Poster No. 463

Construction of Two BAC Libraries of the *Leymus cinereus* X *L. triticoides* Hybrid

Steve R. Larson [1], Chantel Scheuring [2], Bradley S. Bushman [1], Ivan W. Mott [1], N. Jerry Chatterton [1], Jianmin J. Dong [2], Yang Zhang [2], Xiaojun Zhang [2], Hong-Bin Zhang [2], and Richard R.-C. Wang [1*]

[1] USDA-ARS, FRRL, Logan, UT. [2] Texas A & M University, College Station, TX.

ABSTRACT

Leymus cinereus and *L. triticoides* are two wildrye grasses with many contrasting morphological and agronomic traits. The interspecific hybrid *Leymus cinereus* X *L. triticoides* and its progenies had been used to develop linkage maps of traits and molecular markers. To generate genomic DNA sequence information for these important wildrye species, we have developed two bacterial artificial chromosome (BAC) libraries with *Bam*HI or *Mbo*I from the nuclear DNA of the interspecific hybrid. The *Bam*HI library consists of 313,728 clones arrayed in 817 384-well microplates and has an average insert size of 155.0 kb. The *Mbo*I library contains 92,160 clones arrayed in 240 384-well microplates and has an average insert size of 135.4 kb. Therefore, the combined libraries consist of 405,888 clones arrayed in 1,057 384-well microplates and have an average insert size of 150.5 kb. Since the genome of the hybrid is estimated to be about 10,000 Mb in size, the combined libraries are equivalent to approximately 6.1 × haploid genomes. All of the clones of the libraries have been printed on nylon membranes and screened with several sets of Overgo probes to identify BAC clones containing candidate genes for traits of agronomic importance.

Richard Wang, (435) 797-3222, Richard.Wang@ars.usda.gov

INTRODUCTION

Perennial Triticeae grasses serve as important gene pools for forage and cereal crops (Dewey 1984). Efficient utilization of this vast gene pool in germplasm enhancement programs requires the development of genomic tools for identifying and isolating desirable functional genes or their molecular markers.

Bacterial artificial chromosome (BAC) library is an invaluable genomic resource for physical mapping (Zhang and Wu 2001; Wu et al. 2005), gene cloning (Faris et al. 2003; Zhang 2007), comparative mapping and genome evolution studies (Dubcovsky et al. 2001; Sorrells et al. 2003). It will also be useful to develop polymorphic markers for targeted genome regions (Cregan et al. 1999). BAC libraries have been constructed for sorghum (Woo et al. 1994; Lin et al. 1999), rice (Zhang et al. 1996; Nakamura et al. 1997; Yang et al. 1997; Tao et al. 2002), and sugarcane (Tomkins et al. 1999). In the tribe Triticeae, BAC libraries have been constructed for *Aegilops tauschii* (Moullet et al. 1999), *T. monococcum* (Lijavetzky et al. 1999), *Hordeum vulgare* (Yu et al. 2000), *T. aestivum* cv. 'Hartog' (Ma et al. 2000), 'Glenlea' (Nilmalgoda et al. 2003) and 'Chinese Spring' (Allouis et al. 2003; Shen et al. 2005), and *T. turgidum* (Cenci et al. 2003).

Although molecular maps have been developed for *Leymus* using full-sib backcross progenies from an interspecific hybrid (Wu et al. 2003; Hu et al., 2005; Larson et al., 2006; Larson and Mayland, 2007), genomic tools such as EST and BAC libraries were not available for perennial Triticeae. USDA-ARS Forage & Range Research Laboratory (FRRL) scientists undertook a major effort to develop these genomic resources by using two separate cooperative agreements with university collaborators. Here, we report results of one collaboration, i.e., the construction and utilization of BAC libraries from the *Leymus cinereus* X *L. triticoides* hybrid.

MATERIALS AND METHODS

Plants were transplanted from field to greenhouse and re-grown. When new shoots were regenerated, they were harvested and stored at –80°C before use. Megabase-size DNA was isolated from the frozen tissues according to Zhang et al. (1995) with minor modifications (Wu et al. 2004; Ren et al. 2005). The BAC vector pECBAC1 was used, and the BAC library construction and characterization followed the procedure that we developed previously (Zhang et al. 1996; Wu et al. 2004; Ren et al. 2005; He et al. 2007). To further verify their genome coverage and test their utility, the BAC libraries were printed robotically in a 4 x 4 format, with a duplicate for each clone and screened with the hybridization technology of Overgos designed from the candidate genes for the traits of agronomic importance.

RESULTS

BAC Library Construction

We constructed two BAC libraries for the *L. cinereus* x *L. triticoides* hybrid with two restriction enzymes, *Bam*HI and *Mbo*I, to minimize the bias of the library genome coverage that is often encountered for a BAC library constructed with a single enzyme. The *Bam*HI library consisted of 313,728 clones and was arrayed in 817 384-well microplates. A sample of 93 clones randomly selected from the BAC library were analyzed by pulsed-field gel electrophoresis and shown to have an average insert size of 155.0 kb (Figs. 1A and 2). The *Mbo*I library contained 92,160 clones and was arrayed in 240 384-well microplates. A sample of 64 clones randomly selected from the library were analyzed on pulsed-field gels and shown to have an average insert size of 135.4 kb (Figs. 1B and 2). Therefore, the combined libraries consist of 405,888 clones arrayed in 1,057 384-well microplates and have an average insert size of 150.5 kb. Since the genome of the hybrid is estimated to be about 10,000 Mb in size (inferred from data for allotetraploid *L. triticoides*; Vogel et al., 1999), the combined libraries are equivalent to approximately 6.1 × haploid genomes.

Library Screening

To further validate their genome coverage and test their utility, the BAC libraries were double-spotted onto nylon filters. We first screened 402,432 clones of the libraries equivalent to 6.0 x using a single Overgo pair designed from the *Lax* gene of rice (Komatsu et al. 2003). Eleven positive clones were obtained for this Overgo probe (Figure 3). Seven of these were confirmed and partially sequenced. They were also mapped to *Leymus* group 3 chromosomes, 3Ns and 3Xm, using the mapping population (Figure 4). Then, we screened 405,504 clones of the libraries equivalent to 6.1 x using a bulked pool of 24 Overgo pairs designed from 22 genes. A total of approximately 152 positive clones were obtained from the 24 overgos representing 22 genes, giving an average of 6.9 positive clones per gene probe. Although the positive clones of the 22 genes need to be hybridized with individual Overgo pair to determine the clones corresponding to each gene, this result and that obtained with the above single gene Overgo probe support the genome coverage of the libraries estimated by calculation based on insert size, number of clones and the genome size. These results have demonstrated the utility of the libraries for cloning and characterization of genes of agronomic importance in the forage crop.

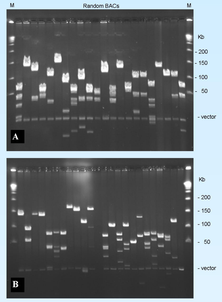

Figure 1 BACs randomly selected from the *Bam*HI (A) and *Mbo*I (B) libraries. BAC DNA was isolated, digested with *Not*I to release the *Leymus* DNA inserts from the BAC cloning vector, subjected to pulsed-field gel electrophoresis and photographed. M – lambda DNA ladder.

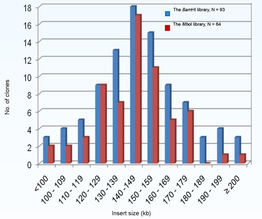

Figure 2 Insert size distribution of clones randomly selected from the *Leymus Bam*HI and *Mbo*I BAC libraries. BAC DNA was isolated, digested with *Not*I to release the *Leymus* DNA inserts from the cloning vector, fractionated on pulsed-field gels, photographed and estimated in insert size.

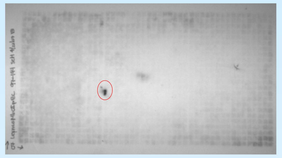

Figure 3 *Leymus* BAC library screening using Overgo primers for the *Lax* gene of rice as a probe. The rice *Lax* gene is orthologous to the maize barren stalk1 (*ba1*) gene (Gallovottie et al., 2004), and both genes are responsible for the initiation of lateral meristems. An orthologue of these rice *Lax* and maize *ba1* genes colocalizes with the *Leymus* LG3a and LG3b rhizome QTLs. The clone circled is an example of the positive BACs of the probe. Since the library was printed on the filters in duplicate, the positive clone should be double-signals.

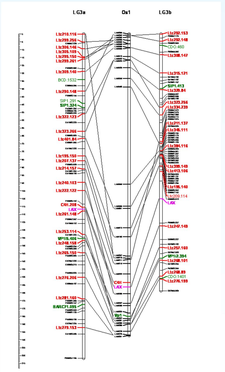

Figure 4 BACs containing the orthologous *Lax* rice gene were mapped on group 3 chromosomes of the Ns and Xm genomes in the allotetraploid *Leymus* hybrid that are homoeologous to rice chromosome 1.

CONCLUSIONS

- Molecular AFLP markers and QTLs had been mapped to 14 linkage groups using the *Leymus cinereus X L. triticoides* hybrid progenies.
- Now we have constructed two bacterial artificial chromosome (BAC) libraries from this hybrid, having an average insert size of 150.5 kb and covering 6.1 times of the genome.
- The usefulness of these BAC libraries was demonstrated with identification of BACs containing the targeted gene sequence and subsequent mapping of these BACs to *Leymus* linkage groups homoeologous to the rice chromosome that carries the gene, *Lax*.
- These *Leymus* BAC libraries will allow us to identify BACs containing genes of interest and then map them physically to chromosomes using fluorescence *in-situ* hybridization (FISH).
- Selected BACs can be sequenced to clone genes of agronomic importance.

References

Allouis S, et al. 2003. Cereal Res Commun 31:331-338.
Cenci A, et al. 2003. Theor Appl Genet 107: 931-999.
Dewey DR. 1984. In: *Gene manipulation in plant improvement,* Gustafson, J.P. (Ed.) Plenum, New York, pp. 209-279.
Dubcovsky J, et al. 2001. Plant Physiol 125:1342-1353.
Faris JD, et al. 2003. Genetics 164:311-321.
Gallovotti A, et al. 2004. Nature 432: 630-635.
He L, et al. 2007. In: *Aquaculture Genome Technologies*. Z. Liu (ed.). Blackwell Publishing, Ames, Iowa, USA, pp 215-244.
Hu Z.-M, et al. 2005. Euphytica 141: 263-280.
Komatsu K, et al. 2003. PNAS 100: 11765-11770.
Larson SR, Mayland HF. 2007. Mol Breeding 20:331-347.
Larson SR, et al. 2006. Crop Sci. 46: 2526-2539.
Lijavetzky D, et al. 1999. Genome 42:1176-1182.
Lin YR, et al. 1999. Mol Breed 5:511-520.
Ma Z, et al. 2000. Nucleic Acids Res 28(24):e106.
Moullet O, Zhang H-B, Lagudah ES. 1999. Theor Appl Genet 99:305-313.
Nakamura S, et al. 1997. Mol Gen Genet 254:611-620.
Nimalgoda SD, et al. 2003. Genome 46:870-878.
Ren C, et al. 2005. In: *The Handbook of Plant Genome Mapping: Genetic and Physical Mapping*. Meksem K and Kahl G (eds.). Wiley-VCH Verlag GmbH, Weinheim, Germany, pp 173-213.
Shen B, et al. 2005. Theor Appl Genet 111:1489-1494.
Sorrells ME, et al. 2003. Genome Res 13:1818-1827.
Tomkins JP, et al. 1999. Theor Appl Genet 99:419-424.
Tao Q, Wang A, Zhang H-B. 2002. Theor. Appl. Genet. 105:1058-1066.
Vogel,KP, Arumuganathan A, Jensen KB. 1999. Crop Sci. 39: 661-667.
Woo SS, et al. 1994. Nucleic Acids Res. 22:4922-4931.
Wu C, et al. 2005. In: *The Handbook of Plant Genome Mapping: Genetic and Physical Mapping.* Meksem K and Kahl G (eds.). Wiley-VCH Verlag GmbH, Weinheim, Germany, pp 257-284.
Wu C, Xu Z, Zhang H-B. 2004. In: *Encyclopedia of Molecular Cell Biology and Molecular Medicine*. Meyers RA (ed.). Vol. 3 (2nd Edition). Wiley-VCH Verlag GmbH, Weinheim, Germany, pp 385-425.
Wu X.-L, et al. 2003. Genome 46: 627-646.
Yang D, et al. 1997. Theor Appl Genet 95:1147-1154.
Yu Y, et al. 2000. Theor Appl Genet 101:1093-1099.
Zhang H-B. 2000. Construction and Manipulation of Large-insert Bacterial Clone Libraries – Manual. Texas A&M University, Texas, USA.
Zhang H-B. 2007. In: *Principles and Practices of Plant Genomics, Vol.: Genome Mapping*. C. Kole and AG Abbott (eds.). Science Publishers, New Hampshire, USA. pp. 229-267.
Zhang, H-B, Woo SS, Wing RA. 1996. *In: Plant Gene Isolation: Principles and Practice.* G. Foster and D. Twell (eds.). John Wiley & Sons, Ltd. England. pp. 75-99.
Zhang, H-B, Wu CC. 2001. Plant Physiology and Biochemistry 39:195-209.
Zhang, H-B, et al. 1995. Plant J. 7:175-184.