
107KB

Guidelines

Note on centrifugation in this protocol:
Maximum x g throughout is determined by both the centrifuge and the tolerances of the plastic labware you are using. If you find that not all of the liquid is moving through the spin column or that tissue debris is not pelleting, you can increase centrifugation times without any detriment to the process. Similarly, if you find plastic labware is breaking during centrifugation (this can happen with certain 0.5 ml deepwell plates), reduce the speed/g of the centrifuge and increase the centrifugation time to compensate.

Note on blocked spin columns:
Large samples and gelatinous samples can occasionally block the spin column. This can be dealt with by:
1. Increasing centrifuge times.
2. Decreasing input material.
3. Increasing the centrifugation time in the debris pelleting stage.

Preparation of grinding tubes and buffer

1 Source steel beads (ball bearings) for tissue grinding (Tungsten beads are not usually necessary). We use hardened 3mm **carbon steel** or **stainless steel bearings from simplybearings.co.uk**. This protocol requires three beads per sample tube.

2 Beads are usually shipped coated in manufacturing oil (especially the carbon steel beads). To remove this, place beads in a borosilicate glass beaker or Duran bottle with the pouring lip and lid removed then bake for at least 12 hours at 250 ^{o}C.

Figure 1: Depending on baking time, carbon steel beads will change colour, this is normal.

3 Prepare **CTAB lysis buffer**:

A	B	C
Reagent	**Required cencentration in buffer**	**Amount per litre**
CTAB	2%	20 g
Polyvinyl pyrrolidone	2%	20 g
Tris-HCl	10 mM	10 ml (of 1M stock solution)

A	B	C
EDTA	20 mM	40 ml (of 0.5M stock solution
Ammonium chloride	1.3M	69.54 g

4 Prepare 5 ml preparation tubes (**e.g. Starlab #E1450-1100**) containing three 3 mm hardened steel beads in batches of 90 tubes.

Preparation of plant material

5 Using forceps (either disposable forceps or ethanol and flame steel forceps between samples), place ~ 50 mg of dried plant material (100 mg if using fresh) into the pre-prepared collection tubes containing the hardened steel ball bearings.

Note

Be sure to label the tube with a sample number and any additional detail necessary for the study.

Alternatively barcode your tubes with preprinted labels and use data collection *software* ***such as epicollect5*** *to record sample IDs and metadata.*

6 Place all sample tubes in zip-lock bags in batches of 90 (to allow for two extraction negatives, a DNA negative and three positive controls).

Freeze at -80°C until ready to proceed with the extraction.

Initial homogenisation of plant material

7 Place tubes while still frozen in tissue homogeniser and grind the plant material in a tissue homogeniser until homogenised.

Note

We use a Geno/Grinder 2010 at full speed (1750 RPM) for 4-5 minutes in 2 min batches using tube holders stored at the same temperature as the grinding tubes but different machines or even tube sizes and sample volumes will require separate optimised settings.

8 To the plant material, add freshly-prepared **CTAB lysis buffer (see below for volumes)**, and place in a tube shaker, vortex mixer or tissue homogeniser (at low speed) to mix.

Note

For dried material add add 700 µl of CTAB lysis buffer as some will be absorbed by the dry material. For fresh material you only need 500 µl.

Note

Ensure that the powdered plant material is thoroughly mixed with the CTAB lysis buffer so that the TNA can dissolve into the supernatant.

9 Incubate lysed samples in CTAB lysis buffer.

55 °C for 60 minutes.

10 Centrifuge at 1500 x *g* for 5 minutes to pellet plant debris.

Note

This step is very important. Plant lysates tend to contain suspended fine particulates. These can block the spin column so it is best to remove as much as possible.

11 Transfer 350 µl of supernatant to a new 96 well filter plate (**NBS biologicals #SD5006**) placed on top of a 1-2 ml deep well plate. Seal the filter plate with a breathable plate seal (**Starlab #E2796-3005**)

Add 350 µl of CTAB buffer to two of your empty wells designated as extraction negatives.

12 Centrifuge at 1500 *x g* for 1 minute to filter and further homogenise the plant material.

Note

Sample can be stored at -20 ^{O}C until ready to proceed.

TNA purification

13 Add 350 µl of 100% Isopropanol to each sample and vortex to mix.

14 Centrifuge at 1500 x *g* for 5 minutes to pellet plant debris.

Note

This step is very important. Plant lysates tend to contain suspended fine particulates. These can block the spin column so it is best to remove as much as possible.

15 For each sample, add as much as possible* of the supernatant from step 14 to a well in a 96-well silica membrane spin column (**we use SD5007 from NBS Biologicals**) sitting on top of a 1-2 ml deep well plate and cover with **a breathable seal**.

Note

** without disturbing any sediment.*

16 Centrifuge at 1500 *x g* for 5 minutes.

17 Discard flow-through or change waste receiver and add 500 µl of 70% Ethanol. Centrifuge at 1500 *x g* for 5 minutes.

18 Repeat step 15 two more times for a total of three washes. After the last wash spin for 10 minutes to dry the spin column.

19 Transfer the spin column to a new 0.5 ml deep well plate and add 50-100 µl of water or 10mM Tris-HCl. Incubate for 10 minutes at room temperature and centrifuge at 1500 *x g* for 1 minute.

The flow-through now contains the extracted TNA.