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High Molecular Weight DNA Extraction From Leaf Tissue

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We use this protocol and it's working

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Abstract

A protocol designed for isolation and purification of high molecular weight genomic DNA from ~1g of vascular plant leaf tissue, for linked and/or long-read sequencing. Used successfully with *Syzygium oleosum* (Myrtaceae), *Rhodamnia argentea* (Myrtaceae), *Telopea speciosissima* (Proteaceae). Sorbitol pre-wash together with higher salt and CTAB concentrations (per Thermo Fisher Application Note 52645, adapted from Inglis et al 2018 doi: 10.1371/journal.pone.0206085), followed by SPRI bead-based purification (adapted from Schalamun et al. 2018 doi: 10.1111/1755-0998.12938) help to ensure sufficient purity for most genomic sequencing applications.

Guidelines

Starting material:
Ideally, fresh young leaf tissue which is immediately frozen in liquid nitrogen upon collection from plant. Time from collection to preservation affects molecular weight of recovered DNA.

Handling genomic DNA (from Ramaciotti Centre sample submission guidelines):

- Avoid over drying of genomic DNA. Allow DNA to air dry and do not use heat.
- When resuspending DNA be gentle. Either carefully invert the tube several times after adding buffer and/or tap the tube gently. Alternatively, allow the DNA to stand in buffer overnight at room temperature (20-22°C) to resuspend.
- Avoid vortexing and harsh pipetting as it can shear genomic DNA. Use wide-bore pipette tips and pipette gently when transferring DNA in solution.
- DNA should be eluted in neutral, buffered solution (e.g. 10 mM Tris Acetate or Tris-HCl, pH 8). Avoid buffers containing EDTA (e.g. TE).
- DNA should be stored at 4°C (short-term) or –20°C / –80°C (long-term).
- Avoid repeated freezing and thawing of genomic DNA, as this will lead to DNA shearing.

Materials

Sorbitol wash buffer (store at 4^{o}C for up to 6 months)

1	Ingredient	Concentration	for 500mL	For 250mL
2	Sorbitol	0.35 M	13.66g	6.83g
3	PVP-40	1 %	5 g	2.5 g
4	Tris-HCl pH 8 (1M)	100 mM	50 mL	25 mL
5	EDTA pH 8 (0.5M)	5 mM	5 mL	2.5 mL
6	β-mercaptoethanol	1 %	(add before use)	(add before use)

Extraction buffer (store at RT for up to 6 months)

1	Ingredient	Concentration	for 500mL	For 250mL
2	NaCl	3 M	87.66 g	43.83 g
3	PVP-40	1 %	5 g	2.5 g
4	CTAB	3 %	15 g	7.5 g
5	Tris-HCl pH 8 (1M)	100 mM	50 mL	25 mL
6	EDTA pH 8 (0.5M)	20 mM	5 mL	2.5 mL
7	β-mercaptoethanol	1 %	(add before use)	(add before use)

Safety warnings

Consult MSDS for each required reagent and handle accordingly.

Before start

Wash magnetic beads according to suppliers' instructions.
Make up chloroform:isoamyl mixture (24:1) and 77 % ethanol; place 77 % ethanol in freezer.
Heat fresh extraction buffer to 65°C.
Add β-mercaptoethanol to wash and extraction buffers.

Extraction

1 Grind ~2 g leaf material in mortar and pestle under liquid nitrogen to fine powder. Make sure material does not thaw. Transfer to 15 mL tubes.

2 Add 10mL sorbitol wash containing β-me to each tube and mix by inversion for 2 min.

3 Centrifuge tubes at 3,000 x g for 5 min. Discard supernatant.

4 Add 6mL pre-warmed extraction buffer containing β-me to each tube, and resuspend pellets by inverting/tapping tubes.

5 Add 30 uL of proteinase K (20mg/mL) to each tube and mix by inversion.

6 Incubate tubes at 65°C for 1 to 1.5 hours, invert tubes every 10-20 min.

7 Cool down tubes for at least 5 minutes.

8 Add 6 mL of chloroform:isoamyl alcohol (24:1) and mix by inversion for 2 min.

9 Spin at 3000 x g RT for 20 min.

10 Transfer water phase to fresh tube, record the volume transferred.

11 Add 5µL of RNAse A (10 mg/mL) and incubate at 37°C for 15 minutes, gently inverting the tubes periodically.

12 Add 1 volume of chloroform:isoamyl alcohol and mix by inversion for 2 min.

13 Centrifuge tubes at 3000 x g for 20 min.

14 Transfer water phase to fresh tube, record the volumes transferred, and add 1 volume room temperature isopropanol. Mix by inversion for 2 min.

15 Leave tubes in fridge overnight.

16 Centrifuge tubes at 3,000 x g for 30 min.

17 Discard supernatant, pipette off residual isOH, and allow pellet to air-dry on bench (take care not to over-dry).

18 Add 1.5 mL ice cold 77 % ethanol. Gently dislodge pellet from bottom of tube, and pour etOH + pellet into new 1.5 mL tube. Leave in fridge for 20 min.

19 Centrifuge tubes at 14,000 x g for 20 min.

20 Discard supernatant. Add 1.5 mL ice cold 77 % ethanol, gently dislodge pellet by inverting/tapping, and leave in fridge overnight.

21 Centrifuge tubes at 14,000 x g for 20 min.

22 Discard supernatant. Add 1.5 mL of 77 % ethanol. Gently dislodge pellet by inverting/tapping, and leave in fridge for 20 minutes.

23 Centrifuge tubes at 14,000 x g for 20 minutes.

24 Discard supernatant, and allow pellet to air-dry on bench (take care not to over-dry).

25 Re-suspend pellet in 100 µL 10mM Tris-HCl pH 8.

26 Evaluate DNA quantity (yield) using Qubit and evaluate DNA purity and integrity via OD260/280, 260/230 (protein, phenol, carbohydrate contamination) and agarose gel (qualitative size, RNA contamination).

SPRI bead cleanup

27 Preheat 10mM Tris-HCl, pH 8 to 50°C. Make fresh 70% EtOH.

28 Add 1x volume of washed beads to 1x volume of DNA.

29 Incubate 10 minutes at room temperature, gently inverting the tubes periodically.

30 Touch spin in desktop centrifuge to draw bead suspension to bottom of tube.

31 Place tube on magnet rack and wait until bead pellet forms on side of tube.

32 Remove and discard supernatant.

33 Add 1mL fresh 70% EtOH and slowly rotate the tubes 360° in the magnet rack to wash bead pellet.

34 Remove and discard supernatant.

35 Repeat EtOH wash (steps 6-7) once.

36 Touch spin in desktop centrifuge to draw residual EtOH to bottom of tube, and remove and discard residual EtOH by pipetting, taking care to avoid disturbing the bead pellet.

37 Allow beads to dry briefly (less than 1 minute), then add 50-100uL (depending on expected/desired DNA concentration) 10mM Tris-HCl, pH 8 (preheated to 50°C).

38 Incubate at least 10 minutes at room temperature to elute. If elution is difficult due to high concentration of high molecular weight DNA (thick, fluffy bead pellet), incubate overnight at 37°C.

39 Place tube on magnet rack and wait until bead pellet forms on side of tube.

40 Transfer supernatant to new tube.

41 Evaluate DNA quantity (yield) using Qubit and adjust concentration as necessary for sample submission guidelines. Evaluate DNA purity and integrity via OD260/280, 260/230 (protein, phenol, carbohydrate contamination) and agarose gel (qualitative size, RNA contamination).