

### Identification of the recombinant scaffolds and definition of the recombinant intervals

In order to warrant the robustness of the physical distances, we focused our recombination study within scaffolds from the v4.4.3 assembly of chromosome 3B (Choulet et al. 2014). We used molecular markers (SNPs and SSRs; Supplementary Table S4) to identify the parental status (Chinese Spring (Cs: A) or Renan (Re: B) in figures 1 & 2 hereafter) at a given physical position (in base pair: bp) in each recombinant line. Two consecutive markers constitute thus an interval of a precise size (in bp) that can be associated to a completely annotated (genes and expression, transposable elements, motifs...) sequence. We identified 73 scaffolds ranging from 101 to 2812°kb with at least 1 CO. A careful analysis of the allelic status (parent A or B) at each marker locus allows identification of allelic changes from one parent to another (Fig. 1) which reflect COs.

|                    | M1                | M2                | M3                | M4                | M5                |
|--------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| <b>Chr 3B bp</b>   | <b>34 682 781</b> | <b>34 843 216</b> | <b>35 515 278</b> | <b>36 438 787</b> | <b>36 580 687</b> |
| bp between markers |                   | 160 435           | 672 062           | 923 509           | 141 900           |
| CsRe-1             | B                 | B                 | B                 | B                 | B                 |
| CsRe-2             | A                 | A                 | A                 | A                 | A                 |
| CsRe-3             | B                 | B                 | A                 | A                 | A                 |
| CsRe-4             | B                 | B                 | A                 | A                 | A                 |
| CsRe-5             | B                 | B                 | A                 | A                 | A                 |
| CsRe-6             | B                 | B                 | A                 | A                 | A                 |
| CsRe-7             | A                 | A                 | B                 | B                 | B                 |
| CsRe-8             | A                 | A                 | B                 | B                 | B                 |
| CsRe-9             | B                 | B                 | B                 | A                 | A                 |
|                    |                   | 6 COs             |                   |                   |                   |

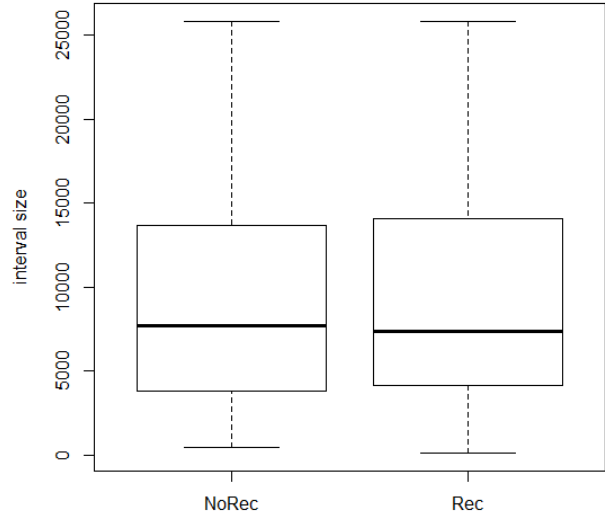
**Fig. 1:** Graphical genotype of nine recombinant inbred lines (CsRe-1-9) for five molecular markers (M1-5) with their physical position along chromosome 3B pseudomolecule (in bp) and the size (in bp) of each interval they define. A: Cs allele (in red) and B: Re allele (in blue). Six COs are detected between M2 and M3 in a 672,072 bp interval.

### Fine mapping of COs and selection of recombinant (Rec) and non-recombinant (NoRec) intervals

For each scaffold showing recombination events, we fine-mapped the COs by increasing the density of markers to achieve a resolution of a maximum of 26kb per interval containing at least one CO which is the size commonly accepted for similar studies (Fig. 2). We thus selected a set of 74 intervals ranging from 108 to 25,848 bp (average: 9,344°bp) (total size 698,591 bp) which constituted our recombinant (Rec) sample.

|                    | M1         | M2         | M6         | M7         | M8         | M9         | M10        | M11         | M12        | M13        | M14        | M15        | M16         | M17        | M18        | M19        | M3         | M20        | M21        | M22        | M23        | M24        | M25        | M26        | M4         | M5         |
|--------------------|------------|------------|------------|------------|------------|------------|------------|-------------|------------|------------|------------|------------|-------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| Chr. 3B            | 34 682 781 | 34 843 216 | 34 922 132 | 34 929 835 | 34 943 212 | 34 951 942 | 34 963 600 | 34 972 542  | 35 038 438 | 35 181 069 | 35 243 506 | 35 305 851 | 35 315 295  | 35 511 537 | 35 513 158 | 35 515 016 | 35 515 278 | 35 651 544 | 35 669 473 | 35 974 238 | 36 125 608 | 36 220 370 | 36 266 055 | 36 279 750 | 36 438 787 | 36 580 687 |
| bp between markers |            | 160435     | 78916      | 7693       | 13387      | 8730       | 11718      | 8882        | 65916      | 142611     | 62437      | 62345      | 9444        | 196242     | 1621       | 1858       | 262        | 136266     | 17929      | 304755     | 151380     | 94762      | 45685      | 13695      | 159037     | 141900     |
| CsRe-1             | B          | B          | B          | B          | B          | B          | B          | B           | B          | B          | B          | B          | B           | B          | B          | B          | B          | B          | B          | B          | B          | B          | B          | B          | B          | B          |
| CsRe-2             | A          | A          | A          | A          | A          | A          | A          | A           | A          | A          | A          | A          | A           | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          |
| CsRe-3             | B          | B          | B          | B          | B          | B          | B          | B           | A          | A          | A          | A          | A           | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          |
| CsRe-4             | B          | B          | B          | B          | B          | B          | B          | B           | B          | B          | B          | B          | B           | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          |
| CsRe-5             | B          | B          | B          | B          | B          | B          | B          | B           | A          | A          | A          | A          | A           | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          |
| CsRe-6             | B          | B          | B          | B          | B          | B          | B          | B           | A          | A          | A          | A          | A           | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          | A          |
| CsRe-7             | A          | A          | A          | A          | A          | A          | A          | A           | A          | A          | A          | A          | A           | B          | B          | B          | B          | B          | B          | B          | B          | B          | B          | B          | B          | B          |
| CsRe-8             | A          | A          | A          | A          | A          | A          | A          | A           | B          | B          | B          | B          | B           | B          | B          | B          | B          | B          | B          | B          | B          | B          | B          | B          | B          | B          |
| CsRe-9             | B          | B          | B          | B          | B          | B          | B          | B           | B          | B          | B          | B          | B           | B          | B          | B          | B          | B          | B          | B          | B          | A          | A          | A          | A          | A          |
|                    |            |            |            |            | NoRec      |            |            | 2 COs (Rec) |            |            |            |            | 2 COs (Rec) |            |            |            |            | NoRec      |            |            |            |            |            |            |            |            |

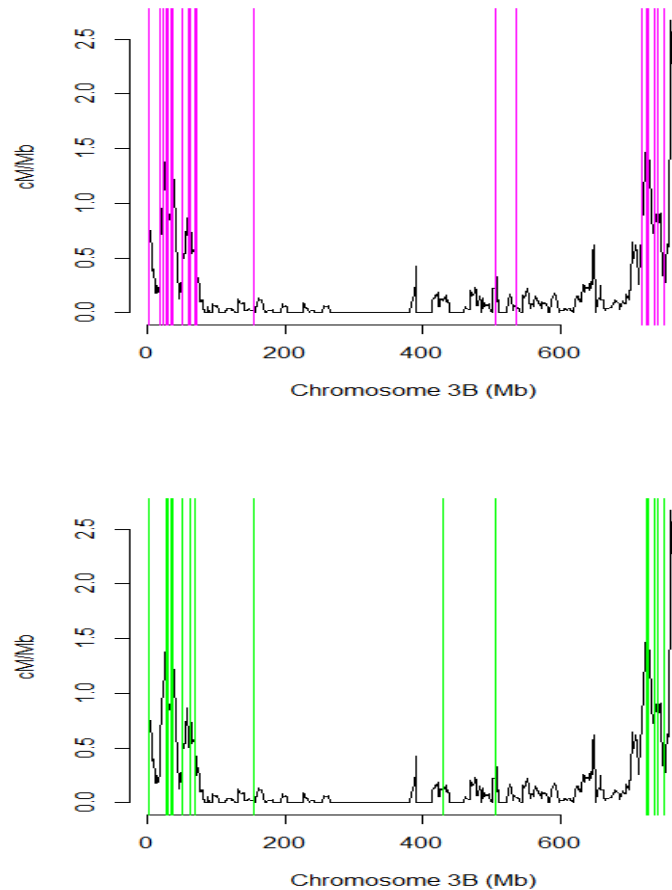
**Fig. 2:** Graphical genotype of the same nine recombinant inbred lines (CsRe-1-9) for the same five markers (M1-5) and 21 additional molecular markers (M6-26 highlighted in grey) with their physical position along chromosome 3B pseudomolecule (in bp) and the size (in bp) of each interval they define. Intervals of a size < 26 kb are highlighted in green. A: Cs allele (in red) and B: Re allele (in blue). NoRec and Rec intervals are framed with black boxes. Four COs are resolute enough: two between M10 and M11 in a 8,882 bp interval and two between M15 and M16 in a 9,444 bp interval. The size of the interval between M11 and M12 for the other two COs remains too large (65,916 bp) and was longer taken into account for further analyses. The same holds true for the CO located in interval between M23 and M24.



**Fig. 3:** Box-plot showing the similarity in size of Rec and Norec intervals.

In order to validate the significance of sequence features associated to recombination events, we conducted a comparative analysis with non-recombinant intervals (NoRec). To

avoid any ascertainment bias that could be due to the partitioning of chromosome 3B in regions showing highly different recombination rates combined to differences in gene and transposable element contents (Choulet et al. 2014), the NoRec intervals were selected in the vicinity of the Rec intervals (Fig. 2) and of a similar size (Fig. 3; 698,852 bp). We thus constituted an unbiased NoRec sample with 74 intervals of a similar total size and located in the same regions (Fig. 4) as the Rec sample.



**Fig. 4:** Distribution of the Rec (purple) and NoRec (green) intervals along the pseudomolecule of chromosome 3B.

#### **Selecting a set of intervals representing the whole chromosome 3B (Overview)**

In order to make a comparative analysis with the general features corresponding to chromosome 3B, we selected a set of 74 intervals without any recombination event and any N, of a mean size of 9,459 bp (total size 737,802 bp) and evenly spaced each ~10 Mb along chromosome 3B pseudomolecule.