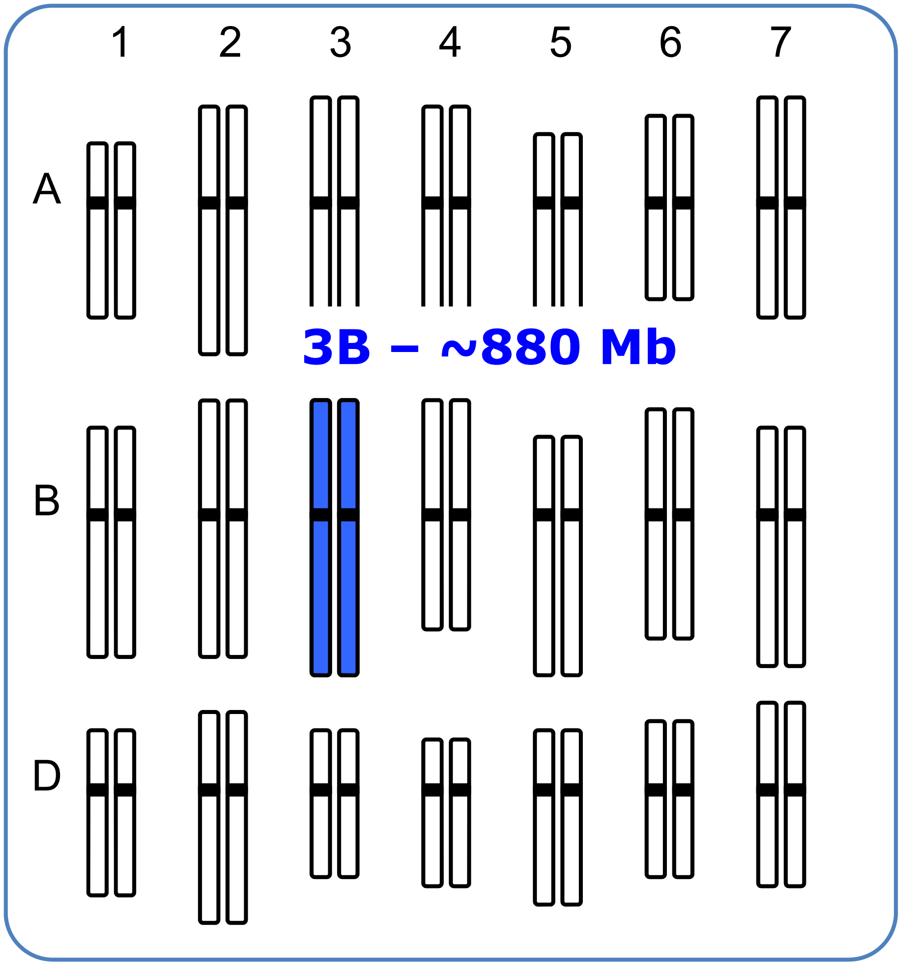
PAG 2014, San Diego

A reference sequence of wheat chromosome 3B

Frédéric CHOULET

INRA GDEC – Clermont-Ferrand, France

❑ Hexaploid wheat genome



- Hexaploid AABBDD
- 17 Gb

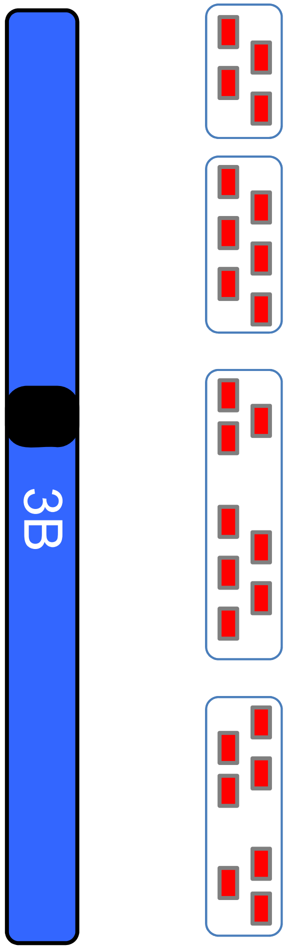
3B

- physical map v2

(Rustenholz et al. 2011)

➔ Reference sequence

❑ 3B MTP-BAC sequencing



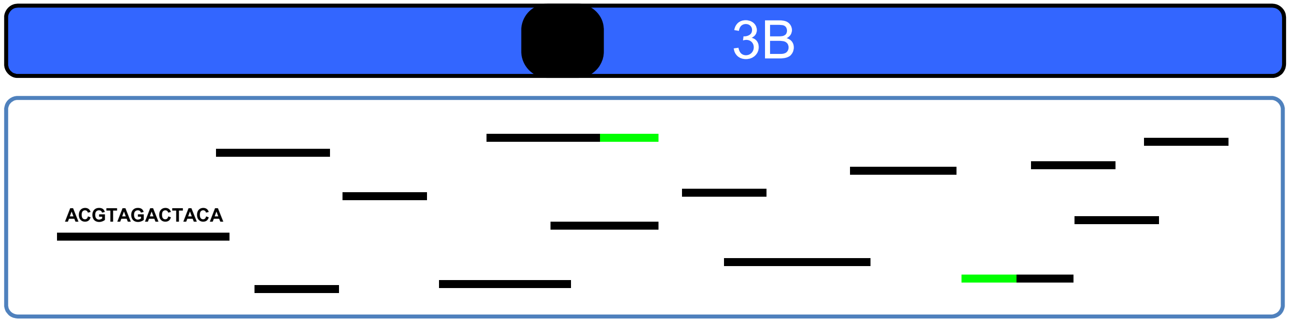
	Sequenced physical map
• Depth	19x
• #BAC-contigs	1282
• #MTP BACs	8452
• #BAC pools	922
• #Roche 8 kb MP lib.	922
• #GS-FLX runs	150
• bp coverage (Roche/454)	36x
• BAC-ends (Sanger)	42,551
• Whole 3B shotgun (Illumina)	82x

❑ RNASeq – Deep transcriptome sequencing

- **30 samples**
  - ○ 5 organs (root, stem, spike, leaf, grain)
  - ○ 3 dev. stages
  - ○ x2 replicates

Presenter: **Lise Pingault**
Session: Analysis of complex genomes – Saturday 5:20 PM

❑ Assembly and scaffolding



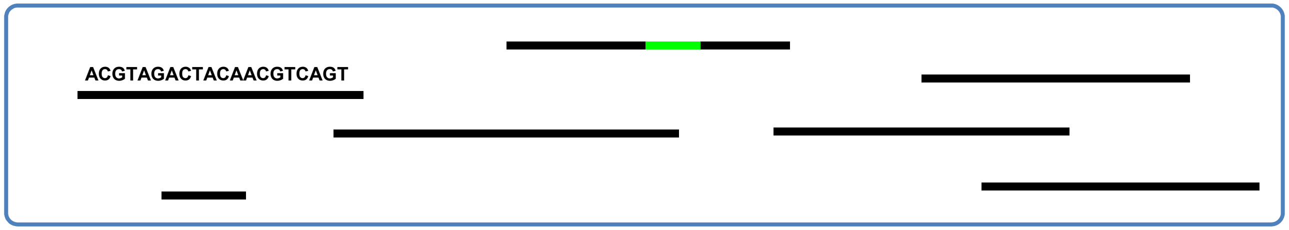
3B-v1

16,136 scaff

1040 Mb

18% Ns

- Manual improvement of the scaffolding
- Gap filling
- Homopolymer error corrections
- Redundancy removal – scaffold merging



3B-v443

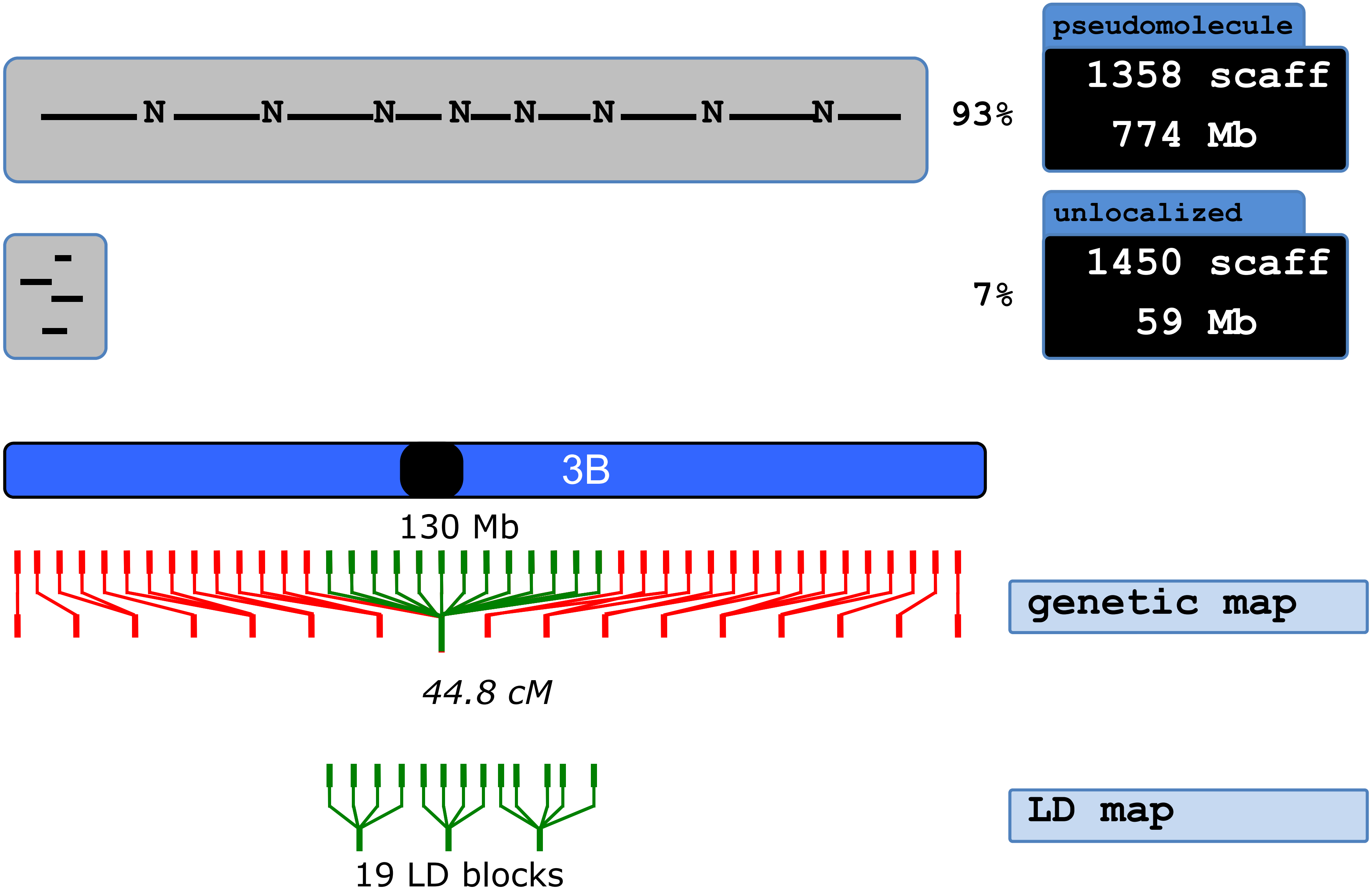
2808 scaff

833 Mb

N50:892 kb

7% Ns

Ordering scaffolds



93%

pseudomolecule

1358 scaff

774 Mb

7%

unlocalized

1450 scaff

59 Mb

3B

130 Mb

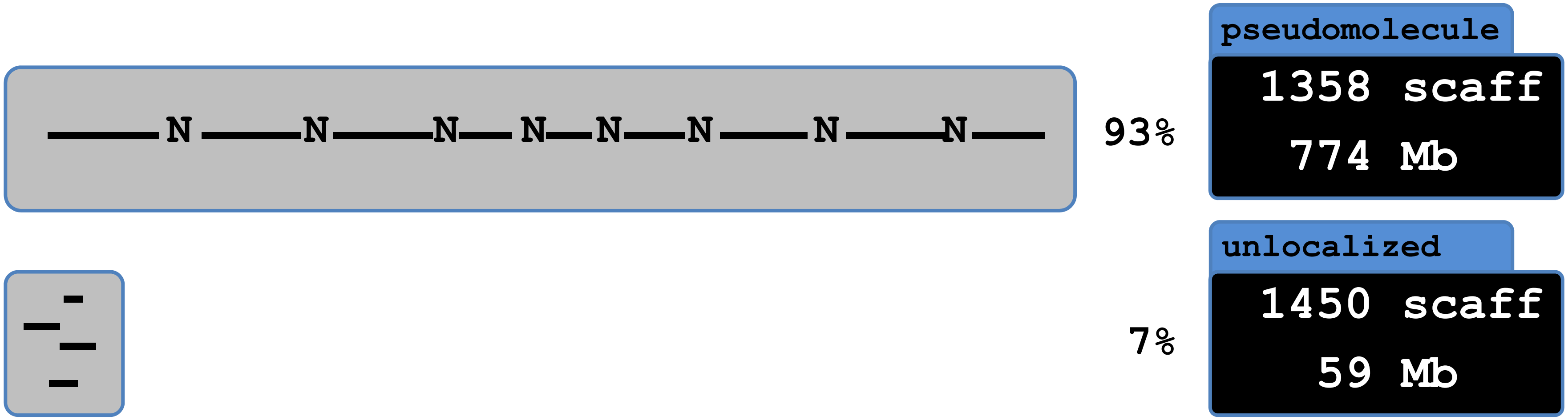
genetic map

44.8 cM

LD map

19 LD blocks

❑ Completeness



93%

pseudomolecule

1358 scaff

774 Mb

7%

unlocalized

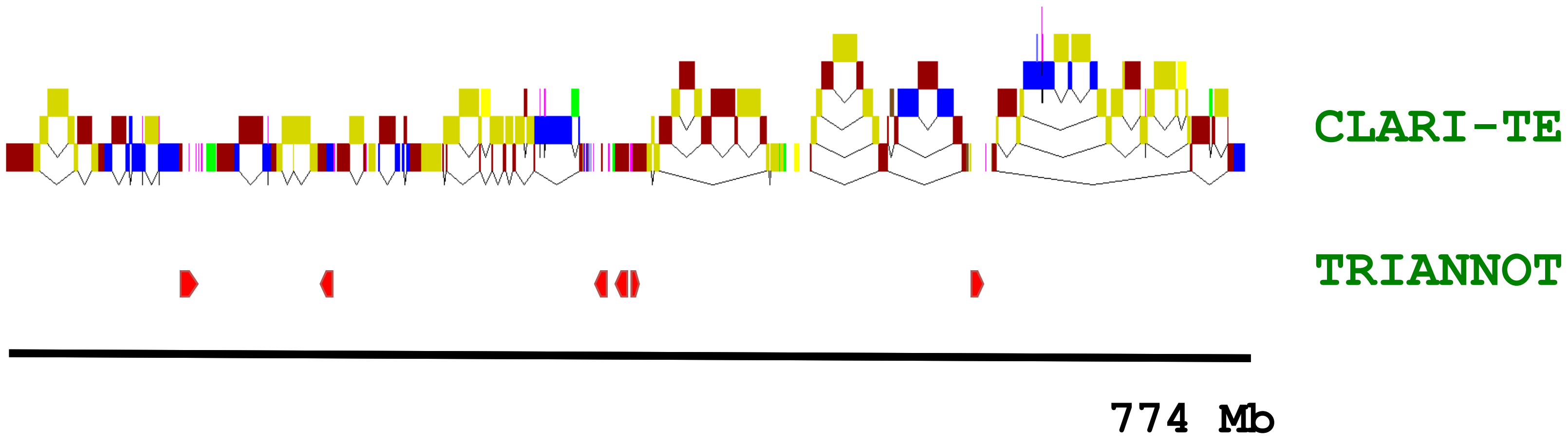
1450 scaff

59 Mb

94%

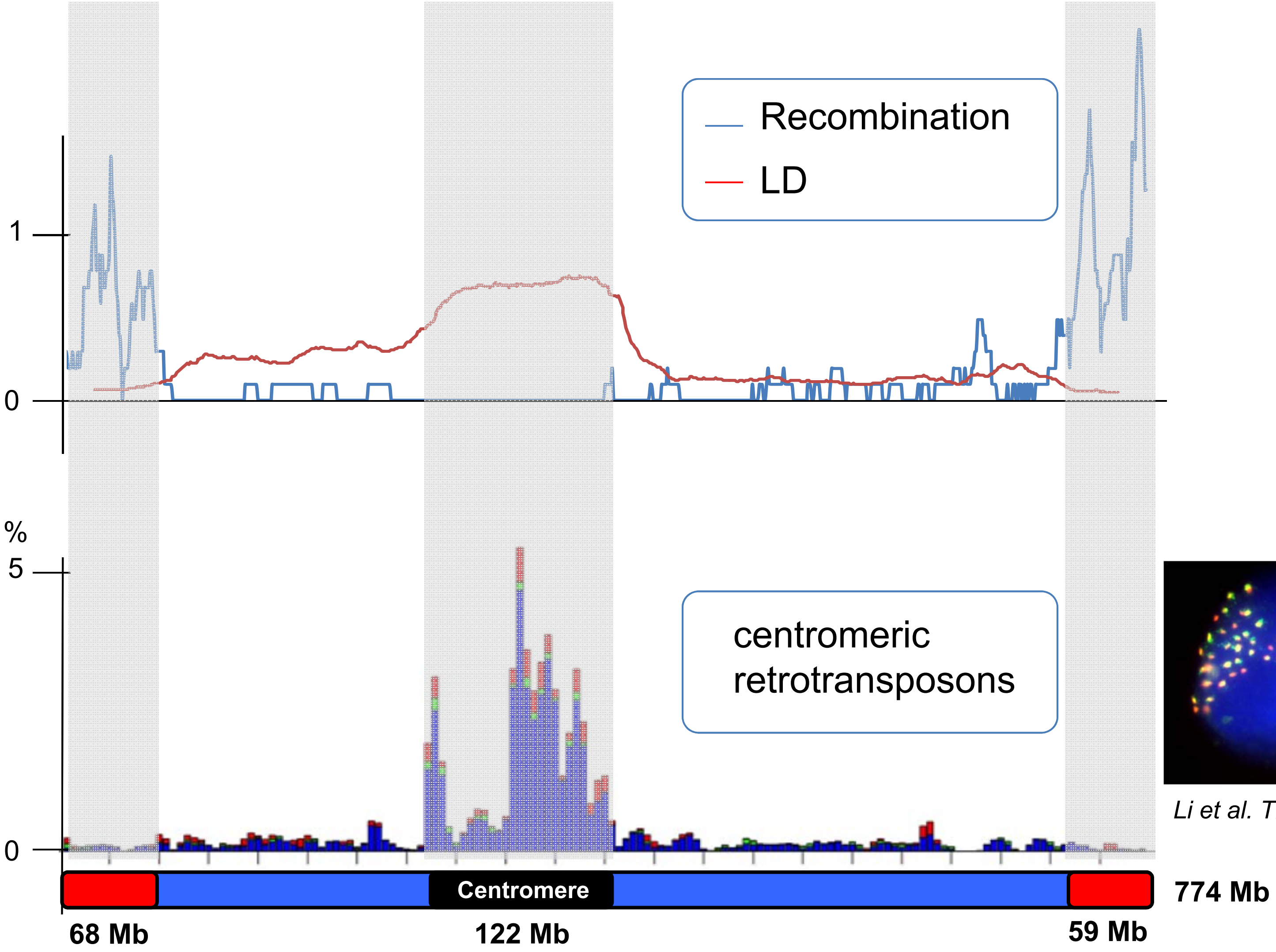
- gaps in read assembly
- gaps in the physical map
- BAC clone contaminations

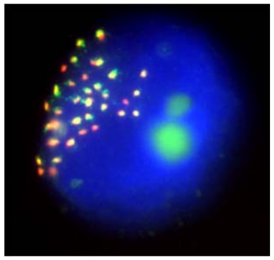
❑ Annotation



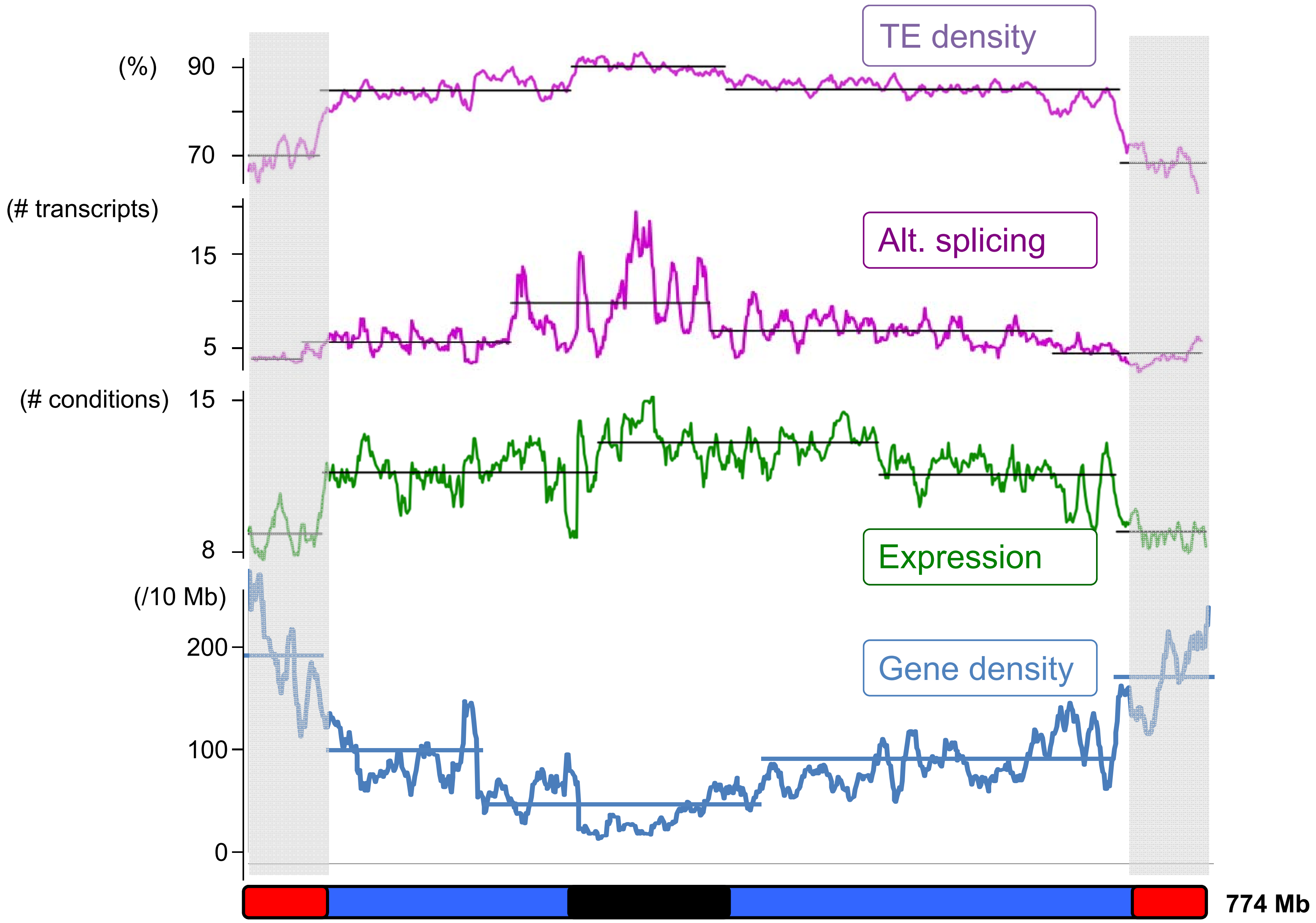
	pseudomolecule	unlocalized scaffolds
• protein coding genes	7264	439
(pseudogenes: 27%)		
• NTRs (RNASeq)	3693	209
• ncRNAs (tRNA, rRNA, snRNA, snoRNAs)	791	37
• transposable elements	234,606	18,553

❑ Chromosome structure





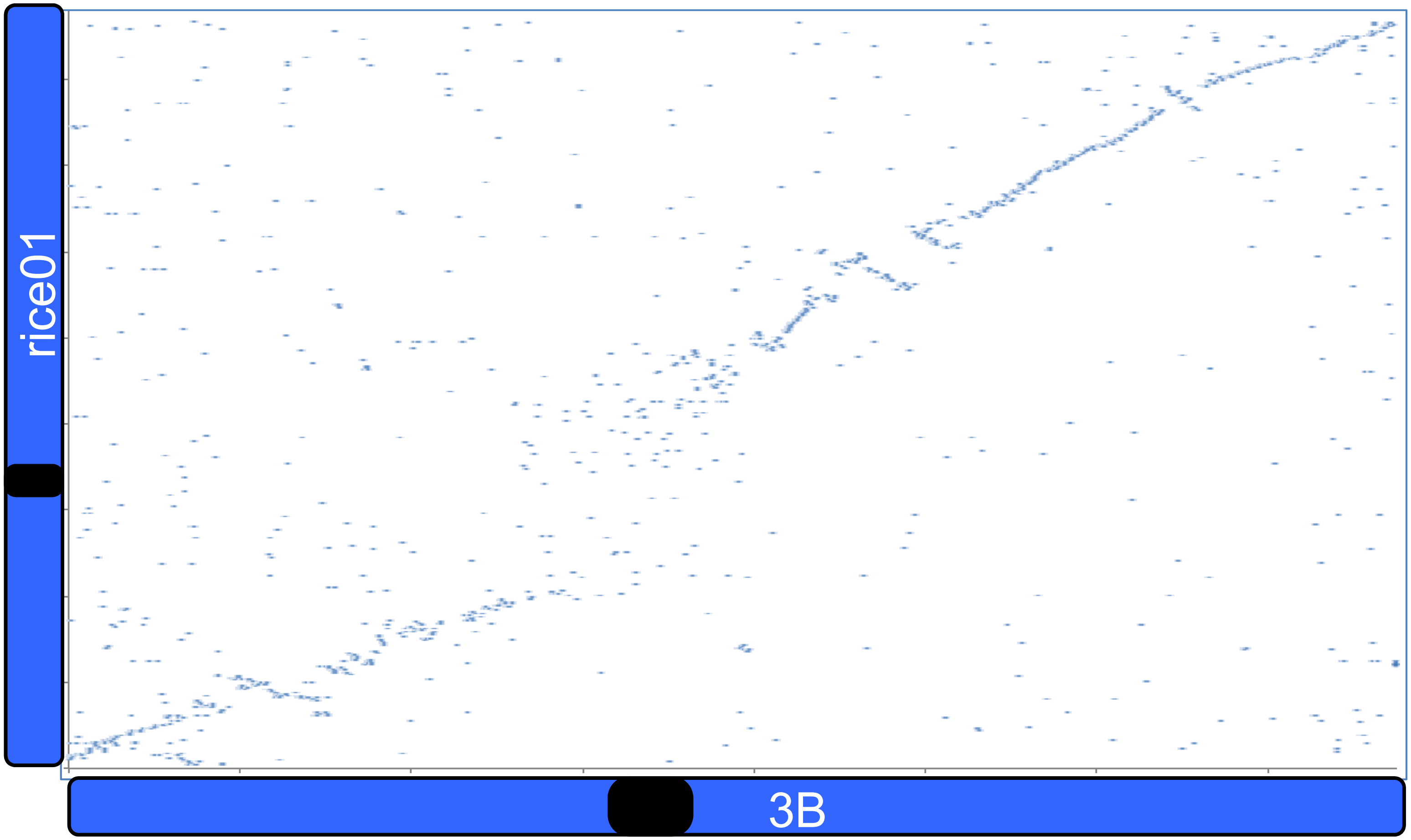
Li et al. TPJ 2013



TE density
(%)
90
70
Alt. splicing
(# transcripts)
15
5
Expression
(# conditions)
15
8
Gene density
(/10 Mb)
200
100
0
774 Mb

❑ Evolution

- ○ Synteny

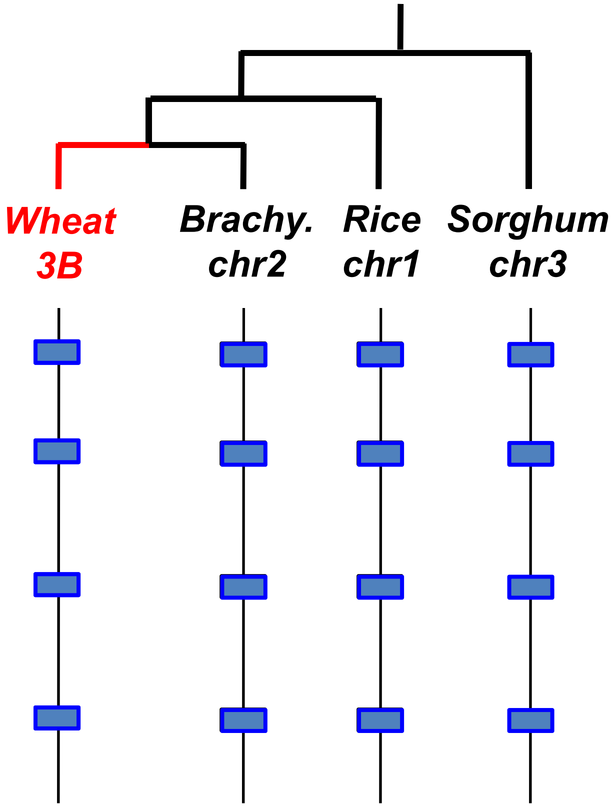


❑ Evolution

○ Gene loss?

Conserved genes Bd-Os-Sb:

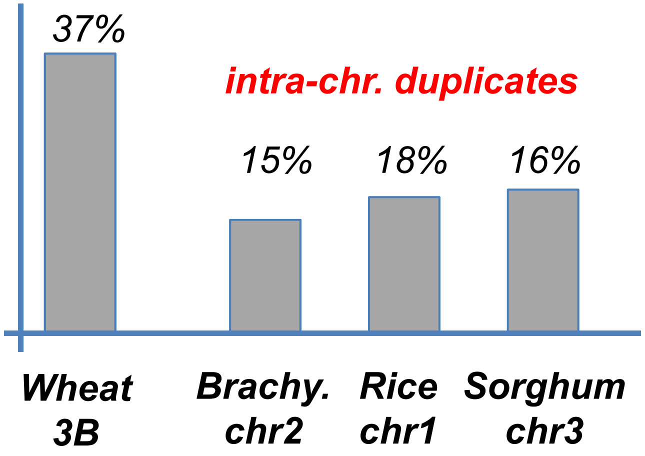
- ➢ 91% found on 3B

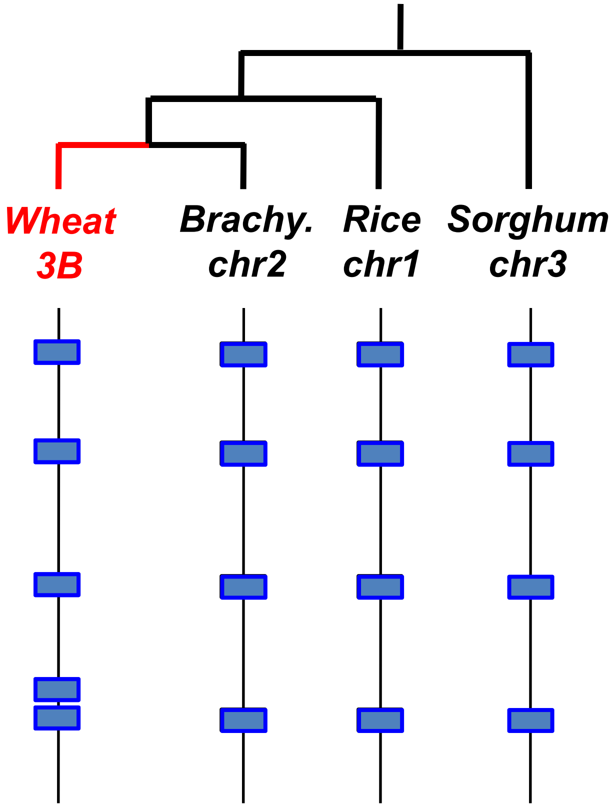


➔ Limited gene loss following polyploidization

❑ Evolution

- ○ Intra-chromosomal duplications

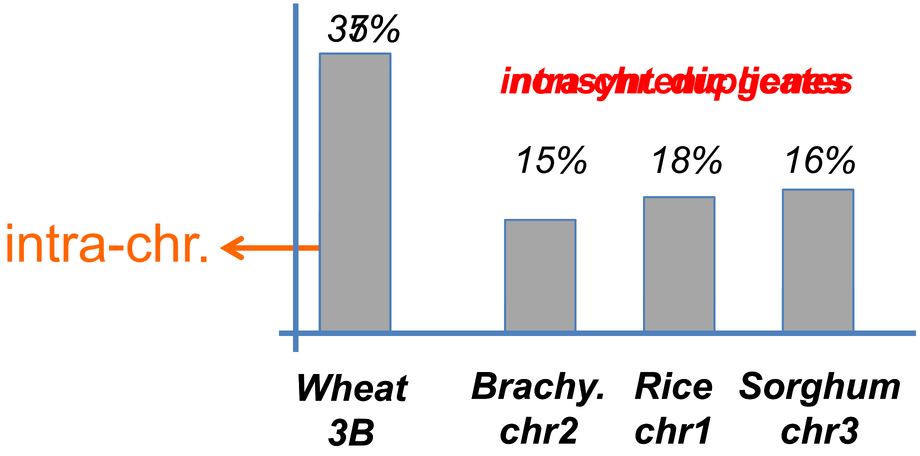


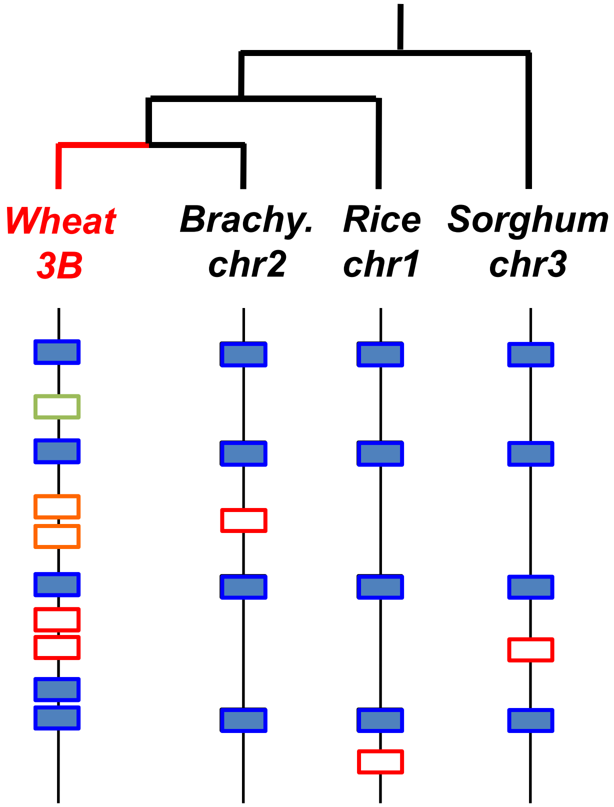


➔ Higher rate of lineage specific intra-chr. gene duplication

☐ Evolution

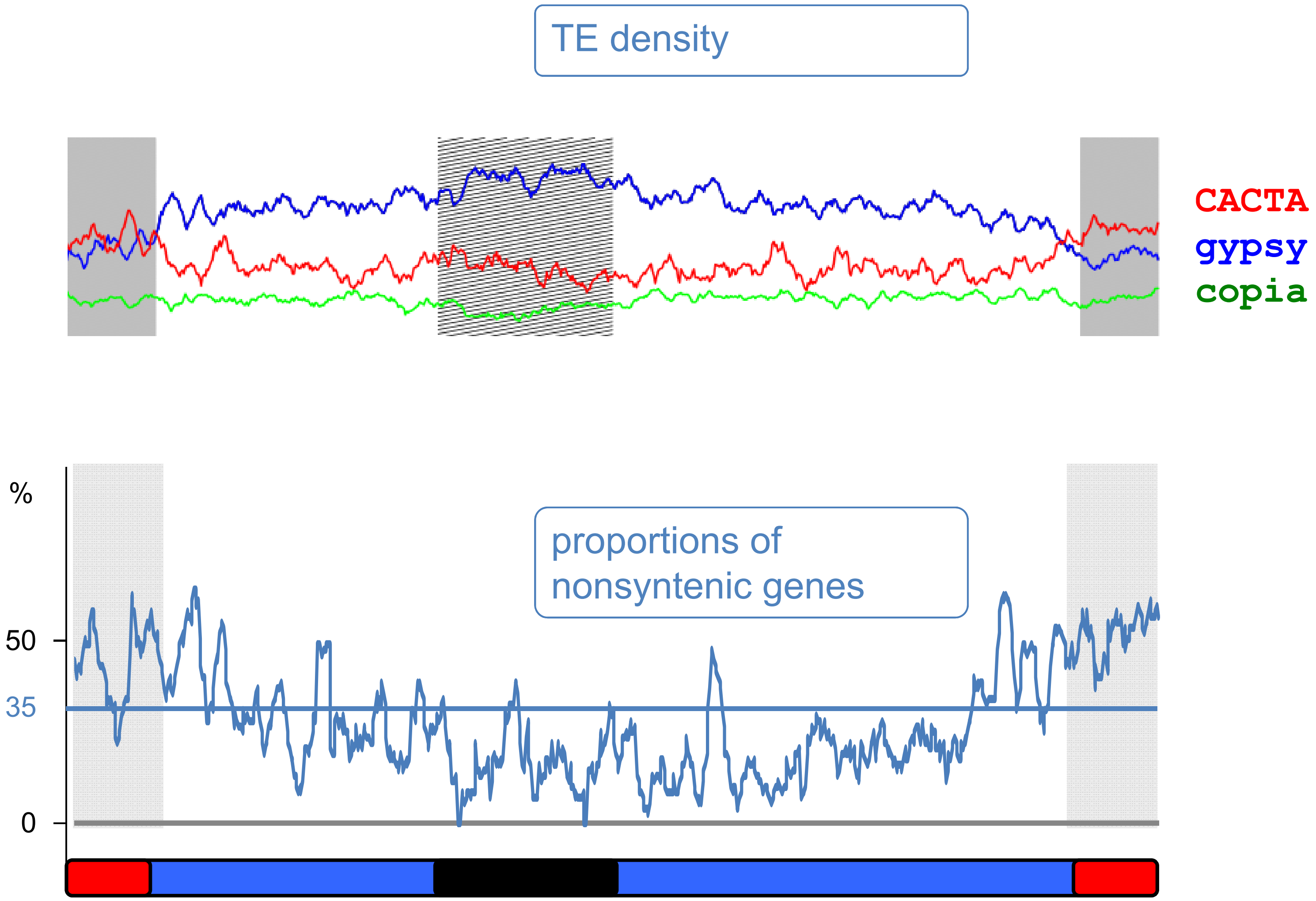
- ○ Intra-chromosomal duplications
- ○ Inter-chromosomal duplications

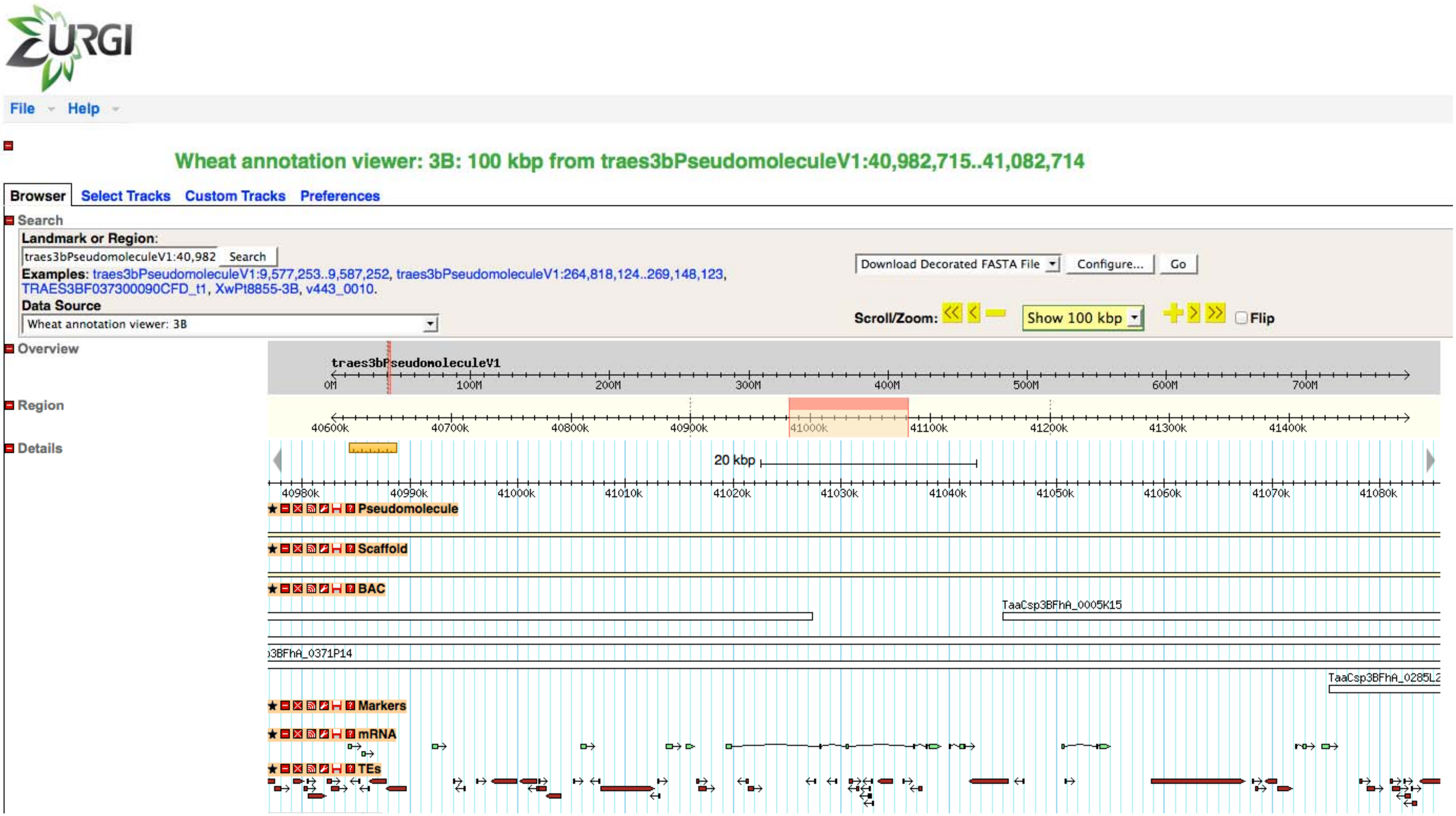




# syntenic genes	3899	3400-3600
# nonsyntenic genes	2065	150-210

➔ Higher rate of lineage specific inter-chr. gene duplication


TE density
CACTA
gypsy
copia
%
proportions of
nonsyntenic genes
50
35
0



Wheat annotation viewer: 3B: 100 kbp from traes3bPseudomoleculeV1:40,982,715..41,082,714

Landmark or Region: traes3bPseudomoleculeV1:40,982

Examples: traes3bPseudomoleculeV1:9,577,253..9,587,252, traes3bPseudomoleculeV1:264,818,124..269,148,123, TRAES3BF037300090CFD_t1, XwPt8855-3B, v443_0010.

Data Source: Wheat annotation viewer: 3B

Scroll/Zoom: Show 100 kbp ☐ Flip

Computer Demo – Saturday 1:50 - 2:10 PM
Presenters: **Michael Alaux, Loïc Couderc**, et al.

Acknowledgments

IWGSC

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VIB
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MIPS
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URGI
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CNRGV
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A. Bellec

IEB
J. Dolezel
J. Safar

TGAC
J. Rogers, M. Caccamo et al.

SAB
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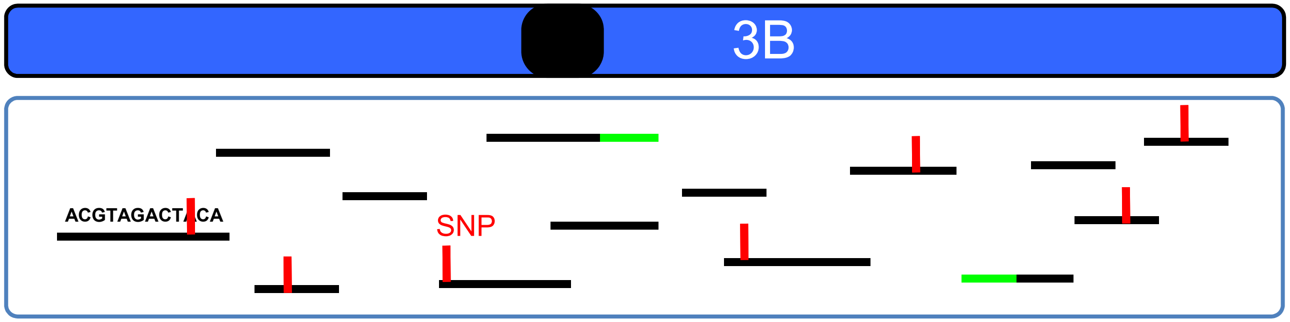
AGGAGCATTGTTGGTGGGTTTCAGTACTTGACGATCACATGTCCAGATATATCCTTTGTTGTCAACAGAGTATGTCAGTATCTCCAAGCACCTCGTGACACTCATTGGTCTGCGGTGAAGTGCATCTTGCGCTACATTCGCTTCAAGTTGTCCTA_DUPLICATED
_GENE_TGGTTTGCACATTCGACCGAACCCCTCTAGGGTCATTTCGGCGTATTCTGATGTGGATTGGGTTGGCAGTCCGGATGACAGGCGATCCACGGGAGGCTCTGCTGTATTTTTTGGTTCTACCTTGATCGCCTGGAGTGCTCGGAAACAGGCTACTGTTTCG
CGTAGCAGTACTGAAGCTGAGTATAAGGCTGTGGCTAATGCAACTGCAGAAATTATTTGGATACAGTCTTTGCTTCAGGAGTTGGGAATATCTAATCACAGTCTCCTGTTCTTTGGTGTGATAACATCGGTGCTACATACCTATCTGCTAATCCGGTATTCCATGC
CCGAATGAAACACAACGAAGTGTACTATCATTTTGTACAGGAACGGGTATCTCAGAAGCAACTACAGATCAAGTTCATTTCTTCCAAGGATCAACTTGGGTAGTCGGCTGCTGCTGCTCTTGTTCGGTCTCTTGGTGCTGACTTTGCGGTCAAAGATCTTGGGCAG

❑ Ordering scaffolds



3B-v1

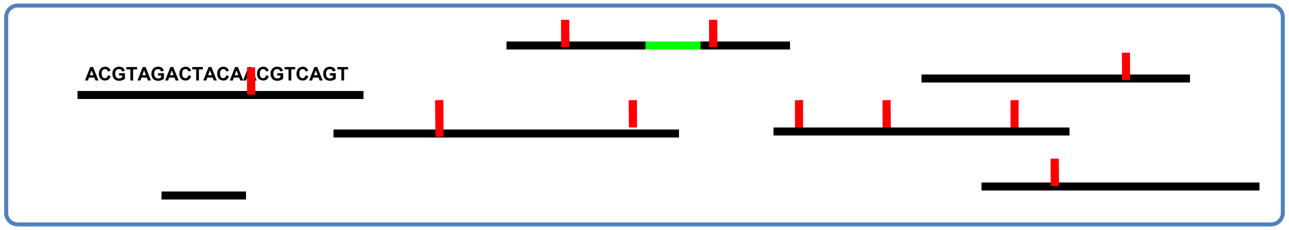
16,136 scaff

1040 Mb

- SNP discovery: **39,077 SNPs** (Cs/Renan)
- genotyping: **3075 SNPs**
  - 284 Cs/Re RILs
  - association panels

genetic mapping

LD mapping

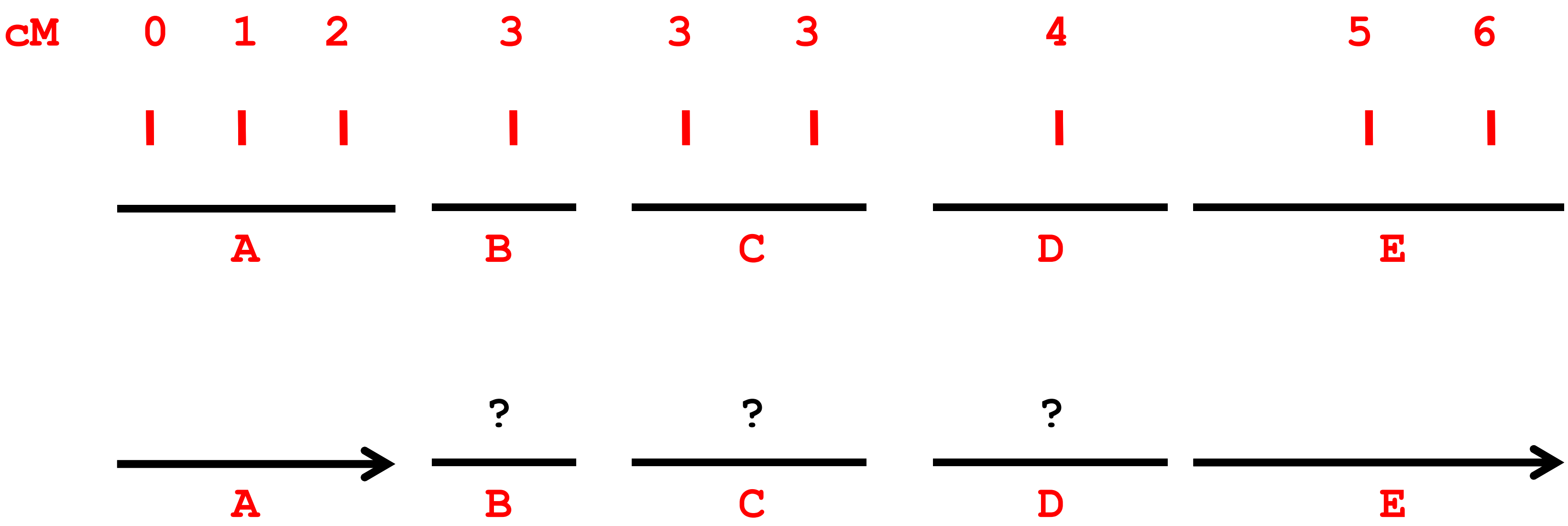


3B-v443

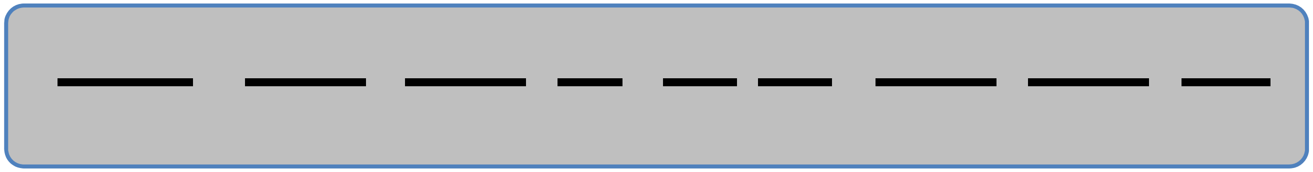
2808 scaff

833 Mb

N50:892 kb



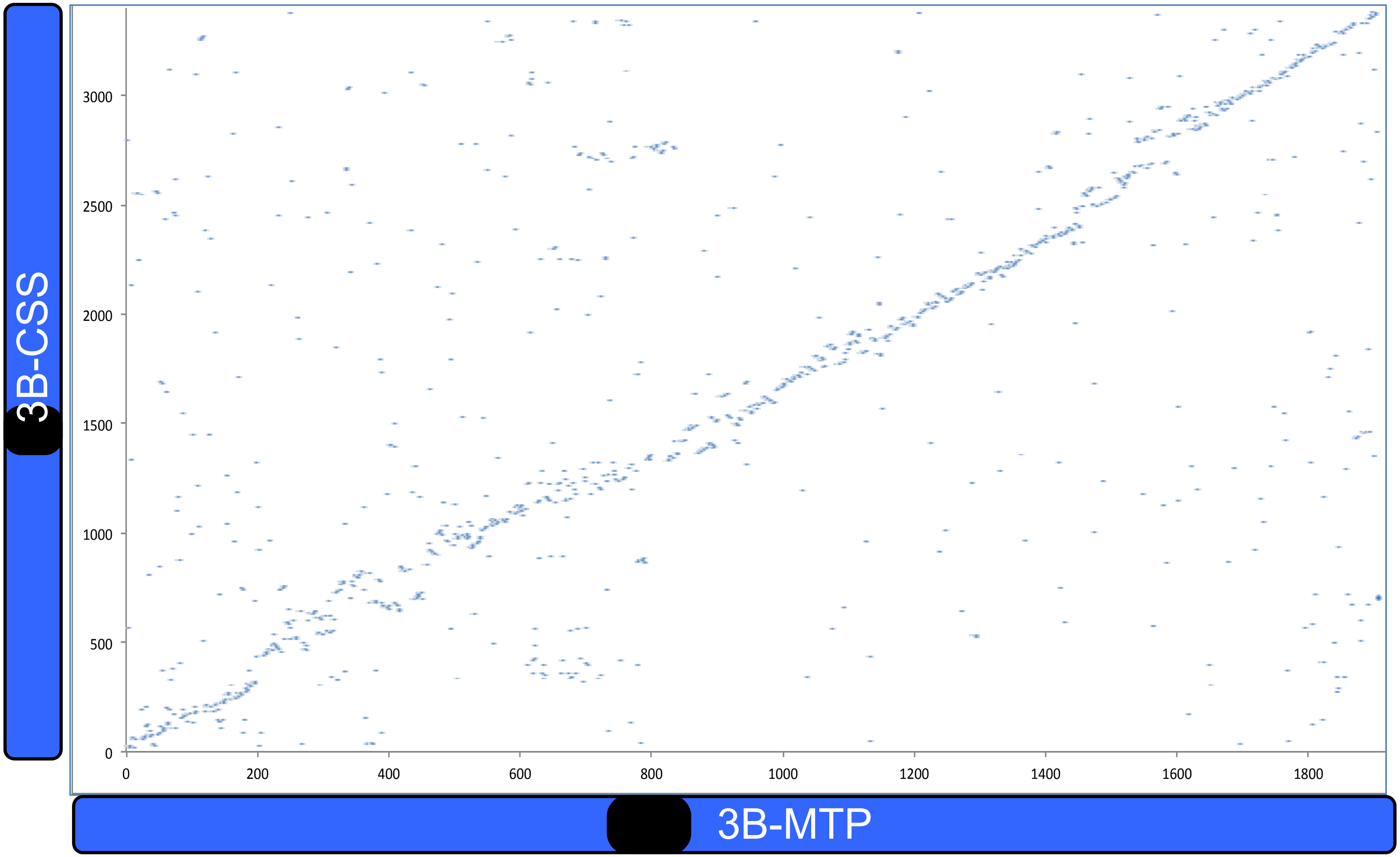
- ○ orientation unknown: **869** scaff (**48%** of the seq.)
- ○ micro-order unknown: **620** scaff



pseudomolecule

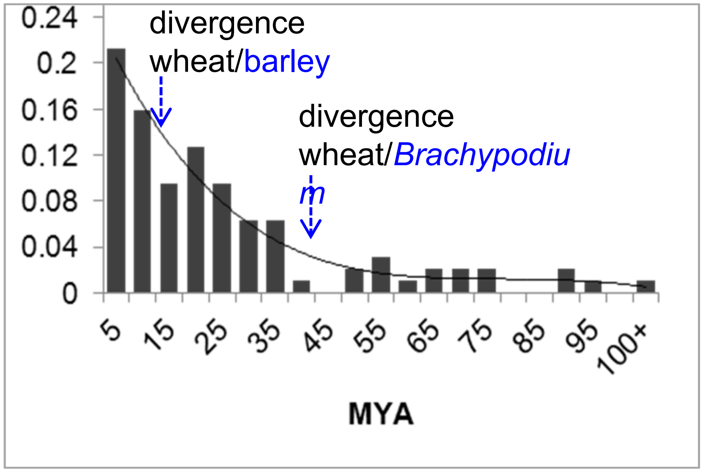
1358 scaff
774 Mb

❑ 3B-MTP *vs* 3B-CSS



	syntenic genes	*nonsyntenic genes*
• pseudogenes	17%	32%
• expressed	82%	69%
• mean genome-wide no. copies	8	19

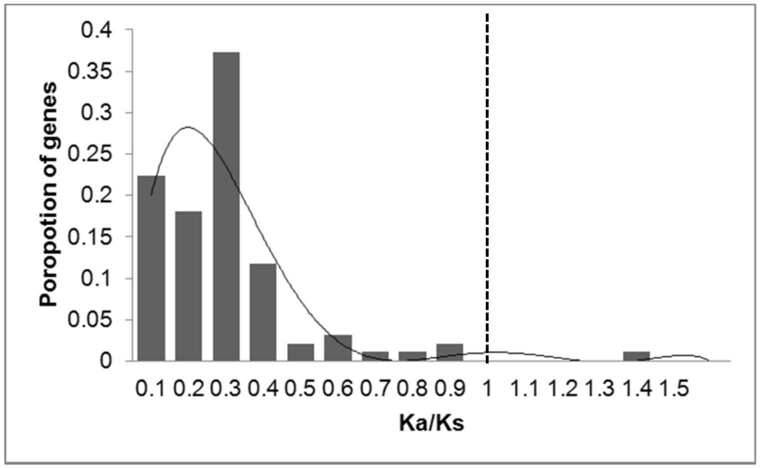
○ Ks



divergence wheat/barley

divergence wheat/*Brachypodium*

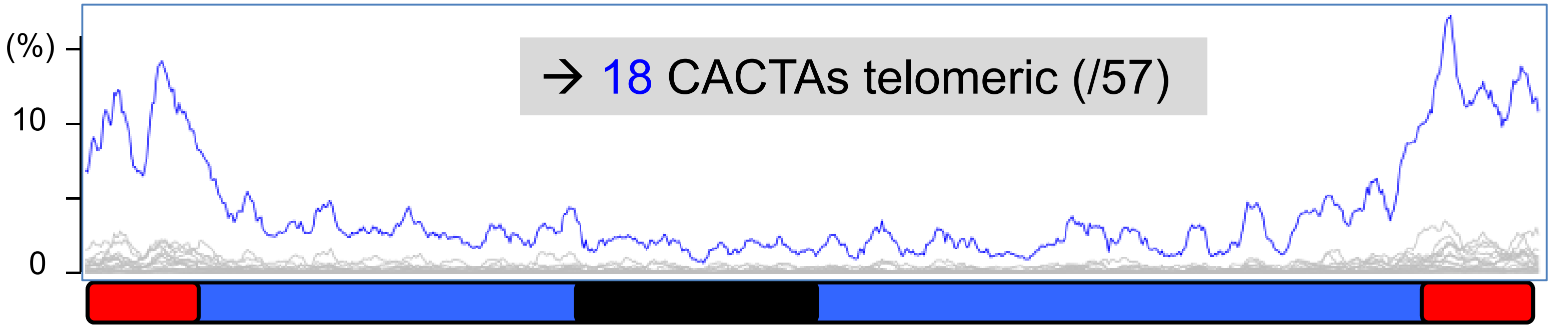
○ Ka/Ks



→ under purifying selection

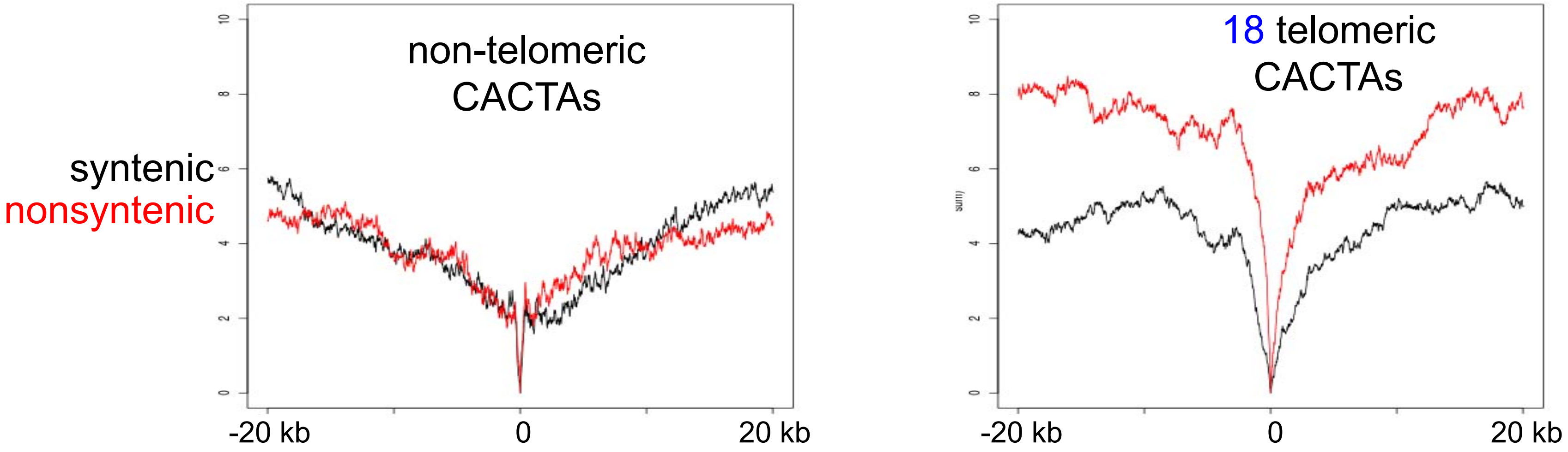
❑ Relation between CACTAs and gene duplications?

- Clustering distributions of 57 CACTA families



→ 18 CACTAs telomeric (/57)

- Abundance in the vicinity of genes



Sub-telomeric regions

- 16% of chromosome length
- 76% of recombination events
- 33% of genes
- Expression specificity
- Variability/Plasticity
- Adaptation