

Divide and Conquer: Chromosomal Approach to Cope with the Wheat Genome

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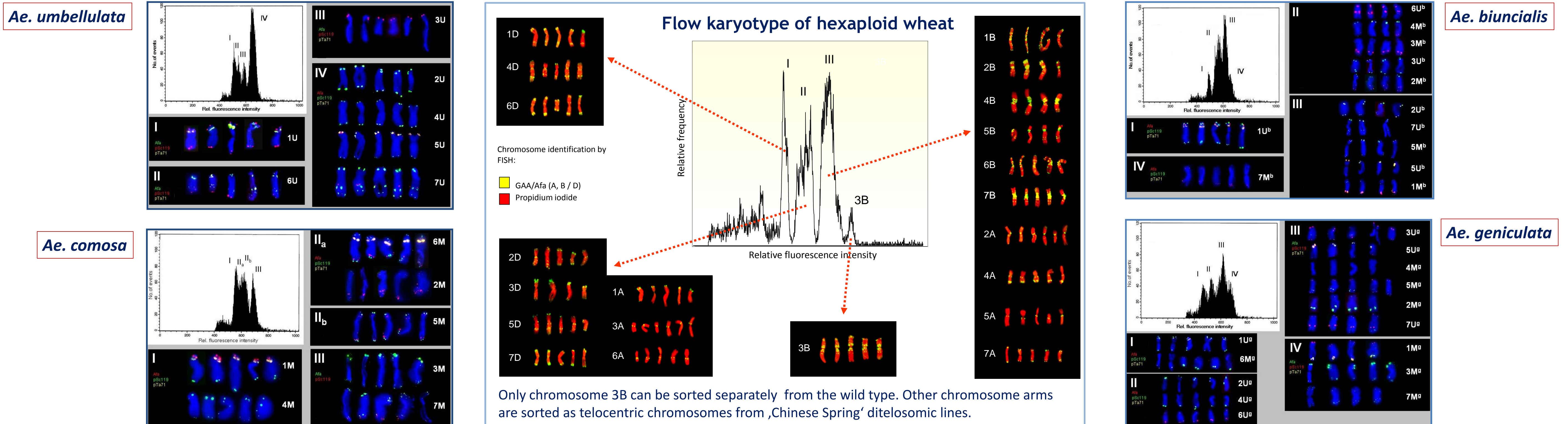
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Bread wheat, a crop with enormously large genome (1C~17 Gbp), substantial fraction of repetitive sequences and three homoeologous subgenomes poses a hard nut to crack for genome researchers. The chromosomal approach, which consists in dissecting the genome into particular chromosomes or chromosome arms by flow-cytometric sorting, helps to cope with these obstacles and facilitates genome analysis, sequencing, as well as positional gene cloning. Wheat chromosomal genomics was established ten years ago and since that time a large portfolio of applications were developed. The chromosome-based approach is not restricted to hexaploid wheat, but can be applied in other species of Triticeae such as barley and rye and even in wild relatives of wheat such as *Aegilops umbellulata*, *Ae. comosa*, *Ae. biuncialis* and *Ae. geniculata*.

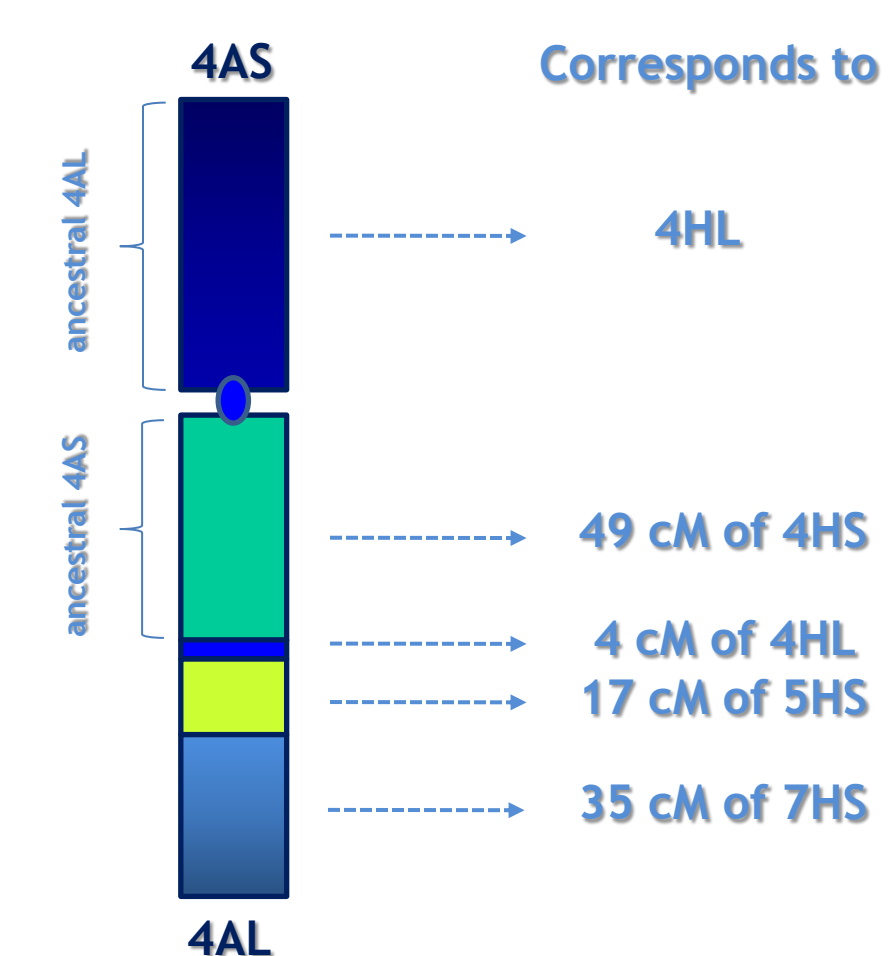


NEXT-GENERATION SEQUENCING of flow-sorted chromosomes

Sequencing 4A

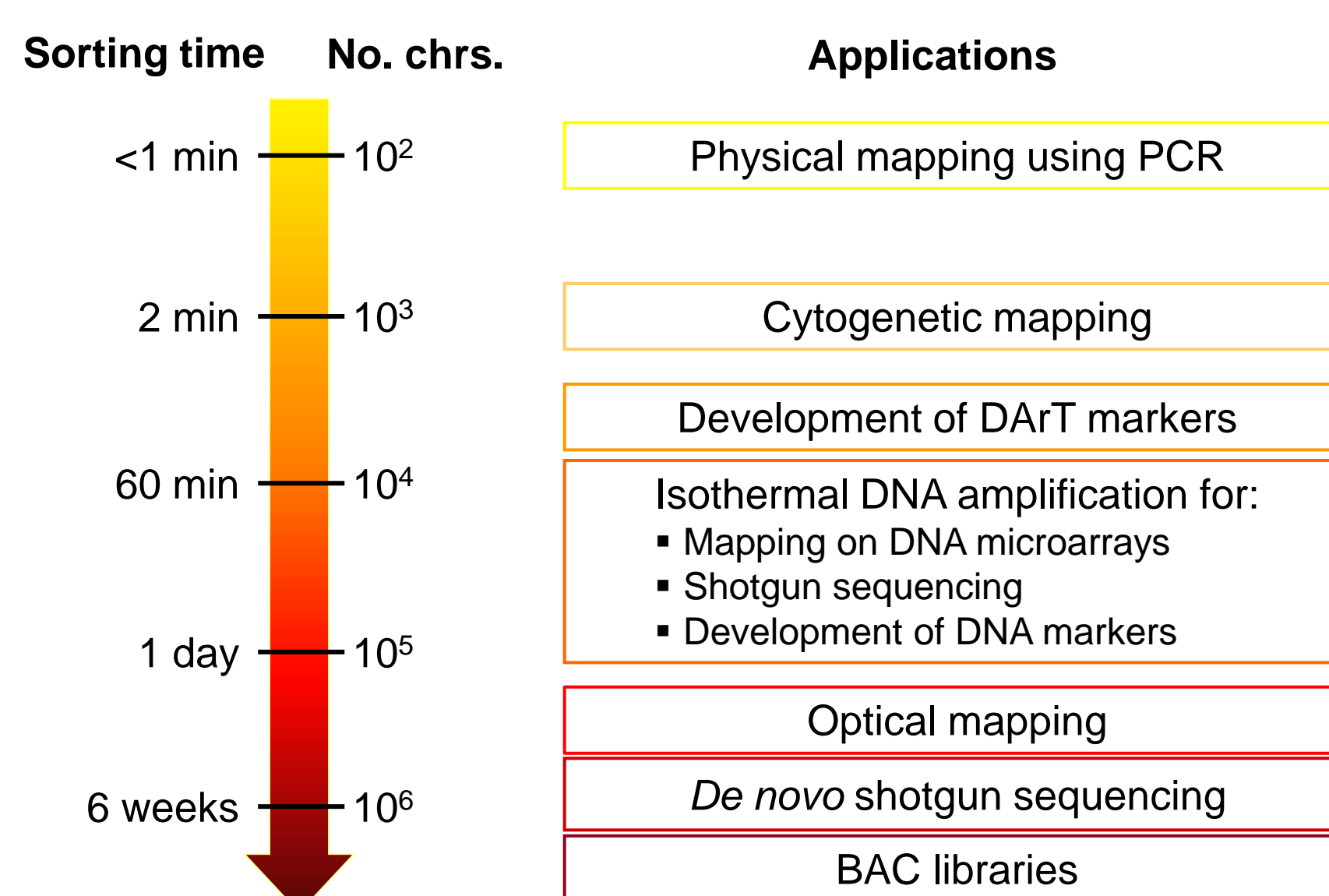
Phi29-amplified DNA of flow-sorted 4AL and 4AS chromosome arms was low-pass sequenced by 454 to a coverage of 1.6x for the 4AL and 2.3x for the 4AS arm. Genic sequence reads were integrated and ordered based on syntenic relationships with genomes of *Brachypodium*, rice and *Sorghum* using 4-way GenomeZipper.

Complicated origin of the chromosome 4A was proposed based on comparison with the diploid genome of barley.



Sequencing of the entire set of wheat chromosomes is in progress.

APPLICATIONS OF FLOW-SORTED CHROMOSOMES



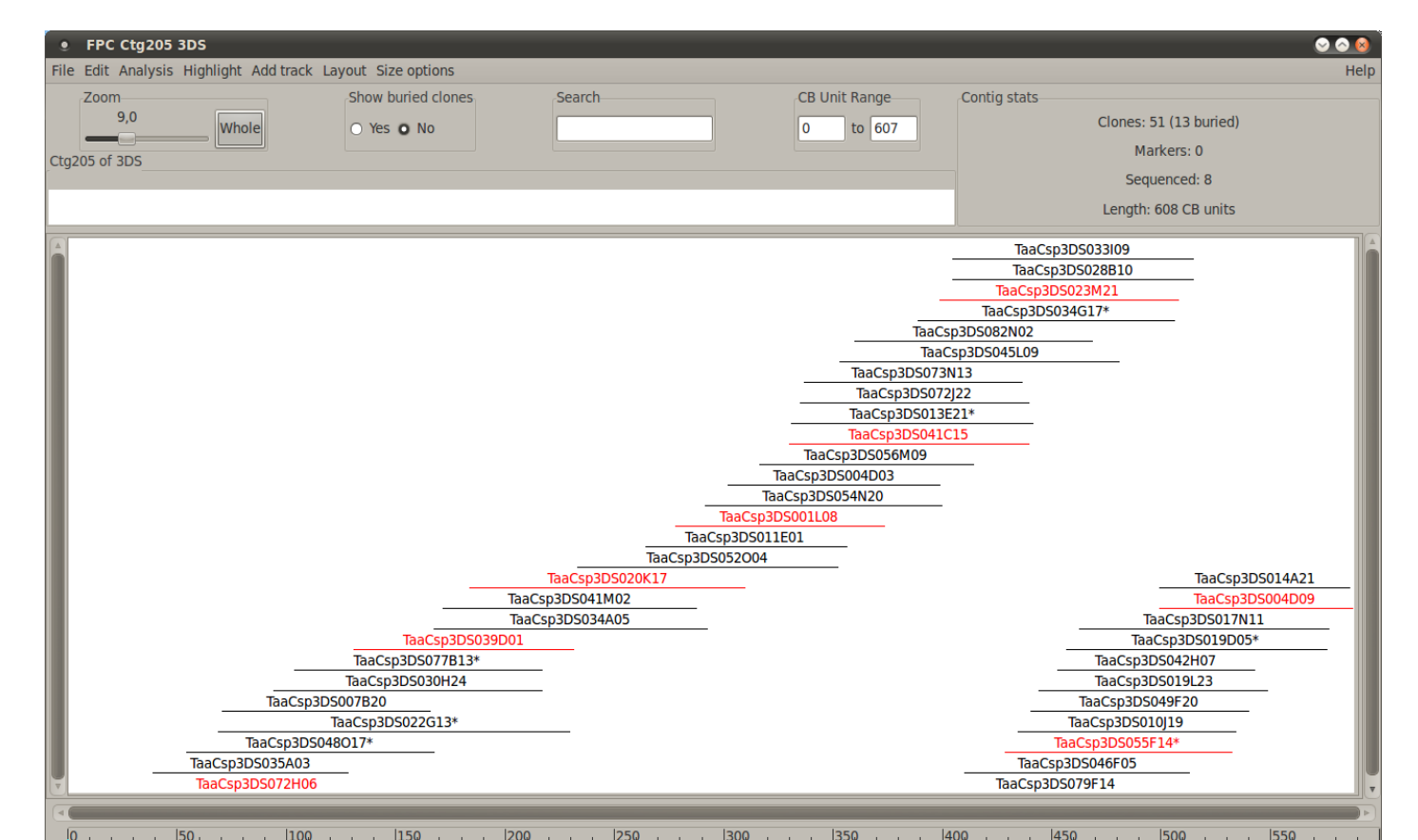
CHROMOSOME/ARM SPECIFIC BAC LIBRARIES

Species	Cultivar	Chromosome/arm	No. clones	Insert size	Coverage
<i>T. aestivum</i>	Chinese Spring	1D, 4D, 6D	148,224	102kb	6.9x
<i>T. aestivum</i>	Chinese Spring	1D, 4D, 6D	138,240	116kb	7.4x
<i>T. aestivum</i>	Chinese Spring	1AL	92,544	106kb	15.7x
<i>T. aestivum</i>	Chinese Spring	1AS	31,104	111kb	11.8x
<i>T. aestivum</i>	Chinese Spring	1BL	92,160	114kb	15.4x
<i>T. aestivum</i>	Chinese Spring	1BS	55,296	113kb	15.7x
<i>T. aestivum</i>	Chinese Spring	2AL	76,800	120kb	15.8x
<i>T. aestivum</i>	Chinese Spring	2AS	56,832	123kb	15.4x
<i>T. aestivum</i>	Chinese Spring	3AL	55,296	106kb	10.2x
<i>T. aestivum</i>	Chinese Spring	3AL	24,576	114kb	5.2x
<i>T. aestivum</i>	Chinese Spring	3AS	55,296	80kb	10.9x
<i>T. aestivum</i>	Chinese Spring	3AS	55,296	115kb	15.9x
<i>T. aestivum</i>	Chinese Spring	3B	67,968	103kb	6.2x
<i>T. aestivum</i>	Chinese Spring	3B	82,176	126kb	9.1x
<i>T. aestivum</i>	Chinese Spring	3DL	64,512	105kb	12.2x
<i>T. aestivum</i>	Chinese Spring	3DS	36,864	110kb	11.0x
<i>T. aestivum</i>	Chinese Spring	4AL	92,160	126kb	17.3x
<i>T. aestivum</i>	Chinese Spring	4AS	49,152	131kb	16.6x
<i>T. aestivum</i>	Chinese Spring	5AL	90,240	123kb	18.3x
<i>T. aestivum</i>	Chinese Spring	5AS	46,080	120kb	16.5x
<i>T. aestivum</i>	Chinese Spring	5BS	43,776	122kb	15.8x
<i>T. aestivum</i>	Chinese Spring	6BL	76,032	130kb	18.0x
<i>T. aestivum</i>	Chinese Spring	6BS	57,600	132kb	15.3x
<i>T. aestivum</i>	Chinese Spring	7AS	58,368	134kb	15.4x
<i>T. aestivum</i>	Chinese Spring	7BS	27,648	182kb	12.5x
<i>T. aestivum</i>	Chinese Spring	7DL	50,304	116kb	14.9x
<i>T. aestivum</i>	Chinese Spring	7DS	49,152	113kb	12.1x
<i>T. aestivum</i>	Hope	3B	92,160	78kb	6.0x
<i>T. aestivum</i>	Pavon	1BS	65,280	82kb	14.5x
<i>S. cereale</i>	Imperial	1RS	36,864	75kb	5.2x
<i>S. cereale</i>	Imperial	1RS	66,816	72kb	8.8x

<http://olomouc.ueb.cas.cz/dna-libraries/cereals>

CONSTRUCTION OF PHYSICAL MAPS of 3DS, 4AL and 7DS chromosome arms

The chromosome-based strategy in the physical map construction simplifies significantly contig assembly and provides an efficient approach to cope with polyploidy. Arm-specific BAC libraries of 3DS, 7DS and 4AL were fingerprinted using SNaPshot-based HICF technology. BAC fingerprints were assembled using FPC with tolerance of 0.4bp. Initial assembly performed at e-75 was subsequently run through single-to-end and end-to-end merges at six successively lower cutoffs finishing at e-45.



Ctg205 from 3DS physical map. Red BAC clones belong to 3DS Minimum Tiling Path (MTP).

Chromosome arm	3DS	7DS	4AL
Chromosome-arm size	321 Mb	381 Mb	539 Mb
No. fingerprinted clones	36,864	49,152	63,174
Useful fingerprints	27,880	39,765	60,144
No. contigs (e ⁻⁴⁵)	1,360	1,767	3,617
Assembly length	310 Mb	468 Mb	942 Mb
N50-contig length	244 kb	308 kb	260 kb
No. MTP clones	3,823	6,022	10,671

ALLOCATION OF DaRT MARKERS to wheat and barley chromosomes/arms

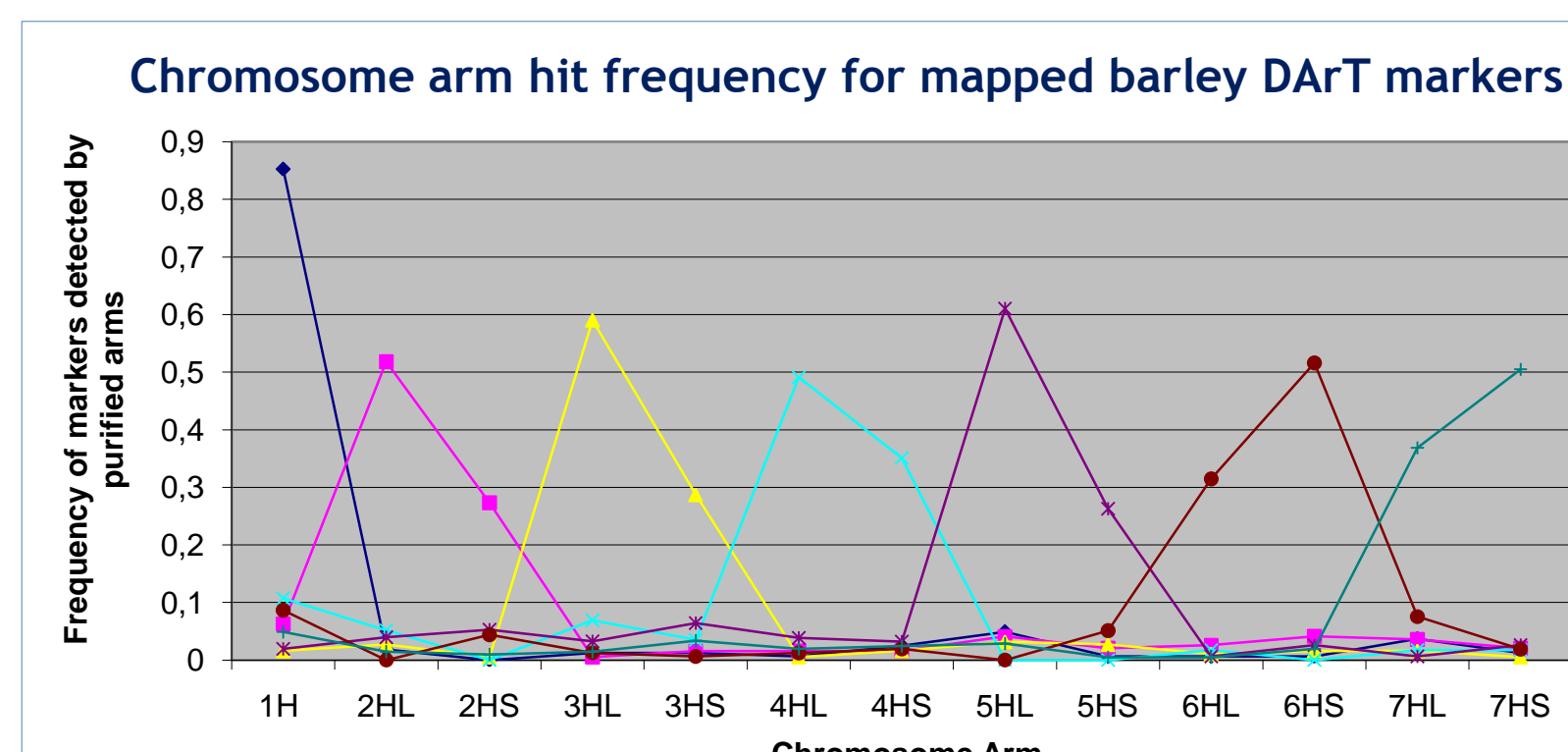
DNAs of individual chromosomes/arms of wheat and barley, respectively, were hybridized with

Wheat DaRT Array version 3.0 (with 7,680 sequenced markers)

> 1,454 markers previously unmapped } unambiguously assigned to a chromosome arm
 > 1,094 genetically mapped }

Barley DaRT Array Version 2.0 (with 3,840 sequenced markers)

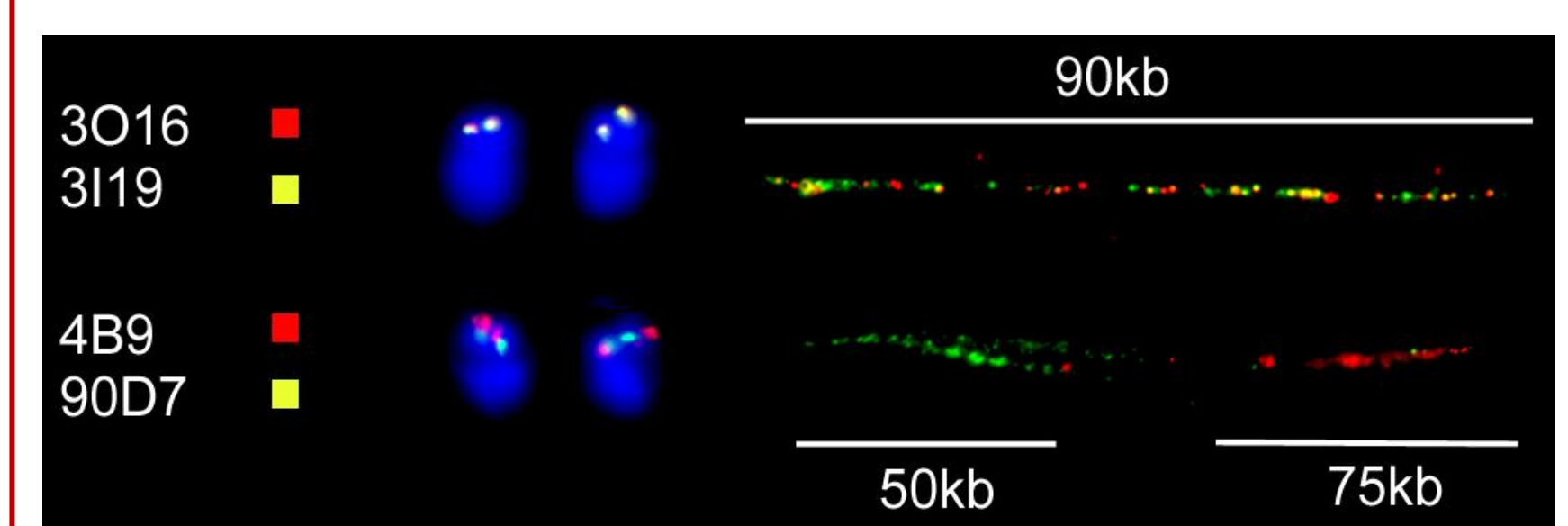
> 733 markers previously unmapped } unambiguously assigned to a chromosome arm
 > 1,187 genetically mapped }



CYTOGENETIC MAPPING

Advantages of the flow-based approach

- > Higher throughput (mapping on large populations of chromosomes)
- > Higher sensitivity (localization of short DNA sequences ~2 kb)
- > Higher spatial resolution



Localization of two couples of BACs on mitotic and stretched telosomes 1BS, respectively.

