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IMPROVING THE TECHNOLOGY FOR FLOW SORTING WHEAT 5A CHROMOSOME ARMS SPECIFIC DNA

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Common wheat (*Triticum aestivum* L. 2n=6x=42 AABBDD) is one of the most important commodity. It is an hexaploid species with a huge genome of 17 Gbp composed for more then 80% of repetitive DNA sequences. Three homeologus genomes and large interdispersed repetitive sequences make genomics of wheat a challenging task. Flow sorting of specific chromosomes and chromosome arms in suspension is a useful and handly approach to dissect complex genomes, on the basis of a smaller amounts accounting for only a few percent of the whole nuclear DNA content. Flow-sorted chromosomes are an invaluable source of DNA that can be used for physical gene mapping, isolation of molecular markers, and construction of chromosome-specific DNA libraries. Here we describe the procedure to obtain 5AS chromosome arm specific DNA by flow cytometry analysis, flow sorting, proteinase K digestion and isothermic DNA amplification. All the primary steps have been optimized in terms of analysis accuracy, time and yield such as: (1) accumulation of cells in metaphase, (2) preparation of chromosome suspensions, (3) flow analysis and sorting, (4) purity control of sorted chromosomes and (5) processing of their DNA. In particular, we have: improved the precision of DAPI chromosome staining; applied a simple and fast FISH method for purity estimation of sorted fractions; adopted a faster processing method to purify and obtain chromosomes specific DNA of high molecular weight. These new achivements will contribute to make easier and more effective the chromosome approach for wheat and other crop.

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