Minipreps of plant genomic DNA

(From Hector Candela 12/19/05)

Solutions:

a. Extraction buffer (final concentrations)

100mM Tris-HCl, pH 8.0
50mM EDTA
100mM NaCl
10mM beta mercaptoethanol (add fresh, 0.7ul/ml extraction buffer)

To make 200ml:
20ml of 1M Tris-HCl, pH 8.0
20ml of 0.5M EDTA
5ml of 4M NaCl
155ml water

b. 10 or 20% SDS
c. 5M potassium acetate (stored -20C)
d. 3M sodium acetate pH 5.2
e. Isopropanol
f. 70% EtOH

Procedure:

1. Wash and blot dry leaves. Cut chunk approx 1" x 0.5".
    a. For grinding with liq N2:
        i. Place in baked mortar. Add liq N2 and grind until a fine powder.
        ii. Transfer to 1.5ml sterile eppie. Add 500ul extraction buffer. Vortex.
    b. For Tissuelyser:
        i. Place in 2.0ml eppendorf safe-lock tube or in qiagen 96 well plate.
        ii. Add tungsten bead, 500ul extraction buffer and cap.
        iii. Place in 24-place holder. Insert holder into tissuelyser. ALWAYS use both holders and ALWAYS balance the tubes within each holder.
        iv. Hit "start" to send off the preset program (30/s, 1 minute).
        v. Send off once more if the tissue has not been destroyed sufficiently.
    c. For processing leaves and storing before DNA isolation:
        i. Place in 2.0ml eppendorf safe-lock tube.
        ii. Leave cap off and place in 50C oven for 2 days to dry down the sample.
        iii. Cap the tube and store at RT. When ready to isolate DNA, add the bead and 500ul extraction buffer and run in the tissuelyser as above.
2. Add 35ul of 20% SDS (or 70ul of 10%SDS) and invert to mix.
3. Inc 10min in 65C in heat block with wells filled w H2O.

4. Add 130ul ice cold 5M KOAc. Invert to mix.
5. Inc on ice 5min.
6. Spin 10min at 13,000rpm.
7. Pipet off supernatant to fresh eppie containing 64ul 3M NaOAC. Vortex.
8. Add 640ul isopropanol. Invert to mix. Inc on ice at least 1 hr or o/n at -20C.
9. Spin 10min at 13,000rpm.
10. Pipet off supernatant. Wash with 1ml 70% EtOH. Invert. Spin 10min at 13,000rpm.
11. Pipet off supernantant. Let air dry or dry in speedvac for 5min w/o heat.
12. Resuspend in 50ul TE + RNAse A (10ug/ml final, add 1:1000 of 10mg/ml stock).
13. Store 4C overnight, or -20C for longer term