Research Center for
Pharmaceutical Nanotechnology
Tabriz University of Medical Sciences
RCPN

DNA extraction from plants

PSR

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Solutions

- Lysing buffer: 2% cetyl trimethyl ammonium bromide (CTAB), 100 mMTris/HCl, 1.4 M NaCl, 2% polyvinylpyrrolidone (PVP), 20 mM disodium salt of ethylenediaminetetra acetic acid (Na_2EDTA), 0.2% LiCl, Total pH 8.

- Chroloform:isoamyl alcohol (CIA), 24:1.

Detailed procedure

1. Pulverize the plant leaves with liquid nitrogen using mortar and pestle.
2. Add a volume of 0.7 mL lysing buffer to prepared sample (1.5 mL tube).
3. Incubated the sample at 55–60 °C for about 1 h.
4. Centrifuge the sample at 9,5009g for 5 min.
5. Transfer the aqueous phase to a fresh tube and add an equal volume of chloroform-isoamilalcohol.
6. Centrifuge the sample at 9,5009g for 5 min and transfer the supernatant to a new tube.

Optional: add 0.2 mL of acetate sodium in this stage in order to have excess quality of extracted DNA.

7. Add an equal volume of isopropanol (-20 °C) to the tube.

Note. The isopropanol should be added drop-wise and mixed gently since the ice cold isopropanol (if added at once) may lead to DNA fragmentation.

8. Keep the sample in -20°C for 30 min.
9. Centrifuge the sample at 11,5009g for 5 min.
10. Remove the supernatant and add 500 µL of 96% ethanol (4 °C).
11. Centrifuge the tube at 7,0009g for 5 min.
12. Remove the supernatant using a pipette and add 500 µL of 70% ethanol (4 °C).
13. Centrifuge the sample at 7,0009g for 5 min to stick the pellet at the bottom of the tube and discard the supernatant.

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14. Dry the pellet at room temperature.

Note. The pellet should not be excessively dried, at which its water solubility may be decreased.

15. Add a volume of 100 µL TE or sterile distilled water to dissolve the DNA pellet.

Reference

Barzegari, A., Zununi Vahed, S., Atashpaz, S., Khani, S., and Omidi, Y. (**2010**) Rapid and simple methodology for isolation of high quality genomic DNA from coniferous tissues (Taxus baccata). *Mol Biol Rep* 37: 833-7.

Good luck!

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