

establishing
chromosome genomics
in grasses

david kopecký

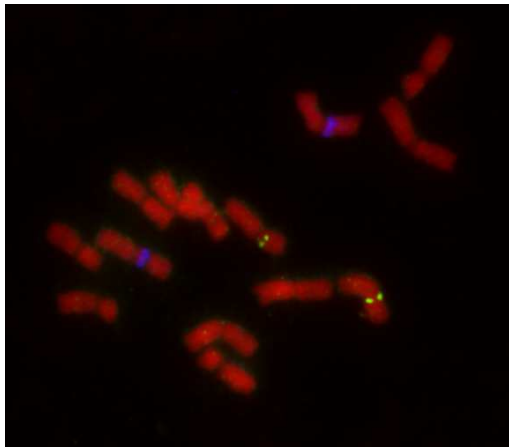
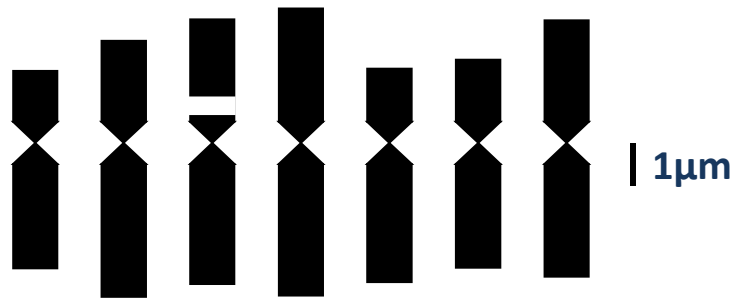
laboratory of molecular cytogenetics and cytometry
centre of the region haná for biotechnological and agricultural research
olomouc, czech republic

grass genome (meadow fescue, italian ryegrass)

- diversification 2,915,537 years and 78 days ago (+/- 300,000 years)
- 1C=3,175Mb (fescue), 2,567Mb (ryegrass)
- karyotypes characterized (7 chromosome pairs)
- genetic maps available: 521 and 881 markers (including DArTs)
- limited sequence information available (fescue)



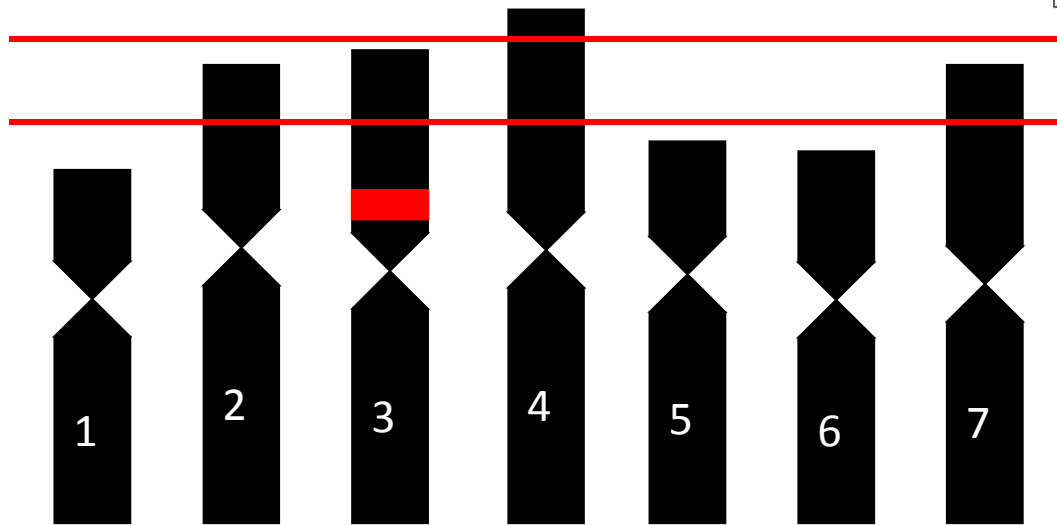
meadow fescue



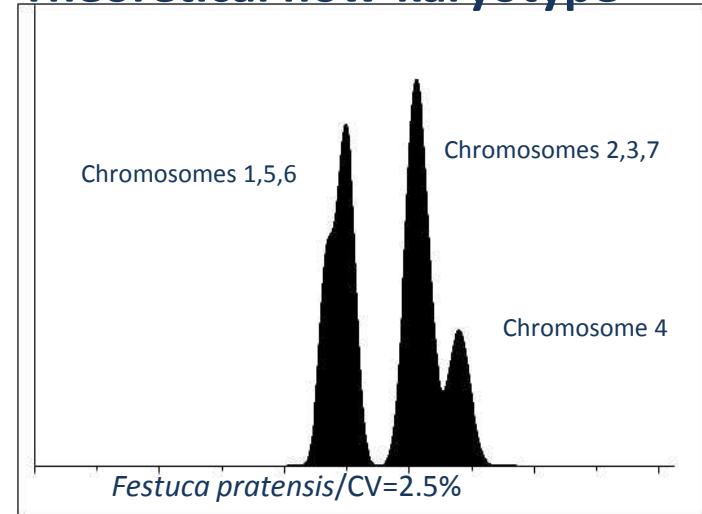
KARYOTYPING OF MEADOW FESCUE

Chromosome	1	2	3	4	5	6	7
Size (Mbp)	373	485	499	543	403	394	484
Cen							
Tel							
45SrDNA							
5SrDNA							
1F21							
1G18							
2N14							
2O16							
1K11							

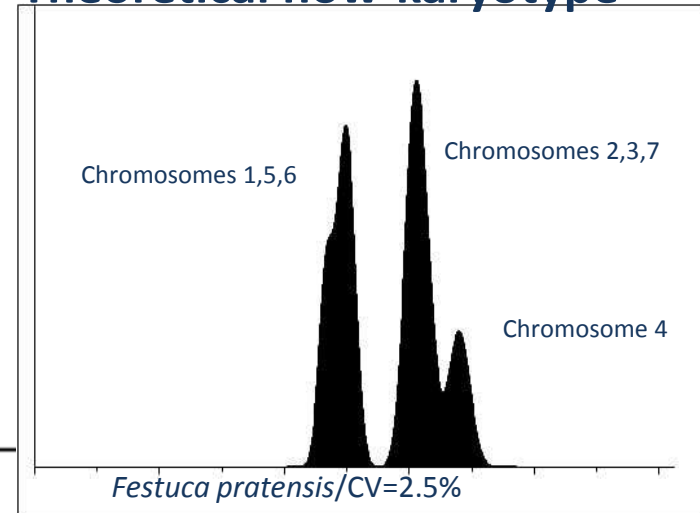
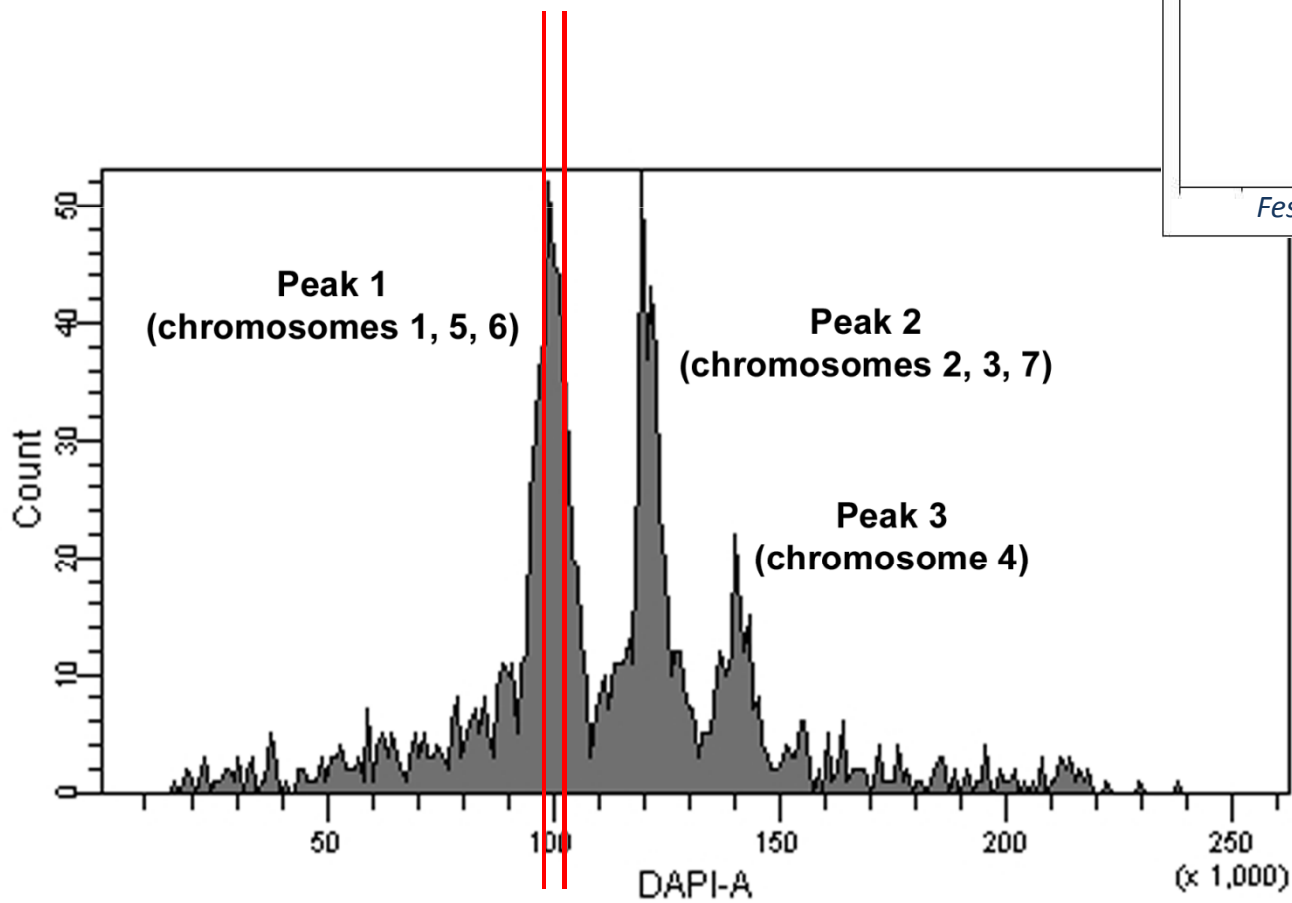
Sorting individual/groups of chromosomes



Theoretical flow-karyotype

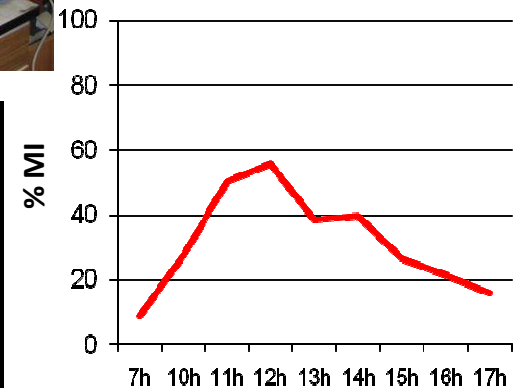
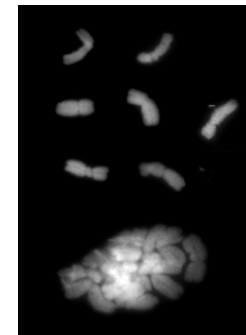
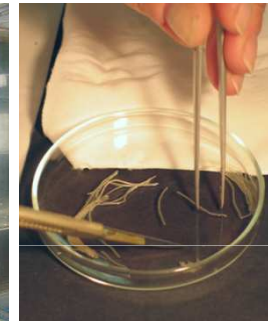
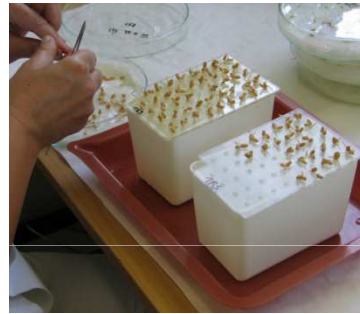
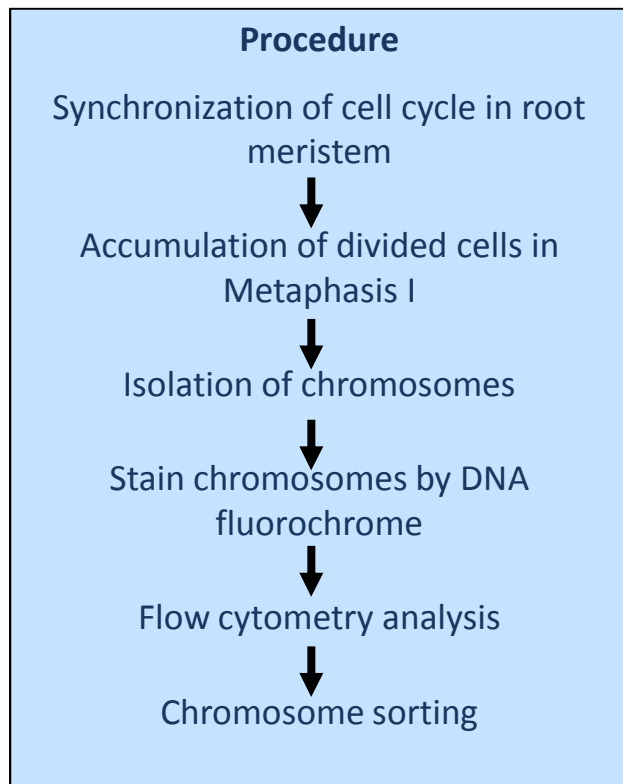


Theoretical flow-karyotype



synchronization of cell cycle and chromosome sorting

sample for sorting is a suspension of intact mitotic chromosomes



18h
1.5mM HU

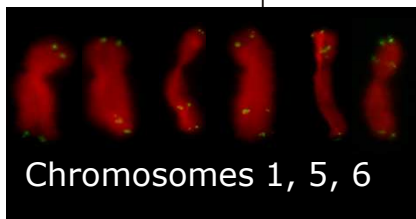
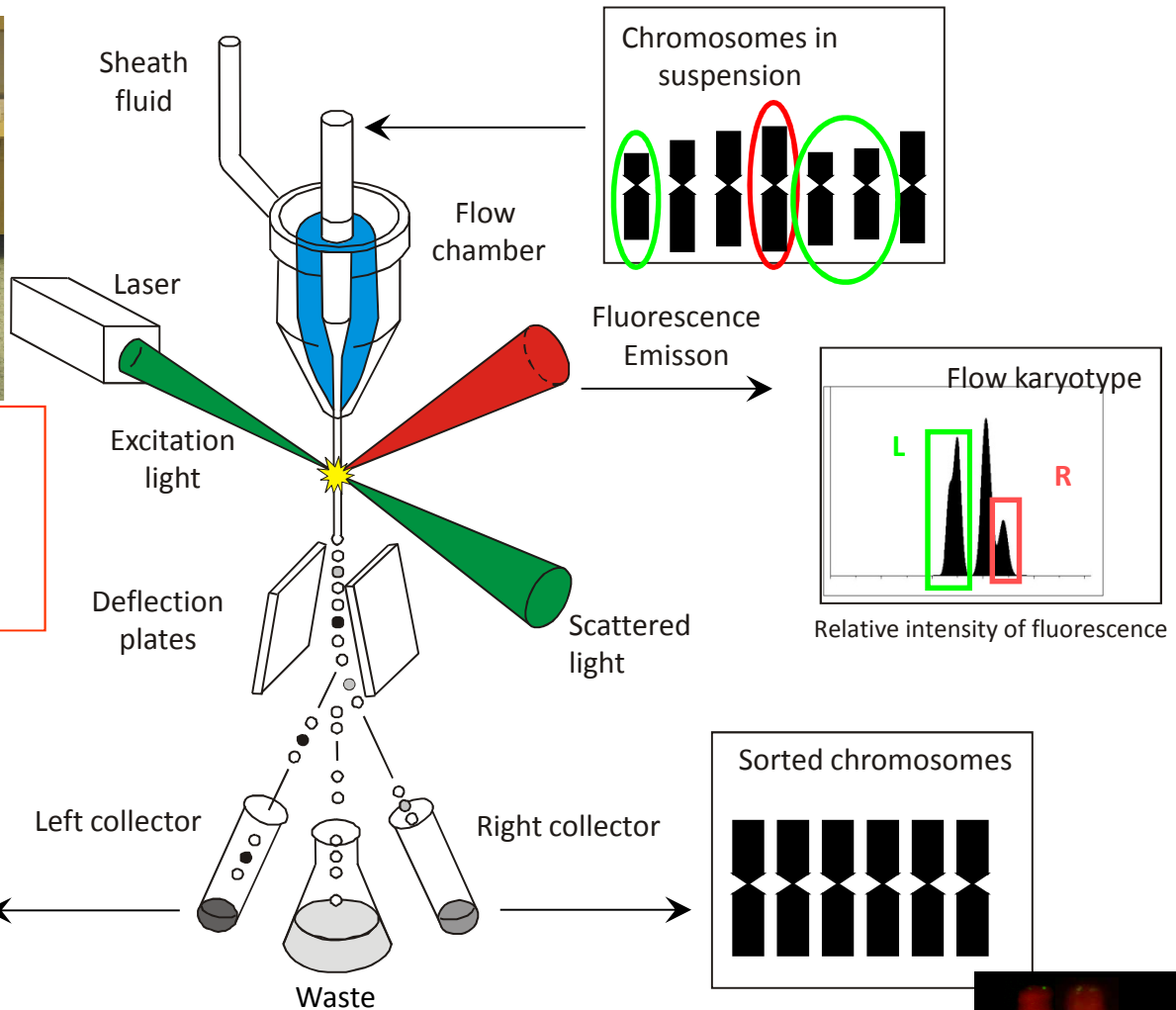
4.5h
Recovery time

2h
5uM oryzalin

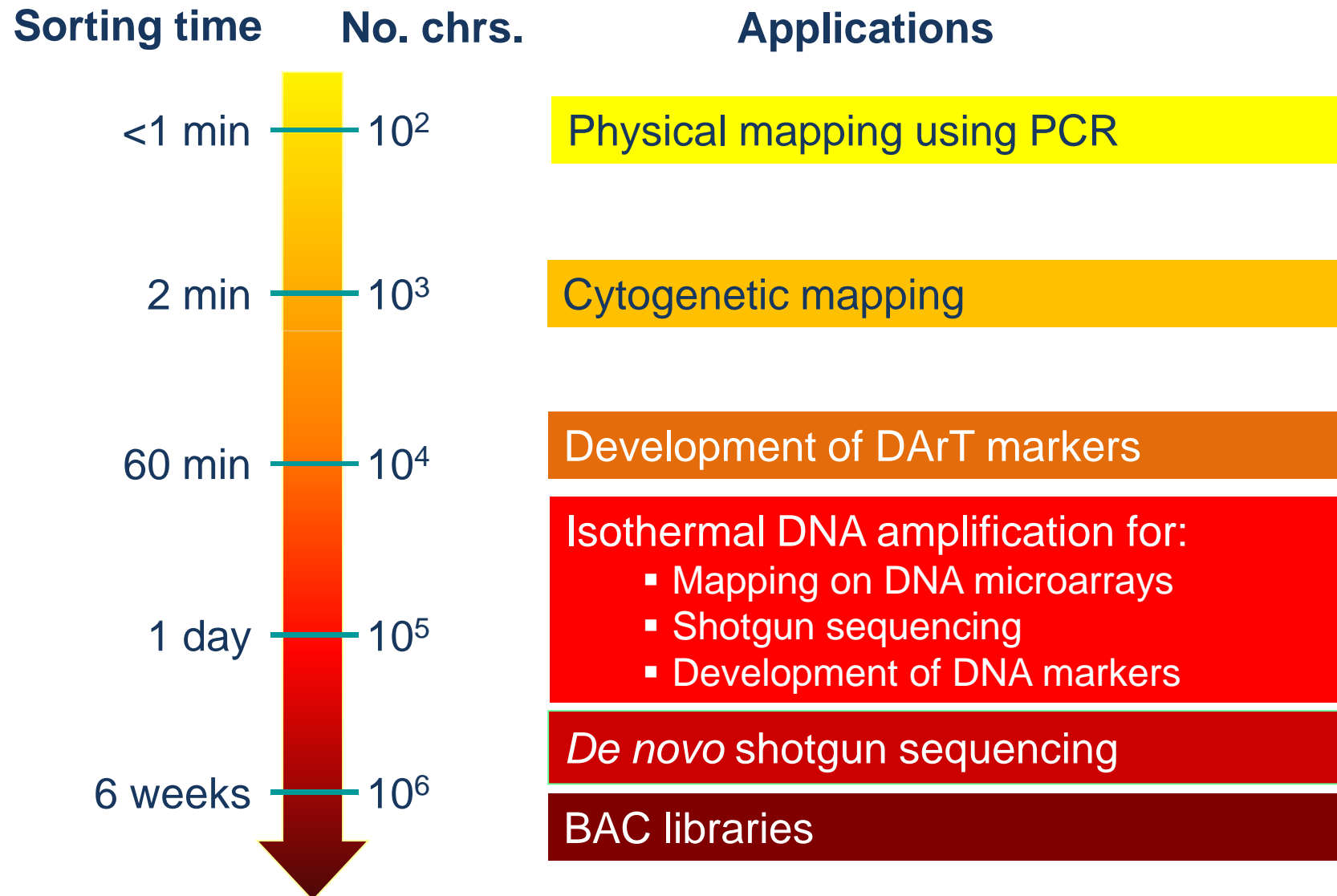
synchronization of cell cycle and chromosome sorting



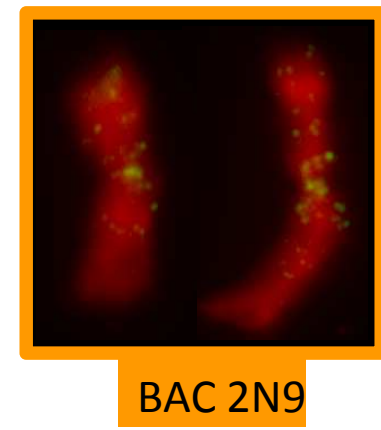
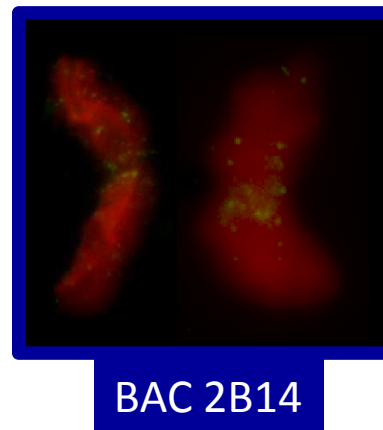
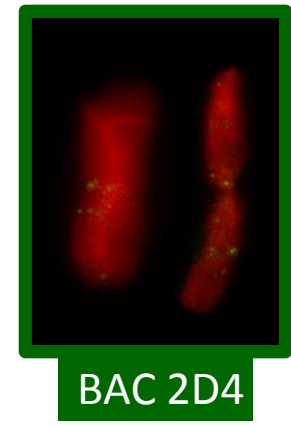
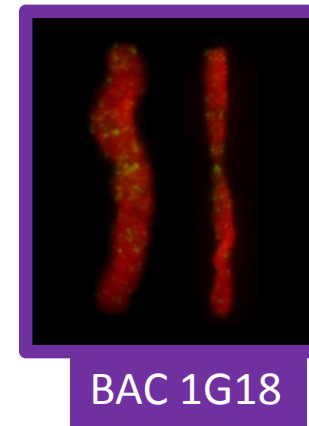
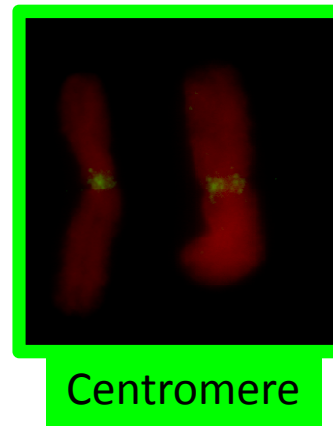
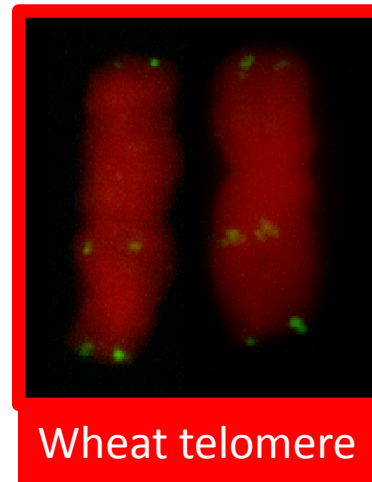
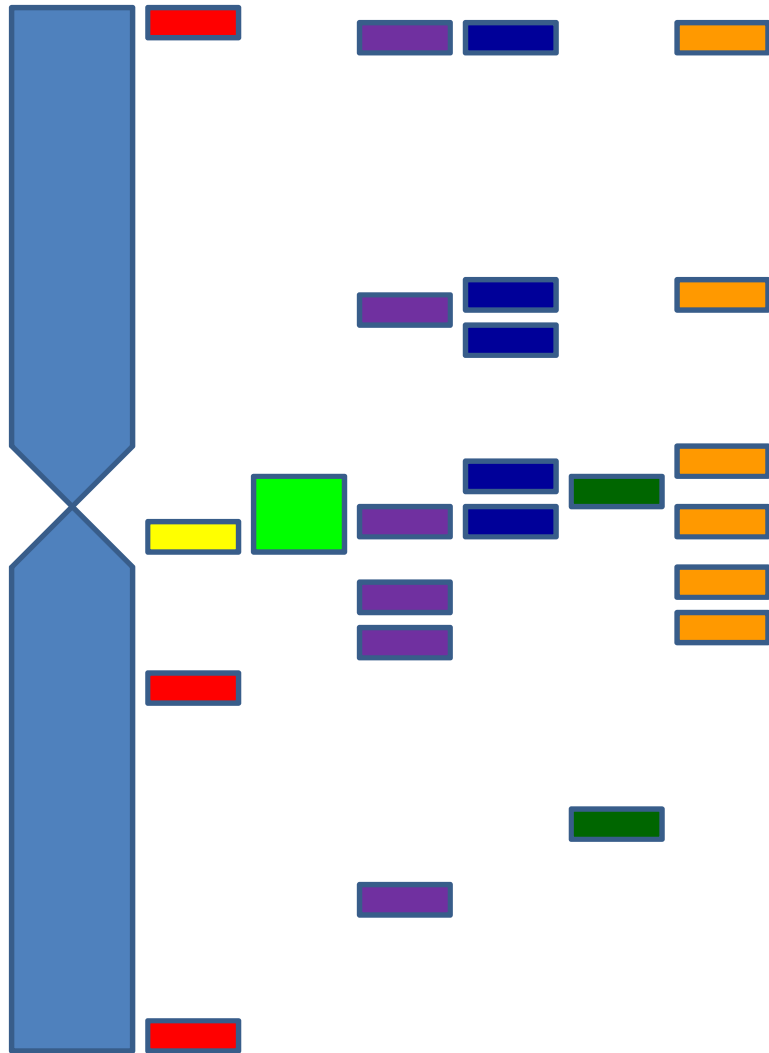
- Speed:
~1000 chromosomes/sec
- Yield per day:
2 - 5x10⁵ chromozómů



what to do with sorted chromosomes?

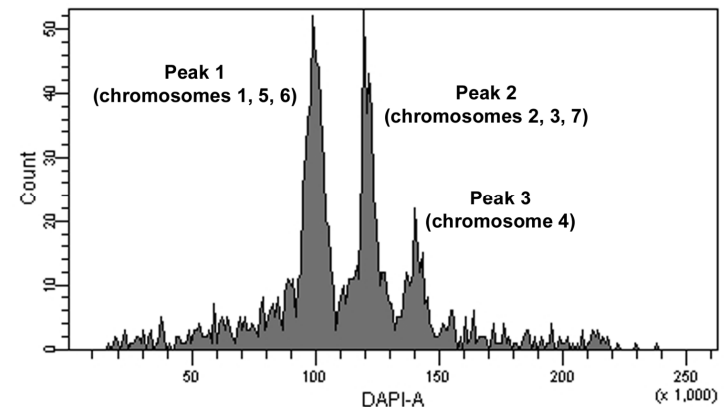


cytogenetics of chromosome 4F



sequencing of chromosome 4F

- purity of sorted chromosome: 92.4%
- 140,000 copies of chromosome 4 amplified using GenomiPhi DNA Amplification Kit (GE Healthcare)
- sequenced by Illumina HiSeq2000 (IGA)
- 28,270 Mbp (over 50x coverage)



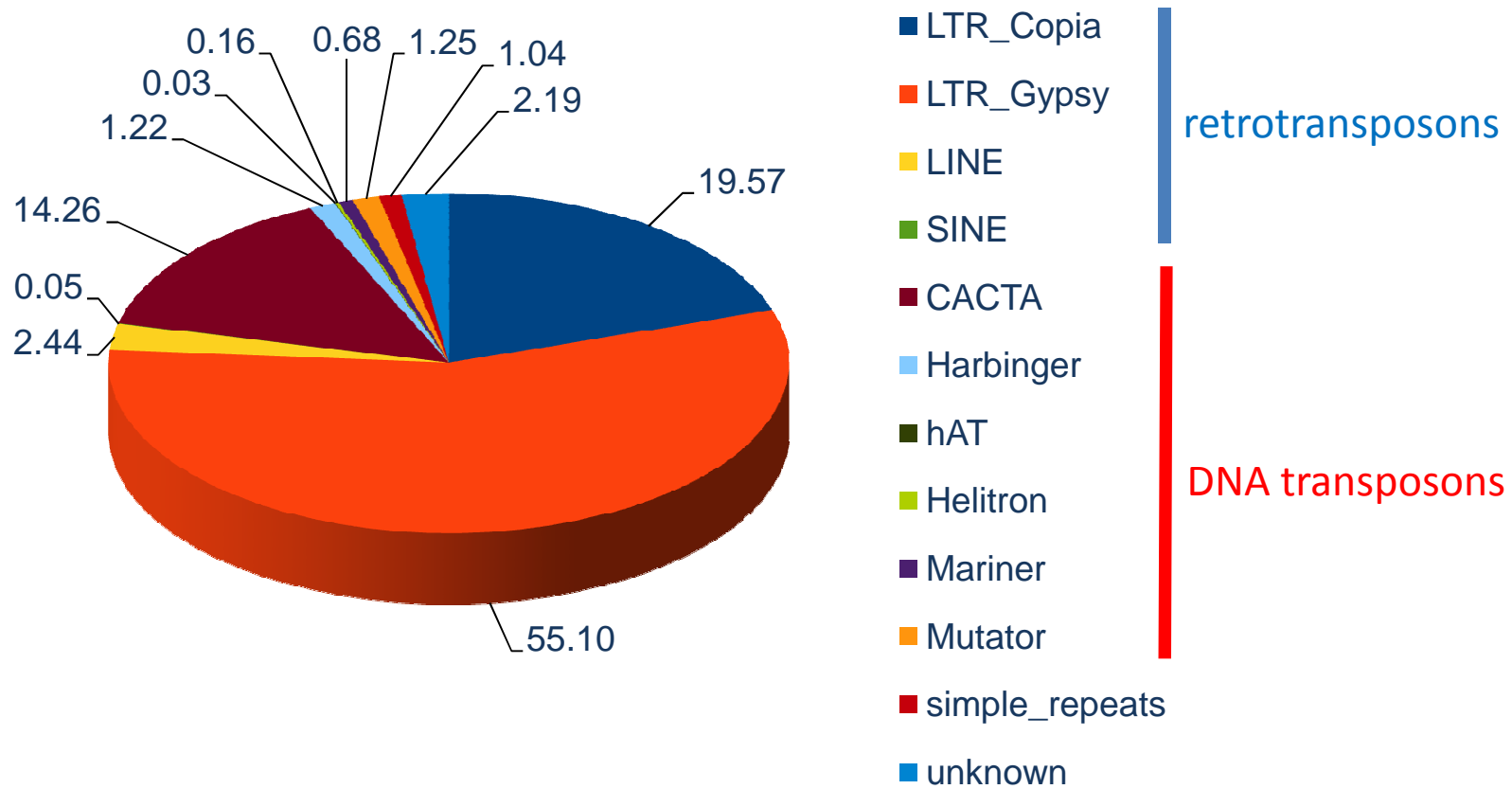
assembly

program SOAPdenovo with k-mer size 70

	4F
contigs	4,057,786
min bp	72
max bp	15,919
average	125.5
L50	132
# bp	509,357,265
GC-content [%]	42.58

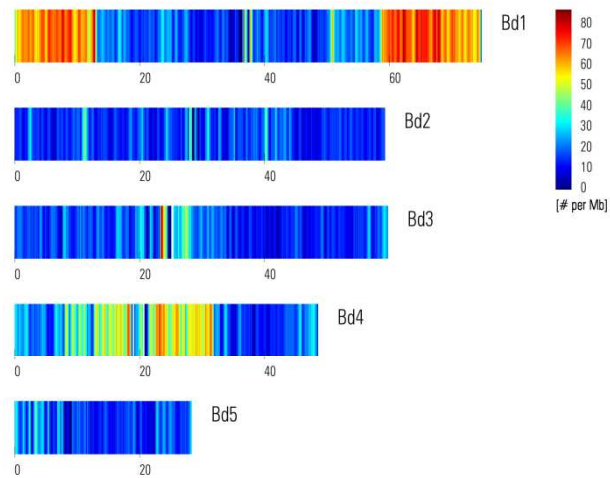
repeats

only **22.05%** sequences with hits in the repeat database

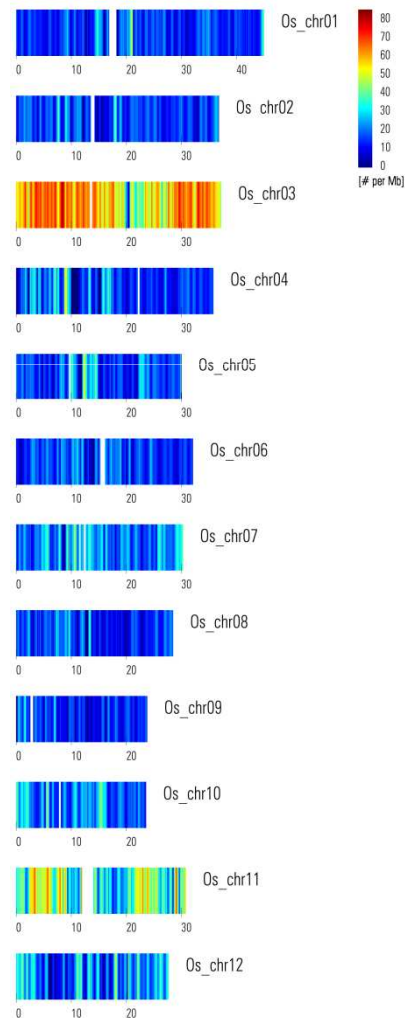


collinearity with brachy, rice and sorghum

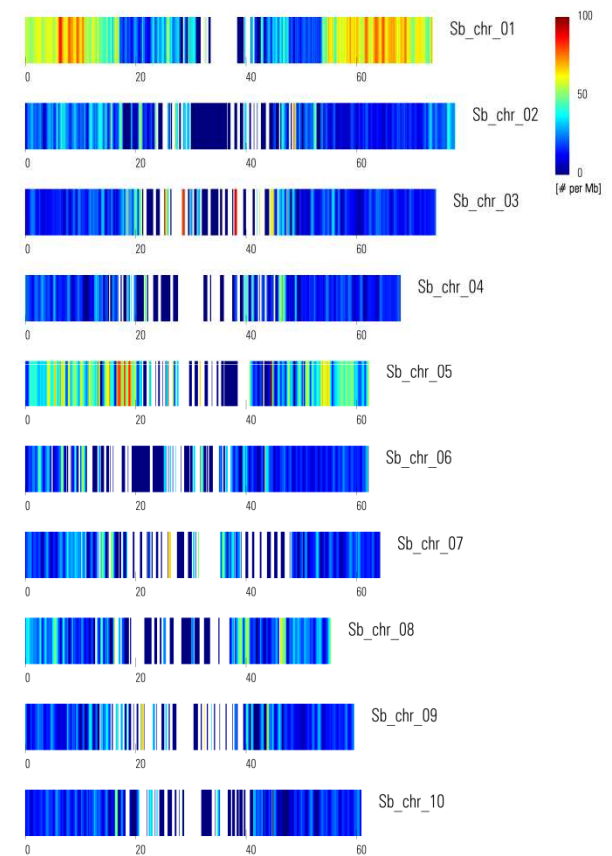
festuca_4f_vs_brachy



festuca_4f_vs_rice

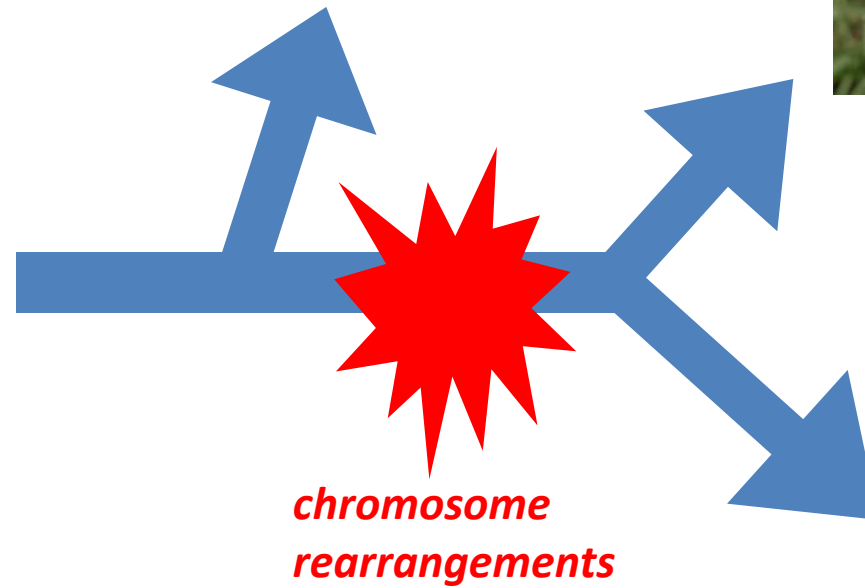
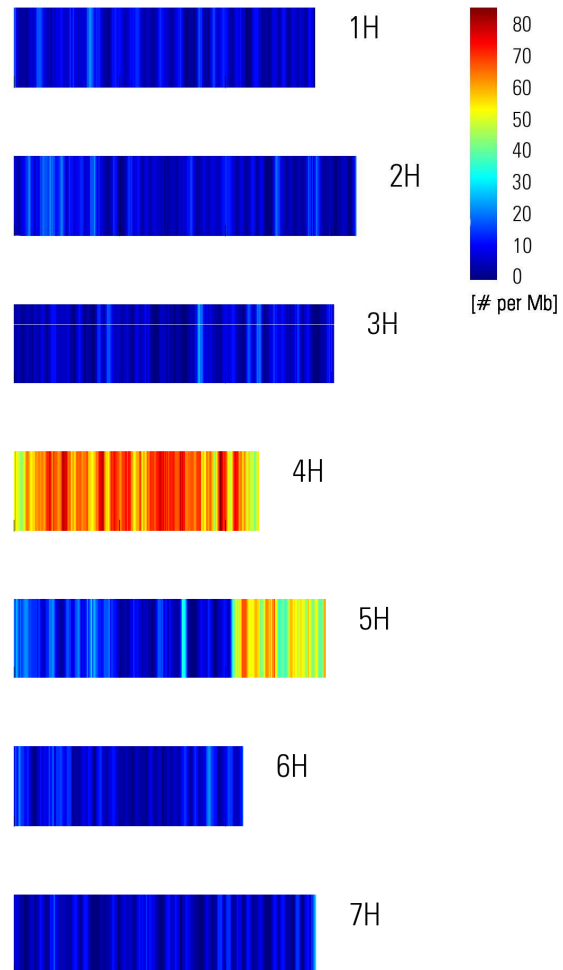


festuca_4f_vs_sorghum



collinearity with barley

festuca_4F_vs_barley



traits associated with chromosome 4 (F, L)

- freezing tolerance
- resistance to crown rust
- bacterial wilt resistance

acknowledgements

synchronization of cell cycle and accumulation of metaphases

- jarmila číhalíková
- jitka kopecká

chromosome sorting

- jan vrána
- petr navrátil

cytogenetics

- mirka havránková
- tomasz książczyk



chromosomal DNA amplification

- hana šimková

sequencing

- federica cattonaro



Czech Science Foundation

genome zipper

- mihaela maria martis
- klaus mayer

HelmholtzZentrum münchen
German Research Center for Environmental Health