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THE BACTERIAL ARTIFICIAL CHROMOSOME (BAC) LIBRARY OF THE NARROW-LEAFED LUPIN (*Lupinus angustifolius* L.)

ANDRZEJ KASPRZAK[1], JAN ŠAFÁŘ[2], JAROSLAV JANDA[2], JAROSLAV DOLEŽEL[2], BOGDAN WOLKO[1]* and BARBARA NAGANOWSKA[1]
[1]Institute of Plant Genetics, Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland, [2]Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Sokolovská 6, CZ-772 00 Olomouc, Czech Republic

Abstract: The narrow-leafed lupin possesses valuable traits for environment-friendly agriculture and for the production of unconventional agricultural products. Despite various genetic and environmental studies, the breeding of improved cultivars has been slow due to the limited knowledge of its genomic structure. Further advances in genomics require, among other things, the availability of a genomic DNA library with large inserts. We report here on the construction of the first DNA library cloned in a BAC (bacterial artificial chromosome) vector from diploid *Lupinus angustifolius* L. cv. Sonet. The high molecular weight DNA used for its preparation was isolated from interphase nuclei that were purified by flow cytometry. The library comprises 55,296 clones and is ordered in 144×384-well microtitre plates. With an average insert size of 100 kb, the library represents six haploid genome equivalents. Thanks to the purification of the nuclei by flow cytometry, contamination with chloroplast DNA and mitochondrial DNA was negligible. The availability of a BAC library opens avenues for the development of a physical contig map and positional gene cloning, as well as for the analysis of the plant's genome structure and evolution.

Key words: BAC, Genomic DNA library, *Lupinus angustifolius*, Narrow-leafed lupin

* Corresponding author: e-mail: bwol@igr.poznan.pl

Abbreviations used: BAC – bacterial artificial chromosome; DAPI – 4',6-diamidino-2-phenylindole; FISH – fluorescence *in situ* hybridization; HMW – high molecular weight PAC – artificial bacteriophage P1 chromosome; PFGE – pulsed field gel electrophoresis, PRINS – primed *in situ* DNA labeling; YAC – yeast artificial chromosome

INTRODUCTION

The genus *Lupinus* Tourn. (Fabaceae) represents an outstanding plant group that includes wild forms and crops of the Old and New World. Lupins grow in highly divergent climates and environmental conditions [1, 2]. Of over three hundred species belonging to the genus, there are three Old World species, *L. albus*, *L. angustifolius* and *L. luteus*, and one New World species, *L. mutabilis*, used as crops in sustainable agriculture. During their evolution, lupins preserved many valuable traits that can be exploited in contemporary agriculture. Their seeds are rich in proteins and can be used for animal feed [3] and for human consumption [4]. Some species may be cultivated on poor and contaminated soils, and are widely used as catch crops for soil enrichment in nitrogen and for the removal of heavy metals from contaminated arable areas [5, 6]. Furthermore, lupins are characterized by a rich secondary metabolism and the production of alkaloids, phytoalexins and flavonoids [7, 8]. Some of these biologically active substances have potential applications in medicine and in pest control [9, 10].

Despite their obvious advantages as a crop, lupins are still of minor importance in Europe. Lupin yield can be improved up to a point by traditional breeding methods, but it is the recent application of genetic engineering and biotechnology that will allow achievements in lupin genetics and genomics to catch up with those of other crops. So far, genetic maps have been constructed for some lupin species using molecular and biochemical markers, as well as morphological and physiological characteristics [11-13], and specific DNA markers closely linked to agriculturally important traits were developed [14].

Earlier cytological studies within the genus *Lupinus* were confined to chromosome counting, analysis of the morphology of mitotic chromosomes, and the examination of meiosis [1, 15, 16]. More recently, rRNA genes and some repetitive DNA sequences were physically mapped to lupin chromosomes via fluorescence *in situ* hybridization (FISH) [17-19]. The first attempts to localize BAC clones by FISH (BAC-FISH) and the use of PRINS (primed *in situ* DNA labeling) in *L. angustifolius* were reported on by Naganowska and Kaczmarek [20]. The nuclear genome size of several *Lupinus* species was also determined [21]. The comparative flow cytometric analysis of nuclear genome size in all Old World species and subspecies revealed a 2.5-fold range of variation [22].

Physical genome mapping, positional gene cloning and sequencing can be greatly facilitated by the availability of DNA libraries with large inserts. Several types of vectors can be used to clone large DNA fragments, such as the yeast artificial chromosome (YAC), the bacterial artificial chromosome (BAC) and the artificial bacteriophage P1 chromosome (PAC) [23]. Bacterial artificial chromosomes have recently proven to be invaluable tools in plant genomics. Their advantages are high transformation efficiency, stability of inserts, low chimerism and simplified manipulation with bacteria [24, 25]. Thanks to the large insert size (10^5 bp), the number of BAC clones needed to cover the genome is relatively low (10^4-10^5 clones). Thus, it is possible to store the clones

individually and create ordered libraries representing whole genomes; these may be used for the selection of specific clones. BAC libraries have been created and used for many model species, such as *Arabidopsis thaliana* [26], rice [27], *Medicago truncatula* [28], and for crop plants, such as wheat [29, 30], maize [31], barley [32], soybean [33], potato [34], common bean [35] and tomato [36].
In this paper, we describe the construction of the first nuclear genome BAC library of the narrow-leafed lupin *Lupinus angustifolius* L. We demonstrate that flow cytometry and sorting are efficient approaches for purifying intact nuclei, which, in turn, can be used to isolate high molecular weight DNA suitable for cloning. Thanks to this purification step, the library is practically free of cytoplasmic DNA.

MATERIALS AND METHODS

Plant material

Seeds of *Lupinus angustifolius* cv. Sonet were obtained from Poznań Plant Breeders, Plant Breeding Station Wiatrowo (Poland). The Sonet is a very early ripening cultivar resistant to viruses, fusarium disease and plant lodging. Its genotype contains some domesticated genes such as: *iuc* (low-alkaloid content), *ta* and *le* (non-shattering pods), *Deter* (self-completing vegetation), *Ku* (early flowering and thermoneutrality – lower vernalization requirements), and *moll* (soft seed coat). Seeds were germinated in the dark at 25ºC on wet filter paper in a petri dish to obtain 2- to 3-cm long roots.

Preparation of high molecular weight DNA

Roots were cut 1 cm from the tip and fixed for 20 min at 5ºC in 2% (v/v) formaldehyde made in Tris buffer (10 mM Tris, 10 mM EDTA, 100 mM NaCl, pH 7.5) with 0.1% (v/v) Triton X-100 [37, 38]. After three 5 min washes in Tris buffer, the root tips were cut into small parts and transferred to a 5-ml polystyrene tube containing 1 ml of ice-cold isolation buffer (IB) [39]. Suspensions of intact nuclei were prepared by mechanical homogenization of root tips (20 root tips per sample) with a Polytron PT1200 homogenizer (Kinematica, Littau, Switzerland) at 9,500 rpm for 10 sec. The crude nucleus suspension was passed through a 50-µm pore-size nylon mesh and stained with 4',6-diamidino-2-phenylindole (DAPI) at a final concentration of 2 µg/ml. The analysis and sorting of the nuclei was done using a FACSVantage flow cytometer (Becton Dickinson, San José, USA). The sorting gates were set on a dot plot of fluorescence pulse area versus fluorescence pulse width to select intact nuclei at the G_1 phase of the cell cycle. Nuclei were sorted at rates of 100 to 200 per second into 200 µl of ice-cold 3.75×IB buffer (final concentration of 0.75×IB). Flow-sorted nuclei were embedded in low melting-point agarose. To make one agarose plug, 5×10^5 nuclei were sorted and pelleted at 200 *g* for 25 min at 4ºC. The nuclei were resuspended in 40 µl of IB warmed to 50ºC and mixed with an equal amount of pre-warmed 2% InCert LMP agarose (BMA, Rockland, USA) made in IB.

BAC library construction

The procedure of the lupin BAC library construction was based on the protocol described by Šáfář *et al.* [40]. Agarose plugs were washed twice for 1 h in TE buffer, cut into small pieces and equilibrated in digestion buffer (1×*Hin*dIII buffer supplemented with 4 mM spermidine) on ice for 60 min. The high molecular weight (HMW) DNA was digested by *Hin*dIII in a series of reactions with enzyme concentrations ranging from 1.5 to 10.0 units per sample (one third of a chopped plug in 0.5 ml digestion buffer). After equilibration on ice for 60 min, the tubes containing plugs were transferred into a water bath and incubated at 37ºC for 15 min. The reactions were stopped by adding 50 µl 0.5 M EDTA, pH 8.0, to each tube. Partially digested DNA was size-selected by pulse-field gel electrophoresis (PFGE) in the following conditions: run in 1% Gold SeaKem agarose gel (BMA, Rockland, USA) at 6 V/cm, 14ºC in 0.5×TBE for 20 h, with a 1.0 to 50 sec switch time ramp and an angle of 120º.

After the first electrophoresis, the region containing non-stained HMW DNA (approximately 100-300 kb) was excised from the gel and subjected to a second round of size selection. For the second round, different parameters of pulse time were used: 2.5 s to 4.5 s for 12 h. The DNA was recovered from the agarose gel by electroelution for 1.5 h in 1×TAE buffer, 4 V/cm at 4ºC) using an ElectroEluter 422 (Biorad, Hercules, USA). The concentration of electroleluted DNA was estimated by agarose gel electrophoresis using a dilution series of lambda DNA as a concentration standard. The commercially available BAC vector pIndigoBAC-5 *Hin*dIII-Cloning Ready (Epicentre, Madison, USA) was used for cloning. Ligations were performed in a 40 µl volume consisting of approximately 150 ng of size-selected DNA, 25 ng of linearized and dephosphorylated vector, 1×ligase buffer and 4.5 units of T4 DNA ligase (New England Biolabs, Beverly, USA) at 16ºC overnight. The ligation solutions were then de-salted and transformed into *Escherichia coli* ElectroMAX DH10B (Invitrogen, Carlsbad, USA) competent cells by electroporation.

Each electroporation mix consisted of 3 µl de-salted ligation solution and 20 µl competent cells. Electroporation was performed using a Gibco BRL Cell-Porator System (Life Technologies, Carlsbad, USA) with the following settings: capacitance 330 µF, voltage 350 V, impedance low ohms, charge rate fast, resistance 4 kΩ. After transformation, the cells were resuspended in 1 ml SOC medium (2% Bacto tryptone, 0.5% Bacto Yeast extract, 10 mM NaCl, 10 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose, pH 7.0) and incubated for 45 min at 37ºC on an orbital shaker at 225 rpm. Aliquots of the SOC medium with recombinant cells were plated on LB plates containing 12.5 µg/ml chloramphenicol, 50 µg/ml X-Gal and 25 µg/ml IPTG, and incubated at 37ºC overnight. White recombinant colonies were picked out using a GeneTac G3 robotic station (Genomic Solutions, Huntingdon, UK) and transferred to 384-

well plates containing 50 µL LB freezing buffer [41]. The plates were incubated overnight at 37ºC, duplicated, and stored at -80ºC. BAC clones that were not ordered in 384-well plates were collected in aliquots as a pooled sublibrary.

BAC library characterization

144 randomly selected BAC clones were used to estimate the average insert size of the library. The clones were inoculated in 3 ml of 2YT medium [42] supplemented with 12.5 µg/ml chloramphenicol. BAC DNA was isolated, digested with *Not*I enzyme to release inserts, and electrophoresed on PFGE (1% agarose 0.5×TBE, pulse time 1 s to 40 s for 16 h, 6 V/cm, angle 120º, and temperature 14ºC). Insert sizes were estimated using the lambda ladder PFG marker (New England Biolabs, Beverly, USA).

The library was spotted on Hybond N^+ 22.2×22.2cm nylon filters (AP Biotech, Little Chalfont, UK) with a GeneTAC G3 robotic workstation. In order to determine the library contamination with organellar DNA, the filters were hybridized with DNA fragments specific to mitochondrial gene *coxII* (250 bp) and chloroplast gene *ndhK* (700 bp). The organellar DNA fragments (25 ng) were labeled using a Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, California) by incorporating 50 µCi of [α^{32}P] dATP. Hybridization was carried out for 16 h at 65ºC in HYBSOL which is composed of 5x SSC (0.75 M NaCl, 0.075 M sodium citrate), 5x Denhardt's Solution (0.1% w/v Ficoll-400, 0.1% w/v polyvinylpyrrolidone, 0.1% w/v BSA), 0.5% w/v SDS [43]. A high stringency wash was performed three times in 0.1×SSC and 0.1% SDS at 65ºC for 10 min. After hybridization, the filters were exposed to imaging plates for 48 h and scanned using a Typhoon Phosphoimager (Amersham/Pharmacia, Uppsala, Sweden).

RESULTS

In total, 16×10^6 nuclei were sorted from 55 samples of nucleus suspensions and used to prepare 32 agarose plugs. The sorting took six working days. Ten plugs were used to set up proper conditions for partial digestion using a restriction enzyme. Fifteen plugs (approximately 28 µg DNA) were used for BAC library construction.

Altogether, about 90,000 clones were obtained. Of these, 55,296 clones were ordered in 144×384-well microtiter plates. Following this, two copies of the library were made. One is stored together with the master copy at the Institute of Experimental Botany, Olomouc (Czech Republic) as a backup copy. The other is stored at the Institute of Plant Genetics, Polish Academy of Sciences in Poznań (Poland) as a working copy. The remaining not-ordered pooled BAC clones are stored in 5 ml glycerol stock tubes at -80ºC. If needed, they can be used to increase the total number of clones in the library and hence the genome coverage.

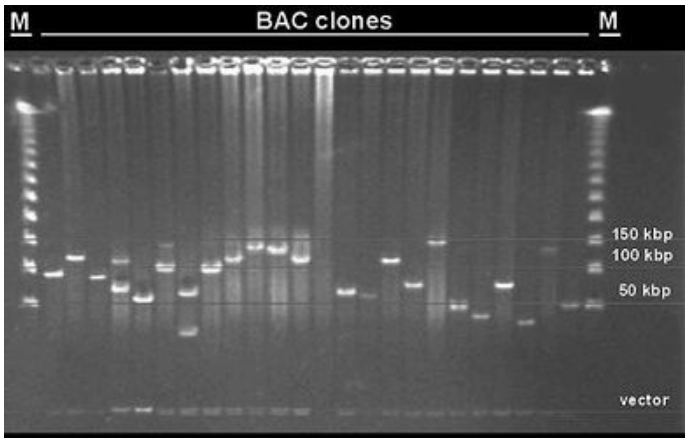

Fig. 1. Pulsed field gel electrophoresis (PFGE) of inserts from randomly sampled BAC clones. A lambda ladder (M) was used as the size marker.

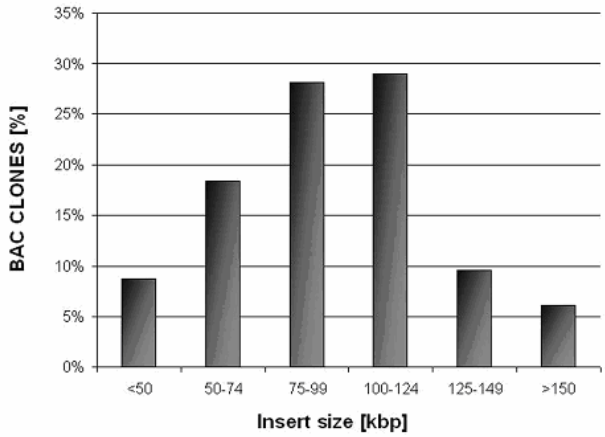

Fig. 2. The distribution of insert size in 144 randomly selected BAC clones.

A representative sample of PFGE for BAC clone size analysis is given in Fig. 1. The size of inserts ranged from 30 kb to 150 kb (Fig. 2). The average insert size in the library was estimated to be 100 kb.

The complete library occupies three filters (4×4 pattern, 18,432 clones on each filter in duplicate). The result of the analysis of contamination with organellar DNA was that the *coxII*, a mitochondrial-specific sequence, gave 10 positives signals in the whole library (Fig. 3); the chloroplast specific sequence *ndhK* gave 25 positive signals. Therefore, contamination of the library with mtDNA and cpDNA was estimated as 0.02% and 0.05%, respectively.

Considering the 2C DNA content of 1.89 pg for *Lupinus angustifolius* [22], which corresponds to a 1C genome size of 924 Mbp (see Doležel *et al.* [44] for

the conversion factor), the average insert size of 100 kb, and the presence of approximately 0.07% BAC clones containing organellar DNA, we estimated that the narrow-leafed lupin BAC library represents 6 haploid genome equivalents of *L. angustifolius*. Using the formula of Clarke and Carbon [45], the probability of recovering any lupin DNA sequence from the library was predicted to be 99.7%.

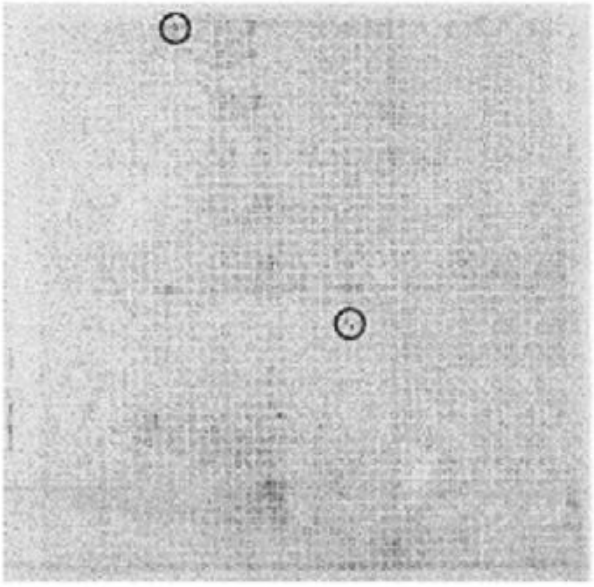

Fig. 3. An example of library screening with a mitochondrial-specific probe; 2 positive signals were found among the 18,432 BAC clones spotted in duplicate on one filter.

DISCUSSION

In selecting a lupin species for the BAC library construction, potential agricultural values as well as genomic traits were considered. We chose *L. angustifolius* because of its relatively low chromosome number (2n = 40), its moderate genome size of 924 Mbp, its economic importance, and its wide range of cultivation, which includes not only the Mediterranean region of species origin, but also Northern Europe and Australia.

BAC library construction involves several steps which determine its quality. One of the crucial problems is obtaining high molecular DNA free of contamination with organellar DNA. When traditional DNA purification protocols are used, the contamination may reach 14% [41]. Šimková *et al.* [39] developed an improved protocol using flow sorting for the purification of nuclei. That resulted not only in an almost complete absence of organellar DNA but also in a very high molecular weight of isolated DNA. The improved protocol was used to create a genomic BAC library of banana with low cytoplasmic contamination [40]. In this study, the same protocol resulted in contamination lower than 0.1%. To the

best of our knowledge, the genomic BAC library we have created is the first library of this type for lupin. The key quality parameters of the library, i.e. the average insert size of 100 kb and genomic coverage of 6×, are comparable to other plant BAC libraries (http://www.genome.arizona.edu/orders/), and make the library suitable for the entire range of genomics applications.

One of the attractive uses for the library is the development of molecular markers closely linked to agriculturally important traits, e.g. disease resistance genes. To address this question, high density colony arrays are screened with a known marker sequence [46]. Selected BAC clones are end-sequenced and specific primers designed. The segregation of polymorphic PCR products is tested using a mapping population and linkage to the gene of interest is determined. If the linkage distance is large, another round of the library screening is performed with the PCR product as a probe. Another set of BAC clones is selected and end-sequenced, and new primer pairs are designed. This chromosome-walking technique is useful for selecting BAC clones carrying the genes of interest and facilitates the contig construction of interesting genome regions and finally the gene cloning.

The new BAC library will be used to integrate genetic and physical mapping in lupin and to study the organization of plant genomes. Molecular cytogenetics can contribute to genome mapping through the assignment of genetic linkage groups to chromosomes [47, 48]. Localization of BAC clones using fluorescence *in situ* hybridization (BAC-FISH) was shown to be an effective approach to physically map specific DNA sequences and develop chromosome-specific cytogenetic markers [49, 50]. Our preliminary results indicate that FISH with BAC clones selected from the lupin BAC library will facilitate physical mapping of important genes. BAC clones selected after hybridization of high density colony arrays with probes for ENOD40 and molecular markers linked to anthracnose or phomopsis resistance genes are being localized on mitotic chromosomes using FISH [20].

The *L. angustifolius* genomic BAC library described here is free of IP issues and is accessible for research collaboration with lupin geneticists and breeders. Its availability should stimulate the development of a physical contig map, positional gene cloning, and further analysis of genome structure in lupin.

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REFERENCES

1. Gladstones, J.S. Lupins of the Mediterranean region and Africa. **Technical Bull. 26, Dept. of Agriculture Western Australia,** 1974.

2. López-Bellido, L., Fuentes, M., Lhamby, J.C.B. and Castillo, J.E. Growth and yield of white lupin (*L. albus*) under Mediterranean conditions: effect of sowing date. **Field Crops Res.** 36 (1994) 87-94.
3. Edwards, A.C. and van Barneveld, R.J. Lupins for livestock and fish. In: **Lupins as Crop Plants: Biology, Production and Utilisation.** (Gladstones, J.S., Atkins, C. and Hamblin, J., Eds) CAB International, London, 1998, 385-411.
4. Bhardwaj, H.L. Evaluation of lupin as a new food/feed crop in the Mid-Atlantic region. In: **Trends In New Crops and New Uses** (Janick, J. and Whipkey, A., Eds) ASHS Press, Aleksandria, 2002, 115-119.
5. Herridge, D.F. and Doyle, A.D. The narrow-leafed lupin (*Lupinus angustifolius* L.) as a nitrogen-fixing rotation crop for cereal production. II Estimates of fixation by field-grown crops. **Austr. J. Agri. Res.** 39 (1988) 1017-1028.
6. Petterson, D.S. and Harris, D.J. Cadmium and lead content of lupin seed grown in Western Australia. **Austr. J. Exp. Agric.** 35 (1995) 403-407.
7. Wink, M., Meißner, C. and Witte, L. Patterns of quinolizidine alkaloids in 56 species of the genus *Lupinus*. **Phytochemistry** 38 (1995) 139-153.
8. Kachlicki, P., Marczak, Ł., Kerhoas, L., Einhorn, J. and Stobiecki, M. Profiling isoflavone conjugates in root extracts of lupine species with LC/ESI/MS^n systems. **J. Mass Spectrom.** 40 (2005) 1088-1103.
9. Wink, M. Chemical defence of *Lupinus*. Mollusc-repellent properties of quinolizinidine alkaloids. **Z. Naturforsch.** 39c (1993) 553-558.
10. Folkman, W., Szrechan, J. and Gulewicz, K. Preparations of alkaloid-rich lupin in plant protection: an effect of the preparations on feeding and development of *Piersi brassicaceae* L. and *Piersi rapae* L. **J. Plant Prot. Res.** 42 (2002) 143-155.
11. Wolko, B. and Weeden, N.F. Linkage map of isozymes and RAPD markers for *L. angustifolius* L. **Proceedings of the VIIth International Lupin Conference**, Evora, Portugal, 1993, 42-49.
12. Kruszka, K. and Wolko, B. Linkage maps of morphological and molecular markers in lupins. **Proceedings of the 9th International Lupin Conference, An Ancient Crop for the New Millenium.** (van Santen, E., Wink, M., Weissmann, S. and Roemer, P. Eds) Klink/Müritz, Germany, 1999, 100-110.
13. Boersma J.G., Pallotta, M., Li, C., Buirchell B.J., Sivasithamparam K. and Yang, H. Construction of a genetic linkage map using MFLP, and identification of molecular markers linked to domestication genes in narrow-leafed lupin (*Lupinus angustifolius*). **Cell. Mol. Biol. Lett.** 10 (2005) 331-344.
14. You, M., Boersma, J.G., Buirchell, B.J., Sweetingham, M.W., Siddique, K.H.M. and Yang, H. A PCR-based molecular marker applicable for marker-assisted selection for anthracnose disease resistance in lupin breeding. **Cell. Mol. Biol. Lett.** 10 (2005) 123-134.

15. Pazy, B., Heyn, C.C., Herrnstadt, I. and Plitmann, U. Studies in populations of the Old World *Lupinus* species. Chromosomes of the East-Mediterranean lupines. **Israel J. Bot.** 26 (1977) 115-127.
16. Carstairs, S.A., Buirchell, B.J. and Cowling, W.A. Chromosome number, size and interspecific crossing ability of three Old World lupins, *Lupinus princei* Harms, *L. atlanticus* Gladstones and *L. digitatus* Forskal, and implications for cyto-systematic relationships among the rough-seeded lupins. **J. R. Soc. West. Aust.** 75 (1992) 83-88.
17. Naganowska, B. and Zielińska A. Physical mapping of 18S-25S rDNA and 5S rDNA in *Lupinus* by fluorescent *in situ* hybridization. **Cell. Mol. Biol. Lett.** 7 (2002) 665-670.
18. Naganowska, B., Doležel J. and Święcicki, W.K. Development of molecular cytogenetics and physical mapping of ribosomal RNA genes in *Lupinus*. **Biol. Plant.** 46 (2003) 211-215.
19. Hajdera, I., Siwińska, D., Hasterok, R. and Małuszyńska, J. Molecular cytogenetic analysis of genome structure in *Lupinus angustifolius* and *Lupinus cosentinii*. **Theor. Appl. Genet.** 107 (2003) 988-996.
20. Naganowska, B. and Kaczmarek, A. Repetitive DNA sequences in cytogenetic studies of the *Lupinus* genome. In: **Mexico, Where Old and New World Lupins Meet. Proceedings of the 11th International Lupin Conference** (van Santen, E. and Hill, G.D. Eds), Guadalajara, Jalisco, Mexico. May 4-9, 2005. International Lupin Association, Canterbury, New Zealand, ISBN 0-86476-165-1 (2005) 27-29.
21. Obermayer, R., Święcicki, W.K. and Greilhuber, J. Flow Cytometric Determination of Genome Size in some Old World *Lupinus* Species (Fabaceae). **Plant Biol.** 1 (1999) 403-407.
22. Naganowska, B., Wolko, B., Śliwińska, E. and Kaczmarek, Z. Nuclear DNA Content Variation and Species Relationships in the Genus *Lupinus* (*Fabaceae*). **Ann. Bot.** 92 (2003) 349-355.
23. Primrose, S.B. **Principles of Genome Analysis. A guide to Mapping and Sequencing DNA from Different Organisms.** Blackwell Sciences Ltd Second Edition, 1998, 54-60.
24. Zhang, H.B., Woo, S.S. and Wing, R.A. BAC, YAC and cosmid library construction. in: **Plant Gene Isolation. Principles and Practice** (Foster G.D. and Twell, D. Eds.), John Wiley and Sons Ltd., 1996, 75-99.
25. Peterson, D.G., Tompkins, J.P., Wing, R.A. and Paterson A.H. Construction of plant bacterial artificial chromosome (BAC) libraries: An illustrated guide. **J. Agric. Genom.** 5 (2000) 1-100.
26. Choi, S., Creelman, R.A., Mullet, J.E. and Wing, R.A. Construction and characterization of bacterial artificial chromosome library of *Arabidopsis thaliana*. **Plant Mol. Biol. Rep.** 13 (1995) 124-128.
27. Yang, D., Parco, A., Nandi, S., Subudhi, P., Zhu, Y., Wang, G. and Huang, N. Construction of a bacterial artificial chromosome (BAC) library and

identification of overlapping BAC clones with chromosome 4-specific RFLP markers in rice. **Theor. Appl. Genet.** 95 (1997) 1147-1154.
28. Nam, Y.W., Penmesta, R. V., Endre, G., Uribe, P., Kim, D. and Cook, D.R. Construction of a bacterial artificial chromosome library of *Medicago truncatula* and identification of clones containing ethylene-response genes. **Theor. Appl. Genet.** 98 (1999) 638-646.
29. Lijavetzky, D., Muzzi, G., Wicker, T., Keller, B., Wing, R. and Dubcovsky, J. Construction and characterization of a bacterial artificial chromosome (BAC) library for the A genome of wheat. **Genome** 42 (1999) 1176-1182.
30. Allouis, S., Moore, G., Bellec, A., Sharp, R., Faivre, P., Montimer, K., Pateyron, S., Foote, T., Griffiths, S., Caboche, M. and Chalhoub, B. Construction and characterisation of a hexaploid wheat (*Triticum aestivum* L.) BAC library from the reference germplasm "Chinese Spring". **Cereal. Res. Comm.** 31 (2003) 331-338.
31. Tomkins, J.P., Frisch, D.A., Byrum, J.R., Jenkins, M.R., Barnett, L.J., Wicker, T., Luo, M. and Wing, R.A. Construction and characterization of a maize bacterial artificial chromosome (BAC) library for the inbred line LH132. **Maize Genet. Coop. News Lett.** (2000) 74 www.agron.missouri.edu/mnl/74.
32. Yu, Y., Tomkins, J.P., Waugh, R., Frisch, D.A., Kudrna, D., Kleinhofs, A., Brueggeman, R.S., Muehlbauer, G.J., Wise, R.P. and Wing, R.A. A bacterial artificial chromosome library for barley (*Hordeum vulgare* L.) and the identification of clones containing putative resistance genes. **Theor. Appl. Genet.** 101 (2000) 1093-1099.
33. Danesh, D., Peňula, S., Mudge, J., Denny, R.L., Nordstrom, H., Martinez, J.P. and Young, N.D. A bacterial artificial chromosome library for soybean and identification of clones near a major cyst nematode resistance gene. **Theor. Appl. Genet.** 96 (1998) 196-202.
34. Song, J., Dong, F. and Jiang, J. Construction of a bacterial artificial chromosome (BAC) library for potato molecular cytogenetic research. **Genome** 43 (2000) 199-204.
35. Vanhouten, W. and MacKenzie, S. Construction and characterization of a common bean bacterial artificial chromosome library. **Plant Mol. Biol.** 40 (1999) 977-983.
36. Folkertsma, R.T., Spassova, M. I., Prins, M., Stevens, M.R., Hille, J. and Goldbach, R.W. Construction of a bacterial artificial library of *Lycopersicon esculentum* cv. Stevens and its application to physically map the *Sw-5* locus. **Mol. Breed.** 5 (1999) 197-207.
37. Doležel, J., Číhalíková, J. and Lucretti, S. A high-yield procedure for isolation of metaphase chromosomes from root tips of *Vicia faba* L. **Planta** 188 (1992) 93-98.
38. Doležel, J., Číhalíková, J., Weiserowa, J. and Lucretti, S. Cell cycle synchronization in plant root meristems. **Methods Cell Sci.** 21 (1999) 95-107.

39. Šimková, H., Číhalíková, J., Vrána, J., Lysák, M.A. and Doležel, J. Preparation of HMW DNA from plant nuclei and chromosomes isolated from root tips. **Biol. Plant.** 46 (2003) 369-373.
40. Šafář, J., Noa-Carrazana, J.C., Vrána, J., Bartoš, J., Alkhimova, O., Lheureux, F., Šimková, H., Caruana, M.L., Doležel, J. and Piffanelli, P. Creation of a BAC resource to study the structure and evolution of the banana (*Musa balbisiana*) genome. **Genome** 47 (2004) 1182-1191.
41. Woo, S.S., Jiang, J., Gill, B.S., Paterson, A.H., and Wing, R.A. Construction and characterization of a bacterial artificial chromosome library of *Sorghum bicolor*. **Nucleic Acids Res.** 22 (1994) 4922-4931.
42. Sambrook, J. and Russell, D.W. **Molecular Cloning. A Laboratory Manual. Third Edition.** Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., USA vol. 3 (2001) A 2.4.
43. Yang, H., McLeese, J., Weisbart, M., Dionne, J.-L. and Aubin R.A. Simplified high throughput protocol for Northern hybridization. **Nucleic Acids Res.** 21 (1993) 3337-3338.
44. Doležel, J., Bartoš, J., Voglmayr, H. and Greilhuber, J. Nuclear DNA content and genome size of trout and human. **Cytometry** 51 (2003) 127-128.
45. Clarke, L. and Carbon, J. A colony bank containing synthetic Col El hybrid plasmids representative of the entire *E. coli* genome. **Cell** 9 (1976) 91-99.
46. Wolko, B., Kasprzak, A., Doležel, J. and Naganowska, B. Screening a Narrow-Leafed Lupin BAC Library to Search for Clones Containing Nodulation and Disease Resistance Genes. **Proc. of the 11th International Lupin Conference: Mexico, Where Old and New World Lupins Meet.** Guadalajara, Mexico, 2005, 49-51.
47. Mozo, T., Dewar, K., Dunn, P., Ecker, J.R., Fischer, S., Kloska, S., Lehrach, H., Marra, M., Martienssen, R., Meier-Ewert, S. and Altmann, T. A complete BAC-based physical map of the *Arabidopsis thaliana* genome. **Nat. Genet.** 22 (1999) 271-275.
48. Islam-Faridi, M.N., Childs, K.L., Klein, P.E., Hodnett, G., Menz, M.A., Klein, R.R., Rooney, W.L., Mullet, J.E., Stelly, D.M. and Price, H.J. A molecular cytogenetic map of sorghum chromosome 1: fluorescence *in situ* hybridization analysis with mapped bacterial artificial chromosomes. **Genetics** 161 (2002) 345-353.
49. Suzuki, G., Ura, A., Saito, N., Do, G.S., Yamamoto, M., Mukai, Y. and Seo, B.B. BAC FISH analysis in *Allium cepa*. **Genes Genet. Syst.** 76 (2001) 251-255.
50. Zhang, P., Li, W., Fellers, J., Friebe, B. and Gill, B.S. BAC-FISH in wheat identifies chromosome landmarks consisting of different types of transposable elements. **Chromosoma** 112 (2004) 288-299.