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Genetic Resources

Construction of an Arabidopsis BAC Library and Isolation of Clones Hybridizing with Disease-Resistance, Gene-Like Sequences

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Abstract: A bacterial artificial chromosome (BAC) library consisting of 9,000 clones (representing a 4.5 genome equivalents) with an average DNA insert size of 60 kb was constructed from arabidopsis nuclear DNA. We have demonstrated the usefulness of this library by identifying one BAC clone that hybridizes with the arabidopsis *PHYB* gene and seven clones, representing four distinct classes, that hybridize to a putative disease-resistance gene. This library represents an additional resource for map-based cloning and physical mapping in arabidopsis.

Arabidopsis thaliana is a widely used experimental organism in plant biology due to its short generation time, small genome size, and small amount of repetitive DNA (Meyerowitz et al., 1994). Efforts to construct a contiguous set of clones spanning the entire arabidopsis genome are underway (Schmidt and Dean, 1993; Schmidt et al., 1995). Completion of this project will greatly facilitate gene isolation using positional cloning strategies and will lead to new insights into genome organization. The availability of genomic libraries with large DNA inserts is essential to these efforts. Yeast artificial chromosome (YAC) libraries have made valuable contributions to the production of

Abbreviations: BAC, bacterial artificial chromosome; *PHYB*, gene encoding phytochrome B; PFGE, pulse-field gel electrophoresis; YAC, yeast artificial chromosome.

physical maps of the arabidopsis genome and to the isolation of many genes (Arondel et al., 1992; Putterill et al., 1993). YAC libraries, however, have some problems including chimeric and unstable clones (Neil et al., 1990; Green et al., 1991; Libert et al., 1993; Umehara et al., 1994, Schmidt et al., 1994). In addition, pinpointing the gene of interest on the large DNA insert of a YAC clone can be time-consuming (Tanksley et al., 1995).

Bacterial artificial chromosome (BAC) and P1 cloning systems were developed as a supplement to the YAC system (Shizuya et al., 1992; Sternberg, 1990). These systems have several advantages over the YAC system, such as high efficiency of transformation, low frequency of chimeric clones, and easy manipulation and maintenance of the cloned DNA. The P1 system is based on a bacteriophage vector and has successfully been used to construct a contig covering 600 kb in the region of the *cer9* locus of arabidopsis (Liu et al., 1995).

The BAC system utilizes an F-factor–based vector and is capable of maintaining large genomic DNA fragments (>300 kb) (Shizuya et al., 1992). Recently, BAC libraries of sorghum, rice, and arabidopsis have been constructed (Woo et al., 1994; Wang et al., 1995; Choi et al., 1995). The availability of the rice BAC library facilitated positional isolation of the *Xa21* disease-resistance locus (Wang et al., 1995; Song et al., 1995). In this article, we report the construction of an arabidopsis BAC library consisting of 9,000 clones with an average insert size of 60 kb. This library represents a useful supplement to the YAC (Ecker, 1990; Ward and Jen, 1990; Grill and Somerville, 1991; Creusot et al. 1995), P1 (Liu et al., 1995), and BAC libraries (Choi et al., 1995) that have been described previously. The library is arrayed in 384-well microtiter plates and can be used for cloning and physical analysis of arabidopsis genes.

Materials and Methods

Preparation of high molecular weight DNA, partial digestion, size fractionation, ligation, transformation, filter preparation and hybridization screening was carried out as previously described (Wang et al., 1995). Nuclei derived from leaf tissue of arabidopsis (Col-0 ecotype) were embedded in agarose plugs (approximately 5 µg DNA/80 µl plug). DNA was released from the agarose matrix by melting the plugs before partial digestion with *Hind* III to ensure exposure of all the DNA to the enzyme (Martin et al., 1992; Wang et al., 1995). The optimal amount of *Hind* III was determined empirically. One to five units produced the maximum amount of DNA in the 250- to 350-kb range. Three units of *Hind* III per plug yielded the highest percentage of white colonies after

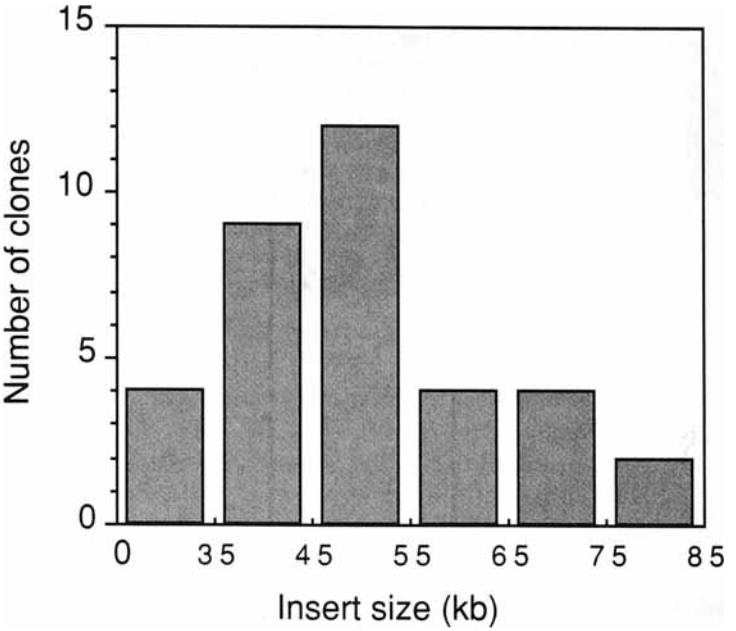

Fig. 1. Size distribution of clones in the arabidopsis BAC library. Thirty-five randomly isolated arabidopsis BAC clones were isolated, digested with *Not* I and sized using PFGE.

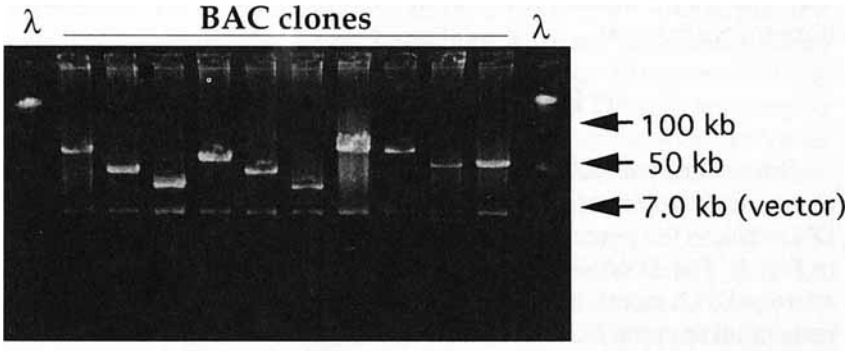

Fig. 2. Digestion of DNA inserts by *Not* I. Ethidium-stained agarose gel showing ten randomly isolated arabidopsis BAC clones digested with *Not* I and separated by PFGE. *Not* I releases the entire insert from the BAC vector. Arrows indicate the sizes of DNA fragments deduced from λ concatamer size markers and the 7-kb pBelo BAC11 vector. The size of the BACs range from 20 to 85 kb.

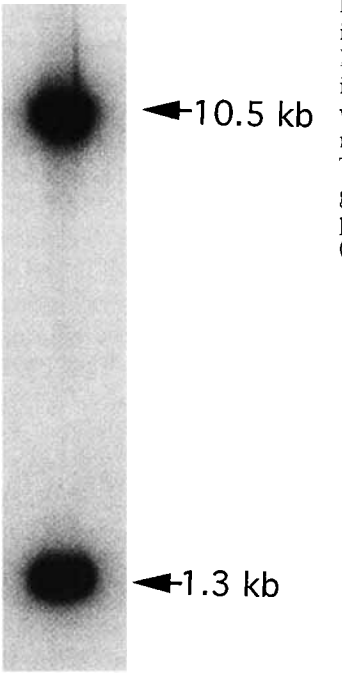

Fig. 3. Autoradiogram showing the BAC clone insert DNA hybridizing with the *PHYB* gene. Plasmid DNA, isolated from the bacterial colony identified by colony hybridization, was digested with *Hind* III and the DNA fragments separated by standard agarose gel electrophoresis. The gel was blotted and hybridized with the gene to confirm the identity of the clones. The probe hybridized with two DNA fragments (10.5- and 1.3-kb, respectively).

ligation to the pBelo BAC11 vector (gift of H. Shizuya and M. Simon), and transformation; therefore, this enzyme concentration was used for the library construction.

Results and Discussion

The arabidopsis BAC library consists of 9,000 clones. Out of 37 BAC clones randomly chosen from 7 plates of the library, 35 clones contained DNA inserts (95 percent). The size distribution of these clones is shown in Fig. 1. The DNA insert sizes ranged between 20 to 85 kb, with an average DNA insert of 60 kb. Ten of these clones were digested with the restriction enzyme *Not* I, and the DNA fragments were separated using pulse-field gel electrophoresis (PFGE) (Fig. 2).

Isolation of BAC clones that hybridize to *PHYB*

In order to test the completeness of the library, we used colony hybridization to screen the entire library for clones containing *PHYB*, which is

a single-copy gene in arabidopsis (Sambrook et al., 1989; Wang et al., 1995; Wanger and Quail, 1995). The probe was a 2164 bp *Kpn* I-*Nco* I fragment taken from the *PHYB* cDNA, encompassing nearly all of exon I (gift of J. Tepperman and P. Quail). One clone was isolated that hybridizes to *PHYB*. DNA of the clone was isolated using an alkaline lysis procedure and analyzed by restriction digestion and Southern hybridization (Sambrook et al., 1989). This 20-kb clone contains two *Hind* III fragments that hybridized to the *PHYB* probe (Fig. 3). Fragments of the same size are detected in genomic DNA from arabidopsis ecotype Col-0 (data not shown), indicating that this clone contains *PHYB*.

Isolation of BAC clones that contain disease-resistance gene-related sequences

We also screened the BAC library with an arabidopsis *EST* (expressed sequence tag) clone that we believe encodes a member of a family of disease-resistance genes. This clone (ATTS0477) displays significant similarity to the disease-resistance genes *RPM1* and *RPS2* (Grant et al., 1995; Bent et al., 1995), and co-segregates with the disease-resistance gene *RPS5* (Siminoch and Innes, 1995; E. Drenkard and F. Ausubel, personal communication). The hybridization probe was made from a 288-bp DNA fragment encoding part of the leucine-rich repeat region, which is a motif found in several recently isolated disease-resistance genes in plants (Staskawicz et al., 1995). We screened the entire BAC library at low stringency [membranes were hybridized and washed at 50°C using the buffers described by Church and Gilbert (1984)] in order to identify clones containing the ATTS0477 sequence, and ATTS0477-like sequences. We wished to identify the latter clones because many disease-resistance genes are members of clustered multi-gene families (Martin et al., 1993; Song et al., 1995), and we wished to determine if ATTS0477 was similar in this regard. Hybridization of the ATTS0477 probe to arabidopsis genomic DNA reveals at least seven *Hind* III fragments using the conditions of low-stringency hybridization conditions described above (data not shown). We identified a total of seven clones that hybridized to the 288-bp probe. The inserts in these clones ranged in size from 9- to 55-kb, and were thus smaller than expected, based on the sizes of randomly selected clones (Fig. 1). The seven clones contain five different fragments that hybridize to the ATTS0477 probe. Three clones contain a 9.0-kb *Hind* III fragment, two clones contain a 4.1- and 4.9-kb *Hind* III fragment, and the remaining two clones are unique, containing 8.0- and 1.8-kb *Hind* III fragments. None of these clones contained the ATTS0477 sequence, which hybridizes to a 3.2-kb *Hind* III fragment in arabidopsis genomic

DNA. Although we were unsuccessful in identifying a BAC clone containing ATTS0477, we were able to isolate several members of the ATTS0477 family.

The usefulness of the BAC library for genome analysis

With an average insert size of about 60 kb, this library theoretically contains 4.5 haploid genome equivalents. The average insert size of BAC clones in this library is relatively small compared with other BAC libraries, probably because only one size-selection of partially digested DNA was conducted (Woo et al., 1994; Wang et al., 1995; Choi et al., 1995). However, 60-kb inserts are useful for dissection of chromosome regions as a supplement to YAC and to other BAC libraries.

Recent developments in technological and analytical procedures are shifting the chromosome walking strategy of gene isolation to "chromosome landing" (Tanksley et al., 1995). This strategy takes advantage of high-volume marker technologies that allow thousands of loci to be assayed for linkage in a short time. DNA markers tightly linked to a gene of interest are used to screen a genomic library. In this case, small insert genomic libraries have advantages over larger insert libraries since, once a clone is identified containing the marker gene and gene of interest, less subcloning is required to pinpoint the gene. Since BAC DNA inserts can be introduced into binary vectors by subcloning or *Cre-lox*–mediated recombination (Shizuya et al., 1992), the clones can be rapidly adapted for arabidopsis transformation. This library should serve as an additional resource for map-based cloning efforts with arabidopsis. To this end, the library is available for distribution to academic researchers through the Arabidopsis Biological Resource Center at Ohio State University.

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