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A modified protocol for rapid HMW DNA isolation from plant tissues using CTAB

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Protocol status: Working

I use this protocol to isolate high molecular weight DNA for PacBio sequencing.

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Materials

CTAB, also known as hexadecyltrimethyl ammonium bromide (Calbiochem, cat. no. 219374).

Extraction buffer To make 250 ml, mix 25 ml of 1 M Tris, pH 8.0, 70 ml of 5 M NaCl, 10 ml of 0.5 M EDTA pH8 and 5 g of CTAB, and bring the final volume to 250 ml with H₂O. Autoclave the buffer.

Note

Add 0.5–1% (v/v) of β ME to the extraction buffer immediately before use to decrease the possibility of oxidation.

5PRIME Phase Lock Gels (PLG Heavy), QuantaBio.

RNase A, DNase and protease-free (10 mg/mL), Invitrogen (Cat. EN0531).

Troubleshooting

- 1 Collect the tissue sample in a 50 mL Falcon tube and snap freeze in liquid nitrogen.
- 2 Preheat extraction buffer in a 65 °C water bath before proceeding with tissue grinding.
- 3 Pour a small amount of liquid nitrogen into a mortar that is precooled to -70 °C. Place the frozen sample into the mortar containing the liquid nitrogen.
- 4 Start slowly and increase the intensity of grinding as the liquid nitrogen completely evaporates. If necessary, add more liquid nitrogen and continue to grind until the sample is a fine powder.
- 5 When grinding is complete, carefully pour the frozen sample powder into 2-ml polypropylene tubes. It may be necessary to use a spatula, precooled in liquid nitrogen, to guide the sample into the 2-ml tube to prevent thawing. Immediately place the microfuge tubes containing ground powder into liquid nitrogen.
- 6 Add 1.2 ml of the preheated extraction buffer to the frozen tissue powder and invert the tube for 5–10 s to mix thoroughly.
- 7 Incubate at 65 °C for 30 min in an incubator or water bath and invert tubes every 5–10 min to allow mixing.
- 8 Centrifuge at 13,500*g* for 10 min at RT to remove nonsoluble debris. Supernatant will be saved for step 10.
- 9 Dispense 800 µl of phenol:chloroform:isoamyl alcohol into 2-ml Phase Lock Gel tubes (PLG Heavy). Prepare one tube per sample.
- 10 Transfer the supernatant from Step 8 to the 2-ml Phase Lock Gel tubes containing phenol:chloroform:isoamyl alcohol that you prepared in Step 9.
- 11 Close the tubes tightly and mix gently by inverting tubes for 20 min at 20–22 °C (RT). A nutator mixer is recommended for this step.
- 12 Separate the phases by centrifuging the mixture at 13,500*g* for 10 min at RT in a microfuge. The Phase Lock Gel will eliminate interphase contamination of nucleic acid solution.
- 13 Carefully transfer the aqueous (upper) layer to a new 2-ml microfuge tube containing 800 µl cold isopropanol (stored at -20 °C) and mix by inverting the tube and then



- incubate at RT for 10 min to precipitate the DNA.
- 14 Centrifuge the mixture at 13,500*g* for 10 min at RT.
 - 15 Remove the supernatant using a micropipette and then gently resuspend the pellet in 250 µl TE at RT.
 - 16 Pipette 2.5 µl of DNase-free RNase A (10mg/mL) into sample mixture and incubate at 37 °C for 30 min.
 - 17 Pipette 25 µl of 3 M NaAc, pH 5 and mix. Add 600 µl of precooled 100% ethanol (−20 °C), mix and incubate at −20 °C for 20 min to precipitate the DNA.
 - 18 Centrifuge the mixture at 13,500*g* for 10 min at RT.
 - 19 Carefully remove the supernatant with a micropipette and then add 500 µl of cold (−20 °C) 70% (v/v) ethanol. Do not dislodge the pellet.
 - 20 Centrifuge at 13,500*g* for 5 min at RT and remove the 70% ethanol supernatant, taking care not to disturb the pellet.
 - 21 Carefully remove the supernatant with a micropipette and then add 500 µl of cold 70% ethanol. Do not dislodge the pellet.
 - 22 Centrifuge at 13,500*g* for 5 min at RT and remove the 70% ethanol supernatant, taking care not to disturb the pellet.
 - 23 Discard supernatant with pipette, do not disturb the DNA pellet.
 - 24 Quick spin to gather the residual ethanol at the bottom of the tube and carefully remove with a P20 tip.
 - 25 Let DNA pellet air dry for 5 min at RT, taking care not to over dry.
 - 26 Resuspend the DNA pellet in 100 µL 10 mM Tris-HCl, pH 8. Incubate at 4°C with gently mixing overnight to resuspend. Store at 4°C for use within one week, or store at −80°C for long-term storage.

