Radiation Hybrid Mapping: High Resolution Maps of D- Genome Chromosomes of Hexaploid Wheat

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Wheat Genome Sequencing

BAC BY BAC

Chromosome Sorting
40,000 BACs / chromosome

Next Generation Sequencing

16,000,000,000 bp @ 500 bp/sequence
= 80,000,000 sequences @ 5x coverage
=400,000,000 sequences to assemble

Every genome sequence needs a good map

Harris A. Lewin, Denis M. Larkin, Joan Pontius, et al.

Genome Res. 2009 19: 1925-1928 originally published online July 13, 2009
Access the most recent version at doi:10.1101/gr.094557.109

Genetic vs Physical

- **~25-30% of the chromosome around the centromere represents about 1% of recombination on the genetic maps**
- **~30% of the genes are in recombination poor regions**
- **Requirement of polymorphic markers**

Mapping methods that do not rely on meiotic recombination are needed for BAC contig alignment prior to ‘complete’ genome sequencing

Radiation Hybrid (RH) Mapping

Physical mapping based on radiation induced chromosome breakage and a reconstruction of marker order based on their co-retention pattern

- ✓ **Non-Polymorphic markers**
- ✓ **Independent of recombination event**
- ✓ **Small mapping population size and resolution can be controlled through radiation dosages**

Objectives: RH Mapping Project

1. **Develop RH populations for D- genomes of diploid *Aegilops tauschii* and hexaploid wheat Chinese Spring**

2. **Characterize RH panels and selection of most informative lines**

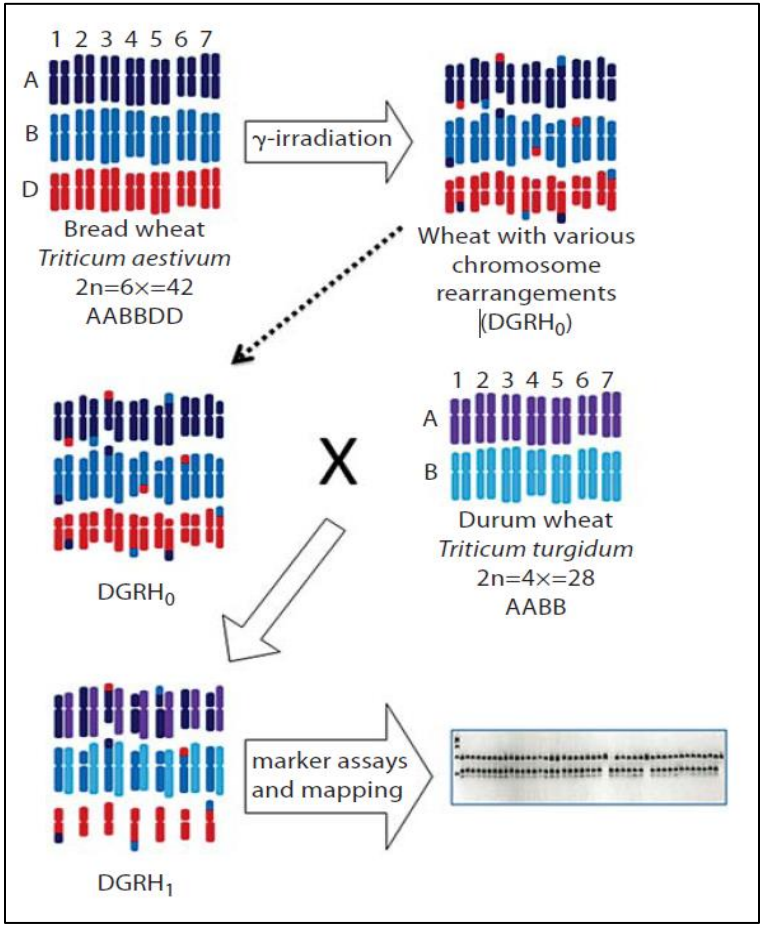
3. **Develop the necessary volume of markers**

4. **Optimize a high-throughput genotyping approach**

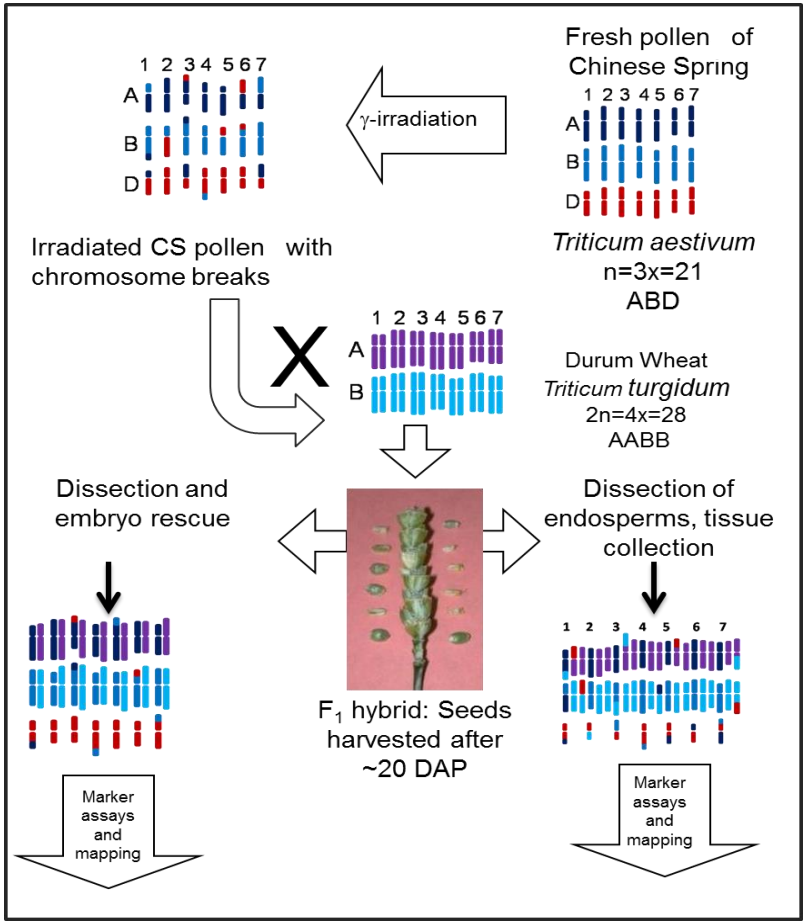
5. **Genotype the selected lines**

6. **Construct RH maps and order BAC contigs**

Radiation Hybrid Panels Developed for Hexaploid Wheat 'Chinese Spring'



SEED IRRADIATION BASED PANEL



POLLEN IRRADIATION BASED PLANT PANEL

POLLEN IRRADIATION BASED ENDOSPERM PANEL

Riera-Lizarazu, Tiwari et al, 2010; Kumar et al, 2012

Tiwari et al, 2012

CS-RH Resource Generated

Type of RH Panel	Dosages (Krad)	Number of lines or samples	*Average marker retention (%)	Number of lines tested	Number of lines with at least one break	Number of selected lines
Seed irradiation based plant panels	15.0	261	99.0	94	9	-
	30.0	285	98.4	94	15	10
	35.0	1583	97.0	752	220	204
	40.0	276	96.6	94	29	29
	45.0	160	92.0	94	39	39
	Total	**2565**		**1023**	**312**	**282**
Pollen irradiation based panels	1.0 (Pollen plant)	102	90.0	94	68	-
	1.5 (Pollen plant)	400	84.0	372	315	188
	Total	**502**		**466**	**383**	**188**
	1.5 (Endosperm)	1500	80.0	94	83	-
	2.0 (Endosperm)	1000	65.0	564	540	160*
	Total	**2500**		**658**	**623**	**160**

*Based on 14 markers; 2 per chromosome

Further Characterization of CS-RH Panels

- Genotyping of the RH panels –DArT Genotyping (Previous results)

  - **35Krad-seed panel:**

    - ✓ a random set of 94 RH lines was genotyped
    - ✓ ~950 D-genome specific markers
    - ✓ Not enough mapping information

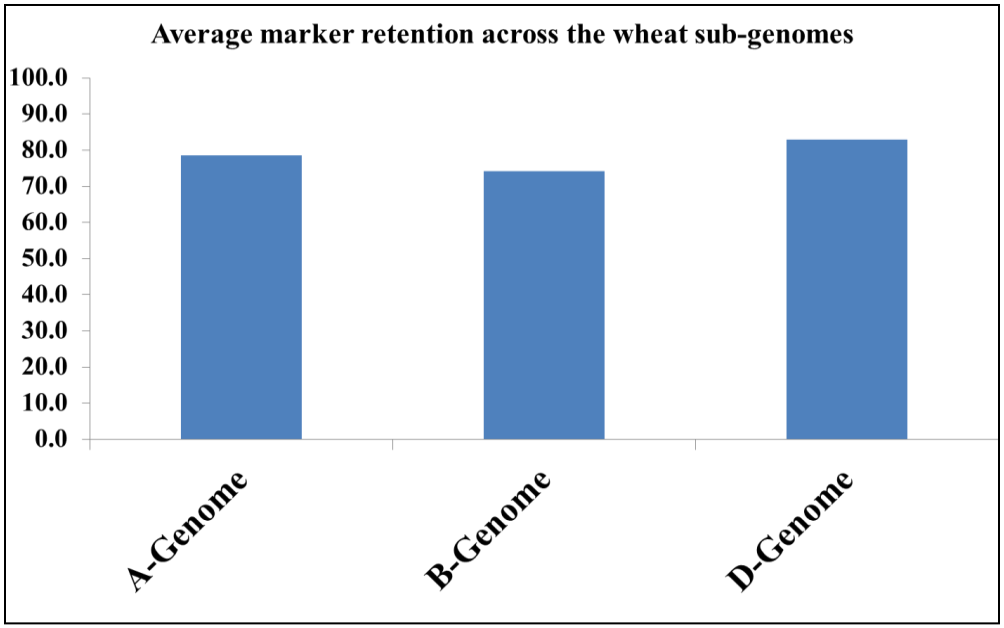
  - **2.0Krad endosperm panel:** (Tiwari et al; 2012)

    - ✓ Endosperm tolerance of paternal aneuploidy allowed us to develop most informative RH panel reported so far
    - ✓ A set of 81 RH samples were used for DArT genotyping
    - ✓ ~940 D- genome specific markers
    - ✓ High resolution maps were constructed for D- genome
    - ✓ Average map resolution: 600 Kb

Genotyping of a Pollen Plant Panel Using High-Density 90K SNP Array

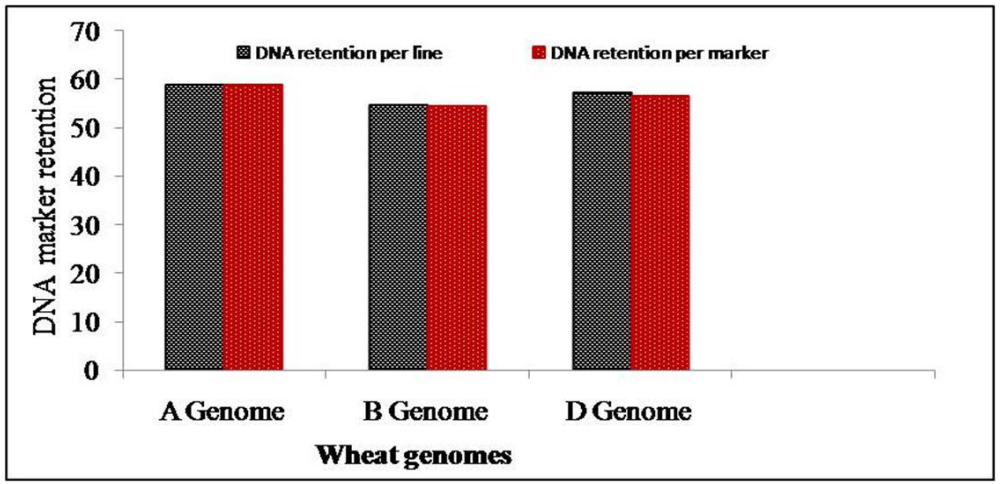
- CS-RH -1.5Krad Pollen Plant Panel: a set of 94 lines + parental lines
- A total of 90,59 SNPs were found to be informative for RH mapping
- LOD 10 and TD of 0.3 were used for grouping
- ~8,300 SNPs mapped on A, B and D- genomes
- Total 48 RH groups

A SUB-GENOME OR WHOLE GENOME PANEL???



- ✓ ~5,500 SNPs were mapped on D- genome chromosomes
- ✓ ~ 2,800 SNPs were mapped on A and B- genome chromosomes

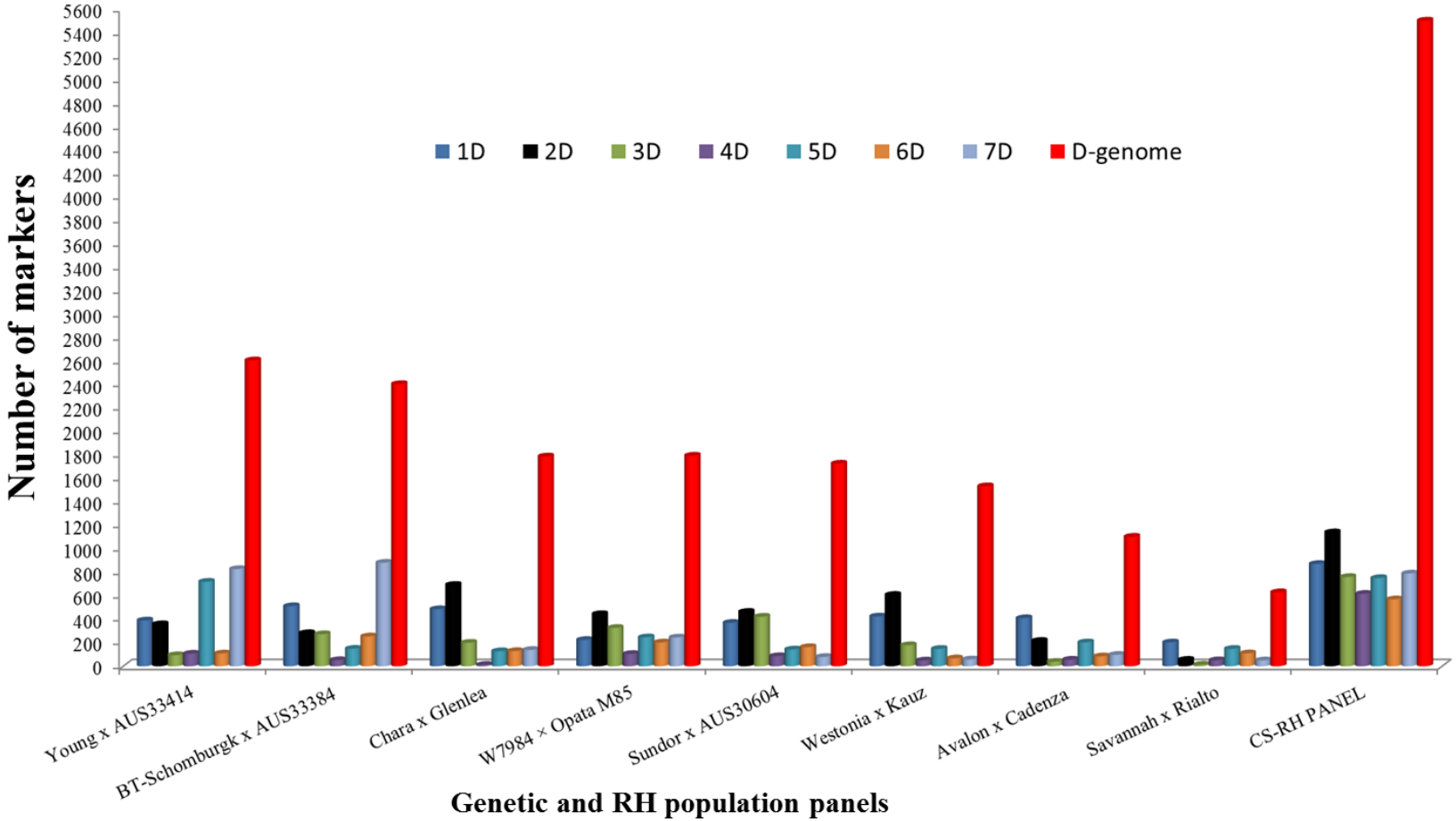
WHOLE GENOME RH PANEL



Similar to what we observed from the DArT genotyping of 2Krad endosperm panel

(Tiwari et al, 2012)

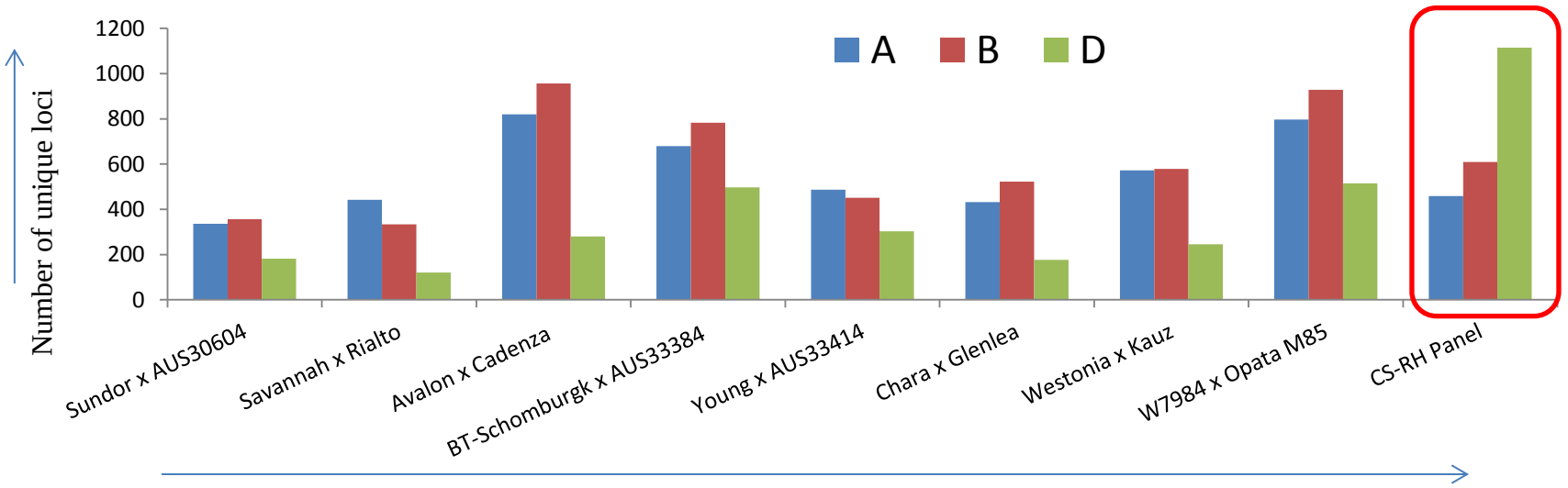
Number of markers mapped on D- genome on genetic and RH populations



Most number of markers were mapped on CS-RH panel

Similar distribution of markers on all D- chromosomes on CS-RH panel

Number of unique loci mapped on the A, B and D- genomes



*Genetic Populations	For CS-RH Panel
Range for A- genome: 336-797	A- genome: 458
Range for B-genome: 333-928	B-genome: 609
Range for D-genome:120-515	D-genome: 1115

- **RH panel has the most number of unique loci mapped on D-genome**
- **Number of unique loci on A and B- genome are comparable between the RH panel and the genetic populations**

Wang et al, 2014

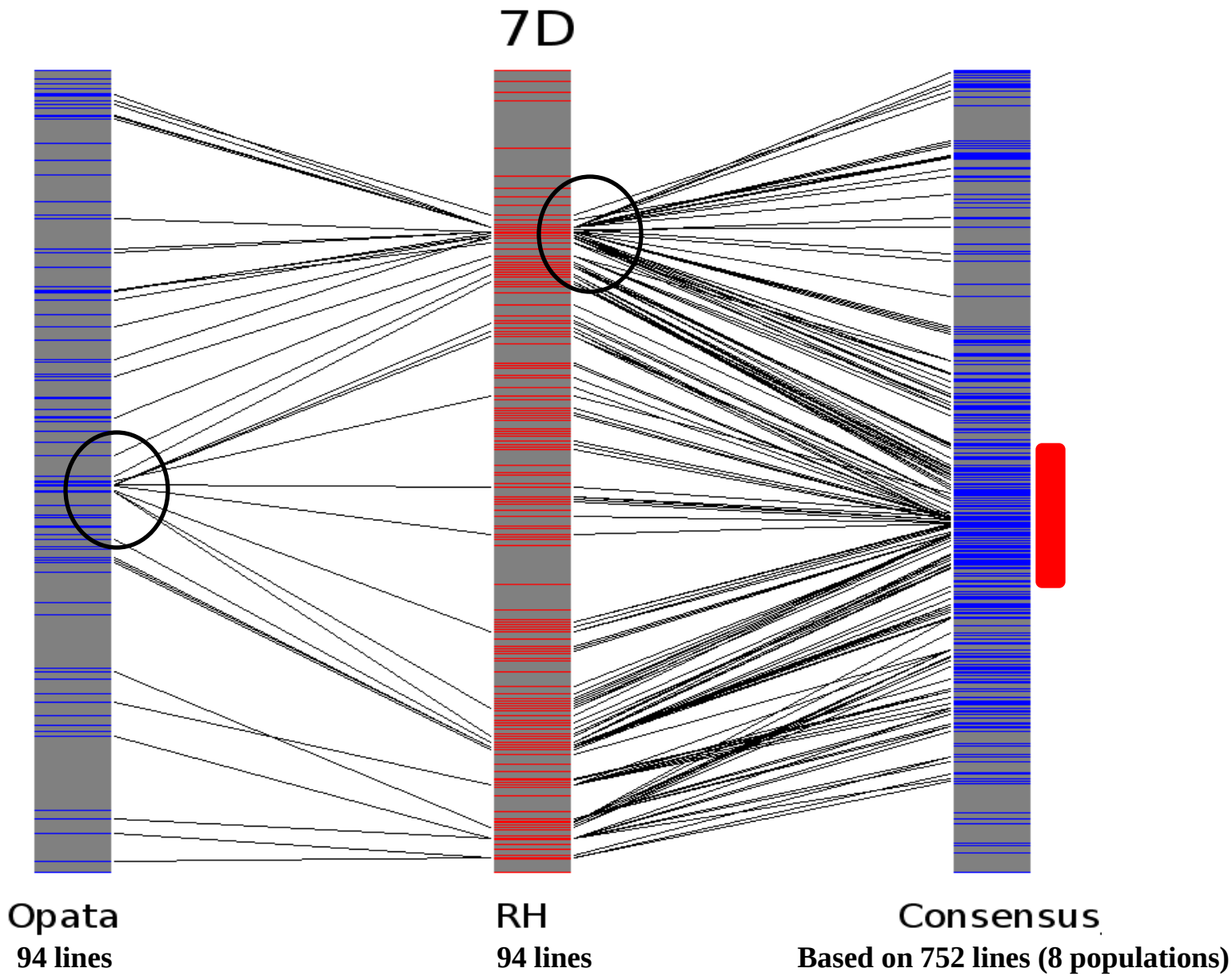
Map Based Characteristics of D-Genome Chromosomes

Chromosome	Chromosome arm	Total number of markers mapped	Total number of unique loci	Map length (cR_{1500})	Total number of obligate breaks	Average marker retention of the map (%)
1D	1D-short	316	41	377.8	96	86.0
	1D-long	555	94	672.3	177	85.0
2D	2D-short	453	91	584.9	202	78.0
	2D-long	688	98	565.2	170	82.0
3D	3D-short	288	86	522.7	163	82.0
	3D-long	472	83	644.8	194	81.0
4D	4D-short	225	46	358.3	101	84.0
	4D-long	392	124	847.5	367	70.0
5D	5D-short	231	55	501.8	131	85.0
	5D-long	520	61	453.7	103	87.0
6D	6D-short	287	76	456.3	100	76.0
	6D-long	283	39	310.0	58	89.0
7D	7D-short	392	61	508.2	153	81.0
	7D-long	398	82	588.7	165	84.0
Total		5500.0	1037.0	7392.2	2180.0	82.1

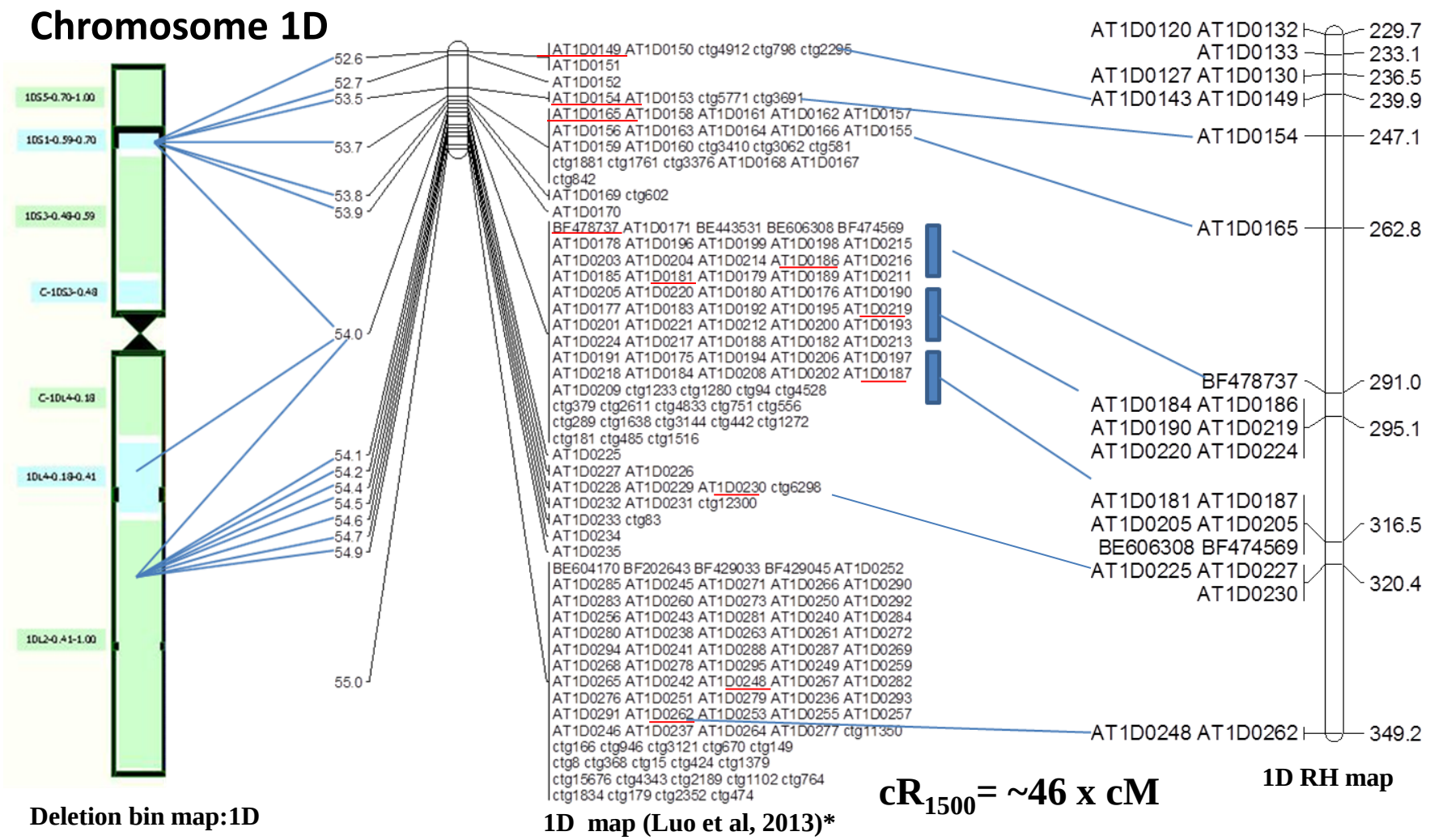
- ~1000 markers detect ~2200 deletion breaks across the D-genome
- ~6600 markers would be needed to have at least one marker in each bins
- A and B genome maps were also constructed

*Groups with less than 100 markers were not included in the table

Comparison of Different Maps Using Common Markers



Ordering BAC Contigs



Deletion bin map:1D

1D map (Luo et al, 2013)*

cR_{1500}= ~46 x cM

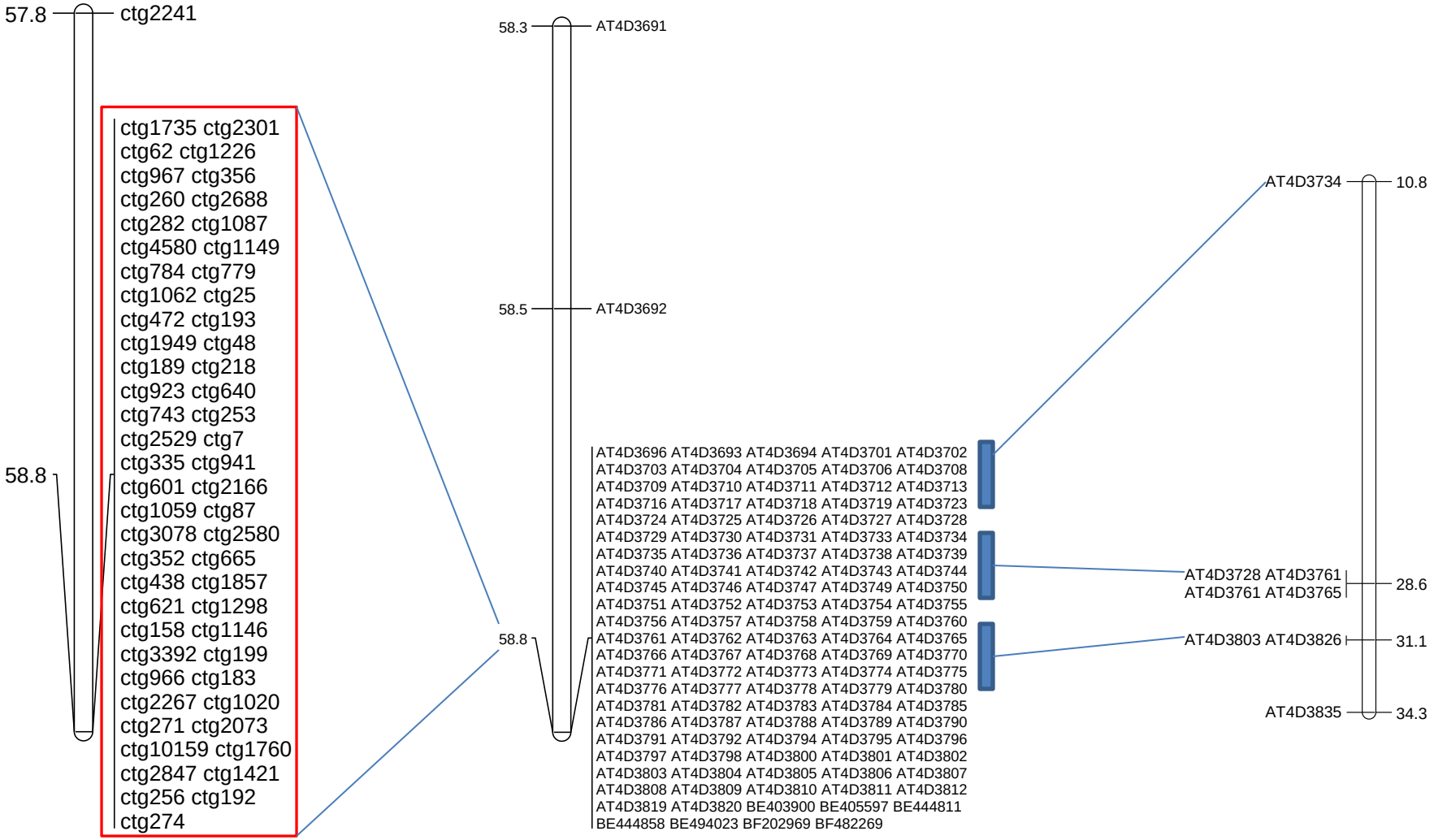
1D RH map

RH map offers ~45 fold higher resolution than the genetic map in this region

- ~5,000 SNPs from the 90,000 SNP array came from the *Ae. tauschii* physical mapping project
- We have similar results for the other six D- genome chromosomes indicating RH maps are useful in ordering contigs in low recombination regions

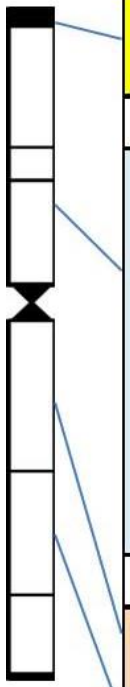
*** Based on 1200 lines**

Ae. tauschii Contigs on Chromosome 4D



Map Resolution: RH vs Physical

Chromosome 2D of hexaploid wheat (RH) vs *Aegilops tauschii* map



Markers (map order)	Position	cM (GM)	cR (RHM)	Mb	Mb shared	Mb/cM	Mb/cR
AT2D0989	5312500	4.75	0	5.31	16.21	0.64	0.35
AT2D1056	21522500	30.17	46.7	21.52			
		25.42	46.7	16.21	16.21	0.64	0.35
AT2D1266	108216000	89.44	67.7	108.22	4.36	5.97	0.32
AT2D1271	112571500	90.17	81.5	112.57	1.06	3.31	0.23
AT2D1274	113630000	90.49	86.1	113.63	3.02	11.18	0.53
AT2D1280	116647500	90.76	91.8	116.65	2.78	4.34	0.50
AT2D1285	119428000	91.4	97.4	119.43	3.42	2.28	0.22
AT2D1293	122846500	92.9	112.6	122.85	0.33	6.54	0.11
AT2D1296	123173500	92.95	115.6	123.17	3.89	7.77	0.32
AT2D1304	127060500	93.45	127.8	127.06			
		4.01	60.1	18.84	18.84	4.70	0.31
BE442788	427232500	110.27	0	427.23	1.50	11.55	0.02
AT2D1774	428734000	110.4	60.3	428.73	55.85	5.14	1.37
AT2D1906	484582500	121.26	101.2	484.58	6.12	3.62	0.14
BE490763	490702500	122.95	145.8	490.70			
		12.68	145.8	63.47	63.47	5.01	0.44

Based on 188 RH pollen plant lines

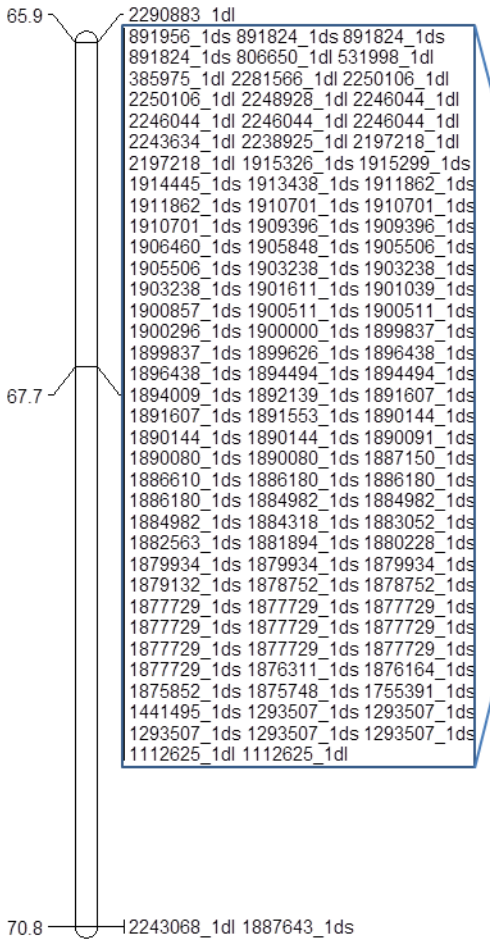
Ordering of Next Generation Sequencing (NGS) Based Contigs from D- Genome of Chinese Spring[$] and *Aegilops tauschii*

Type/Name of the mapping panel (D-genome)	Number of the Chinese Spring-sequenced contigs ordered (D-genome)	Number of the *Aegilops tauschii* sequenced contigs ordered (D-genome)
***Opata Maps**	725	1118
***Consensus Maps**	2727	3278
RH Maps	**2722**	**3510**

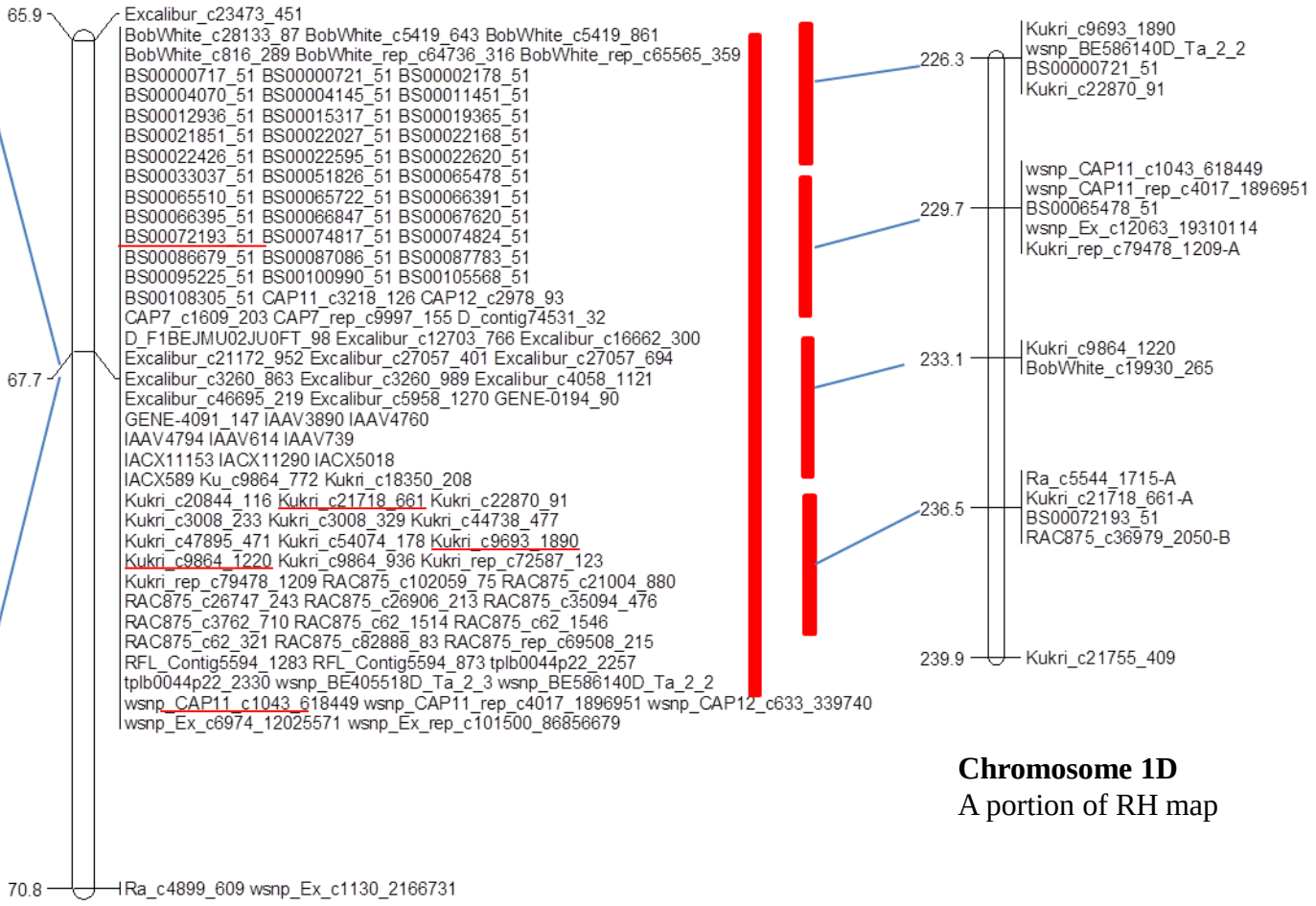
[$-] IWGSC

* Wang et al. 2014

NGS Based ‘Chinese Spring’ Contigs Ordered on Consensus and RH Maps



Chromosome 1D
Ordered contigs based on 90K concensus map



Chromosome 1D
SNPs on 90K concensus map used to order 1D contigs

Chromosome 1D
A portion of RH map

1.5Krad CS-RH panel

- Informative as whole genome RH panel
- Map resolution up to ~200Kb is possible
- Can used for any genotyping platform
- DNA quantity is not a limiting factor

Status of the Entire CS-RH Project

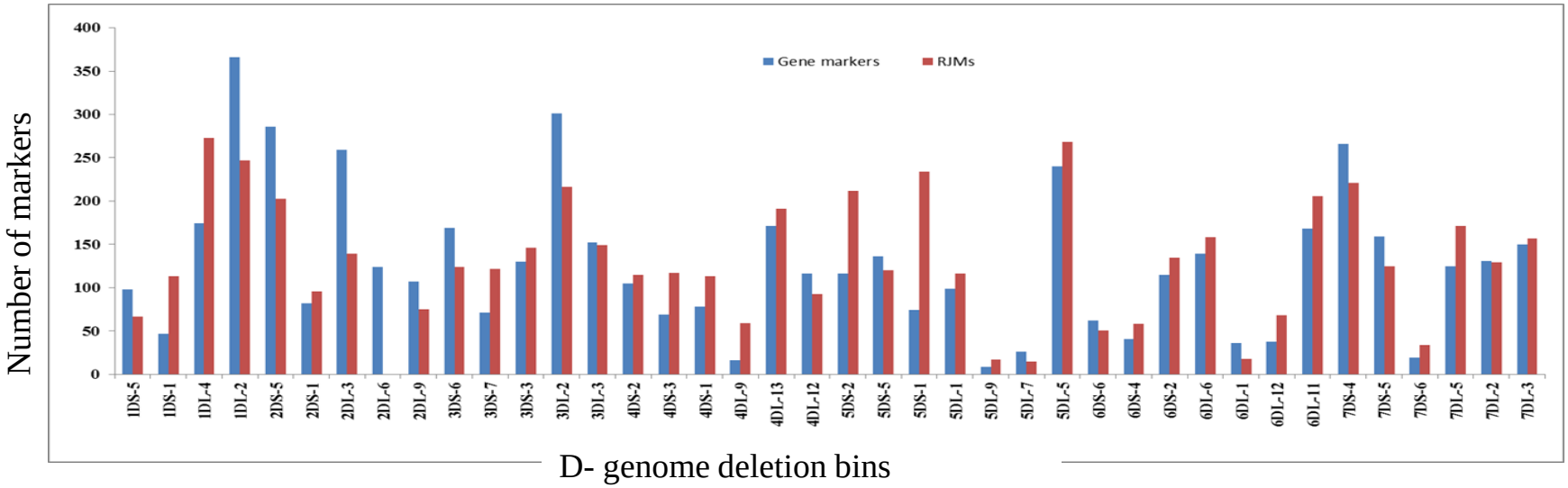
Development and application of Nimblegen genotyping array

- Tested on Nullisomic-tetrasomic lines of D- genome chromosomes
- 40 deletion bin lines of D- genome chromosomes and ditelosomic lines of D-genome chromosomes were genotyped on this array
- All the probes were replicated in three sets and all the RH lines/samples were hybridized in three replications

Chromosome	Size (Mb)#	RJMs mapped on nullisomic lines	Gene based probes on nullisomic lines	Unique gene markers	Total number of markers/ probes-RJM+GM	RJM/ Mb (~)	Gene marker/ Mb (~)	RJ +Gene (unique) / Mb (~)
1D	604	3921	1561	743	4664	6.5	1.2	7.7
2D	727	4146	1923	923	5069	5.7	1.3	7.0
3D	770	4453	2153	1031	5484	5.8	1.3	7.1
4D	648	4366	1412	672	5038	6.7	1.0	7.8
5D	748	4491	2062	982	5473	6.0	1.3	7.3
6D	712	3551	1298	618	4169	5.0	0.9	5.9
7D	727	4265	2008	956	5221	5.9	1.3	7.2
D-genome	4936	29193	12417	5925	35118	5.9*	1.2*	7.1*

Mapping and Distribution of Gene Markers as well as RJMs on Deletion Lines of D- Genome Chromosomes

Chromosome	Short arm (deletion bins)	Long arm (deletion bins)	Chromosome size included (Mb)	Gene Marker	RJ Markers	Gene marker / Mb (~)	RJ marker / Mb (~)	Marker (RJM+GM) / Mb (~)
1D	1DS1-0.59-0.70 to 1DS5-0.70-1.00	1DL4-0.18-0.41 to 1DL2-0.41-1.00	404.26	685	700	2	2	3
2D	2DS1-0.33-0.47 to 2DS5-0.47-1.00	2DL3-0.49-0.76 t0 2DL9-0.76-1.00	421.33	858	513	2	1	3
4D	4DS1-0.53-0.67 to 4DS2-0.81-1.00	4DL9-0.31-0.56 to 4DL12-0.71-1.00	395.63	823	757	2	1	3
3D	3DS3-0.24-0.55 to 3DS6-0.55-1.00	3DL2-0.27-0.81 to 3DL3-0.81-1.00	571.73	555	688	1	1	2
5D	5DS1-0.67-0.73 to 5DS2-0.78-1.00	5DL1-0.60-0.74 to 5DL5-0.76-1.00	206.32	700	982	3	5	8
6D	6DS2-0.45-0.79 to 6DS6-0.99-1.00	6DL6-0.29-0.47 to 6DL11-0.74-0.80	276.15	599	694	2	3	5
7D	7DS5-0.36-0.61 to 7DS4-0.61-1.00	7DL5-0.30-0.61 to 7DL3-0.82-1.00	303.16	850	837	3	3	6
	All 40 deletion bins		2578.58	5070	5071	2	2	4



Genotyping of CS-RH Panels on Nimblegen Array

- Genotyping of RH lines is completed
  - 94 seed lines (15-45Krad)
  - 188 pollen plant lines (1.5Krad)
  - 118 endosperm samples (2.0Krad)
- Analysis and filtering of the data is completed
- RH mapping of the D- chromosomes is in process
- Final results: PAG 2015

The team involved with construction of high-resolution radiation hybrid based physical maps of wheat

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Oscar Riera-Lizarazu
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Robert Zemetra

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Gerrard Lazo

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Catherine Feuillet
Etienne Paux
Frederic Choulet
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