

Plant HMW DNA Extraction:

Updated November 2023

Kit: Cytiva Nucleon PhytoPure Genomic DNA Extraction Kit**Reagents:**

- Sorbitol wash (D-Sorbitol 0.35 M, PVP-40 000 1%(w/v), Tris-HCl pH8 100 mM, EDTA pH8 5 mM) (from Jones and Schwessinger, 2020)
- DTT (1,4-dithiothreitol).
- Chloroform.
- RNase A.
- Isopropanol.
- 70% Ethanol.
- Nuclease free dH₂O.

Adapted Cytiva Nucleon PhytoPure Genomic DNA Extraction Kit Protocol**Sampling and Sorbitol Wash****Sampling:**

1. Cut plant leaf material into a mortar filled with liquid nitrogen.
2. Pulverize (±45 young leaves per 30 ml Nalgene tubes) of plant tissue using liquid nitrogen. (Grind for at least 25 min until leaf material is a smooth, homogenous fine powder). Transfer 1 g of tissue to a 30mL Nalgene extraction tube.

Sorbitol Wash:

3. Prepare the sorbitol wash solution (D-Sorbitol 0.35 M, PVP-40 000 1%(w/v), Tris-HCl pH8 100 mM, EDTA pH8 5 mM) as per Jones and Schwessinger, (2020).
4. Prepare ~170 mL sorbitol wash per 1g tissue prepared for extraction. Add 3x tiny scoops of DTT to 50 ml of the sorbitol wash in a 50 mL falcon tube solution just before use.
5. Resuspend 1 g of ground tissue in 25 mL sorbitol wash solution. Make sure the tissue is homogenised in the sorbitol solution via shaking. Make sure all the tissue is in solution without any lumps.
6. Centrifuge at 5000g, room temperature for 5 min. Pour off the supernatant and repeat step 5 until supernatant is clear (minimum of 6X).
7. During the final sorbitol wash, resuspend the tissue in exactly 15 mL of sorbitol wash solution. Once tissue is homogenous in solution, aliquot 1.5 mL of tissue using a P1000 pipette and wide bore pipette tips in sorbitol solution to each 1.5 mL DNA LoBind Tube (Prepare ten 1.5 mL DNA LoBind Tubes per 1 g tissue prepared for extraction).
8. Centrifuge at 5000g, room temperature for 5 min, remove the supernatant.

Cytiva Nucleon PhytoPure Genomic DNA Extraction Kit**Cell Lysis:**

9. Pre-mix Reagent 1 (Cell wall degrading buffer) and RNase A (20 ml Reagent 1 and 40 µL (20 mg/ml) RNase A) in a 50 mL Falcon tube.
10. Add 600 µL of the pre-mixed Reagent 1. Mix using a P1000 pipette and a wide bore tip by pulsating the pipette and flicking the tube until the solution is homogenous.
11. Incubate at 37°C for 30 minutes (Possible to use luke warm water).
12. Transfer 220 µL of Reagent 2 per reaction to a 50 mL Falcon tube and add 1% BME of total reagent 2 volume.
13. Add 200 µL of the Reagent 2 mix (Cell lysis buffer, work gently from this point on) to each DNA extraction tube.
14. Gently mix through ONE SINGLE pipette pulse using a P1000 pipette and a wide bore pipette tip and invert gently to obtain a homogenous solution.

15. Incubate at 65°C in a water bath for 10 minutes. Mix every 2 minutes through gentle inversion.
16. Remove samples from water bath and gently invert to tubes 3X to get everything into solution.
17. Place sample at -20 for ~4 minutes to cool down.
18. Place samples at 4 °C for 16 min (Total of 20 min cooling time). This will reduce the cold sock of the chloroform in the next step.

DNA Extraction:

19. Add 500 uL of ice-cold chloroform to samples.
20. Add 100 uL of Nucleon PyhtoPure DNA extraction resin suspension. Shake well to get everything homogenous and place on Hulamixer with 360° rotation at room temperature for 10 min. Centrifuge for 10 min @1300g, room temperature.
21. Transfer the aqueous phase using a P200 and wide bore pipette tips and slow pipetting to a 1.5 mL DNA LoBind Eppendorf tube.
22. Add 500 uL of ice-cold chloroform slowly starting with drops to prevent cold shock to samples.
23. Add 100 uL of Nucleon Pyhtopure DNA extraction resin suspension. Shake well to get everything homogenous, place in Eppi rack with lid and invert rack (gently shake) at room temperature for 10 min. Centrifuge for 10 min @1300g.
24. Transfer the aqueous phase using wide bore pipette tips and slow pipetting to a 1.5 mL DNA LoBind Eppendorf tube.
25. Add an equal volume (700 uL) of isopropyl alcohol previously cooled to -20 °C. Mix gently by inversion for 1 min. Place the samples at -20 °C. for 1h.
26. Centrifuge for 5 min at 4000 g, room temperature (place eppis with back of tube facing to the back of the rotor to help locate pellet). Discard the supernatant carefully, be careful not to lose your pellet, some may be transparent and stuck to the site of the tube.
27. Resuspend the pellet in 1 mL of 70% ethanol previously cooled to – 20°C. Mix gently by inverting tubes 5X.
28. Centrifuge for 5 min at 4000 g, room temperature (place eppis with back of tube facing to the back of the rotor to help locate pellet). Discard the supernatant gently and place upside down on clean tissue paper to remove excess ethanol.
29. After a quick spin down, immediately take off excess ethanol using a pipette. Allow to dry for max 30 seconds.
30. Resuspend the pellet in 15 µL of nuclease free dH₂O (or preferred buffer for sequencing library preparation).
31. Proceed to Quality Control steps. Do not freeze DNA, store at 4 °C.

Link to sorbitol wash solution:

Ashley Jones, Benjamin Schwessinger 2020. Sorbitol washing complex homogenate for improved DNA extractions. [protocols.io https://dx.doi.org/10.17504/protocols.io.beuvjew6](https://dx.doi.org/10.17504/protocols.io.beuvjew6)