VERSION 1
NOV 08, 2017

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.kpwcvppe

Protocol Citation: Alex Harkess 2017. Modified Samberg Phenol:Chloroform HMW DNA prep for (some) plants. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.kpwcvppe>

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Protocol status: Working

Created: Nov 08, 2017

Last Modified: Mar 17, 2018

PROTOCOL integer ID:
8662

Modified Samberg Phenol:Chloroform HMW DNA prep for (some) plants V.1

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High molecular weight DNA extraction from all kingdoms
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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 dirtier than others, and overloading enzyme seems to help.

SAFETY WARNINGS

! May cause emotional distress.

BEFORE START INSTRUCTIONS

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 ddH₂O to 50ml, add stir bar, spin over low/med heat to dissolve, good on the benchtop for 1-2 weeks

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

- 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 ddH₂O to 50ml, add stir bar, spin over low/med heat to dissolve.

- 3 Vortex at full speed for 10 seconds

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

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

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

- 7 Spin at 3000 – 4500 x g for 30 minutes

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

- 9 Remove aqueous top layer slowly and divide equally into 2 x 15 ml conicals, again dispensing very slowly
- 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.
- 11 Spin for 30 minutes at 3000 – 4500 x g.
- 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.
- 13 Remove supernate very slowly and gently and combine into a single 50ml conical
- 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.
- 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.
- 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.
- 17 With another glass pipette, gently move the DNA clump off of the crook and into a fresh 1.5ml

eppendorf tube

- 18** Add 1ml 70% ethanol, flick tube, rotate end-over-end gently for 1 minute
- 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.
- 20** Add 150ul of ultrapure H₂O. Let sit overnight at 4 degrees C.
- 21** Using a cut-off 10ul tip, draw 10ul from near the bottom of the tube and dilute into 90ul of ddH₂O. Spin on hula mixer gently for an hour to somewhat homogenize the sample. Test on Nanodrop.
- 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.
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