

Second-generation chromosome-specific BAC resources in wheat

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BACKGROUND

Bread wheat, a crop with an enormously large genome (1C~17,000 Mb), substantial fraction of repetitive DNA sequences (>85%) and presence of three homoeologous genomes (A, B and D) poses a hard nut to crack for genome researchers. Flow-cytometric chromosome sorting can be used to simplify the genome analysis by dissecting the genome into small fractions – chromosomes or chromosome arms that represent only a few percent of the hexaploid genome. The standard wheat karyotype enables sorting of chromosome 3B only but all wheat chromosome arms can be sorted from special cytogenetic stocks, mainly ditelosomic lines. Flow-sorted chromosomes (arms) can be used for a variety of applications, the BAC library construction being the most attractive of them.

NEW-GENERATION BAC LIBRARIES

Previously, the flow sorting enabled construction of several subgenomic wheat BAC libraries including a composite library from chromosomes 1D, 4D, 6D, from chromosome 3B, and chromosome arms 1BS, 1RS and 3AS. Although being invaluable resources for wheat physical mapping and positional cloning, these libraries had lower insert size (typically 75-85 kb). This was a consequence of a limited amount of DNA obtained after a time-consuming sorting, which enabled only one size-selection step. Recently, we increased the efficiency of BAC library construction, which made the second size-selection step feasible. This improvement led to a significant increase in insert size. The improved protocol was used to construct BAC libraries specific for chromosome arms 3AL, 3DS, 3DL, 7DS and 7DL and to enlarge libraries from chromosomes 1D, 4D and 6D and from 3B, respectively (Fig. 1). The average insert size in these libraries reaches 100 -125 kb. Further, BAC library specific for chromosome arm 1BL is under construction and several other arm-specific libraries are in pipeline.

CUSTOMIZED BAC LIBRARIES

The increased efficiency of the procedure also enabled construction of a special type of library – customized library – constructed from a smaller number of chromosomes under less stringent size selection. Such libraries can be constructed for positional cloning of genes that are not present in cv. Chinese Spring, which

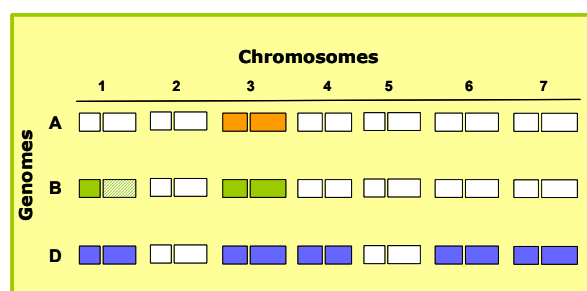


Fig. 1 Chromosome-specific BAC resources presently available for hexaploid wheat

is used for development of a physical framework map of wheat.

Library of this type was constructed from chromosome 3B of cv. Hope. Whereas about 3 millions of chromosomes or 6 millions of chromosome arms are usually used for classical BAC library construction, in this case only 1 million chromosomes were used providing a library with 6x coverage.

CONCLUSIONS

New-generation arm-specific BAC libraries combining high coverage (typically 10-15x) with a relatively small number of clones (typically 36-55,000), which can be easily managed, provide an attractive tool for wheat genomics. The availability of these genomic resources opens avenues for developing physical maps, positional cloning, comparative analyses and sequencing of the wheat genome.

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