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Sequencing and Assembling a Reference Sequence of the 1 Gb Wheat Chromosome 3B

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The 3B sequence

□ Whole 3B Shotgun



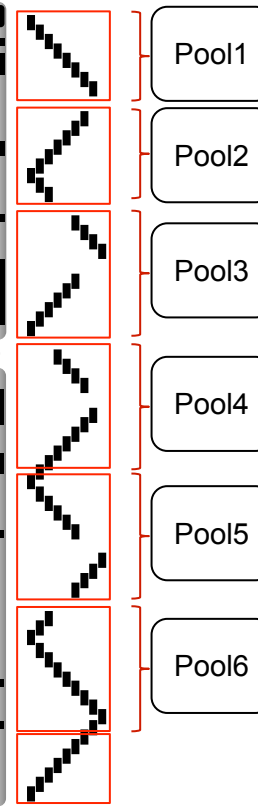
3B DNA

Illumina GAII
PE 2 x 108 nt



82 Gb

□ MTP BAC Sequencing



8452 BACs

932 pools
MP 8kb

454 GS-FLX



40 Gb

□ BAC-ends

❑ BAC pool raw assemblies



| | | |
|--------------------|-------|-------|
| <i>#scaffolds</i> | 16136 | scaff |
| <i>Cumul. size</i> | 1 | Gb |
| <i>Gaps</i> | 18 | % |
| <i>N50</i> | 275 | kb |

➔ Large scaffolds but small contigs (Newbler fragmentation)

□ Finishing

➔ v2.1



V. Barbe, S. Mangenot

Manual

Manual Curation of the scaffolding

Consider : BAC-ends

Mate pair info (Newbler)

➔ v3.0



Automated

Gap closer

Homopolymer correction

JM. Aury, A. Couloux

➔ v4.0



| | | #scaff | Mb | N50 |
|-------------|-----------------------|--------|------|--------|
| v2.1 | Raw assemblies | 16136 | 1040 | 275 kb |
| v4.0 | Finishing + gapCloser | 5109 | 993 | 463 kb |

BAC pool – *Issues*

- ❑ Deal with ~8000 *E. coli* clones
- ❑ Assignment scaffolds ↔ BACs
- ❑ Incomplete representation of chr.
- ❑ Redundancy
- ❑ Misassembled BACs
BAC contamination

Pooling/tagging

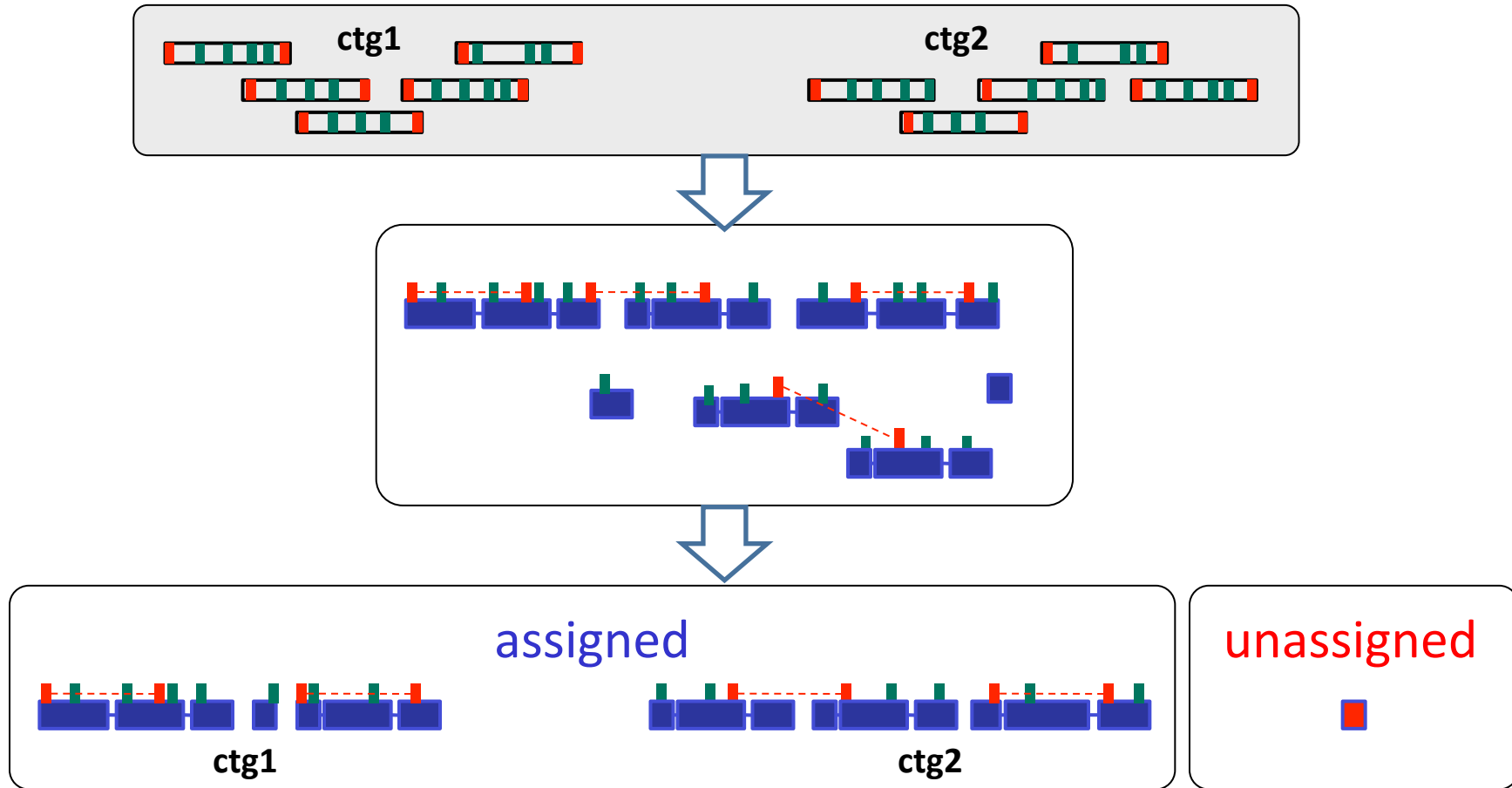
Quality of the
physical map

Assigning scaffolds ↔ BAC-contigs

➤ BAC Ends

➤ Whole Genome Profiling tags

(Philippe *et al.* BMC Genom. 2012)



➔ v4.1

957 Mb

36 Mb

❑ Incomplete representation of the chr.

Full map *Sequenced map*

| | | |
|----------------------|---------------|------|
| • Fingerprinted BACs | 133,000 (19x) | - |
| • #BAC contigs | 1717 | 1282 |
| • #MTP BACs | 9216 | 8452 |

❑ Incomplete

- MTP scaff compared to "survey" contigs

✓ Match 87%
✓ Absent 13%

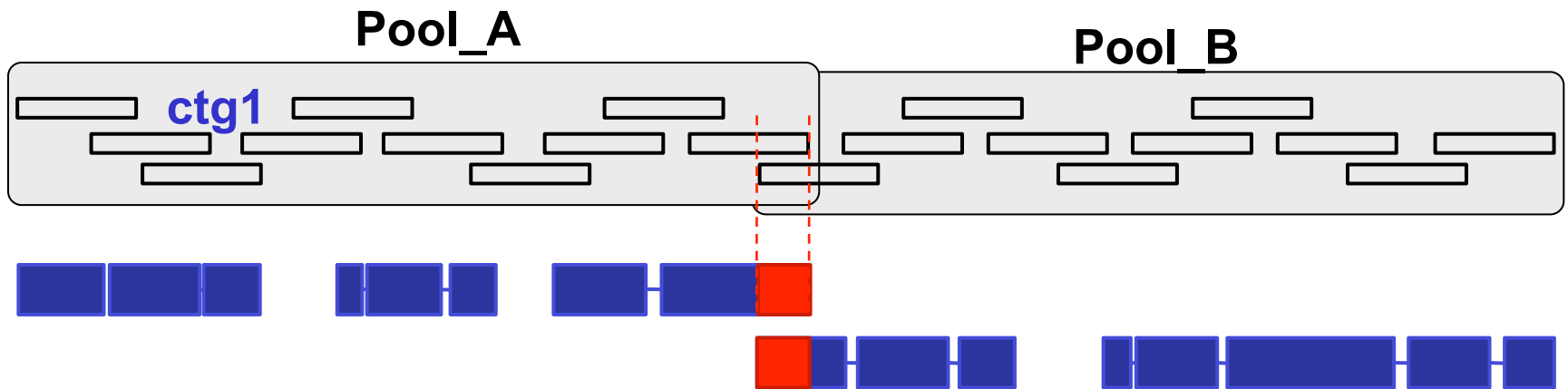
→ Gaps = 6%
→ non-3B DNA = 7%

- Inversely (using ~30,000 exons)

✓ Full 89%
✓ Partial 7%
✓ Absent 4%

□ Redundancy

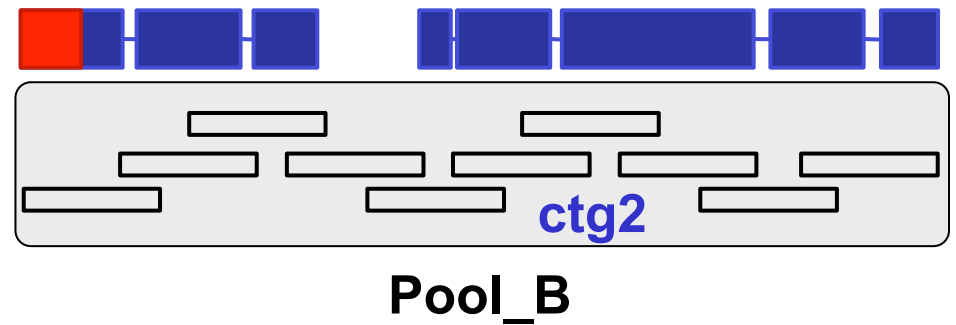
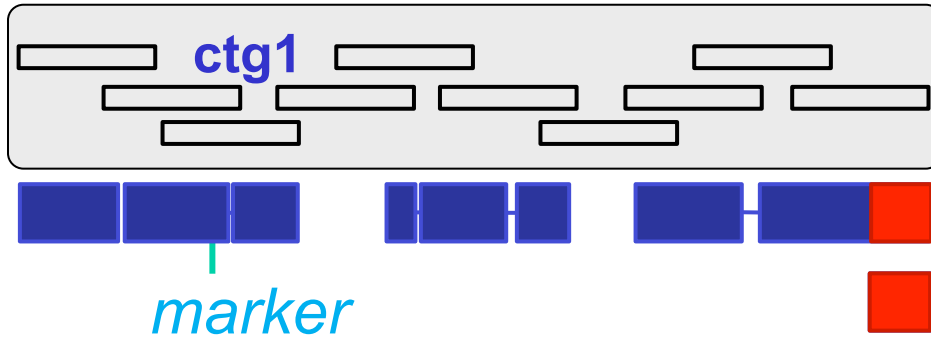
- Expected redundancy



Redundancy

- Unexpected redundancy

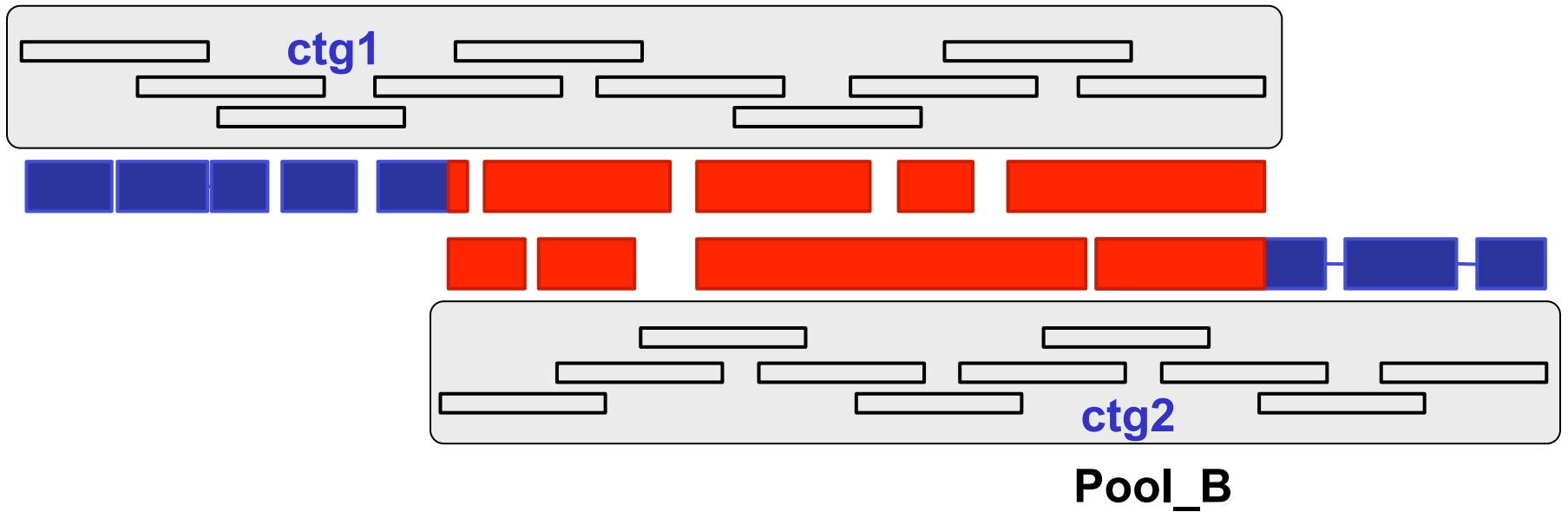
Pool_A



Redundancy

- Unexpected redundancy

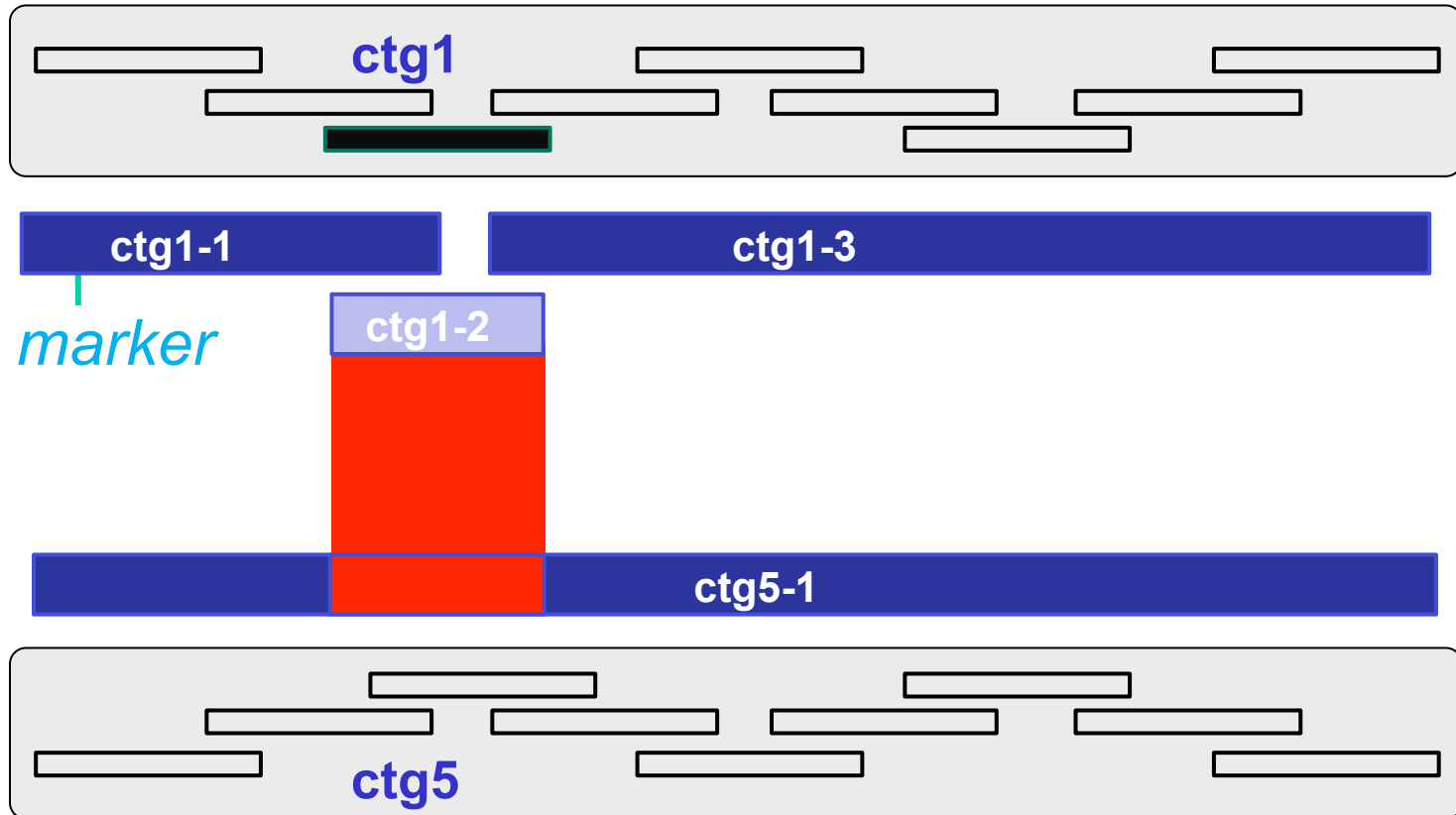
Pool_A



➤ `scaffAssembler.pl`

❑ Redundancy

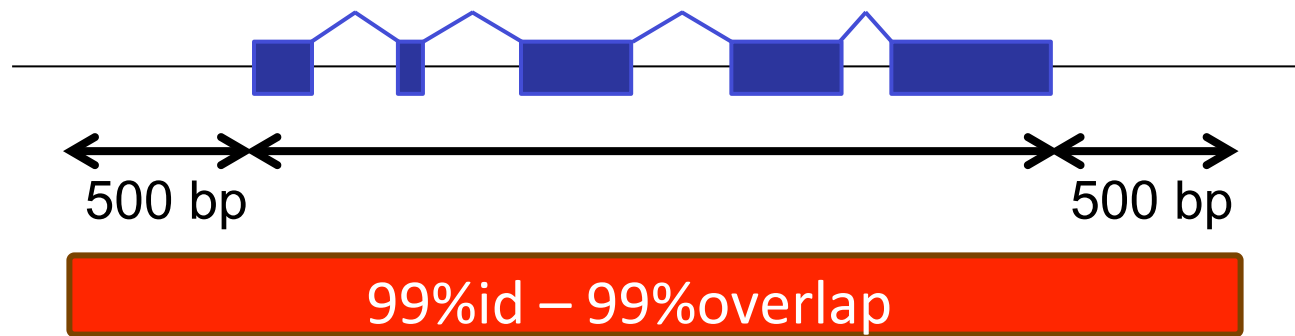
- Misassembled BACs / contaminations



→ Produce wrong associations betw contigs

□ Redundancy

- Estimate redundancy with annotated genes



17% redundant gene copies (unexpected)

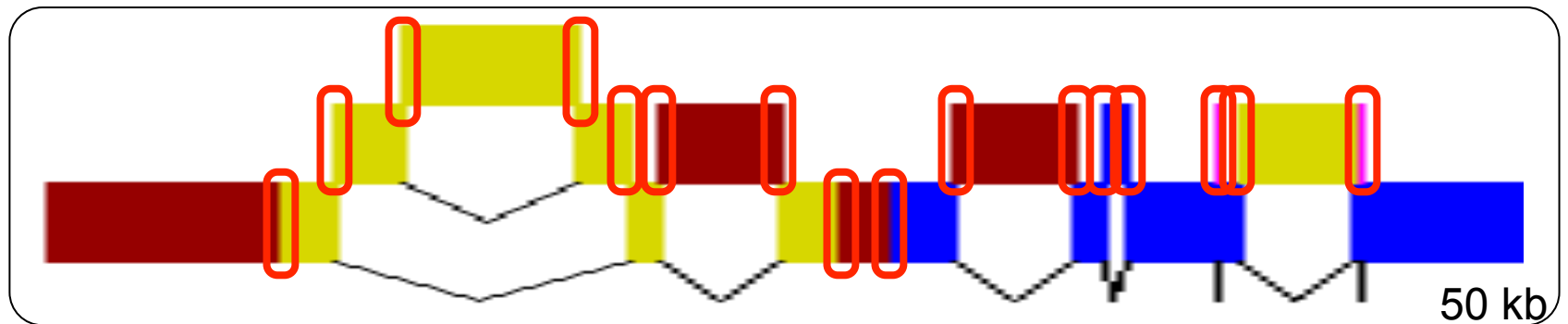
□ Redundancy

- Unexpected redundancy

- Problem: "all by all" alignment (1 Gb vs 1 Gb)

- ~~Solution1: Mask TEs?~~

- Solution2: Compare TE junctions=ISBPs



➔ Search for shared ISBPs between scaffolds

| | | #scaff | Mb | N50 |
|---------------|--------------------------|--------|------|--------|
| v2.1 | Raw assemblies | 16136 | 1040 | 275 kb |
| v4.0 | Finishing + gapCloser | 5109 | 993 | 463 kb |
| v4.1.2 | Assigning scaff ↔ ctg | - | - | - |
| v4.2.2 | Merging expected overlap | 4747 | 981 | 495 kb |
| v4.4.3 | Merging all vs all | 2808 | 833 | 892 kb |

v4.4.3

redundancy

6%

- Misassemblies
- Duplicated regions

➔ work on a set of **non-redundant genes**

□ Ordering scaffolds

➤ How many scaffolds with genes?

with genes → 675 Mb (81%)

wo genes → 158 Mb (19%)

➤ SNP discovery (Agilent-*SureSelect*®)

Bait



39,077 SNPs

❑ Ordering scaffolds

➤ Genetic mapping (*P. Sourdille INRA-GDEC*)

Pseudomolecule

○ Cs-Re map
(**1891** SNPs)

804 sc 599 Mb 72%



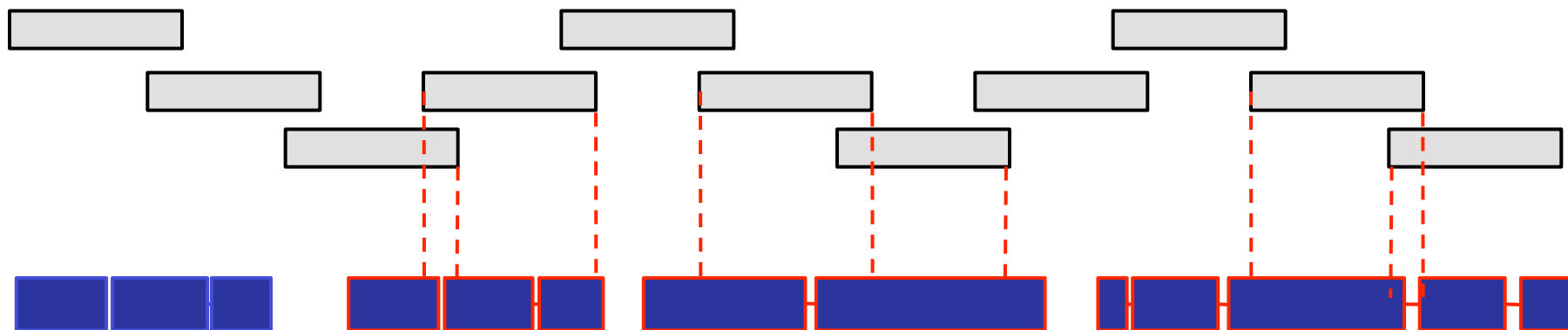
○ Neighbor map
(**3865** markers)

964 sc 679 Mb 82%



○ Use phys. map
info to infer scaff
position

pseudomolecule



SNP₁

pseudomol.pl

❑ Ordering scaffolds

➤ Genetic mapping

Pseudomolecule

○ Cs-Re map
(**1891** SNPs)

804 sc **599** Mb **72%**



○ Neighbor map
(**3865** markers)

964 sc **679** Mb **82%**

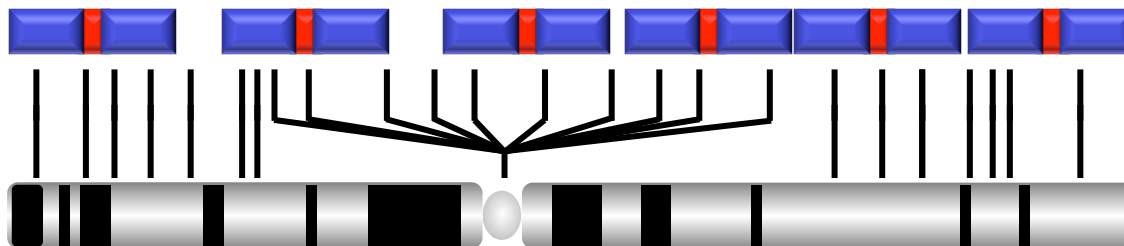
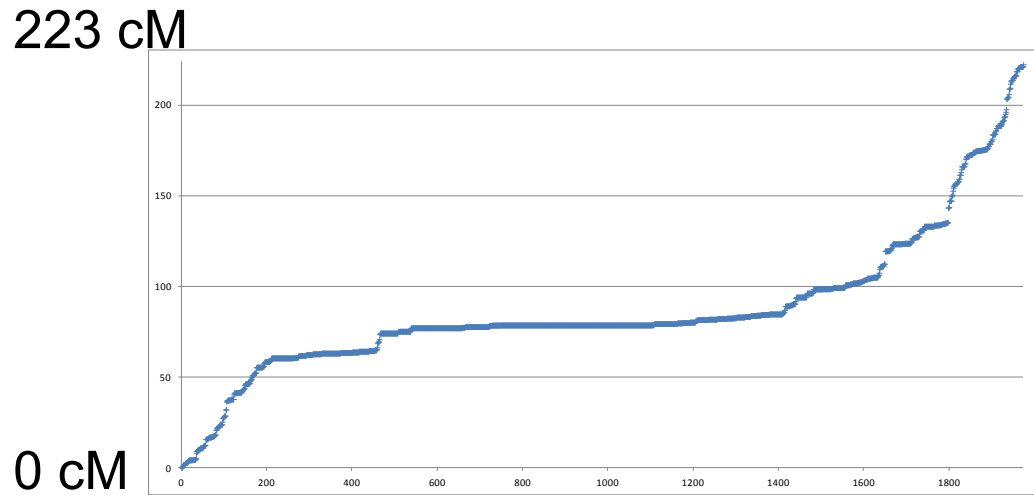


○ Use phys. map
info to infer scaff
position

1295 sc **760** Mb **91%**

Ordering scaffolds

Genetic mapping



1295 scaff

366 bins

Ordering scaffolds

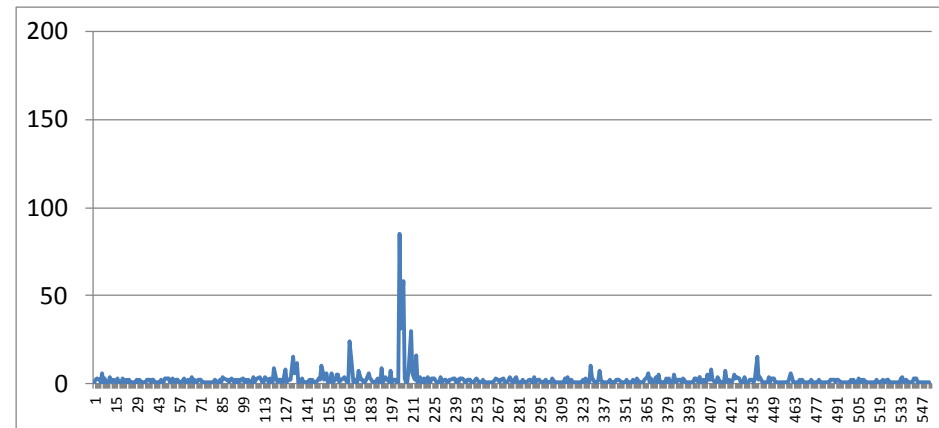
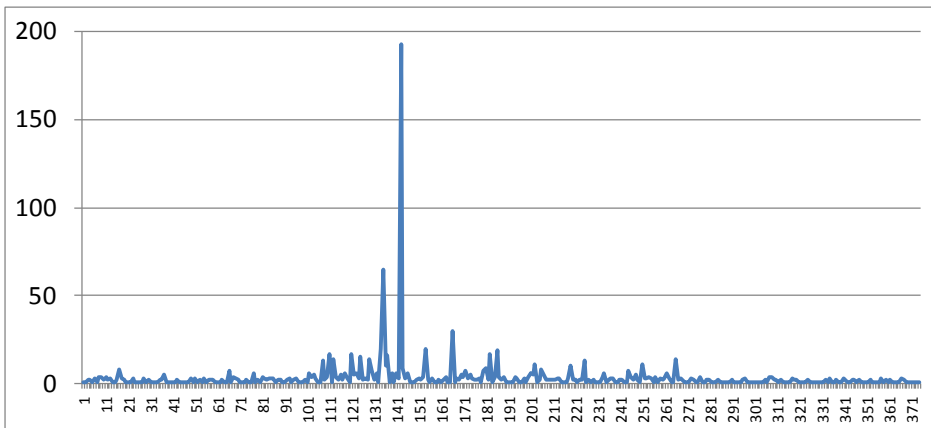
➤ LD mapping (*F. Balfourier - INRA-GDEC*)

1295 scaff
366 genetic bins



1295 scaff
554 genetic+LD bins

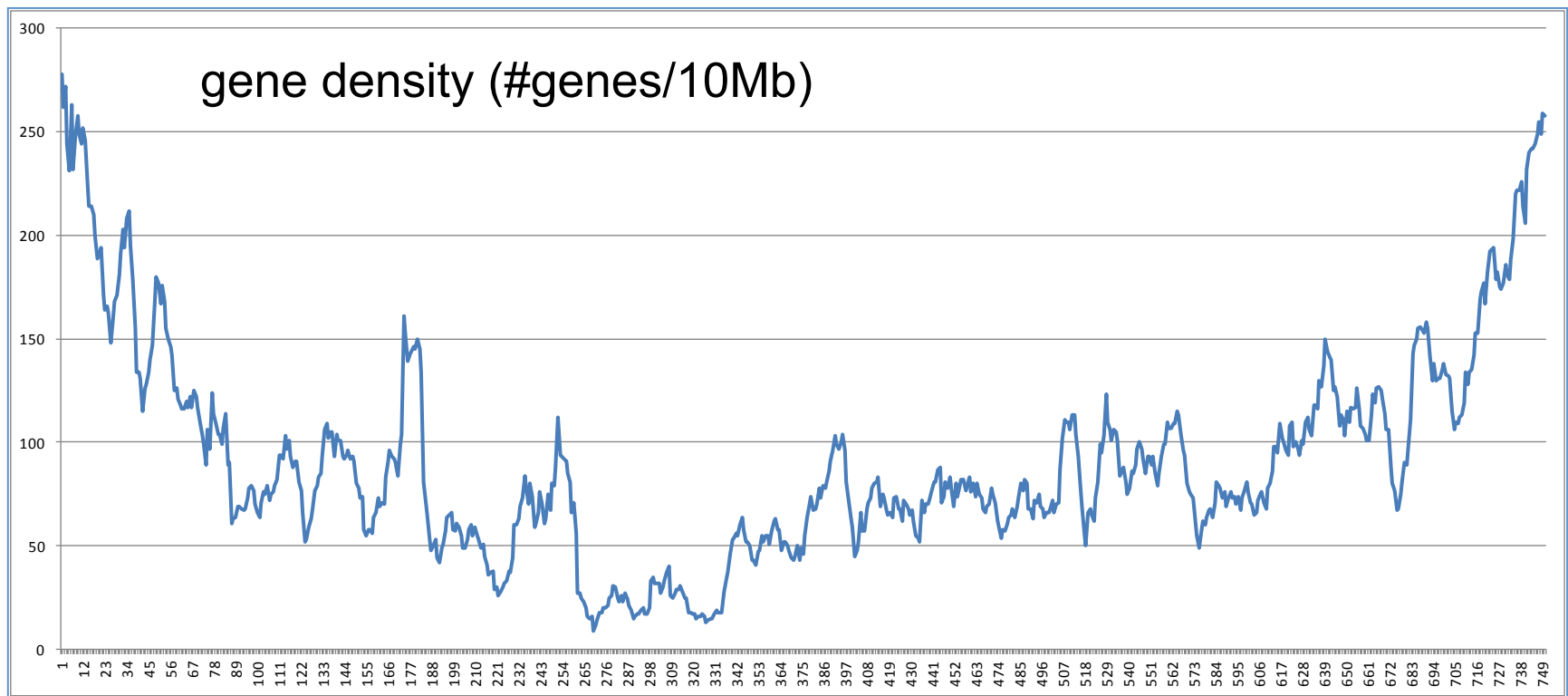
#scaff per bin



3B pseudomolecule

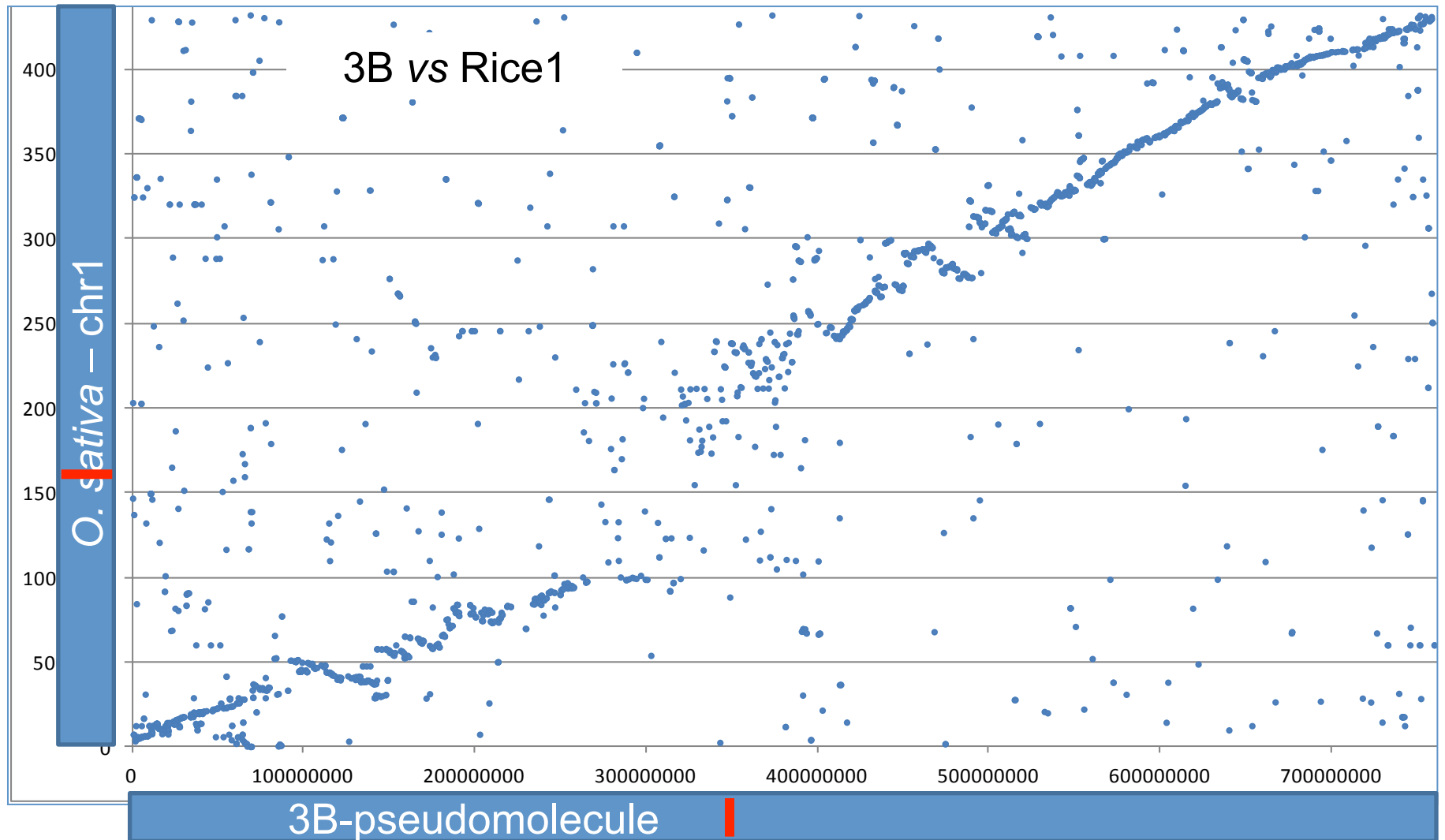
TriAnnot pipeline

7703 prot-coding genes



3B-pseudomolecule

3B pseudomolecule



□ Ongoing studies

- Gene space
 - Comparative genomics (duplicated genes!)
 - TE
 - Gene expression
 - Structural variations
 - Recombination studies
- + 15 map-based cloning projects on 3B (collab.)



INRA Clermont-Ferrand

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E. Paux J. Daron
P. Sourdille N. Glover
P. Leroy S. Theil
N. Guilhot



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INRA Toulouse

H. Berges et al.



Inst. Experimental Botany

J. Dolezel et al.



FranceAgriMer

