An efficient method of genomic DNA isolation from plant tissues*

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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).

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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

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 × 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 × 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 μg/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 pBl101.2 [1]. To confirm that the emerging kanamycin resistant hairy roots were transformed, PCR analysis of DNA extracted

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 μl PCR reactions. Sequences of the used oligo-primer corresponded to the sequence of the drug resistance gene aminoglycoside phosphotransferase III; 5'ACCTATGATGTTGGGACGGGAA 3' and 5'GTCAACACCATGTCGACCCCT 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 μl 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).
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.

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REFERENCES


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 μg 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 x SSC, 1 x Denhards solution [4], 0.3% SDS and 100 μg/ml of salmon sperm DNA, 16 h, 64°C) with the 32P-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, EcoRl/SalI; lane 2, BamHI/SalI; lane 3, BamHI/EcoRl; M, DNA markers (in base pairs — bp).

from 5–10 mm pieces of roots of plants 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 A260/280 ranging between 1.75–1.9), which were fully digestible by all restriction nucleases.