# An Integrated Map of Arabidopsis thaliana for Functional Analysis of Its Genome Sequence

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#### ABSTRACT

The genome of the model plant species *Arabidopsis thaliana* has recently been sequenced. To accelerate its current genome research, we developed a whole-genome, BAC/BIBAC-based, integrated physical, genetic, and sequence map of the *A. thaliana* ecotype Columbia. This new map was constructed from the clones of a new plant-transformation-competent BIBAC library and is integrated with the existing sequence map. The clones were restriction fingerprinted by DNA sequencing gel-based electrophoresis, assembled into contigs, and anchored to an existing genetic map. The map consists of 194 BAC/BIBAC contigs, spanning 126 Mb of the 130-Mb Arabidopsis genome. A total of 120 contigs, spanning 114 Mb, were anchored to the chromosomes of Arabidopsis. Accuracy of the integrated map was verified using the existing physical and sequence maps and numerous DNA markers. Integration of the new map with the sequence map has enabled gap closure of the sequence map and will facilitate functional analysis of the genome sequence. The method used here has been demonstrated to be sufficient for whole-genome physical mapping from large-insert random bacterial clones and thus is applicable to rapid development of whole-genome physical maps for other species.

RABIDOPSIS thaliana is a model system for geno- $\Pi$  mic studies of plant species (MEINKE *et al.* 1998). To facilitate genome research of the species, chromosome- and genome-wide physical maps were developed from large-insert yeast artificial chromosome or bacterial artificial chromosome (BAC) libraries (HwANG et al. 1991; SCHMIDT et al. 1995; ZACHGO et al. 1996; CANIL-LERI et al. 1998; MARRA et al. 1999; MOZO et al. 1999). Using these maps as frameworks, the genome of A. thaliana was sequenced (ARABIDOPSIS GENOME INITIATIVE 2000). However, a number of gaps still exist in the sequence map, especially in the heterochromatic regions surrounding the centromeres of its chromosomes. These gaps are intractable to closure by the conventional chromosome walking approach using the ends of BACs adjacent to the gaps as probes because of repetitive sequences. This piecemeal approach is also time-consuming. Therefore, to rapidly close most, if not all, of the gaps in the sequence map, it is necessary to construct a whole-genome physical map from a new DNA library that complements the Texas A&M University (TAMU) and the Institut für Genbiologische Forschung (IGF) BAC libraries of the sequence map and to integrate the new map with the existing sequence map.

Sequence analysis has indicated that the genome of A. thaliana contains  $\sim$ 25,498 genes. However, the functions of >90% of the predicted genes remain to be characterized experimentally (ARABIDOPSIS GENOME INITIATIVE 2000). Experimental determination of the functions of these genes and related sequences has been targeted as a goal for the coming decade (SOMERVILLE and DANGL 2000). To this end, several methods have been developed, including T-DNA-based (AZPIROZ-LEE-HAN and FELDMANN 1997; SUSSMAN et al. 2000) or transposon-based (MARTIENSSEN 1998) gene tagging, DNA microarray or gene chip analysis (SCHENA et al. 1995; DESPREZ et al. 1998; RUAN et al. 1998), and genetic transformation (Feldmann and Marks 1987; Chang et al. 1994; LIU et al. 1999). Because transformation of A. thaliana via Agrobacterium is efficient and can be accomplished without tissue culture procedures (FELD-MANN and MARKS 1987; KONCZ et al. 1989; BECHTOLD et al. 1993), this method facilitates functional analysis of the genome sequence. Therefore, a whole-genome, binary, clone-based map that is integrated with the existing sequence map will be significant for accelerated experimental determination of the functions of every segment of the genome sequence. However, none of the Arabidopsis physical and sequence maps developed to date contains clones that can be directly transformed in plants. The TAMU and IGF BAC clones of the existing physical and sequence maps were cloned in general DNA cloning BAC vectors (CHOI et al. 1995; Mozo et al. 1998), which are incompetent for direct transforma-

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tion in plants via A. tumefaciens. For functional analysis of the genome sequence by genetic transformation, these clones must be subcloned into a plant-transformationcompetent binary vector. However, the process of subcloning is often tedious. Furthermore, the goals of Arabidopsis genome research are to identify every gene and determine the function(s) of every gene of this model species. Although individual clones for functional analysis by genetic transformation could be isolated from a plant-transformation-competent binary bacterial artificial chromosome (BIBAC) library using the corresponding sequences as probes, this process would be inefficient for isolation of a large number of BIBAC clones for functional analysis of different segments of the genome sequence. Therefore, it is desirable to develop a physical map from a large-insert BIBAC library that is competent for plant transformation and to integrate it with the existing sequence map.

In this study, we developed a whole-genome integrated physical and genetic map of the A. thaliana ecotype Columbia from a new plant-transformation-competent BIBAC library and integrated it with the existing sequence map of the species. The integration of the new map with the sequence map will significantly accelerate genome research of the model species in many aspects. The complementarity of the new BIBAC library to the source libraries of the sequence map and the competency of the BIBACs for plant transformation will facilitate gap closure of the sequence map and large-scale functional analysis of the genome sequence. Furthermore, because the new map was constructed using a DNA sequencing gel-based fingerprinting method (TAO et al. 2001) that differs from those used for construction of the existing physical maps of Arabidopsis (MARRA et al. 1999; Mozo et al. 1999), it may provide a tool to further verify the existing physical and sequence maps in which errors have been recently reported (STUPAR et al. 2001). This study has also demonstrated that the DNA sequencing gel-based fingerprinting method is powerful for rapid development of whole-genome physical maps from large-insert random bacterial clones.

#### MATERIALS AND METHODS

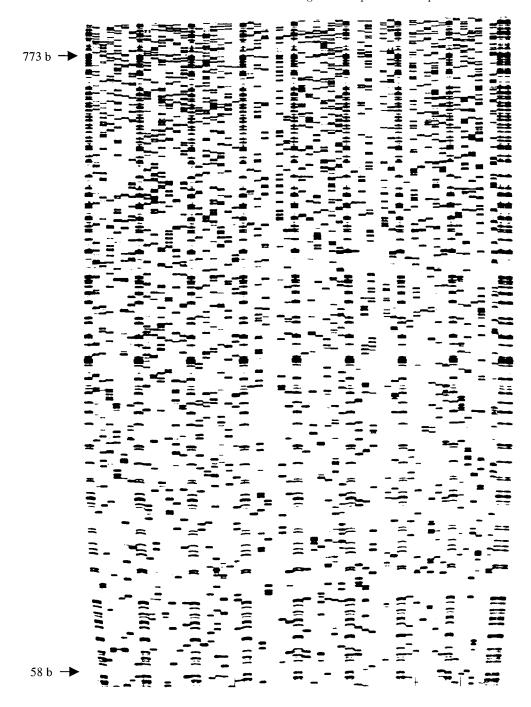
**BAC and BIBAC libraries:** A new plant-transformation-competent binary library (the clones are hereafter referred as to BIBACs; Y.-L. CHANG, K. MEKSEM, H.-W. CHUANG, C. SCHEUR-ING and H.-B. ZHANG, unpublished data) and the TAMU (CHOI *et al.* 1995) and IGF (Mozo *et al.* 1998) BAC libraries of the *A. thaliana* ecotype Columbia were used to develop the integrated physical map. The average insert sizes of the BIBAC library and the TAMU and IGF BAC libraries are 110, 100, and 100 kb, respectively. These BAC and BIBAC libraries are publicly available at the GENE*finder* Genomic Resources (formerly the Texas A&M BAC Center; http://hbz.tamu.edu).

The TAMU and IGF BAC libraries are the source libraries of the existing physical and sequence maps of *A. thaliana* (MARRA *et al.* 1999; MOZO *et al.* 1999; ARABIDOPSIS GENOME INITIATIVE 2000). The BIBAC library was cloned in the bacterial P1-based binary vector pCLD04541, which was designed

for Agrobacterium-mediated transformation in plants (JONES *et al.* 1992; TAO and ZHANG 1998). The ability of BIBACs to transform plants via Agrobacterium (JONES *et al.* 1992; BECH-TOLD *et al.* 1993; BENT *et al.* 1994; WU *et al.* 2000; Y.-L. CHANG, K. MEKSEM, H.-W. CHUANG, C. SCHEURING and H.-B. ZHANG, unpublished data) will facilitate functional analysis of the genome sequence by genetic transformation. Furthermore, the BIBAC library was constructed with a restriction enzyme (*Bam*HI) differing from those used for the TAMU (*Hind*III; CHOI *et al.* 1995) and IGF (*Eco*RI; MOZO *et al.* 1998) BAC libraries. The *Bam*HI sites are G/C-rich, whereas the *Hind*III and *Eco*RI sites are A/T rich. These differences were expected to allow cloning of the regions that are not represented in the existing BAC libraries and thus to close the gaps in the existing Arabidopsis sequence map.

Fingerprinting and contig assembly: BAC and BIBAC DNA were isolated and fingerprinted according to TAO et al. (2001) with modifications in which [33P]dATP was used to label the digested BAC DNA fragments and 3.5% (w/v) denaturing DNA sequencing gels were used to fractionate the DNA fragments. The fingerprints were scanned into image files using a UMAX Mirage D-16L scanner and edited using Image 4.0 of the FingerPrinted Contig (FPC) package (SODERLUND et al. 1997). The resolvable bands of each fingerprint ranged from 58 to 2225 nucleotides in size. Considering the lower resolution (probably >1 nucleotide) of the bands in the higher-molecular-weight portion at the top of each autoradiograph, only the fragments ranging from 58 to 773 bases were used for contig assembly, on average, 36 bands per fingerprint. The bands derived from the BAC vectors were deleted from the data files, while the bands of the BIBAC vector pCLD04541 were not present in the fingerprint range (e.g., see Figure 1). The clones that had no inserts or produced four or fewer bands were excluded during fingerprint image editing because the number of bands per fingerprint was insufficient to be included for contig assembly. Consequently, 9389 of the clones, being equivalent to  $7.2 \times$  Arabidopsis haploid genomes, were used to assemble the physical map contigs. To construct the physical map, we first assembled automatic contigs using the FPC3.8 program (SODERLUND et al. 1997) and then merged the automatic contigs or singletons with the automatic contigs using a lower comparison stringency. The automatic contigs were assembled at tolerance 2, cutoff  $10^{-12}$ , DiffBury 0.1, and MinBands 8. The mergence of contig-contig and singleton-contig was conducted at tolerance 2, cutoff  $10^{-7}$ DiffBury 0.1, and MinBands 8.

Library screening: The source clones of the physical map were double spotted on Hybond N+ membrane in a format of  $3 \times 3$  by using the Biomek 2000 Robotic Workstation (Beckman, Fullerton, CA), and the high-density colony filters were prepared according to ZHANG et al. (1996). To determine the genome origin of the BAC/BIBAC contigs, three chloroplast DNA probes, *adhA*, *psbA*, and *rbcL*, which are  $\sim$ 50 kb apart on the chloroplast genome, were used to screen the source BAC and BIBAC libraries, and the IGF BACs derived from mitochondrial DNA were used to search the database of the new physical map. To verify the accuracy of the contigs and anchor them to Arabidopsis genetic maps, 77 restriction fragment length polymorphism (RFLP) markers were selected from the Arabidopsis genetic map (LIU et al. 1996; see Table 2), obtained from the Arabidopsis Biological Resource Center (Ohio State University), and used as probes to hybridize the high-density BAC and BIBAC filters. The probes were prepared using the PCR Dig-Probe synthesis kit as described by its manufacturer (Roche Molecular Biochemicals, Indianapolis). In addition, nine Arabidopsis cDNAs were selected from the Arabidopsis expressed sequence tagged (EST) set obtained from the Arabidopsis Biological Resource Center (Ohio State University) and positioned to the integrated map by colony hybridization.



## RESULTS AND DISCUSSION

**Fingerprinting the BAC and BIBAC clones:** To construct the whole-genome physical map that is integrated with the existing sequence map (ARABIDOPSIS GENOME INITIATIVE 2000), we selected 1536 IGF BACs (4 384-well plates), 3072 TAMU BACs (8 384-well plates), and 6144 BIBACs (16 384-well plates) from their libraries. The 10,752 BAC and BIBAC clones were fingerprinted on 224 autoradiographs using the DNA sequencing gelbased restriction fingerprinting method (TAO *et al.* 2001). Figure 1 shows an autoradiograph of the BAC and BIBAC fingerprints.

Assembling the BAC/BIBAC map: We scanned the

FIGURE 1.—Autoradiograph of the Arabidopsis BIBAC fingerprints used for contig assembly of the new BAC/BI-BAC-based map. DNA was isolated, double digested with HindIII/HaeIII, end labeled with [33P]dATP, electrophoresed on a 3.5% (w/v) denaturing DNA sequencing gel, and exposed to an X-ray film. λDNA/Sau 3AI markers labeled with [33P]dATP were used in the first lane and every seventh lane thereafter. Note that the bands derived from the cloning vector of the new BIBAC library, pCLD04541 (JONES et al. 1992; TAO and ZHANG 1998), were not present in the fingerprint range, which facilitated the map contig assembly. However, one or two bands derived from the cloning vectors of TAMU and IGF BACs were present in the range and were deleted manually from the fingerprint image files. The fragments ranging from 58 to 773 bases were used for contig assembly.

clone fingerprints into image files and edited with the Image program of the FPC package (SULSTON *et al.* 1988; SODERLUND *et al.* 1997). During editing (see MATERIALS AND METHODS), 1363 clones were deleted from the data files because they had no inserts or produced four or fewer bands in their fingerprints, which were insufficient to be included in contig assembly. As a result, the data from 9389 clones with an average of 36 bands per clone were used to assemble contigs with the FPC program (SODERLUND *et al.* 1997). Of the 9389 clones (7.2 × genome equivalents), the clones equivalent to 2.1 × haploid genomes were from the TAMU BAC library, the clones equivalent to  $1.0 \times$  haploid genomes

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# TABLE 1

BAC/BIBAC contigs of A. thaliana

Contig	Clone	kb	Contig	Clone	kb	Contig	Clone	kb	Contig	Clone	kb
ctg1	161	162	ctg51	5	177	ctg1025	63	990	ctg4006	10	159
ctg2	108	564	ctg52	5	120	ctg1026	148	2,220	ctg4007	20	324
ctg3	87	939	ctg53	5	48	ctg1027	119	1,665	ctg4008	3	111
ctg4	32	318	ctg54	5	96	ctg2001	91	1,371	ctg4009	45	747
ctg5	27	369	ctg55	5	171	ctg2002	39	579	ctg4010	12	174
ctg6	24	270	ctg56	5	159	ctg2003	42	429	ctg4011	230	3,255
ctg7	22	201	ctg57	4	69	ctg2004	37	441	ctg4012	31	522
ctg8	22	219	ctg58	4	99	ctg2005	89	1,029	ctg4013	37	555
ctg9	22	252	ctg59	4	135	ctg2006	13	237	ctg4014	148	2,202
ctg10	20	297	ctg60	4	99	ctg2007	55	747	ctg4015	16	309
ctg11	19	390	ctg61	4	75	ctg2008	29	357	ctg4016	150	2,325
ctg12	16	219	ctg62	4	150	ctg2009	12	246	ctg4017	128	1,764
ctg13	14	186	ctg63	3	132	ctg2010	200	2,292	ctg5001	37	597
ctg14	13	252	ctg64	3	111	ctg2011	45	585	ctg5002	181	2,412
ctg15	13	240	ctg65	3	120	ctg2012	72	924	ctg5003	15	297
ctg16	13	177	ctg66	3	57	ctg2013	26	321	ctg5004	41	654
ctg17	13	99	ctg67	3	90	ctg2014	51	639	ctg5005	99	1,452
ctg18	12	171	ctg68	3	108	ctg2015	66	852	ctg5006	16	315
ctg19	12	141	ctg69	2	114	ctg2016	13	180	ctg5007	48	828
ctg20	12	222	ctg70	2	126	ctg2017	21	348	ctg5008	40	630
ctg21	11	81	ctg71	2	84	ctg2018	63	972	ctg5009	62	582
ctg22	11	243	ctg72	2	93	ctg2019	97	1,086	ctg5010	74	1,056
ctg23	10	108	ctg73	2	75	ctg2020	294	4,581	ctg5011	32	402
ctg24	10	90	ctg74	2	102	ctg3001	31	714	ctg5012	9	225
ctg25	9	96	ctg75	2	84	ctg3002	55	954	ctg5012	15	303
ctg26	9	138	ctg76	2	132	ctg3003	25	528	ctg5014	25	498
ctg27	9	108	ctg1001	205	3,009	ctg3004	395	4,899	ctg5015	27	381
ctg28	9	150	ctg1001	99	1,338	ctg3005	333 7	129	ctg5016	31	459
ctg29	8	51	ctg1002	123	1,800	ctg3006	, 155	2,112	ctg5017	85	951
ctg30	8	105	ctg1003	29	360	ctg3007	97	1,626	ctg5017	67	711
ctg31	8	141	ctg1001 ctg1005	50	500 774	ctg3008	49	642	ctg5019	45	657
ctg32	8	192	ctg1005	66	675	ctg3009	11	294	ctg5020	24	327
ctg33	7	57	ctg1000	148	2,166	ctg3010	77	810	ctg5020	9	201
ctg34	7	156	ctg1007	10	159	ctg3010	39	345	ctg5021	8	183
ctg35	7	72	ctg1000	48	573	ctg3012	112	1,308	ctg5022	63	870
ctg36	7	81	ctg1005	111	1,668	ctg3012	24	354	ctg5024	8	159
ctg37	7	105	ctg1010	75	990	ctg3014	52	651	ctg5025	157	2,157
ctg38	6	75	ctg1011	55	882	ctg3014	97	1,152	ctg5025	59	900
ctg39	6	135	ctg1012	55 71	864	ctg3015	25	342	ctg5027	132	1,977
	6	156	ctg1013 ctg1014	114	1,335	ctg3017	33	510	ctg5027	72	1,131
ctg40	-		0			~			~		612
ctg41 ctg42	6     6	81 81	ctg1015 ctg1016	11 91	$144 \\ 1,071$	ctg3018 ctg3019	$\frac{79}{140}$	$1,164 \\ 2,118$	ctg5029 ctg5030	$\frac{39}{104}$	1,362
	6	75	ctg1010 ctg1017	18	234	ctg3019	88	1,086	ctg5030	29	483
ctg43 ctg44	6	129	ctg1017	116	1,668	ctg3020	9	213	ctg5031	29 44	465 642
		93	ctg1018	30	375		26	492	ctg5032	16	300
ctg45 ctg46	6 6	93 93	ctg1019	30 130	1,749	ctg3022 ctg4001	20 73	492 1,116	ctg5035 ctg5034	10 22	300 489
ctg46		93 75		130 25	1,749 414	ctg4001	73 80	1,116 942	C1g5054	44	409
ctg47	6		ctg1021			ctg4002		942 570			
ctg48	5	120	ctg1022	212	3,243	ctg4003	38				
ctg49	5	126	ctg1023	10	237	ctg4004	29	507			
ctg50	5	63	ctg1024	84	909	ctg4005	113	1,401			

Contig 1 (ctg1) was derived from chloroplast DNA, contig 2 (ctg2) from the mitochondrial DNA, and the remaining 194 contigs from nuclear DNA. Of the 194 nuclear DNA clone contigs, ctg3 through ctg76 had not been anchored to chromosomes and the 120 remaining contigs were anchored to the chromosomes of their origin. The anchored contigs are numbered using four-digit numbers, with the first one identifying the chromosome to which the contig was anchored and the last two specifying the contig.



FIGURE 2.—Comparison of a BAC/BIBAC contig (ctg1026) of the integrated map developed in this study (A) *w*. the corresponding BAC contig of the AGI sequence map (B; ARABIDOPSIS GENOME INTIATIVE 2000) anchored to chromosome 1. The clones highlighted in green are the positive clones of the DNA marker mi425. The IGF and TAMU BAC clones shared by these two contigs are highlighted in blue. Note that the content and order of the clones in the two contigs are identical, indicating that the contigs assembled from the fingerprints generated by the DNA sequencing gel-based restriction fingerprinting method are accurate.

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B07124H	F01D14 B0	P16" F04		TO1C07H B09116 B14F07" B04L0		B04C10" B11K13" P1C01" B16022"
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BO7H01 B04P06" B04P06" B13D02 B14C21H T0	I) <u>Clear All</u> 1, runder of eleve мi279 Во9Е24ж <u>Во9Е24ж</u> <u>Во2Сонж</u> 81301 Во2Сонж 8137 Во2Сонж	Merge         Analysis           220, sequenced         6           Bi6L03"         F02           Bi6E188         F02           Bi1001-         F02	2006" B05B05 B11823" B08D06" T06H16+	ne: #i112 #i330 To2 <u>B15</u> To2 <u>B15</u> To2 <u>B15</u> To2 <u>B06E17</u> To2 <u>B05</u> To2 <u>B05</u> To2 <u>B05</u> To2 <u>B15</u> To2 <u>To2</u> To2 <u>To2</u> To2 To2 To2 To2 To2 To2 To2 To2	н14" В То <u>бЕ12"</u> К21ж Вов.	03610# B08J15" B03E14= B03E14= B07M15"
BO2HO1 BO4PO6" B13B02 B14C21H TO B037124H TO B037124H FO	B09E24H B09E24H B12013 B02E04H B02E04H B02E04H B02E04H B02E04H B02E04H B05E17 B05E17 B05E17 B05E17 B05E17 B05E17 B05E17 B05E17 B05E17 B05E17 B05E17 B05E17 B05E17 B05E17 B05E24H B05E24 B05E24H B05E24	Merge         Analysis           220, sequenced         6           B16L03"         Formation           To1C20=         Formation           B06F188         B11001=           P16"         F14008	2006" B05B05 B11823" T05A01= B06H05 T05A01= B06H05	re: F03A19" T08 T02 <u>B15</u> " T08 <u>B05H</u> T06 <u>C07</u> # E09116 B14F07" E09106 E16C07 E14F13	н14" В То <u>бЕ12"</u> К21ж Вов.	03610# B08J15" B03E14= B03E14= B07M15"
BO7H01 B04P06" B13D02 B14C21# T0 B14C21# B13D02 B14C21# T0 B14C21# T0	I) [Clear All] I. Number of plows N1279 B09E24H B12013 F10011 B02I04H	Merge         Analysis           220, sequenced         60           B06F18         F02           B11C01=         F04           B14D05#         F04           B015#         B14D05#           B015#         T04H24=	EDOG** BOSROOT BilB23** TOSBOS Boold Toscole Boold Toscole	ne: F03019" T08 T02 <u>815</u> " T08505H D06E17H T06 D06E17H T06 D06 D06 D06 D06 D06 D06 D06 D	H14" DoE12" BO8 K21* B10013" F0 B10013" F0 B14009# B02 B07C22 B02	03610# B08315" B03E14= B03H5" B04C10" B11K13" 1001" B4022
BO7H01 B04P06" B13D02 B1465 Mi129 B1465 Mi129 B1465 Mi129 B1465 B13D02 B14621H TO T06024 T03H06H F0 T06324" B160 B04F05 T02H24* B1264	ВО9Е24ж В12013 В10011 В02048 В12013 В10011 В0213 В10011 В0213 В10011 В0213 В10011 В0213 В10011 В0213 В10011 Б0114 В0 361374 В163074 В14 ГО00948 В141 ТО00948	Merge         Analysis           230, sequenced         60           B16L03"         F02           B06F188         F02           B11C01"         F02           P16"         B14005"           B04515H         F04H14=           B04515H         T04H14=           B161.01"         T04H14=           B161.01"         T07H18H	igure Display         Clo           igure Display         Clo           igure Display         Clo           ni260         Ni260           BliB23"         Do6806           Boone         To6801=           Boone         To5501=           Boone         To3co1=           Boone         Billsco           To5801=         To3co1=           Boone         To3co1=           To800         To3co1=	me:         ni112 mi330           F03819"         T08 mi330           T02815"         T06800H D06E17H         T06 mi330           T01007*         B0815 mi4F07"         T06 mi4F10"           B16070"         B04L0 B14P10"         B04013 B0401           F06F07"         B04013 F04F11"         B0410 B04013           K118         F04F11"         B03114' F04F11"           F094         T02B20"         B0314'	H14" Def12" K21* B10013" F0 B14009# F0 B007C22 F0 B03F02 F0	03610# B08315" B03E14= B07H15" H014 B07H15" H014 B04C10" B11H13" H004 B00H24+ T04116= H021 B00H24+ T04116= H02 B00H24+ T04102# B00H23= T04L02# J009F08 T060L9 B15H24+
BO7H01           B07H01           B04P06"           B04P06"           B13D02           B14C21#           T05006#           B03P24#           T06324"           B04P068           F06496           F02424#           T08U05#           F02402#           F02402#           F02402#           F02402#           F02402#           F02402#           F02402#           F02402#           F02402#	I. Clear All I. NAME of close N1279 B09524H B02104H B02104H B02104H B02104H B02104H B05107H B16507H T05100 B141 T069004H P04324H P04324H B0604 P04324H B0604 B	Merge         Analysis           230         sequenced           230         sequenced           10020=         10020=           806F180         F04           81400=         F04           81400=         F04           81400=         F04           81400=         F04           8140=         B0602=           08"         100114=           16"         T04N14=           16"         F04H03	igure Display         Clo           0         Number of Markeri           N1260           2006"         B05B05           B11823"         To6H16+           B06H15"         To5H01=           B02B220"         B13K20           T05H08"         To3C01=           B02B420"         B13K20           T05H08"         To3C01=           B02H08"         F03C01=	не: ні112 ні330 То2 <u>815</u> То <u>8505</u> н То <u>2815</u> То <u>8505</u> н То <u>1007</u> <u>Воент</u> То <u>8</u> <u>Воент</u> То <u>8505</u> н <u>Воент</u> <u>Воент</u> <u>То<u>8</u> <u>Ві4</u>но<u>7</u> <u>Воент</u> <u>Воент</u> <u>Ві4</u>но<u>7</u> <u>Воент</u> <u>Воент</u> <u>Ві4</u>но<u>7</u> <u>Воент</u> <u>Воент</u> <u>Ві4</u>но<u>7</u> <u>Воент</u> <u>Воент</u> <u>То<u>8</u> <u>Кі19</u> <u>Гозбон</u> <u>Гозбон</u> <u>Торделя</u> <u>Тордоб</u></u></u>	H14" B T06E12" 6 B14003# F0 8007C22 F0 903F02 F1 B16015# B1615	03610# D08J15" D04 B03E14= B07H15" 1001* B04C10" B11K13" 1001* B4024* T04116= B04K23* T04L02# B04K23* T04L02# B04K23* T04L02# 1004C2* B09F08 100F08 1
B07H01           B07H01           B04P06"           B13D02           B14C21H           T06065           T06105           T05244           T05245           F01H08           F01H08           B04F08           T05244           B04265           F01H08           F01H08           B04F08           F01H08           B04F08	1]         Clear All           1.         Number of olone           N1279           B09E24H           B12013         B10011           B02104H         B0011           B02104H         B0011           B02104H         B0117           F01104         B0017H           T06107H         B1407H           F04009H         B141           F04009H         B144           F04009H         B144           F04029H         B145           T06427H         B154           T070H19=         B154	Merge         Analysis           220, sequenced         60           B06F188         F02           B06F188         F04           B14D05*         F04           B215*         B06D02*           T04H24=         00**           B14D05*         B14D17*           T07H18*         B16_117*           16**         F04H03           D14**         B16_113*           T04H02**         B10_113*           D4**         F04402**	2006" B05B05 B11823" T06H16+ B06B05" T05H16+ B06B05" T05H16+ B06H15" T05A01= B06H15" T05A01= B06H15" T01503+ T05H08" T01503+ F01516H B13H20 T05H08 F021 B14H04H F021 T05K23 B12H	ne: ni112 ni330 T02 <u>B15"</u> T03 <u>B19"</u> T03 T02 <u>B15"</u> T08 <u>B06H</u> <u>B06E17M</u> T06 <u>B16C07</u> B34 <u>P19</u> B04L0 <u>B16C07</u> B34 <u>P19</u> B04L0 <u>B16C07</u> B31 <u>H4</u> F04 <u>P11"B03114</u> F04 <u>P11"B03114</u> F04 <u>P11"B03114</u> F04 <u>P11"B03114</u> F04 <u>P11"B03114</u> F04 <u>P11"B03114</u> F04 <u>P11"B03114</u> F04 <u>P11"B03114</u> F04 <u>P11"B03114</u> F04 <u>P11"B0304</u> F03 <u>P16</u> T05 <u>D15"</u> T03 <u>P06"</u> B04 <u>D16</u>	H14" D6E12" K21H B10013" F0 6 B14009H F0 B07C22 F0 B03F02 F1 B16615H B11K3 T01F	03610# B08315" B03E14 B07H15" B04E B07H15" B04C10" B11H13" 1001 B10224 B06H244 T04116= N2E B04H24 T04106= B06H244 T04106= B09H264 B09F08 T06L02# T06L12= T08L12= B09324 19 T06J032
B07H01           B07H01           B04P06"           B04P06"           B13N02           B14C21H           T03H06H           F0422H           T03H06H           F04204H           F04204H           F04204H           F04204H           F04204H           F04204H           F01404           F01405           F01406H           F01404           F01405           F01406H           F01408           F024084           F01408           F024084           F024084           F024084	I. Clear All           I. Number of plows           N1279           B09E24H           B12013           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B0417           F01D14           B06002"           F04009H           B14006"           B12806"           T064024"           B12806"           T070119           B16604           B16604           B16604	Merge         Analysis           230         centercod         c           Bi6L03"         F02           B06F188         F02           B11C01=         F04           B14D05H         B06D02=           D0F188         T04H24=           00H         T07H18H           116"         F04H03           01H         T04H02H           04"         B06L20"	2006" B05B05 B11823" T06H16+ B06B05" T05H16+ B06B05" T05H16+ B06H15" T05A01= B06H15" T05A01= B06H15" T01503+ T05H08" T01503+ F01516H B13H20 T05H08 F021 B14H04H F021 T05K23 B12H	mi112 mi330           F03019"         Tog mi330           TogB15"         TogB05H TogB05H B06E17M         Tog mi330           TogE15"         TogB05H B06E17M         B04L0 B09D13           F04101         B09D13         B09D13           K11#         F04F12"         B03114'           F03D04A         F02D20"         B03114'           F04F12"         B03114'         F03D04A           F03D04A         F02D20"         TogD20"           F03D04A         F02D16F         T03D04G           T05D15"         T03D04A         F02D16F           T05015"         T03D04A         F03D04A           S         F02L33A         F03L3A	H14" D6E12" K21H B10013" F0 6 B14009H F0 B07C22 F0 B03F02 F1 B16615H B11K3 T01F	036100 B08315" B03E14= B07H15" 1014 B07H15" 1001" B10121" 1001" B10122" 1001" B10122" 1001" B10123" 1001" B10124 10012 B10124 100124
BO7H01           B07H01           B04P06"           B04P06"           B13D02           B14C21H           T05002           B07124H           T06025           T06124           D02H24H           T06125           T06126           F0106           B04F08           B048	I. Clear All           I. RANGER OF DIGME           N1279           B12013           B12013           B12013           B12013           B12013           B12014           B02104H           B02104H           B12013           F0101           B02104H           B12013           F0107H           B123           B060102           F04009H           B12006H           F04022           B1500H           T07H19=           B1500H           B064003           B06404           B06404	Merge         Analysis           230         centerroad         centerroad           Bi6L03"         F02           T01C20=         B06F188         F02           B06F188         B11001=         F04           B1554         B06B02=         T04H03=           T07H184         B15013*         F04H03           D01         F04H02*         F04224           D06L20*         T02E104         T02E104	igure Display         Clo           igure Display         Clo           on muchaer of markers         mi260           isizes         Bo5B05           Bi1B23**         T06H16+           Bo2B05**         T06H16+           Bo2B05**         T06H16+           Bo2B05***         T05H01=           Bo2B05***         T05H02**           Bo2B05***         T05H02**           Bo2B05***         T05H02**           Bo2B05***         T0103**           T05H02***         B12H04***           T06H02****         B12H04****           T06H02***********************************	me: mi112 mi330 To2 <u>B15"</u> To2 <u>B15"</u> To2 <u>B15"</u> To2 <u>B15"</u> To2 <u>B16"</u> To <u>1007</u> B14F07" To <u>1007</u> B14F07" B14F10" B14F10" B14F10" B14F10" B14F10" B14F10" B14F11"	H14" B TOGE12" BOG; 6 B14009W F0 8 B14009W F0 8 B14009W B02 8 B07C22 F0 8 B14009W B11K3 8 B16615W T04F 8 B14613W B02F07 T04E13 B09E13 8 B09E13 8 B14000 8 B14000 8 B14000	D3610#         D08,315"           b03510#         B03214=           b014         B037815"           b017         B14133"           1004*         B04124*           T04116=         B04123*           B04423*         T04106*           B04423*         T04106*           1004*         B09408           1009708         B15424*           4"         T06102           T0500         B09324           19         T06003*           F02101         B15F10*           7         T08518*           508         T08518*
BO2HOI           mi465         mi128           mi465         mi128           B04P06"         mi128           B04P06"         mi128           B04P06"         mi128           B04P06"         mi128           B04P06"         mi128           B04P06"         mi128           B04F08         T06005           F04P08         F0104           B04F08         B01104           B04F08         B01104           B04F08         B11D11"           B06110         B02806"           F04H08         B01104           B061109         B02805"           F04K09*         B05806"           B06109*         B062F13"           F04K09*         B05806"           B06105         B05806"	I. Clear All           I. RANGER OF OLONG           N1279           B09524H           B2013           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B04007H           F01014           B06002H           F04009H           B04009H           B04009H           B04009H           B04009H           B04009H           B04009H           B0417           B04009H           B04009H           B04004H	Merge         Analysis           230         sequenced         6           231         sequenced         6           232         sequenced         6           233         sequenced         6           234         sequenced         6           235         sequenced         6           236         sequenced         60           237         sequenced         60           233         sequenced         60           233         sequenced         60           234         sequenced         60           235         sequenced         60           236         sequenced         80           237         sequenced         70           238         sequenced         70           239         sequenced         70           230         sequenced         70	igure Display         Clo           igure Display         Clo           or muster of markers         *           wi260         *           2006"         B05B05           B11823"         T06H16+           B06B06"         T05A01=           B06H15"         T05A01=           B06H15"         T01C03+           T04B04"         T01C03+           F01C16#         B13K20           T05H06"         T01           T05H03*         B12N           T06H23*         B12N           T06H22*         B05J10#           T04E20*         B05J10#           T04E20*         B12N           T06H22*         T05J22*           T07H14#         T07H14#           T07H14#         T07H14#	F03019"         T08           T02B15"         T08B05H           T02B15"         T08B05H           T0407"         B0410           B14F07"         B0410           B16P10"         B09013           K11"         F08F16"           B16P10"         B09013           K11"         F08F17"           F04P11"         B09114           F04P11"         B03114'           F04P11"         B03114'           F04D20"         T03D04H           F03D04H         F02D16H           B04015"         T03P06"           B050114''         B06103H           B02L11+         B06023"           P04009"         B12010           B12010         B12010	H14" B08; K21H B09; 6 B14009H F0 8 B14009H F0 8 B14009H B02 8 B07C22 F0 8 B03F02 F1 8 B16015H T01F 8 B16015H T01F 8 B16015H B02F0 T04E13 B09E13 8 B10908 B14400 9 B13011	D3610#         D08,315"           b03E14=         B07H15"           b014         B07H15"           b017         B141313"           1004*         B06H24+           T0410*         B14133"           B06H24+         T04106=           N24         B09F08           T06019         B15H24+           4"         T06102#           T060019         B15H24+           4"         T06102#           T06003         B15F10+           7         T08004#           509324         19           T060004#         B15F10+           7         T08K18"           508         T06204           509         T08K21           50         T06204           F03H17=         B04504"           F03H17=         B04504"
BO7H01           B07H01           B04F06"           B13D02           B13D02           B13D02           B13D02           B13D02           B13D02           B13D02           B13D02           B04F08"           F06155           T06124"           B04F08           F01406           F01405           F01405           F01405           F01405           F01405           F01405           F01405           F01405           B0610           B06105           F046094           F046094           B05F094           B05F104           B05F104           B05F104           B05F104           B05F104	I. Clear All           I. Rister of close           N1279           B09E24H           B12013           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B06117           F01D14           B06002**           F04009H           B123           B06000**           B0417**           B04004**           B04004**           B06006**	Merge         Analysis           230         sequenced           230         sequenced           101220=         To1220=           Bo6F188         F04           B14L01=         F04           B14L01=         F04           B14L01=         F04           B14L01=         F04           00*         B14254           00*         F04H03           116*         F04H03*           01*         F04H02*           04*         F04L2*           04*         T02E14           105E06         B           105E06         B           008011         T03003*           B058011         T042=	igure Display         Clo           igure Display         Clo           igure Display         Clo           wi260         wi260           BilB23**         To6Hi6+           BogB05**         To6Hi6+           BogB05**         To6Hi6+           BogB05**         To6Hi6+           BogB05**         To6Hi6+           BogB05**         To5Ho1+           BogB05**         To5Ho1+           BogB05***         To5Ho1+           BogB05***         To5Ho1+           BogB05***         To5Ho1+           BogB05****         Bi3K20           To5Ho0****         Fo2           To5Ho0*****         Fo2           To5Ho0*****************         Fo2           To5Ho0***********************************	F03A19"         T08           T02B15"         F03A19"         T08           T02B15"         T08505H         T06           T01007#         B09116         T06           T01007#         B09116         T06           B14F07"         B0410         B09013           K119         F03D044         F09013           F04P11"         B03114'         F03D044           F03D044         F02D16F         F02D16F           F03D044         F02D16F         F02D16F           B050113"         B06103"         B05023"           S0         F02D13F         F06109"           F01P20         F04009"         B16010           B12010         B16010         B16010           B12010         B16010         B14607           B15022         T06P12=         T06P12=	H14" D K21× B10013" B06 6 B14009× F0 8007C22 F0 8007C22 F0 8007C22 F0 814009× F02 814013" B02F0 T04F13 B02F0 1009F09 B14400 1009F09	03510# B08315" B03E1# B07H15" B04E1# D07H15" B04E2# T04116= B04E2# T04116= B04E2# T04102# B04E2# T04102# B04E2# B09F08 T06019 B15H24# 4" T03D02 B09124 T06J03= B15H24# 4" T03D02 B09124 T06J03= B15H24# T06J03= B15H24#
BO7H01           B07H01           B04P06"           B13D02           B13D02           B13D02           B13D02           B13D02           B14C21#           T03H06#           B04P06"           B04P06           B04P06           B04P06           B04F06#           B04F06#           B04F06#           B04F06#           B04F06#           F01406           F01406#           B04E08#           B1010           B04E08#           B06H10*           B06H10*           B06E19#           B06E19#           B05H20*	I. Clear All           I. RANGER OF DIGME           N1279           B12013           B12014           B05104H           B12015           F0107           B123           B06002H           F04009H           B141           T06P04B           F04009H           B15400H           T00P04B           F04003H           B15400H           B04E03H           B04E0	Merge         Analysis           230         sequenced           230         sequenced           01         ToiC20=           B06F188         Foi           B11001=         Foi           P16**         B11001=           P16**         B06B02=           00**         B14.017*           106**         Foid           01*         ToiH04           01*         FoidH03           01*         ToiH02*           04**         FoidC24           B06L20**         Toi           4**         ToigE05           B09011         Toi           009011         Toi           1005003=         B1           1005003=         B1	igure Display         Clo           igure Display         Clo           wi260         wi260           Bil83"         To6H16+           BogBos"         To6H16+           BogBos"         To6H16+           BogBos"         To5H16+           BogBos"         To5H18+           BogBos"         To5H18+           Foilciew         Bil2N           To6H22         BogL12*           BogH12*         To1E10*           BogH12**         To7H14#           To2H09         To7H14#           To2H09         Fo4J01	F03A19"         T08           T02B15"         T08B05H           T02C07"         T09016           T01C07"         B09116           B14F07"         B09102           B14F07"         B09116           B16F10"         B09013           K119         F03D04H         F02D16F           F03D04H         F02D16F         F02D16F           M10         B09E07         B06J03H           B02L114         B06J03H         B06J03H           B02L115"         F04D09"         B06J03H           B02L114         B06J03H         B06J03H           B12D10         B16C10         B14H6T           B15022         T06P12=	H14" D K21* P10013" F0 B10013" F0 B14009* F0 B007C22 F0 B007C22 F0 B14013* T04F B14013* B02F0 T04F13 B02F0 T04F13 B09F13 B10P00 B14000 F02P12 B13011	03610# B08315" B03E14= B15H01# D04 B07E15" B04K23 B07H5" B06H24# T04116= N21 B04K23 D01P16" B06H24# T04116= N21 B04K23 D01P16" T06L12# T06L12# B09324 19 F02101 B15H24# 4" T08L12# T06J03= B09324 19 F02101 B15F10# 7 F06H3" B15H0# 7 F06H3" B02P06" F64K21 B02P06" F64K21 B02P06" F06K13" B02P06" F04K21 F03H17= B04D04" B02B04" T03L5#
B07H01           B07H01           B04F06"           B04F06"           B13H02           B14C21H           T03H06H           F04C015           T03H06H           F0106           B04F06           T03H06H           F0106H           B04E08H           F0106E           F0106E           B04E08H           F0108E           F0108E           B06115           B06105           F0108E	I. Clear All           I. Rister of close           N1279           B09E24H           B12013           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B06117           F01D14           B06002**           F04009H           B123           B06000**           B0417**           B04004**           B04004**           B06006**	Merge         Analysis           230         sequenced           230         sequenced           101220=         To1220=           Bo6F188         F04           B14L01=         F04           B14         B06           P16**         B14           B14         B06           00**         B14           01**         F04H03           01**         F04H02*           04**         F04L2*           04**         T02E14           *         T07B03           *         T03003*           *         B09811           10402=         B031           *         T03003*           *         B05404	igure Display         Clo           igure Display         Clo           or number of markers         n           wi260         n           Postor         Bo5B05           BilB23**         T06H16+           Bo8D06**         T05H16+           Bo8D06**         T05H16+           Bo8D06**         T05H16+           Bo8D06***         T05H16+           Bo8D06***         T05H16+           Bo8D06****         B13K20           T06H08***         F02           T06H08****         F02           T06H08***********************************	F03A19"         T08           T02B15"         F03A19"         T08           T02B15"         T08505H         T06           T01007#         B09116         T06           T01007#         B09116         T06           B14F07"         B0410         B09013           K119         F03D044         F09013           F04P11"         B03114'         F03D044           F03D044         F02D16F         F02D16F           F03D044         F02D16F         F02D16F           B050113"         B06103"         B05023"           S0         F02D13F         F06109"           F01P20         F04009"         B16010           B12010         B16010         B16010           B12010         B16010         B14607           B15022         T06P12=         T06P12=	H14" B K21× To6E12" Bog. 6 B14009× FG 8 B14009× B02 8 B07C22 FG 8 B03F02 FG 8 B1403 B02FG 104E13 B02FG 104E13 B09E13 810P08 B14603 1069F09 B13G11 1009F09 B13G11 1009F19 1009F19	03610# В08315" 101# В07E14= В07H15" 101# В04610" В15H01# 101# В04F23" В04F02" 1001" В16022" 1001" В16022" 1004/23" В04F02 1004/23" В09F08 1004/23" В09F08 1004/23" В09F08 1004/23" В09F08 1004/23" В09F08 1004/24 100505 100504 100505 100504 100505
B07H01           B07H01           B04P06"           B04P06"           B13H02           B14C21H           T03H06H           F04C015           T03H06H           F01224H           T03H06H           F01224H           T03H06H           F0100E           F02H021H           F0100E           F02H021H           F02H02H           F0	I)         Clear All           I)         ruster of olone           Ni279           B09E24H           B12013         B10011           B02104H         B0011           B02104H         B0117           B02104H         B0117           B02104H         B0117           B02104H         B0117           B0317H         B16307H           B123         B06002*           F04009H         B144           F04009H         B144           F04020H         B145           F04020H         B144           F04020H         B144           F04020H         B144           F04020H         B145           F04020H         B145           F04020H         B145           B04404*         B15606H           B050066         B060066           B050066         B060066           B166064         B166064           B166064         B065065           B055509*         B07302	Merge         Analysis           230         sequenced           230         sequenced           101220=         To1220=           Bo6F188         F04           B14L01=         F04           B14         B06           P16**         B14           B14         B06           00**         B14           01**         F04H03           01**         F04H02*           04**         F04L2*           04**         T02E14           *         T07B03           *         T03003*           *         B09811           10402=         B031           *         T03003*           *         B05404	igure Display         Clo           igure Display         Clo           or marker of markers         mi260           mi260         mi260           2006"         B06B06"           B01823"         T06H16+           B06B06"         T05H16+           B06B06"         B13K20           B02B20"         B13K20           B04B06"         T05           B02B06"         B13K20           B04B06"         T00           B04B06"         F021           T05H08"         B12H0           T05H08"         B12H0           T04H22"         B05310#           B12H0         B12H0           T05H22"         T07H14#           T02H09         T07H14#           T02H09         F04301           B1H08         B1H08	F03A19"         T08           T02B15"         T08B05H           T02B15"         T08B05H           T01007H         B09116           B14F07"         B09116           B14F07"         B09116           B14F07"         B09116           B14F07"         B09116           B14F07"         B09116           B14F07"         B09118           F04F11"         B09114           F03D04H         F02D16+           F03D04H         F02D16+           F04B20"         F06J03H           B02L11+         B06503"           B02L11+         B06503"           B02L11+         B05023"           F04D20         B12C10           B04B16"         B05023"           F04D20         B16C10           B04B16"         B06912=           B15022         T06912=           B050424         T04	H14" B K21* To <u>6</u> E12" Bog 6 B14009* Fo 8 B14009* Bog 8 B07C22 Fo 8 B07C22 Fo 8 B14009* B1140 8 B16615* To1F 8 B16615* B09E12 8 B10P08 B14400 1008F09 B13011 1008F09 B13011 1009F09 B13011 1009F09 B13011	03610# В08315" 101# В07E14= В07H15" 101# В04610" В15H01# 101# В04F23" В04F02" 1001" В16022" 1001" В16022" 1004/23" В04F02 1004/23" В09F08 1004/23" В09F08 1004/23" В09F08 1004/23" В09F08 1004/23" В09F08 1004/24 100505 100504 100505 100504 100505
BOZHOI           B0ZHOI           B04P06"           B04P06"           B13D02           B14C21H           T03H06H           F04C015           T03H06H           F0224H           T03H06H           F0224H           F03H06H           F01H06           F01H07           F03H08H           F01H08           F04K09H           F04K09H           F04K09H           F02E15H           F01E20           F02E15H           F02E08           F02E15H           F02E08           F02E15H           F02E08           F02E08           F02E08 <tr< td=""><td>I. Clear All           I. Rister of close           N1279           B09E24H           B12013           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B06117           F01D14           B06002**           F04009H           B123           B06000**           B0417**           B04004**           B04004**           B06006**           B06006**</td><td>Merge         Analysis           230         sequenced           230         sequenced           101220=         To1220=           Bo6F188         F04           B14L01=         F04           B14         B06           P16**         B14           B14         B06           00**         B14           01**         F04H03           01**         F04H02*           04**         F04L2*           04**         T02E14           *         T07B03           *         T03003*           *         B09811           10402=         B031           *         T03003*           *         B05404</td><td>igure Display         Clo           igure Display         Clo           or number of markers         mi260           mi260         Ni260           igure Display         To6H16+           B08D06**         To6H16+           B08D06**         To6H16+           B06D06**         To6H16+           B06D06**         To6H16+           B06D06**         To6H16+           B06D06***         To6H16+           B06D06***         To6H16+           B06D06****         B13K20           T06H08****         To1C03**           B06D06******         B12H0           T06H08***********************************</td><td>F03A19"         T08           T02B15"         F03A19"         T08           T02B15"         T08505H         T06           T01007#         B09116         T06           T01007#         B09116         T06           B14F07"         B0410         B09013           K119         F03D044         F09013           F04P11"         B03114'         F03D044           F03D044         F02D16F         F02D16F           F03D044         F02D16F         F02D16F           B050113"         B06103"         B05023"           S0         F02D13F         F06109"           F01P20         F04009"         B16010           B12010         B16010         B16010           B12010         B16010         B14607           B15022         T06P12=         T06P12=</td><td>H14" B K21× To6E12" Bog. 6 B14009× FG 8 B14009× B02 8 B07C22 FG 8 B03F02 FG 8 B1403 B02FG 104E13 B02FG 104E13 B09E13 810P08 B14603 1069F09 B13G11 1009F09 B13G11 1009F19 1009F19</td><td>03610# В08315" 101# В07E14= В07H15" 101# В04610" В15H01# 101# В04F23" В04F02" 1001" В16022" 1001" В16022" 1004/23" В04F02 1004/23" В09F08 1004/23" В09F08 1004/23" В09F08 1004/23" В09F08 1004/23" В09F08 1004/24 100505 100504 100505 100504 100505</td></tr<>	I. Clear All           I. Rister of close           N1279           B09E24H           B12013           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B02104H           B06117           F01D14           B06002**           F04009H           B123           B06000**           B0417**           B04004**           B04004**           B06006**	Merge         Analysis           230         sequenced           230         sequenced           101220=         To1220=           Bo6F188         F04           B14L01=         F04           B14         B06           P16**         B14           B14         B06           00**         B14           01**         F04H03           01**         F04H02*           04**         F04L2*           04**         T02E14           *         T07B03           *         T03003*           *         B09811           10402=         B031           *         T03003*           *         B05404	igure Display         Clo           igure Display         Clo           or number of markers         mi260           mi260         Ni260           igure Display         To6H16+           B08D06**         To6H16+           B08D06**         To6H16+           B06D06**         To6H16+           B06D06**         To6H16+           B06D06**         To6H16+           B06D06***         To6H16+           B06D06***         To6H16+           B06D06****         B13K20           T06H08****         To1C03**           B06D06******         B12H0           T06H08***********************************	F03A19"         T08           T02B15"         F03A19"         T08           T02B15"         T08505H         T06           T01007#         B09116         T06           T01007#         B09116         T06           B14F07"         B0410         B09013           K119         F03D044         F09013           F04P11"         B03114'         F03D044           F03D044         F02D16F         F02D16F           F03D044         F02D16F         F02D16F           B050113"         B06103"         B05023"           S0         F02D13F         F06109"           F01P20         F04009"         B16010           B12010         B16010         B16010           B12010         B16010         B14607           B15022         T06P12=         T06P12=	H14" B K21× To6E12" Bog. 6 B14009× FG 8 B14009× B02 8 B07C22 FG 8 B03F02 FG 8 B1403 B02FG 104E13 B02FG 104E13 B09E13 810P08 B14603 1069F09 B13G11 1009F09 B13G11 1009F19 1009F19	03610# В08315" 101# В07E14= В07H15" 101# В04610" В15H01# 101# В04F23" В04F02" 1001" В16022" 1001" В16022" 1004/23" В04F02 1004/23" В09F08 1004/23" В09F08 1004/23" В09F08 1004/23" В09F08 1004/23" В09F08 1004/24 100505 100504 100505 100504 100505
B07H01           B07H01           B04P06"           B04P06"           B13H02           B14C21H           T03H06H           F04C015           T03H06H           F01224H           T03H06H           F01224H           T03H06H           F0100E           F02H021H           F0100E           F02H021H           F02H02H           F0	I)         Clear All           I)         ruster of olone           Ni279           B09E24H           B12013         B10011           B02104H         B0011           B02104H         B0117           B02104H         B0117           B02104H         B0117           B02104H         B0117           B0317H         B16307H           B123         B06002*           F04009H         B144           F04009H         B144           F04020H         B145           F04020H         B144           F04020H         B144           F04020H         B144           F04020H         B145           F04020H         B145           F04020H         B145           B04404*         B15606H           B050066         B060066           B050066         B060066           B166064         B166064           B166064         B065065           B055509*         B07302	Merge         Analysis           230         sequenced           230         sequenced           101220=         To1220=           Bo6F188         F04           B14L01=         F04           B14         B06           P16**         B14           B14         B06           00**         B14           01**         F04H03           01**         F04H02*           04**         F04L2*           04**         T02E14           *         T07B03           *         T03003*           *         B09811           10402=         B031           *         T03003*           *         B05404	igure Display         Clo           igure Display         Clo           or marker of markers         mi260           mi260         mi260           2006"         B06B06"           B01823"         T06H16+           B06B06"         T05H16+           B06B06"         B13K20           B02B20"         B13K20           B04B06"         T05           B02B06"         B13K20           B04B06"         T00           B04B06"         F021           T05H08"         B12H0           T05H08"         B12H0           T04H22"         B05310#           B12H0         B12H0           T05H22"         T07H14#           T02H09         T07H14#           T02H09         F04301           B1H08         B1H08	Billi2         Billi2           Billi2         Billi2           To2B15"         To8B06H           To2B15"         To8B06H           To2D215"         To8B06H           To2D20"         BildF07"           BildF07"         Bo3013           BildF07"         Bo3013           F03D04H         F02D20"           F03D04H         F02D20"           F03D04H         F02D20"           F03D04H         F02D20"           B02H11         B06B023"           B04B16"         B12010           B04B16"         B16C10           B15022         T06P122           B03H16"         B16C10           B15022         T06P12E           B03H24         T	H14" B TOGE12" BOG: 6 B140094 F0 8 B140094 F0 8 B07C22 F0 9 B07C22 F0 9 B07C2 F1 1460154 T04F1 B146154 T04F1 B146154 B09F11 B140908 B14400 109F09 B13011 F02P12 B09H18 22P09	03610# В08315" 101# В07E14= В07H15" 101# В04610" В15H01# 101# В04F23" В04F02" 1001" В16022" 1001" В16022" 1004/23" В04F02 1004/23" В09F08 1004/23" В09F08 1004/23" В09F08 1004/23" В09F08 1004/23" В09F08 1004/24 100505 100504 100505 100504 100505

were from the IGF BAC library, and the clones equivalent to  $4.1 \times$  haploid genomes were from the new BIBAC library. A total of 196 contigs were assembled, each consisting of at least 2 clones (Table 1 and Figure 4), whereas 279 clones remained as singletons due to the insufficient numbers of bands (<10 bands) in their fingerprints.

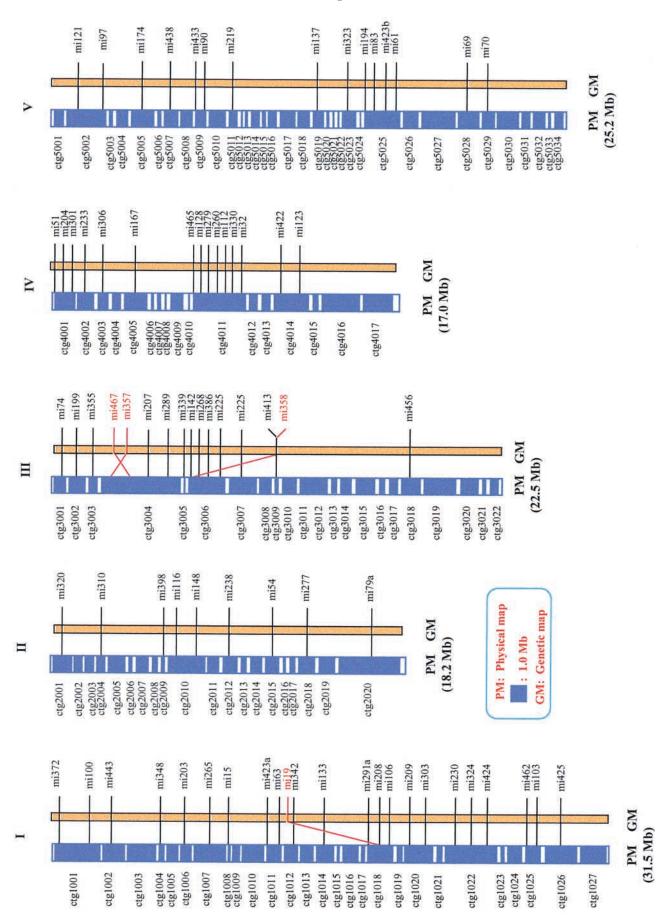
Origin of the 196 contigs was investigated by colony hybridization using chloroplast DNA as probes and by analyzing the mitochondrial DNA-derived clones of the IGF library (Mozo et al. 1998) against the BAC/BIBAC contigs. Contig 1 (ctg1 in Table 1) was shown to derive from chloroplast DNA. It consists of 161 clones and was estimated to be 162 kb in length. The size of the contig is close to the 153-kb chloroplast genome size (PALMER et al. 1994). Contig 2 (ctg2 in Table 1) was shown to derive from the mitochondrial DNA. It consists of 108 clones and was estimated to be 564 kb in length. The length of the contig is much greater than the size of either the cytoplasmic mitochondrial genome (372 kb; KLEIN et al. 1994) or the mtDNA inserted into the nuclear genome revealed by sequencing (270 kb; LIN et al. 1999), but close to the size of the mtDNA inserted into the nuclear genome revealed by fiber-fluorescence in situ hybridization (618  $\pm$  42 kb; STUPAR et al. 2001). STUPAR et al. (2001) demonstrated that the size of the mtDNA inserted into the nuclear genome was underestimated in the sequence map (LIN et al. 1999) due to errors of the physical and sequence map assembly. This result indicated that it is necessary to further verify the existing physical and sequence maps using an approach differing from those used in the development of the existing maps. The 194 remaining contigs were derived from nuclear DNA (Table 1). The FPC program showed that these 194 contigs consisted of 42,119 unique bands, each band representing a 3.0-kb fragment. Therefore, the nuclear DNA clone contigs were estimated to span >126Mb in length.

**Verifying the map:** To test the accuracy of the new map, we compared the BAC/BIBAC contigs constructed in this study with the BAC contigs of the existing physical (Mozo *et al.* 1999) and sequence (ARABIDOPSIS GENOME INITIATIVE 2000; http://www.arabidopsis.org) maps using the

TAMU and IGF BACs shared between the contigs. The comparison showed that  $\sim 95\%$  of the new BAC/BIBAC contigs were consistent with the contigs of the existing physical and sequence maps in both clone content and order (Figure 2), but 5% of the contigs were different. To further verify the accuracy of the new BAC/BIBAC contigs, we screened them with 77 DNA markers selected from the genetic map (LIU et al. 1996) and nine cDNA clones selected from the Arabidopsis EST set. The results showed that all positive clones of every marker or cDNA probe used were located at a restricted fragment of a single contig (see Table 2 and Figure 3). This indicates that the contigs assembled in this study are accurate. The cause of the 5% difference between the contigs of the new map and the existing physical and sequence maps needs to be further investigated as errors were recently reported in the existing physical and sequence maps (STU-PAR et al. 2001). We have also investigated the orders of markers in the contigs that contained three or more of the DNA markers. The result showed that they were consistent with those in the genetic map (see Figure 4), except for four DNA markers (mi19, mi467, mi357, and mi358). To explain this inconsistency, we further investigated the orders of the four DNA markers in the existing physical and sequence maps (Mozo et al. 1999; http://www.arabidopsis.org). The investigation showed that the orders of the four DNA markers in the new physical map were consistent with their orders in the existing physical and sequence maps, indicating that these DNA markers might be mismapped in the genetic map. All above results strongly indicate that the BAC/BIBAC contigs constructed in this study are accurate and that the fingerprinting method used is reliable for whole-genome physical mapping from random BACs and/or BIBACs.

Anchoring the physical map contigs to the genetic map: To anchor the BAC/BIBAC contigs to a public genetic map of *A. thaliana*, we used the above screening results of the contig BACs and BIBACs with the 77 mapped DNA markers and the database of the TAMU and IGF BACbased maps of the IGF (Mozo *et al.* 1999) and the Arabidopsis Genome Initiative (AGI; ARABIDOPSIS GENOME INI-TIATIVE 2000). Of the 194 nuclear DNA clone contigs, 120 were anchored to the five chromosomes of Arabidopsis

FIGURE 3.—BAC/BIBAC contig of the Arabidopsis BAC/BIBAC-based, integrated genetic, physical, and sequence map (ctg4011 in Table 1). (A) Integration of the new map with the existing physical and sequence maps of *A. thaliana* (http://www.arabidopsis.org) and distribution of TAMU BACs, IGF BACs, and BIBACs in the map. The contig (ctg4011) contains 230 clones and has a length of 1084 unique bands, being equivalent to 3255 kb (see Table 1). The clones prefixed with letter T were from the TAMU BAC library, the clones prefixed with F from the IGF BAC library, and the clones prefixed with B from the new BIBAC library (see http://hbz.tamu.edu). Each clone is specified with a library letter (T, F, or B), plate number (two-digit number), row letter, and column number (two-digit number). For instance, the clone B07M01 was from the new BIBAC library, plate 7, row M, and column 1. (B) Accuracy verification and anchoring of the BAC/BIBAC contigs to the Arabidopsis chromosomes. The contig 4011 was anchored to chromosome 4 by seven linked-DNA markers (mi465, mi128, mi279, mi260, mi112, mi330, and mi32) mapped to linkage group 4 of the Arabidopsis genetic map (LIU *et al.* 1996). The clones highlighted in blue indicate the positive clones of each marker below and above them. Note that all positive clones of each marker were located to a single location of the contig and the order of the seven markers in the contig is completely the same as that in the genetic map (see Figure 4). Asterisk (\*) indicates a parent clone that covers one or more clones.



(Figure 4 and Table 2). These 120 contigs collectively span 114 Mb in length. Of them, 27 were anchored to chromosome 1, spanning 31.5 Mb in length; 20 to chromosome 2, spanning 18.2 Mb; 22 to chromosome 3, spanning 22.5 Mb; 17 to chromosome 4, spanning 17.0 Mb; and 34 to chromosome 5, spanning 25.2 Mb (see Table 1 and Figure 4).

Potential applications of the new integrated map for accelerated genome research of Arabidopsis: This new integrated map has provided a platform for accelerated genome research of Arabidopsis in many aspects. To test the utility of the integrated map for gap closure in the sequence map, we attempted to close four gaps in the sequence map using the new map. One of the gaps was between clones T2P3 and F2G19 in the sequence map of chromosome 1, and three gaps were between ctg714-ctg719ctg731-ctg11 in the sequence map of Arabidopsis chromosome 3 (http://genome.wustl.edu/gsc/arab/arabidopsis. html; communicated with Dr. Christopher Town, The Institute for Genome Research). The TAMU and IGF BACs in the contigs were used to search the new BAC/ BIBAC contigs. One contig (ctg1024) was identified from the new map to span the gap between T2P3 and F2G19 by two BIBACs (B05G22 and B09C04). The two BIBACs have been used to close the gap of the existing sequence map (C. Town, personal communication). Similarly, we searched the contigs of the new map that span the three gaps between ctg714-ctg719-ctg731-ctg11 in chromosome 3. As a result, from the new map we identified three contigs, ctg3010 (810 kb), ctg3011 (345 kb), and ctg3012 (1308 kb). To further determine whether ctg3011 and ctg3012 overlap, we further analyzed the fingerprints at the contig ends under less comparison stringency and screened the BACs and BIBACs of the new map using one IGF BAC clone (F03O21) at a ctg3012 end as a probe. As a result, eight positive BIBAC clones at one of the ctg3011 ends were identified. The hybridization and fingerprint analysis results suggested that the ctg3011 and ctg3012 overlapped and thus were merged. The merged contig (1618 kb) seems to span the gaps between ctg719, ctg731, and ctg11. Therefore, of the four gaps in the AGI sequence map, three were likely to be closed using the new map.

Whether the gap between ctg714 and ctg719 in the sequence map can be closed by ctg3010 and ctg3011 remains to be determined. Similarly, other gaps in the existing sequence map could also be closed using the new map.

Experimental determination of the function of the genes and related sequences predicted by the genome sequence analysis of A. thaliana will be a significant challenge. Since A. thaliana can be readily transformed via Agrobacterium (Feldmann and Marks 1987; Koncz et al. 1989; BECHTOLD et al. 1993), genetic transformation and subsequently transgenic plant analysis will provide an alternative tool for experimental determination of the functions of the predicted genes and related sequences in the genome sequence map. The binary vector pCLD04541 used for the new BIBAC library was designed for plant transformation via Agrobacterium (JONES et al. 1992). It has been widely used in Arabidopsis genetic complementation studies (e.g., BENT et al. 1994; http:// www.jic.bbsrc.ac.uk/staff/ian-bancroft/vectorspage/htm). Although further investigation will be needed to transform large DNA fragments using this vector in Arabidopsis and other plant species, it has been shown recently that a 135-kb clone of Brassica DNA in pCLD04541 was stable in Agrobacterium (Wu et al. 2000) and transformed into Brassica (Y.-Z. Wu and Y.-P. ZHANG, personal communication). We transformed a 120-kb clone of soybean DNA in the vector into A. thaliana by the vacuum-infiltration method (BECHTOLD et al. 1993; Y.-L. CHANG, K. MEKSEM, H.-W. CHUANG, C. SCHEURING and H.-B. ZHANG, unpublished data). Furthermore, using the same vacuuminfiltration method and a similar vector, LIU et al. (1999) successfully transformed a DNA fragment of 80 kb into A. thaliana and showed that the transformation efficiency was not affected substantially by the sizes of introduced T-DNA. By transforming two clones of 75 and 80 kb carrying the SGR1 locus into Arabidopsis, LIU et al. (1999) were able to complement the sgr1 mutant and thus identify the functional sequence of the SGR1 gene. Transformation of DNA fragments of 150 kb via Agrobacterium was also documented in tobacco (HAMILTON et al. 1996) and tomato (HAMILTON et al. 1999). These studies have indicated that high-molecular-weight DNA

FIGURE 4.—The BAC/BIBAC-based, integrated genetic, physical, and sequence map of *A. thaliana* ecotype Columbia. Since the clones of the map equivalent to  $3.1 \times$  Arabidopsis haploid genomes were from the TAMU and IGF BAC libraries used in the sequence map (http://www.arabidopsis.org), the new map is integrated with the sequence map (see Figure 3). The contigs containing DNA markers were anchored to the chromosomes of their origin using the screening results of the contig BACs and BIBACs with the DNA markers. The contigs not containing DNA markers were anchored to the chromosomes of their origin by using the database of the TAMU and IGF BAC-based maps of the IGF (Mozo *et al.* 1999) and the AGI (ARABIDOPSIS GENOME INITIATIVE 2000). The collective length of the contigs anchored to each chromosome is given below its physical map (PM) in mega base pairs (Mb) in parentheses. The sizes of gaps between neighboring contigs were not determined in this study; most of the neighboring contigs may overlap even though the extent of their overlaps could not be detected by the FPC program under the conditions used in this study. Although the orders of the four DNA markers mi19, mi467, mi357, and mi358, highlighted in red on the BAC/BIBAC-based physical map, are different from those on the genetic map (GM; LIU *et al.* 1996), they are consistent with their orders on the physical map of the IGF and the AGI (http://www.arabidopsis.org).

# TABLE 2

Probe	Clone	Contig <sup>a</sup>
mi15	B08O03, B09B02, B14O12, F03C06, F04M13, T01H23, T05M04	1008
mi19	B04P08, T04P06	1018
mi32	B10P08, B14C13, B16G15, F02P12, T02P09, T04E13, T08P09	4011
mi51	B13B12, T07N12	4001
mi54	B05C06, B08A08, B14E08, B16L19, F03G21	2015
mi61	B01P12, B02C13, B03H10, B05H09, B08A17, B14O01, B14E21, F02F17, F04O21, T02A13, T04P10	5025
mi63	B03L12, B04M03, B09C18, B15D09, T02E10, T08K04	1011
mi69	B01D23, B12A21, B12P09, B13D03, B14G05, F01L09, T06O12	5028
mi70	B08I09, B09G04, B11A10, B15N07, T04K24, T05J16, T08F08	5029
mi74	B01A10, B06O10, B06L08, B07D06, F01C09, F01J06, F02P08, T01E08	3001
mi79a mi83	B06H12, B14O14, F03G08, T06G23 B01C02, B12N03, T04H07, T07N93	$2020 \\ 5025$
mi90	B01G03, B13N03, T04H07, T07N23 B05P13, B11B07, T04L22	5025 5009
mi90 mi97	B05F15, B11B07, 104L22 B06O24, B15A03, B16L13, F01G19, F04I15, T04L12	5009 5002
mi100	B10O23, B10P23, F01A17, F03E19, T08D18	1001
mi103	B01M16, B02L04, B15I24, B16D23, F02N11, T04G12	1001
mi106a	B02E01, B04H04, B05E22, B06F14, B08F21, B12L16, F02J10, F03F06, T05N18, T06G11	1018
mi106b	B06D07, F04[19	1010
mi112	B04B16, B06E17, B09E07, B09M24, B14F07, B16P10, F02M02, F02L13, F03A19, F03D04, F04G09, T02D20, T07B04	4011
mi116	B06J02, B06P14, B12F02, B12H05, B12N23, F01P15, F02P06, F03C16	2010
mi121	B04K04, B08K11, B10K15, B10F02, F01H15, T01E03, T02I20	5002
mi123	F02L16, F03P01, F04G17	4014
mi128	B02A05, B03A20, B05F08, B07I24, B07D24, B08F13, T06G15	4011
mi133	B02[11, B16N18, F02G10, T02A12, T04M23, T05L02, T06M18, T06F11, T07L04, T08D08	1014
mi137	B04I14, B06C18, B09A17, T08D21	5019
mi142	B04E22, B12M12, B12A16, B12H10, F03B08, F03P10, F03J21, T02C08, T03C23, T04K14, T05G03, T06K16	3006
mi148	B04E16, B08E08, B16G12, T02C02, T02G12, T05D24	2010
mi167	B03L03, B09O05, B10E11, B13P09, B16D13, T01H20, T04P21	4005
mi174	B14M01, F01A23, T04J18	5005
mi194	B01M09, B02E20, B03L21, B08E21, B11D20, B13E17, B13D08, B14D19, B14D20, F04J06, T05J02	5025
mi199	B07I02, B08N19, B09M17, B10M17, T04P02, T05N20, F04J14, T08J15	3002
mi203	B02O04, B04L19, B06G01, B09J17, B10F21, B14M17, F03D08, F04C20, F04E19, F04N04, T06A18, T07J22, T08O20	1006
mi204	B07D03, B08A18, B12K16, F03C15, T08M24	4001
mi207	B05L07, B08G16, B08F05, B14L03, B15D14, T01A09, T03I04, T03O05	3004
mi208	B02N17, B03P13, B04K24, B08B01, T06G18	1018
mi209	B07F14, B12F05, B14E04, B16O23, T05A14	1020
mi219	B02C02, B03D21, B08B10, B11C06, B16P06	5011
mi225	B04C03, B13A04, F02A23, F02P22, F03M05	3007
mi230	B08G24, B09G21, B11L05, B12L09, B16O01, F02M24, T01N23, T02H02	1022
mi233	B02E06, T03B24, B14I12, B16M08, F04F14, T07P21 B07P02, B08D20, B09H01, B15E01, B16M17, F02H05, F02P11, T07N05, T08A08	4002
mi238 mi260	B07F02, B08D20, B09H01, B15E01, B10M17, F02H05, F02F11, 107N05, 108A08 B02A20, B06H15, B08D05, B11K12, B11B23, F01C16, T04F22, T05N08, T06K23	$2012 \\ 4011$
mi265	B14[09, F01C02, T06M11, T07G13	1007
mi268	B13G21, B15O19, F01M24, F03K10, T04M20	3006
mi277	B02K08, B06G11, B06L19, B12C14, B14I07, T04E23	2018
mi279	B02M13, B05L15, B09O04, B12O13, B15A04, F03K17, T06G17	4011
mi289	B02I12, B09A21, B10F12, T05D07	3004
mi291a	B02B10, B11016, B15017, B15N19, B16H16, T03F24, T05I04	1018
mi301	B14E10, B14I01, T05A19, T07B07	4001
mi303	B04O17, B07G22, B08K24, B08L14, B10C11, B10E01, B13A13, B14B04, B15A06, B16F06, F01K04, F03G17, T06H22	1020
mi306	B05G06, B07O07, T02B23, T06F14, T06F18, T07G22, T07N06	4003
mi310	B04K11, B09I20, B15N02, F04M17, T01C04, T01F17, T02J01, T03K03, T03J06, T04E14, T05B06, T06P09	2004
mi320	B01D22, B02N13, B15C12	2001

(continued)

### TABLE 2

Probe	Clone	Contig <sup>a</sup>
mi323	B01A09, B06J14, B14A23, F04O02, T05G18	5023
mi324	B02K22, B08N24, B09G12, B11H02, B14O24, F02K11, F04L13, T01N14, T03K11	1022
mi330	B09I16, B14P19, T06P12, T08B05	4011
mi339	B01H13, T04L24, T07H05	3005
mi342	B01117, T03120, T07A21, T08A20	1012
mi348	B10B09, B16J06, F02C14, F02O23, T02E17	1004
mi355	B14G08	3003
mi357	B02K21, B04B09, B04D04, B05C23, B09G02, B12D12, F01I03, F03K15, T01O12, T04C01, T08A07	3004
mi358	B04E22, B12M12, B12A16, B12H10, F03B08, F03P10, T02C08, T03C23, T04K14, T05G03, T06K16	3006
mi372	B01H03, B02O17, B04I08, B04K19, B06C08, T03F10, T06L08, T08I17	1001
mi386	B07H15, B13K17, T05D03	3006
mi398	B02P18, B08L05, B08N21	2009
mi413	B03B12, B03J05, B08I07, B11C22, T07I19	3009
mi422	B07L03, B14B15, T03P20, T04J24, T06I01	4014
mi423a	B09L02, B12O24, F04L20, T07F05	1011
mi423b	B10A02, B11D07, B12I11, B14I23, B14H05, B14F13, B15M08, F04A18	5025
mi424	B01P05, B02K05, B04N05, B13A17, B14C01, F02A01, T06J21, T08L05	1022
mi425	B04I16, B05N20, B07D11, F02H22, F03E06	1026
mi433	B03P15, B04A01, B05P13, B07F18, B11B07, T04L22	5009
mi438	B01K12, B04B10, B07C05, B08I04, B08F11, T02B06, T03C20, B16K16	5007
mi443	B04F07, B04D19, B05A19, B09C16, T04A12, T04F13, B14F16, B15F22, B16O12, F02J24	1002
mi456	B02E14, B07A08, B10A17, B15K10, F03C22	3018
mi462	B15P17, F02N03, F03G06, F03G19, T02K22	1025
mi465	B12P06, T01E20, T08A01, T08I09	4011
mi467	B02E19, B06K16, B08O01, F01P10, F03B23, F03L24, F04N16, T01A06, T01J13, T05L22	3004
AtAF1	B02O23, B03E03, B11M24, F02H17, F03K08, F04P07, T08A06	2019
AtNAM	B05G19, B05B02, B07P05, B09D02, B09H20, B11O22, B14P18, B16D19, F03H05, F04F05, T02M02, T03O02, T03O22	1022
AtS20-1	B01F01, B10F03, B11G08, B13B05, T01I07, T02C06, T02A24, T08K19	1020
AtS20-2	B02H12, B02J24, B03I18, B07A24, B07E19, B11M13, B11M19	3004
AtS478	B01F23, B03M15, B03B22, B04K21, B05L13, B09C01, B11H15, B12J02, B12J05, B14G15	3014
Oleosin	B06114, B09L01, T03K09, T03N19, T08K24	2020
PEI1	B03M16, B08A24, B10N07, F03M10, F03J19, T08M16	3019
C11	T04O08, B15I06, B15I07, B15F24	1022
C12	B02K06, B02J04, B05J08, B06J17, B07M22, B10O15, B14G01, F01G19, T01C21, T02I01, T06I09, T07E22, T08I24	5002
C13	B01O10, B06H05, B08I18, B08J17, B13L08, B16F07	3008

<sup>*a*</sup>Each contig is named using a four-digit number, with the first one identifying the chromosome to which the contig was anchored and the last two specifying the contig. The clones were named as described in Figure 3.

could be transformed into plants via Agrobacterium. Since the BAC/BIBAC-based map developed here is integrated with the existing sequence map, the BIBAC sequences could be deduced from their overlapping BACs and used for large-scale functional analysis of the genome sequence by genetic transformation.

In addition to their utility in gap closure and functional analysis of the Arabidopsis genome sequence, the new integrated map and fingerprint database have provided a platform for numerous other studies of not only *A. thaliana*, but also of many other plant species. We have already received a number of inquiries, including those from the laboratories of the AGI. These studies include gap closure in the sequence map (see above), isolation of genes by positional cloning, identification of the functional sequence of centromeres, studies of gene regulation, engineering of a cluster of genes at a locus, and comparative genomics research between *A. thaliana* and crop plants. Since errors have recently been identified in the existing sequence map (STUPAR *et al.* 2001), this new integrated map may provide a tool to identify and correct the errors in the sequence map because it was developed with a method different from those used for development of the existing maps. Furthermore, this study, along with the recent development of the whole-genome physical map of rice (TAO *et al.* 2001), has demonstrated that DNA sequence electrophoresis-based restriction fingerprint analysis is a reliable and high-throughput method for rapid genomewide physical mapping of large, complex genomes from large-insert random bacterial clones.

Accessing the integrated map: The integrated map

and the new BIBAC library have been posted at http:// hbz.tamu.edu (Physical Mapping-Arab Map) and made available to the public. Users can access the map using any of the following approaches: clone-FPC hitting; clone-graphic contig map; clone-fingerprint map; contig no.-graphic contig map; or marker/EST-positive clones-contig/PFC hit/ fingerprint matches. The contigs, clones, and libraries can be requested at http:// hbz.tamu.edu-BAC Library-Library List.

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