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Development of a BAC library in Common Bean Genotype BAT93

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Several types of large-insert DNA libraries have been developed. These include principally yeast artificial chromosome (YAC), bacterial artificial chromosome (BAC), and P1-derived artificial chromosome (PAC) libraries. Because these libraries typically include inserts of sizes from several 100,000 bps to several 1 Mb for YACs (Burke et al. 1987) and 100-300 kb for BACs and PACs (Shizuya et al. 1992), they are able to bridge the gap between genetic and physical distance, either individually or as an ordered, linear group (contig). Genetic distances are generally of the order of several 100 kb to Mb per unit of recombination (cM) whereas earlier cloning vectors (plasmid, lambda, and cosmid) only accepted inserts below 50 kb. Thus, these large-insert libraries are essential tools for the isolation of and cloning of individual genes or genome regions by map-based cloning. They have also been used to in genome evolution analysis, physical mapping, comparative mapping, high-resolution mapping, determining the relationships between genetic and physical distance, and constructing transcription maps.

In spite of their advantage in terms of insert size, YAC libraries have several disadvantages such as low transformation efficiency, the difficulty to separate and isolate YAC DNA from the yeast chromosomal background, genomic instability, and a high frequency of chimerisms. Hence, alternative library types have been developed, the PAC and BAC libraries (Shizuya et al. 1992). The latter system is the one now generally used to clone large plant DNA fragments. BAC libraries have been developed for model species such as *Arabidopsis*, *Lotus japonicus*, and *Medicago truncatula*, and crop species such as barley, common bean, lettuce, rice, sorghum, soybean, and tomato. The BAC vector is an F-factor-based plasmid vector, which is maintained as a single-copy plasmid in a recombination-deficient *E. coli* strain to limit sequence rearrangement. Compared to YAC libraries, BAC libraries have the advantages of ease of isolation of BAC DNA and low DNA instability and chimerisms.

Establishing a BAC library in itself has become a relatively routine operation. BAC libraries have been established for quite a number of species (*e.g.*, Clemson University Genomics Institute: http://www.genome.clemson.edu/lib_frame.html; Texas A & M BAC Center: <http://hbz.tamu.edu/bacindex.html>), including common bean (Vanhouten and Mackenzie 1999).

We constructed a BAC library for *P. vulgaris* cv. BAT93, a breeding line resulting from the breeding program of S. Temple, formerly at the Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia. Its pedigree is (Veranic x Tlalnepantla 64) x (Jamapa x Tara). BAT93 harbors genes controlling several host-microorganism interactions including resistance to Bean Common Mosaic Virus (BCMV; *I* gene), *Xanthomonas campestris* pv. *phaseoli* (causal agent of common bacterial blight), *Uromyces appendiculatus* (causing rust), and *Colletotrichum lindemuthianum* (causing anthracnose). These resistances are correlated with low nodulation (Nodari et al. 1993; Tsai et al. 1998). In addition, BAT93 is one of the parents of the BJ recombinant inbred population, with which most of the linkage maps of common bean have been correlated (Freyre et al. 1998). In contrast with cv. Sprite, the genotype used by Vanhouten and Mackenzie (1999), which is an Andean genotype, BAT93 is Mesoamerican.

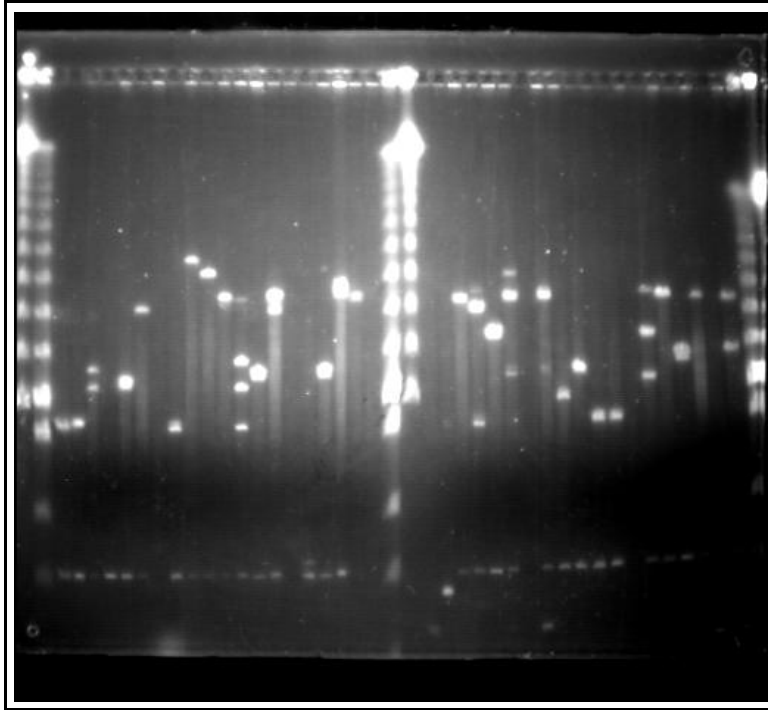


Figure 1. Pulsed field gel electrophoresis of BAC clones of common bean cv. BAT93. Lanes 1, 24, and 44: molecular weight markers: lambda concatemer ladder (multiples of 48.5 kb); lanes 2, 23: combination of a lambda *Hind*III digest and lambda concatemers. The faint band at the bottom is the vector (around 7 kb). 1% Agarose in 0.5X TBE; Switch Time ramped 1 to 40 sec, 200 volts for 20 hours.

The library was constructed using the methods and protocols outlined in Lijavetzky et al. (1999). Several different types of nuclei isolation buffers were tested but gave variable results and low yields of intact nuclei. The method of Kuehl (1964) gave more consistent results in our hands and yielded sufficiently high concentrations of clean, intact nuclei for BAC library construction. Ligations from BAC vector and high molecular weight DNA yielded approximately 100 white colonies per μ l of ligation mix. The average insert size was 165 kb (Fig. 1). Based on a haploid genome size of 635 Mbp (Aramuganathan and Earle 1991), we would have at least 10 genome equivalents. The library needs to be characterized further, however, with regard to content of cytoplasmic clones and coverage of specific sequences.

Common bean provides an excellent model to investigate evolutionary issues. The phylogeny

of the genus *Phaseolus*, in general, and the phylogeography of *P. vulgaris*, in particular, are well-known (Delgado-Salinas et al. 1993, 1999; Kami et al. 1999). The divergence between Andean and Mesoamerican gene pools is well-documented (Gepts 1993), as is the inheritance of the domestication syndrome of common bean (Koinange et al. 1996). The availability of BAC libraries from phylogenetically significant genotypes, such as representatives of the Andean and Mesoamerican gene pool will help us understand the molecular basis of phenotypic divergence in the respective evolutionary lineages, such as the diversification of the anthracnose (caused by *Colletotrichum lindemuthianum*) resistance gene cluster on linkage group B4. In this cluster, are located disease resistance gene analogues, major genes for resistance, as well as resistance QTLs against both Andean and Mesoamerican strains of the pathogen (Geffroy et al. 1999, 2000).

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