

TECHNICAL REPORT

Identification and map position of YAC clones comprising one-third of the *Arabidopsis* genome

Inhwan Hwang, Takayuki Kohchi, Brian M. Hauge and Howard M. Goodman*

Department of Genetics, Harvard Medical School and Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts 02114, USA

Renate Schmidt, Gerda Cnops and Caroline Dean
Cambridge Laboratory, AFRC Institute of Plant Science Research, John Innes Centre, Colney Lane, Norwich NR4 7UJ, UK

Sue Gibson, Koh Iba, Bertrand Lemieux, Vincent Arondel, Linda Danhoff and Chris Somerville
DOE Plant Research Laboratory, Michigan State University, East Lansing, Michigan 48824-1312, USA

Summary

YAC clones corresponding to 125 *Arabidopsis thaliana* RFLP markers have been identified. At least one YAC clone has been isolated for each of the RFLP markers tested. Based on CHEF gel analysis of 196 clones, the mean insert size of the available *Arabidopsis* YAC libraries is approximately 160 kb. The YACs of known genetic map location encompass about 30% of the *Arabidopsis* genome. The results presented here represent a first step towards assembly of an overlapping YAC library of the *A. thaliana* genome.

Introduction

In a variety of plant species, genes controlling a wide range of developmental and metabolic processes have been identified by mutational analysis and positioned on classical genetic linkage maps. In most cases, virtually nothing is known about the biochemical nature of the gene products. Consequently, the cloning of these genes is generally accomplished by methods which depend solely on the mutant phenotype and genetic map position. A general approach for the isolation of such genes is the technique of chromosome walking. The first step toward cloning genes by chromosome walking is to identify DNA probes residing within a few hundred kilobases of the locus of interest. Typically this is achieved by analyzing the meiotic segregation of the mutation with respect to restriction fragment length polymorphisms (RFLPs). Once linked RFLPs have been identified, cloning the gene necessitates

the isolation of cloned DNA fragments bridging the intervening gap between the nearest RFLP marker and the gene of interest. While it is possible to undertake chromosome walking using overlapping cosmid or bacteriophage λ clones, in practice, the procedure is extremely labor intensive and ill-suited for large projects where more than a few steps are required. The recently developed techniques for cloning and stable maintenance of large DNA fragments into yeast artificial chromosome (YAC) vectors present a more efficient alternative for chromosome walking (Burke *et al.*, 1987; Coulson *et al.*, 1988).

Arabidopsis thaliana has gained increasing popularity as a model system for the study of plant biology. Its short life cycle, small size and large seed output make it well suited for classical genetic analysis. Mutations have been described affecting a wide range of fundamental developmental and metabolic processes (reviewed in Meyerowitz, 1989) and a genetic linkage map consisting of some 90 loci, most of which display visible phenotypes, has been assembled (Koornneef, 1987). *Arabidopsis* offers the additional advantages of having a very small genome and an atypically low content of interspersed repetitive DNA (Leutwiler *et al.*, 1984; Pruitt and Meyerowitz, 1986). The small, relatively simple genome, greatly simplifies the cloning of genes which have been identified by mutational analysis. These features make *Arabidopsis* the plant of choice for undertaking a combined molecular and genetic approach toward understanding many fundamental developmental and metabolic processes.

To facilitate the development of *Arabidopsis* as a model system, efforts are underway to construct a complete physical map of the *Arabidopsis* genome which will ultimately consist of a fully overlapping collection of clones encompassing the five linkage groups. Many of the tools required for mapping the genome have either been developed or are currently being developed. An overlapping cosmid map covering 90–95% of the *Arabidopsis* genome has been constructed (Hauge *et al.*, 1991). Gridded arrays of yeast artificial chromosome libraries are available (Grill and Somerville, 1991; Ward and Jen, 1990) and are being widely used in the *Arabidopsis* community. Approximately 380 RFLP probes (Chang *et al.*, 1988; Nam *et al.*, 1989; Hanley and Goodman, unpublished data; Meyerowitz, unpublished data) are available for correlation of the physical map with the classical genetic linkage map.

In this communication we describe the preliminary results of a collaborative effort to assemble an overlapping

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*For correspondence (fax +1 617 726 3535).

YAC library covering the *Arabidopsis* genome. The overlapping YAC library will serve both to facilitate the cloning of genes by chromosome walking, and provide a minimum set of clones covering the *Arabidopsis* genome. The minimal set of YAC clones will be gridded at high density onto nylon filters which can easily be distributed to the scientific community. The 'polytene blots' will facilitate the mapping of new probes by hybridization to a single filter.

Results and discussion

Three different YAC libraries have been used for this analysis; the ABI and EG libraries were constructed by *Bam*HI partial digestion of genomic DNA from the *Landsberg* (ABI library) and *Columbia* (EG library) ecotypes (Grill and Somerville, 1991). The EW library contains inserts of randomly sheared genomic DNA from the *Columbia* ecotype (Ward and Jen, 1990). The three libraries, ABI, EG and EW contain about 2100, 2700 and 2200 clones, respectively. Based on a mean insert size of 160 kb, the three libraries collectively represent approximately 10 genomic equivalents, which gives at least a 99.9% probability that any given sequence is represented.

Identification of YAC clones corresponding to RFLP markers (Table 1) was performed by yeast colony hybridization using standard methodologies (Coulson *et al.*, 1988). For 196 of the YAC clones identified by colony hybridization the YACs were further characterized by CHEF gel electrophoresis to determine the insert size and to identify colonies containing multiple YACs. The presence of more than one YAC can be attributed to double (multiple) transformants, contamination of a well with more than one clone or instability leading to multiple rearranged clones. Cross-contamination is generally avoided by colony purification prior to subsequent analysis. In many cases colony

hybridizations were further confirmed by Southern blot analysis (indicated in Table 1). Independent validation by Southern blot analysis is essential to eliminate false linkage assignments due to hybridization of the probe to interspersed repeats and multi-gene families. Nevertheless, we have included data in Table 1 which have not been confirmed by Southern blotting in order to provide additional starting points for other laboratories engaged in chromosome walking. For each YAC in Table 1 the criteria of identification are indicated by the information in columns 7 and 8. It should be noted, however, that due to the possibility of cross-hybridization to sequences contained within the RFLP marker some of the positively hybridizing YAC clones may not map to the same position as the RFLP marker. To establish unequivocal linkage it is necessary to demonstrate that the YACs and the RFLP clones used as probes share common bands. Southern blot analysis further reveals potential rearrangements of the insert, at least within the region detected by the corresponding RFLP probe.

To date, the corresponding YAC clones have been isolated for all of the 125 RFLP markers tested. It should be noted, however, that due to technical problems some positives may have been missed. In 15 cases it was necessary to screen more than one library to identify the corresponding YAC clone. As indicated in Table 1, we have identified 296 YACs which hybridize to 125 RFLP markers. Therefore, on average, 2.4 YACs were identified per probe. Assuming approximately 50% overlap between YACs we estimate the average size of a YAC contig to be in the order of 240 kb. Consequently, YACs of known genetic map position encompass 30 000 kb or about 30% of the *Arabidopsis* genome which is currently estimated to be about 100 000 kb (Hauge and Goodman, unpublished results).

Table 1. RFLP markers for which YAC clones have been isolated

RFLP ^a	Chr ^b	MAP ^c	YAC number ^d	Size ^e	Lab. ^f	R ^g	S ^h
488	1	13	EG16C7	70	MSU		
488	1	13	EG8D2		MSU		
322	1	15.7	EG11A5		MSU		
322	1	15.7	EG7A3	175	MSU		
219	1	26.9	EG1G7	220	MSU	Y	
219	1	26.9	EG3G3	210	MSU	Y	
271	1	54.3	EG1D4	180	MSU		
201	1	56.6	EG1D4	180	MSU		
321	1	57.1	EG1D4	180	MSU		
321	1	57.1	EG9B11	150	MSU		
299	1	67.6	EG8C1	170	MSU		
2395	1	71.8	EG5E11		MGH		
2395	1	71.8	EG5H6		MGH		
2395	1	71.8	EG5H9		MGH		
2395	1	71.8	EG8F8		MGH		
NIA2	1	100.5	EG16A7	180	MGH	Y	Y
305	1	104.9	EG13F4	145	MSU		

Table 1. Continued

RFLP ^a	Chr ^b	MAP ^c	YAC number ^d	Size ^e	Lab. ^f	R ^g	S ^h
315	1	111.2	EG2G11	140	MSU	Y	
315	1	111.2	EG4E4		MSU	Y	
315	1	111.2	EG7A7		MSU	Y	
4026	1	121	EG12H10		MGH		
4026	1	121	EG20A7		MGH		
4026	1	121	EG22B1		MGH		
2488B	1	125.9	EG12C8		MGH, MSU	Y	Y
2488B	1	125.9	EG1B5		MSU		Y
2488B	1	125.9	EG1D2	130	MGH, MSU	Y	Y
2488B	1	125.9	EG24E5		MGH, MSU		Y
2488B	1	125.9	EW21B4		MSU		Y
2488B	1	125.9	EW8D4		MSU		Y
532	1	133.3	EG8D6	190	MSU		
237	1	134.8	EG6A3	125	MSU		
237	1	134.8	EG8D6	190	MSU		
237	1	134.8	EG9E5		MSU		
3012	1	135.8	EG13H7		MSU		
3012	1	135.8	EG4E2	210	MSU		
3012	1	135.8	EG9G11		MSU		
4121	1	136.9	EG20E7		MGH		
4121	1	136.9	EG20H9		MGH		
4121	1	136.9	EG21A9		MGH		
4552	1	137.9	EG12F4		MGH		
4552	1	137.9	EG13H3		MGH		
4552	1	137.9	EG6H11		MGH		
132	1	142	EG13H5		MGH		
132	1	142	EG4G5		MGH		
NIA1	1	159.9	EG7D9		MGH	Y	
17311	1	163.9	EG1D10		MGH		
17311	1	163.9	EG1D9		MGH		
17311	1	163.9	EG20A3		MGH		
4553	2	0	ABI7F12		MGH	Y	
4133	2	5	EG9H1	220	MGH	Y	Y
104	2	30.1	EW7B9	150	MGH	Y	Y
216	2	33.5	EW15H11	160	MGH	Y	Y
216	2	33.5	EW6A7	100	MGH	Y	Y
465	2	34.9	EW20F11	130	MGH	Y	Y
6842	2	35.2	EG18D11	260	MGH	Y	Y
6842	2	35.2	EG1H9	200	MGH	Y	Y
6842	2	35.2	EG22G6	180	MGH	Y	Y
6842	2	35.2	EG8C10	170	MGH	Y	Y
251	2	42.8	EG7E2	130	MGH, MSU	Y	Y
ASA2	2	47.2	EW15G1	125	MGH, MSU	Y	Y
ASA2	2	47.2	EW7E2	140	MGH	Y	Y
13808	2	54.6	EG22E4	240	MGH	Y	Y
13808	2	54.6	EG3H2	170	MGH	Y	Y
6825	2	55.1	EG16D12	200	MGH	Y	Y
17288	2	55.1	EG16D12	200	MGH	Y	Y
GPA	2	55.1	EG10A10	180	MGH	Y	Y
GPA	2	55.1	EG1D5		MGH	Y	Y
GPA	2	55.1	EG2A1	170	MGH	Y	Y
GPA	2	55.1	EG2B1	170	MGH	Y	Y
GPA	2	55.1	EG2E1	170	MGH	Y	Y
GPA	2	55.1	EG2G1	170	MGH	Y	Y
GPA	2	55.1	EG10H3	180	MGH	Y	Y
GPA	2	55.1	EG16C6	120	MGH	Y	Y
6191	2	55.6	EG7C11	80	MGH	Y	Y
6191	2	55.6	EW22C12	190	MGH	Y	Y
1789	2	59.5	EG12C11	200	MGH	Y	Y
1789	2	59.5	EG3D2	190	MGH	Y	Y
1789	2	59.5	EW13G11	160	MGH	Y	Y

Table 1. Continued

RFLP ^a	Chr ^b	MAP ^c	YAC number ^d	Size ^e	Lab. ^f	R ^g	S ^h
4514	2	60.3	EG12C11	200	MGH	Y	Y
220	2	66.1	EG4E8	90	MGH	Y	Y
220	2	66.1	EG9D12	190	MGH	Y	Y
323	2	70.6	ABI10C5		MGH	Y	
323	2	70.6	ABI16D12	140	MSU	Y	Y
323	2	70.6	ABI4C8	200	MSU	Y	Y
323	2	70.6	ABI5E9		MGH	Y	
323	2	70.6	EW13D8	150	MSU	Y	Y
551	2	76.9	EG9D6	240	MGH	Y	Y
551	2	76.9	EW10A11	150	MGH	Y	Y
551	2	76.9	EW21F8	160	MGH	Y	Y
P3-5	2	?	EW22F12	170	MGH	Y	Y
302	3	0	EG3H5	100	MGH	Y	Y
302	3	0	EG4H8	145	MGH	Y	Y
583	3	5.6	EG12H9		MSU	Y	
243	3	8.1	EG12B12		MSU	Y	
243	3	8.1	EG13G6	195	MSU	Y	
243	3	8.1	EG4A9		MSU	Y	
243	3	8.1	EG6A12		MSU	Y	
17341	3	9.5	EG21A11	160	MGH	Y	Y
17341	3	9.5	EG21B11		MSU	Y	
17341	3	9.5	EG28D3		MSU	Y	
17341	3	9.5	EG4H6	160	MGH	Y	Y
HSP70-9	3	11.9	ABI22C2		MSU		Y
HSP70-9	3	11.9	ABI8G6		MSU		Y
17343	3	14	EG11E10		MGH		
17343	3	14	EG11F11		MGH		
17343	3	14	EG13G6	195	MGH		
17343	3	14	EG4A9		MGH		
317	3	18.2	EG16B8		MSU	Y	Y
317	3	18.2	EG19A8		MSU		Y
317	3	18.2	EG2A4		MSU		Y
5970	3	18.6	EG12B12		MSU	Y	
5970	3	18.6	EG13G6	195	MSU	Y	
5970	3	18.6	EG16A2		MSU	Y	
5970	3	18.6	EG16H12		MSU	Y	
5970	3	18.6	EG4A9		MSU	Y	
5970	3	18.6	EG6A12		MSU	Y	
2488A	3	20.8	EG20H11	100	MGH, MSU	Y	Y
2488A	3	20.8	EW17B1	75	MSU		Y
PG26	3	21	EG14A1	110	MSU		Y
PG26	3	21	EG18G6		MSU	Y	Y
4547	3	21.5	EG19H2	130	MGH, MSU	Y	Y
4547	3	21.5	EW14E3		MSU	Y	
4547	3	21.5	EW7E8		MSU	Y	
105	3	24.5	EG7E11	150	MSU		
4708	3	30	EW21C11	170	MGH	Y	Y
433	3	36.6	EG20F9	160	MGH	Y	Y
6220	3	38.1	EG18C6	130	MGH	Y	Y
BWS12	3	47.5	EG15A3	160	MGH	Y	Y
4711	3	48.8	EG15A3	160	MGH	Y	Y
17287	3	52.6	EG15H1	100	MGH	Y	Y
17287	3	52.6	EW23C4	160	MGH	Y	Y
2440	3	58	EG13G5	140	MGH	Y	Y
2440	3	58	EG14E3	80	MGH	Y	Y
2440	3	58	EG4D12	110	MGH	Y	Y
PB3	3	85.5	EG19D8		MGH	Y	Y
4117	3	90	EG7H1	245	MGH	Y	Y
2534	3	90.2	EG7H1	245	MGH	Y	Y
4014	3	100.3	EG5B2	160	MGH	Y	Y
4125	3	112.5	EG12G4		MGH	Y	Y

Table 1. Continued

RFLP ^a	Chr ^b	MAP ^c	YAC number ^d	Size ^e	Lab. ^f	R ^g	S ^h
4125	3	112.5	EG18E6		MGH	Y	Y
4125	3	112.5	EG8D12	205	MGH	Y	Y
4125	3	112.5	EG14B1		MGH	Y	Y
BWS-14	3	118.1	EG11C12		MGH	Y	Y
BWS-14	3	118.1	EW12F8		MGH	Y	Y
BWS-14	3	118.1	EW8A3		MGH	Y	Y
2778	3	122	EG11G4	170	MGH	Y	Y
XDB-G	3	124.1	EW21C8		MGH	Y	Y
6844	4	0	EG19A10	145	CL	Y	Y
6844	4	0	EG8A3	155	CL	Y	Y
456	4	1.4	EG14B5	175	CL	Y	Y
3843	4	4.1	EG17G3	100	CL	Y	Y
3843	4	4.1	EG17G5	75 + 145	CL	Y	Y
3843	4	4.1	EG17H3	100	CL	Y	Y
3843	4	4.1	EG17H5	75	CL	Y	Y
3843	4	4.1	EG5G12	120	CL	Y	Y
448	4	6.3	ABI3B7	140	CL	Y	Y
448	4	6.3	ABI3C7	140	CL	Y	Y
448	4	6.3	EG19H3	260 + 280	CL	Y	Y
448	4	6.3	EG4A7	170	CL	Y	Y
518	4	20.6	ABI10A10	75	CL	Y	Y
518	4	20.6	ABI10A11	75	CL	Y	Y
518	4	20.6	ABI10A2	85	CL	Y	Y
518	4	20.6	ABI10A6	80	CL	Y	Y
518	4	20.6	ABI11F4	75	CL	Y	Y
518	4	20.6	ABI3E6	145	CL	Y	Y
518	4	20.6	ABI6D1	60 + 90	CL	Y	Y
518	4	20.6	ABI6E1	60	CL	Y	Y
518	4	20.6	EG10G1		CL	Y	Y
518	4	20.6	EG13C2	155	CL	Y	Y
518	4	20.6	EG4F2	170	CL	Y	Y
518	4	20.6	EG8E12	90	CL	Y	Y
210	4	24.6	EG5D4	185	CL, MSU	Y	Y
2616	4	25.8	EG15H9	170	CL, MGH	Y	Y
2616	4	25.8	EG6C11	230	CL	Y	Y
326	4	30.1	EG15C10	175	CL, MSU	Y	Y
326	4	30.1	EG22C1	190	CL, MSU	Y	Y
326	4	30.1	EG5D4	185	CL, MSU	Y	Y
455	4	30.1	EG15C10	175	CL, MSU	Y	Y
455	4	30.1	EG22C1	190	CL, MSU	Y	Y
455	4	30.1	EG5D4	185	CL, MSU	Y	Y
580	4	30.6	ABI2A2	110	CL	Y	Y
580	4	30.6	EG22C1	190	CL, MSU	Y	Y
580	4	30.6	EG9D2	135	MSU		
226	4	33.9	ABI3C4	110	CL	Y	Y
226	4	33.9	EG9F3	150	CL, MSU	Y	Y
4108	4	46.8	EG17G9	180	CL	Y	Y
4108	4	46.8	EG5D4	185	CL	Y	Y
6837	4	48	ABI3H5		CL	Y	Y
10086	4	49.2	ABI4D2	100	CL	Y	Y
10086	4	49.2	EG1B10	150	CL	Y	Y
4564	4	51.2	ABI20E3	175	CL	Y	Y
4564	4	51.2	EG12B10	135	CL	Y	Y
4564	4	51.2	EG2G12	160	CL	Y	Y
4564	4	51.2	EG3G4	150	CL	Y	Y
4564	4	51.2	EG3H3	205	CL	Y	Y
4564	4	51.2	EG3H4	205	CL	Y	Y
4564	4	51.2	EG4E1	315	CL	Y	Y
4564	4	51.2	EG8C4	140	CL	Y	Y
3883	4	59.2	EG10C12		MGH	Y	
272	4	65.4	EG4C1	190	MSU		

Table 1. Continued

RFLP ^a	Chr ^b	MAP ^c	YAC number ^d	Size ^e	Lab. ^f	R ^g	S ^h
272	4	65.4	EG7B10		MGH	Y	
8300	4	74.5	EG8G8		MGH	Y	
214	4	75.6	EG1E3	210	MSU		
214	4	75.6	EG3G7	190	MSU		
214	4	75.6	EG7B11	95	MSU		
PG11	4	80.4	EG11F9		MGH	Y	Y
3088	4	87.1	ABI1H4	120	MSU	Y	Y
3088	4	87.1	ABI24E12	140	MSU	Y	Y
3088	4	87.1	EG25G8	110	MSU	Y	Y
3088	4	87.1	EG4C2	220	MSU	Y	Y
4551	4	97.8	EG14F5		MGH	Y	
2486	4	98.2	EG14F5		MGH	Y	
3265	4	101.8	EW11A7	150	MGH	Y	Y
3265	4	101.8	EW16G1	100	MGH	Y	Y
3713	4	101.8	EW11A7	150	MGH	Y	Y
3713	4	101.8	EW16G1	100	MGH	Y	Y
562	5	0	ABI18E8		CL	Y	Y
3715	5	9	EG13G6	195	CL	Y	Y
3715	5	9	EG19A10	145	CL	Y	Y
447	5	12.2	EG15H12	225	CL	Y	Y
447	5	12.2	EG16C2	180	CL	Y	Y
447	5	12.2	EG23G5	90	CL	Y	Y
3837	5	15.6	EG21B12	125	CL, MGH	Y	Y
217	5	15.7	EG4D1	210	CL, MSU	Y	Y
217	5	15.7	EG4E2	200	CL	Y	Y
217	5	15.7	EG4F3	165	CL, MGH	Y	Y
217	5	15.7	EG5H1	210	CL, MSU	Y	Y
6830	5	19	EG21A4	170	CL, MGH	Y	Y
6830	5	19	EG7B5	220	CL, MGH	Y	Y
1326	5	22.2	EG5C3		MGH		
6833	5	22.2	EG10D9	125	CL, MGH	Y	Y
6833	5	22.2	EG12F8	95 + 180	CL, MGH, MSU	Y	Y
6833	5	22.2	EG18H3	240	CL, MGH	Y	Y
6833	5	22.2	EG1C12	160	CL, MGH, MSU	Y	Y
7578	5	22.2	EG13A2	100	MGH		
7578	5	22.2	EG18H3	240	MGH		
7578	5	22.2	EG1C12	160	MGH		
7578	5	22.2	EG4E9		MGH		
7578	5	22.2	EG5C3		MGH		
17551	5	22.2	EG5C3		MGH		
17551	5	22.2	EG5F6		MGH		
5962	5	24.2	EG10F12	170	CL, MGH, MSU	Y	Y
5962	5	24.2	EG15G10	180	CL, MGH, MSU	Y	Y
5962	5	24.2	EG18A7	150	CL, MGH, MSU	Y	Y
5962	5	24.2	EG19D11	110	CL, MGH, MSU	Y	Y
5962	5	24.2	EG3A10	155	CL, MGH, MSU	Y	Y
224	5	24.7	ABI4D1	170	CL	Y	Y
224	5	24.7	ABI4E1	170	CL	Y	Y
CHS2	5	26.8	EG10D9	125	CL, MGH, MSU	Y	Y
CHS2	5	26.8	EG12F8	95 + 180	CL, MGH, MSU	Y	Y
CHS2	5	26.8	EG13A2	100	CL, MGH	Y	Y
CHS2	5	26.8	EG18H3	240	CL, MGH, MSU	Y	Y
21503	5	30	EG1F4	135 + 195	CL	Y	Y
21503	5	30	EG1G3	135	CL	Y	Y
21503	5	30	EG1G4	135	CL	Y	Y
21503	5	30	EG1H4	135	CL	Y	Y
2632	5	34	ABI12C9	115	CL	Y	Y
4111	5	35.7	EG17E4	155	CL	Y	Y
4111	5	35.7	EG19E1	145	CL	Y	Y
4560	5	38	EG12H12	90	CL, MGH	Y	Y
4560	5	38	EG7G2	155	CL	Y	Y

Table 1. Continued

RFLP ^a	Chr ^b	MAP ^c	YAC number ^d	Size ^e	Lab. ^f	R ^g	S ^h
4560	5	38	EG7H2	155	CL	Y	Y
4560	5	38	EW20F9	120	MGH	Y	Y
4556	5	42.1	EG10E12	170	CL	Y	Y
4556	5	42.1	EG20H2	140	CL	Y	Y
6843	5	43.9	ABI1B4	145	CL	Y	Y
6856	5	51.5	EG21F1		CL	Y	Y
GS-L1	5	63.8	EG9D9	150	MGH	Y	Y
225	5	112	EW16D6	155	WJ		
225	5	112	EW6H1	220	WJ		
268	5	124.3	EW3A5	145	WJ		
435	5	132.3	EW10F1	190	WJ		
435	5	132.3	EW6G11	140	WJ		
3878	5	134.3	EG3B2		MGH	Y	
233	5	134.6	EG12G1		MSU		
233	5	134.6	EG1D9		MSU		
233	5	134.6	EW11C10	220	MSU		
233	5	134.6	EW17H8	220	MSU, WJ	Y	Y
233	5	134.6	EW21H10	150	MSU	Y	Y
3844	5	136.7	EG9B5		MGH	Y	
558	5	139.7	EG13G1	190	MSU	Y	
558	5	139.7	EW22H1	160	WJ		
558	5	139.7	EG18H3	250	MSU		
2455	5	145.4	EG12B5		MSU		
2455	5	145.4	EG16F11		MSU		
2455	5	145.4	EG20B7		MSU		
2455	5	145.4	EG2H8		MSU		
17337	5	145.4	EG12B5		MSU		
17337	5	145.4	EG16F11		MSU		
17337	5	145.4	EG20B7		MSU		
17337	5	145.4	EG2H8		MSU		
2368	5	147.6	EG14G9		MGH	Y	
4510	5	152.2	EG8E4		MGH	Y	
4510	5	152.2	EG8H3		MGH	Y	
211	5	155.7	EG12D8		MSU		
211	5	155.7	EG6D9		MSU		

^a RFLP marker (Chang *et al.*, 1988; Nam *et al.*, 1989; Hanley and Goodman, unpublished results; E. Meyerowitz, personal communication).

^b Chromosome number.

^c Map position in accordance with the updated RFLP maps of Chang *et al.* (1988) and Nam *et al.* (1989). The updated RFLP maps (Meyerowitz, unpublished data; Hanley and Goodman, unpublished data) may be obtained from the respective laboratories upon request. It is important to emphasize that the map positions are from the two independently assembled RFLP maps and cannot be directly related to one another. For example, the RFLP markers 6833 and CHS2 are physically linked on chromosome 5 and are separated by approximately 150 kb (Coupeland, unpublished data; Hauge, unpublished data), yet the map positions are given as 22.2 and 26.8 respectively. This apparent discrepancy most probably reflects the fact that the map positions are based on two independent data sets.

^d The source of the YAC libraries used for the colony hybridization experiments are referenced as ABI and EG (Grill and Somerville, 1991) and EW (Ward and Jen, 1990). In most cases the EG library has been screened first. When no positive clones were identified in the EG library, either the EW or the ABI libraries were subsequently screened. The YAC co-ordinates designate the plate number (1–29) and position (A–H reference the rows and 1–12 the columns) of a YAC clone based on a standard 96-well microtiter dish configuration. Some of the data presented are from Grill and Somerville (1991) or Ward and Jen (1990) and are included here for the sake of completeness.

^e Estimated size of the YACs in kb (± 10 kb) based on CHEF gel electrophoresis. In a few cases more than one YAC band has been observed due to spill-over from an adjacent well, a double transformation or instability leading to size heterogeneity.

^f Institutions where analysis was carried out: CL, Cambridge Laboratory, John Innes Centre, Norwich; MGH, Massachusetts General Hospital; MSU, Michigan State University; WJ, Ward and Jen (1990).

^g Y indicates that duplicate hybridizations were performed.

^h Confirmation by Southern blot analysis is indicated by a Y, while a blank indicates that Southern blot analysis was not performed.

Among the 296 YACs listed in the table, there appear to be a few anomalous clones. For example, EG13G6 hybridizes to the adjacent RFLPs 243, 17343 and 5970 and the noncontiguous RFLP 3715. This apparent paradox can be explained by either the presence of repetitive sequences or alternatively that EG13G6 contains a chimeric insert which is non-contiguous in the genome. Further experimentation is required to determine the nature of the relatively few anomalies which have been observed. It should also be noted that of the 125 RFLPs examined, 32 have been shown to be physically linked by one or more adjacent YAC clones. The majority of the linkages have been identified in regions of the genome where concerted efforts have been made to identify YACs corresponding to all of the RFLPs. The numbers of these RFLPs are: 271–201–321, 210–326–455–580–4108, 237–532, 243–17343–5970, 1326–6833–7578–17551–CHS2, 1789–4514, 2455–17337, 2486–4551, 2534–4117, 3265–3713, 4711–BWS12, 6825–17288. Many of these linked RFLPs provide important contact points between the two RFLP maps.

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