Chapter 11

Development of the BAC Physical Maps of Wheat Chromosome 6B for Its Genomic Sequencing

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Genome Sequencing Project for Chromosome 6B

The International Wheat Genome Sequencing Consortium (IWGSC) has been promoting the decoding of the wheat genome sequence to enhance our knowledge of the structure and function of the wheat genome. The vision of the consortium is

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to establish a high quality reference sequence with a strategy to sequence its whole genome using BAC (bacterial artificial chromosome) clones aligned on the physical map of each chromosome. Under the framework of IWGSC, we are in charge of the chromosome 6B with the financial support from the Japanese Ministry of Agriculture, Forestry and Fisheries (“Genomics for Agricultural Innovation” project and “Genomics-based Technology for Agricultural Improvement” project) and Nisshin Flour Milling Inc. The “KomugiGSP” (Genome Sequence Program) (<http://komu-gigsp.dna.affrc.go.jp/index.html>) currently consists of the following four main research subjects: (1) Construction of 6B-specific BAC library and development of DNA markers, (2) comprehensive analysis of transcripts for gene annotation, (3) construction of the BAC physical map and genome sequencing (BAC-by-BAC and whole chromosome survey sequencing), and (4) development of annotation pipeline. Here, we report our current progresses on the construction of a physical map for chromosome 6B using chromosome arms-specific BAC libraries with DNA markers and BAC-based genomic sequencing.

Chromosome 6B-Specific BAC Libraries

Common wheat (*Triticum aestivum* L.) has a large genome, of approximately 17 Gb, and is allohexaploid with three homoeologous genomes ($2n=6x=42$, genome formula AABBDD). Chromosome 6B is one of the largest chromosomes among its 21 chromosomes, with an estimated size of 914 Mb, in which the short-arm (6BS) and long-arm (6BL) contain 415 Mb and 498 Mb, respectively (Šafář et al. 2010). Because of the large size, individual chromosomes or chromosome arms can be isolated using the flow cytometry to reduce the complexity of its genome for the construction of physical maps and genomic sequencing as well. The sorting of single chromosomes or chromosome arms from common wheat cultivar ‘Chinese Spring’ (CS) and its aneuploid lines has enabled the construction of chromosome (arm)-specific BAC libraries (Šafář et al. 2010), that have served as the critical resources for the development of physical maps and map-based genome sequencing by IWGSC. A double ditelosomic 6B line of CS was supplied for the flow sorting of mitotic chromosomes to construct chromosome arm-specific BAC libraries by collecting more than five millions of each chromosome arms. Using the chromosomal DNA extracted from each arm, two BAC libraries with the specificities to 6BS and 6BL, comprising 57,600 and 76,032 BACs with an average insert length of about 130 kb to represent 15.3 and 18 times equivalents of their estimated sizes of each arm, respectively, were successfully constructed.

BAC Contig Construction

For physical mappings of chromosome 3B, chromosome arm 1AL and whole genome of *Aegilops tauschii* Coss., BAC clones were fingerprinted through the method of SNaPshot™ (Paux et al. 2008; Lucas et al. 2013; Luo et al. 2013), which

is an automated fingerprinting technique by sizing restriction fragments of each BAC (Luo et al. 2003). However, restriction fragment sizes shared randomly between two non-overlapping BACs often lead to chimerical contigs or mis-assembled BACs particularly in the large and repetitive genomic regions. Whole Genome Profiling (WGP™) is an alternative method for the establishment of the chromosome physical maps developed based on a next-generation sequencing-based technology (van Oeveren et al. 2011). The efficiency of WGP on physical mappings of wheat chromosomes has been demonstrated by comparison with that of SNaPshot (Philippe et al. 2012).

In our project, chromosome 6B BAC clones were fingerprinted by WGP for a robust physical map. Pooled BAC DNAs were digested with the two restriction enzymes, *EcoRI* and *MseI*, and the restriction fragments with adaptors were sequenced using the Illumina HiSeq2000 sequencer to yield WGP tags. Deconvolution enables the assignment of the WGP tags to individual BACs. We excluded the low quality BACs during the contig building process in order to construct the physical map with high accuracy. After filtering BACs, assembly of the fingerprints representing nine and ten times equivalent of 6BS and 6BL, respectively, was performed with the FingerPrinted Contigs (FPC) software (Soderlund et al. 1997). A stepwise method was used for the assembly to further improve the quality of BAC contigs, which was defined by Paux et al. (2008) during the construction of chromosome 3B physical map and finally modified by Philippe et al. (2012). The physical maps of chromosome 6B have been successfully established to have an estimated chromosomal coverage of more than 90 %. Overlap analysis between the neighboring clones within BAC contigs enabled us to select minimal tiling path clones along each chromosome arm, which were subjected to the genomic sequencing.

Development of DNA Markers for Anchoring BAC Contigs to the Specific Genomic Regions on Chromosome 6B

DNA markers are essential to anchor BAC contigs onto their specific genomic positions. Information of publically available markers was collected from the databases such as “GrainGenes” and “National BioResource Project-Wheat Japan”. There are only about 200 markers such as SSR and RFLP available currently for chromosome 6B within these databases. Considering the chromosome size and number of BAC contigs, it is obvious that this number of markers is far enough to anchor BAC contigs for a physical map with high chromosome coverage. We thereafter used other public marker resources, like PLUG (PCR-based Landmark Unique Gene) marker, that were EST-PCR markers developed by taking advantage of the syntenic gene conservation between rice and wheat (Ishikawa et al. 2007, 2009). The barley GenomeZipper (chromosome 6H) (Mayer et al. 2011) and syntenic relationship among rice (chromosome 2) and *Brachypodium* (chromosome 3) were also used as a good resource with more than 2,200 markers. Insertion Site-Based Polymorphism (ISBP) marker, which is developed using the junction sequences between

transposable elements and their flanking sequences, has been reported as a powerful tool for wheat studies (Paux et al. 2006). Using our survey sequences (<http://wheat-urgi.versailles.inra.fr/Seq-Repository>) obtained from 6BS and 6BL, we identified more than 40,000 ISBP marker candidates from both arm survey sequences, and then a higher success rate of markers assigned to chromosome 6B was exhibited than those of other sequence resources (Kaneko et al. in preparation). All markers assigned to chromosome 6B were used for PCR screening of BAC libraries, leading to anchor BAC contigs on the chromosome 6B, which cover about 86 % of the entire chromosome. In general, the ISBP markers were scattered while genic markers tended to be clustered within the gene-bearing BAC contigs. These results might reflect the features of wheat genome organization that it is predominantly composed of repetitive elements around small genic region, indicating the efficiency of ISBP markers as anchors because of their randomly distributed pattern on the wheat genome.

The BAC contigs have been assigned to their specific genomic regions using the anchoring markers, which is extremely important because the order of the contigs provides essential information to create a pseudomolecule sequence to reach the final goal of the IWGSC. A genetic map using a recombinant inbred line derived from a cross between CS and winter cultivar ‘Mironovskaya 808’ (M808) was already developed (Kobayashi et al. 2010), which provided the good frame map with SSRs (Fig. 11.1). We have added the markers mentioned above showing polymorphism between CS and M808 to the 6B genetic map. DNA markers on the genetic map has led to assign BAC contigs covering about 33 % of chromosome 6B in length. Because the pericentromeric region is devoid of recombination event, the marker density of genetic map around the centromere is very low. Aiming to overcome this problem, we employed a method using the chromosome deletion lines caused by the gametocidal (Gc) system or γ -ray irradiation to map more DNA markers on chromosome 6B. The Gc system-induced chromosomal breakage of 6B was derived from a cross between CS with monosomic addition of a chromosome 2C from *Aegilops cylindrica* Host and a nullisomic 6B-tetrasomic 6A (N6BT6A) line of CS. Chromosome mapping using both the new developed deletion lines and previously produced lines by Endo and Gill (1996) led to localize DNA markers more than on 70 loci (Sakaguchi et al. in preparation). Radiation hybrid (RH) mapping is known as a powerful tool for a high-resolution mapping in wheat (Tiwari et al. 2012). The RH panel was produced through a cross between the N6BT6A and CS with the pollen freshly irradiated by γ -ray (Watanabe et al. in preparation). Using the above RH panel, we have successfully assigned the BAC contigs that represented more than 80 % of the entire physical length of chromosome 6B. From the genic marker information, the contig order along the RH map could provide a virtual gene order on the chromosome 6B. The results thus allow us to study the relationship between the position of genic markers present on the chromosome 6B BAC contigs and the rice gene order on chromosome 2, which will reveal the microscale rearrangements with a higher degree of resolution than previously identified by comparative mapping using EST (La Rota and Sorrells 2004).

Fig. 11.1 A linkage map of M808 and CS constructed using RILs. Linkage analysis was performed using MAPMAKER/EXP version 3.0b. The threshold for log-likelihood score was set at 3.0, and the genetic distances were calculated with the Kosambi function. The linkage map provides 410 loci of SSR markers, and the total map length is 2814.5 cM with average spacing of 6.9 cM between markers

Concluding Remarks

We have successfully established a physical map of chromosome 6B integrating the BAC contigs and genetic or RH maps using a large number of DNA markers. Our physical map has a high quality and a high resolution, and provides important information on the robust BAC contigs necessary for its genomic sequencing to conduct comparative analysis and prediction of the genomic structure. Currently BAC sequencing is underway using the next-generation sequencer. We will provide a high quality reference sequence to offer an unlimited source on marker development and genome organization of wheat chromosome 6B.

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