Progress on DNA Sequencing Project of the Wheat Chromosome 6B in Japan

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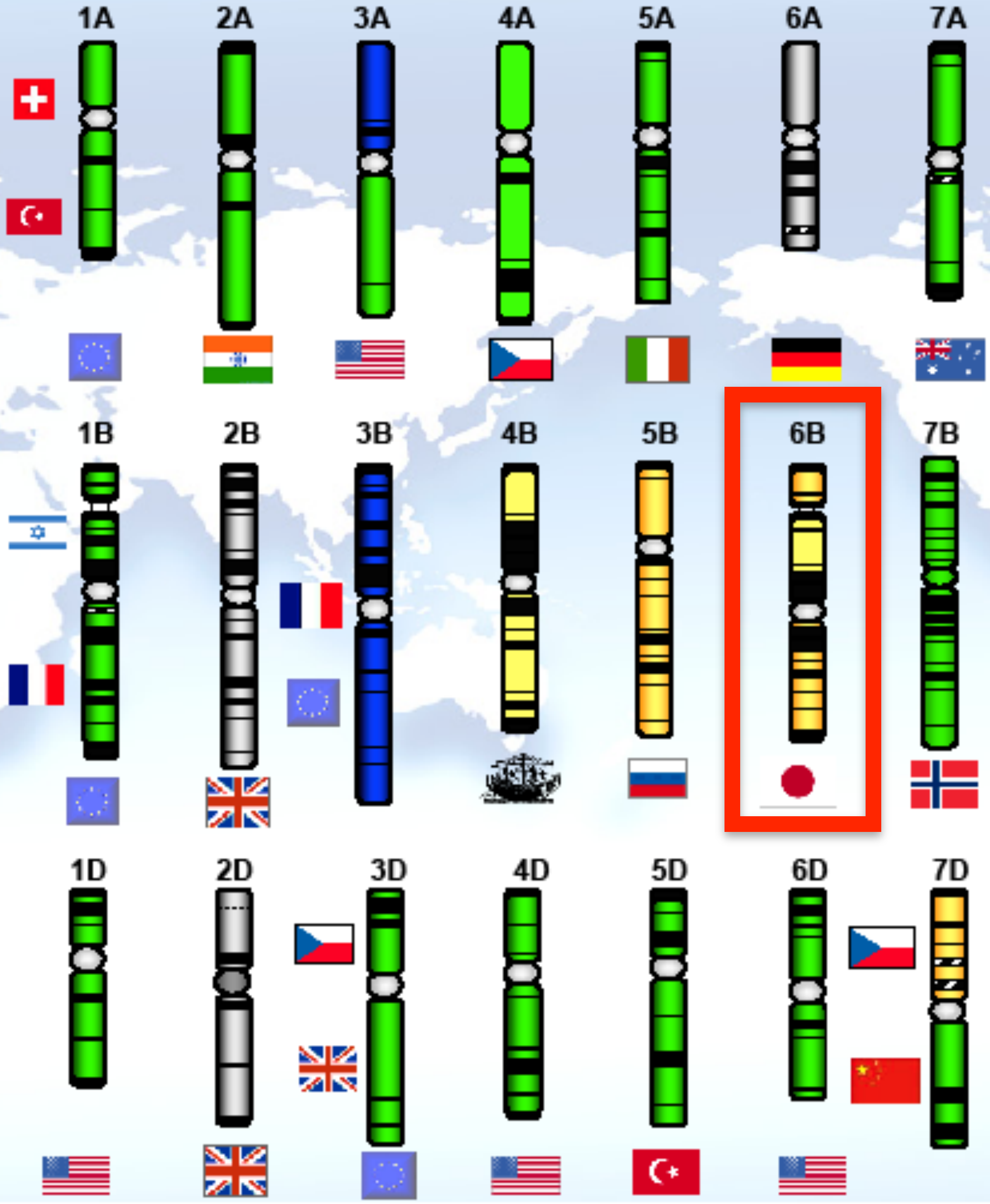
IWGSC Workshop June 24, 2012

IWGSC

Physical mapping of the bread wheat genome



2010

Funded

Some funding

Finished

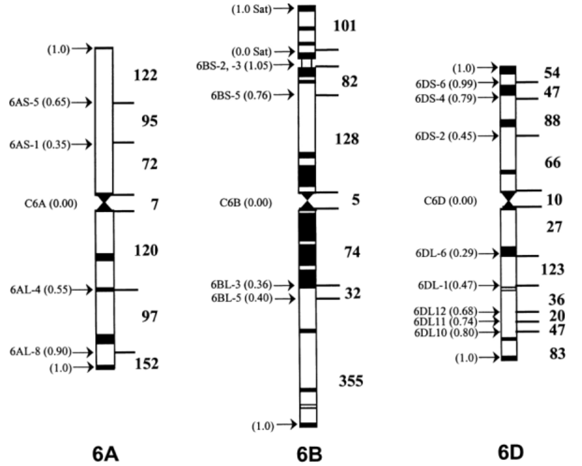
T. aestivum

cv Chinese Spring

www.wheatgenome.org

Wheat chromosome 6B

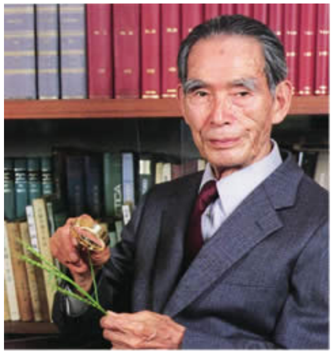
-914 Mb
-Large heterochromatin
- Secondary constriction



Deletion maps of wheat chromosome 6A, 6B and 6D
No. EST loci on the right
Randhawa et al. Genetics 168: 677 (2004)

<u>6BS</u>
rDNA
α/β-gliadin
Disease resistance: *YR36*
Herbicide insensitive: *Su1*

<u>6BL</u>
α-amylase
Disease resistance: *Lr3, Lr9, Sr11*
Pollen killer *Ki*

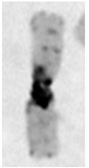


Prof. emer. Hitoshi Kihara
Father of Genome Analysis

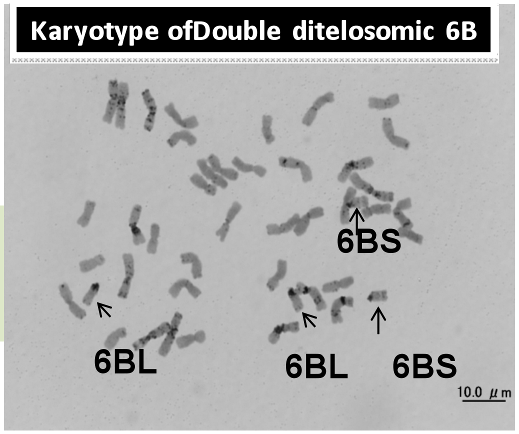
Wheat Genome Sequencing Consortium of Japan

(1) Physical mapping of BAC clones in 6B chromosome and genome sequencing
  Dr. T. Matsumoto, National Institute of Agrobiological Science, Tsukuba
(2) Marker development for BAC screening and touchdown to the chromosome
  Prof. T. R. Endo and Dr. S. Nasuda, Kyoto University, Kyoto
  Dr. S. Takumi, Kobe University, Kobe; Dr. K. Hayakawa, Nisshin Flour Milling Inc. Tsukuba
(3) Transcriptome as touchdown markers and collection of full length cDNAs
  Prof. Y. Ogihara and Dr. K. Kawaura, Kihara Institute for Biological Research, Yokohama C. Univ.
  Dr. J. Kawai, RIKEN, Yokohama
(4) Genome annotation
  Dr. T. Ito and Dr. H. Handa, National Institute of Agrobiological Science, Tsukuba
(5) Construction of 6B chromosome-arm specific BAC library
  Prof. J. Dolezel, Institute of Experimental Botany, Olomouc, Czech

Basic scheme of CS 6B genome sequence

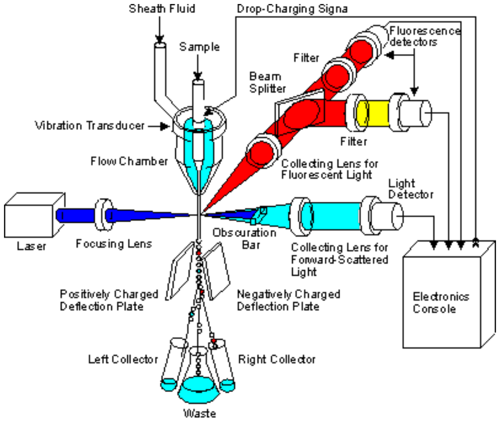


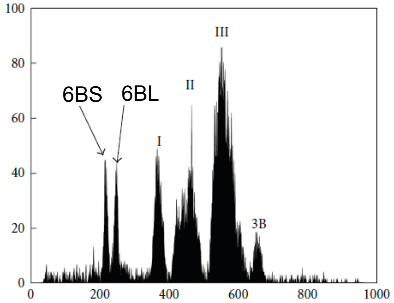
Chinese Spring
Wheat
6B-- 914Mb

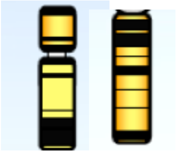


Dr.Endo・Dr.Drezel

flow sorted





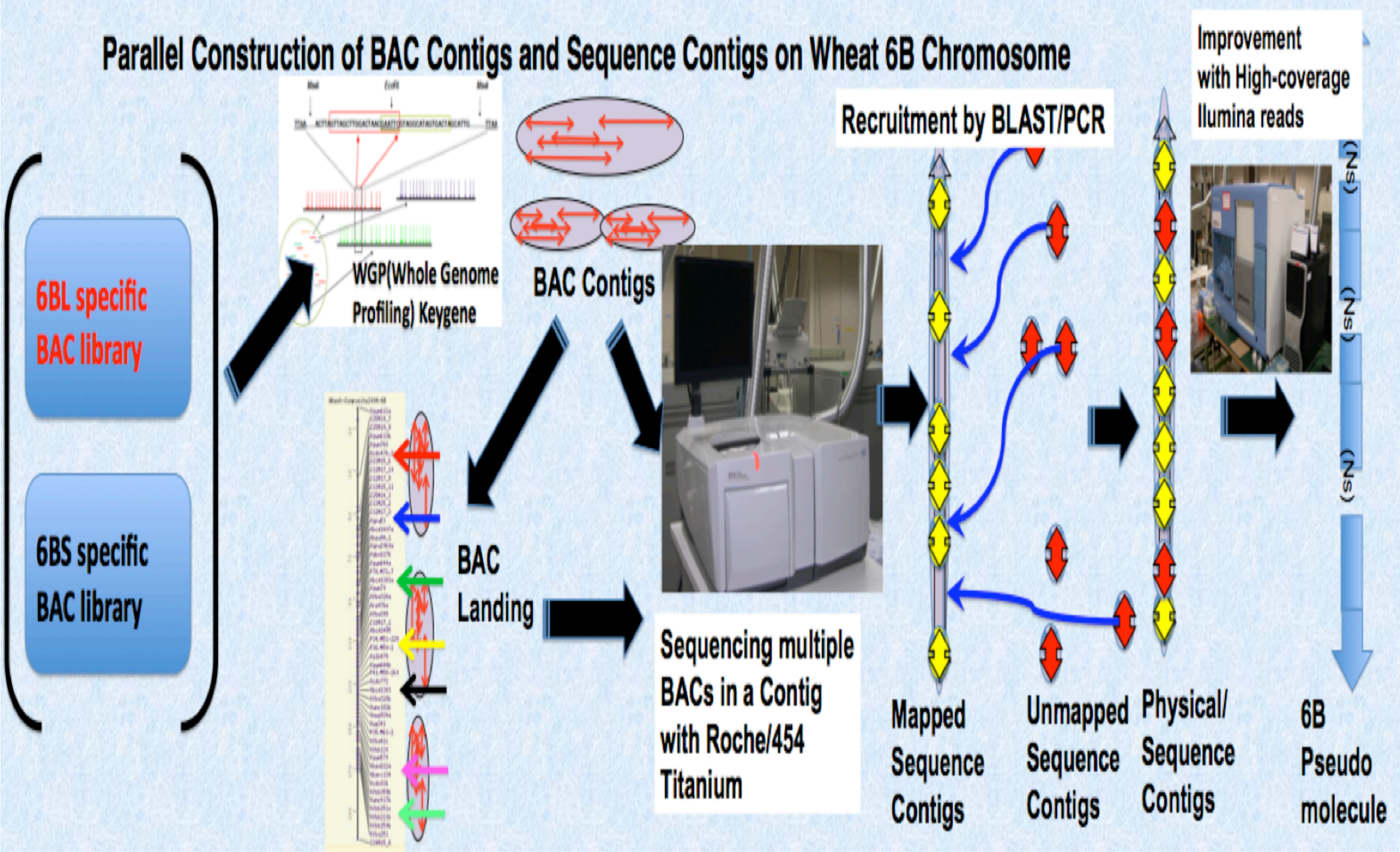


BAC libraries

Arm specific library of chromosome 6B in CS wheat

	6BS	6BL
Library code	TaaCsp6BShA	TaaCsp6BLhA
No. of clones	57,600	76,032
No. of plates (384-well plate)	150	198
Average insert size	132kb	130kb
Chromosome coverage	15.3x	18.0x

1. Construction of physical map of wheat 6B chromosome



Assembly of BAC clones with Whole Genome Profiling

BAC library	Short arm		Long arm	
FPC stringency*	**High**	**Low**	**High**	**Low**
Total No.of BACs in FPC	35,515		45,895	
No. of BACs in contigs	24,694	25,537	34,880	35,012
No. of contigs	2,667	2,449	1,842	1,264
% of BACs in contigs	70	72	76	76
Average contig size (BACs)	9.3	10.4	19	27.7
Average contig size (Mb)	0.29	0.31	0.37	0.50
Total length of contigs (Mb)	769	750	683	630
No. of singleton BACs	10,821	9,978	11,015	10,883
% of singleton BACs	30	28	24	24

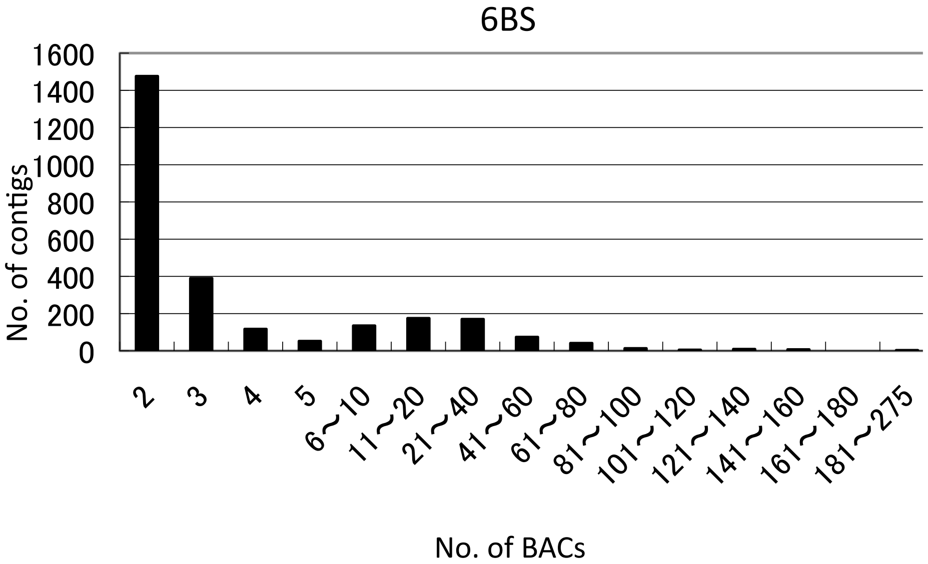
*Stringency: Tolerance=0, cut-off=$1.0E^{-10dq}$ (high); $1.0E^{-7dq+end\ merges\ E-5}$ (low)

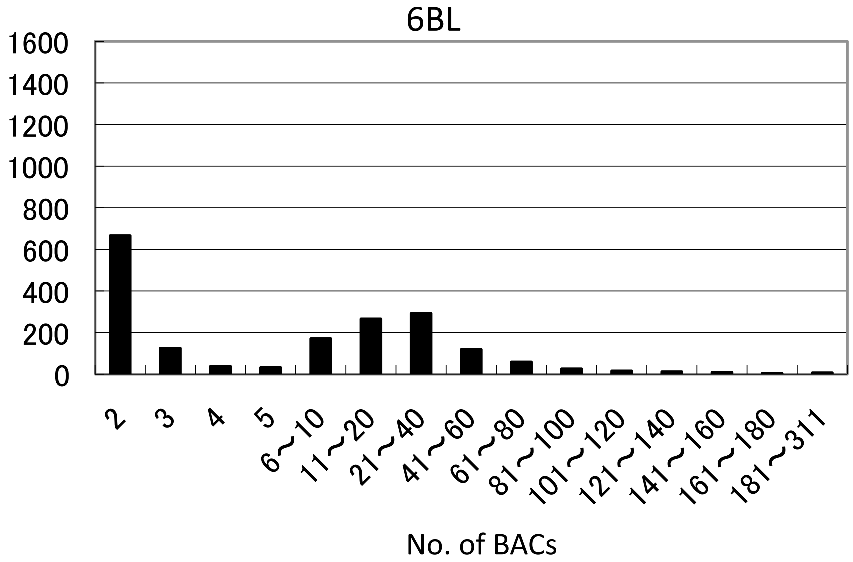
Select minimal tiling path (MTP) clones

	6BS	6BL	6B
No. of contigs	2,478	1,842	4,320
No. of MTP BACs	5,079	4,889	9,968
Size of MTP (coverage)	468Mb (112.8%)	436.8Mb (87.7%)	

Handa et al. ITMI (oral), Kobayashi et al. ITMI (poster)

Contig size distribution (singleton omitted)





Comparison of largest and medium contig size between 6BS and 6BL

	6BS	6BL
Largest contig	ctg45	ctg6
Estimated size (kb)	2621.4	3010.6
No. of BACs	275	311
No. of MTP BACs	28	34
N50 contig size (BACs)	35	44
N50 contig size (kb)	380	590

Handa et al. ITMI (oral), Kobayashi et al. ITMI (poster)

2. BAC screening with DNA markers

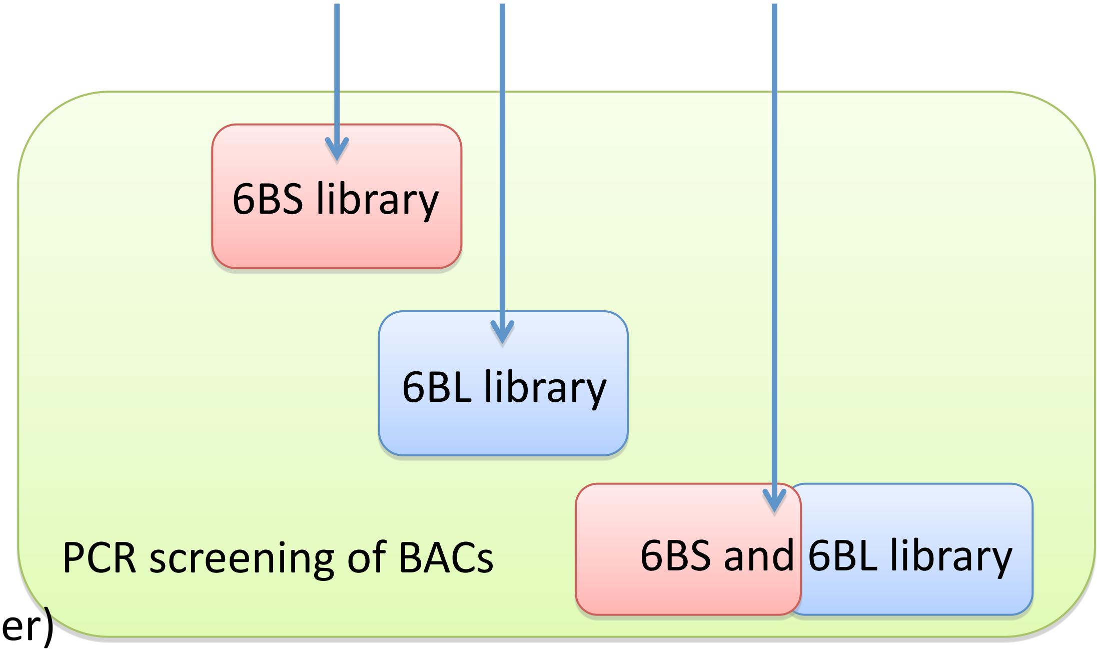
No. markers we designed?

PLUG markers	751
Markers based on GenomeZipper	390
Mapped markers (SSR, RFLP, Locus)	227
Orthologous set of genes (Rice, Brachypodium)	905
Presumed orthologous sequences (FLcDNA, EST)	924
STS derived from RFLP clones	65
ISBP marker	500
Sum	3762

Nasuda et al. ITMI (poster)

2. DNA marker availability from the PCR test using aneuploids of CS wheat

Marker source	No. of markers	6BS	6BL	6BS and/or 6BL	Useful	Not useful
SSR	222	70	64	19	153	69
RFLP	70	15	11	3	29	41
PLUG (gene)	751	170	230	10	410	341
Genome Zipper	390	197	24	8	229	161
Ortholog	1829	404	416	91	911	918
ISBP	500	416	2	21	439	61
Total	3762	1272	747	152	2171	1591



6BS library

6BL library

6BS and 6BL library

PCR screening of BACs

Nasuda et al. ITMI (poster)

BAC screening with DNA markers

2171 markers (57.7%) were useful for the PCR screening of BACs.

	No. of marker
6BS	1272
6BL	747
6BS and/or 6BL	152

BAC screening

Current status of BAC clone screening by DNA markers

	Genic marker	SSR	RFLP	ISBP	Total
6BS	362	40	12	360	774
6BL	250	32	12	4	298
TOTAL	612	72	24	364	1072

Nasuda et al. ITMI (poster)

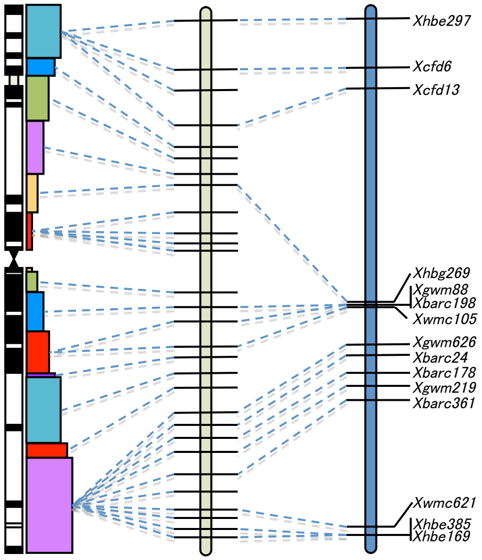
3. Maps of wheat chromosome 6B

Three maps construction for landing the contigs

✧Bin map: ca. 100 deletion lines for chromosome 6B

✧Radiation hybrid (RH) map: ca. 200 RH lines for chromosome 6B
(Nasuda et al., poster presentation)

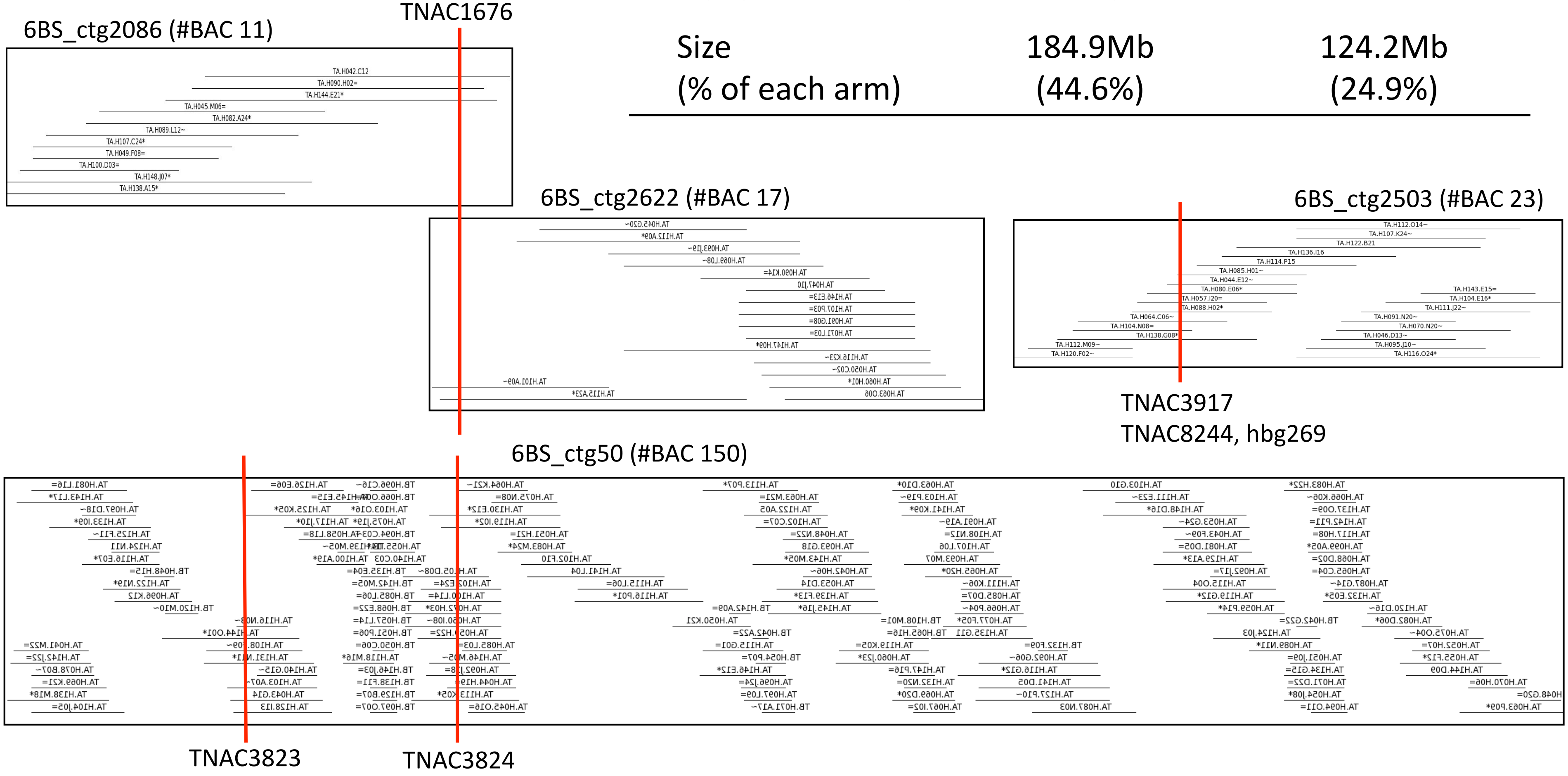
✧Genetic map: 210 RILs (Kobayashi et al. 2010)



Chromosomal assignment of BAC contigs

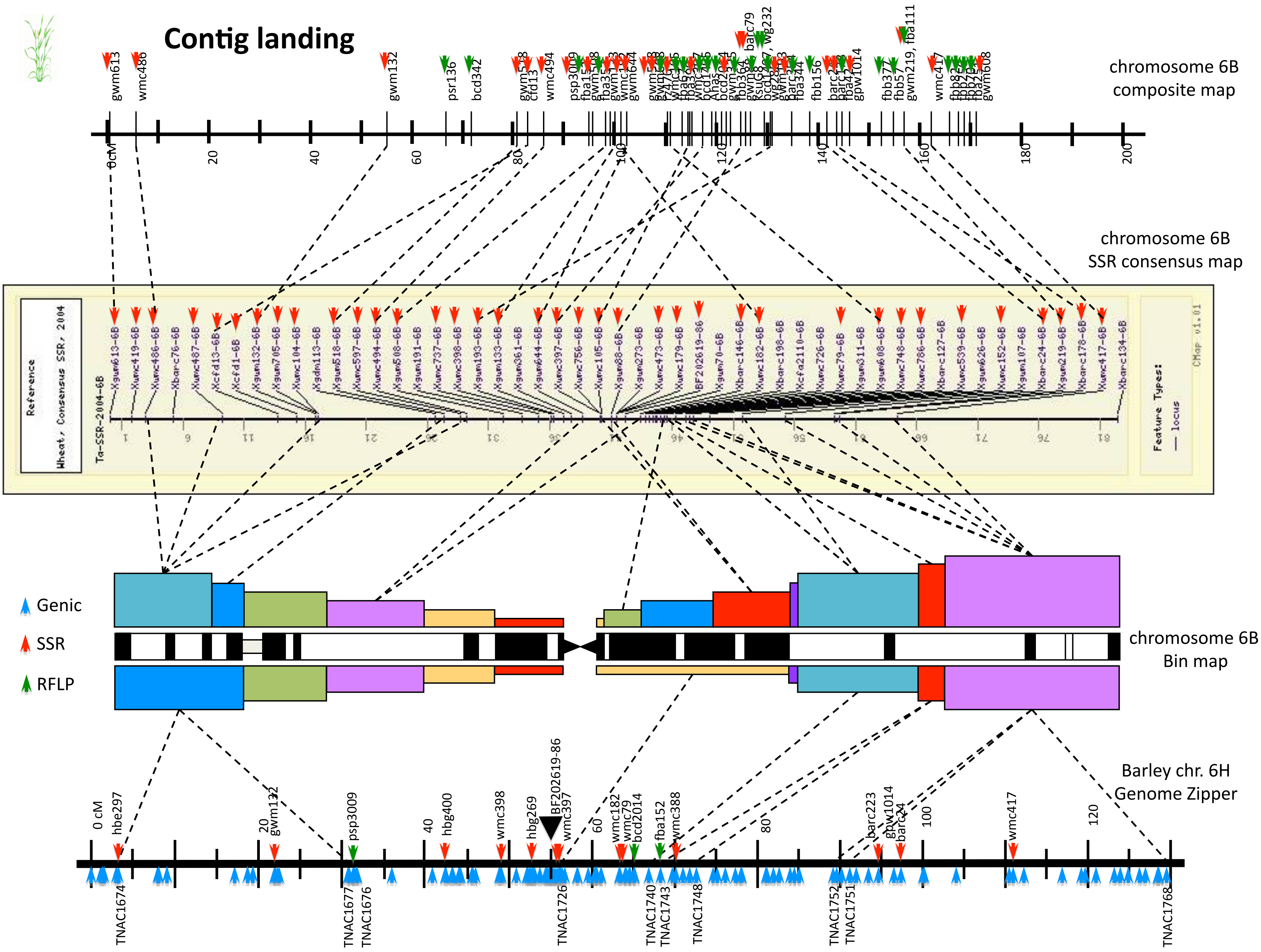
Current status of anchoring the physical map

	6BS	6BL
No. of markers	636	290
No. of contig	414	249
No. of MTP BACs	1749	1224
Size (% of each arm)	184.9Mb (44.6%)	124.2Mb (24.9%)

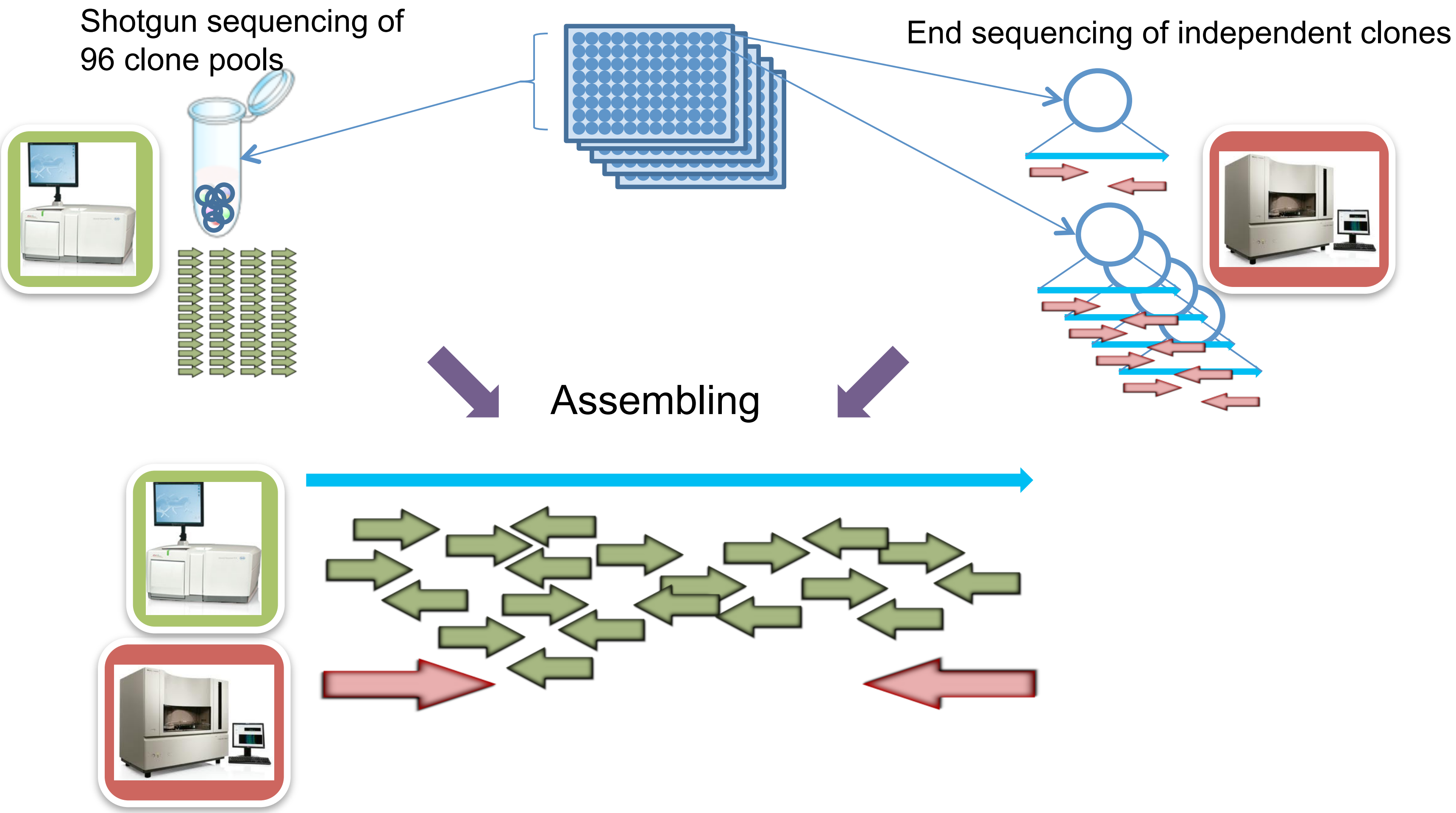


Handa et al. ITMI (oral), Kobayashi et al. ITMI (poster)

Contig landing



Strategy for complete sequencing of full length cDNA clones



Shotgun sequencing of
96 clone pools

End sequencing of independent clones

Assembling

Full length sequences as found both-end sequences of cDNA clones

Shotgun sequencing and assembling for complete sequencing of FL cDNA clones

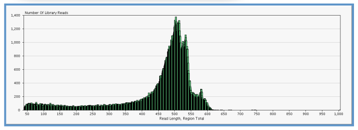
Pilot experiment

FL cDNA (96 clones)

mixed & shotgun sequencing by Roch GS J

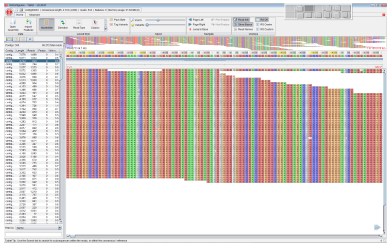
End sequencing by ABI3700

Number of passed filter reads	137,555
Total bases [bp]	61,393,902



Number of reads	192

Assembling by Newbler 2.5



Total contigs	358 contigs (362,588 bp)
Contigs assembled with both end seq	75 (78% of 96 clones)
Contigs with single end seq	87
Contigs without end seq	196

Genome Network Analysis Service, Riken

GeNAS

Shotgun sequencing and assembling for complete sequencing of FL cDNA clones

Data production

FL cDNA (2314 clones)

mixed & shotgun sequencing by Roch GS FLEX

Run	Number of read	Number of base [bp]	Average length [bp]
1st run	951,816	372,083,676	413
2nd run	1,352,035	572,856,417	442
3rd run	1,418,307	678,344,113	492
Total	**3,722,158**	**1,623,284,206**	

End sequencing by ABI3700

Number of reads	4628

Assembling by Newbler 2.6

categoly	statistics
Number of contigs assembled with both end sequence	1678 (73% of 2314 clones)
Average of full length contigs	3550 bp
Longest full length contig	9366 bp

Remarks

- 9968 BACs for MTP
- 4320 BAC contigs were selected for physical map of wheat 6B
- 2171 of 3762 markers were available for BAC screening
- 1072 markers have been screened.
- 871 markers were linked to 663 BACs,
  - 45 % 6BL and 25 % 6BS physical maps
- 17,795 (1,678 additional) full length cDNAs were available.

Kihara Institute for Biological Research
Yokohama City University

Pioneer of Genome Research
Professor Hitoshi Kihara
(1893－1986)

The history of the earth is recorded in the layers of its crust.

The history of all organisms is inscribed in the chromosomes.

1946

H. Kihara

Welcome to the 12th IWGS, Yokohama