Modified Samberg Phenol:Chloforom HMW DNA prep for (some) plants

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Abstract

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Guidelines

Only attempted on Asparagus, Spirodela, and Milkweed. Works reasonably well. Uses a lot of ProtK and RNase H -- I have tested lower amounts with varying degrees of success. Some plants are just dirter than others, and overloading enzyme seems to help.

Before start

Prepare CTAB buffer and use within 1-2 weeks

CTAB buffer (50 ml)

5ml Tris 1M, pH 8.0

2ml 0.5M EDTA

4.15g NaCl

1g CTAB

1g PVP

add ddH20 to 50ml, add stir bar, spin over low/med heat to dissolve, good on the benchtop for 1-2 weeks

Protocol

Step 1.
Grind 3.5 g of young tissue in liquid nitrogen with a mortar and pestle

Step 2.

Add 15 ul of CTAB buffer, plus 30 ul of beta-mercaptoethanol (BME), plus 200ul NEB Proteinase K (800

U / ml)

CTAB buffer (50 ml) (use within 2 weeks)

5ml Tris 1M, pH 8.0

2ml 0.5M EDTA

4.15g NaCl

1g CTAB

1g PVP

add ddH20 to 50ml, add stir bar, spin over low/med heat to dissolve.

ANNOTATIONS
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15 ml CTAB, not ul!

Step 3.
Vortex at full speed for 10 seconds

Step 4.
Incubate in waterbath at 57 C for 30 minutes, gently inverting every 5 or so minutes

Step 5.

Reduce bath temperature to 37 degrees, add 50ul RNase A (20 mg/ml), incubate 30 minutes

Step 6.

Add 17.5 ml chloroform, mix on rocker or inverter gently for 30 minutes

Step 7.
Spin at 3000 – 4500 x g for 30 minutes

Step 8.
Optional but often required: Remove aqueous top layer to new tube with large wide-boar 25ml pipette VERY slowly, add equal volume of chloroform and repeat spin

Step 9.
Remove aqueous top layer slowly and divide equally into 2 x 15 ml conicals, again dispensing very slowly

Step 10.

Add 1 volume of 25:24:1 phenol:chloroform:isoamyl to each, place on rocker or inverter for 30 minutes. Gentle but thoroughly mixed until very milky.

Step 11.
Spin for 30 minutes at 3000 – 4500 x g.

Step 12.

Remove supernate into 2 x 15 ml conicals and repeat steps 10 and 11 for another phenol:chloroform:IAA cleanup. This time the emulsion will not be milky, usually more foamy looking.

Step 13.
Remove supernate very slowly and gently and combine into a single 50ml conical

Step 14.

Add 2.5 volumes 100% ethanol, ice cold. Spin gently end-over-end 20 times until dime-sized (2 cent euro?) precipitate forms. If no visible precipitate clumping out of solution, add 1-2ml of 3M sodium acetate.

Step 15.
Let sit on ice for 10 minutes. Spin tube end-over-end another 10 times, very slowly. This helps to clump up the DNA a little bit more.

Step 16.

Fashion a shepherd's crook from a glass pipette over a flame, fish out the clump of DNA in one piece and gently swirl it in a separate conical filled with 70% ethanol for 1 minute.

Step 17.
With another glass pipette, gently move the DNA clump off of the crook and into a fresh 1.5ml eppendorf tube

Step 18.
Add 1ml 70% ethanol, flick tube, rotate end-over-end gently for 1 minute

Step 19.
Spin at 10,000 x g for 1 minute to pellet DNA, pour off ethanol and prop tube upside down on Kimwipe for 10 minutes or until ethanol almost entirely evaporated.

Step 20.
Add 150ul of ultrapure H20. Let sit overnight at 4 degrees C.

Step 21.
Using a cut-off 10ul tip, draw 10ul from near the bottom of the tube and dilute into 90ul of ddH20. Spin on hula mixer gently for an hour to somewhat homogenize the sample.Test on Nanodrop.

Step 22.

Optional but often: If 260/230 is low (<1.9), potentially salt contamination especially if too much sodium acetate was added. Add 2.5 volumes of ice-cold pure ethanol to sample, spin end-over-end several times slowly, let DNA crash out of solution and repeat steps 19-20.

Step 23.

Warnings

May cause emotional distress.