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A maize bacterial artificial chromosome (BAC) library from the European flint inbred line F2

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Abstract We report here the construction and characterisation of a BAC library from the maize flint inbred line F2, widely used in European maize breeding programs. The library contains 86,858 clones with an average insert size of approximately 90 kb, giving approximately 3.2-times genome coverage. High-efficiency BAC cloning was achieved through the use of a single size selection for the high-molecular-weight genomic DNA, and co-transformation of the ligation with yeast tRNA to optimise transformation efficiency. Characterisation of the library showed that less than 0.5% of the clones contained no inserts, while 5.52% of clones consisted of chloroplast DNA. The library was gridded onto 29 nylon filters in a double-spotted 8 × 8 array, and screened by hybridisation with a number of single-copy and gene-family probes. A 3-dimensional DNA pooling scheme was used to allow rapid PCR screening of the library based on primer pairs from simple sequence repeat (SSR) and expressed sequence tag (EST) markers. Positive clones were obtained in all hybridisation and PCR screens carried out so far. Six BAC clones, which hybridised to a portion of the cloned *Rp1-D* rust resistance gene, were further characterised and found to form contigs covering most of this complex resistance locus.

Keywords Maize · BAC library · Flint · Rp1 resistance gene

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Introduction

Until crop genomes have been completely sequenced, positional cloning represents the most-feasible route to cloning genes underlying traits known only by phenotype. A large number of economically valuable agronomic characters, including abiotic stress tolerance, fruit and seed weight as well as pathogen resistance, lie in this category. The strategy of chromosome landing, originally described by Tanksley et al. (1995) has recently been applied to a large genome cereal, barley (Büschges et al. 1997; Lahaye et al. 1998). We are part of the European Map Maize programme, whose aim is to isolate genes underlying both quantitative and qualitative traits of agronomic importance by the strategy of chromosome landing (O'Sullivan and Edwards 2000). An important aspect of this initiative is to supplement the large insert library resources available for positional cloning in maize through construction of a maize BAC library. The maize Yeast Artificial Chromosome (YAC) library (Edwards et al. 1992) has provided a basis for important advances in our knowledge of the physical constitution and organisation of the maize genome (Civardi et al. 1994; Avramova et al. 1995; SanMiguel et al. 1996). However, if positional cloning of agronomically important genes is to become routine in maize, BAC libraries will be of crucial importance. This is because in contrast to the YAC system, BAC clones are more-stably maintained, are rarely chimaeric and can be easily purified from host DNA (Shizuya et al. 1992). These features mean that their extremities can be easily isolated and sequenced (Ripoll et al. 2000), and overlap relationships of clones easily and reliably established by fingerprinting, even at a whole-genome scale (Ding et al. 1999). Furthermore, BAC clones of interest may be readily subcloned and the entire sequence determined (Boysen et al. 1997).

The number of BAC libraries reported for cereal genomes has been steadily growing such that for the major cereals, BAC libraries are now available not only for rice (Wang et al. 1995; Zhang et al. 1996; Yang et al.

1997) and sorghum (Woo et al. 1994), but more recently also for the diploid progenitors of the "A" and "D" genomes of hexaploid wheat (Lijavetsky et al. 1999; Moullet et al. 1999). Published reports of maize BAC libraries are not currently available, although several laboratories are active in the production of maize BAC libraries, mainly from inbred line B73 (reviewed in O'Sullivan et al. 1999).

The maize inbred line F2, which we have chosen as a template for BAC library construction, originates from the Lacaune maize population in the southwest of France and was released by INRA in 1956. Since this date, it has become widely used as a public breeding line. It is an early flowering, cold-tolerant maize variety, with typical flint kernel characteristics. Additionally, it is known to breeders to combine very well with dent lines to give high yielding hybrids in Northern Europe (Hallauer et al. 1988). Its high mean genetic distance from other inbred lines (Messmer et al. 1992; Burstin et al. 1994; Dubreuil et al. 1996) was one of the reasons for its choice as a parent in the construction of recombinant inbred line populations for genetic mapping of expressed sequences in maize (Causse et al. 1996), as well as in ongoing breeding and mapping programs (A. Charcosset, personal communication).

We report here the construction and characterisation of a 3.2 genome-equivalent BAC library from the inbred line F2, and have demonstrated its usefulness by carrying out PCR and hybridisation screens for a number of gene and molecular-marker sequences, and through the construction of BAC contigs covering most of the physical region corresponding to the complex rust resistance locus *Rp1* (Hulbert 1997).

Materials and methods

Vector preparation

To make a large preparation of the BAC cloning vector, a high copy number construct designated BSBH was made by ligation of pBeloBAC11 and pBluescript, each linearised by *Hin*dIII. The double-vector product was specifically recovered using a double selection on ampicillin (100 µg/ml) and chloramphenicol (12.5 µg/ml). *Hin*dIII digestion of BSBH yielded 7.4-kb and 3.0-kb bands corresponding to the component vector molecules. Gel isolation of the 7.4-kb pBeloBAC11 band, its de-phosphorylation and further purification were as described in Osoegawa et al. (1998). The quality of the vector 5′-overhangs was tested by which self-ligation with and without polynucleotide kinase. A part of the vector self-ligated with the polynucleotide kinase was also digested with *Hin*dIII to check site reconstitution after re-ligation.

High-molecular weight (HMW) DNA preparation

The procedure for HMW DNA isolation was as described in Edwards et al. (1992) with some modifications. Maize seedlings were grown for 1 week at 25°C in light, and in the dark for 3 days. In a typical experiment, 600 seedlings were harvested by excising the shoot just above the hypocotyl to recover de-etiolated leaf tissue. The outer pigmented leaves were discarded. The inner shoot tissue was then finely sliced using a razor blade, and transferred into Petri dishes of protoplasting solution (0.5% Cellulase RS, 0.25% Macerozyme R10, 375 mM mannitol, 50 mM $CaCl_2$, 0.2% MES, pH 5.6). After 3 h at 30°C in the dark without agitation, the contents of each Petri dish were pre-filtered through a double layer of Miracloth (Calbiochem), then through a 53-µm sieve into 50-ml tubes. Protoplasts were pelleted by centrifugation in a swinging-bucket rotor at 1,000 rpm for 6 min. The pellet was gently re-suspended in 40 ml of protoplast wash solution (0.6 M Sorbitol, 10 mM Hepes-KOH, pH 7.5, 2 mM $CaCl_2$) and re-pelleted three times. After the final wash, the protoplasts were resuspended in a minimal volume of wash solution, warmed to 37°C briefly, and an equal volume of 1% InCert (FMC) agarose was added. The protoplast/agarose mixture was transferred to 90-µl plug moulds (BioRad Laboratories) and allowed to solidify. The protoplast plugs were incubated overnight at 50°C in a 0.5 M EDTA pH 8.5, 1% Lithium Dodecyl Sulphate, 1 mg/ml of Proteinase K solution (Sigma). This solution was renewed for a further 24-h incubation. The plugs were then rinsed repeatedly in 500 mM of EDTA pH 8.0, pre-warmed to 37°C before use. Pre-electrophoresis was carried out by loading plugs on a 1% gel, and subjecting to a 90 s pulse time for 6 h at 150 V in the BioRad CHEF-II PFGE apparatus. The plugs were stocked in 50 mM EDTA, 10 mM Tris, pH 8.0, at 4°C prior to use.

Partial digestion and size selection

The DNA plugs (five plugs of 90 µl) were first washed three times for 30 min by incubation in 50 ml of $TE_{10:0.5}$ + PMSF [10 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, pH 8.0, with 1 mM of Phenyl-MethylSufonyl Fluoride (PMSF) added just before washing] on ice and twice for 1 h on ice in 25 ml of 1× *Hin*dIII restriction buffer. Once washed, the plugs were incubated for 4 h on ice in 400 µl of 1× restriction buffer containing 100 µg/ml of BSA and increasing amounts of restriction enzyme. The tubes were then transferred to 37°C for 1 h and loaded on a pulsed-field electrophoresis gel.

The partially digested DNA was separated by pulsed field electrophoresis at 170 V for 16 h with a linear pulse ramp from 0.5 s to 40 s with a CHEF-DR II (BioRad). After migration, the sides of the gel containing the markers were stained with ethidium bromide. By comparison between the stained parts containing the markers and the unstained part containing the partially digested DNA, slices of agarose containing DNA of sizes from 80 to 100 kb and from 100 kb to 120 kb were excised. The DNA from the agarose slices was then electroeluted into dialysis tubing (Life Technologies 15961-014) for 3 h in a cold room at 3 V/cm and drop-dialysed, on ice, against $TE_{10:0.5}$ twice for 1 h on Millipore filters (VSWP05000). The DNA concentration was estimated by gel migration and comparison with Lambda DNA standards.

Ligation and transformation

The purified partially digested DNA was ligated with the *Hin*dIII-linearised and de-phosphorylated pBeloBAC11 vector. The input genomic DNA concentration was fixed after optimisation at 0.25 ng/µl and the ratio vector/insert used was either 10:1 or 15:1. The ligation was done in 1× T4 DNA ligase buffer with 1 Weiss unit of T4 DNA ligase (Roche) for a 50-µl ligation volume at 16°C overnight. The ligations were then drop dialysed twice for 1 h on ice against $TE_{10:0.5}$. Four microlitres of the ligation product and 1 µl of yeast tRNA at 1.25 mg/ml were co-transformed to 27 µl of ElectroMAX DH10B competent cells (Life Technologies) by electroporation using a Gene Pulser apparatus (BioRad). Transformation was perfomed using 15 kV/cm, 100 Ω and 25 µF with a 1-mm cuvette. The electroporated cells were then re-suspended in 500 µl of SOC medium and incubated at 200 rpm and 37°C. After 45 min the cells were plated on LB-agar Genetix plates (200 × 200 mm) containing 12.5 µg/ml of chloramphenicol and incubated at 37°C. After 24 h at 37°C, the clones were picked into 96-well microtitre plates filled with 100 µl of LB containing 12.5 µg/ml of chloramphenicol. After overnight growth at 37°C, 100 µl of LB medium containing 40% glycerol was added and the plates stocked at –80°C.

Library screening by hybridisation

For quality control purposes, a representative subset of clones from the entire library was set up as follows: one clone from each of the 905 plates that constitute the library was randomly picked into a new set of ten 96-well microtitre plates (henceforth referred to as the "cross-section" plates). The clones from these ten cross-section plates were inoculated robotically onto nylon filters (Hybond NX, Amersham, UK) in a 4 × 5 double-spotted array using a Beckman Biomek 2000 robot.

For full library screening, the 86,858 clones that compose the library were robotically inoculated onto 8 × 12-cm nylon filters in a double-spotted 8 × 8 array pattern. In this manner, a set of 29 filters each containing up to 3,072 arrayed clones was generated. The inoculated filters were incubated for 12–16 h at 37°C. Colony DNA transfer and hybridisation screening of the filters were performed according to standard procedures (Sambrook et al. 1989).

Library screening by PCR

The 905 microtitre plates constituting the library were organised in three series of pools, each pool containing a maximum of ten plates. X-pools were made by pooling series of up to ten consecutive plates starting with the plate 1 (1 to 10, 11 to 20, etc.), giving 90 pools of ten plates and 1 pool of five plates. The Y-pools were made by pooling series of up to ten plates with an incrementation of ten starting with plate 1 (1, 11, 21…91; 2, 12, 22…92, etc), giving 90 pools of ten plates and five pools of 1 plate. Finally the Z-pools were made by pooling series of a maximum ten plates with an incrementation of 100 starting with plate 1 (1, 101, 201…901; 2, 102, 202…902, etc.), giving five pools of ten plates and 95 pools of nine plates. These 286 pools (91 X, 95 Y and 100 Z) were the X, Y and Z co-ordinates of a 3-dimensional block of plates with a base area of ten plates by ten plates (Fig. 1). To make these pools, all 905 plates were replicated into a new set of 96-well microtitre plates with 200 µl of LB containing 12.5 µg/ml of chloramphenicol in each well. These plates were incubated overnight at 37°C with agitation at 150 rpm. Each plate was harvested in a 50-ml tube with a specially made vacuum device (design details given on request). At this stage, 5 ml of pooled bacterial culture from each plate were used to constitute the three series of pools. The bacterial pools were centrifuged and stocked at –80°C until the DNA extractions. DNA extractions were carried out with the Wizard Purefection plasmid DNA purification system (Promega) at the midiprep scale. The only modification to the Promega protocol was that the clear lysates were obtained by filtration through sterile cotton wool inside 10-ml syringes. The total yield from these 50-ml extractions was approximately 12 µg, and 2 µl (approximately 20 ng) of the pooled DNA was used for each primary screening reaction. The primary screening (286 PCR reactions) gave the coordinates of the plates containing positive clones. To isolate the positive clones from the positive plates, a secondary screening needed to be carried out on the 96 clones from each positive plate. It should be noted that this pooling scheme has been designed with the aim of isolating single-copy or duplicated marker sequences/genes. In this case each positive plate was duplicated and grown overnight at 37°C at 150 rpm, each well containing 100 µl of LB containing 12.5 µg/ml of chloramphenicol. The plates were then centrifuged, the LB discarded, and the bacteria re-suspended in 100 µl of $TE_{10:0.5}$ and transferred into a 96-well PCR plate to be heated at 100°C for 15 min. The PCR plates were then centrifuged to pellet the cellular debris and 2 µl of supernatant was used for the secondary screening. Positive clones thus identified were grown and their DNA extracted by alkaline precipitation minipreps and the PCR carried out a last time for verification. The primary and secondary screening reactions were carried out in 96-well PCR plates in a total volume of 25 µl. PCR-screening reaction conditions were adapted to the size of the amplicon and the annealing temperatures of the primers. Generally, 40 cycles of PCR were carried out with 0.2 µM of each primer, 0.2 µM of dNTPs and 1U of *Taq* polymerase per 25-µl reaction.

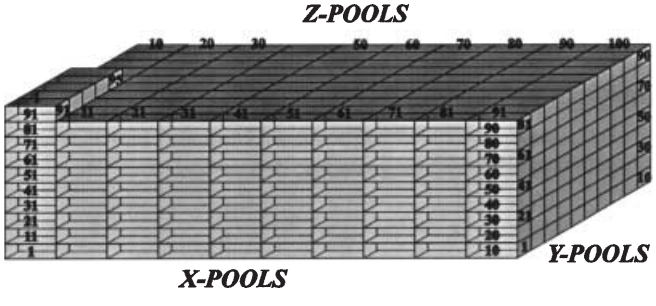

Fig. 1 The 3-dimensional BAC DNA pooling scheme. Each of the 91 X-pools (*light grey*) comprise DNA from pooled cultures of lines of ten or five (only pool X91) microtitre-dishes deep. Each of the 95 Y-pools (*medium grey*) comprise DNA from pooled cultures of rows of ten or one (only pools Y91 to Y95) microtitre-dishes across. Likewise, the Z-pools numbered from 1 to 100 each comprise DNA from pooled cultures of stacks of nine or ten (only pools X1 to X5) microtitre dishes

DNA probes

The chloroplast and knob-specific probes were those described by Edwards et al. (1992). The *les22*, *lls1*, *Zmk*1 and *Vp1* probes were generated via specific PCR-amplification of primer sets designed from published sequences (Gray et al. 1997; Hu et al. 1998; McCarthy et al. 1991; Philippar et al. 1999). AF13, VIP3, Tub8 and the ribosomal protein cDNA are sequenced maize cDNA inserts provided by A. Forsyth and D. Stevenson (IACR-Long Ashton, UK). KSU3A, umc1026, umc1448, T14732 and MSKJE665695 are publicly available RFLP or SSR markers for which source materials can be located at Maize dB (http://www.agron.missouri.edu).

BAC fingerprinting and contig construction

Putative overlapping clones from the *Hin*dIII library identified with a single probe were digested with *Hin*dIII and migrated under the following conditions to generate a restriction fingerprint for each clone: approximately 300 ng of each clone was digested to completion either with *Hin*dIII, or with *Hin*dIII and *Eco*RI. A 30-cm long 1% agarose gel was loaded with the digestions and migrated in a 0.5 × TBE at 60 V for 16 h. The gel was stained with ethidium bromide and bands visualised over UV light. The probe used to isolate the clones (see Table 2) was also used as a probe for Southern blots of the purified BAC clones using standard procedures (Sambrook et al. 1989). Overlaps between clones were determined by compiling data on shared fingerprint fragments (three shared fragments was the threshold for declaring an overlap), and shared *Rp1*-hybridising fragments.

Results and discussion

BAC library construction

The library was generated from a total of eight size selection experiments. In general, size fractions from 80 to 100 kb and from 100 to 120 kb were ligated and transformed for each experiment, and 12 of these 16 ligations yielded significant numbers of colonies (reported in Table 1). The mean insert size obtained from the 80–100-kb size fraction (ligations 2,4,7,8,10,11; Table 1) was 80.5 kb, while the mean insert size for the higher gel

Table 1 Composition of the library

Experiment number	Ligation number	Size selected	Number of clones	Average insert size (kb)
1	1	120–150	627	120
2	2	80–100	1,437	72
3	3	100–120	5,141	91
	4	80–100	187	90
4	5	100–120	4,574	98
5	6	100–120	10,321	98
	7	80–100	6,705	88
6	8	80–100	11,712	88
7	9	100–120	15,062	102
	10	80–100	28,896	77
8	11	80–100	828	68
	12	100–120	1,368	103

slice (100–120 kb; ligations 3,5,6,9,12; Table 1) was 98.4 kb. Among 288 clones analysed, just one clone (0.35%) without an insert was observed. In most cases so far reported, the average size of the insert obtained is much lower than the size of the DNA selected. For example, Salimath and Bhattacharyya (1999) selected partially digested soybean DNA 200–350 kb in size, and yet obtained an average insert size (109 kb) less than half the mean size of the input DNA. Likewise, Vinatzer et al. (1998) obtained a mean insert size of 120 kb from a gel slice containing 350–440-kb apple DNA fragments. On the other hand, Lijavetsky et al. (1999) and Moullet et al. (1999), both selecting DNA from 125 to 200 kb in size, obtained average insert sizes of 115 and 122–128 kb respectively, both close to the lower limit of the size fraction selected. In contrast to the work of Lijavetsky et al. (1999) and Moullet et al. (1999), we used a single-step size selection to fractionate partially digested genomic DNA for construction of our maize library, and yet the average insert size of the clone obtained for each of the fractions used was within, or close to, the range of molecular weights selected.

The second factor which we defined as important for obtaining high cloning efficiency was a low-input DNA concentration. After a series of experiments varying the input of genomic DNA concentration while holding the vector:insert ratio constant, it was established that the overall efficiency was at least 10-fold greater at 0.25 ng/µl than at 0.5 ng/µl (data not shown).

A recent report that co-transformation with tRNA enhanced BAC transformation efficiency (Zhu and Dean 1999) was also exploited. In our hands, addition of the tRNA at 0.25 µg/µl to the transformation mix increased the number of colonies per electroporation at least seven-fold.

Using these modifications to previous BAC cloning protocols, a total of 86,858 BAC clones were generated from these 12 ligations and picked into 905 96-well microtitre plates either manually or using a Q-bot colony picking robot.

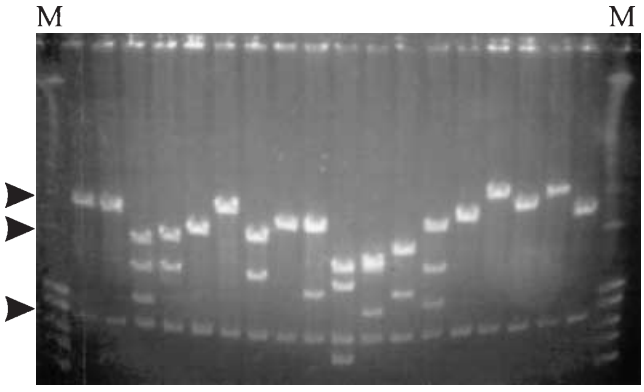

Fig. 2 *Not*I digests of 18 randomly chosen BAC clones from a typical ligation. Marker lanes (*M*) contain both lambda concatamer-ladder (NEB) and 1 kb (Promega)-ladder size standards. The *arrowheads to the left* indicate (from top to bottom respectively) the 97.0-kb and 48.5-kb bands of the lambda ladder, and the position of the 7.0-kb pBeloBAC11 *Not*I vector fragment

Insert-size estimation and quality control

The estimation of mean insert size for each ligation was based on pulsed-field gel analysis of 24 randomly chosen clones digested with the *Not*I restriction enzyme (see Table 1). *Not*I digestions of a sample of 18 BAC clones taken from a typical ligation are shown in Fig. 2. The weighted average of insert sizes for individual ligations was used to calculate the overall average insert size as 88.7 kb. With the average insert size being I = 88.7 kb, the number of clones N = 86,858 and the genome size GS = 2,440,000 kb we deduce that the probability (P) to have a particular sequence represented in this library is $P = 1\text{-}e^{N[\ln(1\text{-}I/GS)]} = 0.9574$ (Clarke and Carbon 1976). Nylon filters containing one clone from each microtitre plate in the library were hybridised with probes derived from different classes of tandem and dispersed repetitive DNA elements from the maize genome to provide an initial assessment of the overall quality of the library. A total of 5.52% of clones were detected by a chloroplast probe; a level of contamination comparable with a previous maize YAC library (Edwards et al. 1992) where leaf protoplasts were used as a source of HMW DNA, but higher than that observed in cases where isolated nuclear DNA was used. For example, using isolated nuclei as the starting material, 0.14% and 1.4% inserts carrying chloroplast DNA were reported by Lijavetsky et al. (1999) and Nam et al. (1999) respectively. The ribosomal RNA genes and the knob sequences are both classes of high copy number, tandemly repeated DNA which give rise to cytologically observable structures (nucleolar organiser regions and ‘knobs’ respectively). Probes derived from repetitive units of each of these tandem repeats were used to probe the cross-section filters in order to check that these sequences were not over-represented in the library. At 0.22% and 0.11% detection rates, they may actually be under-represented in our library, although it is not possible to say what percentage of the genome the knob repeat should represent, as the number of knobs is

Table 2 Probes and markers for hybridisation and PCR screening

Hybridisation screens

Type	Probe designation	No. of positive clones	Clone sizes
Single-copy genes	AF13	2	40 kb , 115 kb
	les22	2	105 kb, 115 kb
	lls1	3	44 kb, 65 kb, 75 kb
	Zmk1	2	nd
	Vp1	2	nd
Gene families	Ribosomal protein cDNA	18	nd
	Tub8	35	nd
	KSU3A	8	nd
	Rp1-D	6	68 kb, 70 kb, 72 kb, 75 kb, 100 kb, 121 kb

PCR screens

Probe type	Probe designation	No. positive clones	Sizes of clones
SSR	MSKJE665695	2	nd
SSR	umc1026	3	65 kb, 75 kb, 100 kb
SSR	umc1448	3	80 kb, 85 kb, 115 kb
EST	T14732	3	50 kb, 50 kb, 70 kb
EST	VIP3	1	95 kb
		Average 2.3 positives per single-copy probe	Average insert size of positive clones : 80.4 kb

known to vary from line to line. The size of the maize NOR has, however, been reported as varying between 5,000 and 23,000 units of 8.5 kb, i.e. between 1.7 and 7.8% of the genome (Buescher et al. 1984).

Finally, a maize YAC left-arm rescue plasmid containing a high-copy number dispersed retrotransposon sequence was used as a probe; 33.0% of the clones hybridised to this probe. None of the clones detected by the retrotransposon, chloroplast, ribosomal or knob-derived probe sequences were positive for more than a single class of these repeat sequences, giving a preliminary indication that the level of chimaerism in the library was low.

Test screening

Since both gridded colony filters and a 3-dimensional DNA pool array (Fig. 1) have been generated for this library, screening by both hybridisation and PCR was possible. These screening methods are highly complementary. Manipulation of the stringency of hybridisation makes the hybridisation screen a powerful way of isolating maize genes using heterologous probes. The same principle applies to the simultaneous isolation of all members of a gene family. Screening by PCR, on the other hand, allows the highly specific isolation of clones even when the marker sequence is of a repetitive nature (e.g. SSR markers) or has dispersed homologous copies (e.g. gene families). As shown in Table 2, we have used both screening methods to test the library coverage.

PCR primer sets corresponding to both mapped SSR markers and a unique EST sequence were used, and in each case a number of clones consistent with the predicted library coverage were obtained. A total of 78 clones were identified by hybridisation screening with nine distinct probes representing both known single-copy genes as well as known gene families. Again, the number of clones isolated in each case was consistent with the number of copies expected and the calculated three-times genome coverage of the library. We therefore conclude that this library will provide useful material for a range of applications including fluorescent in situ hybridisation (FISH), which in maize can only be reliably performed using DNA fragments greater than 15 kb, and positional cloning. Further applications include isolation of the genomic sequence and regulatory elements surrounding transcribed sequences of interest (e.g. ESTs), and study of the physical organisation of interesting loci.

Construction of contigs spanning the F2 *rp1* locus

The *Rp1* maize rust resistance locus is a genetically complex locus to which several independent recombinable resistance specificities have been mapped, suggesting that the locus harbours a cluster of functionally related genes (Hulbert 1997). Recently, the *Rp1-D* resistance specificity has been transposon tagged and its molecular characterisation has revealed that it is a member of a genetically clustered family of NBS-LRR-type genes (Collins et al. 1999). In order to facilitate investigation of the physical structure of this resistance gene cluster, and to establish the order and spacing of NBS-LRR and other genes in the region, we attempted to reconstruct the genomic context of this interesting family of genes

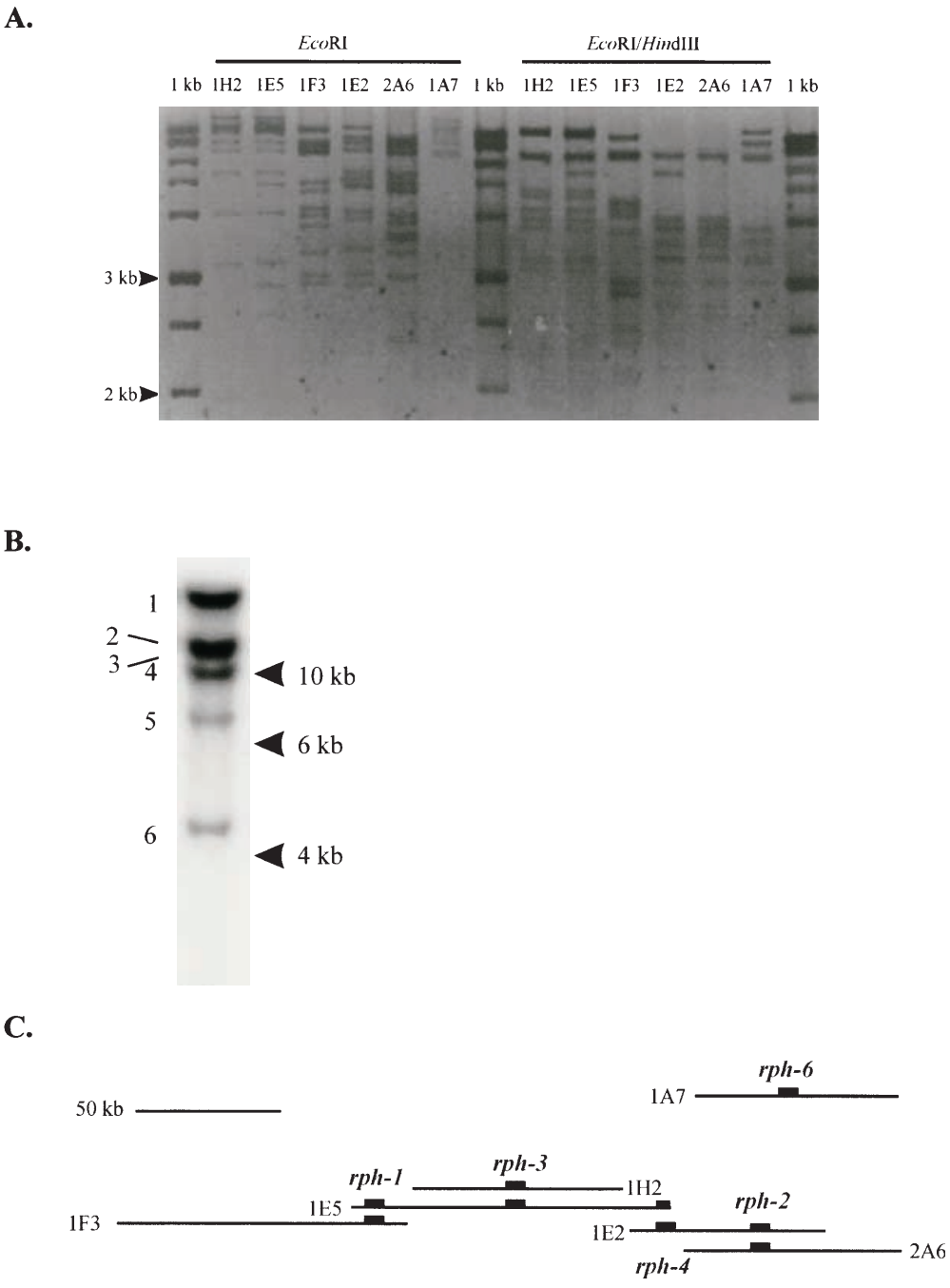

Fig. 3A–C A *Hin*dIII and *Eco*RI-*Hin*dIII fingerprint digests of *Rp1*-hybridising BAC clones. 1F3, 1E5, 1H2, 1E2, 2A6 and 1A7 are the designations of six *rp1*-positive BAC clones. The 1st, 8th, and 15th lanes contain a 1-kb ladder size standard (Promega). The image is shown in negative for clarity. **B** *Eco*RI genomic Southern analysis of the F2 *rp1* array using a portion of the *Rp1*-D gene. *rph* = *Rp1*-D homologous fragment. *rph Eco*RI fragments are labelled *1 to 6* in descending size order. **C** BAC clone coverage of the *Rp1* rust resistance gene array. *Grey bars* indicate the approximate positions of *rph* genes on each BAC clone, while the correspondence of each gene to the *Eco*RI Southern blot shown in **B** is denoted by a number (e.g. *rph-1*)

through the contig assembly of *Rp1*-hybridising BAC clones from the F2 library.

As shown in Table 2, a total of six clones were isolated from the locus. Based on comparing the Southern profiles of BAC clones with that of genomic DNA revealed by the *Rp1* probe, we predict that 5 out of 6 *Rp1* homologues (*rph*s) that are present in the F2 inbred line (Fig. 3B) were isolated. *Hin*dIII and *Eco*RI-*Hin*dIII fingerprints (Fig. 3A) were generated for these six BAC clones, and compiled with the *Rp1* RFLPs to establish the 260-kb contig shown in Fig. 3C. One hybridising band (*rph-5)*, which can be seen on the F2 genomic Southern blot, is not present on any of the BAC clones isolated. This could represent a breakdown in the coverage of the library; indeed, as discussed above, there is a 5% probability that a given sequence would not be represented in this library. However, the failure to isolate a clone carrying *rph*-5 may also reflect the fact that of all six copies, it is the most-weakly hybridising band relative to its size. Clone 1A7 is a singleton; that is, it does not overlap with the 260-kb contig.

These clones can form the basis for answering a number of important questions relating to the *Rp1* rust resistance locus. Firstly, the precise order and spacing of *rph*s within the 260-kb contig can be established and compared to the sequence divergence between the differ-

ent copies. Furthermore, if other genes are found interspersed among these resistance gene analogues, they may be detected by large-scale sequencing.

The maize F2 BAC library described here was constructed as part of the European Framework IV programme, Map Maize. As part of the Map Maize programme, the library is being used for the positional cloning of genes involved in lignification, male sterility and maturity dates. However, we envisage that the library will have many further uses. Since it is the first large insert genomic library resource described from a flint maize genotype, it should prove of particular interest to European maize researchers and those interested in the evolutionary adaptations which have accompanied the divergence of flint and dent heterotic groups. Together with other maize BAC libraries, the F2 BAC library will be a valuable long-term tool for maize genome studies. It is archived at IACR-Long Ashton Research Station and related data will be made available through Maize dB (http://agron.missouri.edu).

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