Construction and Characterization of a BAC Library for the Soybean Cultivar A3244

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Introduction

The construction of a physical framework for the soybean genome requires the use of large insert genomic libraries. The bacterial artificial chromosome (BAC) cloning system appears to offer advantages over other large insert cloning systems as discussed previously (Shizuya et al., 1992; Woo et al., 1994). Therefore, the BAC system is very appealing as a vehicle for advanced genome analysis in soybean. At the present time, the construction of three soybean BAC libraries has been reported in the literature (Marek and Shoemaker, 1997; Danesh et al., 1998; Tomkins et al., 1999). Estimated coverage for these libraries range between 3 and 9 haploid genome equivalents. In order to develop a comprehensive physical framework for soybean using fingerprinting (Marra et al, 1997) and BAC end sequencing technologies, a deeper coverage BAC library providing at least 10 genome equivalents will be required. Our objective was to develop a deep coverage soybean BAC library for the purpose of creating a comprehensive physical framework of the soybean genome.

Materials and Methods

The single copy BAC vector, pBeloBAC11, was obtained from Dr. Hiroaki Shizuya (Shizuya et al., 1992) and prepared as described by Woo et al. (1994). Megabase soybean DNA embedded in agarose plugs was obtained as described by Zhang et al. (1996) using young greenhouse grown leaves from the commercial cultivar A3244. Partial digests of megabase DNA were performed as follows: chopped plugs were distributed in 100 ul aliquots and

incubated on ice for 30 min with 14 ul 10x enzyme buffer, 14 ul 40mM spermidine, and 1.4 ul BSA. After a second 30 min incubation with 2 units HindIII on ice, digestion reactions were allowed to proceed at 37° C for 30 min. Digestions were stopped by placing on ice and adding 1/10 vol .5 M EDTA. Partially digested megabase DNA was subjected to two size selections by pulsed field electrophoresis (CHEF mapper apparatus, BIO-RAD). Initial size selection conditions were; 1% low gelling temperature agarose, 1-50 sec linear ramp, 6 volts/cm, 12° C, 22 hour run time, and 0.5x TBE buffer. Two fractions between 120 and 350 kb were cut from the gel based on a 50 kb lambda ladder reference (NEB). Gel slices were transferred to a second CHEF of similar composition and run at a constant 4 sec switch time under similar time and temperature conditions. Two gel slices were excised and DNA was removed from the agarose by electroelution using the BIO-RAD Electro-Eluter (Model No. 422) system. Ligations were performed in 150 ul reactions using 30 ng vector and 300 ng DNA and allowed to proceed for 16 h at 16°C. After desalting ligations, transformations were performed using 2 ul ligation reaction and 20 ul competent cells (DH10B, Gibco/BRL). Electroporations were performed on a cell porator with voltage booster (Gibco/BRL) using 320 volts at a resistance of 4 KW. Transformed cells were diluted immediately with 0.5 ml SOC (Sambrook et al., 1989) and incubated at 37° C for 60 min before being plated on selective medium (LB, Luria-Bertani medium) with 12.5 ug/ul chloramphenicol, 0.55 mM IPTG, and 80 ug/ml X-Gal. After a 20 h incubation at 37° C, the plates were placed at room temperature in the dark for an additional 20 h to allow stronger color development of nonrecombinant colonies. After determining insert sizes of clones, ligations derived from the 225 to 300 kb gel fraction were utilized for additional transformations to construct the library. Recombinant white colonies were picked robotically (Genetix Q-bot) and stored individually in 538 384-well microtiter plates (Genetix) containing 50 ul freezing broth (Woo et al., 1994). After incubation overnight, microtiter plates were stored at -80° C. Three copies of the library were made and stored in separate -80° C freezers.

To prepare BAC DNA for clone characterization, 3 ml LB chloramphenicol (12.5 ug /ul) cultures were grown overnight in 6-cell autogen tubes and miniprepped robotically (Autogen 740 plasmid isolation system). To estimate insert size and determine distribution of clone size, a total of 372 BAC preps were performed from clones selected at random throughout the library. The BAC DNA was digested with 7.5 units (10 h at 37° C) of NotI and analyzed

by pulsed field electrophoresis in 1% agarose gels (6 v/cm, 5-15 sec switch time, 15 h run time, 14° C).

Results and Discussion

We have constructed a BAC library for soybean A3244 which is suitable for constructing a comprehensive physical framework of the soybean genome. HindIII was used as the cloning enzyme because complete digests with soybean DNA produced fragments - 30 kb. The library consists of 206,592 clones stored in 538 384-well microtiter plates. Approximately 1.5% of the clones do not contain inserts as judged by random analysis of BACs sampled from the library. A random sampling of 372 BACs taken from the library indicated an average insert size of 148 kb with a range of 40 to 370 kb. Based on a haploid genome size of 1,115 mb (Arumaganthan and Earl, 1991), the coverage of the library is approximately 27.4 genome equivalents.

To determine the size distribution of BAC clones in the library, the 372 BACs analyzed with NotI digests were grouped by insert size and the frequency of each group of clones represented in the library was determined. Based on this analysis, 95% of the clones in the library have an average insert size equal to or greater than 100 kb. Of the clones larger than 100 kb, 67% are equal to or greater than 125 kb. Interestingly, the library also contains a high frequency of very large BAC clones with 14% being equal to or greater than 200 kb.

The soybean A3244 BAC library is well suited to construct a comprehensive physical framework of the soybean genome due to it's high redundancy and large average insert size. The physical framework for A3244 will be constructed by HindIII fingerprinting and BAC end sequencing (forward and reverse) all clones in the BAC library. Reagent costs for these efforts will be maximized due to the very low percentage of BACs not containing inserts.

Acknowledgements

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References

Arumaganthan, K. and Earle, E. 1991. Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208-218.

Danesh, D., Penuela, S., Mudge, J., Denny, R., Nordstrom, H., Martinez, J. and Young, N. 1998. A bacterial artificial chromosome library for soybean and identification of clones near a major cyst nematode resistance gene. Theor Appl Genet 96:196-202.

Marek, L. and Shoemaker, R. 1997. BAC contig development by fingerprint analysis in soybean . Genome 40:420-427.

Marra, M., Kucaba, T., Dietrich, N., Green, E., Bownstein, B., Wilson, R., McDonald, K., Hillier, L., McPherson, J. and Waterston, R. 1997. High throughput fingerprint analysis of large-insert clones. Genome Res 7:1072-1084.

Sambrook, J., Frisch, E. and Maniatus, T. 1989. Molecular Cloning: A laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sciumbato, R. 1993. Soybean disease loss estimates for the southern US during 1988-91. Plant Dis 77:954-956.

Shizuya, H., Birren, B., Kim, U., Mancino, V., Slepak, T., Tachiiri, Y. and Simon, M. 1992. Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in Escherichia coli using an F-factor-based vector. Proc Natl Acad Sci USA 89:8794-8797.

Tomkins, J.P., R. Mahalingham, H. Miller-Smith, J.L. Goicoechea, H.T. Knapp, and R.A. Wing. 1999. A soybean bacterial artificial chromosome library for PI 437654 and the identification of clones associated with cyst nematode resistance. Plant Molecular Biology 41:25-32.

Woo, S., Jiang, J., Gill, B., Paterson, A. and Wing, R. 1994. Construction and characterization of a bacterial artificial chromosome library of sorghum

bicolor. Nucleic Acids Res 22:4922-4931.

Zhang, H., Choi, S., Woo, S., Li, Z. and Wing, R. 1996. Construction and characterization of two rice bacterial artificial chromosome libraries from the parents of a permanent recombinant inbred mapping population. Mol Breeding 2:11-24.