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

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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 H_2O. Autoclave the buffer.

Note

Add 0.5–1% (v/v) of βME 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).

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 4ºC with gently mixing overnight to resuspend. Store at 4ºC for use within one week, or store at -80ºC for long-term storage.