HMW DNA extraction for plants (adapted by Manuel)

Tissues must be immediately frozen in liquid nitrogen after harvest and stored at -80°C. The tissues are grinded in fine powder with liquid nitrogen in a pre-cooled mortar or using an automated grinder. The finest the powder is, the better the yield, the purity and the integrity will be.

1. Grind ~ 1 g of frozen leaves to a very fine powder in mortar and pestle. Add small amounts of liquid nitrogen, as necessary, to maintain sample, and mortar and pestle at cryogenic temperatures.

2. Transfer the powder to a falcon tube. Before the extraction, place the tube to -20° C for 10-30 min (this makes it easier to lyse).

3. The following protocol is based on the "QIAGEN Genomic DNA Handbook" available. For 1 g of ground leaf tissue prepare enough Lysis Buffer for 4 x 100/G, or 1-2 x 500/G tip columns:

    - per 100/G column: 9.5 ml Buffer G2 + 19 µl RNase A (100 mg/ml) in a 50 ml tube
    - per 500/G column: 19 ml Buffer G2 + 38 µl RNase A (100 mg/ml) in a 50 ml tube

4. Add the Lysis buffer to the ground powder.
   Immediately homogenate by shaking the tubes several times. It is very important to quickly homogenate the lysate to neutralize the DNAse. Homogeneity of the lysate is also important for the overall yield. **Do not vortex** the samples at any stage because the DNA will be sheared!

5. Add Proteinase K (Qiagen Catalog #19131):
    - 500 µl per 100/G column or 1000 µl per 500/G column

6. Incubate > 3.5 h at 50°C. Mix gently 20 times every 15 minutes.
   The lysate must be homogenous. Aggregates of material can decrease the lysis and that will therefore reduce the yield of the extraction.
   Note: The tube can easily leak. Seal the lid with parafilm and check the tube condition periodically.

7. Preheat buffer QF in falcon tube in a 50 °C waterbath:
   5 mL per 100/G column or 15 mL per 500/G column.

8. Centrifuge the 50ml tubes containing the samples for 15 minutes at room temp at maximum speed (3220g / 4000 rpm) with acceleration and deceleration at maximum (=9).

    - After centrifugation minimal debris should be in the supernatant
    - If large amounts of debris still present repeat centrifugation up to 15 min

9. Calibrate the 100/G or 500/G columns with QBT buffer:

    - Put the column on top of a 50ml Falcon tube
    - Add 4 mL of buffer QBT per 100/G column
    - Add 10 mL of buffer QBT per 500/G column
    - All QBT needs to go through the column. This step is quite quick.

10. Transfer supernatant in the pre-calibrated Genomic tip column and allow the supernatant to pass through the column:
    - Use 1000 uL pipette, **do it slowly and cut the pipette tips**!
    - This is a slow step, it takes about on average 10-20 mins for the supernatant to elute through the column.

11. Add 1 ml of wash buffer QC by running along the walls of the tubes in order to precipitate any debris stuck on the walls. If debris still present wash with another 1 mL of QC buffer.

12. Wash the column twice with buffer QC:

    - 2 x 7.5 mL of buffer QC 100/G column.
    - 2 x 15 mL of buffer QC 500/G column.
    - This can take at least 15 mins for each wash.

13. Transfer the column to a clean 50 ml Falcon tube.

14. Elute with elution buffer QF previously pre-heated at 50°C:

    - 5 mL of buffer QF per 100/G column
    - 15 mL of buffer QF per 500/G column
    - This step usually takes 15 min but can take up to 1 hour per sample.

15. Remove column and precipitate the DNA by adding 2-propanol (isopropanol) along the wall of the tube:
    - 3.5 mL per 100/G column
    - 10.5 mL per 500/G column

16. Mix gently by slowly inverting the tube to form a visible mass of HMW DNA floating in solution (a "jellyfish"). Incubate the tube at -20°C for ~20 minutes.

    (OPTIONAL: Incubate the tube at -20°C overnight. This will increase the amount of precipitated DNA, but can also cause the accumulation of salt).

17. Centrifuge at 3500 x g for 15-45 min at 4°C (mark tube to find the pellet) and remove supernatant.

18. Add 4 mL of **ice-cold fresh** 70% ethanol to the pelleted DNA and invert the tube 10 times.

19. Centrifuge at 3500 x g for 10 minutes at 4°C.

20. Carefully **pour off supernatant**, do not re-suspend. Drain at 45° on a paper towel to dry.

21. Wash two more times (steps 18-20).

22. Centrifuge at 3500 x g for 10 minutes at 4°C.

23. Discard the supernatant and air dry for 1 minute, allowing most of the ethanol to evaporate.

24. Resuspend the DNA in >100 µl of TE buffer and incubate at room temperature, typically overnight.

DNA precipitation and washing with 2mL Eppendorf tubes

15. Distribute 1 ml of eluted DNA per 2 ml eppendorf tube.
16. Precipitate DNA by adding 700 µl of RT isopropanol (2-propanol), mixed by inverting tubes 10-20 times. Incubate at -20°C for ~20 minutes.
17. OPTIONAL: Incubate the tube at -20°C overnight.
18. Centrifuge at 14'000 rpm (3500 x g) for 15-45 min at 4°C (mark tube to find the pellet) and remove supernatant.
19. Add 400 µl of **ice-cold fresh** 70% ethanol to the pelleted DNA and invert the tube 10 times.
20. Centrifuge at 14'000 rpm (3500 x g) for 10 minutes at 4°C.
21. Discard the supernatant without disturbing the pellet.
22. Repeat ethanol wash (steps 19-21).
23. Air dry 10 min (could be done at 65°C for 2-3 minutes).
24. Resuspend the DNA in 25-50 µl of Buffer AE (or TE buffer) overnight.
25. Use Nanodrop to check the quality:
    - A 260/280 ratio of 1.8-2.0
    - 260/230 ratio of 2.0-2.2
26. Electrophoresis on 0.5% agarose with λ-PstI

References:

- ONT_gDNA_Arabidopsis_leaves_V2.pdf (from Oxford Nanopore community)
- Workflow for generating HMW plant DNA for third generation sequencing (from LeafGO; https://doi.org/10.1186/s13059-021-02475-z)
- HMW_DNA_for_Biscutella (from Christian Parisod)