# Sequencing and Assembling 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, Adriana A. Alberti, et al.

2013 April 3rd
IWGSC workshop, Evry
Frédéric Choulet

# The 3B sequence

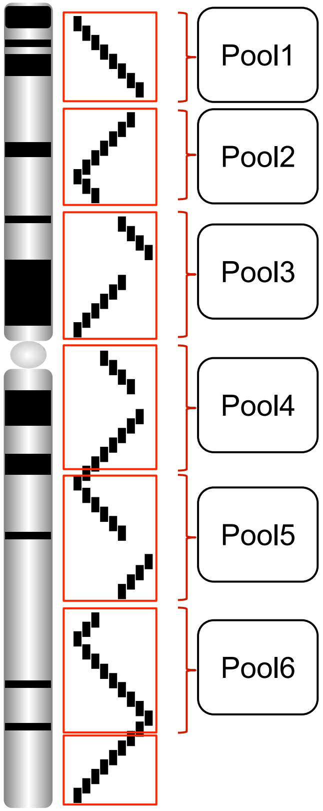
## □ Whole 3B Shotgun

3B DNA

Illumina GAII
PE 2 x 108 nt

82 Gb

## □ MTP BAC Sequencing



**8452** BACs

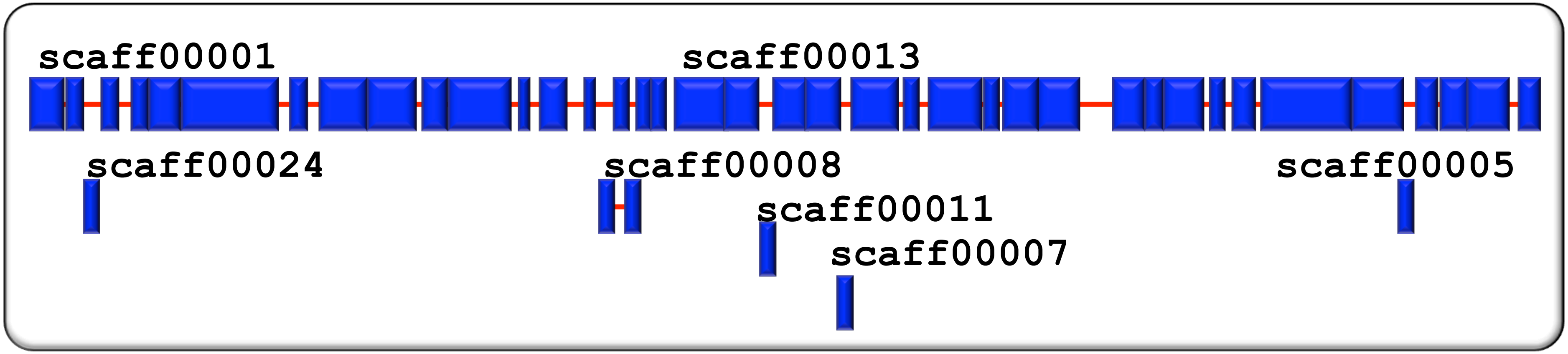
**932** pools
MP 8kb

454 GS-FLX

40 Gb

## □ BAC-ends

# ❑ BAC pool raw assemblies

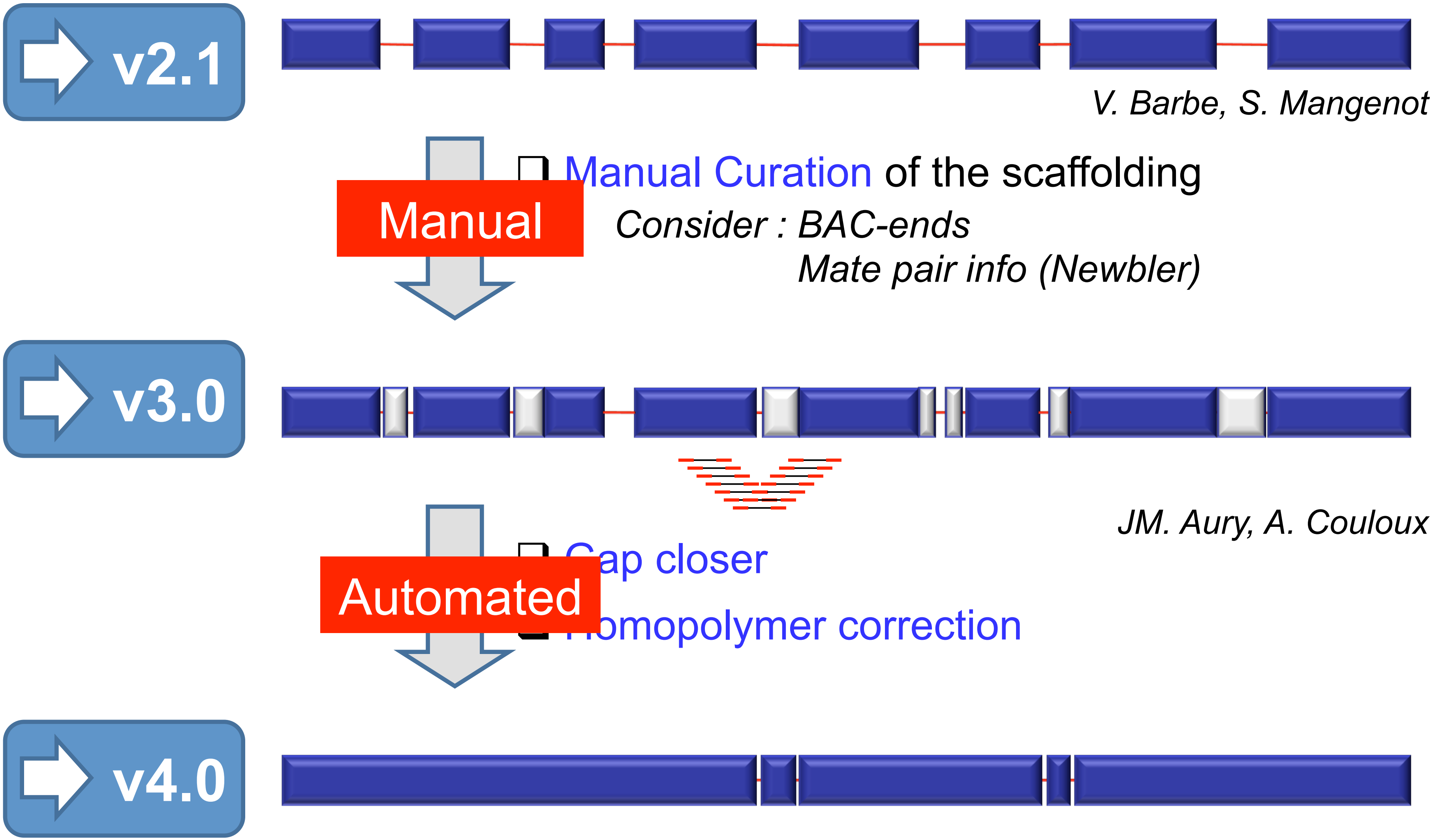


➔ v2.1

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

➔ Large scaffolds but small contigs (Newbler fragmentation)

# □ Finishing



*V. Barbe, S. Mangenot*

□ Manual Curation of the scaffolding

*Consider : BAC-ends*

*Mate pair info (Newbler)*

*JM. Aury, A. Couloux*

□ Gap closer

□ Homopolymer correction

|  |  | #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

Pooling/tagging

❑ Incomplete representation of chr.

❑ Redundancy

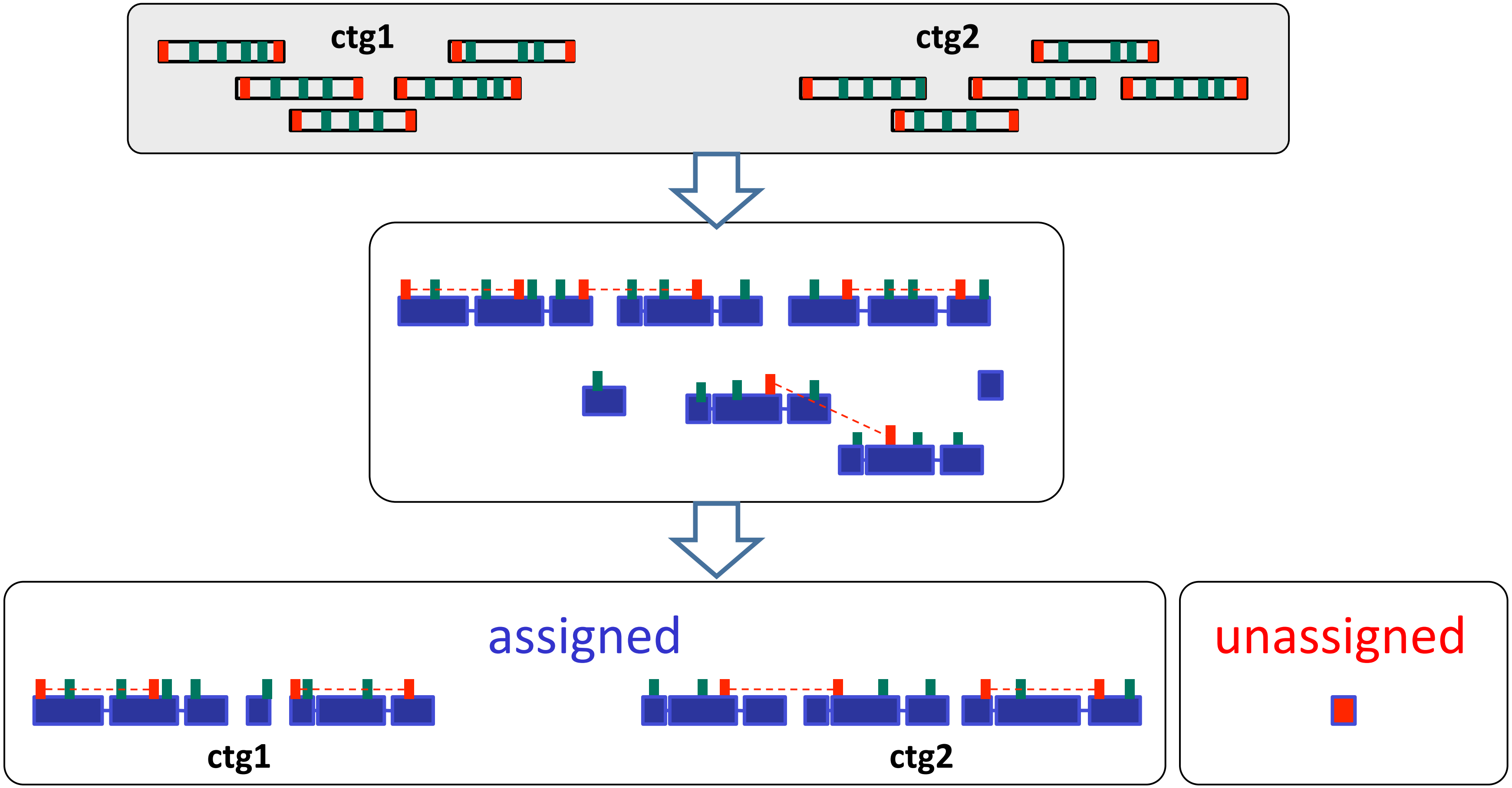
❑ Misassembled BACs
BAC contamination

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

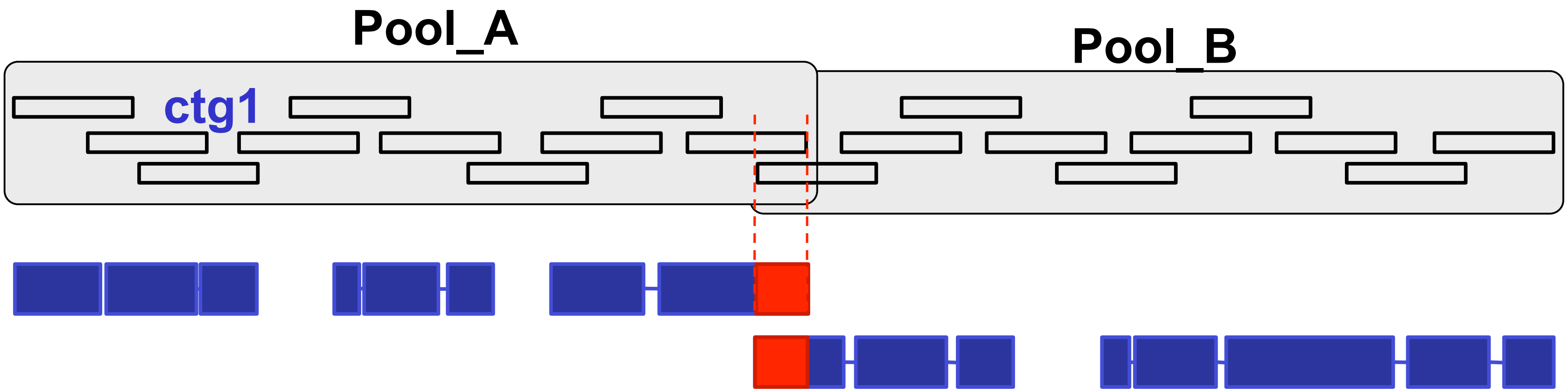
| | |
|---|---|
| ✓Match | 87% |
| ✓ Absent | 13% |

➔ Gaps = 6%
➔ non-3B DNA = 7%

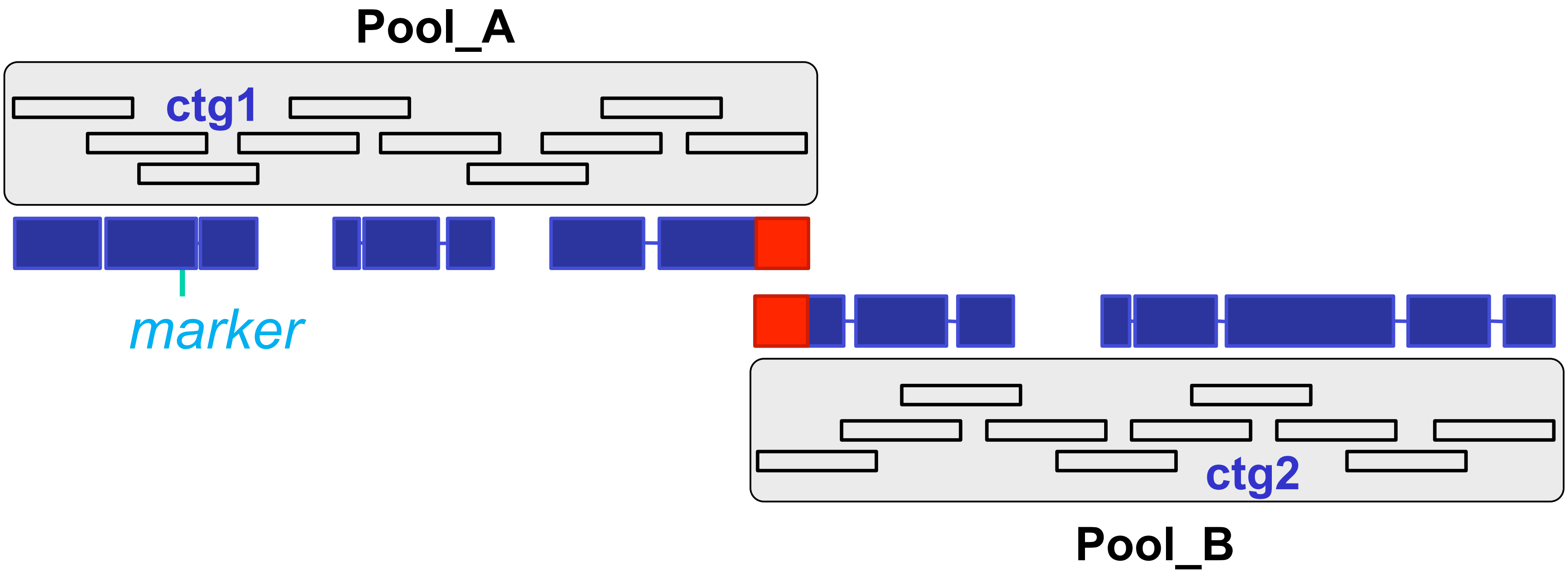
- Inversely (using ~30,000 exons)

| | |
|---|---|
| ✓Full | 89% |
| ✓ Partial | 7% |
| ✓ Absent | 4% |

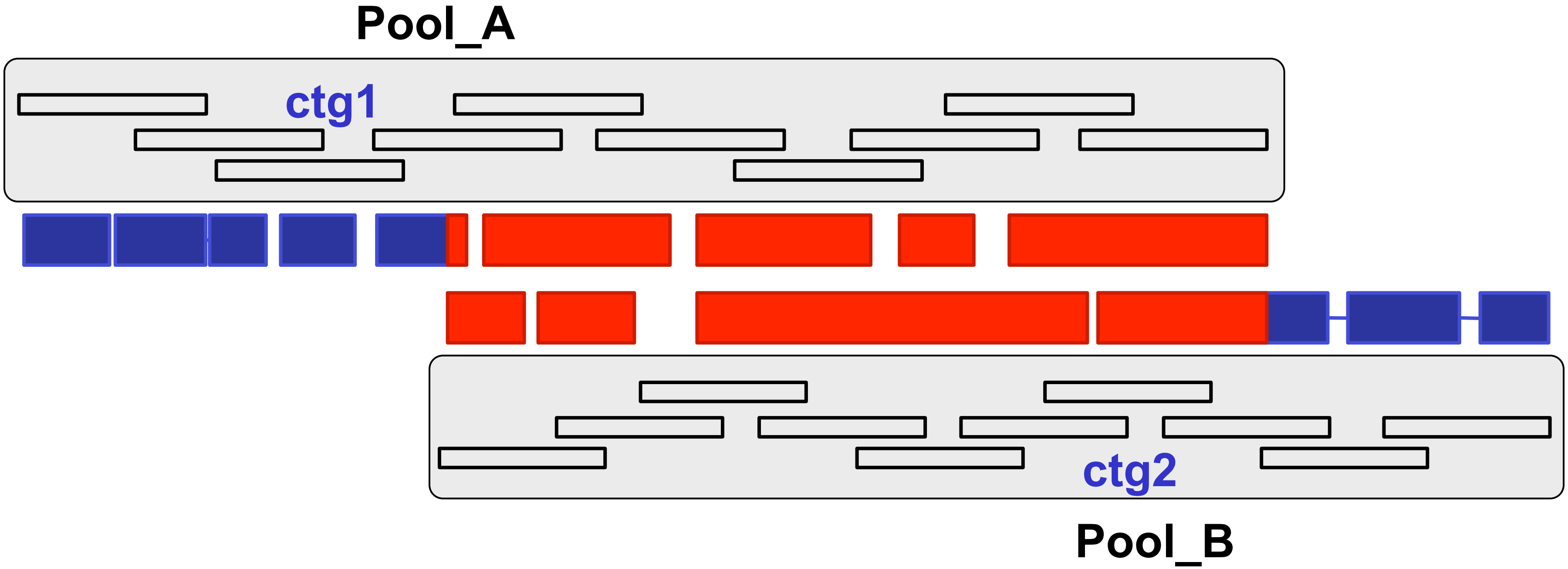
- Redundancy
  - Expected redundancy



- Redundancy
  - Unexpected redundancy

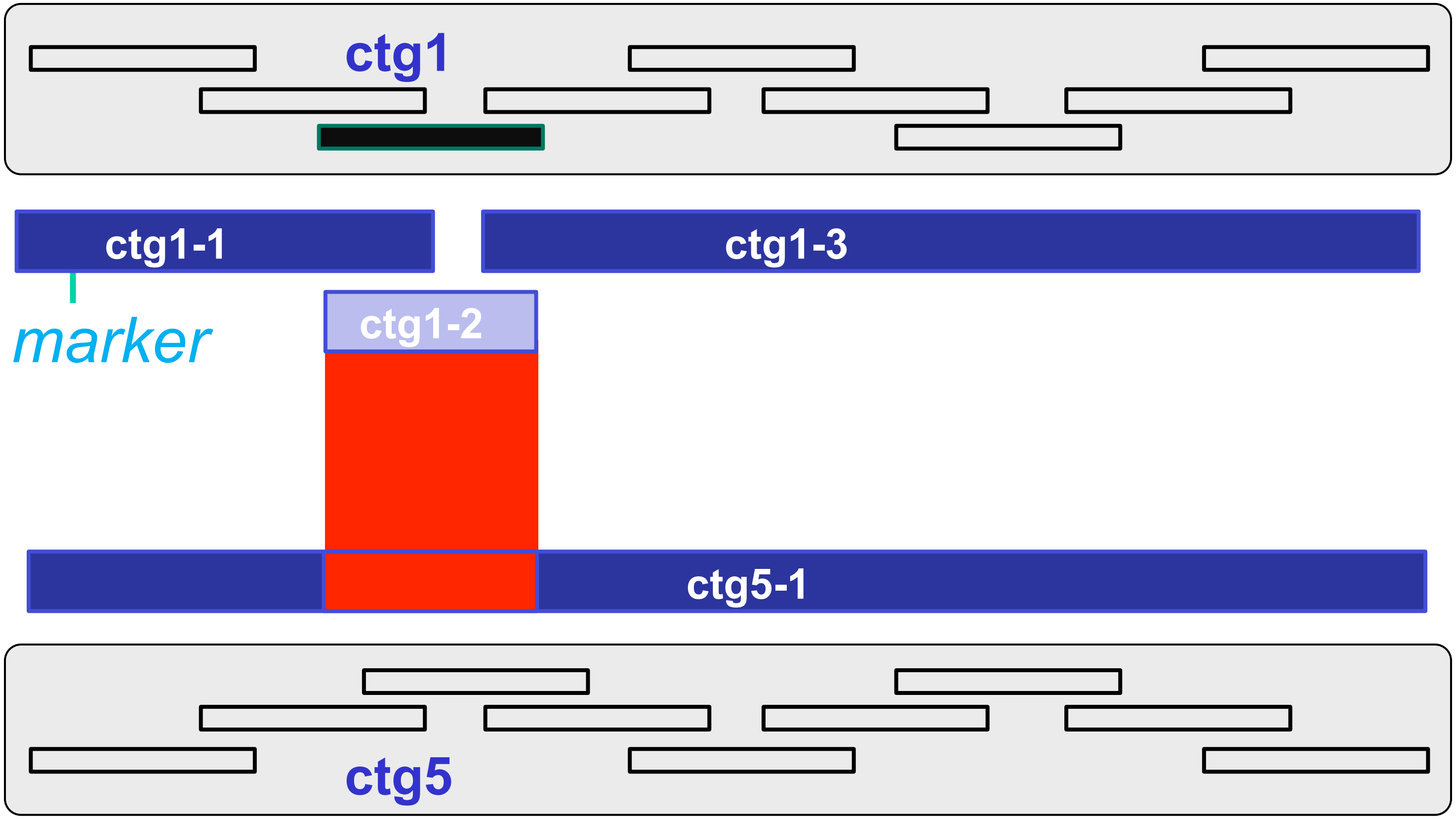


- Redundancy
  - Unexpected redundancy



- `scaffAssembler.pl`

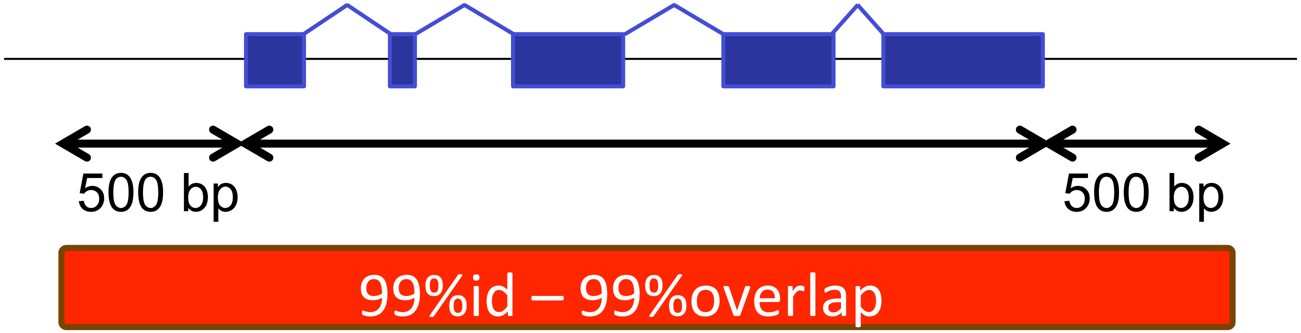
- Redundancy
  - Misassembled BACs / contaminations



→ Produce wrong associations betw contigs

## Redundancy

- Estimate redundancy with annotated genes



500 bp

500 bp

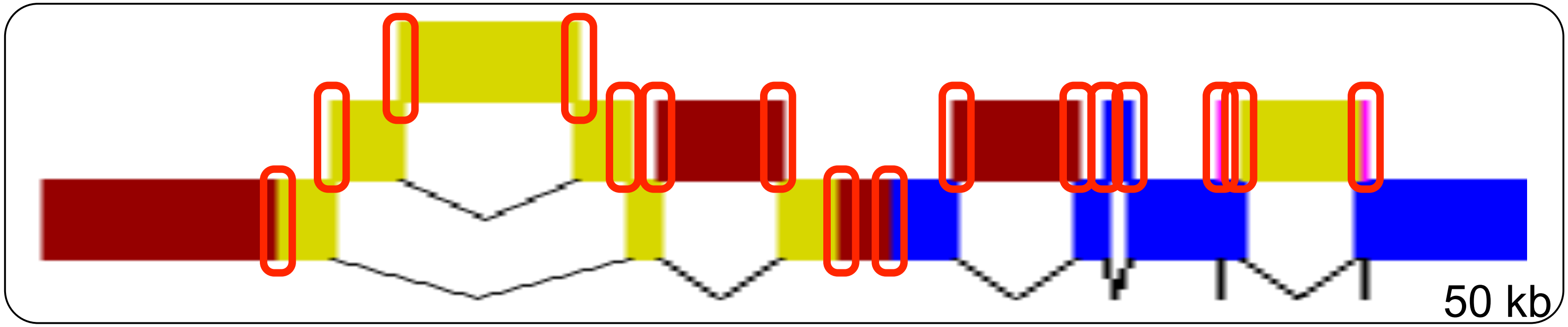
99%id – 99%overlap

**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**

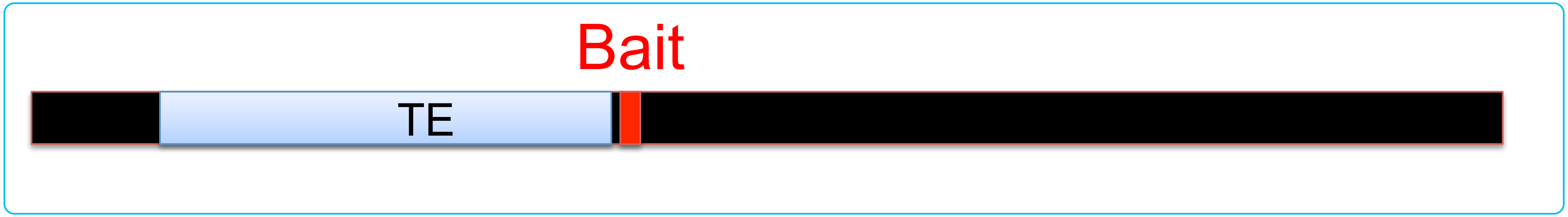
## ❑ Ordering scaffolds

- How many scaffolds with genes?

  with genes → 675 Mb (81%)

  wo genes → 158 Mb (19%)

- SNP discovery (Agilent-*SureSelect*®)

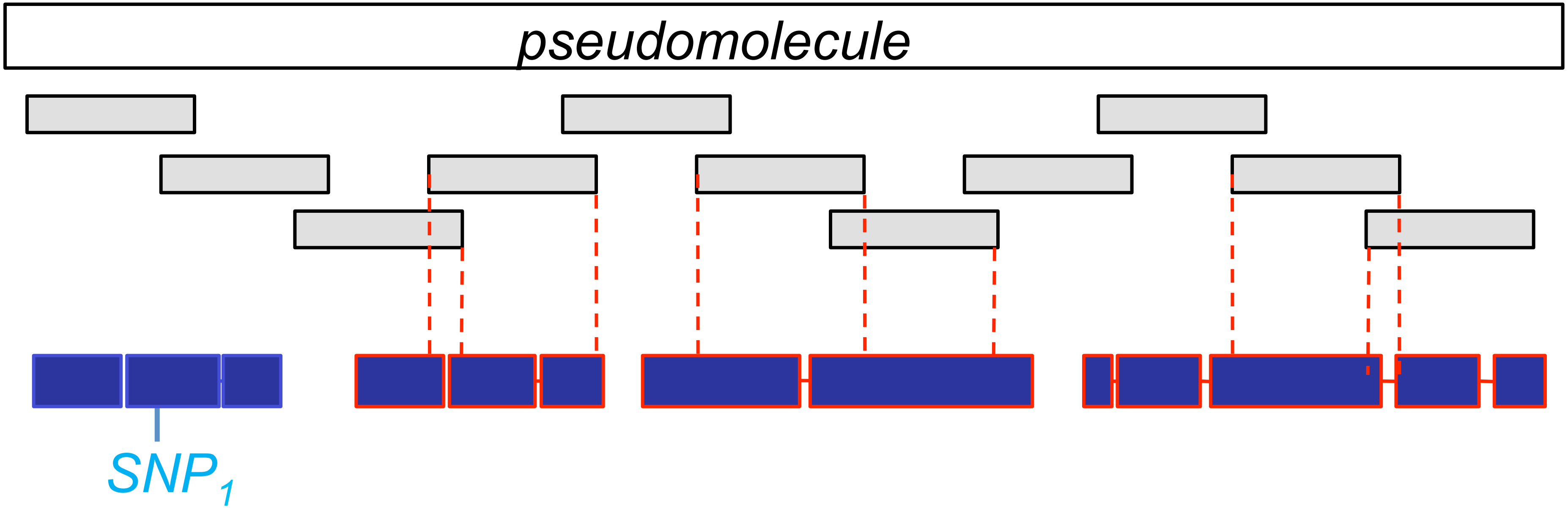


39,077 SNPs

# ❑ 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 | | | |



**pseudomol.pl**

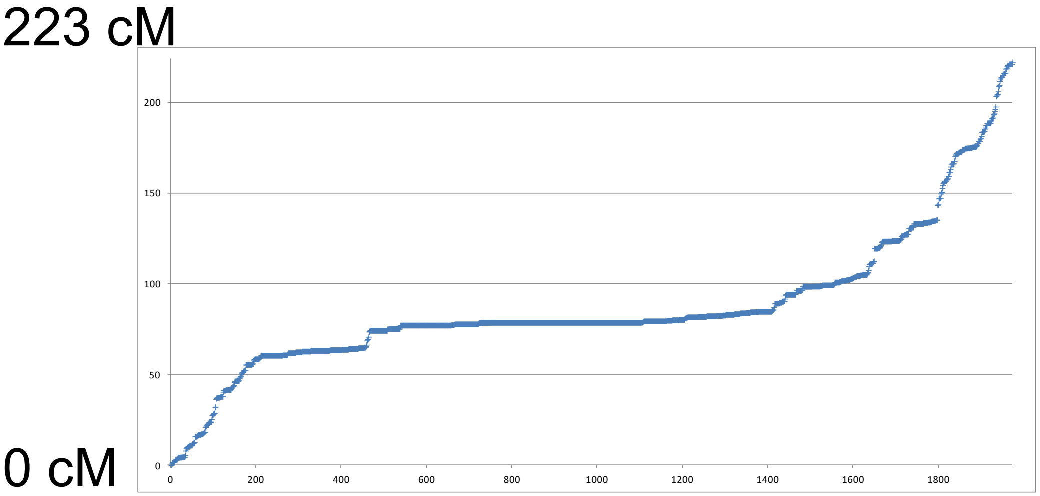
## ❑ Ordering scaffolds

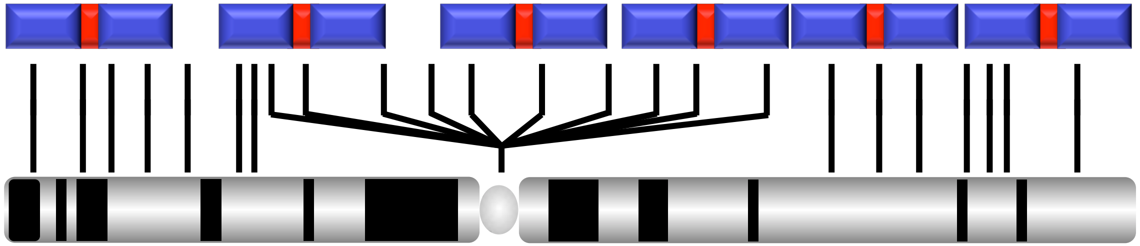
### ➢ Genetic mapping

| | 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

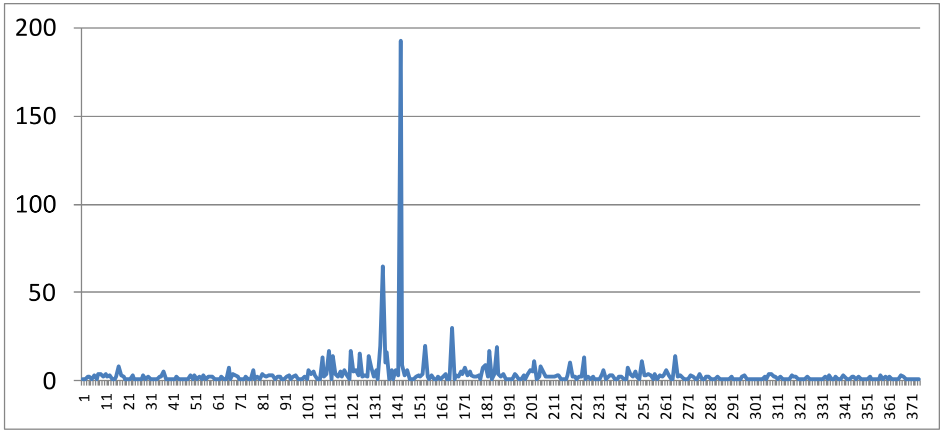
## ➢ LD mapping *(F. Balfourier - INRA-GDEC)*

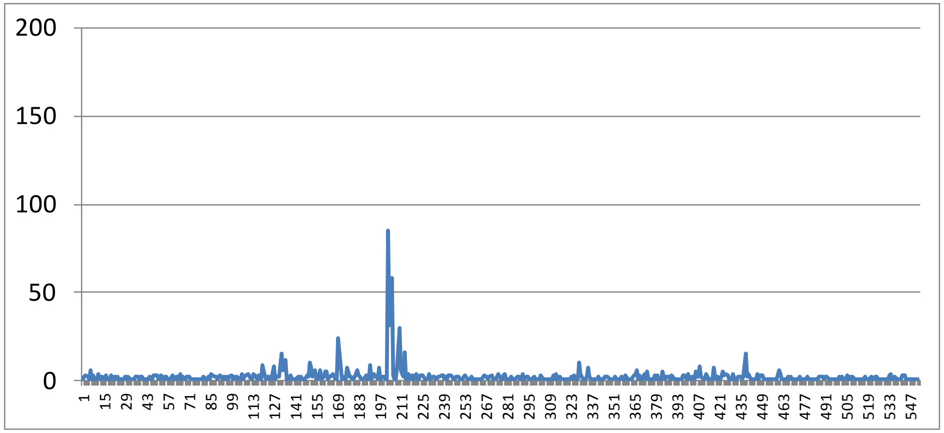
1295 scaff
366 genetic bins

⇨

1295 scaff
554 genetic+LD bins

#scaff per bin

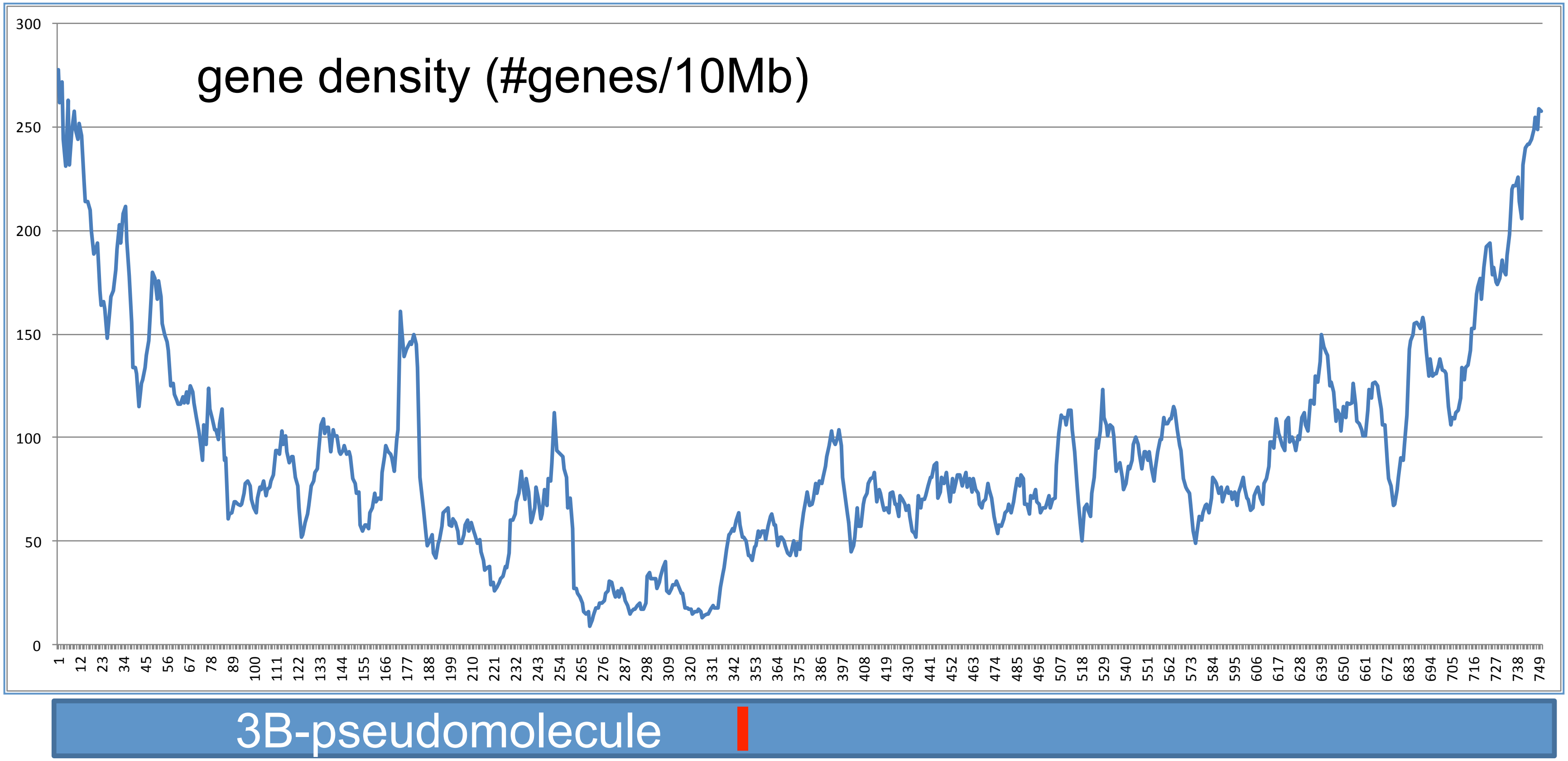




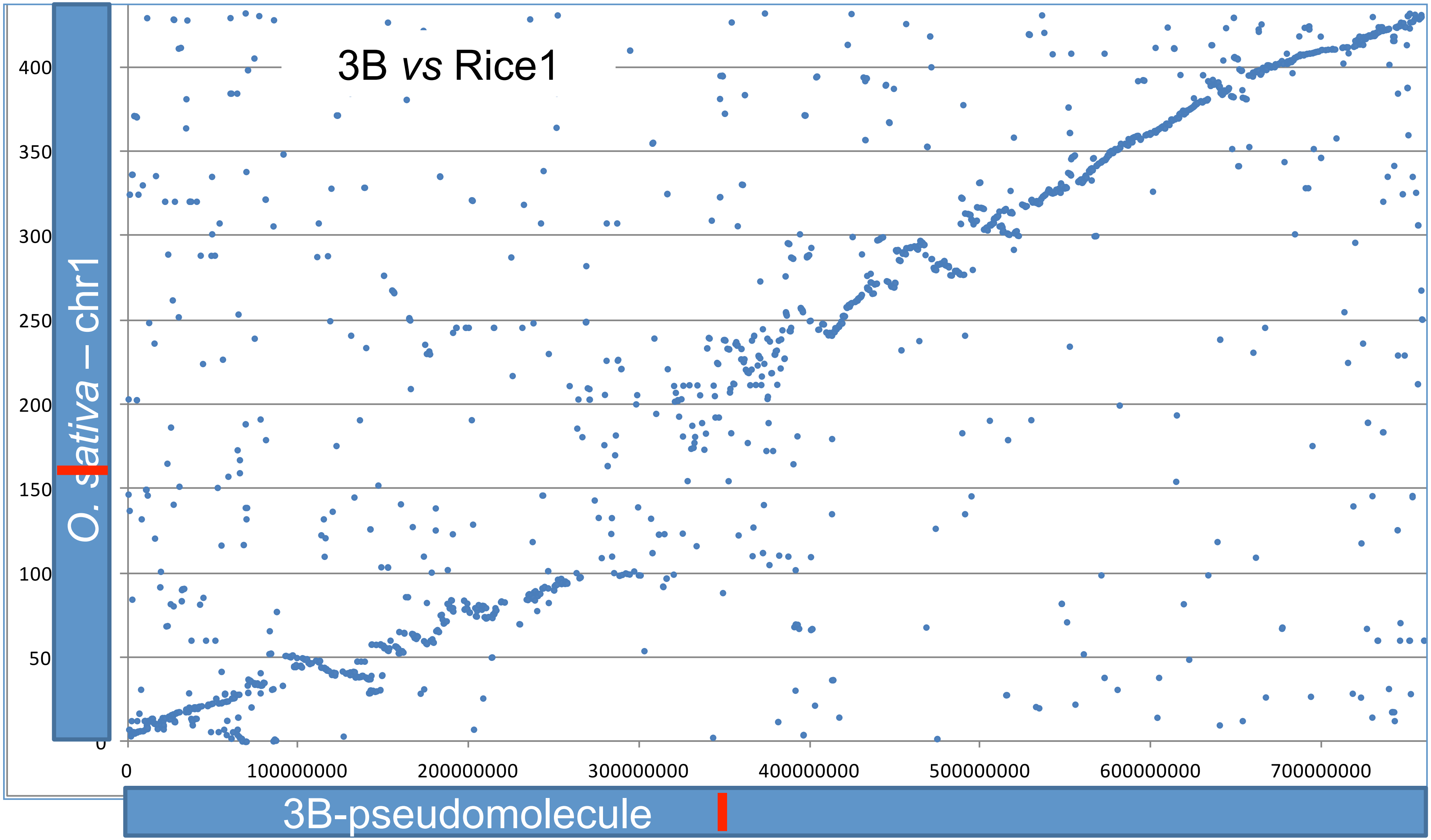
## ❑ 3B pseudomolecule

➢ **TriAnnot pipeline**

**7703** prot-coding genes



# ❑ 3B pseudomolecule



## ❑ Ongoing studies

- ○ Gene space
- ○ Comparative genomics (duplicated genes!)
- ○ TE
- ○ Gene expression
- ○ Structural variations
- ○ Recombination studies

+ 15 map-based cloning projects on 3B (collab.)

GDEC

### INRA Clermont-Ferrand

C. Feuillet
E. Paux
P. Sourdille
P. Leroy
N. Guilhot
L. Pingault
J. Daron
N. Glover
S. Theil

GENOSCOPE
Centre National de Séquençage

### Genoscope, Evry

P. Wincker
A. Alberti
V. Barbe
S. Mangenot
JM. Aury
A. Couloux

URGI

### INRA Versailles

H. Quenesville
M. Alaux
et al.

CNRGV
CENTRE NATIONAL DE RESSOURCES GÉNOMIQUES VÉGÉTALES

### INRA Toulouse

H. Berges et al.

### Inst. Experimental Botany

J. Dolezel et al.

Agence Nationale de la Recherche
ANR

FranceAgriMer

Céréales Vallée

INRA
SCIENCE & IMPACT

I W G S C