Some notes on the extraction of genomic DNA from transgenic Lotus corniculatus

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There has been increasing interest in the use of *Lotus corniculatus* as a model transgenic legume. This species can be readily transformed with wild type strains of *Aqrobacterium rhizoqenes* to yield transgenic root cultures that may then be regenerated to whole plants with few morphological abnormalities (Webb *et al.* . 1990). Foreign gene constructs can be assembled in *E.coli.* transferred into *A.rhizoqenes* and then introduced into *L. corniculatus.* In a number of cases (Stougaard *et al.* . 1986; Forde *et al.*. 1989) such gene constructs have been both introduced and expressed in transgenic *Lotus* plants. Therefore these plant systems are proving to be useful for fundamental and strategic studies relating to plant sciences and legume breeding.

However our group and others have noted problems with the efficient extraction of good quality genomic DNA from *L.corniculatus.* an important consideration in the genetic analysis of transgenic plants. We report here some adaptations to the genomic DNA extraction protocol of Dellaporta *et al.* (1983), which we have found to be convenient for the processing of transformed and non-transformed *Lotus* plant material in this laboratory. We also include a consideration of optimal tissue types to be used as a source for extraction of genomic DNA from transgenic Lotus.

Protocol for extraction of genomic DNA

1. Place 0.5-1.5 g of tissue into a mortar precooled at -70° C and cover with liquid nitrogen. Grind to fine powder.

2. Pour powder into a 50 ml centrifuge tube containing 15 ml of extraction buffer (100 mM Tris, 10 mM EDTA pH 8.0) and mix well.

3. Add 1 ml of 20% SDS and mix, and then 10 µl of 2-mercaptoethanol. Incubate at 65° C for 10 min.

4. Add 5 ml of 5 M potassium acetate and mix, incubate on ice for 20 min.

5. Centrifuge at 18,000 g for 20 min at 4° C. Filter supernatant through Miracloth into a 50 ml polycarbonate centrifuge tube containing 10 ml of isopropanol. Mix and leave on ice for at least 20 min.

6. Spin at 18,000 g for 20 min at 4° C, decant off supernatant and discard. Allow

pellet to air dry for 10 min and resuspend in 700 µl of TE (10 mM Tris, 1 mM EDTA).

7. Transfer to Eppendorf tube and add 500 *iil* of Phenol/chloroform (1 vol : 1 vol) and vortex vigorously, then spin for 5 min at 12,000 g in bench microfuge.

8. Remove supernatant to fresh Eppendorf tube and add 70 µl of 3 M Na acetate pH 6.0 and 500 µl isopropanol. Incubate on ice for 20 min and then spin for 10 min at 12,000 g in bench microfuge. Discard supernatant and resuspend pellet in 1 ml of 80 % EtoH, spin for 5 min, discard supernatant and allow pellet to air dry.

9. Dissolve pellet in 100 µl of TE + 50 µl/ml RNAase A. Add 5 µl of 5 M NaCl and 250 µl EtOH and respin. Wash with 80% EtOH, spin and allow pellet to air dry and then resuspend in 50 µl TE. Quantify DNA yield fluorimetrically using Hoescht 33258 dye and check integrity and shear size of genomic DNA preparation on 0.8% agarose gel. Some typical results using this method on a range of Lotus tissues are shown in Table 1 and Figure 1.

Table 1. Yields and quality of genomic DNA from a range of transgenic Lotus tissues.

	Typical yield µl/g FW	Cuts with 1 Unit EcoR1, 1 hour at 37° c
Root culture (14 day)	23.4	Yes
Root culture (22 day)	38.5	Yes
Callus tissue	0	NA
Shoot culture (14 day)	14.2	Yes
Shoot culture (22 day)	11.4	Yes
Regenerated shootlets	103	Yes
Regenerated plant root	0	NA
Regenerated plant stem	6.2	No
Regenerated plant leaf	48.9	Sometimes

Growth conditions of plant material were as outlined in Robbins et al. (1992).
Root cultures; basal B5 liquid medium +3% sucrose shaken 120 rpm in dark.
Callus; root tips de-differentiated on solid basal B5 medium + 4mg/1 2,4D.
Shoot cultures; basal B5 liquid medium + 2% glucose shaken 100 rpm in light.
Regenerated shootlets; spontaneously regenerated Lotus shoot tissue rooted in basal MS agar +3% glucose in light.
Regenerated plant material produced after transfer to soil, in this study all regenerated plant tissue was greenhouse grown.

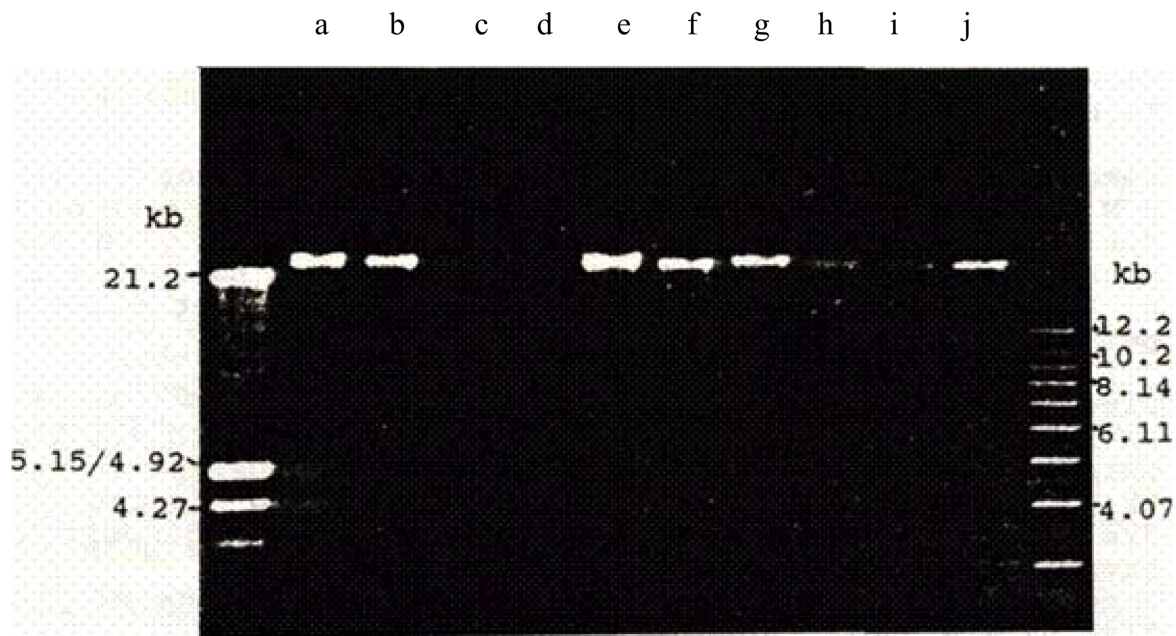

Figure 1. Agarose gel of genomic DNA preparations from transgenic Lotus. 1 µl genomic DNA applied per track.

a) root culture, 14 d; b) as a; c) root culture, 22 d; d) as c; e) shoot culture, 14 d; f) shoot culture, 22 d; g) shootlet; h) regen.plant stem; i) regen.plant leaf; j) regen.plant leaf [75° C extraction]

<u>Comments on protocol</u>

All solutions and plastic-ware etc. should be freshly autoclaved prior to use. Extraction of less than 0.5 g tissue is not really feasible due to low DNA yields. We have tried alternative grinding methodologies including Wareing blender and Mikro-dismembranator (B.Braun Medical) but neither improved yield or quality, though increasing the temperature of extraction from 65° C to 75° C did appear to produce a modest improvement in yield (data not shown).

Addition of 5% PVP and or 5% Dowex 1x2 (antiphenolic components) in initial extraction buffer may give more restrictable DNA but will decrease final yield of DNA. Inclusion of 100 mM sodium diethyldithiocarbamate or 100 mM phenylthiocarbamide (both are peroxidase inhibitors) neither increased yield nor improved restrictability of genomic DNA.

Tissue cultured roots and shoots reproducibly gave yields of genomic DNA in the range 10-30 µl/g FW, although older root cultures often gave partially degraded preparations (Fig. lc,d). Higher yields were noted when regenerating shootlets were used, but all cultured plant material gave genomic DNA that readily digested with Eco Rl and

other restriction enzymes whereas regenerated plant material rarely gave genomic DNA suitable for Southern analysis.

References

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