The standard protocol for high molecular weight DNA extraction in plant genomic studies

Note: This protocol starts with 2 g of fresh and young leaves. Usually, the end product of one extraction process is enough for using 5~6 times of library making for the Nanopore sequencing.

I. PREPARATION OF SOLUTIONS

1. Preparation of nuclei isolation buffer (IB) (for ten reactions)

- For 200 ml of nuclei isolation buffer, dissolve the following in ca. 100 ml water
  - Tris-HCl (1 M stock; ph 9.5) 3 ml (Final conc.: 15 mM)
  - EDTA (0.5 M stock) 4 ml (Final conc.: 10 mM)
  - KCl 1.94 g (Final conc.: 130 mM)
  - NaCl (5 M stock) 0.8 ml (Final conc.: 20 mM)
- Gradually add 16 g of PVP-10 while rapidly rotating the solution with a magnetic stir bar.
- Fill with water to 200 ml.
- Add 0.05 g of spermine and 0.07 g of spermidine (Store IB at 4 ℃).
- After mixing the IB with the ground sample, add Triton X-100 and B-mercaptoethanol to final concentrations of 0.1% and 7.5%, respectively (this constitutes IBTB).

(e. g., add 40 ul of Triton X-100 and 3 ml of B-mercaptoethanol to 40 ml of IB).

Note: Store in a 4 ˚C refrigerator until use. Two weeks will be safe.

2. Preparation of Carlson lysis buffer (Carlson et al., 1991)

- Carlson lysis Buffer = 2X CTAB (Cetyltrimethylammonium Bromide) buffer + 1% PEG 6000
- For 100 ml of Carlson lysis buffer
  - Tris-HCl (1 M stock ph 9.5) 10 ml (Final conc.: 100 mM)
  - EDTA (0.5M stock) 4 ml (Final conc.: 20 mM)
  - NaCl 8.2 g (Final conc.: 1.4 M)
  - CTAB 2 g (Final conc.: 2 %)
  - PEG 1 g (Final conc.: 1 %)

Note: Store in a 4 ˚C refrigerator until use. Two weeks will be safe.

3. Tris-EDTA buffer (TE) (1 X)

- TE buffer = Tris-HCl (pH 8.0) 10mM + EDTA 1mM

Note: Store in a 4 ˚C refrigerator until use.

II. GRINDING AND NUCLEI ISOLATION (modified from Peterson et al., 1997)

1. Cooldown mortar and pestle at a -80 ℃ deepfreezer before the experiment starts. Ground 2 g of fresh young leaves in liquid nitrogen until they become a fine powder.
2. Add 2 g of ground leaves into 20 ml of IB buffer in a 50 ml conical tube and mix by inverting.

   Note: Over-grinding is a negative factor in getting HMW DNA. Grinding until the temperature increases would be fine: you don't need to add additional liquid nitrogen.

   Note: Increase the sample amount for succulent plants and increase the IB buffer when the mixture becomes viscous.
3. Immediately add 20 ul of Triton X-100 and 1.5 ml of B-mercaptoethanol and mix by inverting.
4. Keep on ice for 10 min.

   Note: this step should be conducted inside a fume hood because the IBTB contains B-mercaptoethanol, which is toxic.

5. Filter mixture through a vacuum-aid cell strainer (pore size: 100 ㎛) seated on a 50 ml conical tube to collect nuclei suspension (Fig. 1).

Note: To aid filtration, gently scrape stacked plant tissue on the filter with a top side of 1,000 ul pipette (blue) tip. The filtrate should look light green.

6. Repeat the filtering step again with 40 ㎛ pore-sized cell strainer.
7. Add 200 ul Triton X-100.

Note: This is a step to lyse cell and organelle membranes except nuclear membrane.

8. To pellet the nuclei, centrifuge for 10 min. with 3,000g at 4 ℃.
9. Discard the supernatant.

III. EXTRACT NUCLEAR DNA USING CTAB BUFFER (modified from Doyle and Doyle, 1987)

1. Add 5 ml of Carlson lysis buffer and 12.5 ul of B-mercaptoethanol to the tube and resuspend nuclei pellet with brief tapping.

Note: Incomplete resuspension could reduce yield since many nuclei are not lysed by CTAB. Brief pipetting of the pellet with an end-cut 1,000 ul pipette tip and gentle vortexing may help resuspension.

2. Incubate at 65 ℃ for 15 min. (maximum 2 hours)

Note: If the pellet is not completely resuspended after incubation, a brief centrifugation (3,000 g for 5 min) followed by only the use of the supernatant will help speed up processing.

3. Transfer suspended nuclei pellet to 15 ml polypropylene tube and add equal volume (5 ml) of chloroform:isoamyl alcohol (24:1) solution.
4. Invert several times to mix them.
5. Centrifuge (3,000 g) for 10 min. at 4 ℃.
6. Transfer the aqueous upper phase to a new tube using P1000 pipette.

Note: Taking only 9/10 of the supernatant is safe to avoid the inclusion of cellular debris. This step is highly correlated to the quality of extracted DNA.

Note: If it is viscous, pipetting slowly will help avoid sucking up the plant tissue.

7. Repeat 3~6 (optional but highly recommended).
8. Add 1/10 volume of 3 M sodium acetate, mix gently, add the same volume of isopropanol (room temperature), and gently invert several times.

Note: ex) For 4.5 ml of supernatant, add 0.45 ml of 3 M sodium acetate and 4.95 ml of isopropanol.

9. Precipitate at -20 ℃ for more than an hour.

Note: In case of aggregates are visible, moving to the next step is possible for fast processing.

10. Centrifuge (3,000 g) for 10 min. at 4 ℃.
11. Discard supernatant.
12. Wash pellets with 70% cold ethanol (ca. 20 ml per tube).
13. Centrifuge (3,000 g) for 10 min. at 4 ℃.
14. Discard supernatant.

Note: Keep the tube inverted for a min., and wipe out the tube wall with the Kimwipe.

15. Dry the pellet completely.

Note: This step is very important for the quality of DNA. Alcoholic small is a good indicator of incomplete drying.

IV. RNASE A AND PROTEASE K TREATMENT

1. Dissolve pellet with 2 ml of TE buffer

Note: If it is difficult to dissolve, incubate it 50 ℃ water bath for less than 10 min.

Note: Crushing pellet with pipette tip might be helpful for fast dissolving but never to vortex the sample. If the pellet is not completely resuspended after incubation, brief centrifugation (3,000 g for 5 min) followed by only the use of the supernatant will help speed up processing.

2. Add 20 ul (10 ul/ml) of RNase A (10 mg/ml conc.)
3. Incubate 37 ℃ 5 min.
4. Add 20 ul (10 ul/ml) of proteinase K (>600 units/ml conc.)
5. Incubate 50 ℃ 15 min.
6. Add equal volume (2 ml) of chloroform:isoamyl alcohol (24:1)
7. Invert several times to mix
8. Centrifuge (3,000 g) for 10 min. at 4 ℃.
9. Transfer the aqueous upper phase to a new 15 ml tube.

Note: Taking only 9/10 of the supernatant is safe to avoid the inclusion of cellular debris. This step is highly correlated to the quality of extracted DNA.

10. Repeat 6~9 (optional).
11. Add 1/10 volume of 3 M NaOAc, mix gently, add an equal volume of room temperature isopropanol, and gently invert several times.

Note: ex) For 3.5 ml of supernatant, add 0.35 ml of 3 M NaOAc and add 3.85 ml of isopropanol.

12. Precipitate at -20 ℃ for more than an hour.

Note: In case of aggregates are visible, moving to the next step is possible for fast processing.

14. Centrifuge (3000 g) for 10 min. at 4 ℃.
15. Discard supernatant.
16. Wash pellets with 70% cold ethanol (ca. 5 ml per tube).
17. Centrifuge (3000 g) for 10 min. at 4 ℃.
18. Discard supernatant.

Note: Keep the tube inverted for a min., and wipe out the tube wall with the Kimwipe.

19. Completely drying.

Note: This step is very important for the quality of DNA. Alcoholic small is a good indicator of incomplete drying. 20. Add 50~ 500 ul of water to each tube to dissolve the pellet.

Note: If it is difficult to dissolve, incubate it 50 ℃ water bath for less than 10 min.

Note: Crushing pellet with pipette tip might be helpful for fast dissolving but never to vortex the sample. If the pellet is not completely resuspended after incubation, brief centrifugation (3,000 g for 5 min) followed by only the use of the supernatant will help speed up processing.

21. Check the quality (A260/280 and A260/230) and the quantity of extracted DNA using the Nanodrop (ND2000USCAN; Thermo Fisher Scientific, Massachusetts, USA) and the Qubit. 22. Check length distribution using the Femto Pulse System (Agilent).

V. SPECIAL REAGENTS AND CONSUMABLES

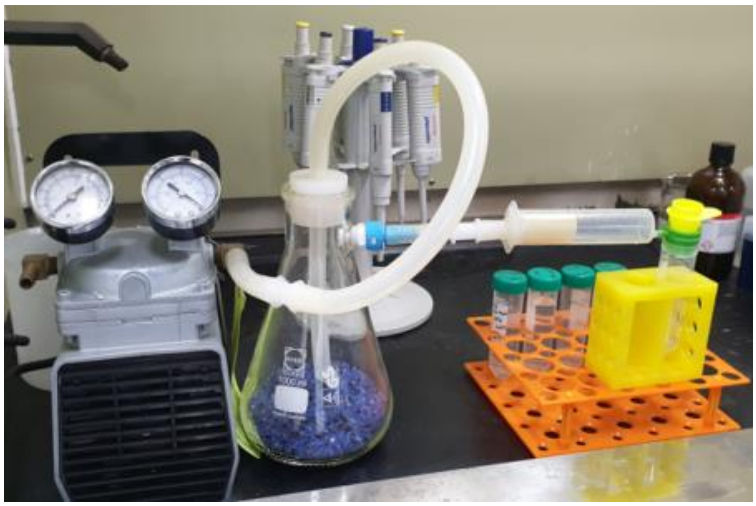
1. Reagents

- PVP-10: Sigma-aldrich CAS 9003-39-8
- spermine: Sigma-aldrich S2876
- spermidine: Sigma-aldrich S2501
- Triton X-100: Sigma-aldrich T8787
- PEG 6000: Sigma-aldrich 81260
- RNase A: Sigma-aldrich R6513
- Proteinase K: Sigma-aldrich P2308

2. Consumables

- Vacuum-aid cell strainer (100 μm): pluriSelect 43-50100-51 yellow 100 μm
- Vacuum-aid cell strainer (40 μm): pluriSelect 43-50040-51 blue 40 μm
- Connector ring: pluriSelect 41-50000-03

Fig. 1. The extraction time can be reduced by using vacuum-assisted filtration.



References

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