RICHARDS’S PLANT DNA EXTRACTION (Large Scale)

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* This method consistently yields good quality, high molecular weight DNA.

1. EXTRACTION BUFFER 100mM Tris-Cl, pH 8 100mM EDTA
   250mM NaCl 100 ug/ml-1 proteinase K

2. PROCEDURE
   (i) Harvest 10 to 50 g fresh plant tissue. * Plants may be placed in the dark for 1 to 2 days prior to harvest to reduce the starch content in the tissues.

   (ii) Freeze tissue with liquid nitrogen and grind to a fine powder in a mortar and pestle. * Keep the tissue frozen throughout this procedure by occasionally adding liquid nitrogen.

   (iii) Transfer frozen powder to a 250 ml centrifuge bottle and immediately add 4 ml extraction buffer per gram of fresh plant tissue. Mix gently to disperse tissue. (iv) Add 10% sarkosyl to achieve a final concentration of 1%. (v) Incubate 1 to 2 h at 55oC. * The lysate should be clear, green and slightly viscous. From this point on, the solutions should be handled gently to reduce shearing of the DNA.

   (vi) Centrifuge lysate 10 min in a Sorvall RC3C rotor at 4,000rpm, 4oC, to pellet debris. Filter through "Miracloth" into Oak Ridge tubes. (vii) Add 0.6 vol isopropanol to the supernatant and gently mix. A nucleic acid precipitate should be visible, if not place at -20oC for 30 min. Centrifuge 10 min in a Sorvall SS34 rotor at 8,000rpm, 4oC. (viii) Resuspend pellet in 9 ml TE buffer. Incubate at 55oC to aid resuspension if necessary. Add 9.7 g solid CsCl and mix gently until dissolved. * For preps over 20 g, dissolve pellet in 18 ml TE and add 19.4 g CsCl.

   (ix) Incubate lysates on ice for 30 min. Centrifuge 10 min at 8,000rpm (in SS34 rotor) at 4oC. Collect the supernatant and filter through "Miracloth" into fresh tubes. (x) Add 0.5 ml of 10 mg/ml-1 ethidium bromide and incubate on ice for 30 min. * For larger preps add 1.0 ml of 10 mg/ml-1 ethidium bromide.

   (xi) Centrifuge 10 min at 8000rpm (in a Sorvall SS34 rotor) at 4oC. A very soft, large RNA pellet should form. Filter through "Miracloth" into fresh tubes. (xii) Transfer the supernatant to two, 5 ml ultracentrifuge tubes. * Use an 18 ml ultracentrifuge tube for larger preps to avoid overloading. (xiii) Ultracentrifuge overnight at 50,000rpm, 15oC in a Beckman TV865 or TV865B rotor. (xiv) Collect DNA band using a large-bore needle and syringe. (xv) Remove the ethidium bromide by repeatedly extracting the collected DNA with isopropanol that has been equilibrated over a CsCl-saturated aqueous phase. (xvi) Add 2 vol water and 6 vol ethanol to the DNA solution and mix. Incubate 1 h at -20oC. Transfer to a silanised glass Corex tube and centrifuge 10 min at 8,000rpm (in SS34 rotor) at 4oC. The DNA may precipitate
immediately as a single white mass, which can be collected using a Pasteur pipette or centrifugation. (xvii) Resuspend pellet in TE buffer and reprecipitate DNA by adding 0.1 vol of 3M Na acetate, pH 5.2, and 2 vol ethanol. Incubate at -20°C if precipitate is not visible and collect DNA by centrifugation. (xviii) Briefly air dry the pellet and resuspend in TE buffer.

* DNA yields should be between 5 and 20 ugg-1 of leaves, although efficiency drops with increasing amounts of tissue.