

A Flow-Based Toolkit for Dissection of *Triticeae* Genomes

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Abstract: Although some species of the tribe *Triticeae* possess small genomes, the genomes of barley (~5000 Mb/1C), rye (~8000 Mb/1C), durum wheat (~13,000 Mb/1C) and bread wheat (~17,000 Mb/1C) are complex, consisting mainly of various classes of repetitive DNA sequences. In addition, the recent evolution of wheat involved two episodes of polyploidization giving rise to progenitors of allotetraploid durum wheat and allohexaploid bread wheat. These features hamper physical mapping and gene cloning. Purification of individual chromosomes by flow cytometry can simplify these tasks by providing small and defined genome fractions. Unfortunately, only one chromosome can be discriminated and sorted in each of the four species due to small differences in DNA content. We have demonstrated that this problem can be overcome by means of cytogenetic stocks from which particular chromosomes and chromosome arms can be discriminated and sorted using flow cytometry. The use of telosomic lines of bread wheat facilitates sorting 40 out of 42 chromosome arms. The remaining two arms, 3BL and 5BL, can be purified as isochromosomes. In durum wheat, the use of telosomic lines facilitates sorting any of the 28 chromosome arms. Flow cytometric fractionation of the genomes of barley and rye relies on the capacity of polyploid wheat to maintain chromosomes and chromosome arms of other genomes of *Triticeae* as addition lines. Flow cytometric analysis of wheat-barley telosome addition lines revealed that they may be used to sort any of the fourteen barley chromosome arms. A similar approach can be used to fractionate the genome of rye. We have found that rye chromosomes 2R–7R, which cannot be sorted from a standard karyotype, could be discriminated and sorted from individual wheat-rye addition lines. To conclude, we have developed a universal flow-based platform that is suitable for dissecting the genomes of wheat, barley and rye. Because the DNA of sorted chromosomes is intact, they are suitable for a range of applications including construction of subgenomic DNA libraries and molecular cytogenetic mapping.