Theor Appl Genet (2000) 101:1093–1099

Y. Yu · J.P. Tomkins · R. Waugh · D.A. Frisch
D. Kudrna · A. Kleinhofs · R.S. Brueggeman
G.J. Muehlbauer · R.P. Wise · R.A. Wing

A bacterial artificial chromosome library for barley (*Hordeum vulgare* L.) and the identification of clones containing putative resistance genes

Received: 20 September 1999 / Accepted: 25 March 2000

Abstract Modern cultivated barley is an important cereal crop with an estimated genome size of 5000 Mb. To develop the resources for positional cloning and structural genomic analyses in barley, we constructed a bacterial artificial chromosome (BAC) library for the cultivar Morex using the cloning enzyme *Hin*dIII. The library contains 313344 clones (816 384-well plates). A random sampling of 504 clones indicated an average insert size of 106 kbp (range=30–195 kbp) and 3.4% empty vectors. Screening the colony filters for chloroplast DNA content indicated an exceptionally low 1.5% contamination with chloroplast DNA. Thus, the library provides 6.3 haploid genome equivalents allowing a >99% probability of recovering any specific sequence of interest. High-density filters were gridded robotically using a Genetix Q-BOT in a 4×4 double-spotted array on 22.5-cm^2 filters. Each set of 17 filters allows the entire library to be screened with 18432 clones represented per filter. Screening the library with 40 single copy probes identified an average 6.4 clones per probe, with a range of 1–13 clones per probe. A set of resistance-gene analog (RGA) sequences identified 121 RGA-containing BAC clones representing 20 different regions of the genome with an average of 6.1 clones per locus. Additional screening of the library with a P-loop disease resistance primer probe identified 459 positive BAC clones. These data indicate that this library is a valuable resource for structural genomic applications in barley.

Key words Barley · BAC library · P-loop genes · Resistance-gene analog (RGA)

Communicated by M.A. SaghaiMaroof

Y. Yu, J.P. Tomkins (✉), D.A. Frisch, R.A. Wing (✉)
Clemson University Genomics Institute,
BAC/EST Resource Center, Room 100 Jordan Hall, Clemson, SC 29634, USA
e-mail: jtmkns@clemson.edu
e-mail: rwing@clemson.edu

D. Kudrna · A. Kleinhofs · R.S. Brueggeman
Department of Crop and Soil Sciences,
Washington State University, Pullman, WA 99164, USA

R. Waugh
Scottish Crop Research Institute, Invergowrie, Dundee, DD2 2DA, UK

G.J. Muehlbauer
Department of Agronomy and Plant Genetics,
University of Minnesota, St. Paul, MN 55108, USA

R.P. Wise
USDA-ARS, Corn Insects and Crop Genetics Research Unit,
Department of Plant Pathology, Iowa Stae University, Ames, IA 50011-1020, USA

Introduction

Modern cultivated barley (*Hordeum vulgare* L.) is a diploid crop species having an estimated haploid genome size of 5000 Mb (Arumuganthan and Earle 1991). An extensive history of genetic, mutagenic, and cytogenetic studies has resulted in a large store of mutants, cytogenetic stocks, and knowledge about the genes that control barley functions, such as malting quality, yield, stress response and disease resistance. Molecular-marker linkage maps have been developed using RFLP, AFLP, SSR, isozyme and protein markers (GRAINGENES database; Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993; Becker et al. 1995; Ellis et al. 1997; Kleinhofs 1997; Waugh et al. 1997). All barley maps appear to be co-linear and can easily be integrated with only minor differences in genetic distances between markers. In addition, a barley morphological-marker linkage map containing over 200 loci has been constructed (Franckowiak 1997) and is being integrated with the molecular-marker maps (Kleinhofs 1996; Castiglioni et al. 1998). Moreover, the molecular-marker linkage maps have been useful for identifying quantitative trait loci (QTLs) for many agronomic and quality traits (Hayes et al. 1993). While many important traits have been mapped in barley, physical mapping, structural analysis and map-based cloning has been limited due to the lack of a complete large-insert genomic library that would be available to the public barley research community.

An essential tool for characterizing genomes is the availability of yeast artificial chromosome (YAC) or bacterial artificial chromosome (BAC) libraries containing large genomic DNA inserts. Plant YAC libraries have been constructed for several organisms including: Arabidopsis (Grill and Somerville 1991), tomato (Martin et al. 1992), maize (Edwards et al. 1992) and barley (Kleine et al. 1993). These libraries have been used for a number of studies but their general use has been limited by the high frequency of chimeric and unstable clones. In contrast, BAC vectors from the mini-F plasmid allows cloning and stable-maintenance of large DNA fragments in *Escherichia coli* (Shizuya et al. 1992). BAC libraries are popular for a number of reasons, including: ease of handling, relatively simple to develop and exhibiting a low frequency of chimeric clones. BAC libraries have been developed for many plant species including: *Arabidopsis*, rice, soybean, sorghum, and sugarcane (Woo et al. 1994; Choi et al., 1995; Wang et al. 1995; Zhang et al. 1996; Marek and Shoemaker 1997; Danesh et al. 1998; Mozo et al. 1998; Tomkins et al. 1999a, b). Lapitan et al. (1997) reported the development of a small BAC library for barley containing 10750 clones with an average insert size of 95 kb that provides less than one genome equivalent. For successful large-scale genomic applications in barley, a larger library providing at least five genome equivalents is needed.

Plant BAC libraries have been used for a number of structural genomic applications. In *Arabidopsis*, BAC libraries have and are being used to develop sequence-ready physical maps (Bevan et al. 1998). In addition, BAC libraries have been used to positionally clone disease resistance genes and other genes known only by their phenotype (cf. Song et al. 1995). Moreover, BAC libraries are useful for examining genomic structure. The genomic structure of the *a1-sh2* region of maize, sorghum and rice has been extensively examined through the sequencing of BAC clones containing these genes (Chen et al. 1997, 1998; Tikhonov et al. 1999). BAC libraries have also been used to develop physical maps for genomic regions containing resistance gene-analog sequences (Marek and Shoemaker 1997).

Of the many uses of BAC libraries, one of our interests is to develop the the resources for the positional cloning of genes and in particular for cloning disease resistance (R) loci from barley. More than a dozen R genes have been isolated from plants and the general nature of these sequences is now apparent. Interestingly, R genes from diverse species and genes that confer resistance to different pathogens have similar protein domains. R genes have been grouped into five general classes based on the conserved protein domains they encode (Hammond-Kosack and Jones 1997). In general, these protein domains consist of a nucleotide-binding site (NBS) and leucine-rich repeats (LRR). Using sequence similarities from the NBS and LRR regions from the R genes, candidate disease-resistance genes from a large set of plants have been isolated and referred to as resistance gene analogs (RGA; Kanazin et al. 1996; Leister et al. 1996; Yu et al. 1996; Aarts et al. 1998; Leister et al. 1998; Ohmori et al. 1998; Seah et al. 1998; Shen et al. 1998; Mago et al. 1999). Mapping studies with these RGA sequences in soybean, lettuce, *Arabidopsis*, rice and barley indicate that they are linked to R genes and tend to cluster at multiple locations in the genome (Kanazin et al. 1996; Yu et al. 1996; Botella et al. 1997; Aarts et al. 1998; Leister et al. 1998; Meyers et al. 1998a, b; Shen et al. 1998). Therefore, identifying RGA-containing BAC clones is a good starting point for positional cloning of R gene-loci.

The primary objective of the present study was to establish the resources and protocols necessary to conduct structural genomics in barley. The specific objectives were: (1) develop a bacterial artificial chromosome (BAC) library providing at least six haploid-genome equivalents, (b) characterize the library for insert size and chloroplast DNA content, (3) screen the library with genetically mapped RFLP markers, and (4) identify RGA-containing BAC clones.

Materials and methods

BAC library construction

BAC library construction was essentially the same as that described by Tomkins et al. (1999a) with the following modifications. The first size-selection of *Hin*dIII fragments used switch times of either a constant 20-s interval or a 1–40 s linear ramp. Fractions between 100 and 300 kbp were cut from the gel and inserted into a second gel and run at a constant 3-s switch time to remove small trapped DNA fragments. After removing appropriate fractions from the second size selection, DNA was either removed from the agarose by Gelase (Epicentre) or by electroelution (Model 422 Electro-Eluter, Bio-Rad). Transformed cells were plated on 200 ml of selective medium (LB, Luria-Bertani medium) in 24×24 cm plates (Genetix) with 12.5 µg/ml of chloramphenicol, 0.55 mM of IPTG, and 80 µg/ml of 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 a stronger color-development of non-recombinant colonies. Plates were either stored at 4°C or used immediately for picking. Recombinant white colonies were picked robotically using the Genetix Q-BOT apparatus and arrayed as individual clones in 384-well microtiter plates (Genetix) containing 50 µl of freezing broth (Woo et al. 1994). After incubation overnight, microtiter plates were stored at –80°C. Two copies of the library were made using the replicating function of Genetix Q-BOT and stored in separate –80°C freezers. BAC clone characterization has been described previously by Tomkins et al. (1999a, b).

BAC library screening and BAC fingerprinting

High-density colony filters for hybridization-based screening of the library were prepared using Genetix Q-BOT. Clones were gridded in double spots using a 4×4 array with six fields per 22.5×22.5-cm nylon (Hybond NT) filter. This gridding pattern allows 18432 clones to be represented per filter. Library screening was performed using 17 filters (labeled A–Q) such that one filter set represented 313344 clones. Colony filters were processed and hybridized using standard techniques (Sambrook et al. 1989). Screening for chloroplast DNA content was performed as described by Woo et al. (1994).

Resistance-gene analog probes

Probes were developed for the barley nucleotide binding site-leucine rich repeat (NBS-LRR) resistance-gene analog sequences b2, b3, b4, b5, b6, b7, b8 and b9 (Leister et al. 1998) using as a template a spike-derived RNA from the barley cv Chevron. In short, RNA was isolated from Chevron spikes at anthesis following the TRIzol reagent (GIBCO-BRL) RNA-isolation procedure. RT-PCR was conducted according to Bauer et al. (1994). cDNA synthesis was performed on the RNA sample using RT (GIBCO-BRL) and a poly (dT) primer. cDNA was PCR-amplified with the following b2–b9-specific primers: b2–5′ primer, 5′-TGTGGATCTGTGTATCCA-3′; b2–3′ primer, 5′-CTAGGATGGTGCTCACAGTTG-3′; b3–5′ primer, 5′-GACAAACTTCAATATTTCTG-3′; b3–3′primer, 5′-TGCATTCCTTATCTTAAACC-3′; b4–5′ primer, 5′-GAAGCTCAATAGGCTTGT-3′; b4–3′ primer, 5′-CAGGGTCCTCGTTCCATAC-3′; b5–5′ primer, 5′-TGCTCGGCTGACAACTTCTG-3′; b5–3′ primer, 5′-GCTAGTATCATGCAACCAC-3′; b6–5′ primer, 5′-CTTCCCTAATGCTCATGCT-3′; b6–3′ primer, 5′-GCCACGACACCGCTCAACTACAG-3′; b7–5′ primer, 5′-CAAGGGAGGTCTACCGCAAGA-3′; b7–3′ primer, 5′-GGCAGCAGTCCTCAGAACCAA-3′; b8–5′ primer, 5′-GGAAGGTGCATAGCCTCATT-3′; b8–3′ primer, 5′-TTCATCCCTTCCGAGTTAGG-3′; b9–5′ primer, 5′-GGTGTCCAGCAACATTTCCAG-3′; and b9–3′ primer, 5′-CTGCTTGATTCTGCTCAACATC-3′. The resulting PCR products were subcloned into a plasmid and verified by sequencing the insert. The lengths of the b2–b9 probes were 391, 220, 472, 461, 463, 398, 794 and 416 bp, respectively. All b-RGA probes were at least 90% identical at the nucleotide level to the previously published b-RGA sequences (Leister et al. 1998) except for b3 which was 82% identical. The b8 probe contains a 328-bp intervening sequence that is most likely an intron, otherwise the b8 sequence is 98% identical to the previously published sequence. The b2 and b5 probes were 82% identical to each other. We obtained PCR-amplified products of genomic DNA for the barley homologs of the rice *Xa21* (Song et al. 1995) and wheat *Lrk* probes (Feuillet et al. 1997) from V. Kanazin and T. Blake (Montana State University). The primer sequences used to amplify the barley Xa21 probe were: 5′-AGTTTTGAGGC(TCGA)GA(GA)-TGTGA-3′ and 5′-ATCCTTG(AC)(TC)ATGCCAAA(AG)TC-3′. The primer sequences used to amplify the barley Lrk probe were: 5′-GGAGGATTTGGAA(CG)TGT(CGA)TA-3′ and 5′-A(TC)AG-CTCCGGTGCAATGTA-3′. Sequencing of the barley Xa21 and Lrk PCR products demonstrated that they were similar to the rice *Xa21* and wheat *Lrk* sequences. The barley P-loop primer probe was synthesized from the sequence of the nucleotide-binding site of a b9-like sequence. The P-loop primer was a 20-mer with the following sequence 5′ GTSSGGRAAGACRACYCTTG 3′.

Results and discussion

BAC library construction and characterization

We constructed a barley BAC library using the cultivar Morex which is suitable for physical mapping and map-based cloning. The library consists of 313344 clones stored in 816 384-well microtiter plates. Less than 4% of the clones do not contain inserts as judged by random analysis of BACs sampled from the library. A random sampling of 504 BACs taken from the library during the course of library construction indicated an average insert size of 106 kbp with a range of 30 to 195 kbp. Fig. 1A shows 24 randomly selected clones digested with *Not*I to release the insert. The two *Not*I sites in pBeloBAC11 flank the multi-cloning site. Because *Not*I recognizes an 8-bp GC sequence and the barley genome is moderately GC rich, digestion typically generates vector plus 1–4 insert bands per BAC clone. Figure 1B shows a Southern blot of the gel in Fig. 1A probed with total barley genomic DNA. As indicated by the strongly hybridizing lanes, many of the BACs contain highly repetitive DNA. However, 42% of the BAC insert bands contain low-copy DNA as indicated by bands not hybridizing or else weakly hybridizing on the Southern blot.

To determine the size distribution of BAC clones in the library, the 504 BACs analyzed with *Not*I digests were grouped by insert size and the insert size of each clone was plotted against the frequency of each group of clones represented in the library (Fig. 2). Based on this analysis, over 80% of the clones in the library have an average insert size greater than 100 kbp. Of the clones larger than 100 kbp, 40% are between 100 and 130 kbp and 40% are greater than 130 kbp. Based on the average insert size and a haploid genome size of 5000 Mb (Arumuganathan and Earle 1991) the coverage of the library is about 6.3 genome equivalents, resulting in a greater than 99% probability of recovering any specific sequence of interest.

To obtain an estimate of the representation of chloroplast DNA in the library, the filters were screened with three different chloroplast genes spaced equidistantly around the 133-kbp barley chloroplast genome. Results from this screening showed that approximately 1.5% of library sequences are chloroplast DNA (data not shown).

BAC library screening with single-copy and resistance gene analog (RGA)-containing probes

Screening the library with 40 single-copy probes identified 256 confirmed BAC clones for an average of 6.4 and a range of 1–13 clones per probe. The probes were judged to be single copy because they hybridized to a single band with genomic barley DNA, were previously mapped in various barley mapping populations to a single locus, and hybridized to a single identical size *Hin*dIII fragment from all positive BACs. All probes tested to-date yielded at least one BAC clone indicating a robust library.

In order to evaluate the potential of the BAC library to identify genomic regions associated with disease resistance, library screening was performed with an array of disease resistance gene analog (RGA) probes. Probes were developed for b2, b3, b4, b5, b6, b7, b8 and b9 (Leister et al. 1998; see Materials and methods). We also received probes for the barley homologs of the rice *Xa21* and wheat *Lrk* probes from V. Kanazin and T. Blake (Montana State University; see Materials and methods). We screened the BAC library with each probe and verified the number of positive BAC clones identified by each probe using DNA gel blots on *Hin*dIII-digested BAC DNA and probing with a specific RGA probe. We identified 16, 5, 9, 16, 8, 10, 20, 12, 31 and 10 BACs that hybridized to the b2, b3, b4, b5, b6, b7, b8, b9, *Xa21* and *Lrk* probes, respectively (Table 1). The b2 and b5 probes were 82% identical at the nucleotide level and

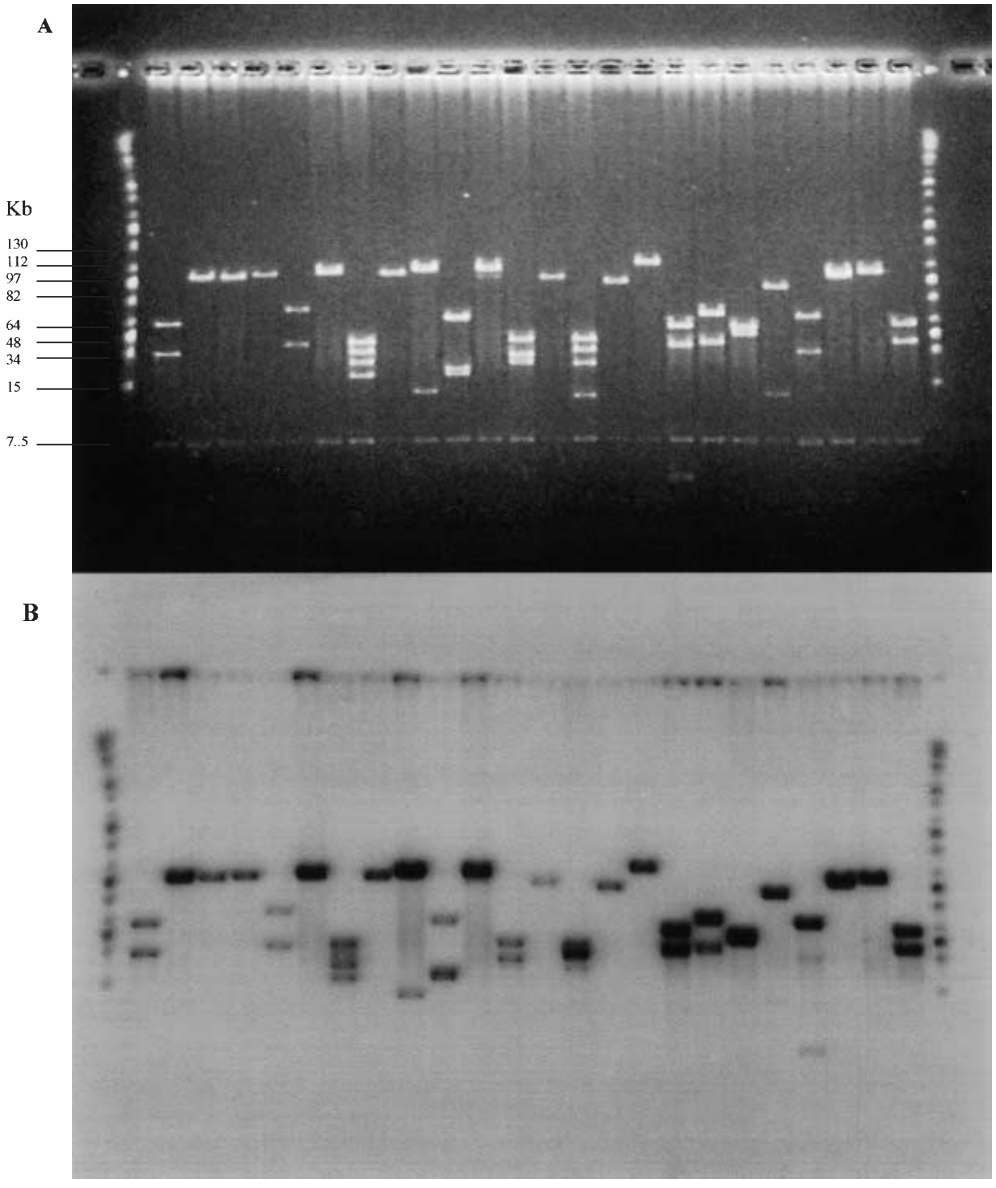

Fig. 1 Analysis of 24 randomly selected barley BAC clones. **A** Ethidium bromide-stained CHEF gel (5–15 s switch time, 14 h) showing insert DNA above and below the common 7.5-kbp pBeloBAC11 vector band. **B** Autoradiograph of gel in **A** after Southern transfer and probing with total barley genomic DNA. Molecular-weight marker in outside lanes is Midrange I (NEB)

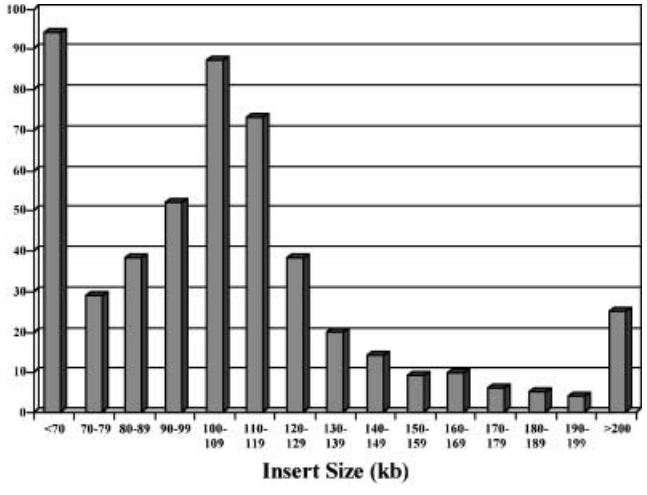

Fig. 2 Insert size distribution of clones in the barley BAC library. To estimate the insert size range, BAC DNA from 504 randomly selected clones was analyzed, as shown in Fig. 1a

identified the same set of 16 clones. The b2 and b5 probes were the only RGA sequences that identified overlapping BAC clones. Taken together, these data indicate that we identified 121 RGA-containing BACs.

We estimated the number of different genomic regions identified by the BAC clones based on our DNA gel-blot analysis. BAC clones exhibiting the same-sized RGA hybridizing *Hin*dIII fragment(s), or combinations of the same fragments, were considered clones covering the same RGA-containing region of the genome. BAC clones that gave different-sized fragments were considered clones covering different RGA-containing regions of the genome. We identified 2, 1, 2, 2, 2, 1, 2, 1, 4, and 5 regions for the b2, b3, b4, b5, b6, b7, b8, b9, *Xa21* and *Lrk* probes, respectively. Added together, these BAC clones account for 20 regions of the barley genome. These data indicate that the barley BAC library is a useful tool for identifying regions of the genome that contain RGA sequences.

Table 1 Number of BACs and genomic regions identified by each RGA probe

Probe	Genomic region	Number of hybridizing BAC clones	Size (kb) of *Hin*dIII-digested fragment(s)
b2	I	3	8
b2	II	13	12, 9, 7.5, 7, 6, 5
b3	I	5	9, 7, 5.5
b4	I	6	6.5
b4	II	3	3, 1
b5	I	3	8
b5	II	13	12, 9, 7.5, 7, 6, 5
b6	I	4	13, 9
b6	II	4	12
b7	I	10	9, 3.2, 1
b8	I	19	13
b8	II	1	5
b9	I	12	4.5, 4
Xa21	I	18	13, 12
Xa21	II	4	4
Xa21	III	4	3
Xa21	IV	5	2.5, 2
Lrk	I	6	13
Lrk	II	1	11, 2.5
Lrk	III	1	10
Lrk	IV	1	7, 4
Lrk	V	1	1.3

Screening with the P-loop probe derived from a b9-like RGA sequence resulted in 459 positive signals. When the BAC clones identified by the P-loop probe were characterized for insert size, the average was the same as that obtained by screening random BAC clones as described previously. In addition to identifying potential R gene-containing regions of the genome, these data helped to further verify that the average insert size of the library was 106 kbp.

Applications of the barley BAC library

There are many uses for the barley BAC library including: physical mapping, positional cloning, genomic structural analysis and comparisons of specific regions in barley to other grasses. Three loci conferring resistance to powdery mildew, have been cloned from barley via a map-based approach. The *Mlo* and *Rar1* genes were cloned via high-resolution AFLP analysis and subsequent chromosome landing utilizing a YAC library from the cultivar Ingrid (Büschges et al. 1997; Simons et al. 1997, Lahaye et al. 1998; Shirasu et al. 1999). The *Mla* resistance cluster was isolated via a combined chromosome-walking strategy using YAC libraries from the cultivar Franka (Kleine et al. 1993, 1997) and the presently described Morex BAC library (Wei et al. 1999). Hence, development of this comprehensive barley BAC library should facilitate the positional cloning of many agronomically important genes.

BAC libraries are also valuable tools to characterize specific regions of genomes that contain R genes. Many R genes and RGA sequences have been genetically characterized in plants. Genetic and molecular analysis of these sequences indicates that they are frequently located in clusters in the genome (Kunkel 1996; Botella et al. 1997; Holub 1997; Meyers et al. 1998a; Michelmore and Meyers 1998; Wei et al. 1999). In barley, RGA sequences are tightly linked to known R genes (Leister et al. 1998). Therefore, identifying RGA-containing BAC clones is a useful first step for characterizing the regions of the barley genome that contain R genes and for the eventual isolation of R genes.

Another useful application of the barley BAC library is for physical mapping of gene-rich regions of barley. There is substantial evidence that the genes in cereal genomes are not distributed evenly throughout the genome, but rather are found in gene-rich regions (Gill et al. 1993; 1996; Civardi et al. 1994; DeScenzo and Wise 1996; Buschges et al. 1997). Examining gene distribution in three Gramineae (barley, maize, and rice) revealed a similar gene distribution in these genomes (Carels et al., 1995; Barakat et al. 1997). Gene space, as these authors refer to as gene-rich regions, occupies 12%, 17% and 24% of the genomes of barley, maize and rice, respectively. Gene-distribution studies at the nucleotide level have revealed similar patterns to those described above. A contiguous 60-kb stretch of barley chromosome 4HL was sequenced and shown to contain three genes (Panstruga et al. 1998). This number of genes in a 60-kb stretch is 6- to 10-fold higher than expected from random gene distribution in the barley genome. Sequencing the *Lrk* loci of barley, wheat and rice revealed one gene per 4–5 kb (Feuillet and Keller 1999). Additionally, sequencing of a 160-kb BAC spanning the barley *Mla* resistance-gene cluster on chromosome 5S (1HS) revealed two major gene islands bridged by nested retrotransposons (Wei and Wise, unpublished data). Although these studies are limited, they are consistent with previous work showing that genes are distributed unevenly across the genome into gene-rich and gene-poor regions. The availability of the barley BAC library will be an important tool for developing physical maps of the gene-rich regions. Because the barley genome is syntenous with other grass genomes, physical maps of the gene-rich regions of the barley genome will be extremely useful for examining microsynteny of particular regions across grass species. In addition, physical maps will be a starting point for positionally cloning genes from any member of the Triticeae.

Acknowledgements Appreciation for technical assistance during the process of library replication and filter production is extended to Michael Atkins, John Bishop, and Jose Luis Goicoechea. We also acknowledge the technical assistance of S. Heinen and D. Martin. This research was funded by grants from the North American Barley Genome Mapping Project, Anheuser Busch Corporation, International Triticeae Mapping Initiative, Japanese Brewers Association, Institut fur Pflanzengenetik und Kulturpflanzenforschung (IPK, Gatersleben), RIS, USDA-ARS Plant Genome Program #93–37300–8768 to R. Wise, NSF Plant Genome Program #98726676 to R. Wing and D. Frisch and by the Coker Endowment at Clemson University.

References

Aarts MG, te Lintel Hekkert B, Holub EB, Beynon JL, Stiekema WJ, Peireira A (1998) Identification of R-gene homologous DNA fragments genetically linked to disease resistance loci in *Arabidopsis thaliana*. Mol Plant Microbe Interact 11:251–258

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Barakat A, Carels N, Bernardi G (1997) The distribution of genes in the genomes of Gramineae. Proc Natl Acad Sci USA 94:6857–6861

Bauer P, Crespi MD, Szecsi J, Allison LA, Schultze M, Ratet P, Kondorosi E, Kondorosi A (1994) Alfalfa *Enod12* genes are differentially regulated during nodule development by Nod factors and *Rhizobium* invasion. Plant Physiol 105:585–592

Becker J, Vos P, Kuiper M, Salamini F, Heun M (1995) Combined mapping of AFLP and RFLP markers in barley. Mol Gen Genet 249:65–73

Bevan M, Bancroft I, Bent E, Love K, Goodman H, Dean C, Bergkamp R, Dirkse W, Van Staveren M, Stiekema W, Drost L, Ridley P, Hudson S-A, Patel K, Murphy G, Piffanelli P, Wedler H, Wedler E, Wambutt R, Weitzenegger T, Pohl T M, Terryn N, Gielen J, Villarroel R, De Clerck R, Van Montagu M, Lecharny A, Auburg S, Gy I, Kreis M, Lao N, Kavanagh T, Hempel S, Kotter P, Entian K-D, Rieger M, Schaeffer M, Funk B, Mueller-Auer S, Silvey M, James R, Montfort, Pons A, Puigdomenech P, Douka A, Voukelatou E, Milioni D, Hatzopoulos P, Piravandi E, Obermaier B, Hilbert H, Dusterhoft A, Moores T, Jones JDG, Eneva T, Palme K, Benes V, Rechman S, Ansorge W, Cooke R, Berger C, Delseny M, Voet M, Volckaert G, Mewes H-W, Klosterman S, Schueller C, Chalwatzis N (1998) Analysis of 1.9 Mb of contiguous sequence from chromosome 4 of *Arabidopsis thaliana*. Nature 391:485–488

Botella MA, Coleman MJ, Hughes DE, Nishimura MT, Jones JDG, Somerville SC (1997) Map positions of 47 *Arabidopsis* sequences with sequence similarity to disease resistance genes. Plant J 12:1197–1211

Buschges R, Hollricher K, Panstruga R, Simons G, Wolter M, Frijters A, van Daelon R, van der Lee T, Diergaarde P, Groenendijk J, Topsch S, Vos P, Salamini F, Schulze-Lefert P (1997) The barley Mlo gene: a novel control element of plant pathogen resistance. Cell 88:695–705

Carels N, Barakat A, Bernardi G (1995) The gene distribution of the maize genome. Proc Natl Acad Sci USA 92:11057–11060

Castiglioni P, Pozzi C, Heun M, Terzi V, Muller KJ, Rohde W, Salamini F (1998) An AFLP-based procedure for the efficient mapping of mutations and DNA probes in barley. Genetics 149:2030–2036

Chen M, SanMiguel P, De Oliviera AC, Woo S-S, Zhang H, Wing RA, Bennetzen JL (1997) Microcolinearity in *sh2*-homologous regions of the maize, rice and sorghum genomes. Proc Natl Acad Sci USA 94:3431–3435

Chen M, SanMiguel P, Bennetzen JL (1998) Sequence organization and conservation in *sh2*/*a1*-homologous regions of sorghum and rice. Genetics 148:435–443

Choi S, Creelman R, Mullet JE, Wing RA (1995) Construction and characterization of a bacterial artificial chromosome library of *Arabidopsis thaliana*. Plant Mol Biol Rep 13:124–128

Civardi L, Xia Y, Edwards KJ, Schnable PS, Nikolau, BJ (1994) The relationship between genetic and physical distances in the cloned *a1-sh2* interval of the *Zea mays* L. genome. Proc Natl Acad Sci USA 91:8268–8272

Danesh D, Penuela S, Mudge J, Denny R., Nordstrom H., Martinez J, 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

DeScenzo RA, Wise RP (1996) Variation in the ratio of physical to genetic distance in intervals adjacent to the *Mla* locus on barley chromosome 1H. Mol Gen Genet 251:472–482

Edwards KJ, Thompson H, Edwards D, de Saizieu A, Sparks C, Thompson JA, Greenland AJ, Eyers M, Schuch W (1992) Construction and characterization of a yeast artificial chromosome library containing three haploid maize genome equivalents. Plant Mol Biol 19:299–308

Ellis RP, Forster BP, Waugh R, Bonar N (1997) Mapping physiological traits in barley. New Phytol 137:149

Feuillet C, Keller B (1999) High gene density is conserved at syntenic loci of small and large grass genomes. Proc Natl Acad Sci USA 96:8265–8270

Feuillet C, Schachermayr G, Keller B (1997) Molecular cloning of a new receptor-like kinase gene encoded at the Lr10 disease resistance locus of wheat. Plant J 11:45–52

Franckowiak J (1997) Revised linkage maps for morphological markers in barley *Hordeum vulgare*. Barley Genet Newslett 26:9–21

Gill KS, Gill BS, Endo TR (1993) A chromosome region-specific mapping strategy reveals gene-rich telomeric ends in wheat. Chromosoma 102:374–381

Gill KS, Gill BS, Endo TR, Boyko EV (1996) Identification and high-density mapping of gene-rich regions in chromosome group 5 of wheat. Genetics 143:1001–1012

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83:250–256

Grill E, Somerville C (1991) Construction and characterization of a yeast artificial chromosome library of Arabidopsis which is suitable for chromosome walking. Theor Appl Genet 226: 484–490

Hayes PM, Liu BH, Knapp SJ, Chen F, Jones B, Blake T, Franckowiak J, Rasmusson D, Sorrells M, Ullrich SE, Wesenberg D, Kleinhofs A, (1993) Quantitative trait locus effects and environmental interactions in a sample of North American barley germplasm. Theor Appl Genet 87:392–401

Hammond-Kosack KE, Jones JDG (1997) Plant disease resistance genes. Plant Mol Biol 48:575–607

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*) Genome 34:437–447

Holub EB (1997) Organization of resistance genes in *Arabidopsis*. In: Crute IR, Burdon JJ, Holub EB (eds) The gene-for-gene relationship in host-parasite interactions, CAB International Press, Wallingford, UK

Kanazin V, Marek LF, Shoemaker RC (1996) Resistance gene analogs are conserved and clustered in soybean. Proc Natl Acad Sci USA 93:11746–11750

Kleine M, Michalek W, Graner A, Herrmann RG, Jung C (1993) Construction of a barley (*Hordeum vulgare* L.) YAC library and isolation of a Hor1-specific clone. Mol Gen Genet 240:265–272

Kleine M, Michalek W, Diefenthal H, Dargatz H, Jung C (1997) Construction of a *Mlu*I-YAC library from barley (*Hordeum vulgare*. L.) and analysis of YAC insert terminal regions. Genome 40**:**896–902

Kleinhofs A (1996) Integrating barley RFLP and classical marker maps. Barley Genet Newslett 27:105–112

Kleinhofs A (1997) Online data set for the Steptoe×Morex barley mapping population. Files available via Internet Gopher, host greengenes.cit.cornell.edu, port70, menu “Grains files to browse”/“Barley Steptoe×Morex map”

Kleinhofs A, Kilian A, Maroof MAS, Biyashev RM, Hayes P, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, Skadsen R, Steffenson BJ (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor Appl Genet 86:705–712

Kunkel BN (1996) A useful weed put to work: genetic analysis of disease resistance in Arabidopsis thaliana. Trends Genet 12:63–69

Lahaye T, Shirasu K, Schulze-Lefert P (1998) Chromosome landing at the barley *Rar1* locus. Mol Gen Genet 260:92–101

Lapitan NLV, Brown SE, Kennard W, Stephens JL, Knudson DL (1997) FISH physical mapping with barley BAC clones. Plant J 11:149–156

Leister D, Ballrova A, Salamini, F, Gebhardt C (1996) A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nature Genet 14:421–429

Leister D, Kurth J, Laurie DA, Yano M, Sasaki T, Devos K, Graner A, Schulze-Lefert P (1998) Rapid reorganization of resistance gene homologues in cereal genomes. Proc Natl Acad Sci USA 95:370–375

Mago R, Nair S, Mohan M (1999) Resistance gene analogs from rice: cloning, sequencing and mapping. Theor Appl Genet 99:50–57

Marek LF, Shoemaker RC (1997) BAC contig development by fingerprint analysis in soybean. Genome 40:420–427

Martin GB, Ganal MW, Tanksley SD (1992) Construction of a yeast artificial chromosome library of tomato and identification of cloned segments linked to two disease resistance loci. Mol Gen Genet 233:25–32

Meyers BC, Chin DB, Shen KA, Sivaramakrishnan S, Lavelle DO, Zhang Z, Michelmore R (1998a) The major resistance gene cluster in lettuce is highly duplicated and spans several megabases. Plant Cell 10:1817–1832

Meyers BC, Shen KA, Rohani P, Gaut BS, Michelmore R (1998b) Receptor-like genes in the major resistance locus of lettuce are subject to divergent selection. Plant Cell 11:1833–1846

Michelmore RW, Meyers BC (1998) Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res 8:1113–1130

Mozo T, Fischer S, Shizuya H, Altmann T (1998) Construction and characterization of the IGF *Arabidopsis* BAC library. Mol Gen Genet 258:562–570

Ohmori T, Murata M, Motoyoshi F (1998) Characterization of disease resistance gene-like sequences in near-isogenic lines in tomato. Theor Appl Genet 96:331–338

Panstruga R, Buschges R, Piffanelli P, Schulze-Lefert P (1998) A contiguous 60 kb genomic stretch from barley reveals molecular evidence for gene islands in a monocot genome. Nucleic Acids Res 26:1056–1062

Sambrook J, Frisch E, Maniatus T (1989). Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Seah S, Sivasithamparam K, Karakousis A, Lagudah ES (1998) Cloning and characterization of a family of disease resistance gene analogs from wheat and barley. Theor Appl Genet 97:937–945

Shen KA, Meyers BC, Islam-Faridi MN, Chin DB, Stelly DM, Michelmore RW (1998) Resistance gene candidates identified by PCR with degenerate primers map to clusters of resistance genes in lettuce. Mol Plant-Microbe Interact. 11:815–823

Shirasu K, Lahaye T, Tan M, Zhou F, Azevedo C, Schulze-Lefert (1999) A novel class of eukaryotic zinc-binding proteins is required for disease resistance signaling in barley and development in *C. elegans*. Cell 99:355–366

Shiyuza H, Birren B, Kim UJ, Manicino V, Slepak T, Tashiiri Y, Simon M (1992) Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in *Escherichi coli* using an F-factor based vector. Proc Natl Acad Sci USA 89:8794–8797

Simons G, van der Lee T, Diergaarde P, van Daelen R, Groenendijk J, Frijters A, Büschges R, Hollricher K, Töpsch S, Schulze-Lefert P, Salamini F, Zabeau M, Vos P (1997) AFLP-based fine mapping of the *Mlo* gene to a 30-kb DNA segment of the barley genome. Genomics 44:61–70

Song W-Y, Wang G-L, Chen L-L, Kim H-S, Pi L-Y, Holsten T, Gardner J, Wang B, Zhai W-X, Zhu L-H, Fauquet C, Ronald P (1995) A receptor kinase protein encoded by the rice disease resistance gene *Xa21*. Science 270:1804–1806

Tikhonov AP, SanMiguel PJ, Nakajima Y, Gorenstein NM, Bennetzen JL, Avramova Z (1999) Colinearity and its exceptions in orthologous *adh* regions of maize and sorghum. Proc Natl Acad Sci USA 96:7409–7414

Tomkins JP, Mahalingham R, Miller-Smith H, Goicoechea JL, Knapp HT, Wing RA (1999a) A soybean bacterial artificial chromosome library for PI 437654 and the identification of clones associated with cyst nematode resistance. Plant Mol Biol 41:25–32

Tomkins, JP, Yu Y, Miller-Smith H, Frisch DA, Woo S, Wing RA (1999b) A bacterial artificial chromosome library for sugarcane. Theor Appl Genet 99:419–424

Wang GL, Holsten TE, Song WY, Wang HP, Ronald PC (1995) Construction of a rice bacterial artificial chromosome library and identification of clones linked to the Xa-21 disease resistance locus. Plant J 7:525–533

Waugh R, Bonar N, Baird E, Thomas B, Graner A, Hayes P, Powell W (1997) Homology of AFLP products in three mapping populations of barley. Mol Gen Genet 255:311–321

Wei F, Gobelman-Werner K, Morroll SM, Kurth J, Mao L, Wing R, Leister D, Schulze-Lefert P, Wise RP (1999) The *Mla* (powdery mildew) resistance cluster is associated with three NBS-LRR gene families and suppressed recombination within a 240-kb DNA interval on chromosome 5 S (1HS) of barley. Genetics 153:1929–1948

Woo SS, Jiang J, Gill BS, Paterson AH, Wing RA (1994) Construction and characterization of a bacterial artificial chromosome library of Sorghum bicolor. Nucleic Acids Res 22: 4922–4931

Yu YG, Buss GR, Saghai-Maroof MA (1996) Isolation of a superfamily of candidate disease-resistance genes in soybean based on a conserved nucleotide-binding site. Proc Natl Acad Sci USA 93:11751–11756

Zhang HB, Choi S, Woo SS, Li Z, Wing RA (1996) Construction and characterization of two rice bacterial artificial chromosome libraries from the parents of a permanent recombinant inbred mapping population. Mol Breed 2:11–24