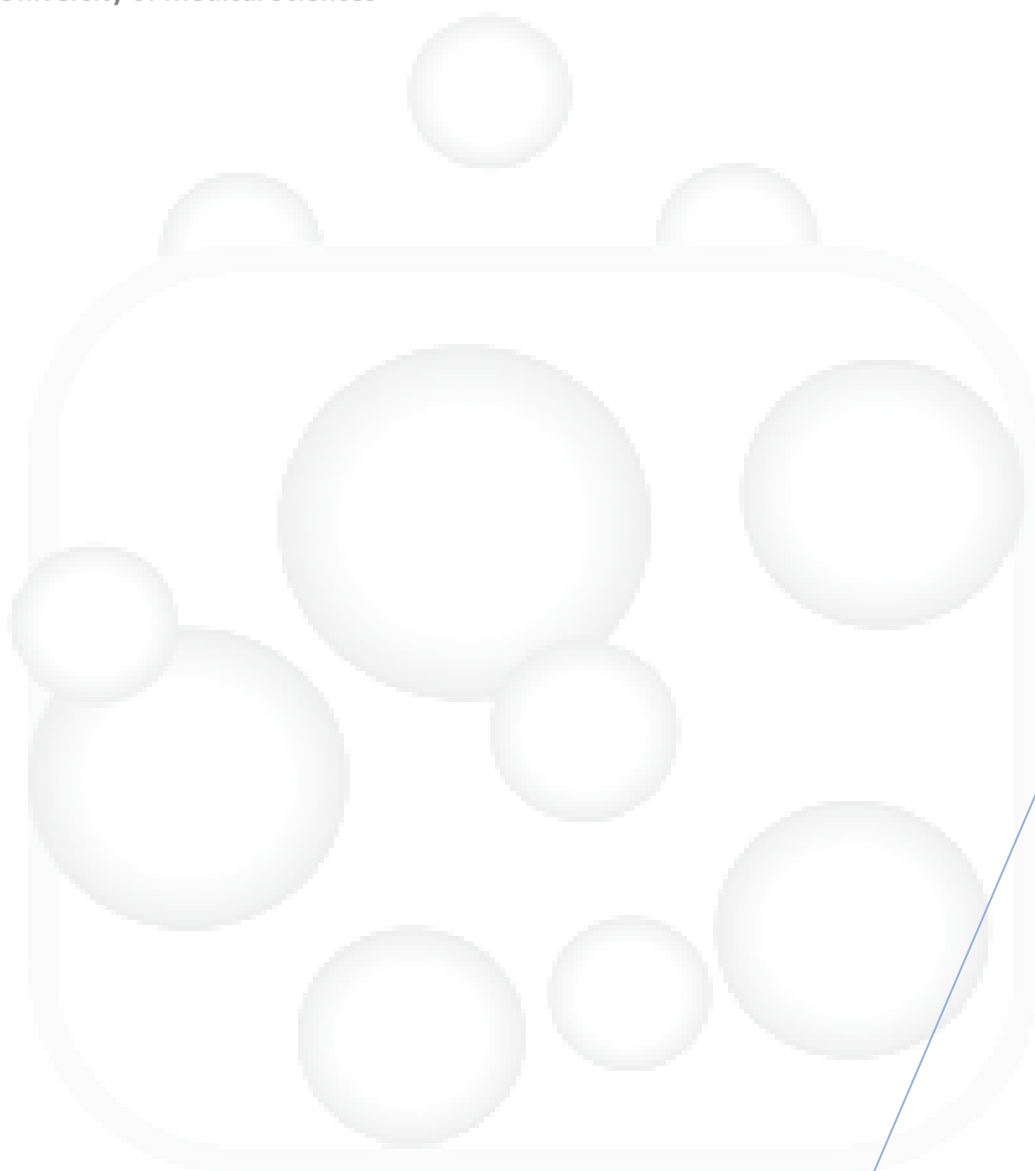




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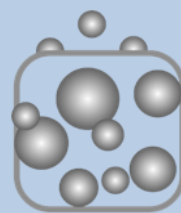
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DNA extraction from plants
PSR

Dr. Zonuoni. S
6/12/2016



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DNA extraction from plants

Solutions

- Lysing buffer: 2% cetyl trimethyl ammonium bromide (CTAB), 100 mM Tris/HCl, 1.4 M NaCl, 2% polyvinylpyrrolidone (PVP), 20 mM disodium salt of ethylenediaminetetra acetic acid (Na₂EDTA), 0.2% LiCl, Total pH 8.
- Choroform: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 9,500g 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 9,500g 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 11,500g for 5 min.
10. Remove the supernatant and add 500 µL of 96% ethanol (4 °C).
11. Centrifuge the tube at 7,000g for 5 min.
12. Remove the supernatant using a pipette and add 500 µL of 70% ethanol (4 °C).
13. Centrifuge the sample at 7,000g for 5 min to stick the pellet at the bottom of the tube and discard the supernatant.

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 μ L TE or sterile distilled water to dissolve the DNA pellet.

Reference

Barzegari, A., Zununi Vahed, S., Atashpaz, S., Khani, S., and Omid, 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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