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A Fast Protocol for the Extraction of High Molecular Weight Nuclear DNA (>50 kbp) from Plants

Note: This method is combined method of nucleus extraction plus CTAB method. It is cp- and mt-genome depleted.

Note: This method is not recommended for samples which produce a viscous solution when ground and mixed with isolation buffer.

I. PREPARATION OF SOLUTIONS

1. Preparation of nuclei isolation buffer (IB) (for 10 reactions)

- For 200 ㎖ of nuclei isolation buffer, dissolve the following in ca. 100 ㎖ water

Tris-HCl (1 M stock; ph 9.5) 3 ㎖ (Final conc.: 15 mM)

EDTA (0.5 M stock) 4 ㎖ (Final conc.: 10 mM)

KCl 1.94 g (Final conc.: 130 mM)

NaCl (5 M stock) 0.8 ㎖ (Final conc.: 20 mM)

- Add 16 g of PVP-10 gradually while spinning the solution fast with a magnetic stir bar.
- Fill with water to 200 ㎖ (enough for 5 extractions).
- Add 0.05 g of spermine and 0.07 g of spermidine (Store IB at 4 ℃).
- After mixing IB with ground sample,

add 0.1 % Triton X-100 and 7.5 % B-mercarptoethanol to IB (this constitutes IBTB)

(e. g., to 40 ㎖ of IB buffer, add 40 ㎕ of Triton X-100 and 3 ㎖ of B-mercarptoethanol).

Note: Keep in 4C refrigerator and use it witin two weeks will be safe.

2. Preparation of Carlson lysis buffer

- Carlson lysis Buffer = 2X CTAB (cetyl trimethylammonium bromide) buffer + 1% PEG 6000
- For 100 ㎖ of Carlson lysis buffer

Tris-HCl (1 M stock ph 9.5) 10 ㎖ (Final conc.: 100 mM)

EDTA (0.5M stock) 4 ㎖ (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: Keep in 4C refrigerator and use it witin a month will be safe.

II. GRINDING AND NUCLEI ISOLATION

1. Cool down mortar and pestle in -80 ℃ before the experiment starts. Grind 2g of tissue to a fine powder under liquid Nitrogen.
2. Add 2 g of ground leaves into 20 ㎖ IB buffer in a 50 ㎖ conical tube and mix by inverting.

   Note: Grinding level (fine/coarse) doesn't seem to appreciably affect to HMW DNA size but insufficient grinding appears to reduce the extraction efficiency and DNA yield.

   Note: Increase amount of ground sample in case of succulent plants
3. Immediately add 20 ㎕ of Triton X-100 and 1.5 ㎖ of B-mercarptoethanol 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-mercarptoethanol, which is toxic.

5. Filter mixture through a 100 ㎛ vaccume-aid cell strainer seated on a 50 ㎖ conical tube to collect nuclei suspension (Fig. 1). To aid filtration, gently scrap unfiltered plant tissue on the filter with top side of 1000 ㎕ pipette (blue) tip. The filtrate should look light green color.
6. Repeat the filtering step again with 40 ㎛ cell strainer.
7. Add 200 ㎕ Triton X-100.

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

8. Centrifuge for 10 min. with 3000g at 4 ℃. to pellet the nuclei (Fig. 2).
9. Discard the supernatant.

III. EXTRACT NUCLEAR DNA USING CTAB buffer

1. Add 5 ㎖ of Carlson Lysis buffer + 12.5 ㎕ B-mercarptoethanol to the tube and re-suspend nuclei pellet with brief tapping.

   Note: Incomplete resuspension could reduce yield since many nuclei are not lysed by CTAB. Pipette pellet briefly with end-cut 1,000 ㎕ pipette tip to help resuspension and vortex sample.

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

   Note: If it is not resuspended after the incubation, centrifuge it (3000g for 5 min.) and just use supernatant for faster processing.

3. Transfer suspended nuclei pellet to 15 ㎖ polypropylene tube and add 5 ㎖ (equal volume) of chloroform : isoamyl alcohol (24 : 1)
4. Invert several times to mix.
5. Centrifuge (3000 g) for 10 min. at 4 ℃.
6. Transfer aqueous upper phase to a new tube.

   Note: Take only 9/10 of supernatant. This step is highly correlated to the quality of extracted DNA.

   Note: If it is viscous, slow pipetting will help not to suck up plant tissue.

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

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

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

   Note: If you can see precipitate, go to the next step for the 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 a tube).
13. Centrifuge (3,000 g) for 10 min. at 4 ℃.
14. Discard supernatant.

    Note: keep tube inverted for a min. and wipe out tube wall with Kimwipe.

15. Air dry pellet completely.

    Note: this step is very important for the quality of DNA.

IV. RNase A and Protease K treatment

1. Dissolve pellet with 2 ㎖ of TE buffer

Note: If it is difficult to dissolve, put the tube in 50 ℃ water bath for a while.

Note: Crushing pellet with pipette tip might be helpful for fast dissolving but never vortex sample. If it is not dissolved after the incubation, centrifuge it (3000g for 5 min.) and just use supernatant for the fast processing.

2. Add 20 ㎕ (10 ul/ml) of RNase A (10 mg/㎖ conc.)
3. Incubate 37 ℃ 5 min.
4. Add 20 ㎕ (10 ul/ml) of proteinase K (>600 units/㎖ conc.)
5. Incubate 50 ℃ 15 min.
6. Add 2 ㎖ (equal volume) of chloroform : isoamyl alcohol (24 : 1)
7. Invert several times to mix
8. Centrifuge (3000 g) for 10 min. at 4 ℃.
9. Transfer aqueous upper phase to a new 15 ㎖ tube .

Note: Take only 9/10 of supernatant. This step is highly correlated to the quality of extracted DNA.

10. Repeat 6~9 (optional). Use 2 ㎖ Eppendorf tube in the final repeat.
11. Add 1/10 volume of 3 M NaOAc, mix gently, add some volume of room temperature isopropanol, and gently invert several times.

Note: ex) For 1.7 ㎖ of supernatant, add 0.17 ㎖ of 3 M NaOAc and add 1.87 ㎖ of isopropanol.

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

Note: If you can see precipitate, go to the next step for the fast processing.

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

Note: keep tube inverted for a min. and wipe out tube wall with Kimwipe.

19. Completely drying.

Note: this step is a critical step for the quality of DNA.

20. Add 50~ 500 ㎕ of water in each tube to dissolve pellet.

Note: If it is difficult to dissolve, put the tube in 50 ℃ water bath for a while.

Note: Crushing pellet with pipette tip might be helpful for fast dissolving but never vortex sample. If it is not dissolved after the incubation, centrifuge it (3,000g for 5 min.) and just use supernatant for the fast processing.

21. Check quality (A260/280 and A260/230) and quantity of extracted DNA using Nanodrop and Qubit.
22. Check length distribution using Femto pulse.

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