Chromatin state affects the DNA breakage/repair mechanism in wheat

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- In radiation hybrid (RH) mapping, the double stranded breaks are repaired by either homology-directed repair or non-homologus end-joining
- RH induced deletion mapping provides an excellent resource for studying the chromosome structure and its correlation with DNA repair
- RH maps have been proposed to provide:
  - higher and more uniform resolution than genetic maps
  - supposed to be independent of the distribution patterns observed for meiotic recombination

Langdon (LDN)
12”+3A”+3B”

Irradiate seed @ various levels

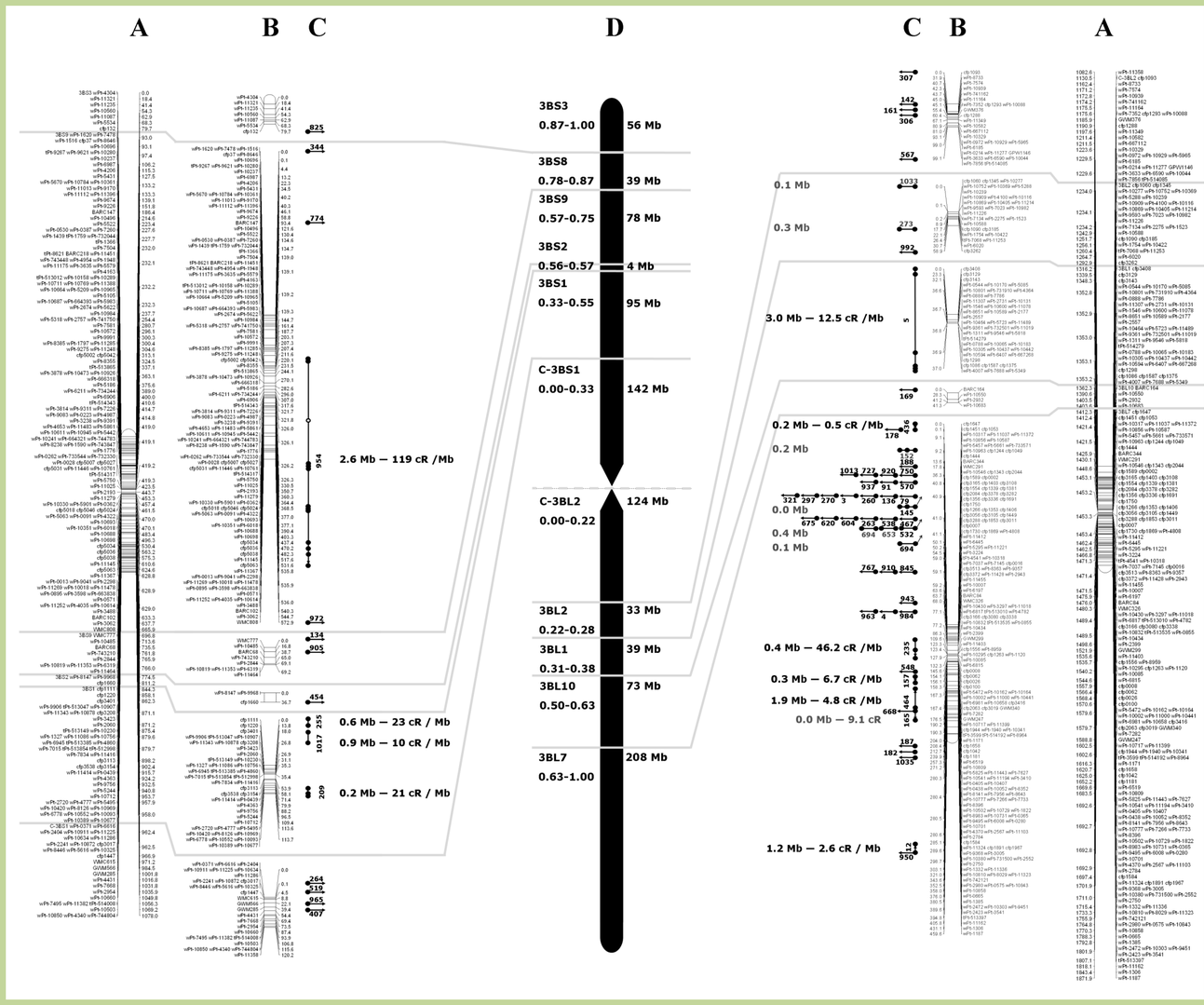
12”+3A”+3D” X 12”+3A”+3B”
LDN 3D (3B) RH^0 generation

12’’+3A’’+3D’+3B’
RH^1 generation

Radiation hybrid panel for single chromosome (3B)

The production of the RH mapping populations for 3B chromosome

- A total of 92 RH_1 selected lines were genotyped with ISBP (cfp), SSR (barc, gwm, and gpw) & DArT (wPt and tPt) markers
- Non-irradiated double monosomic (13” + 3B’+ 3D’) F_1 lines were used as experimental controls.

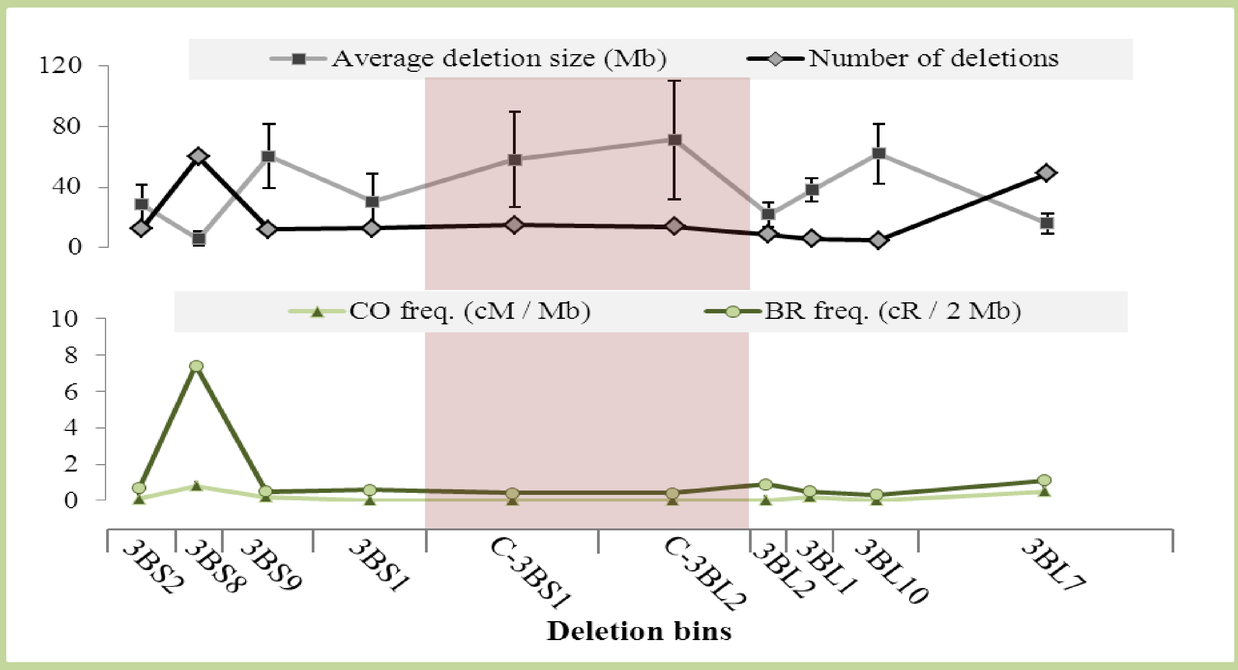


A: RH map of the short (left) and long (right) arm of 3B; map unit is centi-rays (cR). **B:** RH map of divided into deletion bins. **C:** BAC contig distribution as based on anchored markers; contigs for which a breakage was identified are indicated by vertical arrows pointing in the resolved map orientation and cR/Mb resolution within the contig is shown; horizontal lines connecting multiple dots indicate contigs that had no breakage event and the size of the contig is reported; horizontal arrows indicate contig for which only a single marker was mapped. **D:** Deletion bins map of 3B; no anchored markers were available for bin 3BL8-0.28-0.31 and 3BL9-0.38-0.50.

A comparison of radiation hybrid and genetic map of chromosome 3B

Deletion bins	3BS3 -0.87-1.00	3BS8- 0.78-0.87	3BS9 -0.57-0.75	3BS2 -0.56-0.57	3BS1 -0.33-0.55	C-3BS1 -0.33	C-3BL2 -0.22	3BL2- 0.22-0.28	3BL1- 0.31-0.50	3BL10 -0.50-0.63	3BL7 -0.63-1.00	Chr†
No. of marker	7.0	155.0	9.0	3.0	44.0	28.0	27.0	29.0	47.0	4.0	187.0	540.0
Bin size (Mb)	56.0	39.0	78.0	4.0	95.0	142.0	124.0	33.0	39.0	73.0	208.0	992.0
Bin size (%)	5.7	3.9	7.9	0.4	9.6	14.3	12.5	3.3	3.9	7.4	21.0	89.8
Bin saturation (Mb Marker no^{-1})	8.0	0.3	8.7	1.3	2.2	5.1	4.6	1.1	0.8	18.3	1.1	1.8
Map size (cR)	79.7	572.9	69.2	36.7	113.7	115.6	99.1	58.9	37.0	41.3	459.6	1871.9
BR freq (cR $2Mb^{-1}$) §	0.7	7.4	0.5	4.6	0.6	0.4	0.4	0.9	0.5	0.3	1.1	1.0
Resolution (Mb cR^{-1}) ¶	0.7	0.1	1.1	0.1	0.8	1.2	1.3	0.6	1.1	1.8	0.5	0.5
Map size (cM) ‡	5.2	33.1	13.4	--	1.6	0.9	1.6	1.6	6.2	3.5	104.2	179.0
CO freq. (cM Mb^{-1}) §‡	0.1	0.8	0.2	--	0.0	0.0	0.0	0.0	0.2	0.0	0.5	0.2
Resolution (Mb cM^{-1}) ¶	10.8	1.2	5.8	--	61.3	167.1	80.0	21.3	6.3	21.2	2.0	5.5
Map size ratio (cR cM^{-1})	15.3	17.3	5.2	--	73.4	136.0	63.9	38.0	6.0	12.0	4.4	10.45

† Whole chromosome values are not averages for each category but re-calculated for the overall values. ‡ Values in the row are as of Saintenac et al. (2009; Genetics 181: 393 2009). § Frequency of DNA breakage and repair per Mb and frequency of a crossing-over event per Mb. ¶ Resolution is the potential to uniquely resolve two markers at a distance equal or larger than this value..



Comparison of breakage/repair and crossing over frequencies, and deletion sizes across chromosome 3B. The frequency of crossing-over (CO) is as of Saintenac et al. (2009). The breakage/repair (BR) frequency was calculated from 3B RH genotyping data. The pallel lines indicate a good correlation.

CONCLUSIONS

- The 3B-RH panel provided on average a 10.5 folds higher resolution than the genetic map, reaching a max of 136-fold better resolution at the centromere.
- The average resolution, calculated as the total physical size of the chromosome divided by the total map length, was 0.53 Mb cR-1 with a maximum of five-fold deviation from this value (0.1 and 1.8 Mb cR-1) along the chromosome. The RH mapping resolution is six times more uniform than genetic mapping resolution.
- The results suggest that RH mapping potentially relies on poised chromatin regions, probably corresponding to the recombination hot-spot observed in genetic mapping.
- The overall high resolution and consistent physical to cR conversion across the entire chromosome makes this approach the most dependable for the scaffold assembly of genome sequencing initiatives.

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For more details see Kumar et al (2012) BMC Genomics (in press)

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