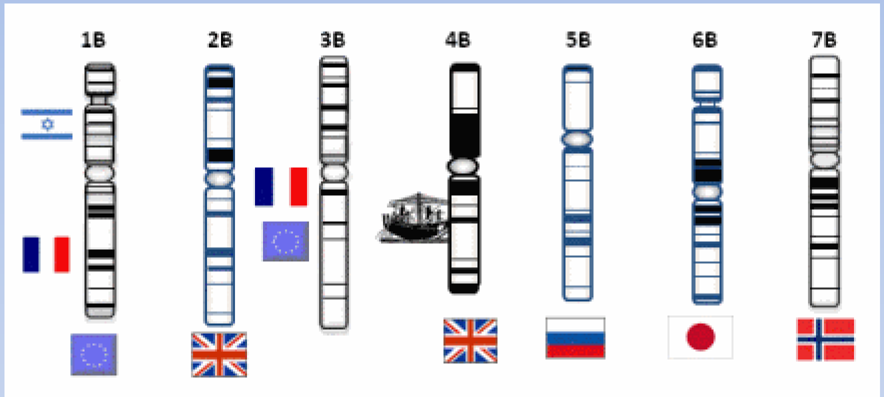
The stages of physical mapping of *Triticum aestivum* cv CS chromosome 5B



<u>Sergeeva E.M.</u>[1], Timonova E.M.[1], Bildanova L.L.[1], Koltunova M.K.[1],

Nesterov M.A.[1], Magni F.[2], Dolezel J.[3], Faris J.[4],

Sourdille P.[5], Feuillet C.[5], Salina E.A.[1]

[1]Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia

[2]Institute of Applied Genomics, Udine, Italy

[3]Institute of Experimental Botany, Olomouc, Czech Republic

[4]USDA-ARS Cereal Crops Research Unit, Fargo, USA

[5]UMR INRA-UBP Genetics, Diversity and Ecophysiology of Cereals, Clermont-Ferrand, France

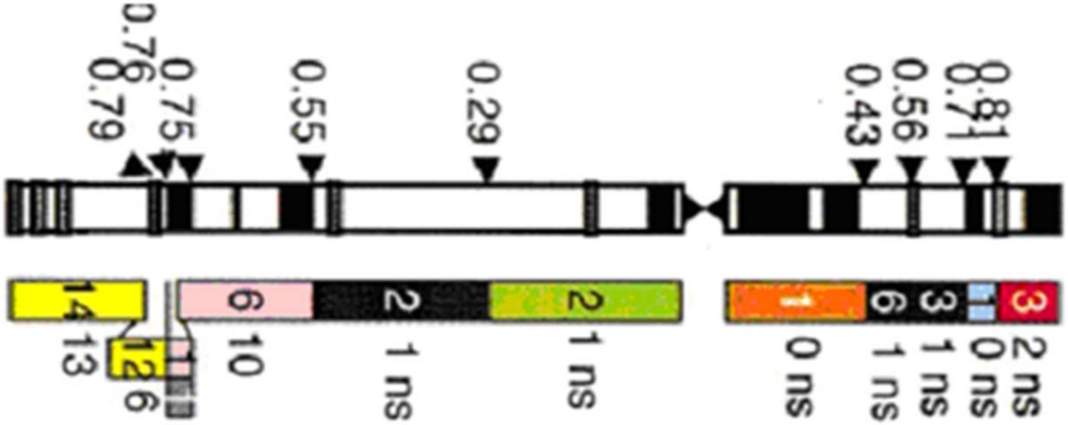
5B chromosome

5BL
580 Mbp

pSc119.2
$(GAA)_m(GAG)_n$
Afa dispersed

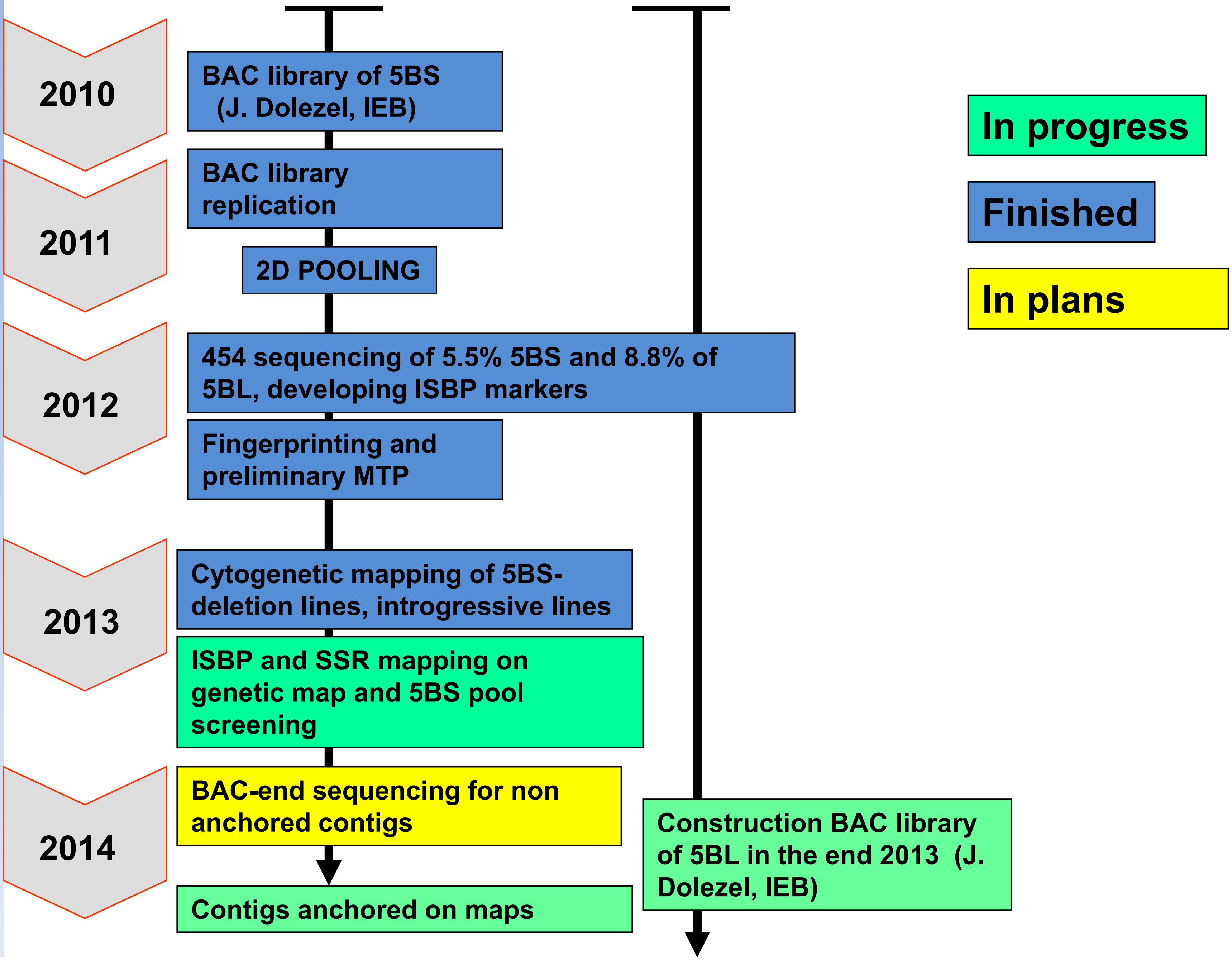
pSc119.2
$(GAA)_m(GAG)_n$
Afa dispersed
5S rDNA

5BS
290 Mbp



(Sorrells et al., 2003)

Steps of 5BS and 5BL physical mapping



2010

BAC library of 5BS (J. Dolezel, IEB)

2011

BAC library replication

2D POOLING

2012

454 sequencing of 5.5% 5BS and 8.8% of 5BL, developing ISBP markers

Fingerprinting and preliminary MTP

2013

Cytogenetic mapping of 5BS-deletion lines, introgressive lines

ISBP and SSR mapping on genetic map and 5BS pool screening

2014

BAC-end sequencing for non anchored contigs

Contigs anchored on maps

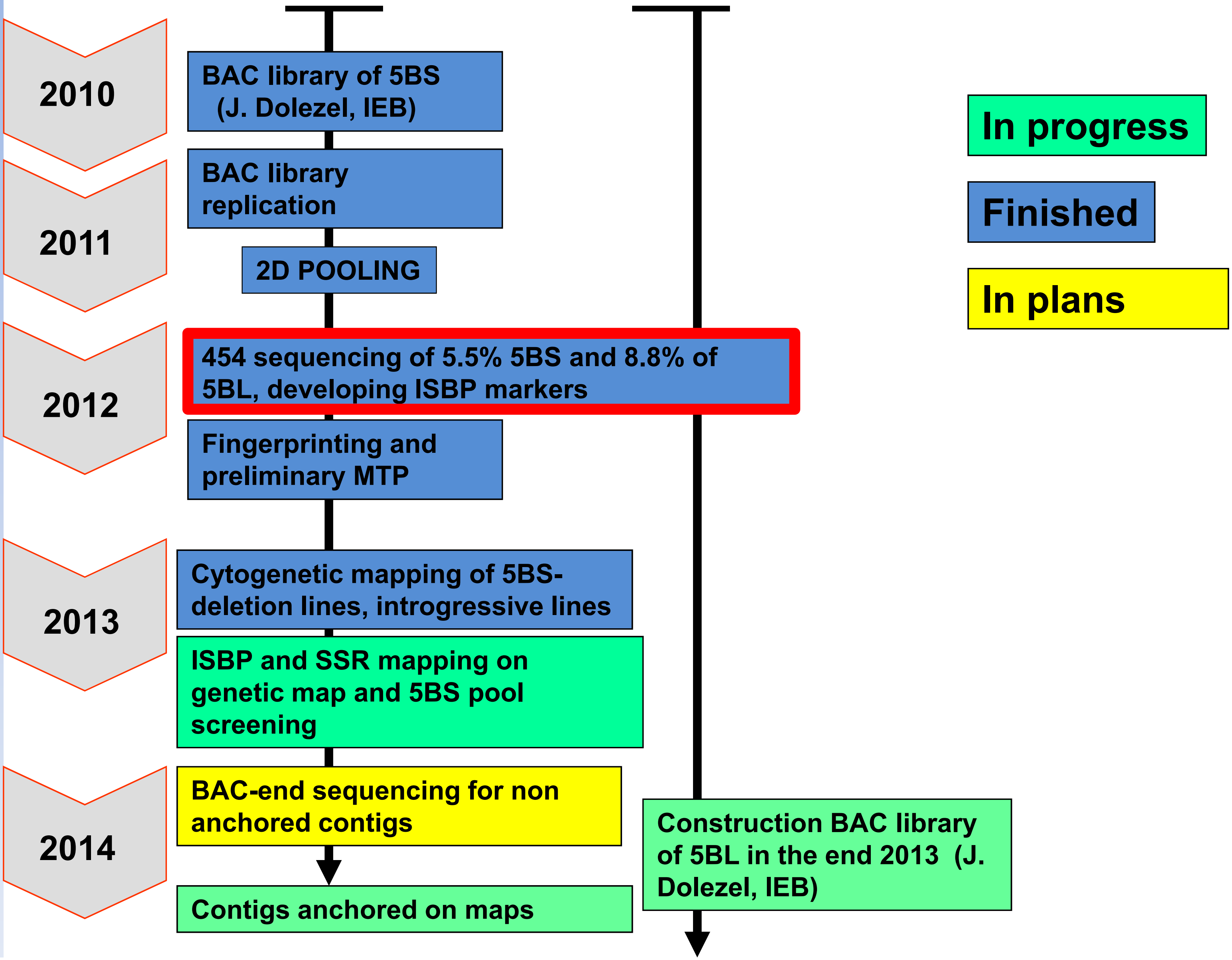
Construction BAC library of 5BL in the end 2013 (J. Dolezel, IEB)

In progress

Finished

In plans

Steps of 5BS and 5BL physical mapping



2010

2011

2012

2013

2014

BAC library of 5BS (J. Dolezel, IEB)

BAC library replication

2D POOLING

454 sequencing of 5.5% 5BS and 8.8% of 5BL, developing ISBP markers

Fingerprinting and preliminary MTP

Cytogenetic mapping of 5BS-deletion lines, introgressive lines

ISBP and SSR mapping on genetic map and 5BS pool screening

BAC-end sequencing for non anchored contigs

Contigs anchored on maps

Construction BAC library of 5BL in the end 2013 (J. Dolezel, IEB)

In progress

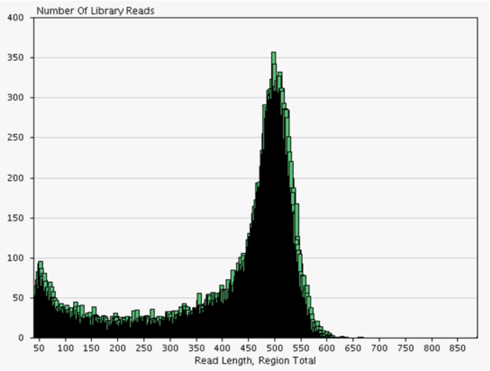
Finished

In plans

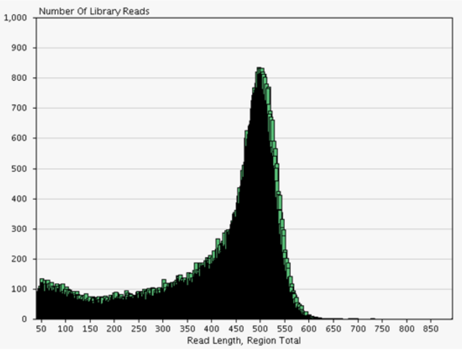
454 sequencing of chromosome 5B and development of ISBP markers

5BS = 290 Mbp

5BL = 580 Mbp



39695 reads
16183252 bp
mean = 475 bp
N50 = 490 bp
6% of total arm length

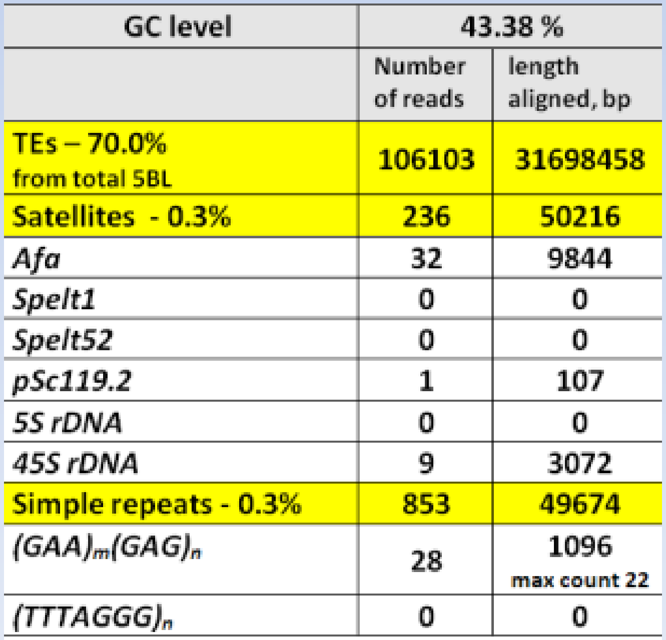


110793 reads
45307076 bp
mean = 466 bp
N50 = 484 bp
8.8% of total arm length

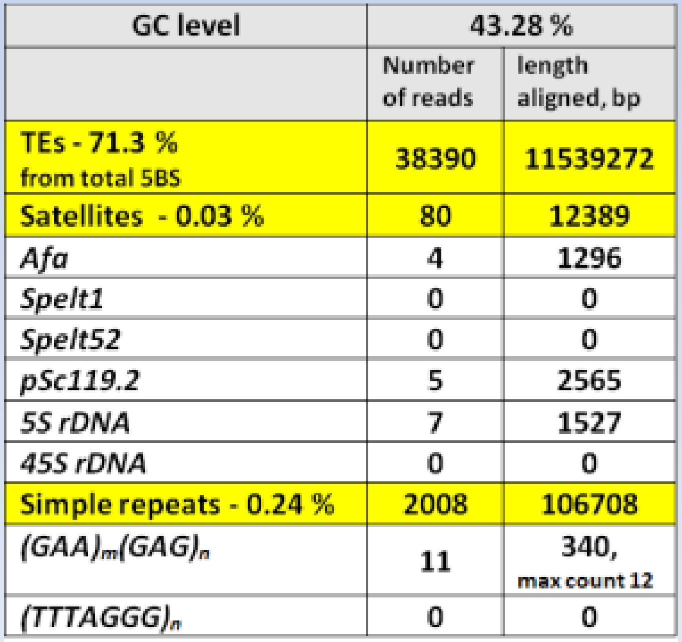
Analysis of 454-sequences. Identification of repetitive sequences

i) no long GAA/GGA tracts and 454- reads consisting of GAA/GGA

ii) no telomeric repeat (TTTAGGG) sequence tracts



GC level	43.38 %	
	Number of reads	length aligned, bp
TEs – 70.0% from total 5BL	106103	31698458
Satellites - 0.3%	236	50216
Afa	32	9844
Spelt1	0	0
Spelt52	0	0
pSc119.2	1	107
5S rDNA	0	0
45S rDNA	9	3072
Simple repeats - 0.3%	853	49674
$(GAA)_m(GAG)_n$	28	1096 max count 22
$(TTTAGGG)_n$	0	0



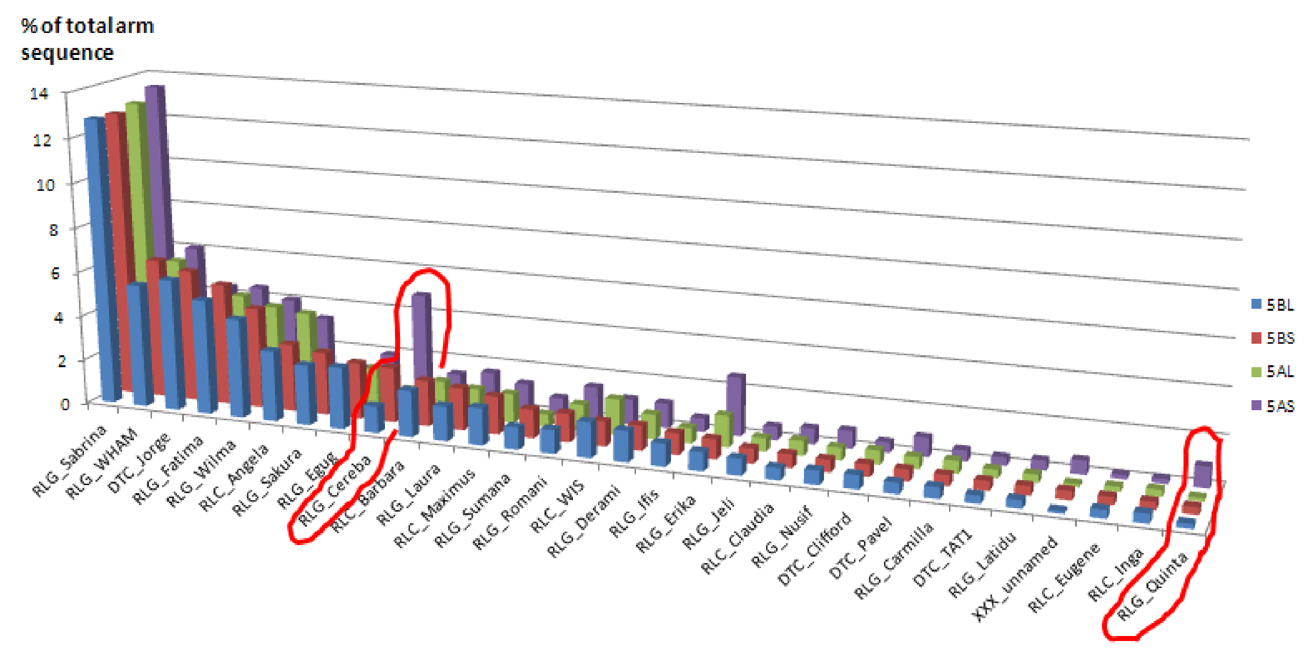
GC level	43.28 %	
	Number of reads	length aligned, bp
TEs - 71.3 % from total 5BS	38390	11539272
Satellites - 0.03 %	80	12389
Afa	4	1296
Spelt1	0	0
Spelt52	0	0
pSc119.2	5	2565
5S rDNA	7	1527
45S rDNA	0	0
Simple repeats - 0.24 %	2008	106708
$(GAA)_m(GAG)_n$	11	340, max count 12
$(TTTAGGG)_n$	0	0

Content of different Transposable Element families

compared the 5BS and 5BL 454-data with 454-sequencing data on 5AS and 5AL (Vitulo et al., 2011)

i) differing content of centromere associated (Li et al., 2013) *Cereba* and *Quinta* retrotransposons between short and long arms

ii) the prevalence of retrotransposons *Fatima* and *Sakura* and DNA-transposon *Jorge* in 5B chromosome comparing to 5A



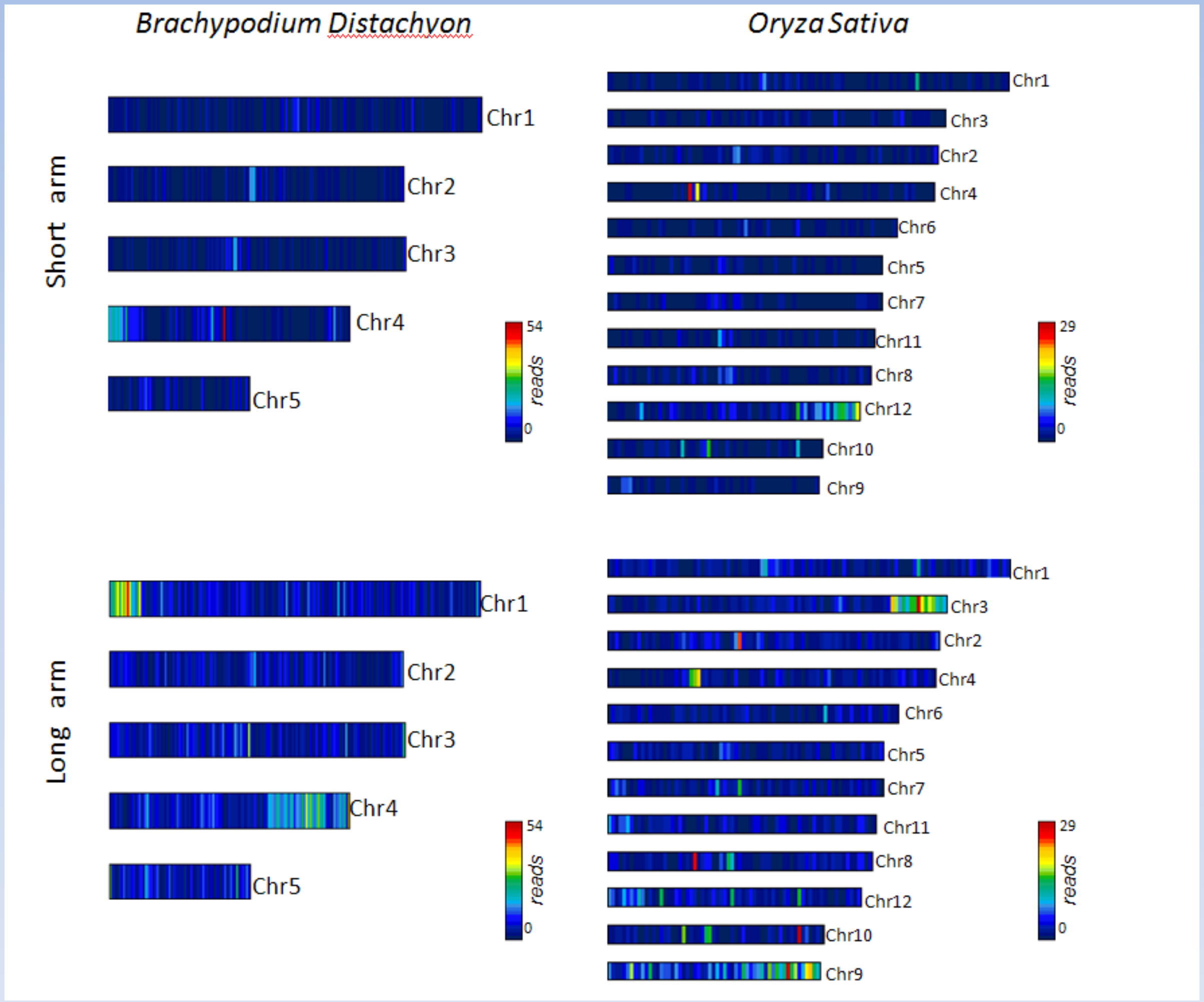
Li B, Choulet F, Heng Y, et al. Plant J. 2013;73(6):952-65.

Vitulo N, Albiero A, Forcato C et al PLoS One (2011) 6(10):e26421.

Mapping 454-reads to rice and Brachypodium chromosomes

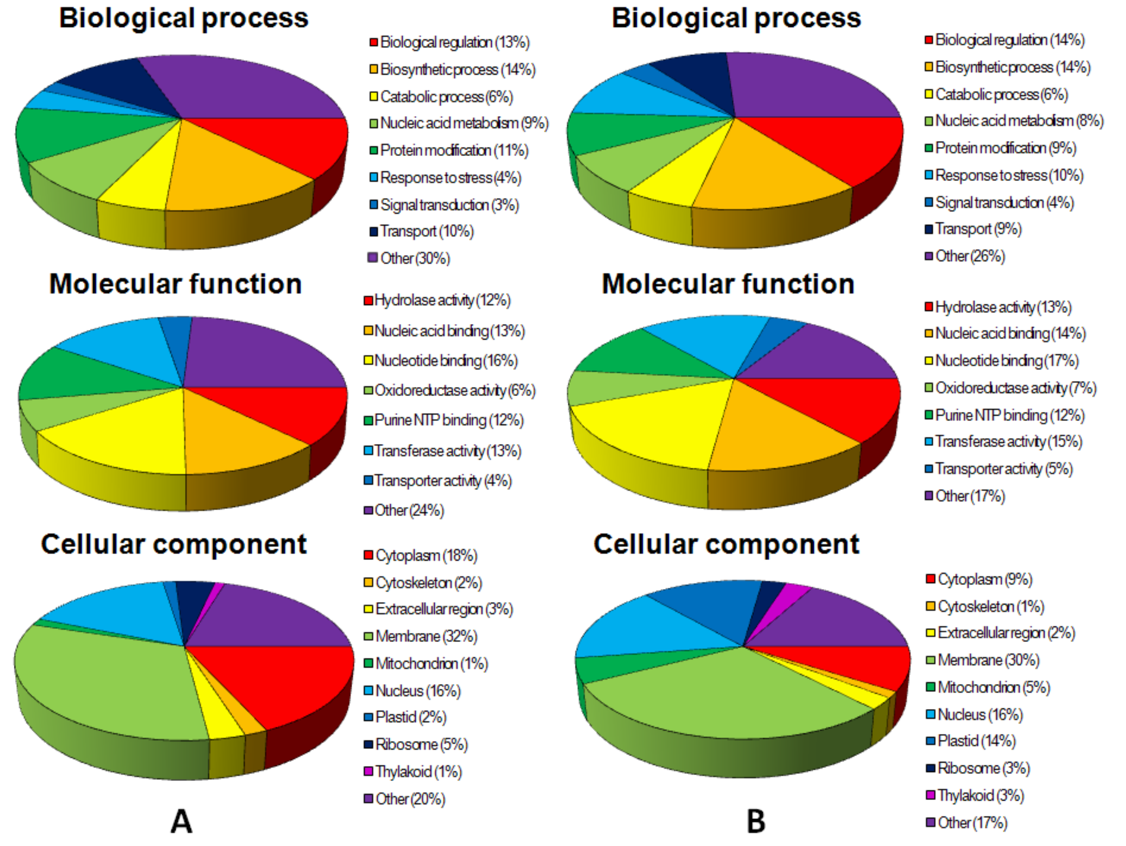
BLASTn

31029 reads matched with *Brachypodium* and 29952 matched with rice.



Results are in agreement with published data on EST mapping of wheat 5B chromosome to the genomes of these grasses (Linkiewicz, 2004, The International Brachypodium Initiative, 2010).

Functional assignment of 5B 454-reads



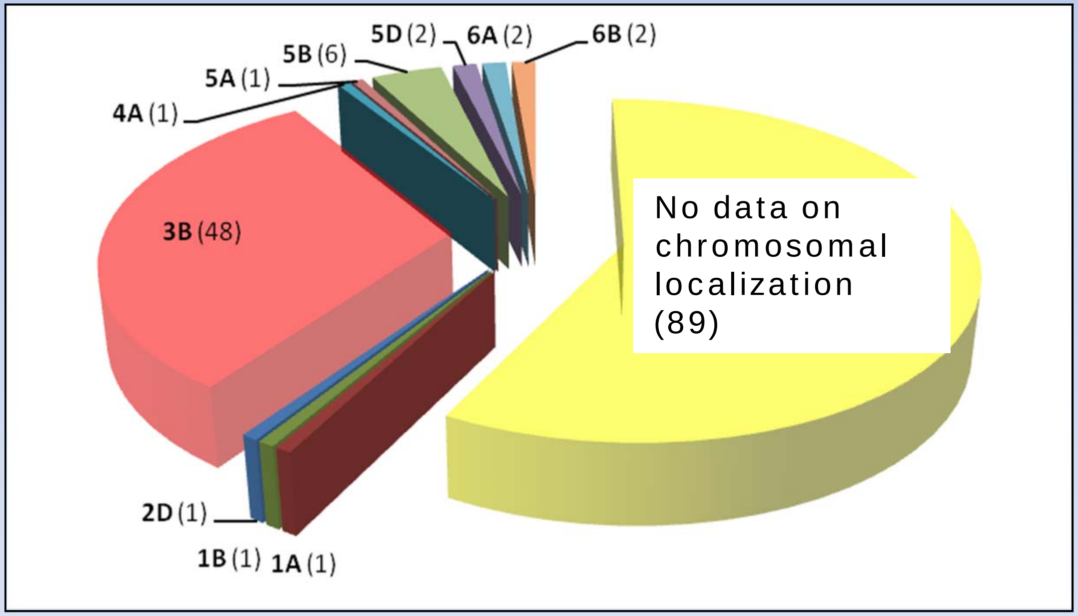
We assigned the mapped 454-reads to Gene Ontology annotations (http://www.geneontology.org/). Our results showed the similar pattern with chromosome 5A (Vitulo et al., 2011).

ISBP development and bioinformatical analysis

Comparison of ISBP marked 454-reads with bread wheat DNA sequences from databases.
The matching of 454-reads of 5B chromosome with the bread wheat genomic sequences (E-value < 1e-50).

5BL:
Total analyzed 3923
421 reads matched (11%)
From them 96 5BL-specific

5BS:
Total analyzed1302
154 reads matched (12%)
From them 6 5BS-specific



Mapping of SSR and ISBP markers on 5B

Population – F2 from cross Chinese Spring and the disomic substitution line CS-5B *dicoccoides* CS –Dic5BxCS

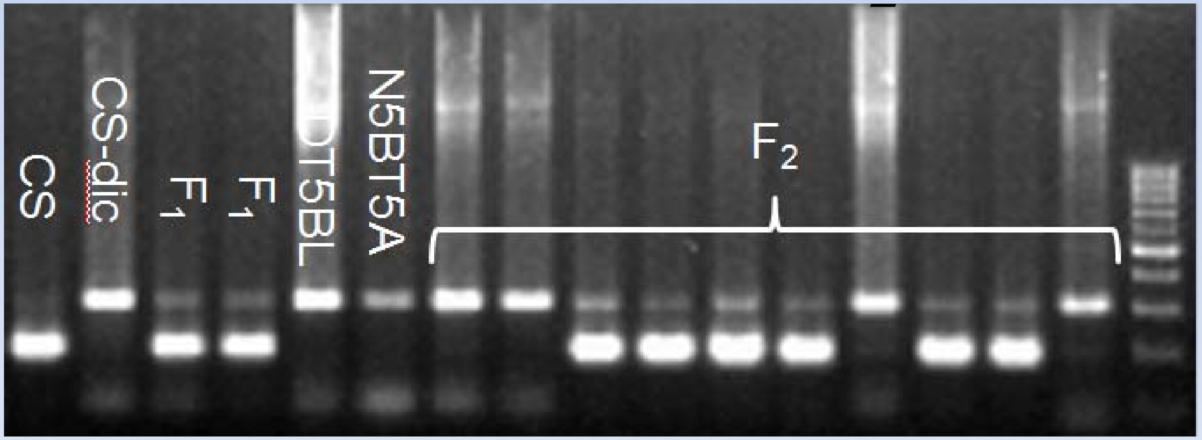
Plants – 366 crossing

Type of marker	SSR		ISBP	
Tested for polymorphism between CS – CS-5Bdic	113		5BS	5BL
			655	-
located on deletion bins of 5B	51 + 5 genes		254	-
Included in genetic map of 5B	5BS	5BL	5BS	5BL
	6	18	39	-

62% of SSR and 7.5% of ISBP revealed polymorphism between CS and CS-5Bdic

SSR markers are more polymorphic between chromosomes 5B of CS and *T.dicoccoides*

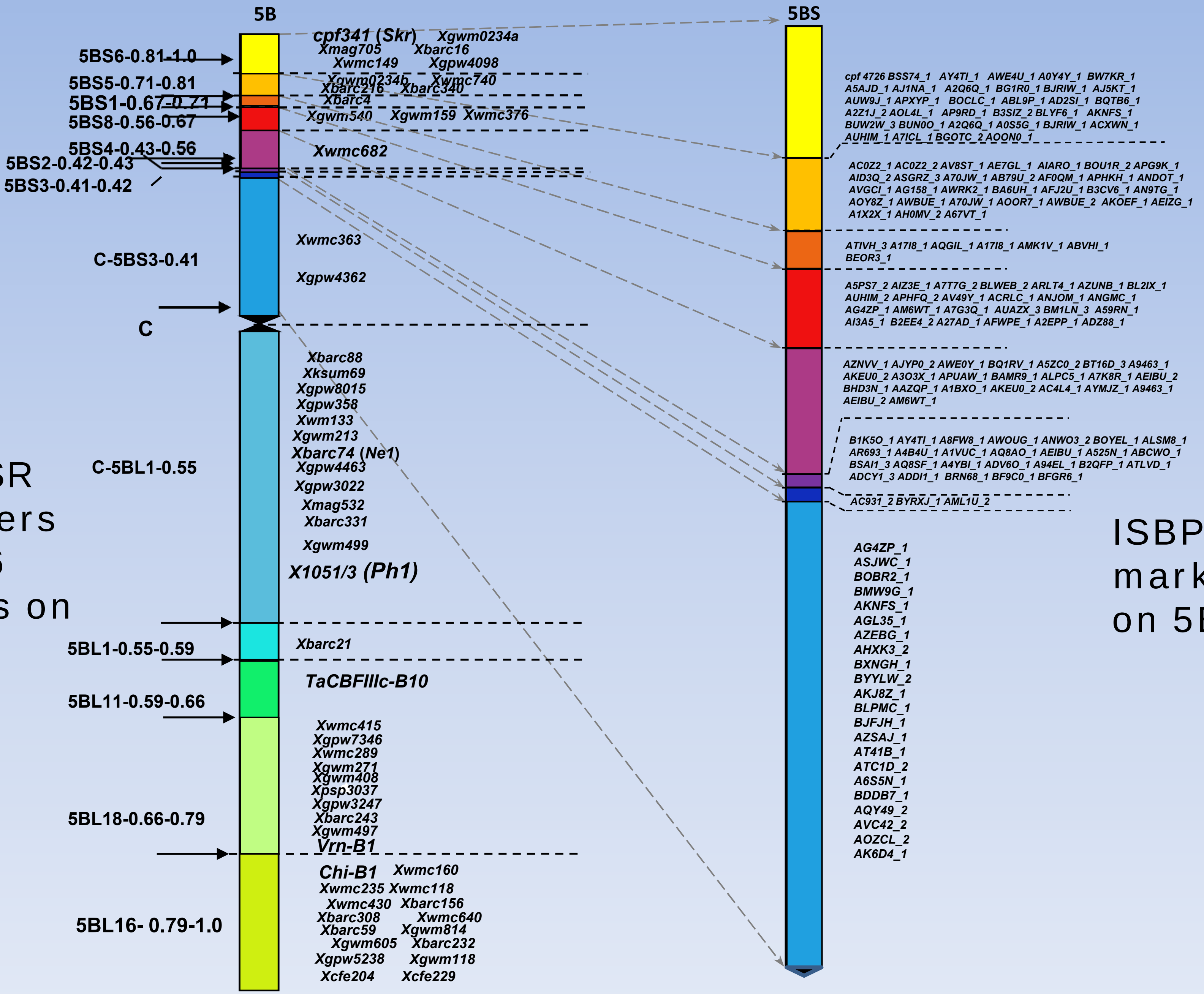
ISBP marker AOON0_1



CS –cultivar Chinese Spring, **CS-dic** – disomic chromosome substitution line in which the 5B chromosom

by the 5B chromosome from *T.dicoccoides*, **F$_1$** – F$_1$ plants from crossing CS×CS-dic, **DT5BL** – di-telosomic line for the 5B long arm, **N5BT5A** – nulli-tetrasomic line, **F$_2$ –** F$_2$ population from crossing CS×CS-dic.

Deletion map of wheat chromosome 5B

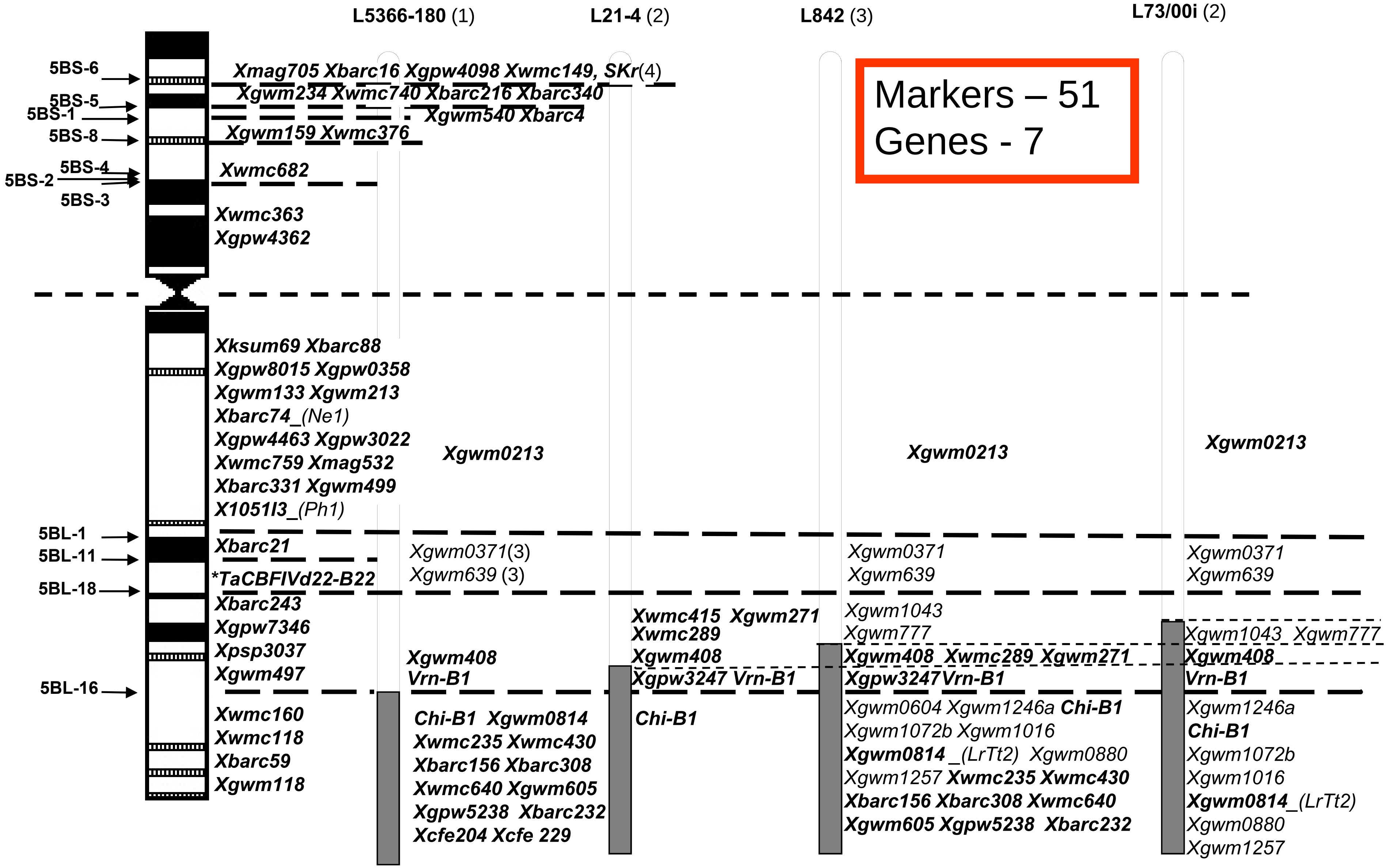


51 SSR markers And 6 genes on 5B

ISBP markers on 5BS

Deletion map of wheat chromosome 5B

Deletion lines CS **Introgressive lines - Saratovskaya 29, Novosibirskaya 29, Rodina**



L5366-180 (1) **L21-4** (2) **L842** (3) **L73/00i** (2)

Markers – 51
Genes - 7

5BS-6
5BS-5
5BS-1
5BS-8
5BS-4
5BS-2
5BS-3

Xmag705 Xbarc16 Xgpw4098 Xwmc149, SKr(4)
Xgwm234 Xwmc740 Xbarc216 Xbarc340
Xgwm540 Xbarc4
Xgwm159 Xwmc376
Xwmc682
Xwmc363
Xgpw4362

Xksum69 Xbarc88
Xgpw8015 Xgpw0358
Xgwm133 Xgwm213
Xbarc74_*(Ne1)*
Xgpw4463 Xgpw3022
Xwmc759 Xmag532
Xbarc331 Xgwm499
X1051l3_*(Ph1)*

Xgwm0213 ***Xgwm0213*** ***Xgwm0213***

5BL-1
5BL-11
5BL-18
5BL-16

Xbarc21
****TaCBFIVd22-B22***
Xbarc243
Xgpw7346
Xpsp3037
Xgwm497
Xwmc160
Xwmc118
Xbarc59
Xgwm118

Xgwm0371(3)
Xgwm639 (3)

Xgwm0371
Xgwm639

Xgwm0371
Xgwm639

Xgwm408
Vrn-B1

Chi-B1 Xgwm0814
Xwmc235 Xwmc430
Xbarc156 Xbarc308
Xwmc640 Xgwm605
Xgpw5238 Xbarc232
Xcfe204 Xcfe 229

Xwmc415 Xgwm271
Xwmc289
Xgwm408
Xgpw3247 Vrn-B1
Chi-B1

Xgwm1043
Xgwm777
Xgwm408_Xwmc289_Xgwm271
Xgpw3247Vrn-B1
Xgwm0604 Xgwm1246a ***Chi-B1***
Xgwm1072b Xgwm1016
Xgwm0814_*(LrTt2) Xgwm0880*
Xgwm1257 ***Xwmc235 Xwmc430***
Xbarc156 Xbarc308 Xwmc640
Xgwm605 Xgpw5238 Xbarc232

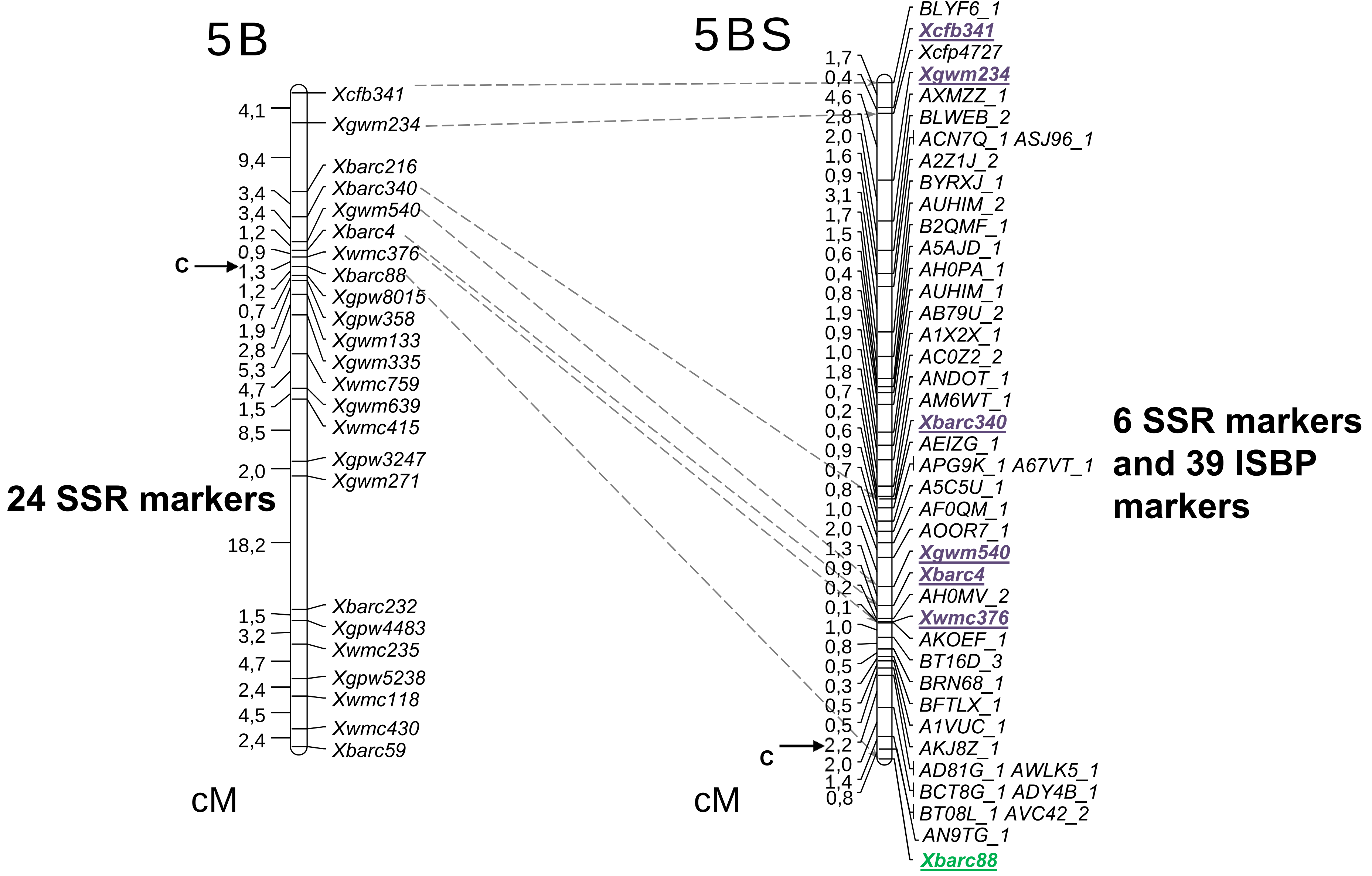
Xgwm1043_ Xgwm777
Xgwm408
Vrn-B1
Xgwm1246a
Chi-B1
Xgwm1072b
Xgwm1016
Xgwm0814_*(LrTt2)*
Xgwm0880
Xgwm1257

****TaCBFIIIc-B10***

1. Timonova et al., 2012; 2. Adonina et al., 2012; 3. Leonova et al., 2011, 4. Alfres et al., 2009

▭ → Introgressive region from wild species

Genetic map of wheat chromosome 5B



24 SSR markers

6 SSR markers and 39 ISBP markers

The genetic map for chromosome 5B was constructed with the help of the MAPMAKER v. 3.0b using the Kosambi mapping function at LOD 3.00

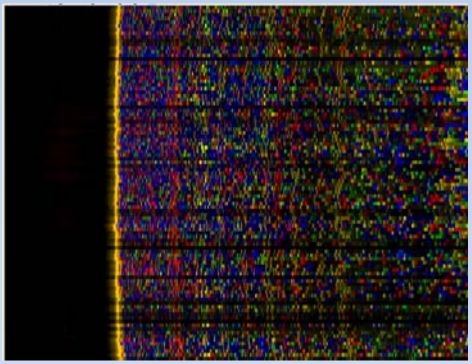
Fingerprinting and processing of data

BAC-library of 5BS chromosome (length 290 Mb) (IEB, Olomouc, Chezh Republic) J.Dolezel)

TaaCsp5BShA – 114 plates, 43776 clones, main insert size 122 Kb, coverage 15,8

Fingerprinting and processing (IGA, Udine, Italy)

- ***BAC-DNA minipreprs***
- ***Hydrolysis and SNaPShot labelling***
- ***Gel electrophoresis ABI3730***
- ***GeneMapper processing***



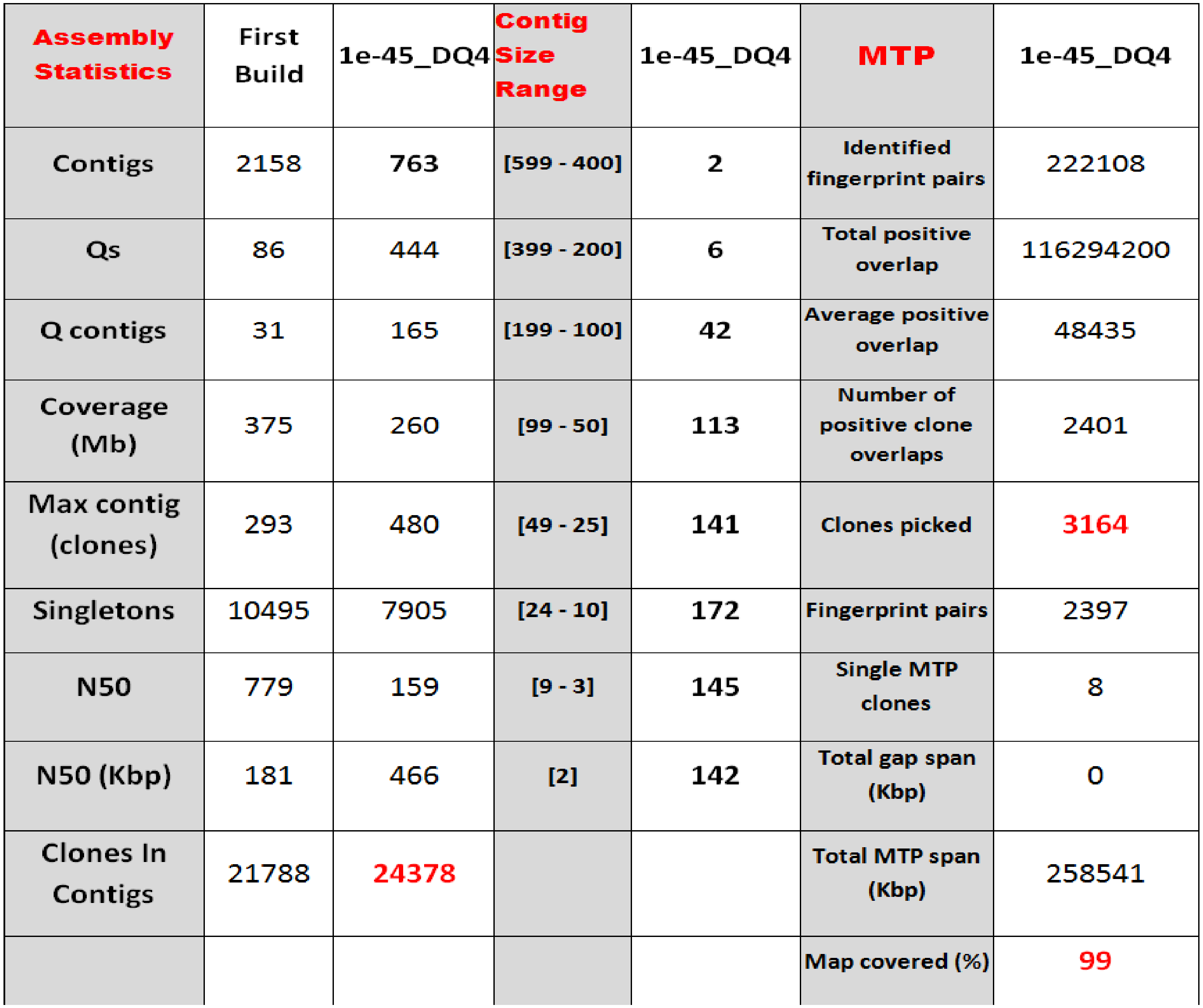
SizingQuality passed 34463 clones (78,7%)

- ***FPB processing***
- ***GenoProfiler removing controls and contaminated clones***

FPB sizes 32575 clones (5,5% no passed)
Contaminated 279 clones (0,9%) +
13 removed controls

FPC Gel Entries – 32283 clones

FPC assembly statistics



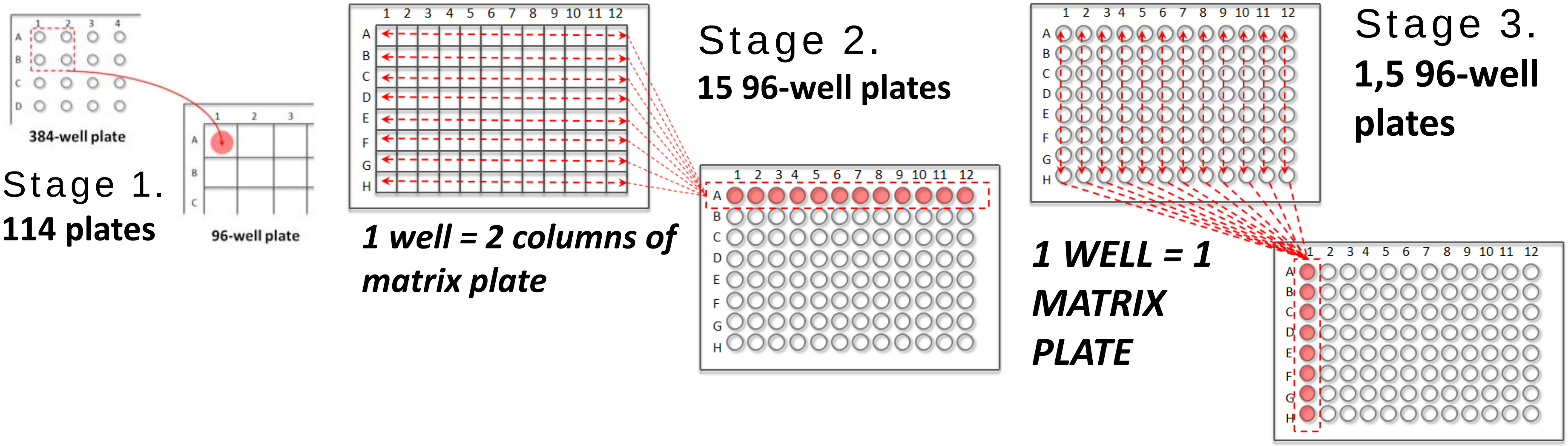
Assembly Statistics	First Build	1e-45_DQ4	Contig Size Range	1e-45_DQ4	MTP	1e-45_DQ4
Contigs	2158	763	[599 - 400]	2	Identified fingerprint pairs	222108
Qs	86	444	[399 - 200]	6	Total positive overlap	116294200
Q contigs	31	165	[199 - 100]	42	Average positive overlap	48435
Coverage (Mb)	375	260	[99 - 50]	113	Number of positive clone overlaps	2401
Max contig (clones)	293	480	[49 - 25]	141	Clones picked	3164
Singletons	10495	7905	[24 - 10]	172	Fingerprint pairs	2397
N50	779	159	[9 - 3]	145	Single MTP clones	8
N50 (Kbp)	181	466	[2]	142	Total gap span (Kbp)	0
Clones In Contigs	21788	24378			Total MTP span (Kbp)	258541
					Map covered (%)	99

FPC Gel Entries
32283 clones

FPC Bands
3315498

Bands/clone
102,7

5BS BAC-library 2D-pooling



Stage 1.
114 plates

1 well = 2 columns of matrix plate

Stage 2.
15 96-well plates

1 WELL = 1 MATRIX PLATE

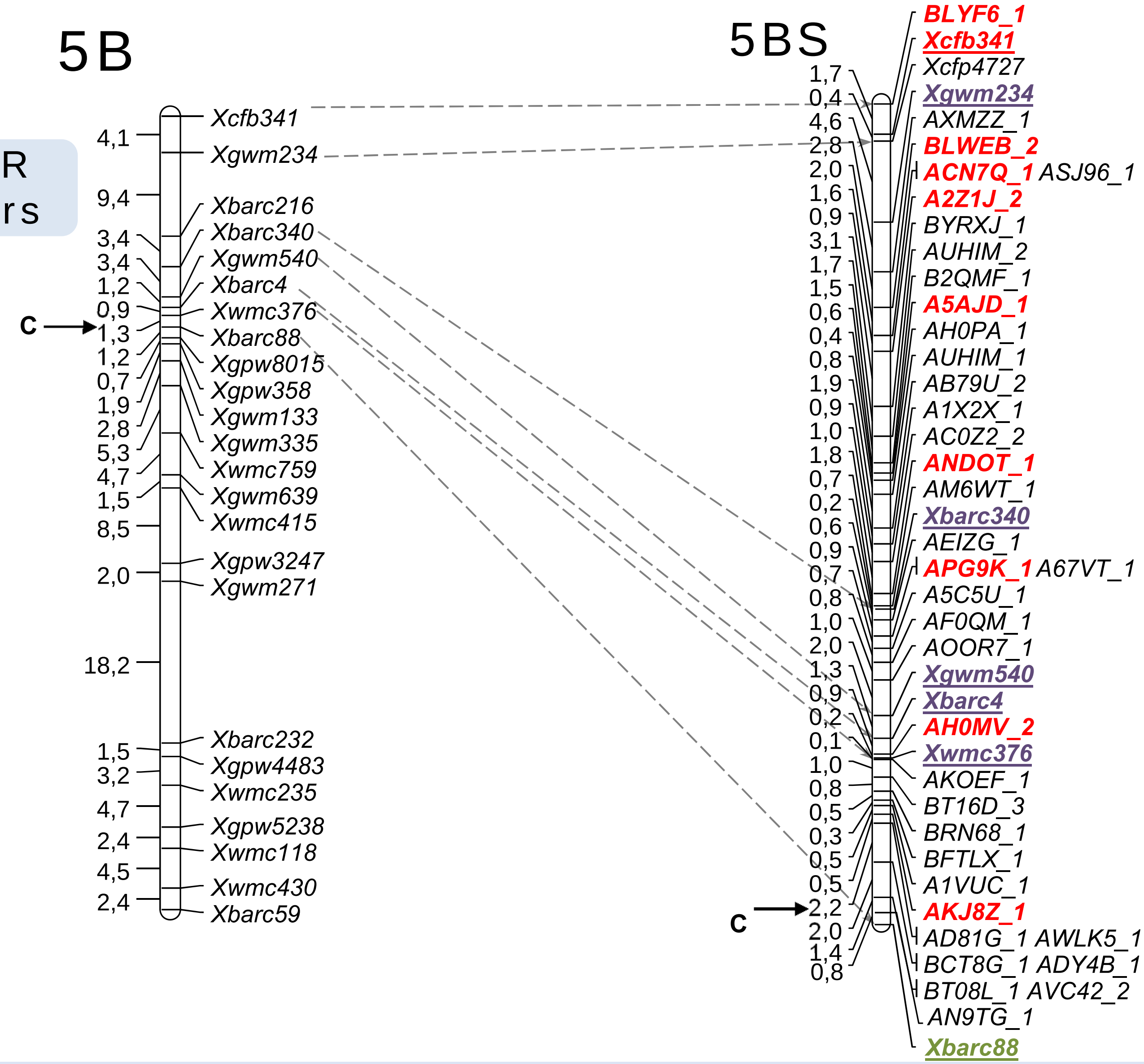
Stage 3.
1,5 96-well plates

BAC library PCR-screening

Type of marker	Number of markers	Number of clones (with FP data)	Number of contigs	Number of singletons	Number of clones in preliminary MTP for selected contigs
Markers to genes (incl 1 genetically mapped SSR)	7 for 2 genes	52	6	1	22
ISBP located in bins	2	8	3	0	15
ISBP in genetic map	9	94	14	6	126
Total	18	154	23	6	163

Genetic map of 5BS

24 SSR markers



6 SSR markers and 39 ISBP markers mapped, 9 ISBP and 1 SSR used for anchoring

MAPMAKER v. 3.0b using the Kosambi mapping function at LOD 3.00.
Common markers located are boldfaced and colored. C – centromere. Connections between common markers are indicated by dotted lines.

Conclusions

- The physical mapping of 5BS is in progress:

1) for saturation of genetic map we developed new 88 SSR markers on the base of 5BS 454-sequences,

2) for include more ISBP markers we will use Real Time PCR and melting curve analysis

3) for optimization of BAC-library screening we will add to the PCR/agarose analysis the Real Time PCR

4) BAC End Sequencing of non-anchored BAC-contigs, and their mapping

Plans

- To finish the physical map of 5BS to the March of 2014
- To start construction of physical map of 5BL from the April 2014

Participants of 5B project

Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia

Salina E.A. organization and coordination of work.

Sergeeva E.M. work with BAC library, fingerprinting, construction of physical map

Timonova E.M. construction of genetic and cytogenetic map

Bildanova L.L. ISBP PCR-testing

Koltunova M.K. development of markers, bioinformatical data analysis

Nesterov M.A. BAC library screening

Institute of Applied Genomics, Udine, Italy

Magni F., fingerprinting, FP analysis

Institute of Experimental Botany, Olomouc, Czech Republic

Dolezel J. construction of BAC library

USDA-ARS Cereal Crops Research Unit, Fargo, USA

Faris J., development of F1 hybrids for CSXCS-5B dic

UMR INRA-UBP Genetics, Diversity and Ecophysiology of Cereals, Clermont-Ferrand, France

Sourdille P., Feuillet C., Poncet C. 454- and ISBP data

Acknowledgement

Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia

Afonnikov D.A. for help in bioinformatical data analysis

Department of Plant Pathology, Kansas State University, USA

Gill B.S. for seeds of 5B chromosome deletion lines and CS-5B-dic