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CTAB DNA extraction of high polysaccharide samples

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We use this protocol and it's working

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Disclaimer

The duration times presented are estimations only.

Abstract

This protocol describes a CTAB HMW DNA extraction for ground samples high in polysaccharides, including an optional sorbitol wash.

Acronyms:
CTAB = cetyltrimethyl ammonium bromide
HMW = high molecular weight

Attachments

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CTAB DNA extraction ...

35KB

Materials

- D-sorbitol (only if performing sorbitol wash)
- PVP40
- Tris-HCl pH 8
- EDTA pH 8
- Nuclease-free water/Milli-Q water
- BME (β-Mercaptoethanol)
- CTAB
- NaCl
- PEG-8000
- Proteinase K
- Pipettes, pipette tips
  - optional: wide-bore p1000 pipette tips if you wish to transfer the DNA extract to a 1.5 ml LoBind tube
- 50 ml tubes
  - 2 ml LoBind tubes if working with smaller non-plant samples
- Vortexer
- Centrifuge
- Thermomixer
- Chloroform:isoamylalcohol (24:1)
- Isopropanol
- 99 % ethanol
- Tube rotator (optional)

Safety warnings

The operator must wear a lab coat, powder-free nitrile gloves and work in a fume hood to perform the laboratory procedures in this protocol. In addition, chloroform-resistant gloves must be worn, and chloroform waste bottles should be stored in the fume hood.

Waste needs to be collected in a suitable container and disposed of in accordance with local regulations.

Liquid waste needs to be collected in a suitable container and disposed of in accordance with local regulations.

See best practices, thawing and handling instructions, and safety instructions for all the kit reagents, as well as instructions and safety data sheets for other reagents used in this protocol **before starting**.

If the tissue sample is ground in cryogenic conditions, take care to follow appropriate safety measures.

Before start

- NB: this protocol only works for ground samples.
- For plants, prepare 20 mL of CTAB lysis buffer in a 50 mL tube, which will accommodate a maximum of 1 gram plant tissue. If low yield is expected, prepare 10 ml extra CTAB lysis buffer per sample.
- For smaller non-plant samples (<50 mg) smaller volumes can be used. Use 2 ml tubes for lysis with a total of 1 ml CTAB lysis buffer.
- Sorbitol wash is usually not employed for non-plant samples, but can be beneficial when high amounts of polysaccharides are expected (e.g., crustaceans, whole insects, etc.)

Reagents used:
CTAB, a cationic detergent, constitutes a long hydrophobic hydrocarbon chain and a hydrophilic head. It forms micelles in water because of the amphipathic nature. During DNA extraction, under aqueous condition, CTAB comes in contact with the biological membrane, captures the lipids, and results in the release of nucleus, which is devoid of membrane.

CTAB at low ionic strength (<0.5M): precipitates nucleic acids and acidic polysaccharides.
CTAB at high ionic strength (>1.4M): binds sugars, denatures proteins and inhibits enzymes.

Buffer Recipes:

Sorbitol wash buffer

	Reagents for Sorbitol wash buffer	Final conc.	Stock	For 1 ml	For 30 ml	For 500 ml
	D-Sorbitol (182.17 g/mol)	0.35 M	powder	0.064 g	1.91 g	31.88 g
	PVP40	1 %	10 %	100 µl	3 ml	50 ml/5 g
	Tris-HCl pH8	100 mM	1M	100 µl	3 ml	50 ml
	EDTA pH 8	5 mM	0.5 M	10 µl	300 µl	5 ml
	NFW/MQW	-	-	to 1 ml	to 30 ml	to 500 ml
	To be added individually:					
	BME	4 %	100 %	40 µl	1200 µl	NA

Recipe for Sorbitol wash buffer

CTAB lysis buffer

	Reagents for CTAB lysis buffer	Final conc.	Stock	For 1 ml	For 20 ml	For 500 ml
	Tris-HCl pH8	100 mM	1M	100 µl	2 ml	50 ml
	EDTA pH 8	25 mM	0.5 M	50 µl	1 ml	25 ml

	Reagents for CTAB lysis buffer	Final conc.	Stock	For 1 ml	For 20 ml	For 500 ml
	CTAB	4 %	-	0.04 g	0.8 g	20 g
	NaCl (58.44 g/mol)	2 M	-	0.12 g	2.34 g	58.44 g
	PEG-8000	3 %	30 %	100 µl	2 ml	50 ml/15 g
	PVP40	1 %	10 %	100 µl	2 ml	50 ml/5 g
	NFW/MQW	-	-	to 1 ml	to 20 ml	to 500 ml
	To be added individually:					
	BME	2 %	100 %	20 µl	400 µl	NA
	Proteinase K	0.4 mg/ml	20 mg/ml	20 µl	400 µl	NA

Recipe for CTAB lysis buffer

Sorbitol Wash

20m

1 Sorbitol wash is done according to **Sorbitol washing complex homogenate for improved DNA extractions** by Jones and Schwessinger with minor changes.

1.1 Transfer 30 ml sorbitol wash buffer to a 50 ml tube, add 1200 µl BME and mix. 2m

1.2 Transfer 0.2-1 gram of frozen ground sample tissue to the tube and immediately shake, invert and vortex to mix thoroughly. 2m

1.3 Centrifuge at 4,700 x g for 00:05:00 at RT. 5m

1.4 Carefully decant the supernatant. Remove as much of the wash solution as possible without losing the pellet (Supernatant should appear slightly cloudy or discolored light yellow or brown.) 1m

1.5 If the supernatant was turbid, viscous or had dark discoloration, repeat the sorbitol wash. 9m

1.6 Proceed to a DNA extraction method of choice by adding lysis buffer to the pellet. 1m

CTAB Extraction

4h

2 Add BME and Proteinase K to individual tubes with CTAB lysis buffer and preheat to 65 °C. 3m

3 Transfer the pre-warmed CTAB lysis buffer from previous step to the sorbitol washed pellet and vortex tube 5 sec. (or as much as needed to get a homogenous suspension). 1m

4 Incubate min. 01:00:00 at 65 °C with agitation (800 rpm). 1h

5 Let the tube cool to RT. 5m

6 Add ~1 volume of chloroform:isoamylalcohol (24:1) and vortex/shake to make sure phases mix properly. 1m

7 Centrifuge at 20 °C, 00:10:00, 4,700 x g with acceleration (9) and deceleration (6). 15m

Note

Centrifugation creates heat and we want to maintain temperature without going below 15 °C.

The slower deceleration will create a more even/horizontal interphase and make clean transfer easier (only applies to swinging bucket rotors).

8 Gently transfer the aqueous phase to a new tube. 1m

Note

If the DNA is hard to transfer after the chloroform spin (big clumps near the interphase), consider breaking up the DNA more by transferring most of it to a new tube and either pipetting or vortexing before doing a second chloroform rinse. If DNA is too HMW it will be hard to pellet and you might lose most of it anyway.

9 For higher yield, add 10 ml pre-warmed CTAB lysis buffer to the organic phase and repeat step 7 and transfer the aqueous phase into the same tube as before. 15m

10 For higher purity, repeat steps 6-8 (if you did step 9, use <1x volume). 15m

11 Add 0.6-0.7 volume of RT isopropanol and gently mix by inversion 10 times. 2m

12 Incubate tube at RT for min. 00:15:00 to allow for better precipitation. 15m

13 Centrifuge at 20 °C, 00:30:00, 4,700 x g. 30m

14 Carefully remove the supernatant by gentle decanting without disturbing the pellet.

1m

Note

For 2 ml tubes, remove the supernatant with a pipette instead.

15 Wash with freshly prepared 70 % EtOH. Gently swirl and invert the tube before leaving the pellet to soak for 00:15:00 at RT or use a tube rotator at 10-20 rpm. 17m

16 Centrifuge 20 °C, 00:10:00 , 4,700 x g. 10m

17 Carefully decant the supernatant without disturbing the pellet. 1m

Note

If transfer to 1.5 ml tube is desirable, you can add 1 ml freshly prepared 70 % EtOH and transfer the pellet to the smaller tube using a wide-bore p1000 tip.

18 Repeat steps 15-17 at least once. 28m

Note

If precipitating with isopropanol, consider a 3rd wash to further remove excess salt.

19 Perform a quick spin to gather the residual ethanol at the bottom of the tube and carefully remove with a P20 tip. Repeat if needed. 5m

20 Let pellet air dry for 5-15 min 00:10:00 at room temperature, taking care not to over dry. 15m

21 Add resuspension buffer (preferred Elution Buffer/lowTE/TE) and leave at RT with gentle mixing overnight to resuspend. No pipetting at this time! Alternatively, leave at 4°C/RT for 3-4 days to increase homogeneity before performing quality control.

Short incubation at 50 °C may promote faster resuspension, especially if pellet has been slightly overdried.

22 It can take several weeks for some samples to rehydrate properly. The higher the concentration and integrity, the longer it takes for the sample to become homogenous.

If needed, the resuspended DNA can be RNase treated and precipitated again.

Note

For HiFi libraries, the DNA can be sheared and cleaned with Ampure beads (0.5X) instead of precipitation.

Protocol references

Sorbitol wash is done according to https://www.protocols.io/view/sorbitol-washing-complex-homogenate-for-improved-d evow1875pr2/v1 with minor changes