Development of BAC Resources for Genomic Research on Wheat

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Abstract: Bread wheat (*Triticum aestivum*, 2*n* = 6*x* = 42) is characterized by a large genome (1C~17,000 Mb) and presence of three homoeologous genomes. These features hamper gene isolation. Various approaches have been devised to overcome this problem. One of them is based on purification of chromosomes or chromosome arms prior to molecular analysis. We have developed a protocol for preparation of suspensions of intact wheat chromosomes and their sorting using laser flow cytometry. Our protocol permitted sorting of up to 500,000 chromosomes in one working day at purities exceeding 90%. DNA of sorted chromosomes was found suitable for cloning into a BAC vector using an improved protocol, which has been optimized for use with small amounts of DNA. Until now, three unique genomic resources for bread wheat were created: a subgenomic BAC library specific for chromosomes 1D, 4D and 6D, a BAC library specific for chromosome 3B, and a BAC library specific for chromosome arm 1BS. The BAC libraries are characterized by large insert size, negligible contamination with DNA of other chromosomes, and excellent genome coverage of individual genome fractions. For example, BAC library specific for the short arm of chromosome 1B (1BS) comprises 65,280 clones with the average insert size of 82 kb. It represents 14.5 equivalents of the 1BS arm with 100% coverage and 90% specificity as confirmed by genetic markers. Our results prove that creation of specific BAC resources representing only few per cent of the whole genome is possible. This work marks the integration of flow cytogenetics and genomics and represents a great leap forward in the wheat genome analysis.