A strategy to assemble a reference sequence of the 1 Gb wheat chromosome 3B

Frédéric Choulet, Sébastien Theil, Josquin Daron, Natasha Marie Glover, Nicolas N. Guilhot, Philippe Leroy, Lise Pingault, Etienne Paux, Pierre Sourdille, Arnaud Couloux, et al.

TGAC/ESF-LESC workshop

Strategies for de novo assemblies of complex crop genomes

Norwich, Oct-9-2012

TGAC
The Genome Analysis Centre™

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Sequencing and assembling wheat chromosome 3B

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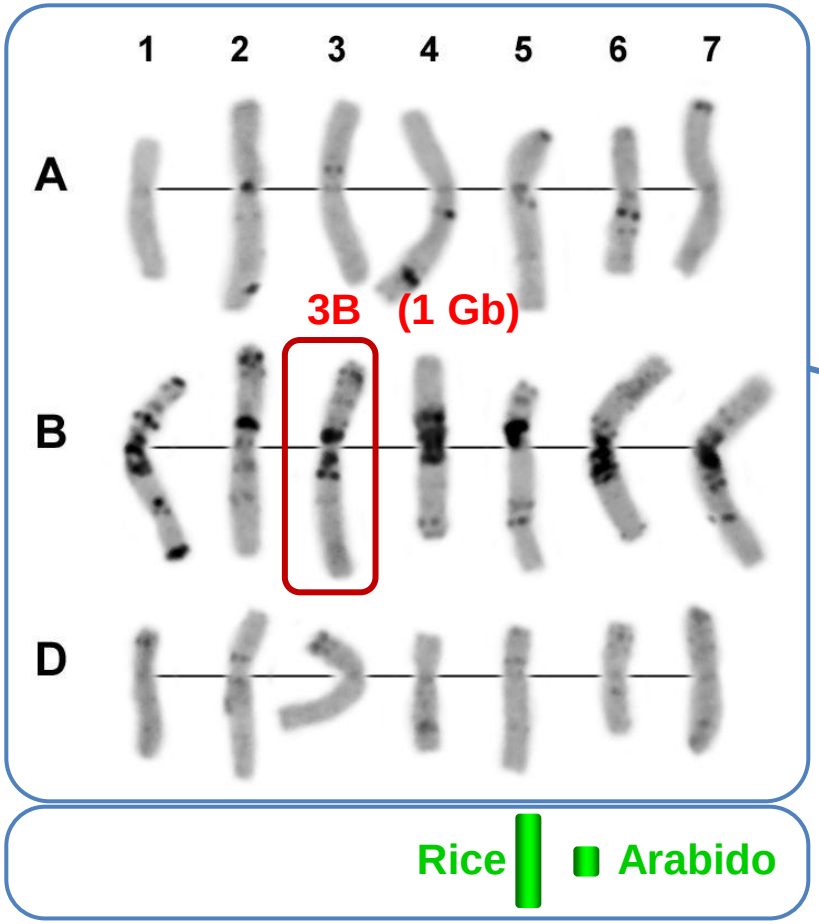
The bread wheat genome



- ✓ Allohexaploid (A, B & D)
- ✓ 17 Gb
- ✓ 85% of sequences derived from TEs

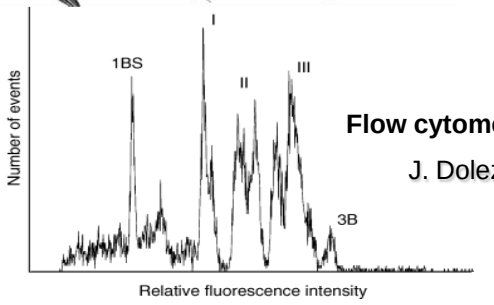
WGS

5x @ *www.cerealsdb.uk.net*

Chromosome-by-Chromosome

⇨ **Survey Seq. chr arms**

⇨ **BAC libraries**



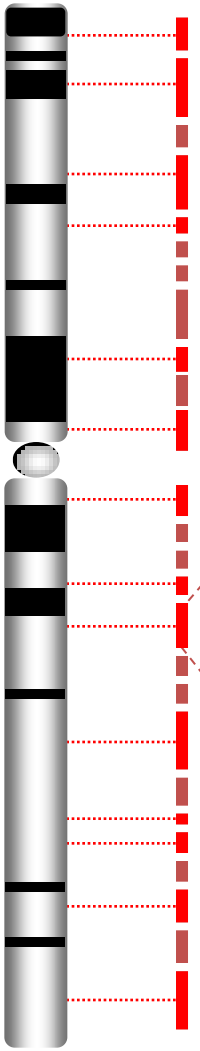
Flow cytometry

J. Dolezel (IEB)

An integrative map of chromosome 3B

Paux et al. Science 2008

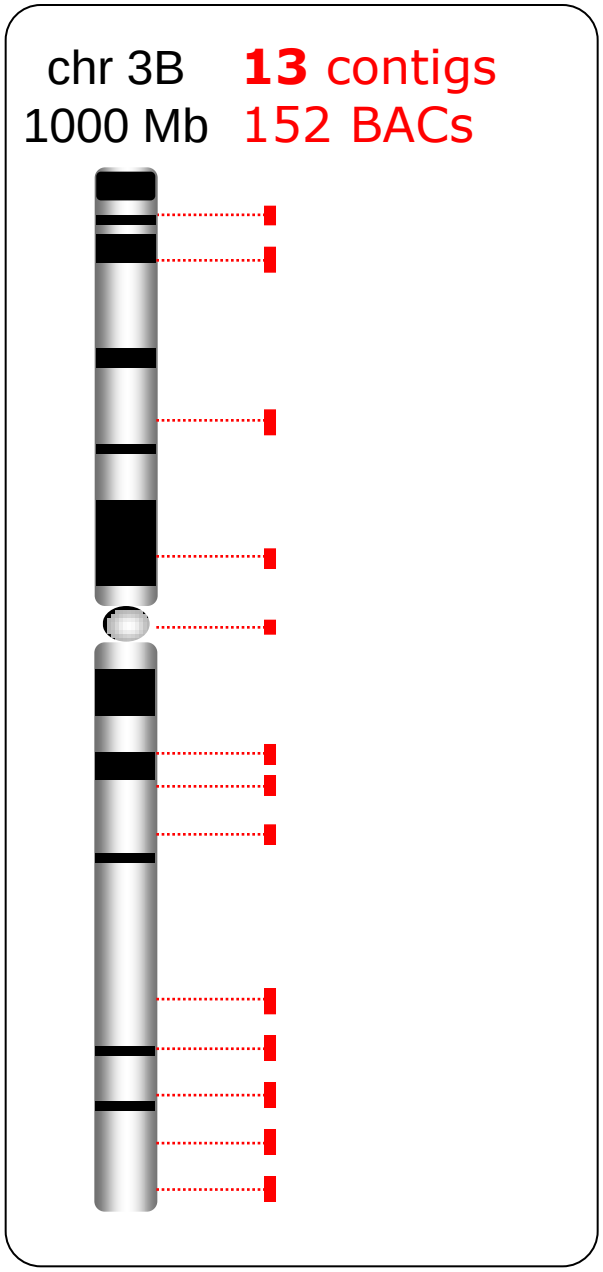
chr 3B
1000 Mb



Physical map

• Fingerprinted BACs	132,000 (19x)
• #contigs	1282
• #MTP BACs	8448
• Markers anchored	4463

3B Pilot (2007-2010)



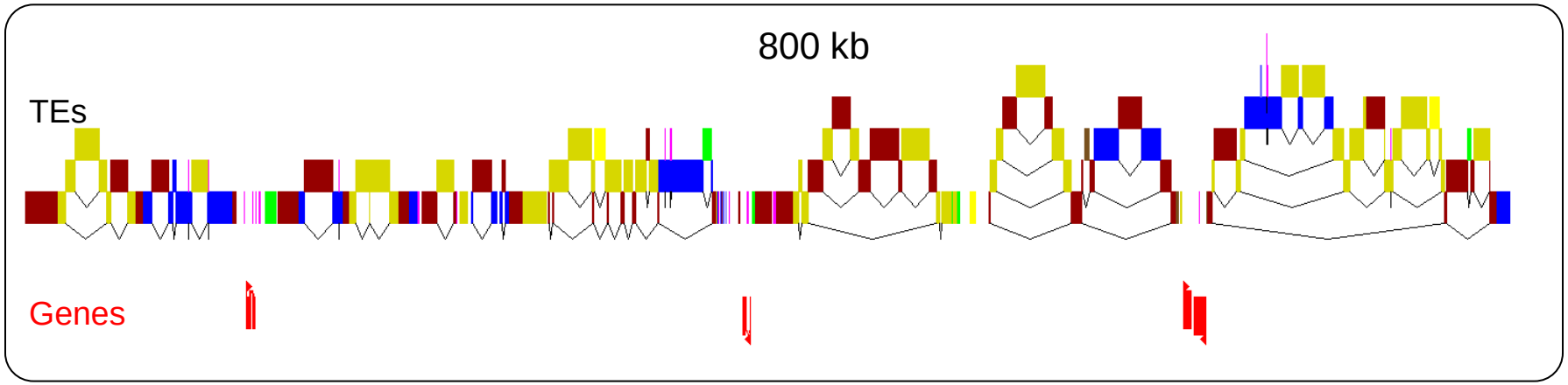
Choulet et al. Plant Cell 2010

18 Mb (**2%** of the 3B)
First set of large contiguous seq.

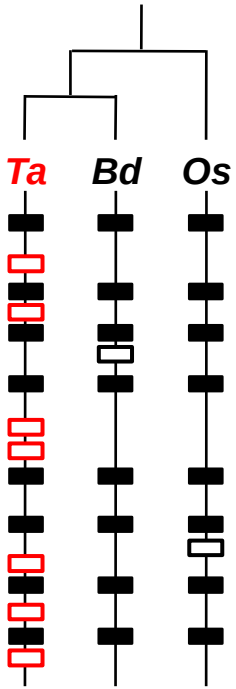
~3000 TEs

~200 protein-coding genes

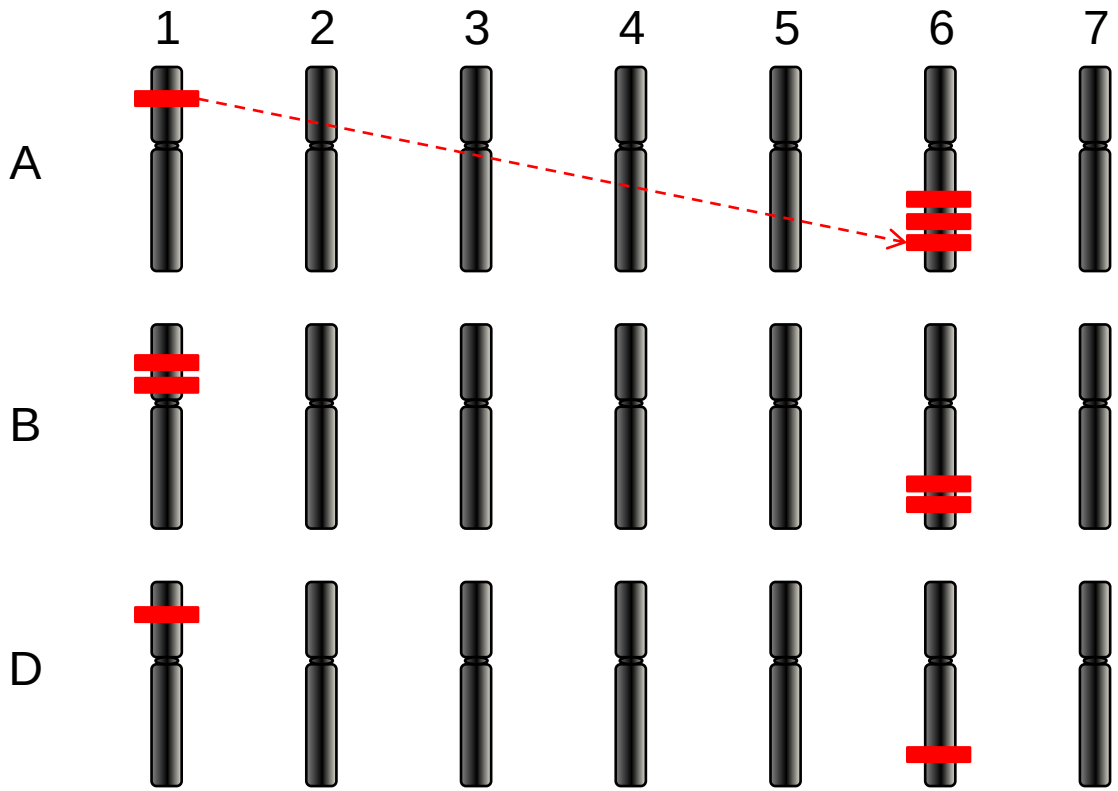
Main results



- 85% **TEs** highly **nested** and still **complete**
- 25% of **pseudogenes**
- 33% of **duplicates**
- No big **gene islands**
- Unexpected **high number of genes**
  (8400 *versus* 5500 expected based on synteny)
- 45% **non-syntenic** genes



Gene redundancy



3 **homoeologous** copies (ABD)

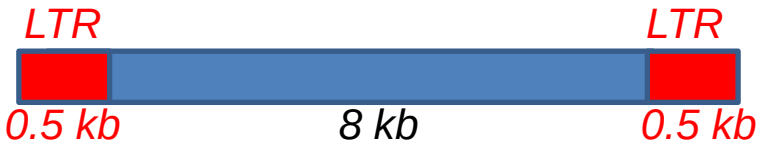
+ paralogs created by **interchromosomal duplication**

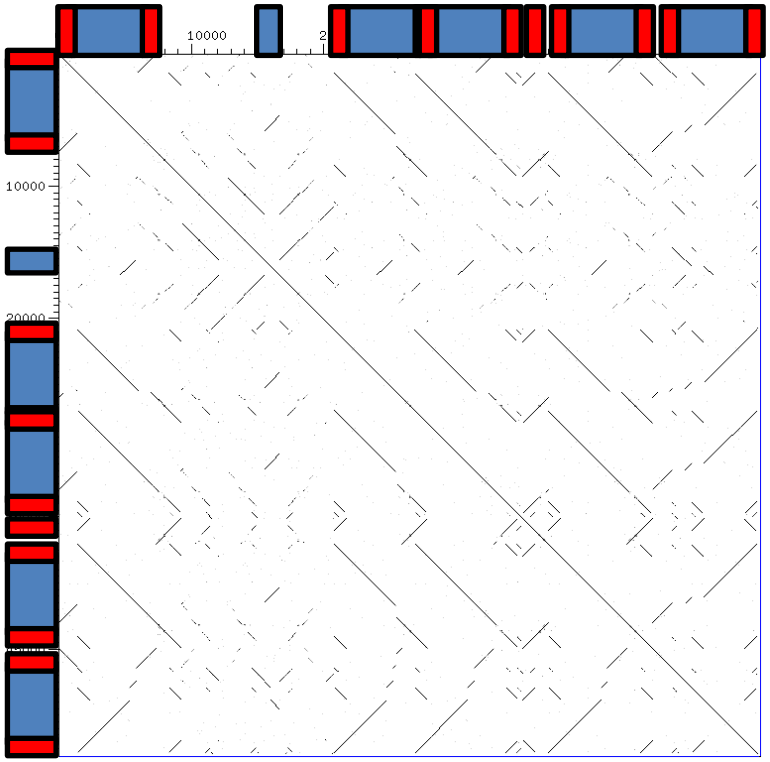
+ paralogs created by **tandem duplication**

/!\ assembly /!\

Repeats

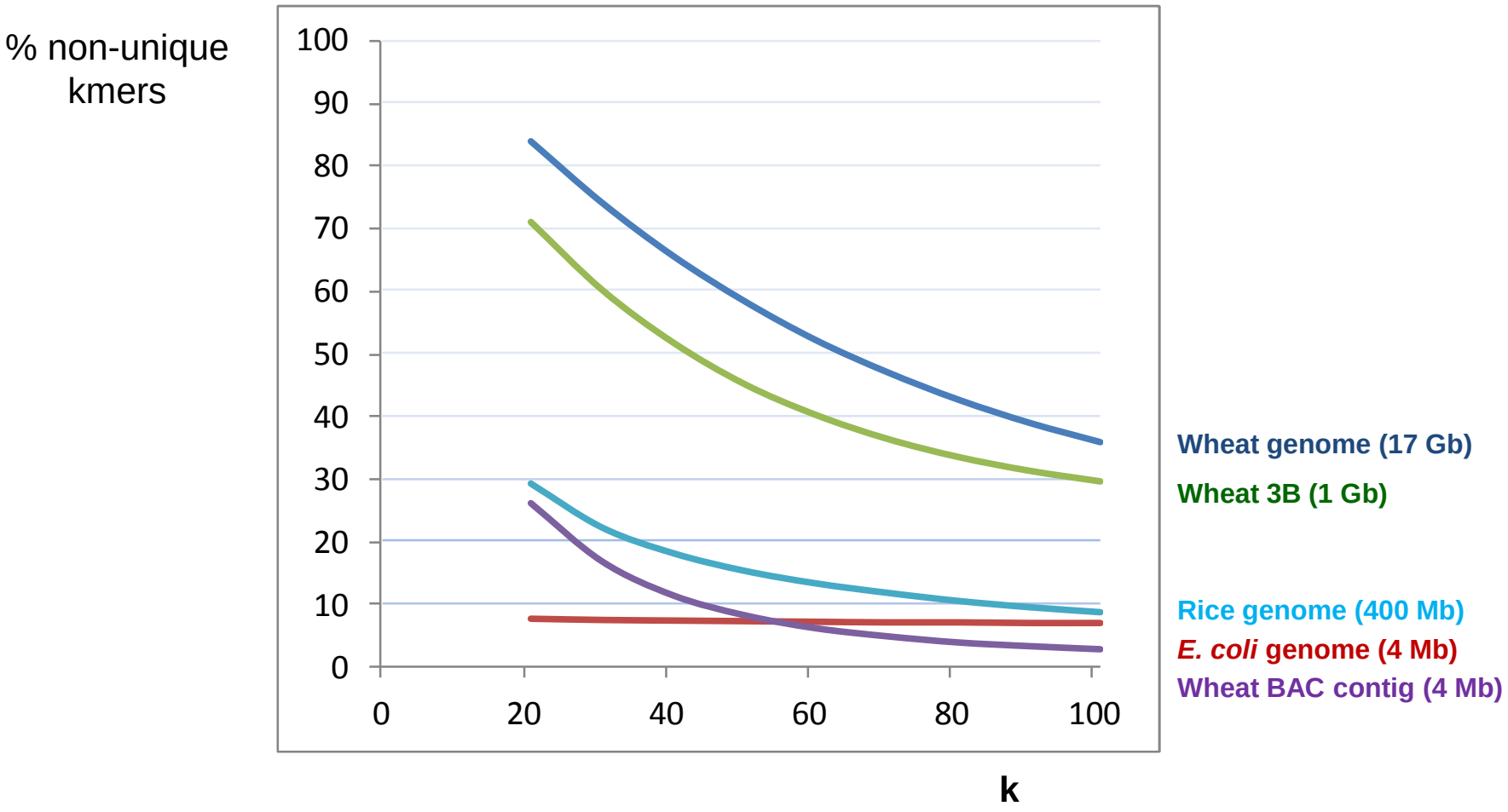
- Genes are repeated
- 85% TEs != 85% repeats
- TEs are made of repeats...





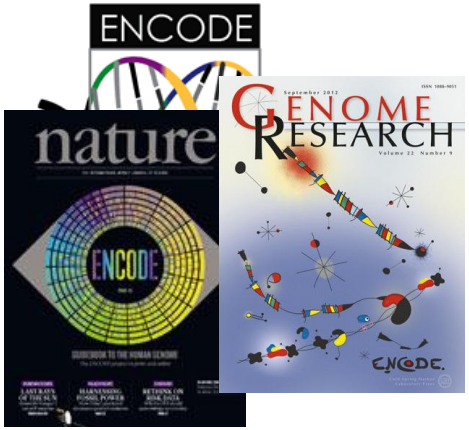
50 kb

kmers


% non-unique kmers
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
k
Wheat genome (17 Gb)
Wheat 3B (1 Gb)
Rice genome (400 Mb)
E. coli genome (4 Mb)
Wheat BAC contig (4 Mb)

A reference sequence? What for?

- Distinguish homoeologous + paralogous copies of gene families
- Solving the assembly of tandem duplicates (e.g. NBS-LRR involved in resistance)
- Study CNVs
- Study genome structure organization & impact on gene expression
- Identify genes under QTLs
- Distinguish genes *vs* pseudogenes
  pseudogenes in cv. Chinese Spring can be functional in others
- Assess what is behind the gene catalog!!!



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Sequencing the 3B

❑ Whole 3B Shotgun (Illumina)

Flow sorted DNA of 3B

contaminations

↓

Multiple Displacement Amplification

bias

↓

Illumina HiSeq2000

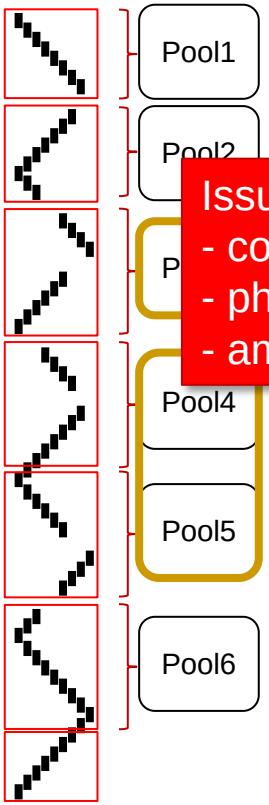
845 M reads (2x108) PE 0.5kb

→ 82X

assembly

❑ MTP Sequencing (Roche/454)

8448 BACs



928 pools of **8-12** BACs

928 libraries: LPE **8 kb**

Issues:
- cost & time
- physical map is incomplete
- amount of work to assemble

~150 runs GSFLX

↓

→ 40x coverage

❑ BAC-Ends of the MTP

Sequencing the 3B

❑ Whole 3B Shotgun (Illumina)

De novo assembly with ABYSS (k=71)

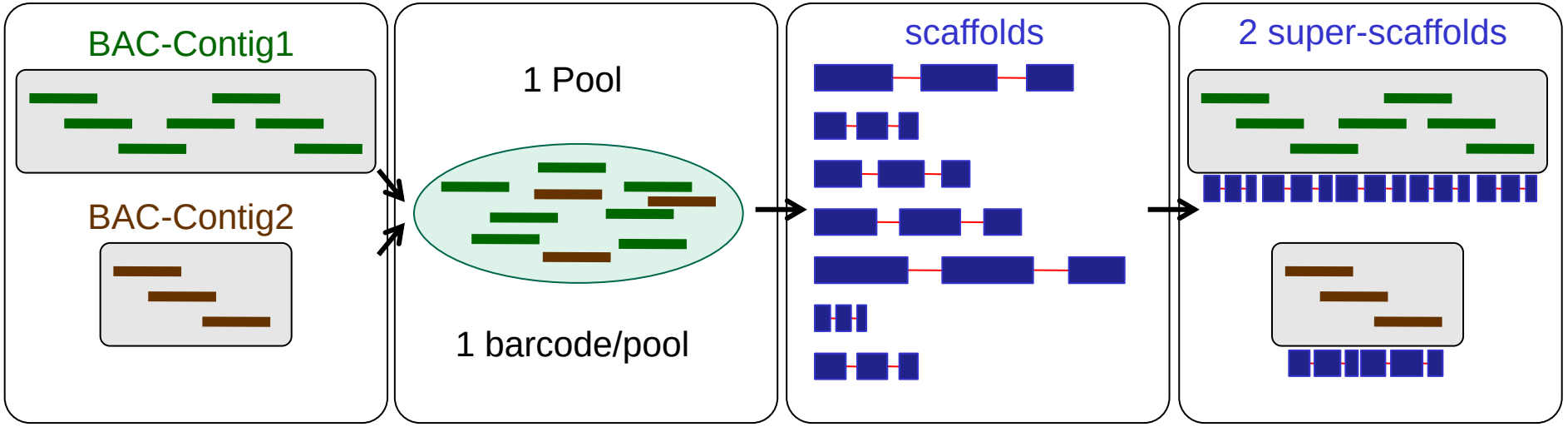
TGAC The Genome Analysis Centre™

J. Wright
M. Caccamo
J. Rogers

	Contigs >200bp	After Masking TEs
# Contigs	546,922	60,319
# bp	639 Mb	125 Mb
N50	2.7 kb	6.7 kb
Max ctg size	48 kb	48 kb

Sequencing the 3B

❑ **MTP Sequencing (Roche/454)**



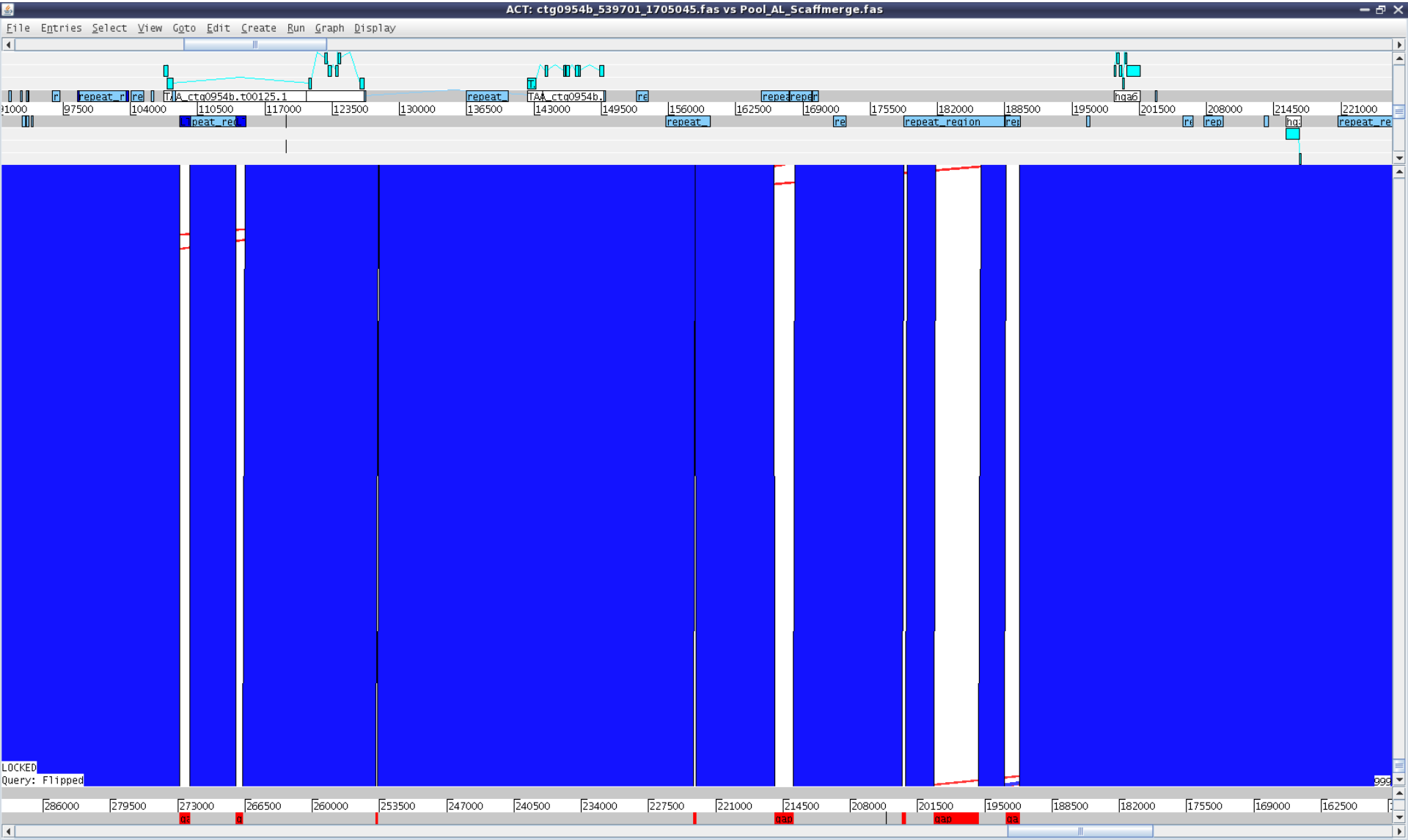
Physical map
x**1282 ctg**

Pooling
x**928 pools**

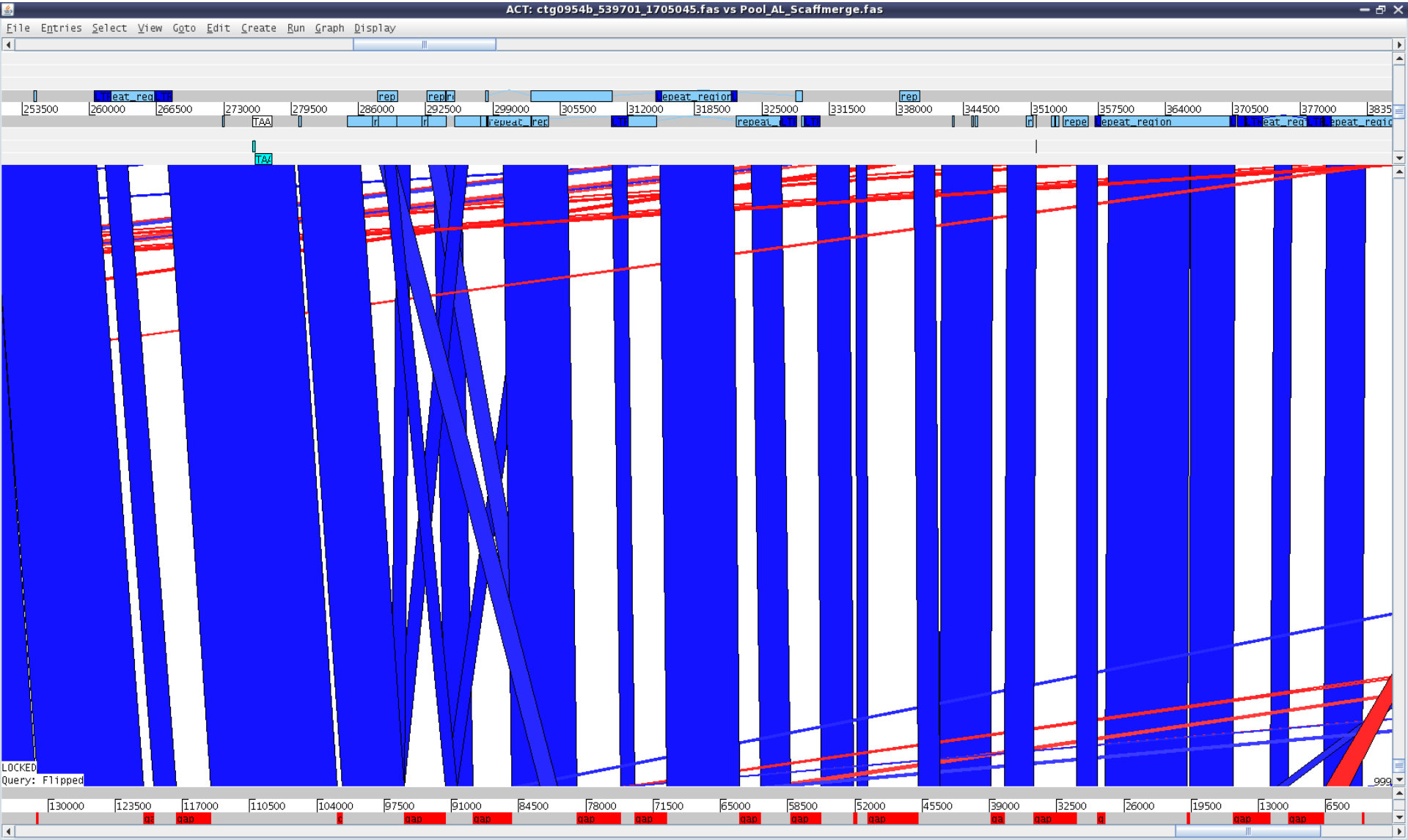
Sequencing +
Assembling

Assigning scaffolds
to physical contigs

Mapping on reference sequence



Mapping on reference sequence



What is missing in the 454-MTP-seq?

- How many sequences present on **Illumina-shotgun contigs** are missing in **MTP scaffolds**?

  - *mask TEs in Illumina contigs*
  - *similarity search against 454 assembly*

✓Match	x%
✓ Absent	x%

➔ Gaps in the physical map (5-10%)
➔ Contamination when flow sorting of 3B chromosomes (5-10%)

What is missing in the Illumina assembly?

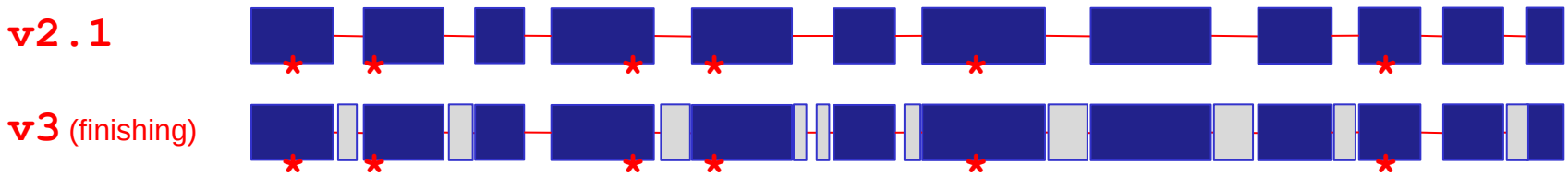
- How many sequences present on **MTP scaffolds** are missing in **Illumina-shotgun contigs**?

  - *extract ~33000 coding exons annotated on 454-scaffolds*
  - *similarity search against Illumina contigs>200 bp*

✓Full	x%
✓Partial	x%
✓ Absent	x%

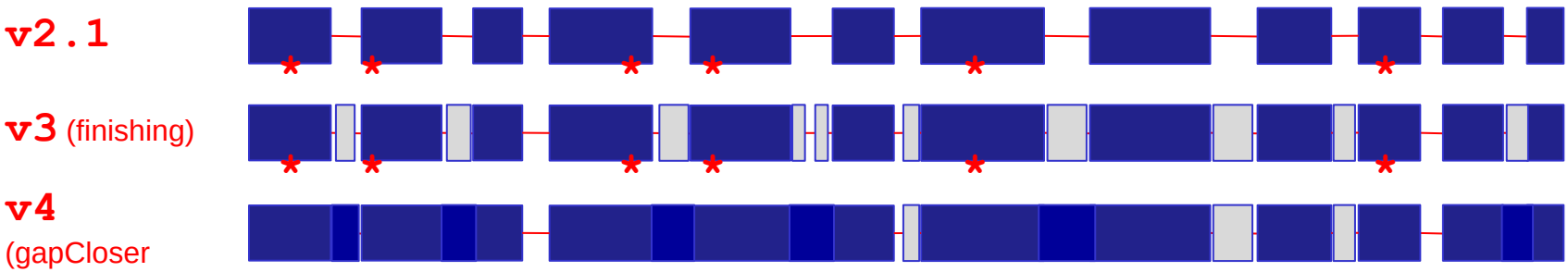
➔ Despite 82X Illumina coverage, x% of coding exons are absent/partial

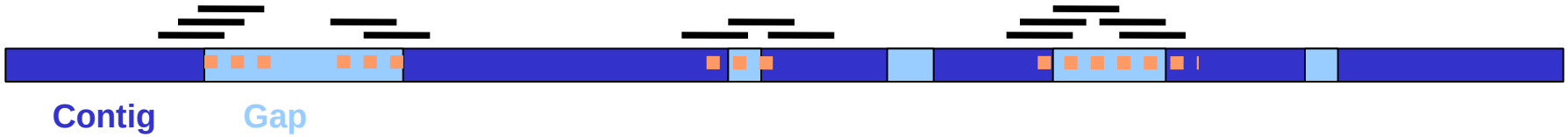
Improve scaffolding by manual curation



- **Curation** of the scaffolding (for the entire project [16000 scaffolds])
  - *Automated parsing of Newbler outputs to flag regions showing ambiguous / nonambiguous pairs*
  - *Decision taken by a curator*
    - ***!!! Time consuming !!!***

Improve 454 assembly with Ill reads





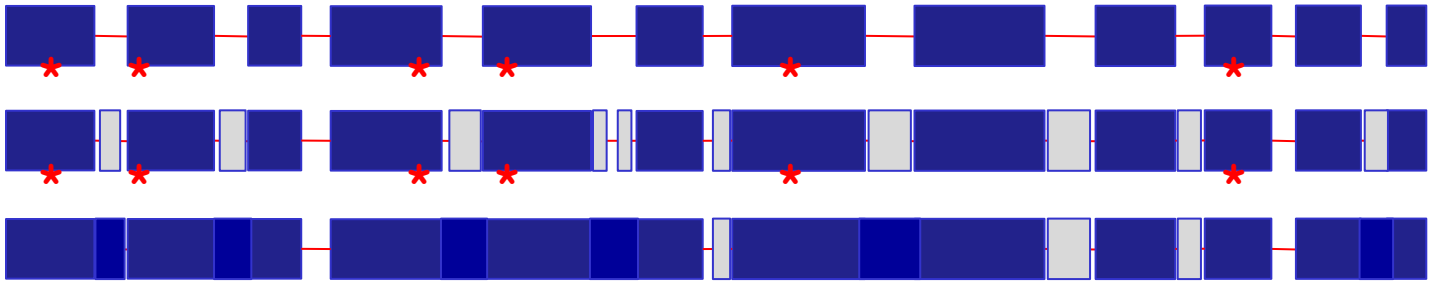
- ❑ **close gaps in the scaffolds**
  With **gapCloser** (JM. Aury et al.)

Gaps:
18% → x%

- ❑ **correct homopolymer errors and mismatches**

xxxxxx bases corrected
(0.1% of low copy regions)

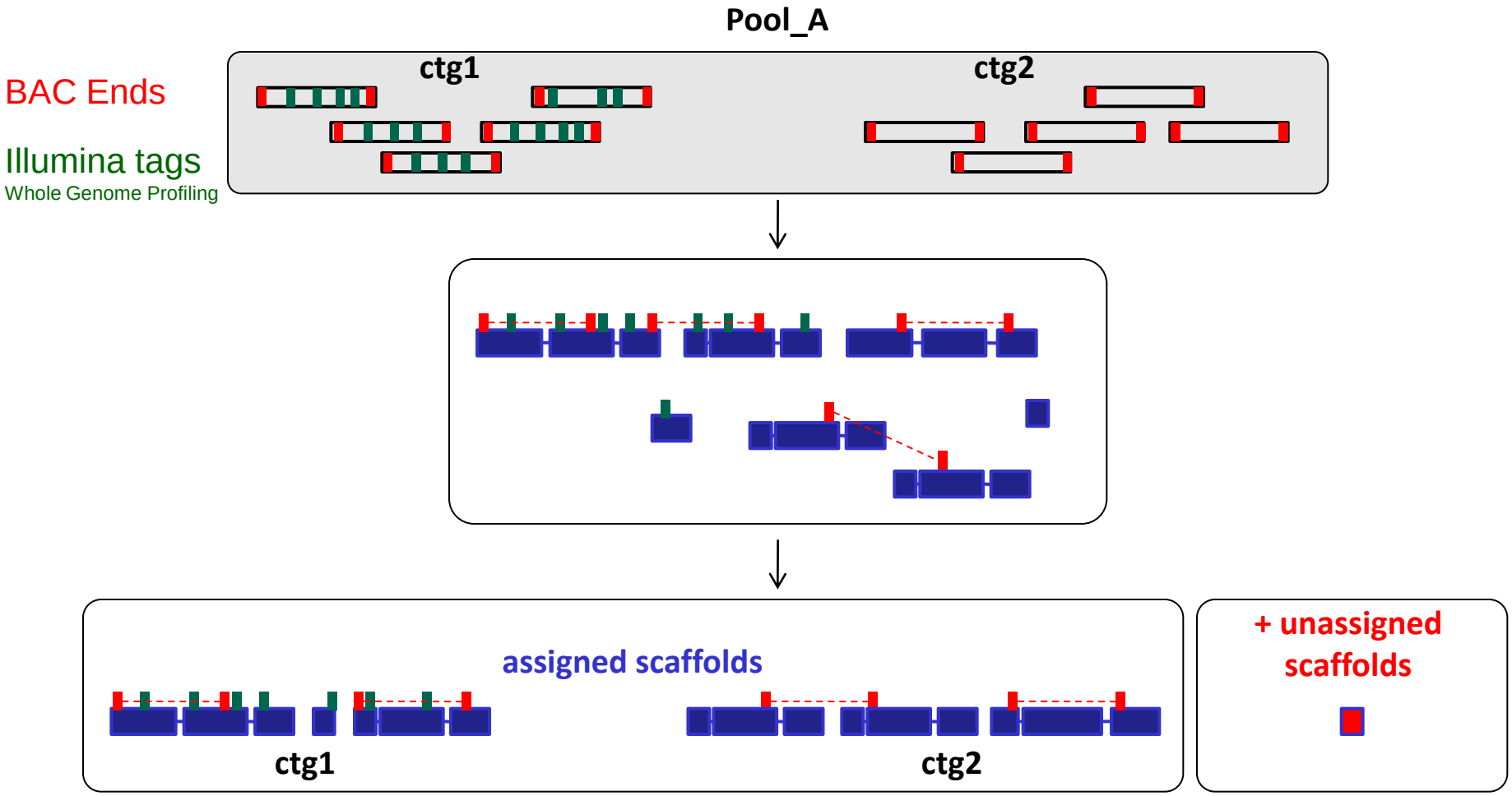
Improve scaffolding



	❑ V2.1	❑ V4	
#scaff	16136 scaff	xxxx scaff	x1/3
Cumul. size	1040 Mb	xxx Mb	
Gaps	18 %	x %	
N50	275 kb	xxx kb	+70%

➡ Route towards a pseudomolecule

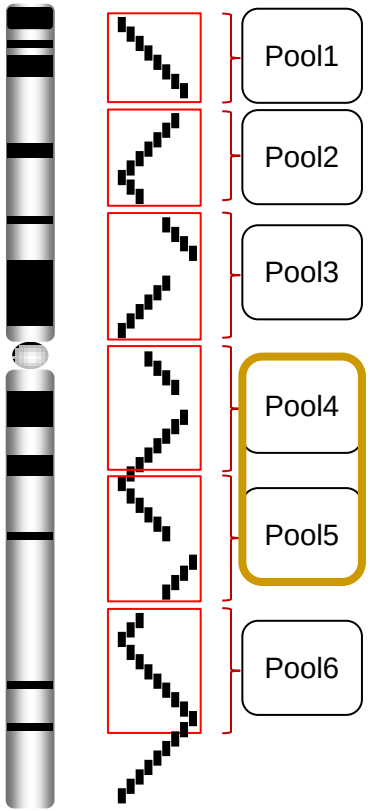
Assignation scaffolds ⇔ BAC-contigs

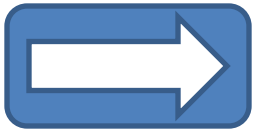


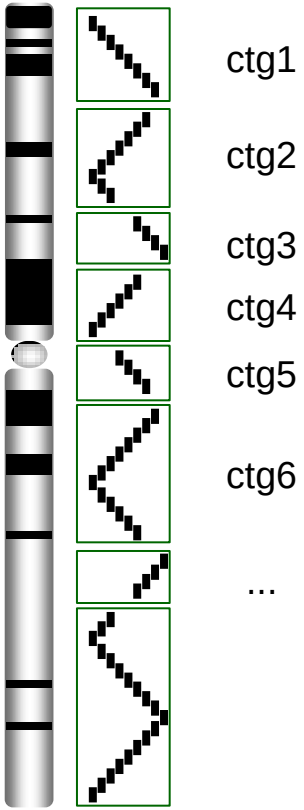
Assignation scaffolds ⇔ BAC-contigs

Assigned: xxx Mb

Unassigned: xx Mb





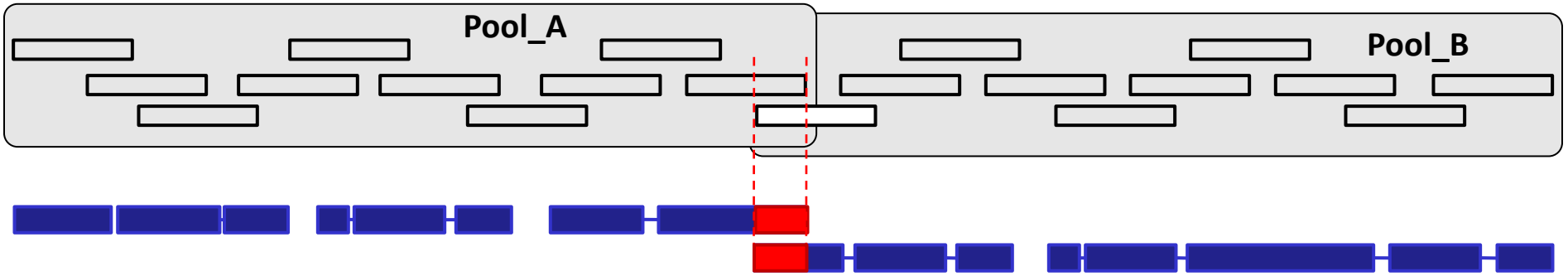


xxxx scaffolds

x scaff / BAC-contig

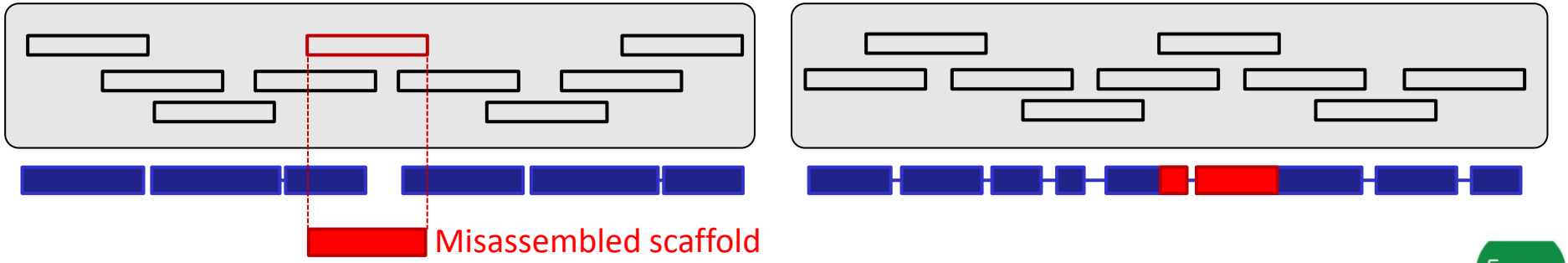
Detect & assemble overlapping scaffolds

❑ Overlapping BAC-contigs



- *Expected overlap*: **525** pools
- *Unexpected overlap*: ???

❑ Misassembled BAC clones



Using *scaffAssembler* (S. Theil)

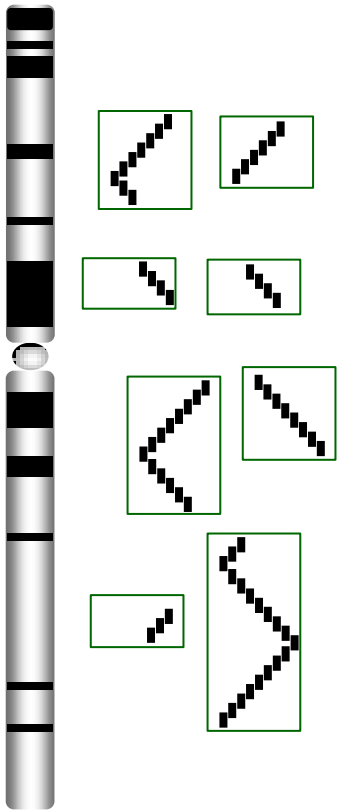
- BLAT --extendThroughNs
- Parse with 99%id & >10kb overlap at extremities
- Merge

1. Merge scaffolds between pools with expected overlap

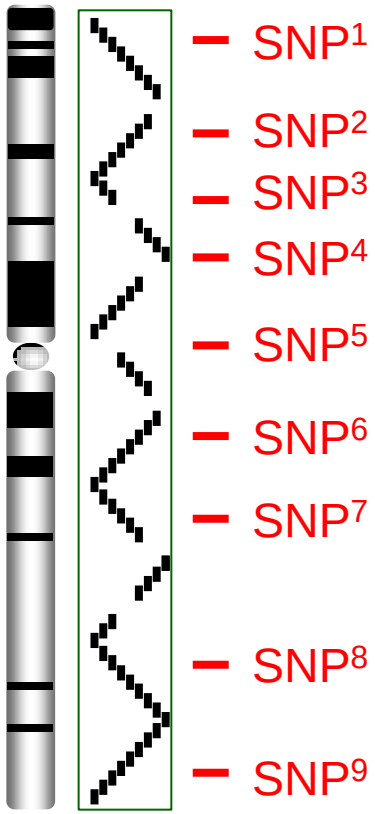
2. Search for overlap between BAC-contigs anchored in the same region

	❑ V4	❑ V4.2	❑ V4.3
#scaff	xxxx scaff	xxxx scaff	xxx scaff
Cumul. size	xxx Mb	xxx Mb	xxx Mb
N50	xxx kb	xxx kb (xxx scaff)	xxx kb (xxx scaff)

Order/Orientate scaffolds with markers



Our objective



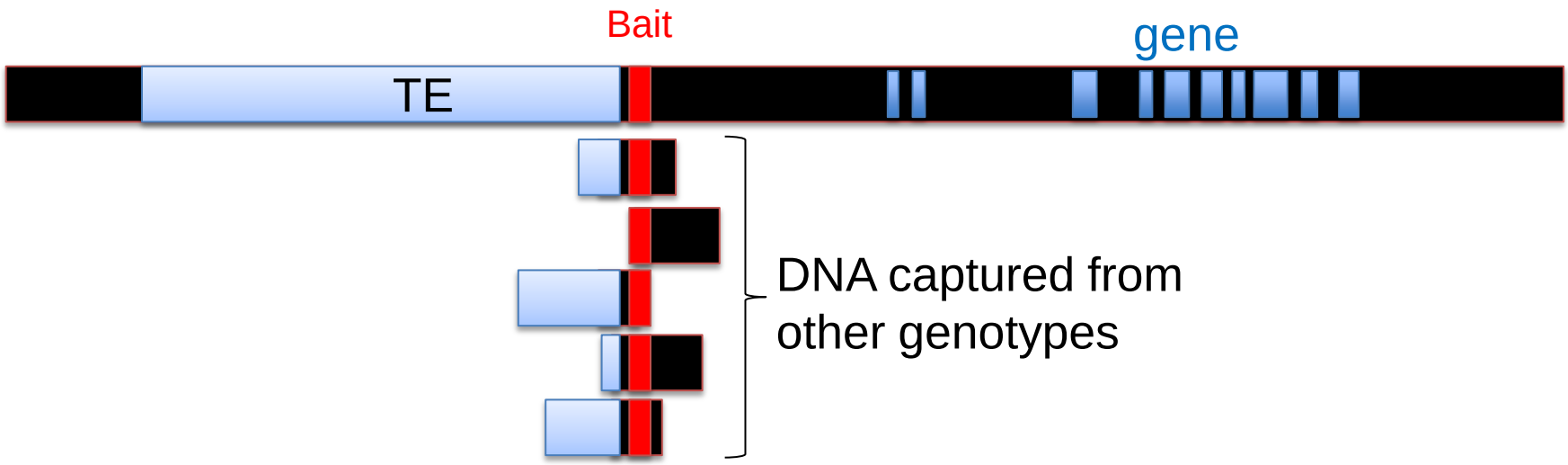
❑ Unordered BAC-contigs assembled into xxxx scaffolds

❑ 1 pseudomolecule

Order/Orientate scaffolds

- SNP discovery with *SureSelect*® Seq. Capture

(*E. Paux, N. Cubizolles, E. Rey*)



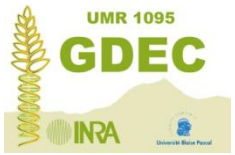
- xxxxx SNPs found on scaffolds accounting for xxx Mb
  - xxxx chr-wide SNPs chosen for genotyping

Biological analyses undertaken

- ❑ Annotation with TriAnnot
- ❑ Comparative genomics
- ❑ TE dynamics
- ❑ RNASeq data analyses
- ❑ CNVs
- ❑ Recombination/LD studies

+ 15 map-based cloning projects on 3B

Acknowledgements



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