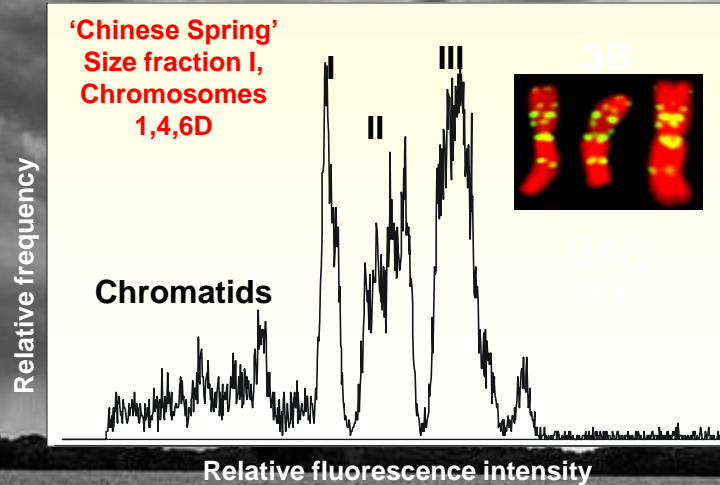


Physical and Genetic Framework of Chromosomes 3A, 1D, 4D, and 6D of Hexaploid Wheat



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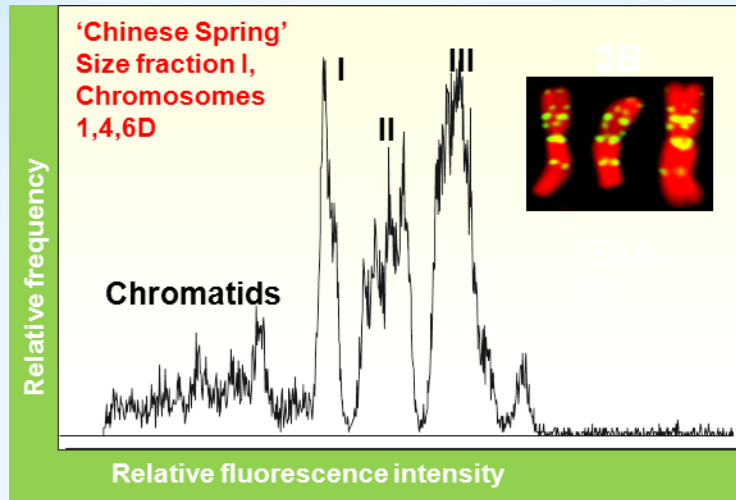


Physical mapping is necessary for complete sequencing of wheat genome primarily because of huge size, largely repetitive DNA, complexity and polyploidy.

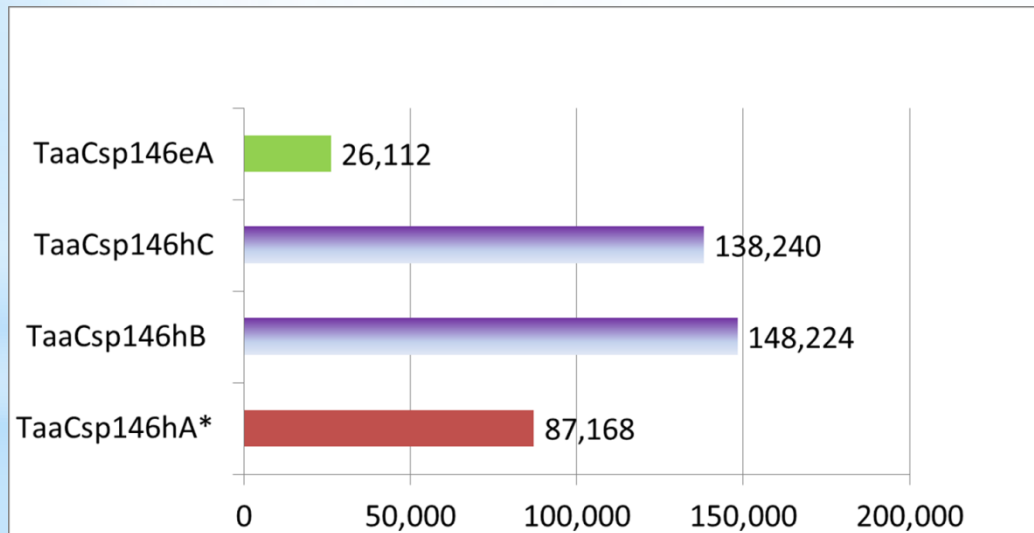
We are making fraction-I physical maps of chromosomes 1D, 4D, and 6D

Chromosome	Size (Mb)	% fraction of wheat genome
1D	604	3.5
4D	648	3.8
6D	712	4.1

BAC Libraries



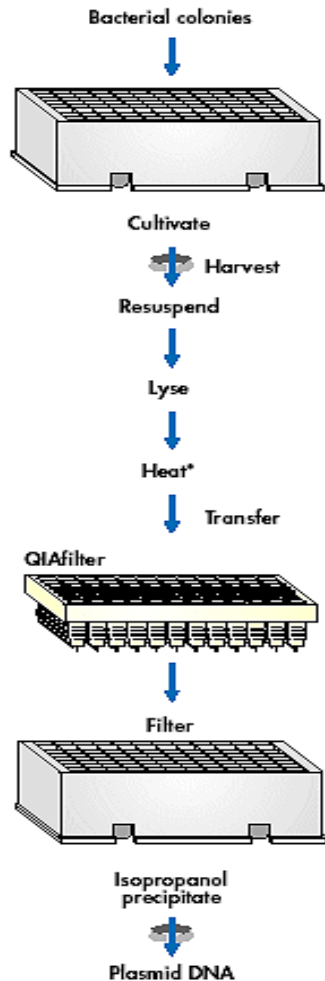
Size: fraction I (1D, 4D, 6D);
fraction II (1A, 3A, 6A, 2D,
3D, 5D, 7D); and fraction III
(2A, 4A, 5A, 7A, 1B, 2B, 4B,
5B, 6B, 7B); in addition to an
individual chromosome 3B



HICF

(a first step in developing a physical map)

BAC-DNA Isolation



SNaPshot BAC fingerprinting technique

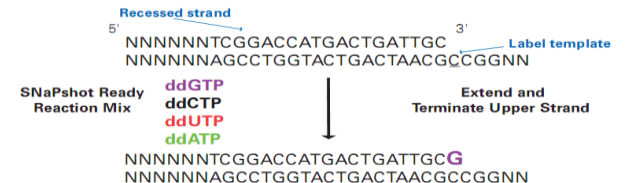


Restriction Endonuclease Digestion

Restriction Endonuclease	Restriction Site	ddNTP	Fluorescent Dye Label	Restriction Fragment Color
EcoRI	G↓AATC	A	dR6G	Green
BamHI	G↓GATCC	G	dR110	Blue
XbaI	T↓CTAGA	C	dTAMRA™	Yellow
XhoI	C↓TCGAG	T	dROX™	Red
HaeIII	GG↓CC	None		

3' end labeling on restricted fragments using SNaPshot chemistry

SNaPshot Reagent Restriction Fragment Labeling



Size exclusion and signal detection on CE instrument like the 3730/3730x1

Data processing



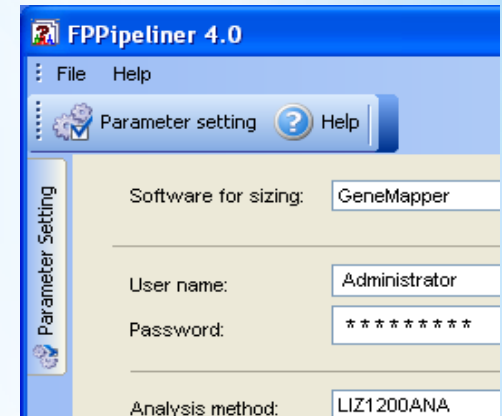
DNA Analyzer

Fragment separation

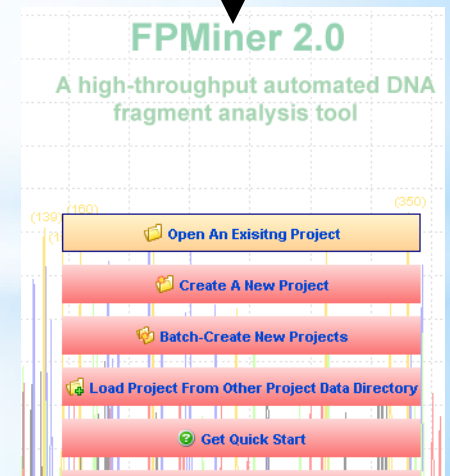


GeneMapper v4.0.Ink

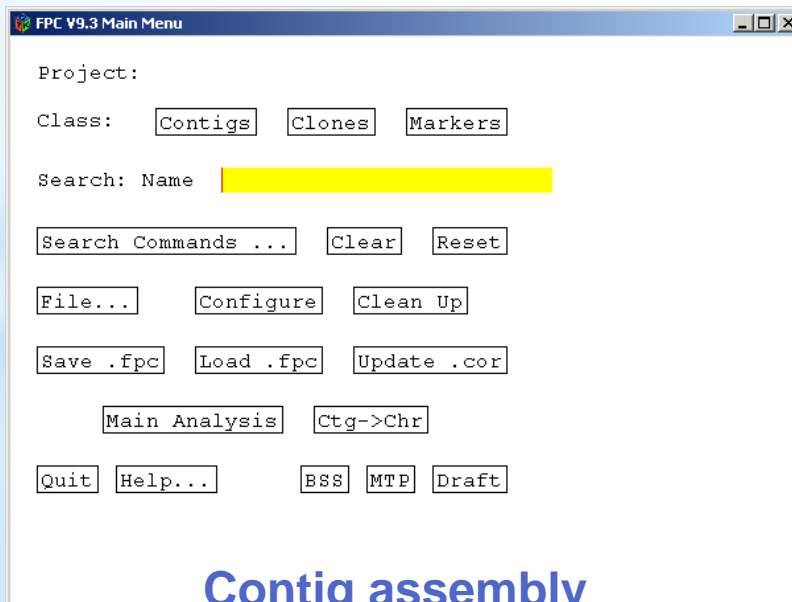
Fragment Sizing



Batch processing



Manual editing, filtering and processing



Contig assembly

FPC Assembly

- 280,000 BAC clones fingerprinted
- High quality fingerprints from 200,069 clones used in FPC assembly

Features	Statistics
Cutoffs	1e-45
Number of clones in contigs	170,368
Singletons	29,701
Total number of contigs (>200 kb)	5,152 (2,411)
Number of contigs >500Kb	1,068
Longest contig (# of clones)	4Mb (517)
400-599clones	4
200-399 clones	89
100-199 clones	350
50-99 clones	714
Average contigs size (Kb)	485
Assembly size (% coverage)	1567Mb (82%)

Selected MTP for Coassembly with *Aegilops tauschii*

A minimum tiling path with MinFPC overlap of 25 was used and 34,350 BACs were identified

Zoom 3.3 Whole

Show buried clones Yes No

Search

CB Unit Range 0 to 2009

Contig stats

Clones: 208 (42 buried)
Markers: 0
Sequenced: 23
Length: 2010 CB units

Ctg1155 of 146d_03_17_2011

146DhB464O23	146DhB411E13	146DhC873E01*	146DhB437D09	146DhC871J16*
146DhC731F20	146DhC955I17	146DhC741P20	146DhC757D01	146DhB375K16
146DhB468F01	146DhB489J12	146DhC851J21	146DhC634M04	146DeA035M18
146DhC825P12*	146DeA047J10	146DhB468L23*	146DhC642I22	146DhC810B06
146DhB391M03	146DhB432J18*	146DhB316K02	146DhC831L04	146DhC834N13
146DhB421J13	146DeA028B08	146DhB387B20	146DhB268A16	146DeA007B07
146DhC622E18*	146DhC901G21*	146DhC758C09	146DhC732H22*	146DeA013B15
146DhC756I05	146DhC914E12	146DhC767J06	146DhC770H02	146DhC663F12
146DeA009O23	146DhC804B15*	146DhB377I07	146DhB455F14	146DhC917B05
146DhB236I01	146DhC818I05	146DhB396B08	146DhC702I01	146DhC726L18
146DhC683I19*	146DhB513C03	146DhC875L01	146DhC770H02	146DhB536M20
146DhB551O05*	146DhC943N16	146DhB553O09*	146DhB532E13	146DhB534I17*
146DhC814J05*	146DhB349O15*	146DhB548D16	146DhC954N15	146DhB449I13
146DhB268A13	146DhB414J05	146DhB289C14	146DhC700N12	146DhB405J08
146DhB476A21	146DhC809F12	146DhC969N17	146DhC735C12	146DhB840S12
146DeA026N16*	146DhB473A11	146DeA047B23	146DhC823N11*	146DhC823N11*
146DhB369P01*	146DhC901G14	146DeA056A23	146DhB415C09*	146DhB401C18
146DhB519A13	146DhB440F05*	146DhC874K16	146DhC701B04	146DhB449C10
146DhC814E18*	146DeA049A11	146DhB370D17	146DhC815H23	146DeA029L07
146DhC649L03	146DhB376P12	146DhC765K10	146DhB421B22	146DeA016D10
146DhC867L22	146DeA032I23	146DhC852I04	146DhB443L12	146DhB240B07
146DeA038P14	146DhC684C20	146DhB433G18	146DhC952D17	146DhB505F07
146DhC892D22	146DhC618D03	146DhB302F04	146DhB379L05	146DhB248L12
146DhC863E13*	146DhB556G01*	146DhC779I04*	146DhB354J18	146DhB461G05*
146DhC901B18	146DeA042J02	146DhC714F09	146DhB525P05	146DhC969L09
146DhC828M10	146DhC677G10	146DhB528O04	146DhC959K20*	146DhB449K03*
146DhB283H12	146DhB234F15	146DhC883E11*	146DhC744E10	146DhB425J11*
146DhC749O20	146DhB503H18*	146DhC693M20	146DhB236A23	146DhB249K11
146DhB250D19*	146DhB327E20	146DeA005H07	146DhB545B22	146DhB525B06
146DhB331A16	146DhC705I10	146DhC804I10*	146DhB516A07*	146DhB401M18
146DeA066F07	146DhB335I12	146DhB248D09	146DhB353K13	146DhB311B15
146DeA065I22	146DhC834C07*	146DhB315N02	146DhC738G10	146DhB262F18
MTP: 146DhC683I19 29160	CB-merge	MTP: expway end	MTP: 146DhB516B14 29160	
MTP: 146DhB268A13 17280	MTP: 146DeA047B23 36720	MTP: 146DhC952D17 32400	MTP: 146DhB318N15 54000	
CB-merge	MTP: 146DhC779I04 22680	MTP: 146DhC744E10 29160	MTP: 146DeA016D10 35640	
MTP: 146DhC814E18 44280	MTP: 146DhB248D09 25920	MTP: 146DhB353K13 41040	MTP: 146DhB311B15 41040	

BAC anchoring

Random BACs 3-D Pools



MTP BACs 3-D Pools



Selected MTP BACs 3-D Pools



Randomly selected 240 384 well plates to develop 3-D BAC pools for anchoring

5x

Total clones= 92,240 clones
Plate Pools= 40
Row Pools= 48
Colum pools=48


High confidence MTP selected for anchoring **and** co-assembly with *Aegilops tauschii*

Total clones= 34,350 clones
Plate Pools= 90
Row Pools= 16
Colum pools=24

Two border clones from each contigs was selected

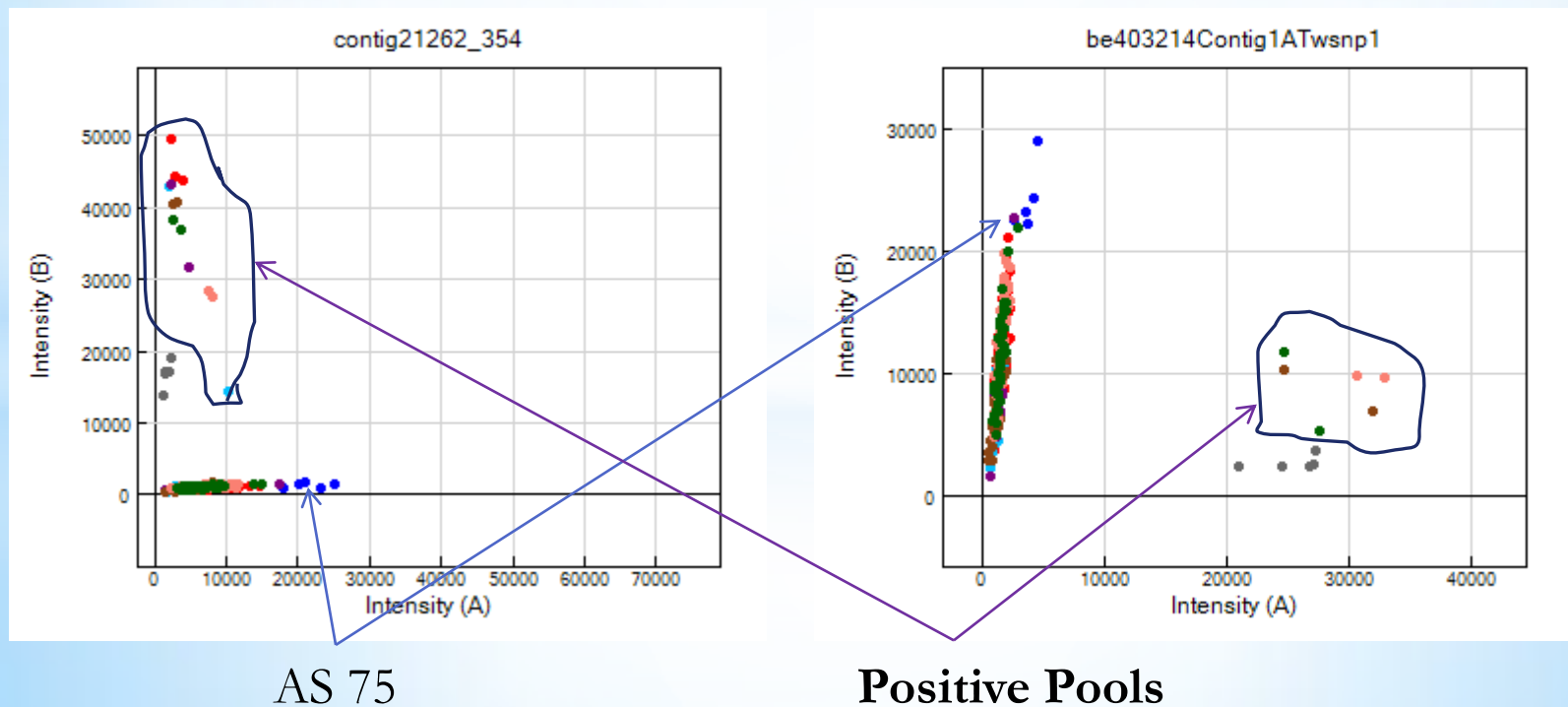
Total clones= 7,064 clones
Plate Pools= 19
Row Pools= 16
Colum pools=24


10K Infinium assay

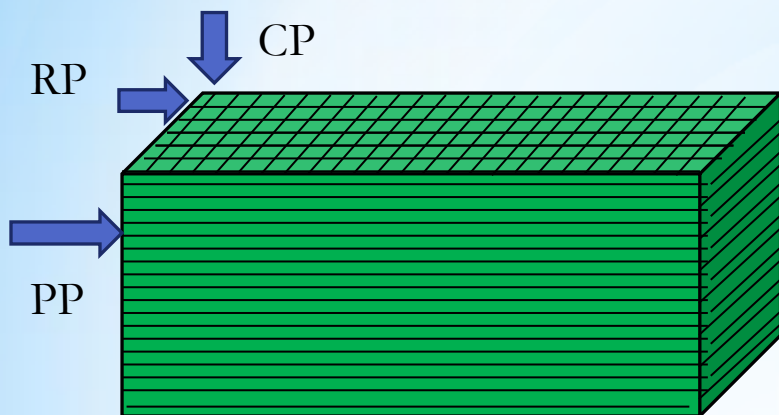

Barcoded BAC pools and Sequenced on HiSeq2000

BAC pool genotyping

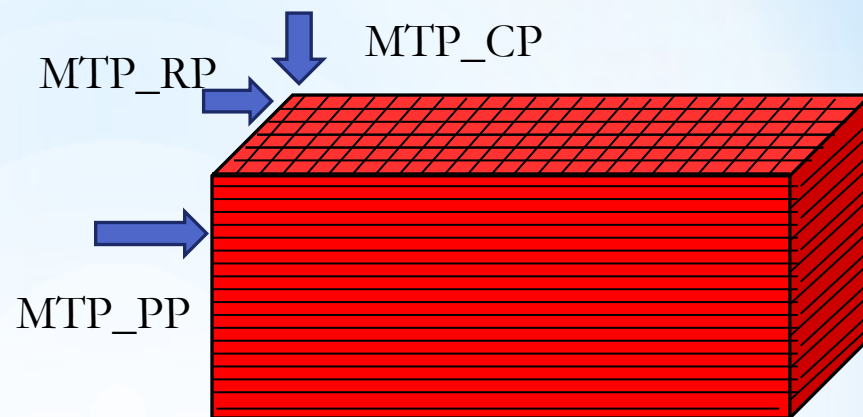
- 10K Infinium was designed based on polymorphism between AL8/78 and AS75 by collaborating group at UC Davis was used for BAC pool genotyping
- SNPs on 1D, 4D, 6D = 2,488
- In absence of target DNA in the pools there was random signal therefore, BAC pools were contaminated with 300 ng of AS75 DNA .



Deconvolution of BAC Pool Genotyping Data



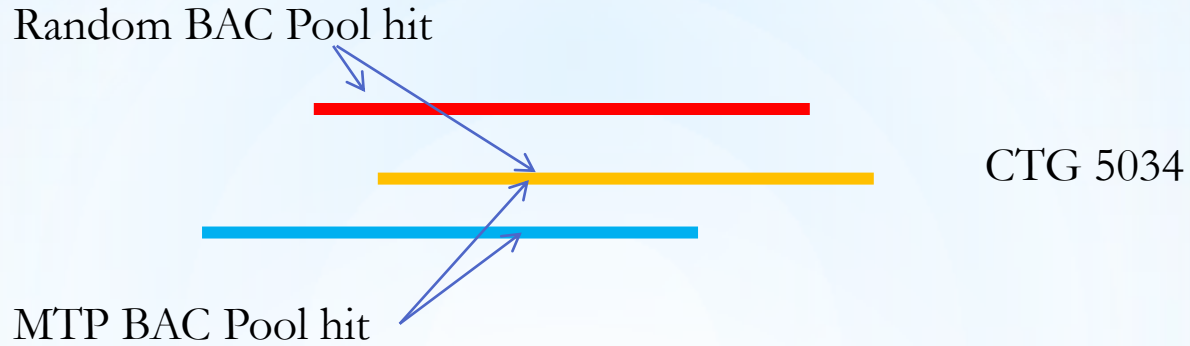
Random BACs 3-D Pools



MTP BACs 3-D Pools

SNP_ID	SNP_Name	Plate Pool hits	Row Pool hits	Column Pool hits	BAC address	Contig
AT6D5706	contig08978_497	RP_PP13	RP_RP26	RP_CP39	146DhB503J15	ctg585
AT6D5706	contig08978_497	RP_PP02	RP_RP38	RP_CP06	146DhC732F06	ctg585
AT6D5706	contig08978_497	RP_PP10	RP_RP33	RP_CP21	146DhC740A21	ctg585
AT6D5706	contig08978_497	RP_PP16	RP_RP35	RP_CP05	146DhC746C05	ctg585
AT6D5706	contig08978_497	RP_PP22	RP_RP47	RP_CP38	146DhC792O14	ctg585
AT6D5706	contig08978_497	MTP_PP82	MTP_RP14	MTP_CP11	146DhC866O20	ctg585

Deconvolution



SNP_ID	SNP_Name	Plate Pool hits	Row Pool hits	Column Pool hits	BAC address	Contig
BE403818	BE403818ATwsnp1	MTP_PP43	MTP_RP08	MTP_CP19	146DhB495N02	ctg3671
BE403818	BE403818ATwsnp1	MTP_PP43	MTP_RP08	MTP_CP05	146DhB495L21	ctg4764
BE403818	BE403818ATwsnp1	MTP_PP81	MTP_RP02	MTP_CP13	146DhC860O01	ctg4784
BE403818	BE403818ATwsnp1	MTP_PP81	MTP_RP08	MTP_CP13	146DhC861F24	ctg5034
BE403818	BE403818ATwsnp1	MTP_PP43	MTP_RP02	MTP_CP05	146DhB495F04	ctg5034
BE403818	BE403818ATwsnp1	RP_PP05	RP_RP22	RP_CP28	146DhB495F04	ctg5034
BE403818	BE403818ATwsnp1	RP_PP11	RP_RP21	RP_CP47	146DhB501E23	ctg5034

SNPs anchored = 1,981 using 1D, 4D, 6D BAC pools

BAC anchoring based on Coassembly

Contigs	No. of contigs	Total length (Mb)
After assembly	3153	4756
After editing	3578	4793
CS BACs in contigs	26,525	

1DAT1D0229_54										1DAT1D0225_54										1DAT1D0227_54										1DAT1D0226_54										1DAT1D0183_54																																																																																																																																																																																																																																																																					
1DAT1D0228_54										2DAT2D1674_107										7DAT7D7020_148										1DAT1D0208_54																																																																																																																																																																																																																																																																															
RI222N06	RI514J18	CS146DC823F0240HD370D22	HD004M24	RI470007	TCM049G03	RI316G13	HD331B18	CS146DC672I12	RI438G04*	TCM	HD430B08	HD439P11	MI235I10613C04I270G17*	CS3D010I11	MI253O13	HD328H17	HD282F1	CS146DC960B15	RI581D15*	RI514N12	HD6C	HD304E14*	HI306E07	HD385E16	RI321E11D243F18	CS146DB376M09	I519A23	CS146DC852N02	MI267D08	RI336I20	RI352H12	HD541I19	HD:	HD282P16	CS146DA049I23	HD258R1231L14HD547C09	CS146DA068D19J573G15*	RI481F07*	RI523P15*	RI276H20	HD260G10	CS146DC664M11	HD5	MI230L20	TCM012F21	CS146DC642H20I11HD298D18*	RI348M14HD055L11	CS146DB430P15	RI310N04	RI306M12	CS146DC635D19	HD276F14	CS146DB	RI462J12	TCM012F20	HI:CS146DB405G085I19	RI405E21	HI327I02	HD338B17	HD612F16*	RI411L16	CS146DB377F24	MI229P20	TET01	CS146DC884M12	RI567I03	TET010R1305H2HD336M23	HD439I20	RI260F11	TET006D04	CS146DB31480722I012*	RI386F17	HD219H18	RI459	HD238L15*	MI247P22	HD578P11D10G08H1201A09*	HD265I18	HI320P21	RI316N19*	RI471B14*	HD055K06	CS146DA002P06	HD419D14	MI228J1	HI261D0C	CS146DA016K11	RI536E13	RI49D07HD262C16*	RI240N15	HD355E19	RI554I15	CS146DB47CS146DB482P13MI287P06	RI227H02	MI227LC	HI291C13	HD402D05	RI330C1638H1099O09	RI595F	CS146DB494G14	HI336J05	HD421H18I635E21	MI241B18	RI467J17*	HD299E2:	RI513F20*	RI527H04	RI406H10D30M225D16	HD397M1RI514E07	CS146DB326J02	HD576M18381P12	RI516P21	HD395M13	MI275H1	HD344C	CS146DB421A06	RI506C095CS146DC962K20	HI228P11E028A14	TET025H22	RI407C2HD062A24	HD378A15	HD220P03	RI352F0:	HD241L05	HD465K02	CS146DC826I09770HD288F03	CS146DB559I2052D15	HI284F02	HD619B18297J23	HD236D14	CS146DA027J04	MI252C2C	CS146DB273B08588E08	CS146DB258I15)368I23	CS7DLO26TCM050K0998C08*	RI441KCS146DB500J18	RI372D13	HD447H18D459D15*	RI332J18	MI282D13	CS146DB346I	CS146DA018C0124ID08	CS146DB540F06H1D1638G12	CS146DA008M174D05	HD405J06	HD32CS146DB314D09	MI250F09	RI549P11	RI323K16	HI226H19	HI224A05	HD018I05	HD4(CS146DC915I02	HD245C	CS146DB519D21	HI285D21	HD004H22RI321K10	HI261M10	HD493F10	CS146DB258I	MI274B01HD370H11*	RI320E20HD295K16	HD209C16MI277P17480A02*	RI396F16	HD580N10	HD365C22	CS146DCCS146DB459H18	HD154H02	RI201B15	RI294H03	RI396L20	RI300N09	MI272B19	HI251C24410B16	HI233O22	HD224D20	HD250F14*	MI:CS146DC649M16	HD151D06	HD452F17	HD126O09	HI226H19	HI224A05	RI369P12	RI216C8D528O02	RI486M21	HI320L17	MI288D18	CS146DB302P10I16	RI560K17	CS146DB427E06	CS146DC790C2	HI241E08*	RI251L08	HI214P08	HI311K12637K12	RI568J06	RI377N23	HD555E16	RI4CS146DB444J19	RI541K17	HI340M20	HD4:CS146	RI282A14	RI636D01	RI261N11*	HD450I23615L18*	HD131F19	RI423O15	HD481N16	RI450O1265P02	TCM049O20	RI216C24	RI422K18	RI396L20	RI300N09	HI273N20*	RI467F0396D22*	RI633D19*	RI209F04*	HD297C17	RI525N0880B08	HI340H24	CS146DB244D12	HD506C2M1:	279O03*	HD331M16	HD437D24	HD534RI305M1CS146DB411P01	HD221O05	HD268K02	RI534E12267N24	HD418L07	CS146DB392I11	HD475N10MI2	320D19*	CS146DC840K08	HD396H05	HD251E11253P04	HD580F04	RI299I	CS146DA032M21	RI201P12007G16	TCM024J08	HD580E17*	CS146DB578L162	501J04*	HD514P06	RI273L19*	HD399E0M012E19	RI451H14	CS146DB233D06M045K14	HD545L18D596B13	HD060H13	RI522I20*	RI589O06	RI5	3DC824H03	HD463N19	CS146DB255N09	RI354D03362K19	HD261J21	HD152P07	RI490L12	MI306I18288K23	CS146DB541K05	HI233I21*	HI212C12	HD	6DB482M07	HD248A07*	HD481L17*	HD429B11211C10HI356O08	HD288O05	RI471B06*	HD440I1451G22*	HD210F20	RI477A05	HD494B09	HD2	D095M	CS146DC751G10	TCM045N05	CS146DA018J179A07HI278J23	RI348J09	HD232O02	RI229M1256L01*	HD367F23	HD490I10	RI536H13	HD2	3D015P0C	CS146DB235I1:CS146DB338J17	RI360D222CS146DB429B14	HD209H	CS146DC699F17	CS146DC644H22504J05*	HD353J08	HD363J21	HD442N10	CS146D

SNP markers anchored on BAC contigs

	Number of markers anchored
Total	2,392
Markers anchored based on 3-D pools	1,984
Markers anchored based on Coassembly with <i>Ae tauschii</i>	408
Chromosome 1D	869
Chromosome 4D	754
Chromosome 6D	685
Other chromosomes	49

FPC assembly statistics

Contigs	No. of contigs	Total length (Mb)	Average length (kb)	<0.2 Mb	0.2-0.5 Mb	0.5-1 Mb	1-2 Mb	2-5 Mb	>5 Mb
Present assembly	3749	1726	460	2146	604	490	393	113	2
Anchored	964	1047	1087	60	176	295	322	108	2
Unanchored	2785	679	265	2086	428	195	71	5	0

1603 contigs with >0.2 Mb is size of 904 contigs have been anchored making 60 % of the large contigs.

1603 contigs have a physical length of 1403 Mb of which 77 % (1,087Mb) is anchored

Manual editing underway

Editing with support of co-assembly is underway

Sequencing MTP BACs for Anchoring



Two border clones from each contigs was selected

7,064 BACs selected

Plate Pools= 19

Row Pools= 16

Colum pools=24

59 Barcoded libraries were prepared and 100 bp PE sequence was generated by running three lanes of HiSe2000

This generated 18, 21, and 16 Gbp of sequence data from three lanes and a total of 75x coverage.

Individual pools based assemblies were developed and preliminary screened for chromosome 1D 4D 6D sequences were done

Pool	Description	Assembled contigs (contamination cleaned)	Note
S95A	RP11(row pool #11)	19,348 (19,215)	Mixture of 456 (24x19) BACs
S95B	RP9 (row pool #9)	20,144 (19,987)	Mixture of 456 (24x19) BACs
S95C	RP7 (row pool #7)	20,185 (19,790)	Mixture of 456 (24x19) BACs
S95D	CP23 (column pool #23)	18,609 (13,916)	Mixture of 304 (16x19) BACs
S95E	PP3 (plate pool #3)	18,458 (16,667)	Mixture of 384 (24x16) BACs
S95F	PP14 (plate pool #14)	17,195 (17,021)	Mixture of 384 (24x16) BACs

Deconvolution and tracing of contigs individual BACs is underway

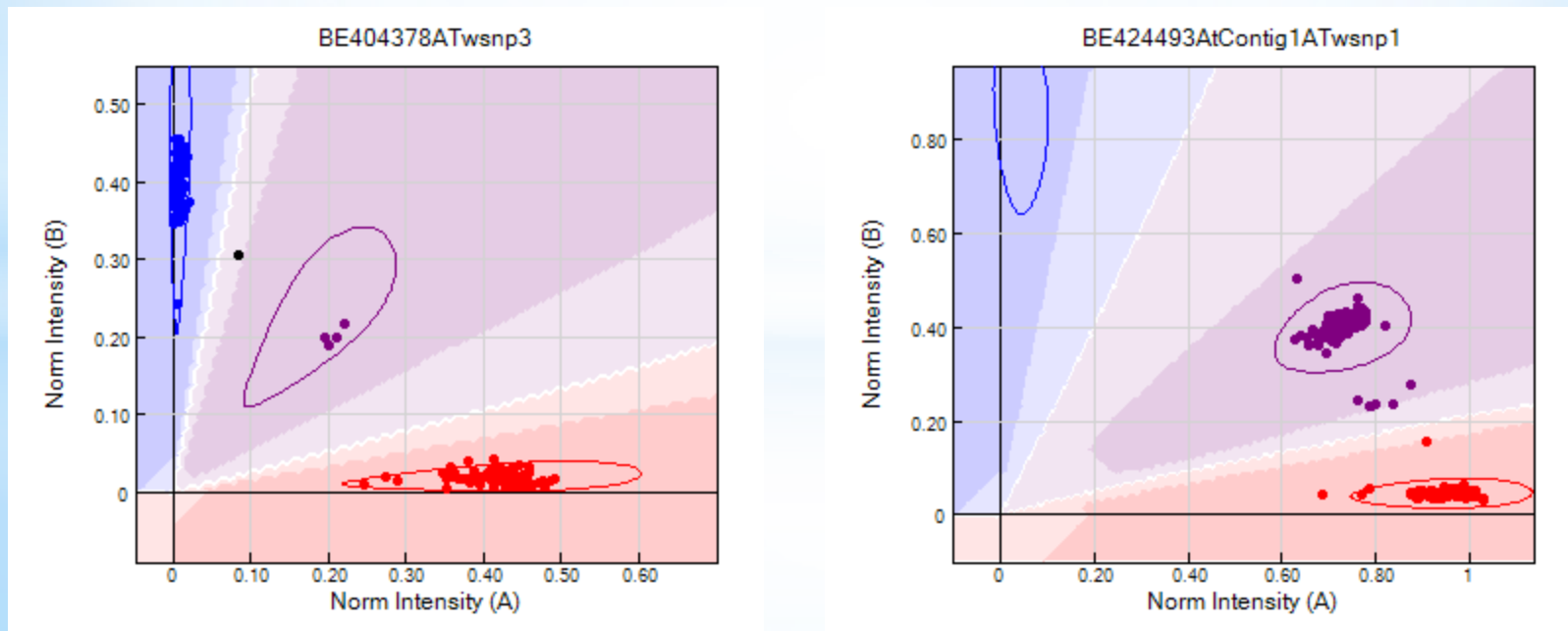
Genetic mapping

- ❖ A recombinant inbred line population of 311 F₆ individuals, derived by crossing the bread wheat cultivar Prelude (TA2988) with a synthetic wheat (TA8051), was used to develop an integrated genetic map of the D genome of bread wheat.
- ❖ 187 RILs were genotyped with Illumina10K Infinium HD array (8,609 SNPs) developed for *Ae. tauschii* physical mapping project.
- ❖ Same set of 187 RILs were sequenced (GBS) on 6 lanes of Illumina HiSeq2000

Infinium SNP genotyping

10K Infinium genotyping data was analyzed

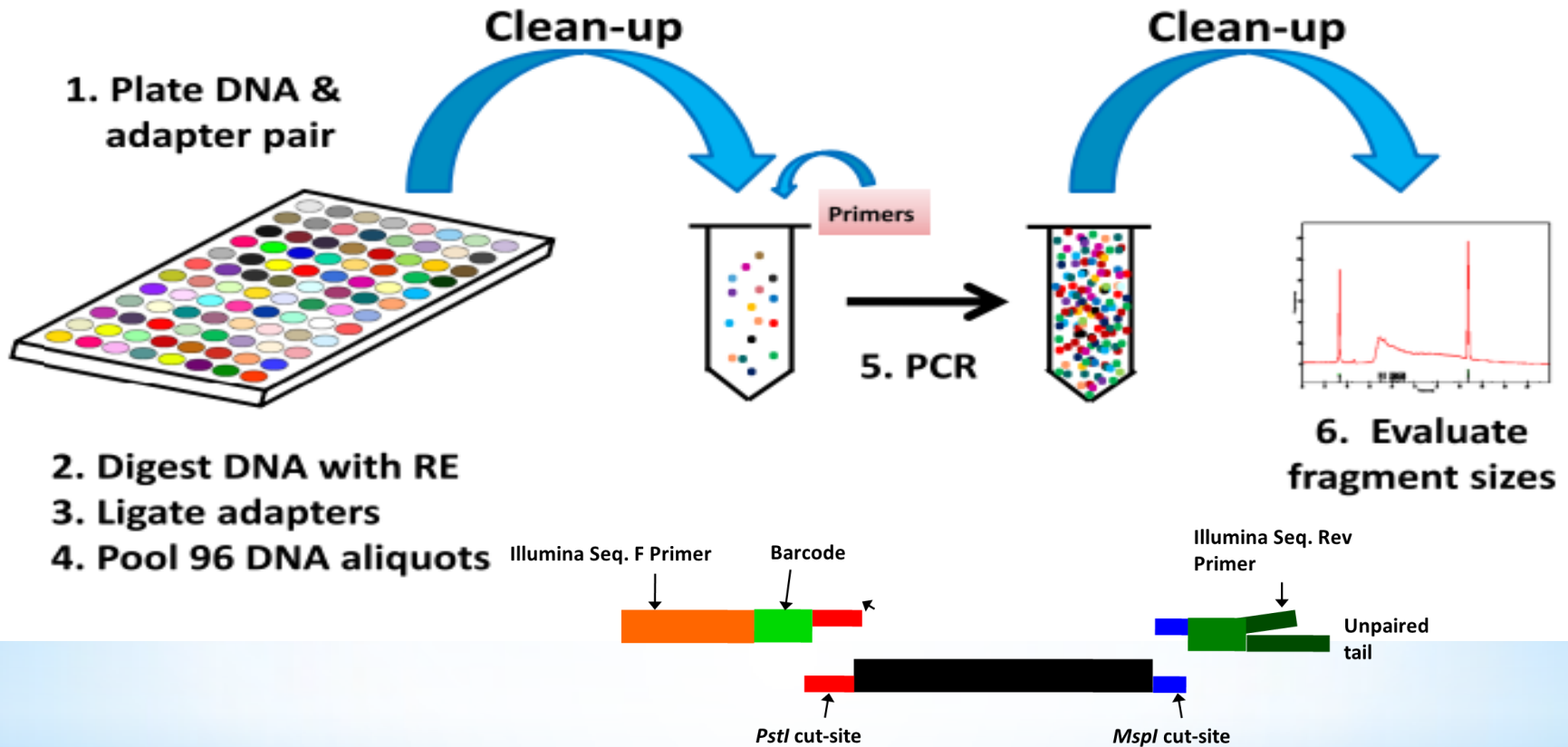
Data for 8,359 SNPs was obtained and 4,768 high quality polymorphic SNPs were identified and used for development of high density genetic map of D- genome wheat



Genetic Map of Wheat D Genome

Chromosome	No. of SNP markers (10K Infinium)	Total distance (cM)
1D	514	209.3
2D	889	288.5
3D	699	322.1
4D	610	200.6
5D	673	243.6
6D	507	202.4
7D	782	266.1
Total	4,674	1732.6

Genotyping By Sequencing



Plant ID	Barcode	Population
11-1-1	TGACGCCA	PreludeTA2988SyntheticTA8051F5P01
11-1-71	CAGATA	PreludeTA2988SyntheticTA8051F5P01
11-1-92	GAAGTG	PreludeTA2988SyntheticTA8051F5P01
11-1-144	TAGCGGAT	PreludeTA2988SyntheticTA8051F5P01
11-1-177	TATTCGCAT	PreludeTA2988SyntheticTA8051F5P01
11-1-216	ATAGAT	PreludeTA2988SyntheticTA8051F5P01
11-1-256	CCGAACA	PreludeTA2988SyntheticTA8051F5P01
11-1-281	GGAAGACAT	PreludeTA2988SyntheticTA8051F5P01
11-1-6	GGCTTA	PreludeTA2988SyntheticTA8051F5P01
11-1-74	AACGCACATT	PreludeTA2988SyntheticTA8051F5P01

6 lane HiSeq 2000

60 Gb of sequence data from
187 RILs and 2 parents

Genetic map of D genome of wheat

More than 30,000 SNPs identified

7,743 SNPs with less than 30% missing data

More than 19,000 mapped to seven D genome chromosomes

Chromosome	No. of SNP markers (Infinium)	No. of SNP markers (GBS)	Total distance (cM)
1D	514	2,104	205
2D	889	2,103	288.5
3D	699	3,781	322.1
4D	610	2,205	200.6
5D	673	3,460	243.6
6D	507	2,768	202.4
7D	782	3,149	266.1
Total	4,674	19,570	1732.6

1D

Integrated Genetic and Physical Map

6D

AT1D0008	0 cM	ctg3191
AT1D0004	0.8 cM	ctg3191
AT1D0009	0.8 cM	ctg3191
AT1D0001	1.1 cM	ctg3191
AT1D0002	1.1 cM	ctg3191
AT1D0003	1.1 cM	ctg3191
AT1D0005	1.1 cM	ctg3191
AT1D0006	1.1 cM	ctg3191
AT1D0007	1.1 cM	ctg3191
AT1D0016	4.7 cM	ctg4040
AT1D0020	4.7 cM	ctg3703
GDEEGVY0	4.7 cM	
AT1D0022	5.4 cM	ctg150
AT1D0024	5.8 cM	ctg150
AT1D0025	6.3 cM	ctg4
AT1D0026	6.3 cM	ctg4
AT1D0031	10.4 cM	ctg50
AT1D0034	12.9 cM	
AT1D0038	16.7 cM	ctg1768
AT1D0036	17.2 cM	ctg1768
AT1D0041	19.6 cM	ctg14106
AT1D0044	24.8 cM	ctg14106
AT1D0048	30.8 cM	ctg3279
AT1D0052	32.3 cM	ctg1544
AT1D0053	32.9 cM	
AT1D0061	35 cM	
AT1D0057	35.6 cM	ctg1704
AT1D0062	37.5 cM	ctg1589
AT1D0066	38.1 cM	ctg241
AT1D0071	42.2 cM	
contig69241	44.9 cM	
AT1D0082	46.4 cM	ctg1699
AT1D0077	47.7 cM	ctg1699
AT1D0076	49.8 cM	ctg14071
AT1D0078	50.6 cM	ctg1699
AT1D0081	50.6 cM	ctg1699
AT1D0084	51.4 cM	ctg14135
AT1D0086	51.4 cM	ctg14135
AT1D0088	51.7 cM	ctg140
AT1D0091	51.7 cM	ctg140
AT1D0090	52.3 cM	ctg13569
AT1D0095	53.7 cM	ctg1403
GB5Y7FA02	53.9 cM	
AT1D0098	54.2 cM	ctg309
AT1D0102	54.7 cM	
AT1D0105	55.3 cM	ctg13988
AT1D0116	57 cM	ctg159
AT1D0123	59.7 cM	ctg1695
AT1D0122	60 cM	ctg1695
AT1D0100	67.4 cM	ctg309
AT1D0099	68.2 cM	ctg309
AT1D0103	68.2 cM	ctg309

	Number of markers physical map	Number of marker on Genetic map
Total	2,392	4,674
Chromosome 1D	869	514
Chromosome 4D	754	610
Chromosome 6D	685	507

AT6D5445	144.5 cM	ctg14081
AT6D5446	144.5 cM	ctg14081
AT6D5447	144.5 cM	ctg14081
AT6D5442	145.5 cM	ctg14081
AT6D5441	146.4 cM	ctg332
AT6D5439	147.5 cM	ctg14150
AT6D5436	148.9 cM	ctg797
AT6D5435	149.2 cM	ctg797
bg262421Cc	149.2 cM	
AT6D5429	149.7 cM	
AT6D5431	149.7 cM	ctg1658
AT6D5424	150 cM	ctg380
AT6D5426	150 cM	ctg380
AT6D5416	150.6 cM	ctg809
AT6D5417	150.6 cM	ctg809
bg495635Cc	150.6 cM	
AT6D5411	150.8 cM	ctg462
AT6D5412	150.8 cM	ctg14
ca643341Cc	150.8 cM	
AT6D5432	154.7 cM	ctg1658
AT6D5433	154.7 cM	ctg1658
AT6D5427	155.3 cM	ctg380
bf483025Col	155.3 cM	
bg604419Cc	155.3 cM	
AT6D5423	155.8 cM	ctg380
AT6D5419	156.1 cM	ctg177
AT6D5420	156.1 cM	ctg177
AT6D5422	156.1 cM	ctg72
AT6D5418	156.3 cM	ctg809
AT6D5413	156.6 cM	ctg809
AT6D5404	158.3 cM	ctg405
AT6D5792	158.6 cM	ctg123
contig30284	159.4 cM	
AT6D5362	161.7 cM	ctg2106
AT6D5371	162 cM	ctg826
AT6D5380	162.8 cM	ctg14043
AT6D5387	162.8 cM	ctg295
be424523Cc	162.8 cM	
AT6D5396	163.1 cM	ctg696
AT6D5393	163.1 cM	ctg1669
contig33076	163.1 cM	
AT6D5401	163.9 cM	ctg1669
GA8KES402	166.3 cM	
AT6D5395	167 cM	ctg300
AT6D5402	167 cM	ctg3293
AT6D5403	167 cM	ctg728
AT6D5391	168.1 cM	ctg1669
AT6D5392	168.1 cM	ctg183
AT6D5394	168.1 cM	ctg474
AT6D5372	168.4 cM	
AT6D5374	168.4 cM	ctg14043
AT6D5376	168.4 cM	ctg14043

61 % of FPC assembly anchored

Deconvolute pooled BAC sequence contigs to individual BACs

Finalize the integrated GBS and 10K Infinium map

Integration of CS data with *Ae. tauschii* data

Final integration of the Physical and Genetic map

Genomic resources

❖ Chromosome 3A: ~827 Mb

❖ 3AS: ~355 Mb,

❖ 3AL: ~472 Mb

Library code	Chromosome Arm	Number of clones	Mean insert size	Coverage
TaaCsp3AShA	3AS	55,296	80 kb	11.2 x
TaaCsp3AShB	3AS	55,296	115 kb	16.1 x
	3AS	110,592		27.7x
TaaCsp3ALhA	3AL	55,296	106 kb	11.4 x
TaaCsp3ALhB	3AL	24,576	114 kb	5.4 x
	3AL	79,872		16.8x

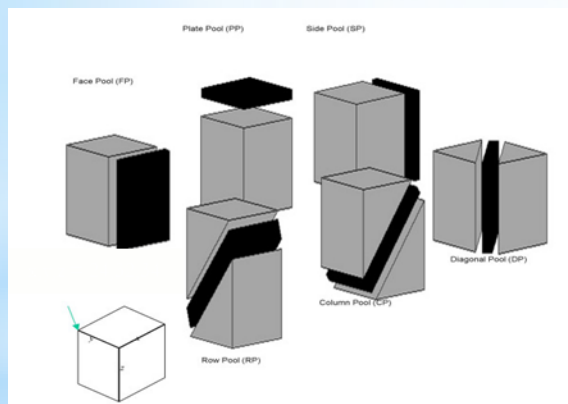
Chromosome 3A FPC assembly

Library	Clones (coverage)	# of clones in contigs	Singletons	Total # of contigs (contigs >500kb)	Longest contig (clones)	Assembly size (% arm coverage with contigs >2clones)
3AS (Phase-I)	55,296 (11.2x)	35,124	11,939	1,677 (199) 1e-15	2.7 kb (417)	327Mb (91 %)
3AS (Phase-II)	65,677 (20.2x)	45,739	11,938	1325 (288) 1e-30	4.99 kb (650)	340Mb (95%)
3AL (Phase-I)	79,872 (16.8x)	51,405	12,064	1417 (273) 1e-25	4.46 (594)	391 Mb (87%)

Chromosome arm	3AS	3AL
FPC assembly	16.8x arm coverage	13.6x arm coverage
Contig assembly	15x arm coverage	12.2x arm coverage
MTP	5,397 clones	5,754 clones
Pools	Six-dimensional random pools Three-dimensional of MTP BAC pools	Three-dimensional of MTP BAC pools

Marker discovery and BAC anchoring

Markers	Identified	Primers designed	Primers tested	Primers amplified (CS)	# of unique contigs anchored
SSRs	1,057	758 180 (class I)	180	156 (88%)	201
ISBPs	2,736	695	96	92 (95%)	172
Genic	519	240	96	84 (85%)	126



6-dimensional pools

Marker	# of primers designed	# of primers amplified (3AS super pool)	# of markers anchored to BAC
NSF-ESTs	198	135	135
ESTs (Barley/Rice/Brachypodium)	1,170	460	366
Total	1,368	595	501

Genetic Mapping

T. monococcum subsp. *aegilopoides* /

T. monococcum subsp. *monococcum*

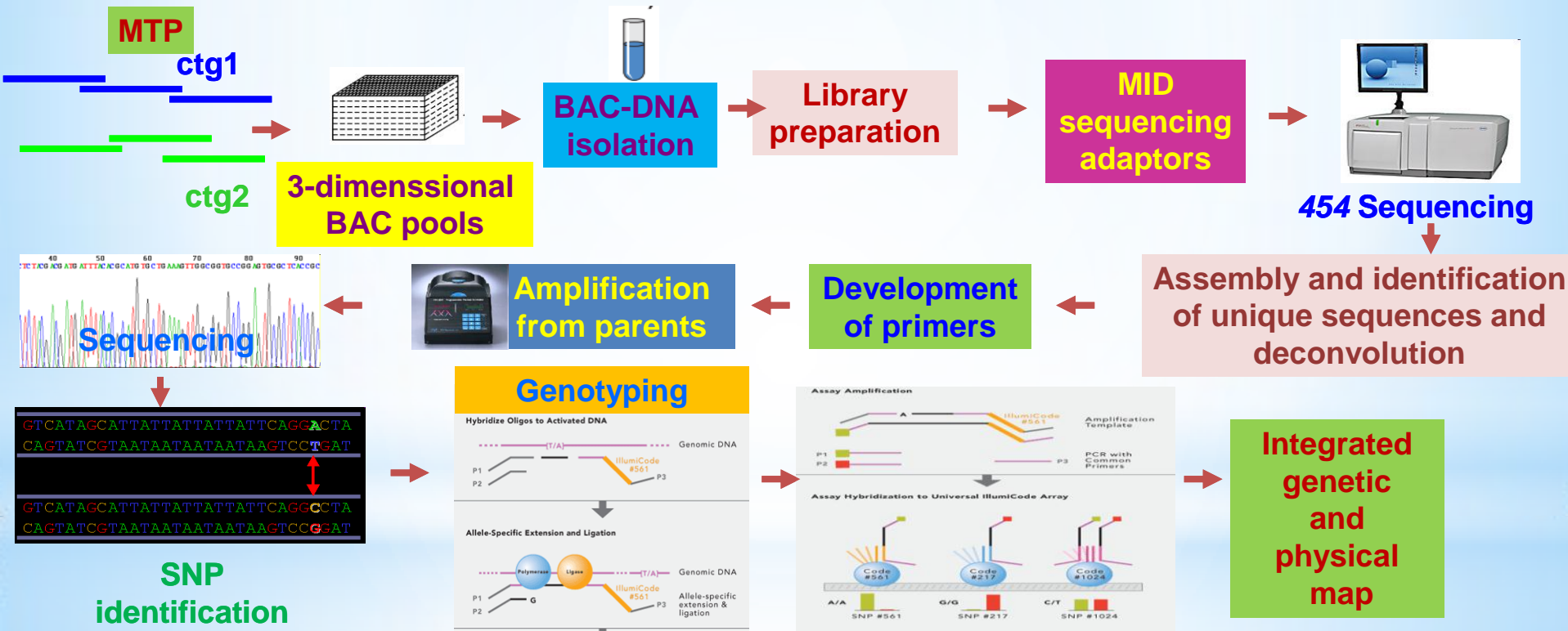
1,200 F₆ RILs

T. monococcum (>100x sequence generated by collaborators at CSHL, PE, MP 2kb 5kb)

T. aegilopoides (~30x sequence being generated at KSU, PE, MP 2 kb, 5 kb, 10 kb)

Marker discovery

2,743 BAC clones (2-6 per contig) from 3A MTP



Marker discovery

The sequences was assembled into 194,000 contigs (228 Mb) and deconvoluted and analyzed for unique sequences.

Nearly, 55% of the 194,000 contigs and over 70% of the gene containing contigs were traced back to individual BAC clones.

From the parents of the diploid mapping population, 2,908 primers (from genic and low-copy sequences) were designed and amplified.

Of these, ~2,100 primers amplifying one unique band were pooled and sequenced on 454 for SNP identification.

We have identified 2,262 SNPs with minimum base consensus ration 0.9 and read depth of 5.

The SNPs were traced back to 750 genes

To further increase the marker coverage we identified *T. monococcum* contigs (20,646) repeated masked shotgun sequence of chromosome 3A and 3B unigenes.

Marker discovery

We sequence the second parent *T. monococcum* subsp. *aegilopoides* depth of 12x

T. monococcum subsp. *aegilopoides* was aligned to *T. monococcum* subsp. *monococcum* contigs and SNPs discovery

Number of SNPs identified	42,021
Number SNPs with minimum 5 reads , 0.9 base consensus ratio and reference length of >150bp	26,211
Number SNPs with no neighboring SNP in 50bp and within or <200bp flanking the gene	1,710
Number of genes covered	1,182

Illumina GoldenGate design is underway for developing high density integrated and genetic map

GBS based SNP map

376 RILs from the mapping population were genotyped by GBS *T. monococcum* subsp. *aegilopoides* / *T. monococcum* subsp. *monococcum*

6 lanes on HiSeq2000 (4more lanes HiSeq 2000 are underway)

61 Gb of sequence data generated

Initial analysis showed 21,454 SNPs with 2,698 SNPs with less than 30% missing data

GBS based map is underway and would be used to integrated 2,743 BACs sequenced (108,000 contigs) from the MTP of chromosome 3A

D-Genome



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Chromosome 3A



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