A Sequence-Tagged Linkage Map of *Brassica rapa*

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ABSTRACT

A detailed genetic linkage map of *Brassica rapa* has been constructed containing 545 sequence-tagged loci covering 1287 cM, with an average mapping interval of 2.4 cM. The loci were identified using a combination of 520 RFLP and 25 PCR-based markers. RFLP probes were derived from 359 *B. rapa* EST clones and amplification products of 11 *B. rapa* and 26 Arabidopsis. Including 21 SSR markers provided anchors to previously published linkage maps for *B. rapa* and *B. napus* and is followed as the referenced mapping of R1–R10. The sequence-tagged markers allowed interpretation of the pattern of chromosome duplications within the *B. rapa* genome and comparison with Arabidopsis. A total of 62 EST markers showing a single RFLP band were mapped through 10 linkage groups, indicating that these can be valuable anchoring markers for chromosome-based genome sequencing of *B. rapa*. Other RFLP probes gave rise to 2–5 loci, inferring that *B. rapa* genome duplication is a general phenomenon through 10 chromosomes. The map includes five loci of FLC paralogues, which represent the previously reported *BrFLC*1, -2, -3, and -5 and additionally identified *BrFLC*3 paralogues derived from local segmental duplication on R3.

The genus *Brassica* includes oilseed, vegetable, fodder, and condiment crops. *Brassica rapa* (syn. *campestris; A genome*), *B. napus* (AC genome), *B. juncea* (AB), and *B. carinata* (BC) contribute ∼12% of the global supply of edible vegetable oil (Labana and Gupta 1993). *B. rapa* and *B. oleracea* (C genome) provide many vegetables that contribute to a healthy human diet, being a valuable source of dietary fiber, vitamin C, and other health-enhancing factors such as anticancer compounds (Fahey and Talalay 1995). The *Brassica* A genome therefore has worldwide importance in agriculture, with the quality and economic value of derived products such as processed oils and kimchi being dependent upon appropriate combinations of alleles. *B. rapa* includes a variety of vegetables crops such as Chinese cabbage, Pakchoi, turnip, and broccoli as well as oilseed crops such as turnip rape and sarson (Gomez-Campo 1999).

The high degree of neutral DNA polymorphisms of most *Brassica* species (Figdore et al. 1988) has facilitated the development of molecular linkage maps, with at least 15 described to date for *B. oleracea* (Slocum et al. 1990; Kianian and Quiros 1992; Lan et al. 2000), *B. rapa* (Song et al. 1991; Chyi et al. 1992; Teutenico and Osborn 1994), *B. nigra* (Lagercrantz and Lydiate 1996), *B. juncea* (Cheung et al. 1997; Pradhan et al. 2003), and *B. napus* (Landry et al. 1991; Uzunova et al. 1995). Where common sets of DNA markers and/or parental genotypes have been used, it has been possible to designate linkage groups according to a common nomenclature (Parkin et al. 1995, 2005; Butruille et al. 1999; Sebastian et al. 2000). Thus for *B. napus* linkage groups N1–N10 representing the A genome correspond to *B. rapa* R1–R10, and linkage groups N11–N19 representing the C genome correspond to *B. oleracea* O1–O9. Bouhou et al. (1996) demonstrated that marker order and linkage group structure had been conserved between the diploid (*B. oleracea*) and amphidiploid (*B. napus*) C genomes. In this study, we generated a detailed linkage map using sequenced EST clones derived from tissue-specific libraries of *B. rapa*. To establish the identity of linkage groups corresponding to R1–R10, we used SSR markers from Suwabe et al. (2002) and Lowe et al. (2004).

The *Brassica* genomes are closely related to the model plant *Arabidopsis thaliana*, diverging ∼20 MYA (Koch et al. 2001), and remain collinear. Comparative mapping of RFLP probes among the three diploid species *B. rapa* (*n* = 10), *B. oleracea* (*n* = 9), and *B. nigra* (*n* = 8) has suggested that genomes of the *Brassica* species are composed of three rearranged variants of an ancestral genome and descended from a common hexaploid ancestor (Lagercrantz and Lydiate 1996). All comparative studies of *Arabidopsis* and *Brassica* to date have revealed extensive duplications, with *Arabidopsis* segments being conserved an average of three times within
BAC clones that represent a triplicated and segmentally duplicated FLC region of *B. rapa* and are homologous with 125 kb of Arabidopsis chromosome 5 (Yang et al. 2006).

In this study we demonstrate the conservation of genome segments within and between chromosomes, on the basis of sequence-tagged markers.

**MATERIALS AND METHODS**

**Population development and DNA extraction:*** F$_2$,$_3$ families (40 F$_3$ seedling) of 134 F$_2$ lines ("JWF3p") were developed from Chinese cabbage F$_1$ cultivar Jangwon (*B. rapa* ssp. pekinensis). These two biennial inbred parent lines were made available courtesy of the former “Seoul Seed” company (Korea). To induce flowering, all seedling plants were vernalized for 35 days in a cold room at 5$^\circ$C with a 16-hr photoperiod. Tissues were collected from F$_2$,$_3$ families after 15 days of growing in greenhouses. Genomic DNA was extracted from the lyophilized tissue following the method of Chio et al. (1994), using 1 ml saturated phenol (BM Co.) for every 1-g sample.

**Gel electrophoresis and Southern blot analysis:*** To screen for polymorphisms, an average of 10 $\mu$g genomic DNA from the inbred parent lines was digested with seven restriction enzymes (*BamHI*, *DraI*, *EcoRI*, *HindIII*, *EcoRV*, *XbaI*, and *ScaI*) and fractionated on 0.9% agarose gels. Electrophoresis and Southern blotting were conducted as described by Chio et al. (1994). *BamHI*, *EcoRI*, *EcoRV*, and *ScaI* enzymes were used for digestion of the segregating progeny populations.

**EST clones used as RFLP probes:*** Four different tissue-specific libraries were used as a source of RFLP probe. These were prepared from mRNA isolated from immature flowers (BIF), anthers (BAN), roots (BR), and dark-grown seedlings (BDS) of *B. rapa* line Jangwon (Kim et al. 1996; Lim et al. 2000). Plasmid DNA preparation and nucleotide sequencing were conducted as described by Lim et al. (2000). We have used less redundant cDNA clones on the basis of their sequence and BLASTN search in GenBank (National Center for Biotechnology Information). Insert DNA was amplified by PCR using T7 and T3 primers and eluted by QIAGEN (Valencia, CA) gel extraction kits. Probe labeling was conducted by random hexamer labeled with $^{32}$P-4CTP (Feinberg and Vogelstein 1983). Hybridization followed the method described by Chio et al. (1994). Hybridized filters were washed with three stringency steps (2x, 1x, and 0.5x SSC with 0.5, 0.1, and 0.1% SDS), respectively, and exposed to X-ray film (Fuji, Stamford, CT) for 2–3 days.

**Genome sequence tag markers used as RFLP probes:*** Genome sequence tags (GSTs) representing 24 genes from Arabidopsis chromosomes 4 and 5 were generated by PCR amplification using Arabidopsis ecotype Columbia genomic DNA. The DNA were cloned and sequenced prior to use as RFLP probes. The 10 GSTs derived from Arabidopsis chromosome (chr)4 were At4RPP5 (At4g16860), At4ML1 (At4g21750), At4TRI (At4g24520), At4CBP2a (At4g25480), At4PRHA (At4g29940), At4CPK5 (At4g35310), At4FAH1 (At4g36220), At4AP2 (At4g36920), At4HL1S1 (At4g37580), and At4CESA2 (At4g39350). The other 14 GSTs derived from Arabidopsis chr5, At5HA72 (At5g4730), At5COR78 (At5g2310), At5PDC2 (At5g4960), At5CIL1 (At5g56650), At5MS11 (At5g58290), At5NPF13 (At5g64330), At5MYB68 (At5g6579), At5LCY (U50738), and 6 R-EST genes containing a cluster of NBS-LRR recognition motif were used. Two flowering-time genes, *AtFCA* (At4g16280) and *AILF* (At5g16850) of Arabidopsis, were developed as probes. As for *B. rapa* genes, 6 flowering-time genes of *B. rapa* *BrFAC* (AY273164), *BrAGL20* (AY345235), *BrBCO* (AY356370), *BrGlI* (AY356369), *BrSVP* (AY356366), and *BrFLC* gene-specific PCR product (forward primer 5'-TTACCGCCCTCTTATATCTTCTC-3'; reverse primer 5'-CATATAACAAAAAAACCCCAATTG-3') were used in this genetic map. The 5 function genes of *B. rapa*, *BrGST*, *BrMyrosinase*, *BrSAM*, *BrSIP*, and *BrDFRI*, were surveyed. Characteristics of genetic markers are summarized in Table 1.

**PCR-based genetic markers:*** Twenty-one previously developed SSR marker assays were selected on the basis of their ability to identify known A genome linkage groups in *B. napus* and *B. rapa* (Suwark et al. 2002; Lowe et al. 2004). Two SSR markers were developed from BAGs containing *BrFLC* sequences. One SSR marker was derived from BAGs containing the *BrMAF* gene. One RAPD marker (operon primer S14) was included since it was codominant in this population.

**Linkage analysis:*** Linkage analysis and map construction were carried out using JoinMap 3.0 (Voorrips et al. 2001). Segregating data were sorted according to locus order for each linkage group using MSExcel. This facilitated detection of errors associated with putative “double-recombinant” events and guided visual checking of original autoradiographs and revision of data points where these had been misscored or typed. All editing operations were recorded and are traceable. Linked loci were grouped on the basis of pairwise LOD values between 5 and 8, and centimorgan distances were estimated with the Kosambi mapping function (Kosambi 1944). Locus order within the LOD grouping was decided through an optimized algorithm using three rounds of linked markers. Multiple segregating loci detected by a probe were indicated by the addition of a suffix (a, b, c, d) to the locus names. Linkage maps were visualized using MapChart (Voorrips 2002) and PowerPoint.

**RESULTS**

**EST marker characteristics:*** A total of 551 cDNA clones from four *B. rapa* tissue-specific EST libraries were screened to obtain informative RFLP markers. Of these 440 were polymorphic between the parents. A high degree of polymorphism with such markers has previously been reported within Brassica subspecies (Song et al. 1988). To obtain segregating genotypes among the progeny, four restriction enzymes were used, with *EcoRV* found to detect the highest level of polymorphism (Table 2).

**The JWF3, B. rapa linkage map:*** This genetic map of *B. rapa* was generated on the basis of 545 markers, 520 RFLPs and 25 PCR-based markers assigned to 10 linkage
groups covering a total map length of 1287 cM with an average 2.4-cM interval. The 10 linkage groups are most likely to correspond to the 10 chromosomes of *B. rapa* (Figure 1). It was possible to assign each linkage group to a previously determined classification (R1–R10) on the basis of evidence from the location of previously published SSR markers designated to A genome linkage groups within the context of *B. napus* (*Parkin et al.* 1995; *Lowe et al.* 2004) and *B. rapa* (*Suwabe et al.* 2002) genetic maps. From a total of 75 mapped A genome SSR markers screened against the parents, 21 displayed polymorphism. Each of the 10 linkage groups had at least one SSR marker that provides an anchor to existing published maps.

The two longest linkage groups R3 (178 cM) and R9 (193 cM) correspond to the two longest groups N3 and N9 of *B. napus* (*Lowe et al.* 2004; *Udall et al.* 2005). Within the Korean Brassica Genome Project (KBGP, http://www.brassica.rapa.org) it had previously been reported that R9 corresponds to cytogenetic chromosome 1 and that R3 corresponds to cytogenetic chromosome 2 (*Lim et al.* 2005). We compared the relative lengths of each linkage group with the length of the corresponding cytogenetic chromosome identified by *Lim et al.* There was good agreement, with a calculated correlation coefficient of 0.87. When the same cytogenetic chromosome lengths were compared with the length of corresponding A genome linkage groups reported for recent genetic maps of *B. napus*, correlation coefficients of 0.66 (*Parkin et al.* 2005) and 0.78 (*Udall et al.* 2005) were obtained.

**Sequence homology:** *B. rapa* probe sequences used to establish marker loci were compared against all sequences in GenBank release using BLASTn. The supplemental table (http://www.genetics.org/supplemental/) lists the *B. rapa* loci for which nucleotide sequence homology was determined with a cutoff of 1E-12, together with the matching GenBank database accession. The similarity data indicated that 422 (77%) of all loci corresponded to genes of known sequence, of which

| Probe        | Marker type          | Source                                      | No. of probes | No. of loci | Status  | Reference                  |
|--------------|----------------------|---------------------------------------------|---------------|-------------|---------|----------------------------|
| RFLP         | EST_BIF              | Immature flower library of *B. rapa* cv. Jangwon | 114           | 157         | pBluescript | *Kim et al.* (1996); *Lim et al.* (2000) |
|              | EST_BAN              | Anther library of *B. rapa* cv. Jangwon     | 176           | 235         | pBluescript |                             |
|              | EST_BDS              | Dark-grown seeding library of *B. rapa* cv. Jangwon | 45            | 55          | pBluescript |                             |
|              | EST_BR               | Root library of *B. rapa* cv. Jangwon       | 24            | 27          | pBluescript |                             |
| At4GTS       | Chromosome 4-located genes of *Arabidopsis* cv. Colombia | 10            | 10          | pGemT      | GenBank                   |
| At5GTS       | Chromosome 5-located genes of *Arabidopsis* cv. Colombia | 14            | 17          | pGemT      | GenBank                   |
| AtGene       | Full length of expressed gene from *A. thaliana*       | 2             | 2           | pGemT      | GenBank                   |
| BrGene       | Full length of expressed gene from *B. rapa*            | 11            | 17          | pGemT      | BrFLC_AY273164; BrAGL20_AY345237, etc. |
| PCR          | SSR R1–R10-located SSR markers                           | 11            | 11          | Primer     | *Lowe et al.* (2004) |
|              | SSR R1–R10-located SSR markers                           | 10            | 10          | Primer     | *Suwabe et al.* (2002) |
|              | BrH80A08_FLC1        | 5’-ttccccaggtgctgttact-3’ 5’-gggattcctctcttgagg-3’ | 1             | 1           | Primer     | *KBrH80A08_AC155344 |
|              | BrH04D11_FLC2        | 5’-ggggcaatataaatgtgtatttt-3’ 5’-ctctctgacagcttgctgg-3’ | 1             | 1           | Primer     | *KBrH04D11_AC155341 |
|              | BrH80C09_MAF         | 5’-cagtggaagttcataaacggcagaga-3’ 5’-cagtggaagttcataaacggcagaga-3’ | 1             | 1           | Primer     | *KBrH80C09_A166741 |
|              | Random primer        | Operon primer S14                           | 1             | 1           | Primer     | BM commercial product       |
| Total        |                      |                                             | 421           | 545         |          |                            |

**TABLE 2**

**Summary of the polymorphism detected by EST clones**

| Source of restriction endonuclease | BIF | BAN | BDS | BR | Total (%)   |
|-----------------------------------|-----|-----|-----|----|-------------|
| EcoRI                             | 32  | 45  | 9   | 6  | 92 (25.6)   |
| EcoRV                            | 37  | 65  | 14  | 8  | 124 (34.6)  |
| BamHI                             | 21  | 29  | 10  | 5  | 65 (18.1)   |
| ScaI                              | 24  | 37  | 12  | 5  | 78 (21.7)   |
| Total                             | 114 | 176 | 45  | 24 | 359 (100)   |

**TABLE 1**

**Characteristics of genetic markers in this linkage map**
Figure 1.—Composite detailed genetic linkage map of *Brassica rapa*.
89 aligned with Arabidopsis expressed, putative, or hypothetical protein-coding sequences. A study of the hit sequence based on their organisms showed that 317 of 422 (75%) had highest sequence homology to the Arabidopsis genome and 97 of 422 (23%) were matched on four species of Brassica, which were *B. rapa* (39/97), *B. napus* (32/97), *B. oleracea* (19/97), and *B. juncea* (7/97). Only 2% (8 of 422) showed the sequence homology on other organisms, including on rice. The probe sequences used to generate the marker loci appeared to represent a wide range of gene classes, including regulatory factors and structural genes involved in membrane transport, signal transduction, cell cycle regulation, carbon metabolism, stress response, DNA synthesis, and fatty acid metabolism.

**Single-locus genes:** From the screening of RFLP probes against parental lines using seven restriction enzymes, 12% of clones gave single hybridization fragments, and 62 of these were incorporated into the linkage map. Probe BAN235 (Figure 2A) mapped to a single locus on R9, and this was confirmed by screening the probe to an 11× genome coverage HindIII BAC library (Park et al. 2005). Positive hybridization signals were detected for 14 BAC clones, and the isolated DNA was digested with HindIII enzyme. The resultant fingerprints were consistent with the BACs forming a single contig by Southern analysis using the same probe, BAN235 (Figure 2B).

The genetic map location of the 62 single-locus markers and their corresponding BLAST comparison data are shown with an asterisk (*) next to their locus name in the supplemental table (http://www.genetics.org/supplemental/). Of these, 26% (17/62) had no sequence similarity to any sequence in GenBank, indicating that these may be Brassica unique genes, and 25% (16/62) displayed highest similarity to sequences corresponding to uncharacterized gene models within the Arabidopsis genome (expressed protein, unknown protein, or full-length cDNA). Single-locus-specific probes are distributed across all *B. rapa* chromosomes with no apparent clustering.

**Duplicated marker and homologous linkage groups:** The remainder of the RFLP probes detected more than one segregating locus, with an average of 1.31 loci per probe (520/396). A total of 102 of the 396 mapped probes gave rise to multiple loci (229), with an average of 2.25 loci per probe. Eighty-one detected 2 loci, 18 detected 3, 2 detected 4, and a single probe detected 5 loci. Of these, 72 probes revealed locus duplication (164 loci) of two or three copies on different linkage groups. The pattern of duplications within the *B. rapa* genome was revealed by comparing ordered clusters of loci derived from common gene probes. At the top of the largest linkage group R3 with five loci duplicated R1 and the middle region of R3 corresponds to sections of R4 and R5, whereas the lower region corresponds to sections represented by 10 loci on R2 and 7 loci on R10. Four marker loci, spanning 19 cM at the top of R4, are also duplicated within R7 and R9 where the four duplicated loci span 17 and 11 cM, respectively. Most chromosomal parts show two or three duplication blocks (Figure 3). These relationships between homeologous chromosome segments provide good evidence for a series of historical segmental duplication events in this genome. However, since all genetic mapping experiments are based on polymorphism of genetic markers, the fine detailed pattern of duplications or triplication is incomplete due to the presence of monomorphic or dimorphic markers.

**Comparison of flowering-time-related genes:** Six genes involved in regulating flowering time isolated from
Figure 3.—Interchromosomal duplication of *Brassica rapa*. Circles show duplicated loci of the same markers. Each linkage group is assigned 10 different colors.
B. rapa (FLC, GI, CO, SVP, AGL20, and VRNI) and two from Arabidopsis (LFY and FCA) were used as RFLP probes. Most probes hybridized to two or three major bands, and some of them were polymorphic between the parents. It was not possible to map any paralogous loci of VRNI due to the presence of three major monomorphic bands when tested with seven restriction enzymes. The BrFLC (AY273164) isolated from B. rapa cv. Maeryuk (F1) detected two polymorphic segregating bands and an additional three monomorphic bands between the two parents. Two distinct polymorphic loci BrFLCa and BrFLCb were mapped 5 cM apart on the long-arm telomere region of R3. BrFLCa was found to correspond to BrFLC3 sequences within the BAC clone KBrH117M18 (AC146875) and BrFLCb to KBrH52008 (AC155342). This was determined by Southern hybridization to BAC HindIII fingerprints. Two SSR markers, BrH80A08_FLCl (KBrH080A08, AC155344) and BrH04D11_FLC2 (KBrH004D11, AC155341) were derived from two individual BAC sequences. They were classified on the basis of results of colony hybridization, HindIII fingerprints, and hybridization pattern using the BrFLC gene as probe. BrH80A08_FLCl was located on the short arm of R10 and BrH04D11 was assigned to the short arm of R2, substantiating the positions determined by Schranz et al. (2002). A synthetic GST probe, designated from the second exon to the fifth exon of BrFLC5, showed a single polymorphic band and was mapped to a position 33 cM away from BrFLC3a. All of the duplicated BrFLC genes are located near telomeres (of R2, R3, and R10), with linked markers in the distal regions usually showing skewed segregation, mostly toward the maternal genotype. Data are consistent with the map location reported in a previous study (Schranz et al. 2002), although we report here one more BrFLC paralogue derived from recent segmental duplication and the reverse map orientation. An SSR marker derived from BrH80C09_MAF (KBrH80C09, AC166741) was mapped to the long arm of R2 and appears to correspond to the VFR1 locus on R2 (Schranz et al. 2002).

**DISCUSSION**

**Genetic linkage map:** A detailed genetic linkage map of the Brassica A genome has been constructed, on the basis of 134 B. rapa F2.3 families. It contains 545 loci and most of them were detected by RFLP analysis using sequenced EST probes from four different tissue-specific libraries (474 loci/359 probes). Additional genespecific probes were derived from Arabidopsis chromosome 4 (10 loci/10 probes) and chromosome 5 (17 loci/14 probes). Several flowering-time genes from Arabidopsis (2 loci/2 probes), B. rapa (7 loci/6 probes), and BrMyrosinase, BrDFRI, BrGST, BrSAM, and BrSLP (10 loci/5 probes) functional genes were mapped on this linkage map. The detection of 1.31 loci per RFLP probe (520/396) closely matches that reported (1.27 and 1.34 from 220 and 269 probes, respectively) for previous B. rapa maps (Song et al. 1991; Chyi et al. 1992). In contrast, Udall et al. (2005) observed a higher level of polymorphism with codominant and dominant segregation in the amphidiploid B. napus, where there are twice as many potential loci.

The accumulated set of sequence-tagged genetic markers provides a valuable source of information for study and navigation of the Brassica A genome, not only in B. rapa but also in the context of B. napus and B. juncea. Since the model dicotyledonous plant, A. thaliana, is closely related to Brassica the genus and share, on average, 87% sequence identity (Cavell et al. 1998), there is an expectation that understanding the genetic control of basic biological processes in Arabidopsis can be transferable to other species (Lagercrantz 1998). However, Brassica EST markers that do not correspond to genes derived from other species are of additional value, as they provide insight into the identity and location of novel gene functions, which may be related to the well-characterized adaptability and plasticity of this crop genus.

We have obtained an average marker density of 2.4 cM. B. rapa has the smallest diploid Brassica genome, estimated at 529 Mb (Johnston et al. 2005). Thus we calculate that the current map provides a genetic marker on average at least every 1 Mb. This information may be exploited in at least two ways. First, within the ongoing B. rapa genome-sequencing project (Yang et al. 2005) 62 single-locus gene markers are now available that will assist in the isolation and confirmation of “seed” BACs, as well as provide anchored markers to span between adjacent BAC contigs to integrate the physical map. Second, there is the prospect of benefiting from the rich source of biological information and genetic resources from Arabidopsis functional genomics research to benefit Brassica crop plants.

**Multiple duplicated FLC genes of B. rapa:** The MADS-box flowering-time regulator FLC, located at the top of chromosome 5 of Arabidopsis, has a repressive function role on flowering time (Michaels and Amasino 1999). There are some differences on the number of orthologous or paralogous BrFLC loci with that of Schranz et al. (2002). Using the BrFLC gene as an RFLP probe, we obtained two polymorphic and three monomorphic bands. These two loci were located at the telomere of the long arm of R3, whereas Schranz et al. were able to assign only one locus to R3. This difference appears to result from the use of a backcross population by Schranz et al., where heterozygote genotypes are not detected. In contrast, the JWF3 is an F3 pooled population (40 seedlings per line) that is able to represent F3 segregation. We were able to detect eight recombinant genotypes in the population between two alleles of BrFLC-a and -b, where one was homozygous for one parent line (genotype A), and the other represented the heterozygote
(H), resulting in a map interval of 5 cM on R3. Other monomorphic BrFLC fragments were detected using SSR markers derived from B. rapa BACs that contain BrFLC genes. Sequences of four BACs, KBrH080A08, KBrH004D11, KBrH117M18, and KBrH52O08 containing BrFLC1, -2, -3a, and -3b, respectively, are collinear to the FLC region at 3.0–3.35 Mb of Arabidopsis chromosome 5 with indels (Yang et al. 2006). This genetic map surrounding BrFLC1, -2, -3a, and -3b shows synteny between each linkage group and with the 3-Mb region of Arabidopsis chromosome 5 (Figure 4). Meanwhile BrFLC5 is identified in a BAC clone KBrH038M21 (not submitted yet) that is collinear with 12.7–12.91 Mb of Arabidopsis chromosome 2 and the genetic position was determined 33 cM away from BrFLC3a on the long arm of R3. From this we infer that this genomic segment was replicated by an insertion within the homeologous region of R3 and this region and mostly shows the homeologous blocks to R4 and R5. Another BAC clone KrH80C09 corresponds to the MAF gene locus (At565050) within the 25.8- to 26.2-Mb region of Arabidopsis chromosome 5 (Yang et al. 2006).
Comparison of flowering-time genes of *B. rapa*, *B. napus*, and *A. thaliana* and QTL of *B. rapa* have been reported (O’Neill and Bancroft 2000; Rana et al. 2004; Lysak et al. 2005). Almost 88% of triplicated genes near the FLC regions returned to a single-copy or a two-copy state by deletion (Yang et al. 2006). Because of this reason, hybridization data using a single EST probe might have limitations for inferring genome duplication. But, overall distributions of duplicate or triplicate regions are detected from the hybridization data of multiloci EST markers (Figure 3), suggesting that the genome-level triplication might have happened in the ancestor of Brassica.

The KBGP is currently underway and is aiming to generate the first complete Brassica chromosome sequence of R9 (cytogenetic chromosome 1) (www.brascic.rapa.org). We have selected nine seed BACs through BAC library screening using single-locus EST markers. FISH and sequence information generally coincided with our expectations. The complete set of 62 locus-specific single-copy EST markers will be valuable markers for the primary anchoring of “seed” BACs for each linkage group.

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