

# The physical map of wheat chromosome arm 3DS

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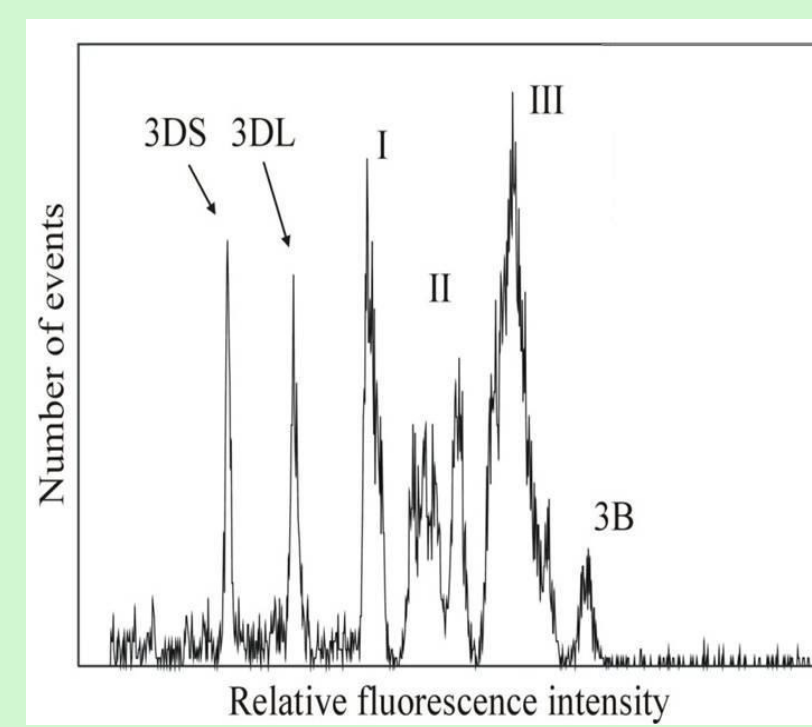
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## Background

Bread wheat is one of the most important crop in the world. Sequencing its genome can provide valuable information leading to its improvement. The wheat chromosome 3DS was sorted using flow cytometry. BAC library specific for wheat chromosome-arm 3DS<sup>1)</sup> was constructed and physical map was build using HIFC technique<sup>2)</sup> using Fingerprinted Contigs software<sup>3)</sup> (FPC). At the end of automated phase of contig building, Minimum Tilling Path (MTP) was selected. Three-dimensional pooling strategy was used to anchor markers.

## Chromosome sorting using flow cytometry and chromosome library



### BAC library parameters

Number of clones  
36,864

Average insert size  
110 kb

Chromosome coverage  
11.0x

Histogram of relative fluorescence intensity obtained from 3DS/3DL double ditelosomic line of wheat cultivar Chinese Spring.

## Sequencing and assembly of "low-copy" portion of 3DS

DNA of sorted chromosome 3DS was sequenced using Roche 454 GS-FLX System to about 3x coverage. 2,081,761 reads were assembled using Newbler 2.5.3

### Assembly results

No. of contigs	46,297
Total length	42,333,654 bp
Average contig size	914 bp
N50 contig size	927 bp
Largest contig size	12,559 bp

## Sequencing of 3D pools of MTP

DNA of 50 BAC pools was sequenced using Illumina HiSeq2000. Reads of individual pools were aligned using MOSAIK 1.1.0021 to three independent sequence data sets: a) GenomeZipper; b) SNPs mapped in *Ae. tauschii*; c) contigs assembled from chromosome-arm 454 sequence. These sequences were then anchored to physical map using a novel *in silico* approach.



### Sequence-Based Anchoring

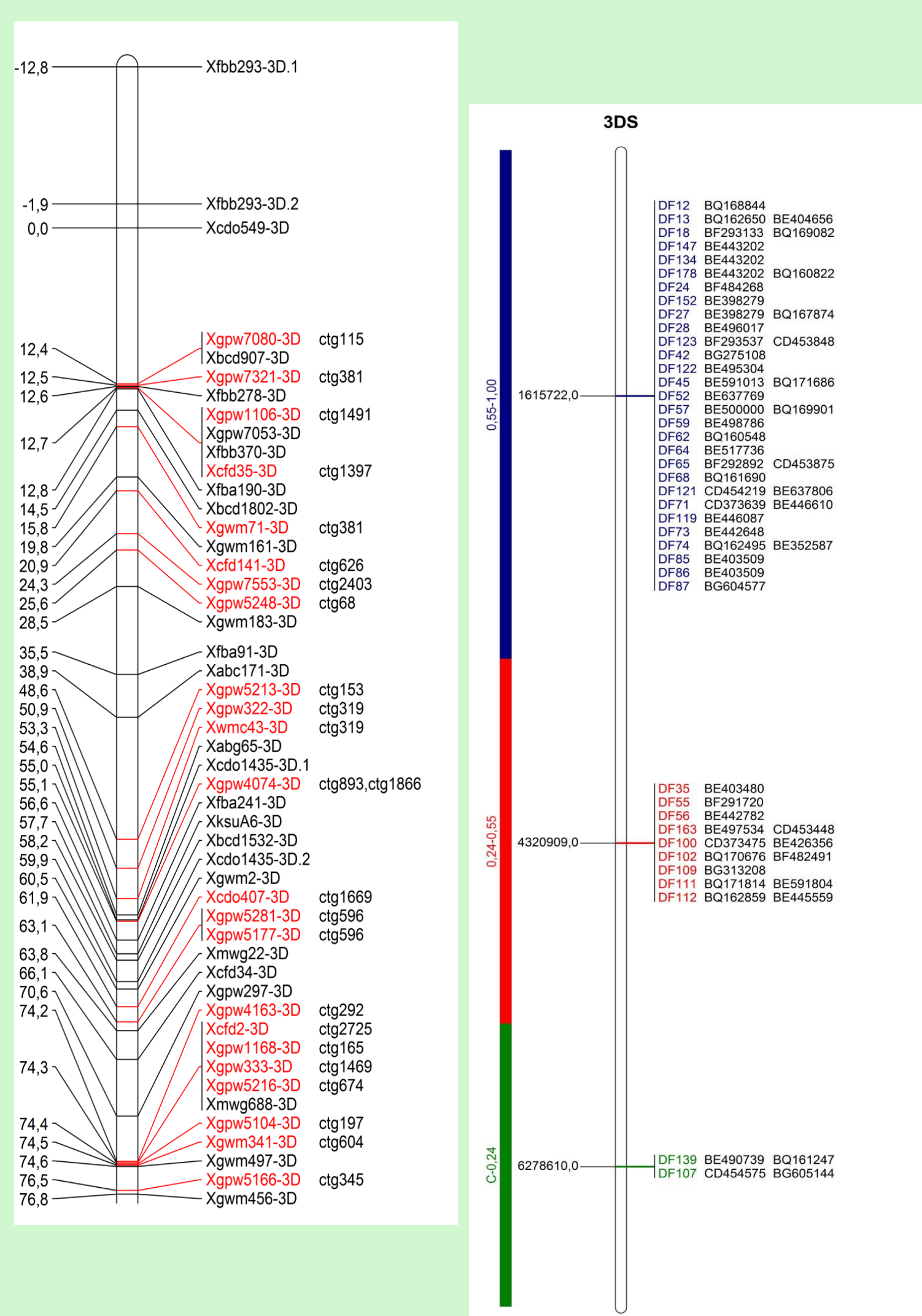
Reference	No. of sequences	No. of BAC addressed seq.
GenomeZipper	594	224
SNP ( <i>Ae. tauschii</i> )	7,152	231
sequence contigs	46,297	20,899



60 sequences, which were anchored *in silico* to the physical map were selected to design primers. These primers were used to check their presence in particular BAC clones to confirm accuracy of this approach.

## Forward physical map anchoring

Part of genetic map of chromosome 3D *Triticum aestivum* - Syntetic/Opata-GPW showing markers (in red), which were mapped to the physical map of chromosome 3DS.



Wheat ESTs dataset available from GenBank was compared to the region of rice chromosome 1 syntenic to wheat chromosome arm 3DS. 144 ESTs were selected for marker development. 42 of these markers were mapped to three bins of wheat chromosome 3DS (C-0.24, 0.24-0.55, 0.55-1.00) and sorted in line with position on the rice chromosome.

## Genome Zipper

GenomeZipper approach was used to identify gene fragments in 454 reads and to order them virtually according to collinearity with Brachypodium, rice and Sorghum syntenic genome regions.

Bradi2g05010.1	Os01g0179300	Sb03g003810.1	ctg1032
Bradi2g05000.1	Os01g0179200	Sb03g003820.1	ctg1032
Bradi2g04980.1	Os01g0179000	Sb03g003830.1	
Bradi2g04970.1	Os01g0178700	Sb03g003920.1	ctg326
Bradi2g04960.1	-	-	
Bradi2g04920.1	Os01g0178600	Sb03g003930.1	
Bradi2g04900.1	Os01g0178400	Sb03g003950.1	ctg2725
Bradi2g04890.1	Os01g0178200	Sb03g003960.1	ctg2725

## The physical map of 3DS

27,880 fingerprints

	1e-75	1e-45	1e-25
Contigs	2,682	1,360	945
Singletons	11,547	8,878	8,878
Q-clones	49	282	575
Assembly lenght (Mb)	404	310	280
Longest contig (kb)	470	1,092	1,595

Markers	454SEQ02875	454SEQ02977	454SEQ03032	454SEQ03741	454SEQ05885	454SEQ09071	454SEQ09050	454SEQ05279	454SEQ05330	454SEQ01002	454SEQ035190	454SEQ021651	454SEQ010789	454SEQ09673	454SEQ05946	454SEQ031032	454SEQ00766	454SEQ001504	454SEQ039857	454SEQ05952	454SEQ019003	454SEQ036797	
BAC clones	TacAg00000001	TacAg00000002	TacAg00000003	TacAg00000004	TacAg00000005	TacAg00000006	TacAg00000007	TacAg00000008	TacAg00000009	TacAg00000010	TacAg00000011	TacAg00000012	TacAg00000013	TacAg00000014	TacAg00000015	TacAg00000016	TacAg00000017	TacAg00000018	TacAg00000019	TacAg00000020	TacAg00000021	TacAg00000022	TacAg00000023

Contig 33, which is shown as example, is composed of 61 clones. 37 markers are anchored to 6 clones in red, which represent MTP.

## Conclusion

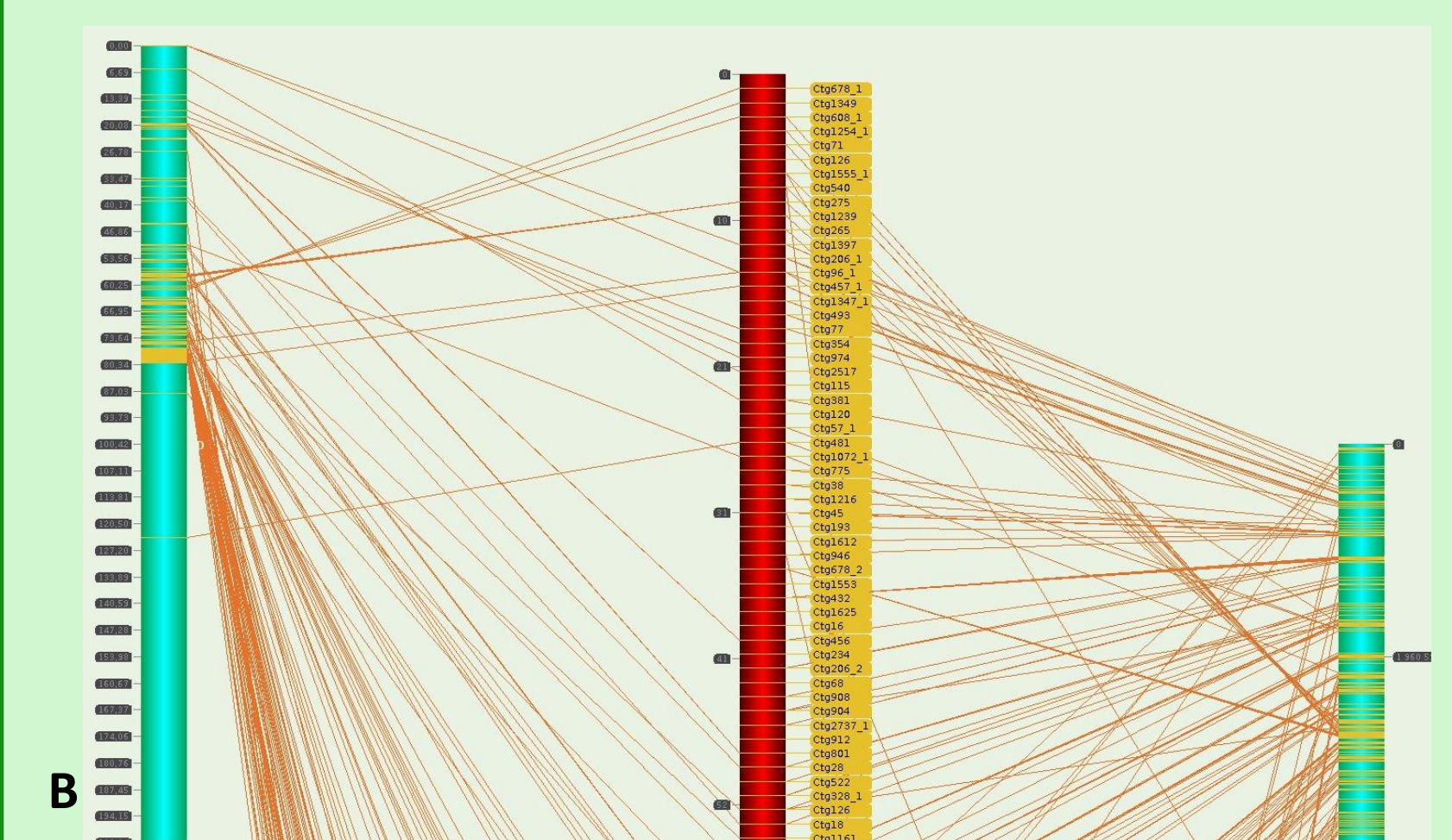
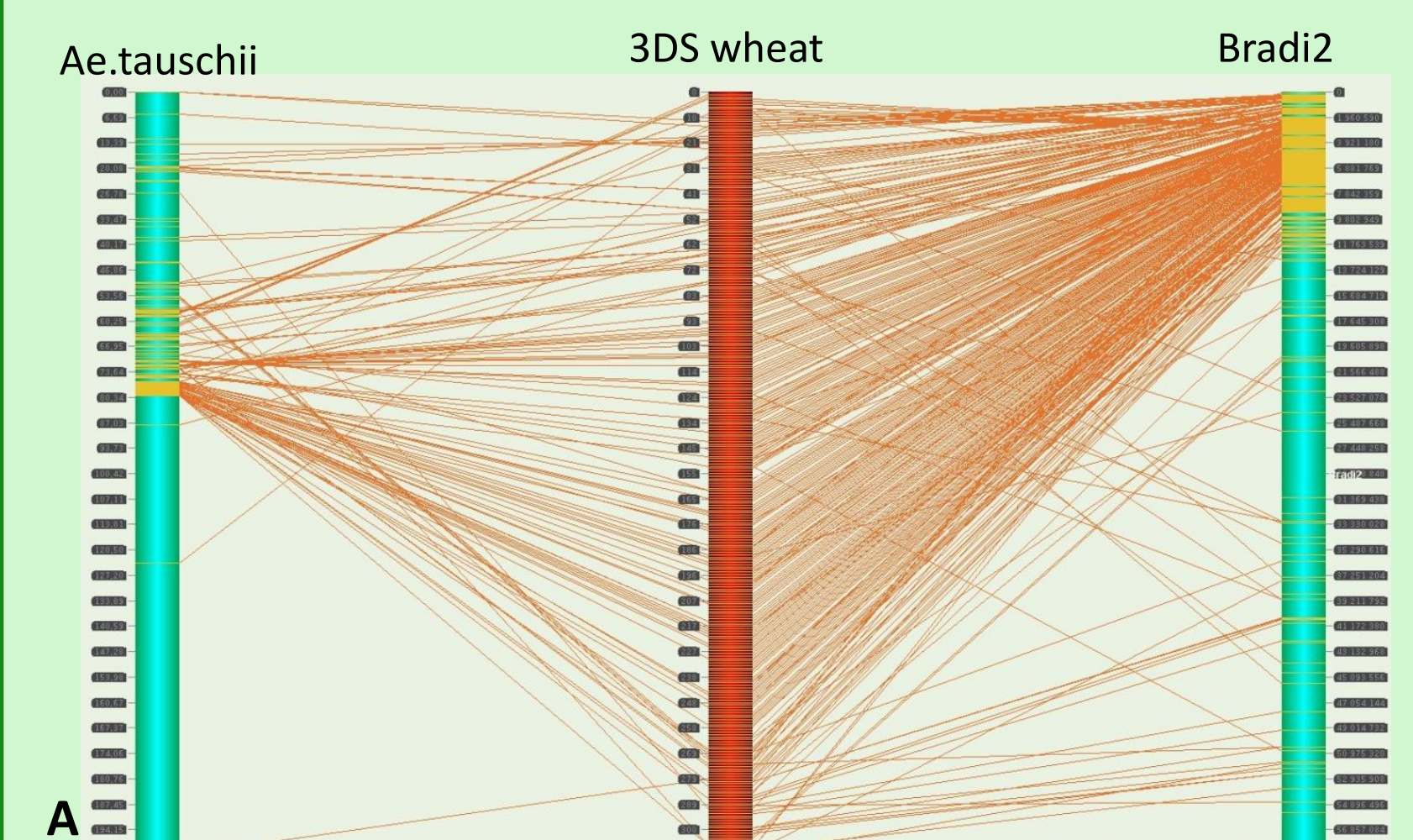
- The physical map of 3DS consist of 945 contigs (87,2% of the chromosome)
- 750 contigs (70%) have at least one marker anchored
- The order of 310 contigs (49%) on 3DS is known
- 95% *in silico* anchored markers were successfully identified on BAC clones using PCR

## References

- Šafář J, Šimková H, Kubaláková M, Čiháliková J, Suchánková P, Bartoš J, Doležel J: Development of Chromosome-Specific BAC Resources for Genomics of Bread Wheat. *Cytogenetic and Genome Research* 2010, 129:211–223.
- Luo M-C, Thomas C, You FM, Hsiao J, Ouyang S, Buell CR, Malandro M, McGuire PE, Anderson OD, Dvorak J: High-throughput fingerprinting of bacterial artificial chromosomes using the snapshot labeling kit and sizing of restriction fragments by capillary electrophoresis. *Genomics* 2003, 82:378–389.
- Soderlund C, Humphray S, Dunham A, French L: Contigs Built with Fingerprints, Markers, and FPC V4.7. *Genome Res.* 2000, 10:1772–1787.

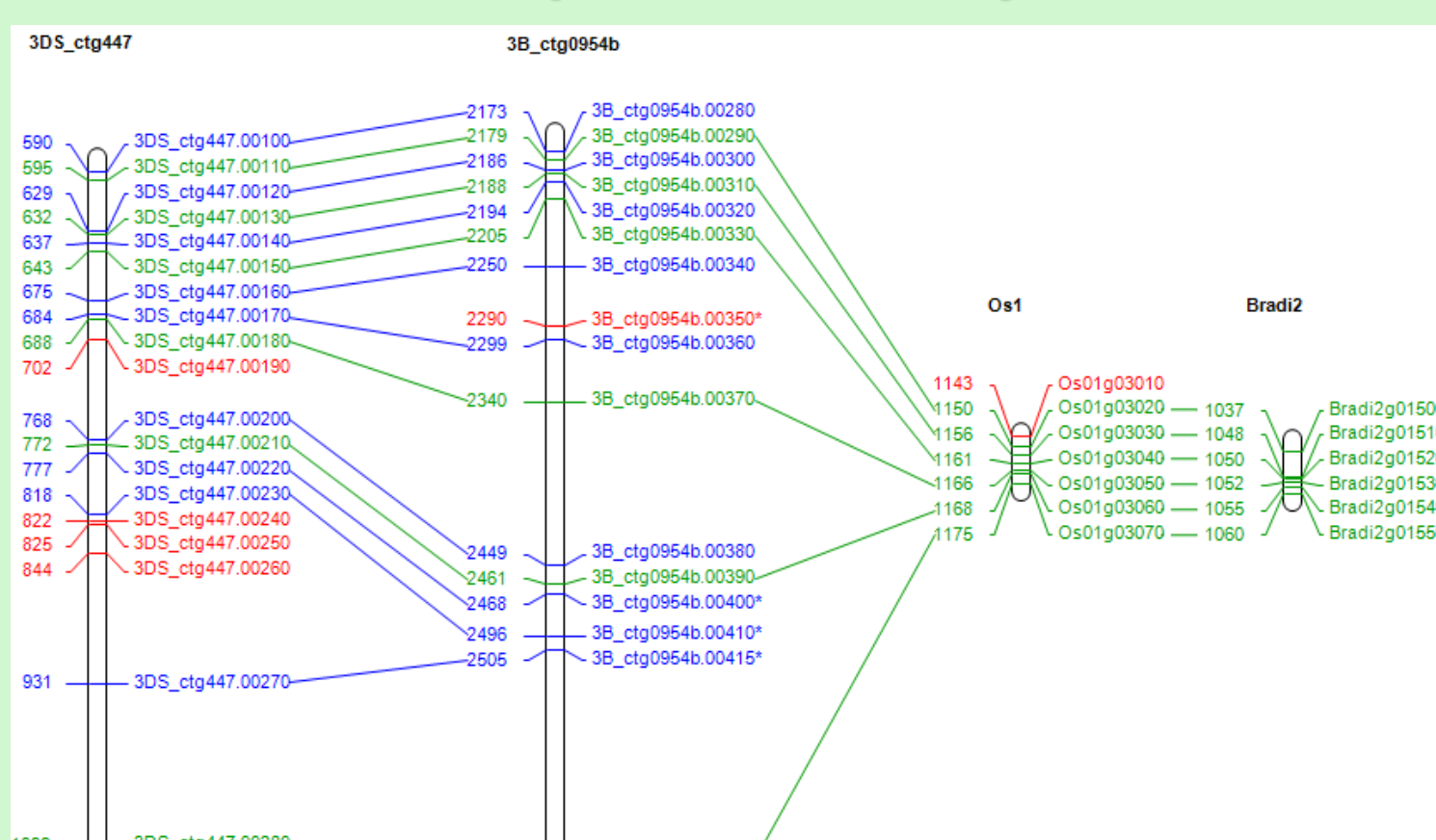
## Contig ordering

Synteny with *Aegilops tauschii*, Brachypodium and Rice genomes was used to order contigs of 3DS physical map.



A) Graphical tool Strudel was used to visualize homology among chromosome 3D of *Aegilops tauschii*, chromosome 2 of Brachypodium, chromosome Os01 of rice and contigs from wheat chromosome 3DS. *In silico*-anchored markers were blasted against the annotated sequences of the three genomes. The contigs of chromosome 3DS were ordered according to the position of genes in rice genome. B) Close-up of a segment of chromosome 3DS showing preservation of synteny.

## Comparative analysis



Comparison of the gene content and order at the Hga locus on 3DS with homologous sequence on wheat chromosome 3B and homologous regions in rice (Os01) and Brachypodium (Bradi2) genomes. Positions of genes in wheat contigs and rice and Brachypodium chromosomes are given in kb.