

An efficient method of genomic DNA isolation from plant tissues*

Paweł M. Stróżycki** and Andrzej B. Legocki

*Institute of Bioorganic Chemistry, Polish Academy of Sciences,
Z. Noskowskiego 12/14, 32–607 Poznań, Poland*

Received: 14 June, 1995

Key words: genomic DNA, isolation, plant tissues, *Lupinus luteus*

The manuscript describes an easy method of isolation of plant genomic DNA. This method allowed us to isolate substantial amounts of good quality DNA from lupin (*Lupinus luteus*) tissues. The described method also appeared to be useful for genomic DNA isolation from tissues of other plants.

Numerous procedures for the isolation of DNA suitable for all kinds of analysis including polymerase chain reaction (PCR) are published each year. Over the years we have checked many approaches trying to isolate reasonable amounts of good quality genomic DNA from different tissues of *Lupinus luteus* plants. The quality of DNA differed from procedure to procedure but yields were always very low. We present here the most efficient method, which gives a very good quality DNA from different plant tissues — roots, shoots and leaves. We have successfully used the DNA, purified with this procedure from tissues of carrot, alfalfa, tobacco and *Arabidopsis*, in number of analyses, including PCR and digestions with many restriction nucleases.

METHODS

"Micro-prep scale" of plant genomic DNA isolation protocol. 5–150 mg of tissue frozen in liquid nitrogen was ground in the microcentrifuge tube using plastic pestles. The samples

were stored in liquid nitrogen until the next step. 300 µl of TE saturated phenol and then 300 µl of extraction buffer A (0.5 M LiCl; with larger amounts of tissue — 0.1 M; 10 mM Tris/HCl, 5 mM EDTA, pH 8.0) were added. The samples were briefly vortexed and incubated for 5 min at 55°C and then for 15 min at RT (being mixed "end-over-end" all the time). 300 µl of chloroform was then added and the samples were mixed for additional 5 min at RT and centrifuged for 10 min with max. speed in an Eppendorf centrifuge. The aqueous phase was collected and reextracted with 600 µl of 1:1 phenol/chloroform mixture followed by 500 µl of chloroform (centrifuged each time as before). 800 µl of 95% ethanol was added and DNA was precipitated at -70°C for 30 min. DNA was recovered by centrifugation in an Eppendorf centrifuge (15 min, 4°C, max. speed), washed twice with 70% ethanol, dried in a Speed Vac for 5 min and resuspended in 100–200 µl of TE supplemented with RNase A (20 µg/ml). 10 µl of DNA solution was used for gel analysis and 4 µl for PCR reactions (Fig. 1).

*The work was supported by the grants 6P20300906 and 6P20405606 from the State Committee for Scientific Research.

**To whom correspondence should be addressed.

Abbreviations used: TE, 10 mM Tris, 1 mM EDTA, pH 8.0; RT, room temperature.

The "preparative" scale protocol for preparation of plant genomic DNA. A 5–10 g sample of 3 day old roots of lupin was ground in a mortar

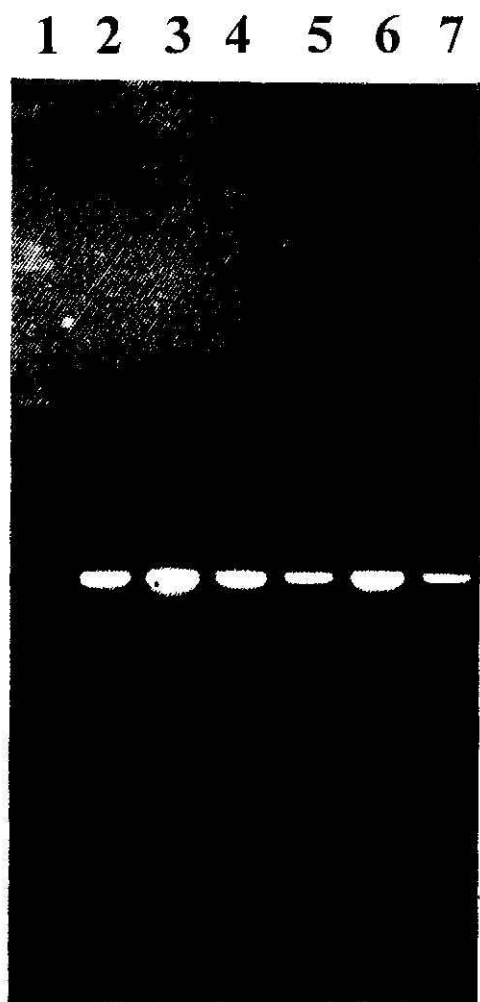


Fig. 1. PCR analysis of genomic DNA isolated from *lotus hairy roots*.

Four microliters of the solution of genomic DNA isolated from individual hairy roots induced by *A. rhizogenes* transformation of *L. corniculatus* [1] was used in 100 μ l PCR reactions. Sequences of the used oligo-primers corresponded to the sequence of the drug resistance gene: aminoglycoside phosphotransferase III; 5'ACCTATGATGTGGAACGGGAA 3' and 5'GTCATACCACTTGTCGCCCT 3' — positions 694–715 and 1194–1173 respectively (PCR product — 501 bp)[2]. The reaction mixture contained 50 mM KCl, 10 mM Tris/HCl (pH 8.8 at 25°C), 1.5 mM MgCl₂, 0.1% Triton X-100, all four dNTPs — 1.125 mM each, 350 ng of each oligo-primer [3] and 2.5 U of Tag DNA polymerase (Promega). 30 cycles of 94°C — 1 min, 30 s, 55°C — 2 min and 72°C — 3 min were performed. 10 μ l samples of each reaction were loaded on 1% agarose gel and DNA products were separated electrophoretically. Lanes: 2–7, products of PCR amplification of *L. corniculatus* DNA isolated from separate hairy roots emerging after *A. rhizogenes* transformation; lane 1, products of the same reaction but with DNA isolated from roots of control plants of *L. corniculatus* (not treated with *Agrobacterium*).

in liquid nitrogen. The powder was transferred using a spatula cooled in liquid nitrogen to six volumes of extraction buffer B (7 M urea, 1% v/v; sarcosyl, 1/15 vol. of phenol; saturated with TE; 10 mM Tris/HCl, 5 mM EDTA, pH 8.0) prewarmed up to 60°C (for lower extraction efficiency two volumes of the buffer are sufficient), dispersed and incubated with gentle shaking at 55°C for 5 min. To the cooled (15 min at RT — with gentle mixing) suspension one volume of 1:1 phenol/chloroform mixture was added and incubation was continued for an additional 10 min at RT as before. Sometimes better results were achieved when chloroform was added separately after 10 min of extraction with TE-saturated phenol alone and then extracted for 10 min more.

The preparation was centrifuged for 15 min 3000 \times g, the aqueous phase was removed and reextracted, first with one volume of phenol/chloroform mixture followed by one volume of chloroform (centrifuged as before). After the addition of 1/10-th of volume of 8 M LiCl, the DNA was precipitated with 2.5 volumes of 95% ethanol at –20°C for 30 min. The DNA was recovered by centrifugation — 15 min, 2500 \times g. The pellet was washed with 70% ethanol, dried carefully, avoiding overdrying, and resuspended (overnight at 4°C) in 1/10-th of the original volume of TE supplemented with RNase A (20 μ g/ml). When the yield is high the volume has to be increased. It is usually recommended to precipitate the DNA once more with three volumes of ethanol with no salt at all (large DNA precipitates easily this way) for one hour at –20°C. Resuspended DNA is kept on ice until used.

The isolation usually results in about 4–7 mg of genomic DNA per 10 g of plant (lupin) tissue used.

RESULTS AND DISCUSSION

This method was originally developed as micro-prep for the purpose of screening root clones after the *Agrobacterium rhizogenes* transformation.

Tissues of *Lotus corniculatus* and tobacco were transformed by *A. rhizogenes* C58 containing plasmid pBI101.2 [1]. To confirm that the emerging kanamycin resistant hairy roots were transformed, PCR analysis of DNA extracted

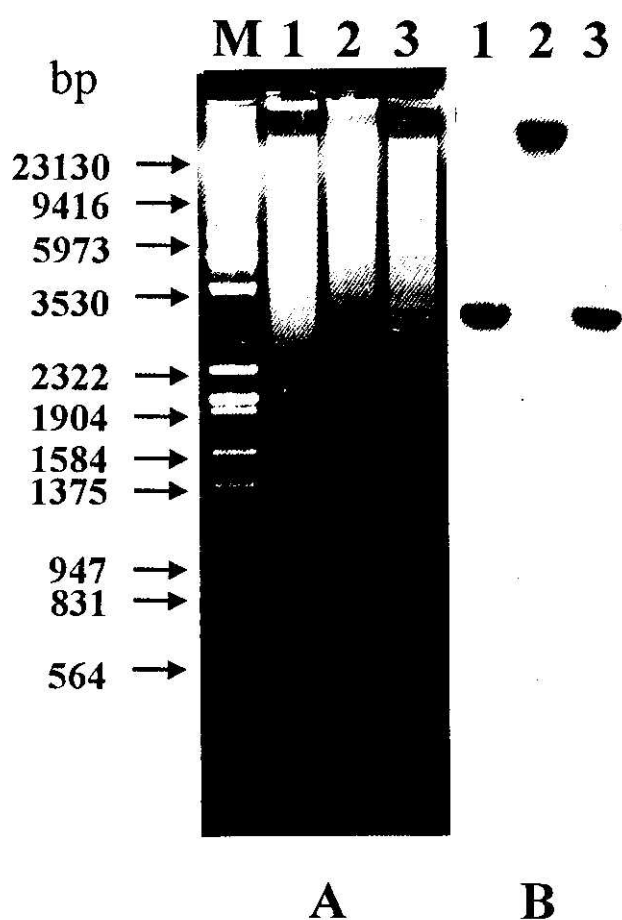


Fig. 2. Southern analysis of lupin genomic DNA. Genomic DNA was isolated from 10 g ("Preparative scale") of 3 day old roots of *L. luteus* (details in text). 40 μ g of the DNA was digested with different restriction enzymes, separated on 0.9% agarose gel and vacuum transferred to GeneScreen Plus filter (A). The filter was hybridized (in $5 \times$ SSC, $1 \times$ Denhards solution [4], 0.3% SDS and 100 μ g/ml of salmon sperm DNA, 16 h, 64°C) with the ^{32}P -labelled 176 bp fragment of 3' noncoding region of leghemoglobin I cDNA (B), amplified in a PCR reaction. The result of the hybridization was as expected from the genomic clones analysis (data in preparation). Digestions: lane 1, *EcoRI/SalI*; lane 2, *BamHI/SalI*; lane 3, *BamHI/EcoRI*; M, DNA markers (in base pairs — bp).

from 5–10 mm pieces of roots was performed (Fig. 1). All plants regenerated from the "positive" root clones were found to be transgenic (not shown).

The scaled-up preparative procedure was also used for successful isolation of DNA for various plant tissues of regenerated transgenic plants (not shown).

Our aim was to develop a convenient procedure for the isolation of genomic DNA from leguminous plants. The isolation of lupin DNA resulted in preparations (with the purity ratio $A_{260/280}$ ranging between 1.75–1.9), which were fully digestible by all restriction nucleases

tested and were used for Southern analysis (Fig. 2). The blot was hybridized with the molecular probe generated by PCR from the lupin leghemoglobin cDNA clone (Stróżycki *et al.* "Leghemoglobins from an evolutionary old legume *Lupinus luteus*." *Plant Science*, in press). The hybridization pattern was confirmed by the sequencing of the leghemoglobin genes (data not shown).

The fact that both methods (micro- and preparative scale) work easily with tissues of different taxonomically distinct plants makes them very useful for studying plant genomes.

The authors wish to thank Ms. Elżbieta Czarnecka for her excellent technical assistance.

REFERENCES

- Petit, A., Stougaard, J., Kuehle, A., Marcker, K.A. & Tempe, J. (1987) Transformation and regeneration of the legume *Lotus corniculatus*: A system for molecular studies of symbiotic nitrogen fixation. *Mol. Gen. Genet.* **207**, 245–250.
- Trieu-Cuot, P. & Courvalin, P. (1983) Nucleotide sequence of the *Streptococcus faecalis* plasmid gene encoding the 3'5'-aminoglycoside phosphotransferase type III. *Gene* **23**, 331–341.
- Hamill, J.D., Rounsley, S., Spencer, A., Todd, G. & Rhodes, M.J.C. (1991) The use of polymerase chain reaction in plant transformation studies. *Plant Cell Reports* **10**, 221–224.
- Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular cloning. A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.