High quality DNA extraction protocol from recalcitrant plant tissues

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Abstract

This protocol was developed in order to remove secondary compounds which inhibited restriction enzyme digestion of *Xanthorrhoea* genomic DNA. It has also been used to successfully obtain high quality, high weight DNA from other recalcitrant plant tissues, including *Eucalyptus*, *Eremophila*, *Spyridium*, and various Malvaceae, Rutaceae, Orchidaceae, and Fabaceae species.

It is based on a modified CTAB protocol from Doyle and Doyle (1990), and includes modifications from Shepherd and McLay (2011) and Tibbits *et al* (2006).

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Guidelines

This protocol requires standard molecular laboratory equipment and chemicals. There are three solution additives which were critical for removing inhibiting secondary compounds from *Xanthorrhoea* tissue; STE, NaCl:BSA, and NaAC. It is possible that not all these additives are required for all plant tissues. However, all three are used in our lab to obtain very clean DNA from a variety of families.

It is possible to include the NaAC wash step with the isopropanol precipitation, but performing it at the end seems to produce better quality DNA.

Before start

Prepare the following reagents:

Tris-HCl pH8 (1M)

EDTA (0.5M)

NaCl (5M)

STE

CTAB

NaCl:BSA 5:1

Sodium acetate (2.4M)

Tris-HCl pH8 (10mM)

70% ethanol

Protocol

Prepare STE (fresh is best, keep for 2 weeks maximum)

Step 1.

Volume for 50 samples	Volume for 25 samples
4g sucrose	2g sucrose
1.5 mL Tris-HCl pH8 (1M)	0.75 mL Tris-HCl pH8 (1M)
5 mL EDTA (0.5M)	2.5 mL EDTA (0.5M)
H_2O to 50 mL	H_2O to 25 mL

Prepare CTAB (fresh is better)

Step 2.

	Start Conc M.	Final conc M.	for 50 mL solution
Tris-HCL pH8	1 M	100 mM	5 mL
NaCl	5 M	1.4 M	14 mL
EDTA	0.5 M	50 mM	5 mL
CTAB	2%		1g
PVP	2%		1g
H_2O			To 50 mL

Mix on a stir plate (unheated) until the solution is clear.

Prepare NaCl:BSA additive

Step 3.

Make 100x (=4%) BSA from powder in H_2O. Combine 5M NaCl with 100x BSA in a 5:1 ratio.

This can be frozen for long-term storage.

Preheat CTAB

Step 4.

Add 600 mL of CTAB per sample to a falcon tube. In fumehood, add 2 µL of BME per mL of CTAB, and 4 µL of Proteinase K per reaction. Warm at 65 °C.

Grind tissue

Step 5.

Grind 20-50 mg of plant tissue using your favourite grinding method. I use liquid nitrogen and grinding sand for particularly sclerophyllous tissue. Place into labelled 1.7 mL eppendorf tube.

At this stage it is useful to label two additional tubes per sample. You could either label them now or later during a centrifugation step.

STE step

Step 6.

Add 1 mL of STE to tissue. Vortex thoroughly so tissue is fully mixed. Spin at 5000 rpm for 10 minutes. Discard supernatent by pouring off.

Addition of CTAB

Step 7.

In a fumehood, add 500 µL of the pre-warmed CTAB solution to each tube. Add 100 uL of NaCl:BSA solution. Vortex. Incubate at 65 C for at least an hour (to overnight). Mix by vortexing or shaking several times through incubation.

First chloroform step

Step 8.

Add 450 µL of chloroform to each tube. *If this volume of chloroform will not fit into the eppendorf tube, split the tissue+CTAB mixture into two separate eppendorf tubes and add 450 µL of chloroform to each tube.

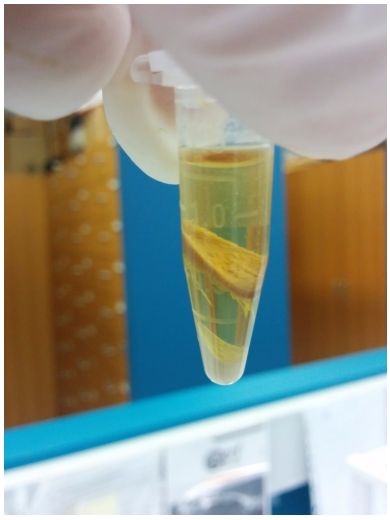
Shake to mix and open tube to release built-up gas using a kimwipe to catch drops. Be careful of cross-contamination at this point.

Spin at maximum speed for 5 minutes.

removing supernatent

Step 9.

using a P200 set to 190 µL, remove the top aqueous phase (usually between 300-400 µL) and put into a new eppendorf tube. Be careful not to include any of the solid layer.



Second chloroform step

Step 10.

Add 450 µL to the removed aqueous phase solution. Shake to mix and open tube to release built-up gas using a kimwipe to catch drops.

Spin at maximum speed for 5 minutes.

Removing supernatant

Step 11.

Using a P200 set to 190 µL, remove the top aqueous phase (usually between 300-400 µL) and put into a new eppendorf tube. Be even more vigilant at this step as there is no solid barrier separating the top aqueous phase from the chloroform.

Isopropanol DNA precipitation

Step 12.

Add 500 uL of room temperature isopropanol. Mix by gently inverting five times.

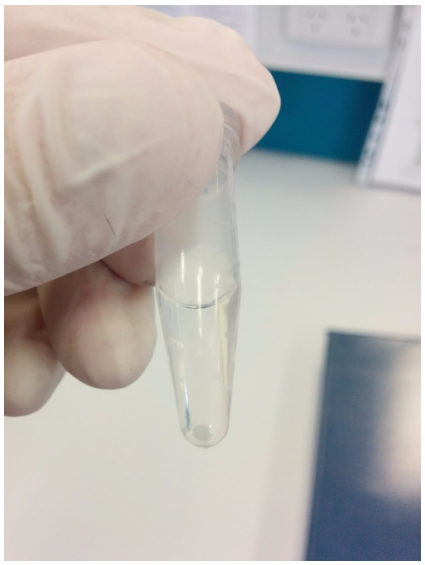
*At this step it is possible to add 110 µL sodium acetate (3M, pH5.2), 500 µL isopropanol, and mix by gently inverting with a 20 mins incubation at room temp. Then follow Steps 13-16. This avoids the need to precipitate again with NaAC after re-eluting the DNA (Steps 17-20) . However, this step did not work with some taxa and resulted in loss of DNA.

Isopropanol centrifugation

Step 13.

Spin for 10 minutes at maximum speed with eppendorf tubes facing inwards (i.e. the hinge is to the outside of the centre of the centrifuge).

A DNA pellet may appear at the bottom of the tube.



Carefully pour off the supernatant.

Ethanol wash and centrifugation

Step 14.

Add 500 µL of 70% ethanol (made fresh). Try to dislodge the pellet from the bottom of the tube through pipetting or flicking the tube.

Centrifuge for five minutes at maximum speed.

Carefully pour off the ethanol.

Drying pellet

Step 15.

Dry pellets for at least two hours to overnight by leaving tubes open on bench/in fumehood, with kimwipes securely placed over them.

Alternatively, you can wick the remaining ethanol away using kimwipes.

Resuspension in Tris-HCl 10mM

Step 16.

Add 50 µL of 10mM Tris-HCl pH8 and allow DNA to resuspend for at least one hour before use.

Sodium acetate wash

Step 17.

Add 200 µL of 100% ethanol and 36.25 µL of NaAC (2.4M) to each tube.

Mix by inverting.

Centrifuge at maximum speed for 10 minutes.

*this step can be incorporated into the isopropanol step by including NaAC so the final concentration is 0.3M, although this did not seem to work for *Xanthorrhoea*.

Ethanol wash

Step 18.

Carefully pour off supernatant. Occassionally the pellet is not stuck to the tube so careful it doesn't come out.

Add 1000 µL of 70% ethanol. Try to dislodge the pellet from the bottom of the tube through pipetting or flicking the tube.

Centrifuge at maximum speed for three minutes.

Drying pellet

Step 19.

Carefully pour off supernatant.

Dry pellets for at least two hours to overnight by leaving tubes open on bench/in fumehood, with kimwipes securely placed over them.

Resuspension of pellet.

Step 20.

Add 50-100 µL of 10mM Tris-HCl pH8 and allow DNA to resuspend for at least one hour before use.

Check quality and quantity using spectrophotometry.

Warnings

General chloroform extraction safety. The inclusion of BME makes a fumehood a requirement.