

## Research Article

# The Selection and Use of Sorghum (*Sorghum propinquum*) Bacterial Artificial Chromosomes as Cytogenetic FISH Probes for Maize (*Zea mays* L.)

Debbie M. Figueroa,<sup>1</sup> James D. Davis,<sup>1</sup> Cornelia Strobel,<sup>1</sup> Maria S. Conejo,<sup>1</sup> Katherine D. Beckham,<sup>1</sup> Brian C. Ring,<sup>1,2</sup> and Hank W. Bass<sup>1</sup>

<sup>1</sup>Department of Biological Science, The Florida State University, Tallahassee, FL 32306-4370, USA

<sup>2</sup>Department of Biology, Valdosta State University, Valdosta, GA 31698-0015, USA

Correspondence should be addressed to Hank W. Bass, bass@bio.fsu.edu

Received 13 August 2010; Accepted 2 November 2010

Academic Editor: Lisa Wiesmuller

Copyright © 2011 Debbie M. Figueroa et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The integration of genetic and physical maps of maize is progressing rapidly, but the cytogenetic maps lag behind, with the exception of the pachytene fluorescence *in situ* hybridization (FISH) maps of maize chromosome 9. We sought to produce integrated FISH maps of other maize chromosomes using Core Bin Marker loci. Because these 1 Kb restriction fragment length polymorphism (RFLP) probes are below the FISH detection limit, we used BACs from sorghum, a small-genome relative of maize, as surrogate clones for FISH mapping. We sequenced 151 maize RFLP probes and compared *in silico* BAC selection methods to that of library filter hybridization and found the latter to be the best. BAC library screening, clone verification, and single-clone selection criteria are presented along with an example of transgenomic BAC FISH mapping. This strategy has been used to facilitate the integration of RFLP and FISH maps in other large-genome species.

## 1. Introduction

Cytogenetics has proven invaluable in eukaryotic genome research, helping to elucidate genome structure in humans and model organisms such as fruit fly (*Drosophila melanogaster*) and maize (*Zea mays*) [1–6]. Historically, the field of cytogenetics, and in particular maize cytogenetics, was greatly advanced by Barbara McClintock's pioneering work in the 1920s and 1930s [7]. Her method for unequivocal identification of individual chromosomes permitted major discoveries regarding the structure and dynamic behavior of the maize genome while also establishing the connection between genetic and physical recombination [8–10]. Subsequent plant molecular cytology built on this foundation by using cloned DNA sequences as probes for *in situ* hybridization in order to visualize their location directly on the physical chromosomes [11–15]. In addition to single-locus detection, fluorescence *in situ* hybridization (FISH) with repetitive sequence probes has been used and combined

with multicolor cocktails to characterize genome structure in polyploid or closely related plant species [16–19]. Modern plant cytogenetics has increasingly contributed to structural, functional, and comparative genomics especially when FISH probes from bacterial artificial chromosome (BAC) clones, which are 100- to 200-Kb genomic fragments, have been used. Individual clones can be arrayed and characterized by fingerprint contig (FPC) mapping and annotated with markers by filter hybridization [20]. Well-characterized libraries can then serve as a resource for whole genome sequencing as was recently done for maize [21].

Among plant crops, maize has great importance for both economics and research. In 2009, maize made up 27% of the United States crop harvest by acreage, more than cotton, sorghum, wheat, barley, and sunflower combined (National Corn Grower's Association, U.S.A. Figures, 2009). Maize is a powerful genetic system, because of its large families and ease of crossing, as evidenced by the historical advances in transmission genetics [22] and epigenetics [23–25], as well

TABLE 1: Sequenced maize CBM-RFLPs.

CBM	Marker/locus	Probe	Accession number	Type	Enzyme(s)	MaizeGDB insert size (bp)	Sequenced RFLP insert size
1.01	tub1	p-tub1	AY987961	Genomic	<i>EcoRI/HindIII</i>	156	156
1.02	umc157(chn)	p-umc157	DQ123890	Genomic	<i>PstI</i>	1220	1250
1.03	umc76(prob a)	p-umc76	AY751079	Genomic	<i>PstI</i>	760	710
1.04	asg45(ptk)	p-asg45	AY771210	Genomic	<i>PstI</i>	350	332
1.05	csu03	p-csu03	DQ123891	cDNA	<i>EcoRI/XhoI</i>	1200	1064
1.06	umc67a	p-umc67	AY771211	Genomic	<i>PstI</i>	650	644
1.07	asg62	p-asg62	DQ001865	Genomic	<i>PstI</i>	500	465
1.08	umc128	p-umc128	DQ123892	Genomic	<i>PstI</i>	740	755
1.09	cdj2	p-csu164	DQ642431	cDNA	<i>EcoRI/XhoI</i>	700	466
1.10	umc107a(croc)	p-umc107	DQ642432	Genomic	<i>PstI</i>	1090	1105
1.11	umc161a	p-umc161	AY771212	Genomic	<i>PstI</i>	700	723
1.12	bnl6.32 <sup>a</sup>	p-bnl6.32		Genomic	<i>PstI</i>	2250	
2.01	bnl8.45a <sup>b</sup>	p-bnl8.45		Genomic	<i>PstI</i>	2100	
2.02	umc53a	p-umc53	AY771214	Genomic	<i>PstI</i>	640	604
2.03	umc06a	p-umc06	AY771213	Genomic	<i>PstI</i>	590	608
2.04	umc34	p-umc34	DQ001866	Genomic	<i>PstI</i>	970	934
2.05	umc131	p-umc131	AY771215	Genomic	<i>PstI</i>	810	859
2.06	umc255a	p-umc255	DQ123893	Genomic	<i>PstI</i>	1050	1013
2.07	umc005a	p-umc005	AY771216	Genomic	<i>PstI</i>	850	830
2.08	asg20	p-asg20	DQ123894	Genomic	<i>PstI</i>	550	337
2.09	umc049a	p-umc049	DQ123895	Genomic	<i>PstI</i>	630	627
2.10	php20581b(tb)	p-php20581	EU190456	Genomic	<i>PstI</i>	1400	1327
3.01	umc032a	p-umc32	DQ001867	Genomic	<i>PstI</i>	990	1019
3.02	csu32	p-csu32	DQ123896	Genomic	<i>EcoRI/XhoI</i>	500	411
3.03	asg24(gts)	p-asg24	AY771217	Genomic	<i>PstI</i>	550	372
3.04	asg48	p-asg48	DQ001868	Genomic	<i>PstI</i>	1600	1617
3.05	umc102	p-umc102	DQ005498	Genomic	<i>PstI</i>	1010	1023
3.06	bnl5.37a <sup>b</sup>	p-bnl5.37		Genomic	<i>PstI</i>	2300	
3.07	bnl6.16a <sup>b</sup>	p-bnl6.16		Genomic	<i>PstI</i>	2450	
3.08	umc17a	p-umc17	AY771218	Genomic	<i>PstI</i>	850	840
3.09	umc63a	p-umc63	DQ123897	Genomic	<i>PstI</i>	620	881
3.10	cyp1	p-csu25	DQ005499	cDNA	<i>EcoRI/XhoI</i>	1100	960
4.01	agrr115 <sup>c</sup>	p-agrr115		Genomic	<i>EcoRI/HindIII</i>	600	
4.02	php20725a <sup>b</sup>	p-php20725		Genomic	<i>PstI</i>	1650	
4.03	umc31a	p-umc31	DQ123898	Genomic	<i>PstI</i>	550	582
4.04	npi386(eks)	p-npi386	DQ007988	Genomic	<i>PstI</i>	1200	1180
4.05	agrr37b <sup>b</sup>	p-agrr37		cDNA	<i>EcoRI/HindIII</i>	949	
4.06	umc156a	p-umc156	AY771219	Genomic	<i>PstI</i>	570	533
4.07	umc66a(lcr)	p-umc66	DQ007989	Genomic	<i>PstI</i>	1020	1036
4.08	umc127c	p-umc127	DQ642433	Genomic	<i>PstI</i>	1210	649
4.09	umc52	p-umc52	DQ123899	Genomic	<i>PstI</i>	1500	824
4.10	php20608a	p-php20608	DQ007990	Genomic	<i>PstI</i>	780	1451
4.11	umc169	p-umc169	DQ123900	Genomic	<i>PstI</i>	670	813
5.01	npi409 <sup>b</sup>	p-npi409		Genomic	<i>PstI</i>	710	
5.02	umc90	p-umc90	DQ642434	Genomic	<i>PstI</i>	1240	1226
5.03	tub4	p-tub4	DQ007991	cDNA	<i>EcoRI/HindIII</i>	200	230
5.04	bnl4.36	p-bnl4.36	DQ642435	Genomic	<i>PstI</i>	2300	2210
5.05	csu93b	p-csu93	DQ015673	cDNA	<i>EcoRI/XhoI</i>	800	677

TABLE 1: Continued.

CBM	Marker/locus	Probe	Accession number	Type	Enzyme(s)	MaizeGDB insert size (bp)	Sequenced RFLP insert size
5.06	umc126a	p-umc126	AY771220	Genomic	<i>Pst</i> I	670	663
5.07	umc108	p-umc108	DQ642436	Genomic	<i>Pst</i> I	970	958
5.08	bnl5.24a <sup>a</sup>	p-bnl5.24		Genomic	<i>Pst</i> I	2500	
5.09	php10017	p-php10017	AY771221	Genomic	<i>Pst</i> I	470	526
6.01	umc85a	p-umc85	AY772450	Genomic	<i>Pst</i> I	600	561
6.02	umc59a <sup>a</sup>	p-umc59		Genomic	<i>Pst</i> I	930	
6.03	npi393	p-G23A-06	DQ015674	Genomic	<i>Pst</i> I	1200	1249
6.04	umc65a	p-umc65	AY772451	Genomic	<i>Pst</i> I	650	691
6.05	umc21	p-umc21	DQ123901	Genomic	<i>Pst</i> I	1050	1062
6.06	umc38a	p-umc38	DQ059316	Genomic	<i>Pst</i> I	1010	1022
6.07	umc132a(chk)	p-umc132	DQ1238902	Genomic	<i>Pst</i> I	500	472
6.08	asg7a	p-asg7	DQ642437	Genomic	<i>Pst</i> I	550	353
7.01	asg8(myb)	p-asg8	DQ642438	Genomic	<i>Pst</i> I	500	320
7.02	asg34a(msd) <sup>a</sup>	p-asg34		Genomic	<i>Pst</i> I	1350	
7.03	asg49	p-asg49	DQ059317	Genomic	<i>Pst</i> I	400	400
7.04	umc254	p-umc254	EU190457	Genomic	<i>Pst</i> I	1050	1000
7.05	umc245	p-umc245	AY772452	Genomic	<i>Pst</i> I	700	665
7.06	umc168	p-umc168	DQ059318	Genomic	<i>Pst</i> I	1080	1072
8.01	npi220a	p-G10F-01	DQ059319	Genomic	<i>Hind</i> III	400	406
8.02	bnl9.11a(lts) <sup>a</sup>	p-bnl9.11		Genomic	<i>Pst</i> I	2400	
8.03	umc124a(chk)	p-umc124	DQ059320	Genomic	<i>Pst</i> I	1160	1162
8.04	bnl7.08a <sup>a</sup>	p-bnl7.08		Genomic	<i>Pst</i> I	2300	
8.05	bnl2.369 <sup>a</sup>	p-bnl2.369		cDNA	<i>Eco</i> RI	700	
8.06	csu31a <sup>a</sup>	p-csu31		cDNA	<i>Eco</i> RI/ <i>Xho</i> I	800	
8.07	npi268a	p-npi268	DQ123903	Genomic	<i>Pst</i> I	710	688
8.08	npi414a	p-npi414	AY772453	Genomic	<i>Pst</i> I	870	893
8.09	agrr21 <sup>a</sup>	p-agrr21		cDNA	<i>Eco</i> RI/ <i>Hind</i> III	899	
9.01	umc109	p-umc109	AY772454	Genomic	<i>Pst</i> I	840	797
9.02	bz1 <sup>a</sup>	p-umc192		cDNA	<i>Pst</i> I	1750	
9.03	wx1 <sup>a</sup>	p-umc25		cDNA	<i>Eco</i> RI	2300	
9.04	csu147 <sup>a</sup>	p-csu147		cDNA	<i>Eco</i> RI/ <i>Xho</i> I	800	
9.05	umc95	p-umc95	AY772455	Genomic	<i>Pst</i> I	680	660
9.06	csu61a	p-csu61		cDNA	<i>Eco</i> RI/ <i>Xho</i> I	500	
9.07	asg12	p-asg12	DQ123904	Genomic	<i>Pst</i> I	700	647
9.08	csu54b <sup>a</sup>	p-csu54		cDNA	<i>Eco</i> RI/ <i>Xho</i> I	1400	
10.01	php20075a(gast)	p-php20075	DQ059321	Genomic	<i>Pst</i> I	1400	1311
10.02	npi285a(cac) <sup>a</sup>	p-npi285		Genomic	<i>Pst</i> I	1250	
10.03	umc130	p-umc130	DQ059322	Genomic	<i>Pst</i> I	640	634
10.04	umc64a	p-umc64		Genomic	<i>Pst</i> I	710	
10.05	umc259a	p-umc259	DQ123905	Genomic	<i>Pst</i> I	550	579
10.06	umc44a	p-umc44	AY772456	Genomic	<i>Pst</i> I	800	794
10.07	bnl7.49a(hmd) <sup>a</sup>	p-bnl7.49		Genomic	<i>Pst</i> I	2100	

<sup>a</sup>Full length RFLP sequence not available.<sup>b</sup>Unable to sequence with vector primers.<sup>c</sup>Poly A tail at both ends of insert sequence.

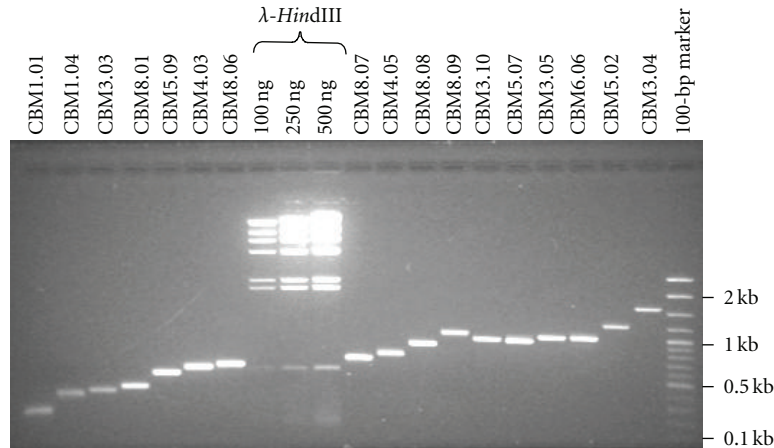


FIGURE 1: EtBr-stained agarose gel of CBM inserts. PCR amplification products for individual prepared CBM RFLP probes (indicated above the lanes), loaded from left to right in order of their predicted size. Mass standards (*HindIII*-digested  $\lambda$  DNA) are labeled, and the total amounts loaded appear above the lanes. Size standards (100-bp marker) are included, and selected band sizes are indicated at right.

as more recent advances in genome structure and evolution [21, 26–34].

The maize genome has been charted in two fundamentally different ways: genetic/linkage maps and physical maps. Linkage mapping is based on recombination and cosegregation frequencies of markers, whereas maize physical maps comprise a larger array of maps most commonly based on molecular cytology or assemblies of contiguous DNA restriction fragments, overlapping clone-based contigs, or DNA sequence assemblies [2, 21, 32, 34–43]. In the last 20 years, the linkage maps and the BAC-based physical contig maps have demonstrated the greatest utility in maize. During the 1980s and 1990s the number of mapped loci on the linkage maps of maize increased markedly, as conventional mutant-based linkage maps were improved or replaced by high-density molecular marker maps [20, 31, 44]. These maps are based on DNA sequence polymorphisms detected with Southern blots of cloned restriction fragment length polymorphism (RFLP) probes or gel electrophoresis of PCR products of microsatellites and simple sequence repeats. These same molecular markers were mapped onto BACs in library arrays for annotation of the physical map with genetic markers [31, 37, 40].

The RFLP probes have featured prominently in the production of the genetic maps in maize (<http://www.maizegdb.org/map.php>). The polymorphic nature of the maize genome makes RFLPs ideal for use in mapping populations made from any two of the hundreds of divergent inbred lines of maize [45]. A select subset of evenly distributed RFLP markers, designated as Core Bin Markers (CBMs), provide a common and standardized set of linkage makers, which conveniently parcel out the maize genome into 100 linkage bins [20]. For example, the first bins on maize chromosome 1, starting from the telomere of the short arm (1S), are designated 1.00, 1.01, 1.02, and so on until the last bin, 1.11, at the telomere of the long arm (1L). By hybridization of the RFLP probes to the arrayed members of the maize BAC libraries used to build the contig maps, the genetic and

TABLE 2: Major steps in the identification and maize marker-based selection of a sorghum BAC for use as a transgenomic FISH probe in maize. The figures and tables corresponding to the steps are indicated.

Step	Procedure
1	Grow maize CBM-RFLP cultures and purify insert (Figure 1; Table 1)
2	Radiolabel CBM insert probes for use in filter library (step 3) or Southern blot (step 5) hybridizations
3	Hybridize probes (Tm-25°C) to <i>Sorghum propinquum</i> BAC library (YRL, 36,000 BACs, $\sim 6 \times$ coverage; Figure 2)
4	Score autoradiographs to identify homologous sorghum BAC and examine location on the FPC map (Figure 2; Table 3)
5	Grow identified overlapping BACs and verify them by Southern blot (Figure 3)
6	Select one BAC for use as a transgenomic FISH probe on maize pachytene chromosome spreads from maize addition lines of oat (Figure 4)

physical maps have been integrated and used to guide the sequencing and assembly of the maize genome [21, 31, 32, 34, 37, 46].

Similarly, efforts to localize RFLP probes, like the CBM probes, on the maize cytogenetic maps have been undertaken but are hindered by the difficulty of detecting the small RFLP DNA segments by FISH. The cytogenetic maps are therefore among the least developed of all the maps of the maize genome, despite the early and prominent work in maize cytogenetics [22]. Although several studies have localized various types of repeat sequences on the chromosomes

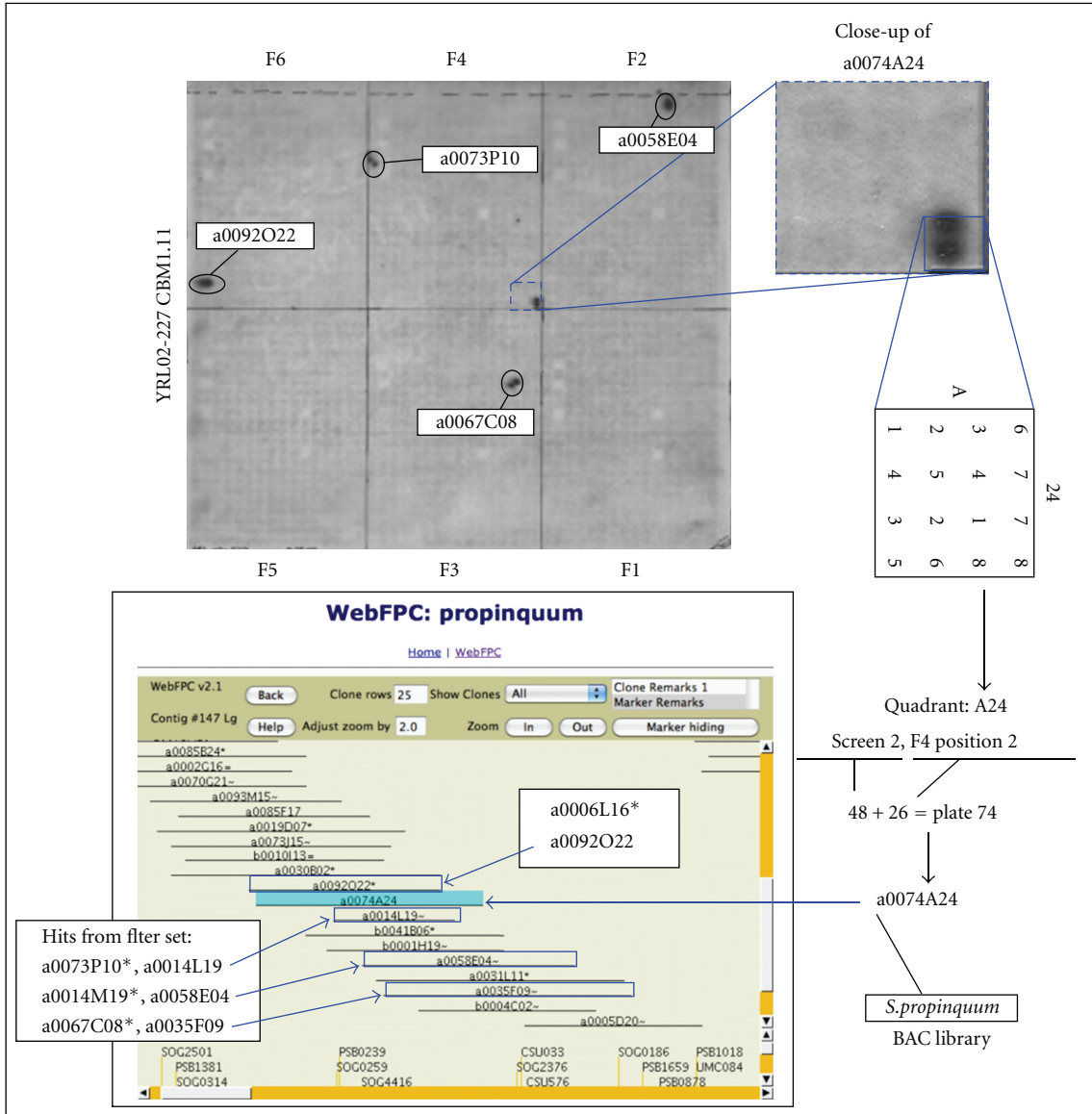


FIGURE 2: BAC filter hybridization, scoring of hits, and location in fingerprint contig (FPC) map for maize CBM1.11 (umc161). A representative filter-screen hybridization (Table 2, step 2) and the autoradiograph from one of the two filters are shown (top). A total of 5 positive signals are seen (circled or boxed), and the scoring scheme for one of them (a0074A24) is illustrated at the right. The autoradiograph is labeled to show the location of the 384-well plate fields 1–6 (F1–F6), and the geometry duplicate spotting of the eight individual plates per field is indicated at the right for quadrant A24. A close-up of one hit (position 2, field 3) is shown with the resulting decoded BAC ID of plate 74 position A-24 (a0074A24). This BAC, along with four other contiguous (arrows) BACs, is indicated within contig number 113 of the *Sorghum propinquum* FPC map (screen capture bottom left). Four additional BACs (asterisks) were detected, but not found to belong to a contig.

of maize, relatively few single-copy sequences have been cytologically mapped on any of the maize chromosomes other than chromosome 9 [15, 16, 36, 38, 42, 47–52]. Because of the extensive use of the CBM loci in maize genetics, along with other RFLPs, these markers have been selected for the development of a cytogenetic map of maize so that connections with the linkage and physical maps can be maximized [35, 53]. These efforts make use of meiotic pachytene-stage chromosomes because they are about 6–25 times longer, providing better axial resolution than their

shorter somatic counterparts [2]. Although the location of the CBM probes on the meiotic chromosome karyotype of maize has been predicted from recombination nodule frequencies, the location of the genetic bins and their boundary CBM loci on the actual chromosomes remain largely unverified [35, 54–56].

The smallest probes and targets that can be localized and reliably detected on maize pachytene chromosomes have averaged 2.4–3.1 Kb in length [38, 42], but the maize CBM-RFLP probes were originally chosen to be small, less

TABLE 3: Maize CBM-RFLP identified sorghum BACs.

CBM	RFLP-probe	No. of BACS indentified	[contig] Member BACs scored	Southern blot comments
1.01	tub1	2	[868] a0039M23 [154] a0053G19	Contiguous BACs not identified
1.02	umc157	3	[154] a0045K08, a0064A06, a0055E08	All BACs confirmed
1.03	umc076	4	[193] a0046J07, a0086C09 [389] a0004B12 [?] a0071N02	BACs a0046J07, 86C09, and 71N02 confirmed
1.04	asg45	5	[490] a0073P09 [536] a0080O07 [573] a0086J05, a0075I08 [967] a0036D16	Contiguous BACs not identified
		7	[150] a0001K22 [187] a0061M20 [557] a0041B19 [?] a0029B11, a0036D16, a0043B12, a0052B23	
1.05	csu003	4	[179] a0001C04, a0026E17, a0052G17, a0094G22	BACs a0001C04, 26E17, 94G22 confirmed
1.06	umc67a	2	[868] a0026H06, a0038A08	
1.07	asg62	5	[550] a0084J12 [537] a0084J14 [443] a0083B09 [542] a0095N15 [567] a0096N18	Contiguous BACs not identified
1.08	umc128	12	[168] a0029P06, a0055L05, a0059H18, a0058G14, a0066G14, a0070C24, a0071B13, a0072H12, a0075H05, a0081P22, a0083A01, a0092C10	Confirmation Hybe failed; only control seen
1.09	cjd2	8	[159] a0011I10, a0023C11, a0039E21, a0056O22, a0081H24, a0091D22, a0093H06, a0093P21	BACs 23C21-93P21 confirmed
1.10	umc107a	8	[154] a0006I08, a0016J24, a0041J08, a0041O17, a0043G04, a0046O22, a0053G19, a0061G12	All BACs confirmed
1.11	umc161a	5	[147] a0014L19, a0035F09, a0058E04, a0074A24, a0092O22	All BACs confirmed; the restriction fragments of BACs 14L19 & 35F09 were smaller than those of other BACs
1.12	bnl6.32	6	[147] a0014L19, a0035F09, a0058E04 [?] a0014M19, a0067C08, a0006L16	
3.01	umc32a	9	[1] a0016H11, a0020E10, a0038G18, a0049H03, a0066I03, a0083B22, a0092J15, a0092J15, a0092K15 [293] a0048H03 [?] a0035E11, a0038G18, a0015L17, a0017E12	All BACs in contig 1 confirmed
3.02	csu32	6	[1332] a0010D09, a0017F16, a0035I06, a0066B14, a0066B20 [?] a0015J10	
3.03	asg24a			Contiguous BACs not identified
3.04	asg48	0		Contiguous BACs not identified

TABLE 3: Continued.

CBM	RFLP-probe	No. of BACS indentified	[contig] Member BACs scored	Southern blot comments
3.05	umc102	9	[898] a0016D10 [884] a0018B20 [394] a0055N13 [246] a0085B11 [894] a0083B12 [1257] a0090I03 [?] a007P18, a0012G07, a0027C12	Contiguous BACs not identified
3.06	bnl5.37a	8	[64] a0010K10, a0042I24, a0043H04, a0045D02, a0047F15, a0047J02, a0058O10, a0096N10	
3.07	bnl6.16a	7 (8)	[56] a0014M18, a0020L03, a0029M08 = a0029M07*, a0050D18, a0085M21 [143] a0051P09 [?] a0052D16	
3.08	umc17a	6	[49] a0002O18, a0041K14 [85] a0074H09 [?] a0074H10, a0096D08, a0035C01	
3.09	umc63a	9	[1292] a0021P11, a0025P06, a0046A02, a0043J05, a0044B15, a0055P13, a0029C04 [?] a0029C03	
3.10	cyp1	6	[1118] a0029L19, a0067D01, a0078E10 [89] a0017J07, a0018N05 [?] a0096D16	All BACs in contig 1118 as well as BAC a0096D16 confirmed
4.01	agrr115a			PolyA at both ends
4.02	php20725a	10	[1] a0016H11, a0092J15, a0092K15, a0049H03 [1292] a0021P11, a0029C03, a0044B15, a0046A02, a0043J02, a0055P13	Confirmation Hybe failed
4.03	umc31a	5	[1320] a0001I16 [3] a0067O16 [?] a0057L09, a0084A23, a0007F05	Contiguous BACs not identified
4.04	npi386	7	[232] a0002B15, a0019A12, a0035B24, a0058L10, a0071D21, a0078C04, a0082H03	All BACs confirmed
4.05	agrr37b	6	[391] a0023C20, a0054H21, a0055K09, a0068N16, a0080D06, a0083G05	All BACs except 55K09 confirmed
4.06	umc156a	3	[280] a0059B12, a0061G01, a0067K14, a0089D08	
4.07	umc66a			Contiguous BACs not identified
4.08	umc127c	3	[290] a0082D10, a0059L11, a0033K19	BACs 59L11 and 33K19 confirmed
4.09	umc32	6	[352] a0030M10, a0045N24, a0050L19, a0056E02, a0069D03, a0072G04	
4.10	php20608	6	[313] a0008B07, a0015N19, a0019O20, a0026C03, a0063A16, a0064O16	All BACs confirmed
4.11	umc169	5	[316] a0012H04, a0017H01, a0038N14, a0040B11, a0094J01	
5.01	npi409	2	[483] a0006F01 [813] a0002O16	Contiguous BACs not identified
5.02	umc90	2	[916] a0003P04 [1120] a0038C23	Contiguous BACs not identified

TABLE 3: Continued.

CBM	RFLP-probe	No. of BACS indentified	[contig] Member BACs scored	Southern blot comments
		4	[154] a0045K08, a0055E08, a0064A06 [?] a0034N18	All BACs confirmed
5.03	tub4	0		
5.04	bnl4.36	8	[118] a0025E21, a0024H23, a0046J09, a0045L08, a0041E24, a0069P23, a0058E11, a0051H03, a0052I15 [314] a0013B03, a0061I09, a0069K04 [385] a0045M19, a0078E12, a0078A08, a0066D12	All BACs confirmed except 13B03
5.05	csu93b	7	[193] a0007P01, a0046J07, a0058O08, a0069L09, a0078B19, a0086C09 [382] a0009P04	
5.06	umc125a	4	[280] a0067K14, a0061G01, a0059B12, a0089D08	All BACs confirmed
5.07	umc108	14	[287] a0095P22, a0061G08, a0063D06, a0050A23, a0052I05, a0054L12, a0046N22 [1108] a0023P17, a0004F02, a006N04 [333] a0020D11, a0029N15 [1288] a0030E06, a0038E06	All BACs in contig 287 confirmed
5.08	bnl5.24a	8	[278] a0042O01, a0020K19, a0023D21, a0095O21, a0095O19, a0081C19, a0063M08, a0002O03 [1017] a0017F09, a0017E09, a0041G23, a0019G02, a0067B22	All BACs confirmed except 63M08
5.09	php10017	6	[275] a0028A23, a0033G16, a0043E09, a0057K02, a0060N20 [?] a0061I19	All BACs confirmed except 43E09
6.01	umc085a	10	[426] a0006I03, a0009K09, a0072P24 [205] a0025E20, a0041O09, a0061M21, a0057H05, a0067N09, a0074E15, a0085D02	All BACs S. blot confirmed except 57H05
6.02	umc059a	9	[366] a0004P17, a0006P16, a0019D19, a0054G04, a0063H16, a0069J20, a0080H11, a0082K02, a0089K05	All BACs confirmed except 19D19 and 54G04
6.03	npi393	6	[759] a0004A06, a0017E10 [528] a0006D09, a0008G11, a0063A20 [?] a0039N21	All BACs confirmed
6.04	umc65a			Contiguous BACs not identified
6.05	umc21	5	[382] a0006P21, a0030G09, a0036H03, a0039E04, a0061C05	
6.06	umc38a			Contiguous BACs not identified
6.07	umc132a	9	[327] a0030L20, a0040J01, a0056F09, a0060H04, a0070I02, a0070K04, a0070K07, a0073H20 [?] a0020O17	All BACs except 20O17 confirmed with cross-Hybe to negative control
6.08	asg7a	6	[323] a0018I04, a0032J21, a0032K17, a0068H17, a0084B19 [486] a0015L19	Confirmation failed
8.01	npi220a	6	[22] a0027H19, a0063B11, a0080P02, a0084E01, a0096C21 [188] a0019O09	All BACs confirmed



TABLE 3: Continued.

CBM	RFLP-probe	No. of BACS identified	[contig] Member BACs scored	Southern blot comments
8.02	bnl9.11	8	[421] a0042P09, a0047M08, a0072L19, a0007N23, a0089I26 = 89I23* [908] a0074H18 [?] a0079F24, a0035C01	
8.03	umc124a	7	[6] a0014C07, a0017N03, a0040E17, a0078K17, a0002I13 = a0002I16(?), a0083P05	
8.04	bnl7.08a			Contiguous BACs not identified
8.05	bnl2.369			Contiguous BACs not identified
8.06	csu31a	13	[45] a0042J03, a0043C22, a0062K14, a0064A02 [233] a0089H10 = a0089G10* [359] a0078O19 = a0078N19* [915] a0085F10 = [866] 77F10* [?] a0035C01, a0044F14, a0053E10 = 51E10*, a0013N08, a0045C22, a0052D10,	
8.07	npi268a	10	[61] a0033M15, a0073N21, a0039H13, a0045D11, a0059E23, a0065K05, a0068E24, a0072D01, a0078K07, a0089A17	Confirmation failed; only positive control seen
8.08	npi414a	9	[58] a0007I19, a0007C07, a0033M05, a0073D02, [1019] a0002I19 = a0007I19* [1125] a0023G22 [223] a0046C21 [1278] a0090D06 [?] a0019G24	
8.09	agrr21			Contiguous BACs not identified

\* Whenever BAC scoring was ambiguous, possible alternate identified BACs were also grown for S. blot verification.

than 1 Kb. Their small size facilitated subsequent single-pass sequencing, but it put them well below the FISH detection limit [2, 20]. In addition, because of the complexity and low gene density of the maize genome, the simple use of maize BAC clones as FISH probes directly on maize chromosomes is not productive without additional steps for the amplification of single-copy sequences within each BAC [38, 52].

In the experiments reported here, we used an approach for indirect FISH mapping of the CBM loci that exploits the recent divergence of maize and sorghum by identifying sorghum BACs, homologous to maize-markers, for use as representative FISH probes [35, 53]. The procedures for the selection, verification, and use of sorghum BACs as transgenomic FISH probes for maize are described. The identity of homologous BAC clones for the CBM loci on several maize chromosomes is presented along with methods and criteria for selecting a single suitable sorghum BAC for each maize marker.

## 2. Materials and Methods

**2.1. Plant Materials.** Plants of a disomic maize chromosome addition line of oat, OMA<sub>d</sub>1.36, carrying a B73 maize

chromosome 1 in an oat genome background [57, 58] were grown in the Mission Road Research Facility greenhouse (Florida State University, Tallahassee, FL, USA) or in Conviron growth chambers under short- and long-day conditions as described previously [57, 58].

**2.2. Full-Length Insert Sequences for CBM and Other Maize RFLP Probes.** Maize RFLP probes were obtained as freezer cultures from the UMC RFLP Lab (G. Davis, T. Muskett, University of Missouri-Columbia, <http://www.maizegdb.org/cgi-bin/displaypersonrecord.cgi?id=97483>). The full-length insert sequences (FLIS) were determined for 66 of the 90 maize CBM RFLP probes by primer walk sequencing across both strands (DNA Sequencing Facility, Department of Biological Science, Florida State University, Tallahassee, FL). The resulting sequence contigs were assembled and analyzed with Sequencher (Gene Codes Corporation, Ann Arbor, MI), inspected for accuracy, and verified by BLAST sequence-similarity searches. Vector sequences were removed, and detailed annotations along with the RFLP-FLIS sequences were submitted to GenBank. An additional 85 non-CBM maize RFLP probes were selected, arrayed as freezer cultures in 96-well plates, and fully sequenced on both strands by primer walking (SeqWrite, Houston, TX). Contig

analysis, verification, and submission were carried out as described above. All of the maize RFLP-FLIS accessions can be retrieved from the supporting data available from the public NCBI Genbank database (<http://www.ncbi.nlm.nih.gov/>) with the query “Bass, maize, RFLP”

**2.3. Maize RFLP Probe-Based Selection of Sorghum Propinquum BACs.** The *Sorghum propinquum* BAC library filter array [59] was screened by hybridization with RFLP probes as previously described [35, 53]. Briefly, insert sequences from plasmids carrying CBM-RFLP probes were obtained from PCR products amplified with insert-flanking vector primers. The PCR products were trimmed of vector sequences by restriction enzymes, gel purified, and used (50 ng) for random-primed labeling with  $^{32}\text{P}$ -dCTP (Rediprime II DNA Labeling System, GE Healthcare). The resulting probes were hybridized at a stringency of  $Tm-25^\circ\text{C}$  to the YRL BAC filters (two  $22 \times 22$ -cm filters per hybridization). The resulting BAC hits were scored, and the individual BAC identities were examined within the *S. propinquum* contig map (<http://www.stardaddy.uga.edu/fpc/WebAGCoL/propinquum/WebFPC/>). Overlapping BACs were then grown, and their DNA was isolated with the Qiagen Spin Miniprep kit (no. 27104, Valencia, CA). The miniprep BAC DNAs were digested with *Hind*III and subjected to Southern blot hybridization for homologous sequence verification with the same probe used in the BAC filter hybridizations.

**2.4. Chromosome Spreading for FISH.** Meiosis-stage florets from oat-maize addition lines were harvested, fixed, and stored, and anthers containing pachytene-staged meiocytes were identified as previously described [35]. Briefly, pre-emerged panicles were fixed in ethanol:acetic acid (3:1) solution for two days and stored in 70% ethanol until staging. For staging of the meiotic cells from fixed florets, single anthers were stained by the aceto-carmin squash method to identify pachytene-staged florets [60]. The remaining two pachytene-staged anthers were then stored in 70% ethanol at  $-20^\circ\text{C}$  until their use for FISH. Meiotic chromosomes were prepared in the initial steps as previously described [61] with modifications used by Danilova and Birchler [38]. Briefly, individual anthers were washed three times with deionized  $\text{H}_2\text{O}$ , washed twice for 5 minutes with citric buffer (10 mM sodium citrate, 10 mM EDTA, adjusted to pH 5 by addition of pH 5 citric acid), and then digested in a pectolytic enzyme solution (0.3% cytohellicase, 0.3% cellulase, 0.3% pectolyase in 30-mM citrate buffer, pH 4.5) for 2 hours. The tubes were then plunged into ice and immediately filled with 10:0.2 TE [(10 mM Tris-HCl (pH 7.6):0.2 mM EDTA-NaOH (pH 8.0))] to stop the reaction. The TE was aspirated, and the anthers were washed twice with 100% ethanol. The ethanol was then aspirated and replaced with 20  $\mu\text{l}$  ice-cold acetic acid:methanol solution (9:1). The anthers were gently macerated in the tube with a straight probe and incubated them on ice for at least 15 minutes to clear the cytoplasm before chromosome spreading. Eight  $\mu\text{l}$  of the cell suspension was then dropped from a micropipettor onto two slides that had been placed in a humid chamber

[16]. The chromosomes were gently spread as described by Koumbaris and Bass [53] by alternating the addition of ice-cold ethanol:acetic acid (3:1) solution with exposure to  $75^\circ\text{C}$  water vapor for a total of three rounds followed by the addition of 100% acetic acid and quick drying at  $65^\circ\text{C}$  for 3 minutes. The slides were then immediately FISH probed as described by Amarillo and Bass [35], but without the slide-aging step.

**2.5. BAC FISH.** Highly purified BAC DNA was isolated with the Qiagen Large Construct purification kit (Qiagen item No. 12462). The BAC DNA was digested with *Alu*I, and 2  $\mu\text{g}$  of the digested BAC DNA was direct-labeled with Alexa-Fluor-546-dCTP for FISH as previously described [35]. The probe cocktail also included centromere and whole chromosome painting probes, prepared and used as previously described [35, 53]. Image collection, chromosome straightening, and FISH chromosome locus determination were also carried out as previously described [53].

### 3. Results and Discussion

We previously developed a strategy using transgenomic BAC FISH to localize genetic marker sequences, such as RFLP probes, on maize chromosomes [53]. This strategy was initially developed to overcome the FISH detection limit so that small RFLP probes could be cytogenetically localized [38, 42]. It also avoided problems associated with direct use of maize BACs as FISH probes, namely, most maize BACs contain relatively few single-copy sequences for site-specific hybridization. The use of transgenomic FISH exploits the general colinearity and genic synteny between related species, supporting homologous cross-mapping in plants with large, complex genomes such as maize [53, 62]. In particular, the use of sorghum BACs as FISH probes on maize chromosomes works well because sorghum and maize diverged  $\sim 12$  mya, preserving genic sequences but not intergenic repetitive DNAs [30, 31, 35, 53, 63, 64].

**3.1. RFLP Probes Are Ideal Markers for Integrating Physical and Linkage Maps of Maize.** Having employed a filter hybridization method for selecting sorghum BACs that correspond to maize genetic markers, we attempted to accelerate the BAC selection process using an *in silico* approach. Many of the widely used maize RFLP probes are not sequenced or have only limited end sequence data. We therefore determined the FLIS of RFLP probes for markers that we selected for possible FISH mapping, including the CBM. Table 1 lists all 90 of the maize CBM-RFLPs along with their primary features and GenBank accession numbers for those that were completely sequenced. The table includes estimated insert sizes before our study as well as the vector-trimmed FLIS sizes that we obtained. A few of the CBM-RFLP probes were difficult to sequence. For example, CBM2.01 and CBM4.05 failed to yield good sequence traces with the vector primers, whereas the CBM4.01 insert is flanked by poly-A sequences on both sides. In addition, we determined the FLIS for an additional 85 non-CBM maize

RFLPs, listed in Supplemental Table 1 in supplementary material available online at doi: 10.1155/2011/386862. For these markers, we chose numerous probes from the “CSU” set [65] because they were derived from B73 cDNA libraries and were therefore expected to be easy to place on the B73 reference genome and to provide excellent probes for high-stringency cross-hybridization to sorghum genomic DNA libraries.

Attempts to use the RFLP sequence information to identify homologous *S. propinquum* BACs *in silico* failed because the limited sequence data for characterized sorghum BAC libraries was insufficient for unambiguous selection of homologous sorghum BACs. The *S. propinquum* physical FPC map is very well annotated with markers, and the clones are available on arrayed filters, but relatively few genomic sequence data were available for BLAST searches [59]. The *S. bicolor* genome sequence could be searched by BLAST, but the sequence was based on whole-genome shotgun sequencing, not directly linked BACs that could be obtained and used for FISH [66]. Although the maize RFLP-FLIS data turned out to be useful for maize genome annotation, we found that the use of the sequence information alone was not as productive as the conventional filter-hybridization method for identification of corresponding sorghum BAC clones.

**3.2. Purification of Maize Restriction Fragment Length Polymorphism (RFLP) Inserts.** The overall procedure for identification, verification, and selection of sorghum BACs to be used for FISH is summarized in Table 2. It begins with the purification of maize RFLP probes for filter hybridization onto the arrayed sorghum BAC library and ends with the selection of a single BAC to be used as a FISH probe. This procedure can be completed for four different RFLP markers in approximately 4 weeks.

First, the bacterial cultures containing the maize CBM-RFLP probes were grown and their inserts amplified by PCR with vector primers. Then the flanking poly-linker sequences were trimmed with the appropriate restriction enzymes, and ethanol precipitated (Table 1, Table 2, step 2, and Supplemental Table 1). The resulting PCR products were redissolved in 10–20  $\mu$ l of 10:0.2 TE and quantified by ethidium bromide (EtBr) staining in agarose gels as shown in Figure 1 for multiple different RFLP probes. The inserts from the maize RFLP probes were loaded from left to right in the order of expected fragment size, and the gel (Figure 1) included standards for mass (*Hind*III  $\lambda$  lanes) and size (100-bp marker lane). The preparations shown yielded inserts of the expected sized, except for CBM8.09, which was subsequently excluded because of additional difficulties in obtaining its FLIS.

The CBM inserts were then radiolabeled with  $^{32}$ P-dCTP and hybridized to the pair of *S. propinquum* BAC filters at Tm-25°C, and the resulting hybridization signals were scored for identification of homologous *Sorghum* BACs. These were located, when possible, within the online FPC BAC map (WebFPC for *S. propinquum*, <http://www.stardaddy.uga.edu/fpc/WebAGCoL/propinquum/WebFPC/>) built from

the same BAC library [64]. This procedure (Table 2, steps 3 and 4) is illustrated with CBM1.11 (umc161a) in Figure 2. A total of eight different sorghum BAC hits were detected, and those (5 BACs) identified on one of the two library filters are shown (Figure 2, circled double dots). All of these BACs were used to query the *S. propinquum* WebFPC, and five of the eight were overlapping in contig number 147 as shown in Figure 3(a) [64, 67]. This outcome exemplifies the successful detection of homologous BACs and is consistent with the sixfold genome coverage of this library [59].

**3.3. Southern Blot Confirmation and Selection of Homologous Sorghum BACs for FISH.** After filter hybridization, the overlapping BACs were grown from a freezer culture replica of the library. Next, BAC DNA was isolated by conventional plasmid minipreps, restriction enzyme digested, and subjected to Southern blot analysis for confirmation that each BAC contained RFLP-homologous sequences (Table 2, step 5). An example of this step is illustrated in Figure 3 with the BACs detected from the previous screen with CBM1.11 (umc161a). Confirmation blots also contain BACs from a separate selection experiment, providing multiple negative control BACs per blot (lanes 1–8, Figures 3(b) and 3(c)).

The confirmation blot for CBM1.11 resulted in the detection of a single cross-hybridizing band for each of the filter-hit BACs (Figure 3(c), lanes 9–13) but no bands for the negative control lanes. As expected, a common restriction fragment was observed for most of the five BACs in question, whereas two of them (Figure 3(c), lanes 9 and 13) showed bands of different sizes. This discrepancy probably resulted from the homologous sequences residing near one end or the other of the BAC, consistent with their size or location within the FPC BAC map. Consequently, these two BACs (a0092O22 and a0014L19) were demoted as candidate BACs to represent the maize CBM1.11 locus.

All together, 58 of the 59 maize CBM probes from maize chromosomes 1, 3–6, and 8 have been used to screen the sorghum BAC filter arrays. Of these, 47 (81%) resulted in the detection of overlapping BACs (Table 2, step 4), and 23 of them have passed the Southern blot confirmation test (Table 2, step 5) as summarized in Table 3. Previous work on maize chromosome 9 has shown that, with regard to this overall approach, once a marker has progressed through the point of Southern blot verification, the prospects for successful transgenomic BAC FISH is quite favorable [35].

The final steps involve the choice of a single BAC, primarily by a combination of four criteria: the BAC should (1) belong to a set of marker-detected, overlapping clones in a contig, (2) be centrally located within the group of detected BACs, (3) possess a cross-hybridizing restriction fragment that is common to other BACs in the group, and (4) grow well and yield at least 2  $\mu$ g of high-quality DNA obtained using a BAC large-scale purification kit. In cases where the first three criteria are met by more than one BAC, we typically choose the first one that meets the fourth criterion, even though such BACs probably share considerable amounts of sequence and any of them may work for FISH.

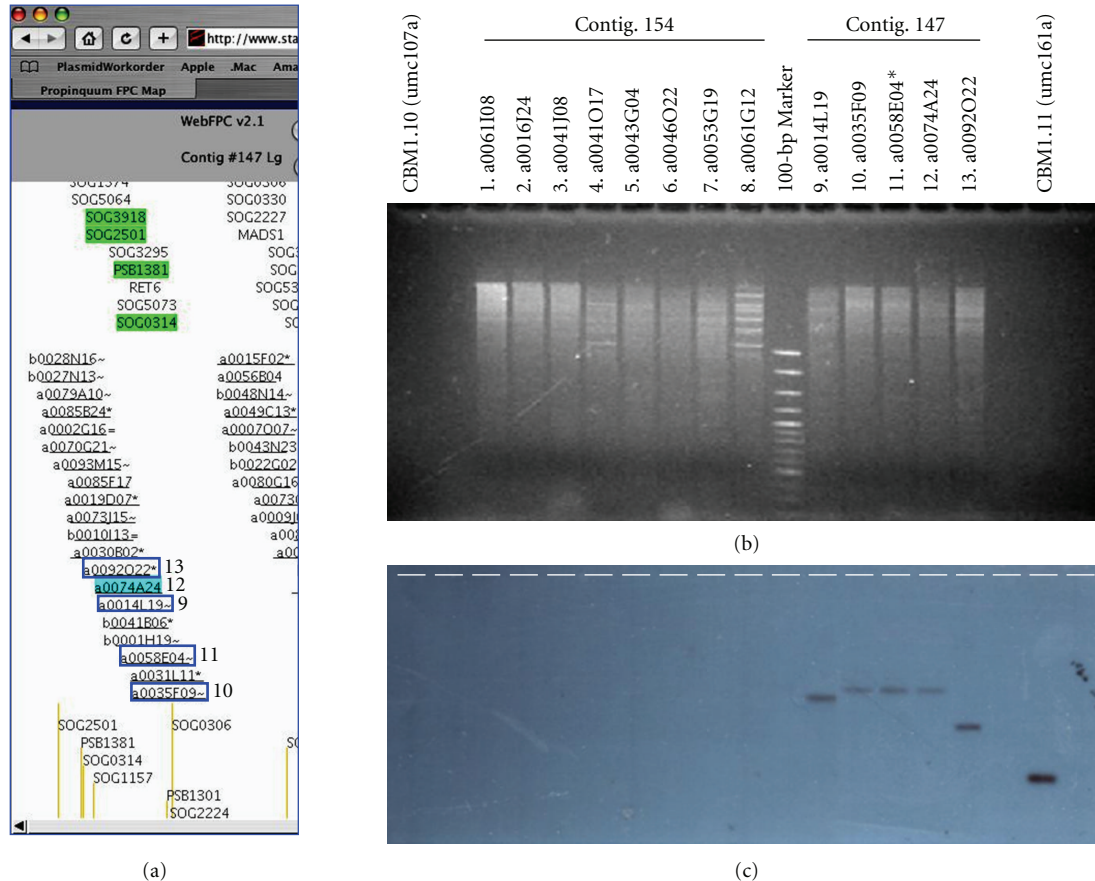


FIGURE 3: Southern blot confirmation of sorghum BACs after filter hybridization. Step 5 (Table 2) of the BAC selection procedure is illustrated with CBM1.11 (umc161a). (a) *Sorghum propinquum* FPC contig map showing contig number 147 and the five contiguous BACs (boxed) detected by filter hybridization with the CBM1.11 probe. Lane numbers are indicated beside the BACs. (b) Ethidium bromide-stained agarose gel of *Hind*III-digested BAC minipreps. This gel includes the BACs under investigation (lanes 9–13) along with another set (lanes 1–8) serving as negative controls. For each confirmation blot, a positive control lane [CBM1.11 (umc161a)] is included. It contains a trace amount of the same insert preparation that was used in this experiment and in the preceding filter hybridization. (c) Autoradiograph after hybridization with the CBM1.11 probe. The asterisk indicates the BAC that was eventually selected as part of step 6 (Table 2).

**3.4. Transgenomic BAC FISH Provides Locus-Specific Cytogenetic Mapping Data.** A maize-marker-selected sorghum BAC was direct-labeled for use as a FISH probe and hybridized, along with a multicolor probe mix, to pachytene spreads from maize chromosome addition lines of oat. These diploid (doubled haploid) lines contain the oat genome plus a single pair of maize chromosomes [57, 58]. Figure 4 illustrates *S. propinquum* BAC (a0053G04) FISH of the CBM1.10 locus on a maize B73 chromosome 1 addition line of oat. The multiprobe mix includes total DNA from the knobless Wilbur's Flint (KWF) line of maize, which paints the entire maize pachytene chromosome (Figure 4(b), FITC image). A centromere-specific probe, centC, first discovered by Ananiev et al. [68] is also included (Figure 4(d), Cy-5 image) along with the specific maize-marker-selected BAC probe (Figure 4(c), Rhodamine image). The nuclei were counterstained with DAPI (Figure 4(a), DAPI image), and 3D deconvolution images were collected as previously described [35, 53]. Images of the pachytene chromosomes were then traced and computationally straightened (Figure 4(f)).

The cytogenetic position was measured in this way for multiple images (Figure 4(g)), and the average location (as fractional distance along the chromosome arm) was determined. In this example, the maize marker CBM1.10 was indirectly FISH mapped, with the sorghum BAC (a0053G04), to 85% of the distance along the long arm. This cytological locus is 15 centiMcClintocks from the telomere [55], and the cytogenetic position is denoted as spb-CBM1.10\_L85 (umc107a) according to nomenclature first described by Koumbaris and Bass [53]. This location is in general agreement with that predicted from recombinant node frequency mapping in maize [54].

Sorghum BAC FISH mapping in maize is precise at the resolution of pachytene FISH even though the FISH signal is derived from sequences adjacent to the RFLP of interest. Furthermore, given the extensive segmental genome duplication in maize, we predict that these sorghum BACs will be useful as FISH probes for both the target CBM region and the corresponding duplicated segment. For example, sorghum BAC a0053G04 (Figure 4) was selected

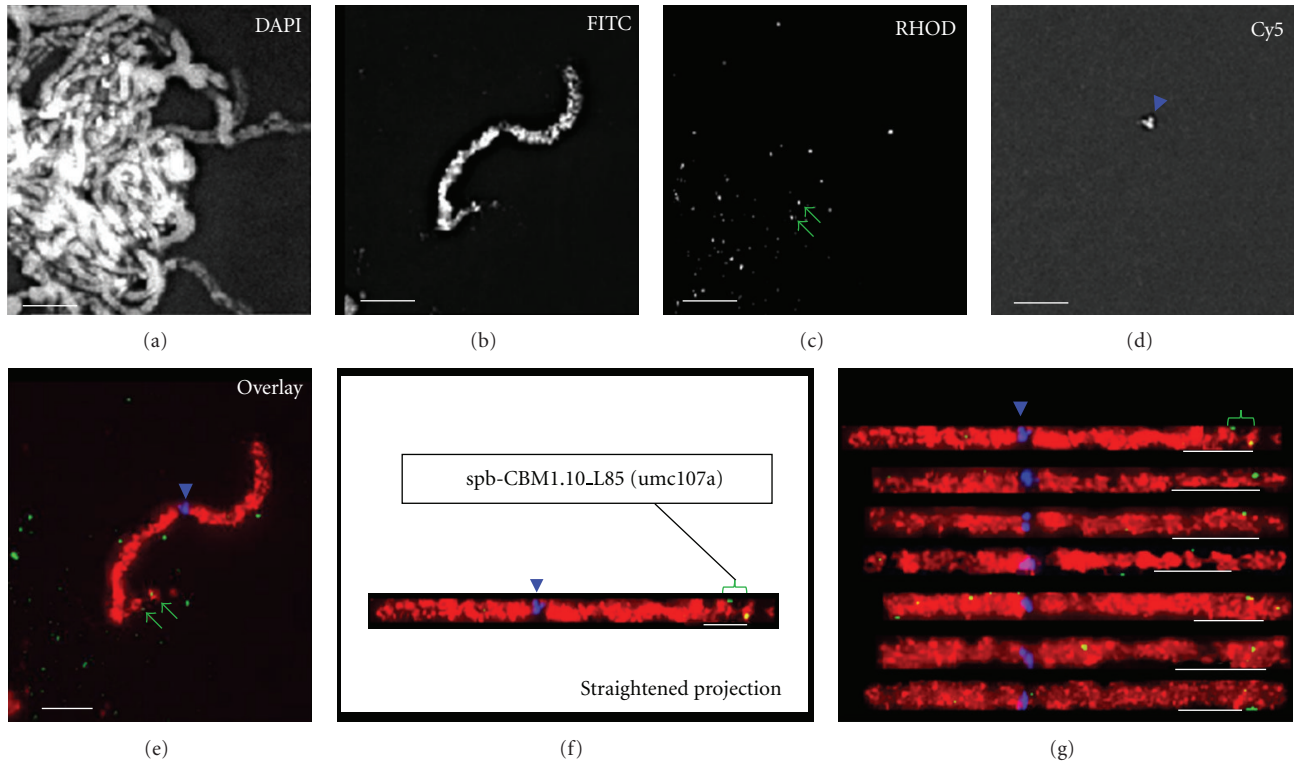


FIGURE 4: FISH mapping of maize CBM1.10 with sorghum BAC a0053G04. (a) A DAPI-stained image of spread pachytene chromosomes from OMAd1.36. (b) FITC image showing maize chromosome 1 direct-labeled with Alexa-488-dUTP-KWF total maize DNA. (c) Rhodamine image from direct-labeled sorghum BAC FISH signals (green arrows). (d) Cy-5 image of centromere FISH signal (blue arrowhead) with direct-labeled CentC. (e–g) Three-color overlay of the FITC (red), rhodamine (green), and Cy-5 (blue) images. (f) Straightened projection of the maize chromosome from panel (e). The locations of the centromere (blue arrowhead) and CBM1.10 BAC FISH signals (green bracket) are indicated along with the resulting cytogenetic locus name (boxed). (g) Straightened projections of six additional chromosomes aligned at their centromeres for comparison. All scale bars represent 5  $\mu\text{m}$ .

with a marker for chromosome arm 1L, but it is expected to hybridize to a region on chromosome arm 5S [21, 69].

#### 4. Conclusions

Generation and integration of the cytological maps with the linkage maps of maize represents a valuable endeavor, contributing to positional cloning, allowing for accurate extrapolation of translocation breakpoints within linkage maps, and helping resolve discrepancies among various maps. In this study, we describe the method for using sorghum BACs to FISH map small widely used maize RFLP probe markers. We have shown that 44 of 59 target maize RFLP loci can be successfully used to screen for homologous and presumably syntenic-region sorghum BACs. So far, half of these have passed the Southern blot verification test, predictive of high success rate in subsequent transgenomic FISH mapping experiments. BACs from small-genome relatives of maize provide gene-rich reagents suitable for single-locus FISH mapping, a strategy that can be employed in other species. This approach hinges on the availability of arrayed BAC libraries and corresponding filters. Numerous such plant BAC libraries exist and are available as filter arrays (CUGI

at <http://www.genome.clemson.edu/>). This technique can be extended therefore to a large number of other plant species. Even in cases where the small-genome relative lacks an FPC physical map (i.e., unable to carry out the last part of step 4 of Table 2), one can still use shared restriction fragments (part of Step 5, Table 2) to identify overlapping BACs. Finally, in addition to solving the FISH detection limit, application of this scheme also generates evolutionarily meaningful links between the genomic and genetic maps of the two related species.

#### Acknowledgments

The authors thank Amy M. Win, Katherine M. Miller, Tabatha M. McHill, and Roberta S. Hill for technical help on this project and Anne B. Thistle, Shaun P. Murphy, Amber N. Brown, and Elizabeth S. Howe for useful comments on the manuscript. This work was supported by the National Science Foundation (DBI-0321639).

#### References

- [1] S. Aulard, L. Monti, N. Chaminade, and F. Lemeunier, "Mitotic and polytene chromosomes: comparisons between

- Drosophila melanogaster* and *Drosophila simulans*,” *Genetica*, vol. 120, no. 1–3, pp. 137–150, 2004.
- [2] D. M. Figueroa and H. W. Bass, “A historical and modern perspective on plant cytogenetics,” *Briefings in Functional Genomics and Proteomics*, vol. 9, no. 2, pp. 95–102, 2010.
  - [3] S. M. Gartler, “The chromosome number in humans: a brief history,” *Nature Reviews Genetics*, vol. 7, no. 8, pp. 655–660, 2006.
  - [4] M. Gatti and S. Pimpinelli, “Cytological and genetic analysis of the Y chromosome of *Drosophila melanogaster*. I. Organization of the fertility factors,” *Chromosoma*, vol. 88, no. 5, pp. 349–373, 1983.
  - [5] E. R. Lozovskaya, D. A. Petrov, and D. L. Hartl, “A combined molecular and cytogenetic approach to genome evolution in *Drosophila* using large-fragment DNA cloning,” *Chromosoma*, vol. 102, no. 4, pp. 253–266, 1993.
  - [6] B. J. Trask, “Human cytogenetics: 46 chromosomes, 46 years and counting,” *Nature Reviews Genetics*, vol. 3, no. 10, pp. 769–778, 2002.
  - [7] N. Fedoroff and D. Botstein, *The Dynamic Genome: Barbara McClintock’s Ideas in the Century of Genetics*, Cold Spring Harbor Press, Plainview, NY, USA, 1992.
  - [8] H. B. Creighton and B. McClintock, “A correlation of cytological and genetical crossing-over in *Zea mays*,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 17, no. 8, pp. 492–497, 1931.
  - [9] B. McClintock, “Chromosome morphology in *Zea mays*,” *Science*, vol. 69, no. 1798, p. 629, 1929.
  - [10] B. McClintock, “The order of the genes C, Sh and Wx in *Zea mays* with reference to a cytologically known point in the chromosome,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 17, no. 8, pp. 485–491, 1931.
  - [11] P. F. Ambros, M. A. Matzke, and A. J. M. Matzke, “Detection of a 17 kb unique sequence (T-DNA) in plant chromosomes by in situ hybridization,” *Chromosoma*, vol. 94, no. 1, pp. 11–18, 1986.
  - [12] P. F. Fransz, M. Stam, B. Montijn et al., “Detection of single-copy genes and chromosome rearrangements in *Petunia hybrida* by fluorescence in situ hybridization,” *Plant Journal*, vol. 9, no. 5, pp. 767–774, 1996.
  - [13] W. J. Peacock et al., “Highly repeated DNA sequence limited to knob heterochromatin in maize,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 78, no. 7, pp. 4490–4494, 1981.
  - [14] J. Xu and E. D. Earle, “Direct and sensitive fluorescence in situ hybridization of 45S rDNA on tomato chromosomes,” *Genome*, vol. 37, no. 6, pp. 1062–1065, 1994.
  - [15] D. L. Shen, Z. F. Wang, and M. Wu, “Gene mapping on maize pachytene chromosomes by in situ hybridization,” *Chromosoma*, vol. 95, no. 5, pp. 311–314, 1987.
  - [16] A. Kato, J. C. Lamb, and J. A. Birchler, “Chromosome painting using repetitive DNA sequences as probes for somatic chromosome identification in maize,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 37, pp. 13554–13559, 2004.
  - [17] J. C. Lamb and J. A. Birchler, “Retroelement genome painting: cytological visualization of retroelement expansions in the genera *zea* and *tripsacum*,” *Genetics*, vol. 173, no. 2, pp. 1007–1021, 2006.
  - [18] M. A. Lysak, P. F. Fransz, H. B. M. Ali, and I. Schubert, “Chromosome painting in *Arabidopsis thaliana*,” *Plant Journal*, vol. 28, no. 6, pp. 689–697, 2001.
  - [19] M. A. Lysak, A. Pecinka, and I. Schubert, “Recent progress in chromosome painting of *Arabidopsis* and related species,” *Chromosome Research*, vol. 11, no. 3, pp. 195–204, 2003.
  - [20] G. L. Davis, M. D. McMullen, C. Baysdorfer et al., “A maize map standard with sequenced core markers, grass genome reference points and 932 expressed sequence tagged sites (ESTs) in a 1736-locus map,” *Genetics*, vol. 152, no. 3, pp. 1137–1172, 1999.
  - [21] P. S. Schnable, D. Ware, R. S. Fulton et al., “The B73 maize genome: complexity, diversity, and dynamics,” *Science*, vol. 326, no. 5956, pp. 1112–1115, 2009.
  - [22] M. M. Rhoades, “The early years of maize genetics,” *Annual Review of Genetics*, vol. 18, pp. 1–29, 1984.
  - [23] M. A. Arteaga-Vazquez and V. L. Chandler, “Paramutation in maize: RNA mediated trans-generational gene silencing,” *Current Opinion in Genetics and Development*, vol. 20, no. 2, pp. 156–163, 2010.
  - [24] K. M. McGinnis, “RNAi for functional genomics in plants,” *Briefings in Functional Genomics and Proteomics*, vol. 9, no. 2, Article ID elp052, pp. 111–117, 2010.
  - [25] N. M. Springer, “Small RNAs: how seeds remember to obey their mother,” *Current Biology*, vol. 19, no. 15, pp. R649–R651, 2009.
  - [26] C. M. Andorf, C. J. Lawrence, L. C. Harper, M. L. Schaeffer, D. A. Campbell, and T. Z. Sen, “The Locus Lookup tool at MaizeGDB: identification of genomic regions in maize by integrating sequence information with physical and genetic maps,” *Bioinformatics*, vol. 26, no. 3, pp. 434–436, 2010.
  - [27] R. S. Baucom, J. C. Estill, C. Chaparro et al., “Exceptional diversity, non-random distribution, and rapid evolution of retroelements in the B73 maize genome,” *PLoS Genetics*, vol. 5, no. 11, Article ID e1000732, 2009.
  - [28] E. S. Buckler, B. S. Gaut, and M. D. McMullen, “Molecular and functional diversity of maize,” *Current Opinion in Plant Biology*, vol. 9, no. 2, pp. 172–176, 2006.
  - [29] A. H. Paterson, M. Freeling, H. Tang, and X. Wang, “Insights from the comparison of plant genome sequences,” *Annual Review of Plant Biology*, vol. 61, pp. 349–372, 2010.
  - [30] Z. Swigonova, J. Lai, J. Ma et al., “Close split of sorghum and maize genome progenitors,” *Genome Research*, vol. 14, no. 10, pp. 1916–1923, 2004.
  - [31] F. Wei, E. D. Coe, W. Nelson et al., “Physical and genetic structure of the maize genome reflects its complex evolutionary history,” *PLoS Genetics*, vol. 3, no. 7, pp. 1254–1263, 2007.
  - [32] F. Wei, J. Zhang, S. Zhou et al., “The physical and genetic framework of the maize B73 genome,” *PLoS Genetics*, vol. 5, no. 11, Article ID e1000715, 2009.
  - [33] J. Yu and E. S. Buckler, “Genetic association mapping and genome organization of maize,” *Current Opinion in Biotechnology*, vol. 17, no. 2, pp. 155–160, 2006.
  - [34] S. Zhou, F. Wei, J. Nguyen et al., “A single molecule scaffold for the maize genome,” *PLoS Genetics*, vol. 5, no. 11, Article ID e1000711, 2009.
  - [35] F. I. E. Amarillo and H. W. Bass, “A transgenomic cytogenetic sorghum (*Sorghum propinquum*) bacterial artificial chromosome fluorescence in situ hybridization map of maize (*Zea mays* L.) pachytene chromosome 9, evidence for regions of genome hyperexpansion,” *Genetics*, vol. 177, no. 3, pp. 1509–1526, 2007.
  - [36] C. C. Chen, C. M. Chen, F. C. Hsu, C. J. Wang, J. T. Yang, and Y. Y. Kao, “The pachytene chromosomes of maize as revealed by fluorescence in situ hybridization with repetitive DNA sequences,” *Theoretical and Applied Genetics*, vol. 101, no. 1–2, pp. 30–36, 2000.

- [37] K. C. Cone, M. D. McMullen, I. V. Bi et al., "Genetic, physical, and informatics resources for maize. On the road to an integrated map," *Plant Physiology*, vol. 130, no. 4, pp. 1598–1605, 2002.
- [38] T. V. Danilova and J. A. Birchler, "Integrated cytogenetic map of mitotic metaphase chromosome 9 of maize: resolution, sensitivity, and banding paint development," *Chromosoma*, vol. 117, no. 4, pp. 345–356, 2008.
- [39] J. Hans De Jong, P. Fransz, and P. Zabel, "High resolution FISH in plants—techniques and applications," *Trends in Plant Science*, vol. 4, no. 7, pp. 258–263, 1999.
- [40] J. Gardiner, S. Schroeder, M. L. Polacco et al., "Anchoring 9,371 maize expressed sequence tagged unigenes to the bacterial artificial chromosome contig map by two-dimensional overgo hybridization," *Plant Physiology*, vol. 134, no. 4, pp. 1317–1326, 2004.
- [41] J. Jiang and B. S. Gill, "Nonisotopic in situ hybridization and plant genome mapping: the first 10 years," *Genome*, vol. 37, no. 5, pp. 717–725, 1994.
- [42] C. J. R. Wang, L. Harper, and W. Z. Cande, "High-resolution single-copy gene fluorescence in situ hybridization and its use in the construction of a cytogenetic map of maize chromosome 9," *Plant Cell*, vol. 18, no. 3, pp. 529–544, 2006.
- [43] J. Jiang and B. S. Gill, "Current status and the future of fluorescence in situ hybridization (FISH) in plant genome research," *Genome*, vol. 49, no. 9, pp. 1057–1068, 2006.
- [44] T. Helentjaris, M. Slocum, S. Wright, A. Schaefer, and J. Nienhuis, "Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms," *Theoretical and Applied Genetics*, vol. 72, no. 6, pp. 761–769, 1986.
- [45] S. A. Flint-Garcia, A. C. Thuillet, J. Yu et al., "Maize association population: a high-resolution platform for quantitative trait locus dissection," *Plant Journal*, vol. 44, no. 6, pp. 1054–1064, 2005.
- [46] E. H. Coe and M. L. Schaeffer, "Genetic, physical, maps, and database resources for maize," *Maydica*, vol. 50, no. 3-4, pp. 285–303, 2005.
- [47] J. Jiang, B. S. Gill, G. L. Wang, P. C. Ronald, and D. C. Ward, "Metaphase and interphase fluorescence in situ hybridization mapping of the rice genome with bacterial artificial chromosomes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 10, pp. 4487–4491, 1995.
- [48] J. Jiang, S. H. Hulbert, B. S. Gill, and D. C. Ward, "Interphase fluorescence in situ hybridization mapping: a physical mapping strategy for plant species with large complex genomes," *Molecular and General Genetics*, vol. 252, no. 5, pp. 497–502, 1996.
- [49] D. H. Koo and J. Jiang, "Super-stretched pachytene chromosomes for fluorescence in situ hybridization mapping and immunodetection of DNA methylation," *Plant Journal*, vol. 59, no. 3, pp. 509–516, 2009.
- [50] J. C. Lamb, J. M. Meyer, B. Corcoran, A. Kato, F. Han, and J. A. Birchler, "Distinct chromosomal distributions of highly repetitive sequences in maize," *Chromosome Research*, vol. 15, no. 1, pp. 33–49, 2007.
- [51] S. S. M. Adawy, R. M. Stupar, and J. Jiang, "Fluorescence in situ hybridization analysis reveals multiple loci of knob-associated DNA elements in one-knob and knobless maize lines," *Journal of Histochemistry and Cytochemistry*, vol. 52, no. 8, pp. 1113–1116, 2004.
- [52] J. C. Lamb, T. Danilova, M. J. Bauer et al., "Single-gene detection and karyotyping using small-target fluorescence in situ hybridization on maize somatic chromosomes," *Genetics*, vol. 175, no. 3, pp. 1047–1058, 2007.
- [53] G. L. Koumbaris and H. W. Bass, "A new single-locus cytogenetic mapping system for maize (*Zea mays* L.): overcoming FISH detection limits with marker-selected sorghum (*S. propinquum* L.) BAC clones," *Plant Journal*, vol. 35, no. 5, pp. 647–659, 2003.
- [54] L. K. Anderson, G. G. Doyle, B. Brigham et al., "High-resolution crossover maps for each bivalent of *Zea mays* using recombination nodules," *Genetics*, vol. 165, no. 2, pp. 849–865, 2003.
- [55] L. K. Anderson, N. Salameh, H. W. Bass et al., "Integrating genetic linkage maps with pachytene chromosome structure in maize," *Genetics*, vol. 166, no. 4, pp. 1923–1933, 2004.
- [56] C. J. Lawrence, T. E. Seigfried, H. W. Bass, and L. K. Anderson, "Predicting chromosomal locations of genetically mapped loci in maize using the Morgan2McClintock Translator," *Genetics*, vol. 172, no. 3, pp. 2007–2009, 2006.
- [57] R. G. Kynast, O. Riera-Lizarazu, M. I. Vales et al., "A complete set of maize individual chromosome additions to the oat genome," *Plant Physiology*, vol. 125, no. 3, pp. 1216–1227, 2001.
- [58] H. W. Rines, R. L. Phillips, R. G. Kynast et al., "Addition of individual chromosomes of maize inbreds B73 and Mo17 to oat cultivars Starter and Sun II: maize chromosome retention, transmission, and plant phenotype," *Theoretical and Applied Genetics*, vol. 119, no. 7, pp. 1255–1264, 2009.
- [59] Y. R. Lin, L. Zhu, S. Ren, J. Yang, K. F. Schertz, and A. H. Paterson, "A *Sorghum propinquum* BAC library, suitable for cloning genes associated with loss-of-function mutations during crop domestication," *Molecular Breeding*, vol. 5, no. 6, pp. 511–520, 1999.
- [60] R. J. Singh, *Plant Cytogenetics*, CRC Press LLC, Boca Raton, Fla, USA, 2nd edition, 2003.
- [61] X. B. Zhong, J. H. De, and P. Zabel, "Preparation of tomato meiotic pachytene and mitotic metaphase chromosomes suitable for fluorescence in situ hybridization (FISH)," *Chromosome Research*, vol. 4, no. 1, pp. 24–28, 1996.
- [62] M. S. Zwick, M. N. Islam-Faridi, D. G. Czeschin et al., "Physical mapping of the liguleless linkage group in sorghum bicolor using rice RFLP-selected sorghum BACs," *Genetics*, vol. 148, no. 4, pp. 1983–1992, 1998.
- [63] B. S. Gaut, M. L. T. D'Ennequin, A. S. Peek, and M. C. Sawkins, "Maize as a model for the evolution of plant nuclear genomes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 13, pp. 7008–7015, 2000.
- [64] J. E. Bowers, C. Abbey, S. Anderson et al., "A high-density genetic recombination map of sequence-tagged sites for Sorghum, as a framework for comparative structural and evolutionary genomics of tropical grains and grasses," *Genetics*, vol. 165, no. 1, pp. 367–386, 2003.
- [65] C. S. Keith, D. O. Hoang, B. M. Barrett et al., "Partial sequence analysis of 130 randomly selected maize cDNA clones," *Plant Physiology*, vol. 101, no. 1, pp. 329–332, 1993.
- [66] A. H. Paterson, J. E. Bowers, R. Bruggmann et al., "The *Sorghum bicolor* genome and the diversification of grasses," *Nature*, vol. 457, no. 7229, pp. 551–556, 2009.
- [67] X. Draye, Y. R. Lin, X. Y. Qian et al., "Toward integration of comparative genetic, physical, diversity, and cytomechanical maps for grasses and grains, using the sorghum genome as a foundation," *Plant Physiology*, vol. 125, no. 3, pp. 1325–1341, 2001.

- [68] E. V. Ananiev, R. L. Phillips, and H. W. Rines, "Chromosome-specific molecular organization of maize (*Zea mays* L.) centromeric regions," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 22, pp. 13073–13078, 1998.
- [69] B. S. Gaut, "Patterns of chromosomal duplication in maize and their implications for comparative maps of the grasses," *Genome Research*, vol. 11, no. 1, pp. 55–66, 2001.



**Supplemental Table 1. Sequenced Maize Probes**

Probe	Bin (loci detected)	GenBank Accession #	Insert Type	Insert Size (bp)	Vector
p-asg15	3.06 (asg15)	EF471910	Genomic	573	pGEM3Zf
p-asg16	3.02 (asg16a), 1.06 (asg16b)	EF471911	Genomic	601	pGEM3Zf
p-asg18	6.07 (asg18)	EF471912	Genomic	381	pGEM3Zf
p-asg63	9.03 (asg63a), 1.09 (asg63b)	EF471913	Genomic	650	pGEM3Zf
p-asg64	3.01 (asg64)	EU728692	Genomic	786	pGEM3Zf
p-asg71	5.05 (asg71)	EU728693	Genomic	879	pGEM3Zf
p-asg72	2.07 (asg72)	EU728694	Genomic	1098	pGEM3Zf
p-asg84	2.07 (asg84a), 5.07 (asg84b), 4.07-4.10 (asg84c)	EF471914	Genomic	571	pGEM3Zf
p-asg85	4.08 (asg85a), 5.07 (asg85b)	EU728695	Genomic	809	pGEM3Zf
p-bnl5.07	4.10 (bnl15.07a), 7.04 (bnl15.07b)	EU445571	Genomic	2008	pUC12
p-bnl5.33	4.05 (bnl5.33a), 3.07 (bnl5.33b, EU445567), 9.03 (bnl5.33c), 8.06 (bnl5.33d), 3.04 (bnl5.33e), 7.02 (bnl5.33g)	EU445567	Genomic	2217	pUC12
p-bnl7.13	9.04 (bnl7.13a), 7.05 (bnl7.13b)	EU445568	Genomic	1072	pUC12
p-bnl7.26	3.09 (bnl7.26)	EU445569	Genomic	2545	pUC12
p-bnl8.44	7.05 (bnl8.44a), 2.08 (bnl8.44b)	EU445570	Genomic	2218	pUC12
p-bnl944	8.03 (bnl9.44)	EU734556	cDNA	2223	pBluescript-SK
p-csu214*	9.03-9.04 (csu214a), 1.03 (csu214b)	EU734557	cDNA	525	pBluescript-SK
p-csu219	9.05 (csu219)	EU734558	cDNA	783	pBluescript-SK
p-csu221	4.01 (csu221)	EU328268	cDNA	756	pBluescript-SK
p-csu222	1.09 (csu222)	EU734559	cDNA	360	pBluescript-SK

p-csu230	3.02 (csu230)	EU328269	cDNA	1850	pBluescript-SK
p-csu252	5.03 (csu252a), 9.03-9.04 (csu252b)	EU328270	cDNA	1372	pBluescript-SK
p-csu266	1.11 (csu266)	EU734560	cDNA	751	pBluescript-SK
p-csu276	10.03 (csu276)	EU328271	cDNA	1975	pBluescript-SK
p-csu328	3.05 (csu328)	EU734561	cDNA	606	pBluescript-SK
p-csu340	5.03 (csu340)	EU734562	cDNA	527	pBluescript-SK
p-csu348	2.02 (csu348a), 2.04 (csu348b)	EU328272	cDNA	834	pBluescript-SK
p-csu351	3.06 (csu351)	EU328273	cDNA	640	pBluescript-SK
p-csu359	10.01 (csu359)	EU328274	cDNA	594	pBluescript-SK
p-csu368	8.00-8.01 (csu368)	EU328275	cDNA	1017	pBluescript-SK
p-csu377	5.04 (csu377b/ubi, EU734563), 4.10 (csu377a/ubi2)	EU734563	cDNA	461	pBluescript-SK
p-csu381	1.11 (csu381)	EU734564	cDNA	455	pBluescript-SK
p-csu382	6.05 (csu382a, EU734565), 8.06 (csu382b), 3.05 (csu382c)	EU734565	cDNA	337	pBluescript-SK
p-csu389	1.04 (csu389)	EU328276	cDNA	1044	pBluescript-SK
p-csu408	3.04 (csu408)	EU734566	cDNA	813	pBluescript-SK
p-csu419	5.03 (csu419)	EU328277	cDNA	1094	pBluescript-SK
p-csu434	5.06 (csu434)	EU734567	cDNA	767	pBluescript-SK
p-csu439	3.05 (csu439)	EU734568	cDNA	674	pBluescript-SK
p-csu440	5.06 (csu440)	EU734569	cDNA	402	pBluescript-SK
p-csu466	9.02 (csu466)	EU328278	cDNA	571	pBluescript-SK
p-csu474	4.05 (csu474)	EU734570	cDNA	408	pBluescript-SK
p-csu475		EU328281	cDNA	1083	pBluescript-SK
p-csu481	6.05 (csu481)	EU734571	cDNA	216	pBluescript-SK
p-csu486	9.02 (csu486a), 7.01 (csu486b)	EU328279	cDNA	1084	pBluescript-SK
p-csu509	4.05 (csu509)	EU734572	cDNA	365	pBluescript-SK
p-csu525	4.06 (csu525)	EU734573	cDNA	442	pBluescript-SK
p-csu536	1.11 (csu536)	EU746366	cDNA	455	pBluescript-SK

p-csu540	6.05 (csu540)	EU746367	cDNA	384	pBluescript-SK
p-csu565	9.05 (csu565)	EU746368	cDNA	387	pBluescript-SK
p-csu567	3.07 (csu567)	EU328280	cDNA	2498	pBluescript-SK
p-csu570	1.11 (csu570b), 5.01 (csu570a)	EU746369	cDNA	584	pBluescript-SK
p-csu590	1.06 (csu590)	EU328282	cDNA	748	pBluescript-SK
p-csu598	5.05 (csu598)	EU746370	cDNA	737	pBluescript-SK
p-csu604	1.11 (csu604a, EU746371), 5.01 (csu604b)	EU746371	cDNA	701	pBluescript-SK
p-csu606	4.01 (csu606)	EU746372	cDNA	285	pBluescript-SK
p-csu610	10.04 (csu610)	EU746373	cDNA	447	pBluescript-SK
p-csu613	10.04 (csu613)	EU328283	cDNA	600	pBluescript-SK
p-csu614	1.07 (csu614)	EU746374	cDNA	336	pBluescript-SK
p-csu615	5.06 (csu615a), 10.06 (csu615b)	EU328284	cDNA	1604	pBluescript-SK
p-G21B-04	9.01 (npi253a), 4.08 (npi253b), 5.07 (npi253c), 6.04 (npi253d), 8.00-8.09 (npi253e), 10.00-10.07 (npi253f)	EU746375	Genomic	529	pUC19
p-G21G-09	4.05 (G21G-09)	EU728673	Genomic	1494	pUC19
p-npi294	2.09 (npi294a), 7.02 (npi294b), 6.05 (npi294c), 2.09 (npi294d), 7.02 (npi294e), 8.04 (npi294f), 4.09 (npi294g), 10.04 (npi294h), 1.12 (npi294i), 4.01 (npi294j)	EU746376	Genomic	594	pUC19
p-php10005	9.01 (php10005)	EU728674	Genomic	771	pUC19
p-php10012	2.04 (php10012)	EU746377	Genomic	613	pUC19
p-php20644	1.06 (php20644)	EU728675	Genomic	1700	pUC19
p-umc1	5.03 (umc1)	EU728676	Genomic	1111	pUC19
p-umc10	3.04 (umc10a), 2.08 (umc10b), 4.11(umc10c)	EU728678	Genomic	1060	pUC19
p-umc104	4.07 (umc104a), 5.08 (umc104b), 1.06 (umc104c)	EU728686	Genomic	769	pUC19
p-umc106	1.10 (umc106a), 5.02 (umc106b)	EU728687	Genomic	754	pUC19
p-umc12	8.05 (umc12a), 2.08-09 (umc12b), 4.07-08 (umc12c), 3.05 (umc12d), 6.01 (umc12e), 10.07 (umc12f), 1.09 (umc12g), 5.02 (umc12h)	EU746378	Genomic	586	pUC19

p-umc121	3.01 (umc121)	EU746381	Genomic	342	pUC19
p-umc125	2.08 (umc125a), 7.04 (umc125b)	EU728688	Genomic	1061	pUC19
p-umc135	2.04 (umc135)	EU728689	Genomic	851	pUC19
p-umc148	9.01 (umc148)	AY642962	Genomic	529	pUC19
p-umc154	3.04 (umc154)	EU728690	Genomic	743	pUC19
p-umc164	8.07 (umc164a), 3.07 (umc164b), 1.01 (umc164c)	EU728691	Genomic	1275	pUC19
p-umc18	3.05 (umc18a), 10.03 (umc18b)	EU728679	Genomic	867	pUC19
p-umc35	7.05 (umc35a), 2.09 (umc35b)	EU746379	Genomic	626	pUC19
p-umc36	2.09 (umc36a), 2.07 (umc36b), 6.01 (umc36c), 8.06-08 (umc36d)	EU746380	Genomic	661	pUC19
p-umc43	5.03 (umc43)	EU728680	Genomic	820	pUC19
p-umc45	7.05 (umc45a), 8.02 (umc45b), 10.04 (umc45c)	EU728681	Genomic	911	pUC19
p-umc61	2.03 (umc61)	EU728682	Genomic	1039	pUC19
p-umc8	1.03 (umc8a), 2.04 (umc8b), 2.05 (umc8g)	EU728677	Genomic	1148	pUC19
p-umc80	7.04 (umc80a), 2.08 (umc80b)	EU728683	Genomic	821	pUC19
p-umc84	1.11 (umc84a), 5.00 (umc84b), 8.06 (umc84c)	EU728684	Genomic	1035	pUC19
p-umc98	2.06-2.07 (umc98a), 7.02 (umc98b)	EU728685	Genomic	1194	pUC19

\* All csu clones are B73 cDNAs from a library as described by Keith et al., *Plant Physiology*, vol. 101: pp. 329–332, 1993.