DNA extraction from plant material using DNAzol

What you need:

1. approx. 100 mg of plant tissue; the younger (smaller cells) the better, e.g. flowers and buds
2. DNAzol, chloroform, 100% ethanol, 75% ethanol, Qiagen Elution Buffer EB, microtube pestle, liquid nitrogen, gloves

What you do:

1. harvest the tissue, weigh, place into microfuge tube
2. freeze in liquid nitrogen (set tube in rack in styrofoam box, pour N_2 over it; make sure the tissue doesn't thaw
3. prechill small blue micropestle in separate microfuge tube
4. use the cold pestle to grind the tissue to powder (you can chill one corner of a test-tube rack with N_2 and use this as a holder for the "mortar" microfuge tube) — Make sure the evaporating N_2 doesn't splash out with your sample!
5. add 300 μl DNAzol and let thaw at RT
6. when thawed, grind/mix some more, remove pestle
7. incubate 5 min with shaking (e.g at 27° with BY-2 cells)
8. add 300 μl chloroform (IN THE FUME HOOD)
9. mix by shaking, incubate 5 min with shaking (as before)
10. spin 10 min @ 12000 x g
11. transfer aqueous (= upper) phase to fresh tube (~300 μl)
12. add 230 μl 100% ethanol, incubate 5 min @ RT
13. spin 4 min @ 5000 x g, discard supernatant
14. add 300 μl of DNAzol+ethanol (200 μl DNAzol + 150 μl 100% ethanol), VORTEX, incubate 5 min @ RT
15. spin 4 min @ 5000 x g, discard supernatant
16. wash pellet with 300 μl 75% ethanol
17. spin 4 min @ 5000 x g, discard supernatant
18. (repeat 16 + 17 if pellet looks greenish)
19. air dry pellet (2 – 5 min) remove remaining ethanol with pipette
20. dissolve pellet in 70 ml EB (or TE, or H_2O)
21. spin 4 min @ 12000 x g, keep supernatant!
22. transfer supernatant to fresh tube + label.

Clean-up:

1. Chloroform should be handled in the hood. All chloroform waste should be stored in the hood. Separate liquid and solid waste.

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updated on 03.07.03 by Andreas