MATCHMAKER
GAL4 Two-Hybrid Vectors Handbook

(PT3062-1)

Information Supplement for:

Catalog #	Product
K1605-1	MATCHMAKER Two-Hybrid System
K1604-1	MATCHMAKER Two-Hybrid System 2
(many)	MATCHMAKER Libraries

FOR RESEARCH USE ONLY

(PR6X890)

Table of Contents

List of Tables

I. Introduction

This Handbook provides vector maps, MCSs, transformation markers, references, GenBank accession numbers (where available), and other pertinent information for the cloning and control plasmids used in the yeast GAL4-based MATCHMAKER Two-Hybrid Systems and Libraries. For ease of use and comparison of the plasmids, much of this information is summarized in the Tables in the Appendix. Complete sequence information for most of the plasmids is available by request from CLONTECH's Technical Support Dept. Selected vector sequences are accessible through our worldwide web site (http://www.clontech.com).

All of the cloning and control plasmids are shuttle vectors that replicate autonomously in both *E. coli* (using the Col E1 or pBR322 origins of replication) and in *S. cerevisiae* (using the 2μ ori). All MATCHMAKER plasmids carry the β-lactamase (*bla*) gene, which confers ampicillin resistance (Amp^r) in *E. coli*, and a nutritional gene (*TRP1* or *LEU2*) that allows yeast auxotrophs transformed with the plasmid to grow on synthetic defined medium lacking tryptophan or leucine, respectively. The nutritional markers also allow selection in certain auxotrophic strains of *E. coli* on M9 minimal medium.

The cloning plasmids generate fusion proteins

All of the cloning plasmids provided in the MATCHMAKER Two-Hybrid Systems are used for the construction and expression of hybrid (fusion) proteins. The GAL4 AD plasmids generate a hybrid that contains the sequences for the yeast GAL4 activation domain (a.a. 768–881) and a cloned protein or cDNA library insert. The GAL4 DNA-BD plasmids generate a hybrid that contains the sequences for the GAL4 DNA-binding domain (a.a. 1–147) and a cloned protein, most often used as the bait protein in a two-hybrid library screening. These vectors have unique restriction sites located in the MCS region at the 3' end of the open reading frame for either the DNA-BD or the AD sequence.

For the construction of a specific hybrid, the gene encoding the protein of interest is inserted into the MCS in the correct orientation and reading frame such that a hybrid protein is generated. In the case of MATCHMAKER Libraries, the inserts may be cloned into the MCS either directionally or nondirectionally (check the Product Analysis Certificate and the User Manual for detailed information on how the library was constructed). The fusion proteins are expressed in yeast cells from the full-length *ADH1* promoter or a modified form of it. See Table I for the relative expression levels observed for the different plasmids; for further information on the promoters, see the Yeast Protocols Handbook (YPH), Chapter II. In all plasmids, transcription is terminated at the *ADH1* transcription termination signal.

The hybrid proteins are targeted to the yeast nucleus by nuclear localization sequences. In the GAL4 DNA-BD cloning plasmids, the nuclear localization sequence is an intrinsic part of the DNA-BD (Silver *et al*., 1984) and is not specifically indicated on the vector maps. In the GAL4 AD cloning plasmids, the nuclear localization sequence from SV40 T-antigen has been cloned into the vector between the *ADH1* promoter and the AD sequence. (For detailed information regarding the construction of this sequence, see Chien *et al*., 1991.)

Some of the cloning vectors also encode the influenza hemagglutinin (HA) epitope in the junction between the GAL4 sequence and the MCS; if present, this element is indicated on the vector map. The HA epitope tag is useful for detecting expressed fusion proteins on Western blots using an anti-HA antibody. GAL4 AD and DNA-BD Monoclonal Antibodies (#5398-1 and 5399-1, respectively) are available from CLONTECH for probing Western blots when using medium- to high-expression level cloning vectors (see Table I). The DNA-BD plasmids pAS2 and pAS2-1 (Harper *et al*., 1993) carry the wild-type yeast CYH^S2 gene, which confers sensitivity to cycloheximide in transformed cells. CYH^S2 can be used to isolate AD/library plasmids in yeast segregants after a two-hybrid library screening, and thus facilitates the elimination of false positives.

I. Introduction *continued*

TABLE I. YEAST PROMOTER CONSTRUCTS IN THE MATCHMAKER GAL4 CLONING VECTORS

Vectors	Promoter	Regulation/ Relative Protein Expression Level	Signal Strength on Western blot [a]
pGAD GH, pAS2, pAS2-1	ADH1 (full-length)	Ethanol-repressed[b]/High	+++
pACT2, pACT	ADH1 (truncated+)[c]	Constitutive/medium	++
pGAD GL	ADH1 (truncated)	Constitutive/low	+/– (weak)
pGAD424, pGAD10, pGBT9		Constitutive/ very low	(not detectable)

[a] Soluble protein extracts were prepared from CG-1945 transformed with the indicated plasmid. Samples equivalent to ~1 OD_{600} unit of cells were electrophoresed and then blotted to nitrocellulose filters. The blots were probed with either GAL4 DNA-BD mAb (0.5 µg/ml; #5398-1) or GAL4 AD mAb (0.4 µg/ml; #5399-1), followed by HRP-conjugated polyclonal goat anti-mouse IgG (Jackson Immunological Research; diluted 1:15,000 in TBST). Signals were detected using a chemiluminescent detection assay and a 2.5-min exposure of x-ray film. Signal intensities were compared to that of known amounts of purified GAL4 DNA-BD or GAL4 AD.

[b] Transcription is repressed in late lag phase by the ethanol that accumulates in the medium as a by-product of yeast metabolism.

[c] The truncated *ADH1* promoter in pACT2 is adjacent to a section of pBR322 which acts as a transcriptional enhancer in yeast.

TABLE II. LIST OF ABBREVIATIONS

AD	GAL4 activation domain (a.a. 768–881)
DNA-BD (or BD)	GAL4 DNA-binding domain (a.a. 1–147)
P	promoter
T	transcription termination sequence
HA	hemagglutinin sequence
AD/library plasmid	Plasmid encoding a fusion of the GAL4 AD and a library insert

II. GAL4 Activation Domain (AD) Cloning Vectors

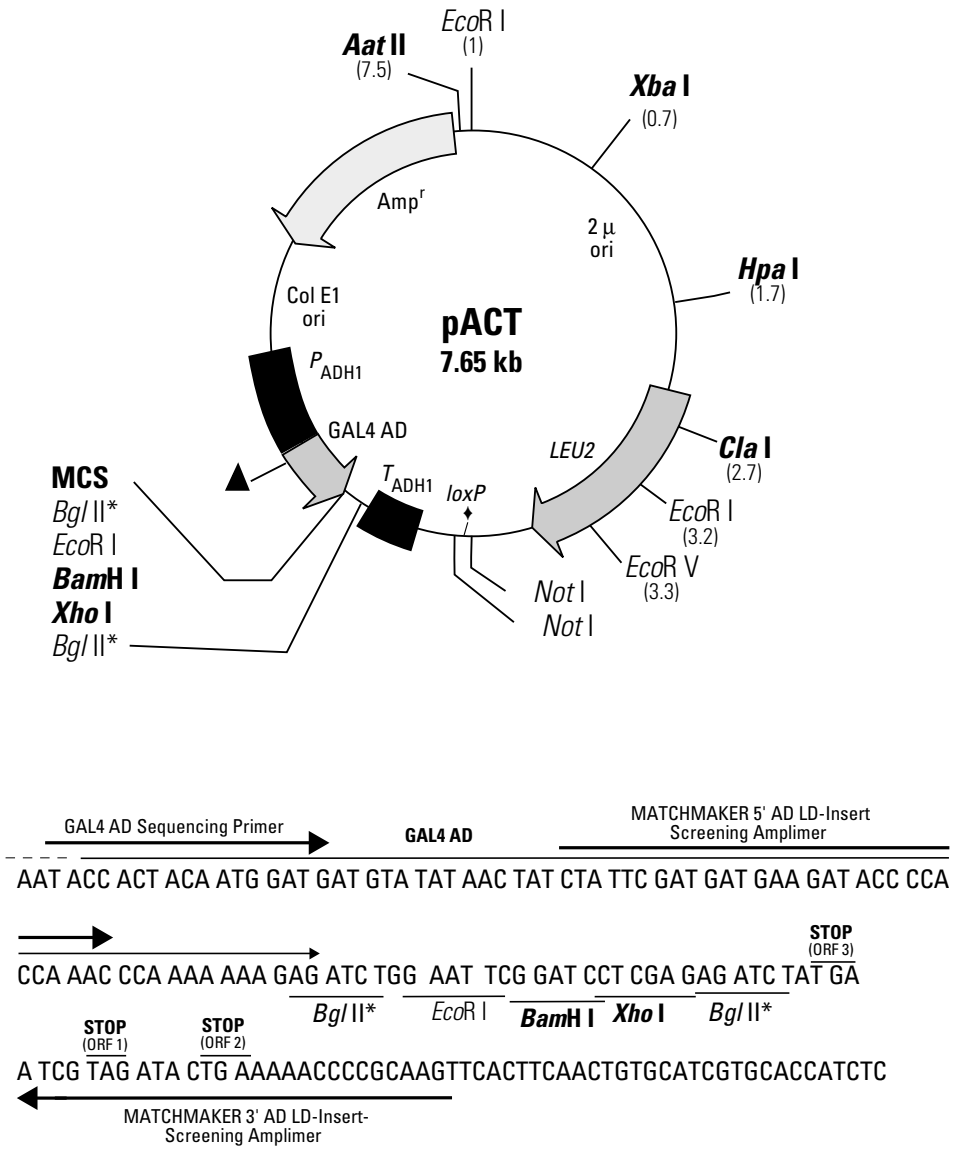


Figure 1. pACT map and MCS. Unique sites are in bold. pACT (Durfee *et al.*, 1993) is used to generate a hybrid containing the GAL4 AD (a.a. 768–881) and another protein of interest (or a protein encoded by a cDNA in a fusion library). The hybrid protein is expressed at medium levels in yeast host cells from the enhanced, truncated *ADH1* promoter and is targeted to the yeast nucleus by the SV40 T-antigen nuclear localization sequence (▲; Chien *et al.,* 1991). pACT contains the *LEU2* gene for selection in Leu⁻ auxotrophic yeast strains. The *Bgl* II (*) sites can be used as a unique cloning site. pACT Libraries from CLONTECH are constructed in λACT; recombinant plasmids are released by Cre-*lox* recombination (see Figure 2). No further pACT sequence information is available.

II. GAL4 Activation Domain (AD) Cloning Vectors *continued*

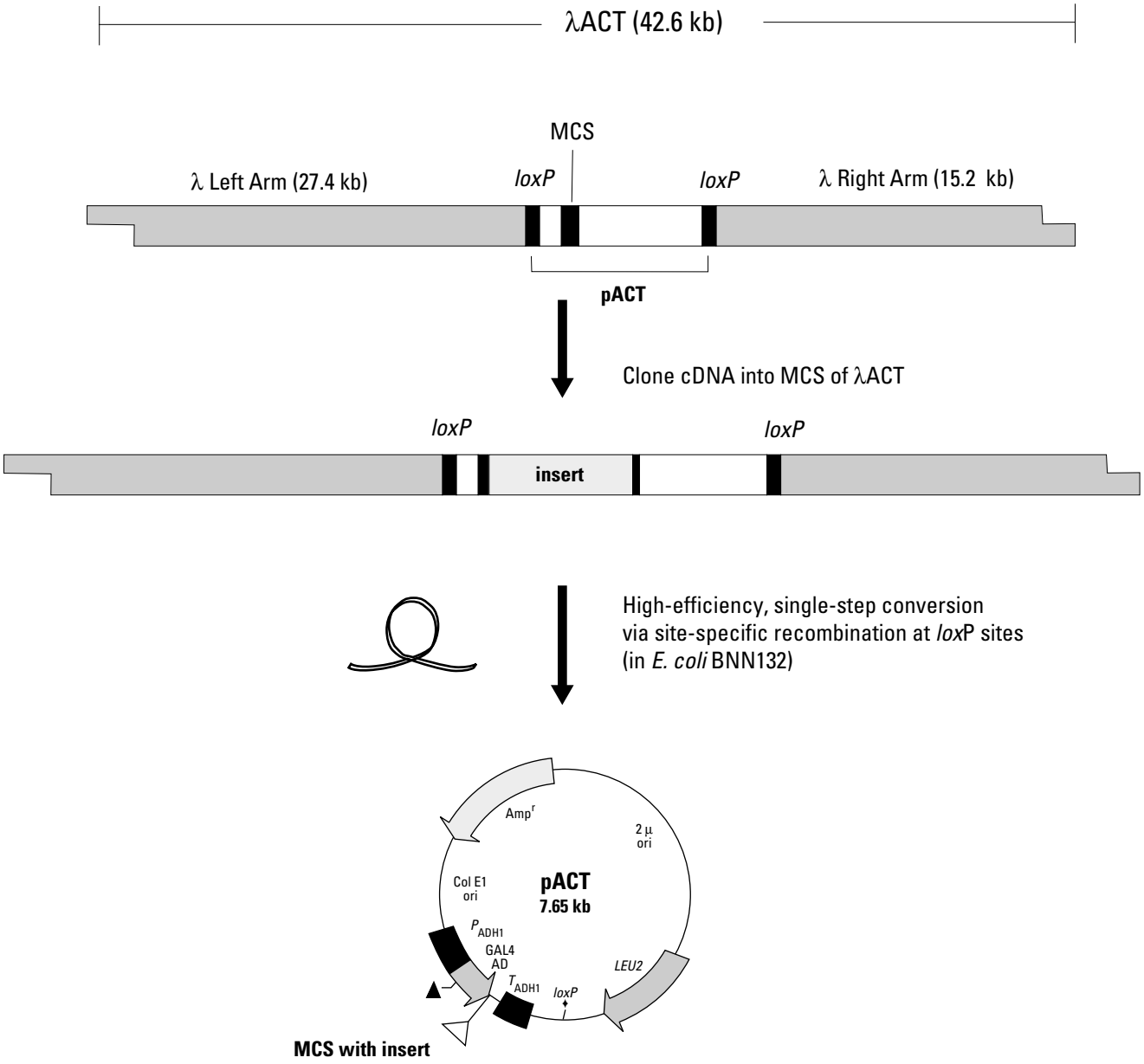
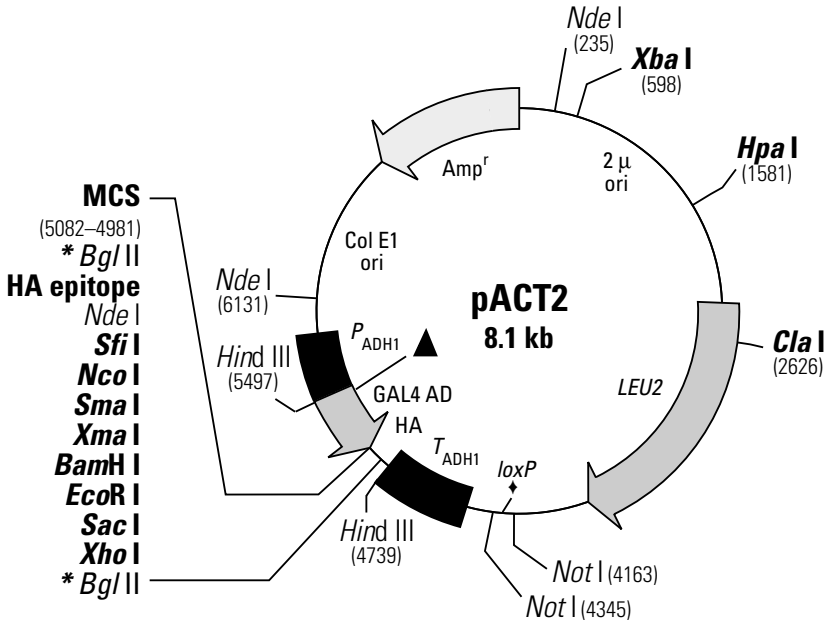


Figure 2. Conversion of a recombinant λACT to the corresponding pACT. The λACT MCS is located within an embedded plasmid, which is flanked by *loxP* sites at the λ junctions. Transduction of a λACT lysate into *E. coli* strain BNN132 promotes *Cre* recombinase-mediated release and recircularization of pACT at the *loxP* sites. Note that the resulting plasmid contains only one *loxP* site.

II. GAL4 Activation Domain (AD) Cloning Vectors *continued*



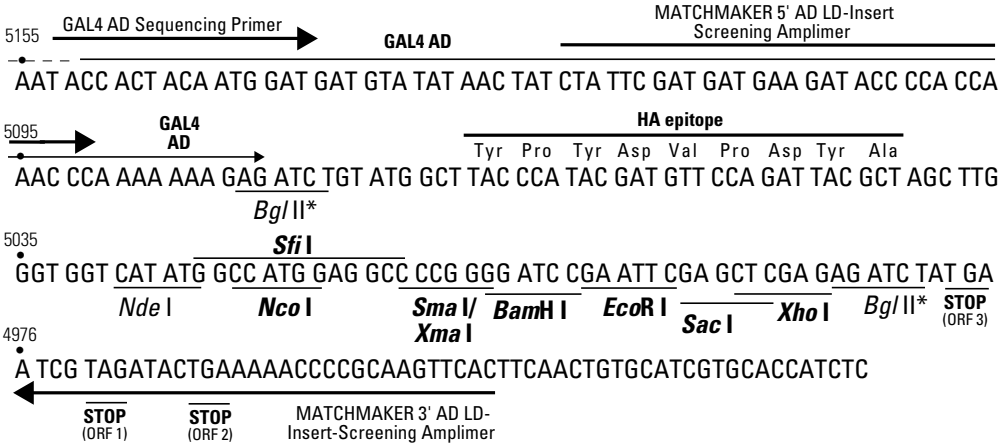
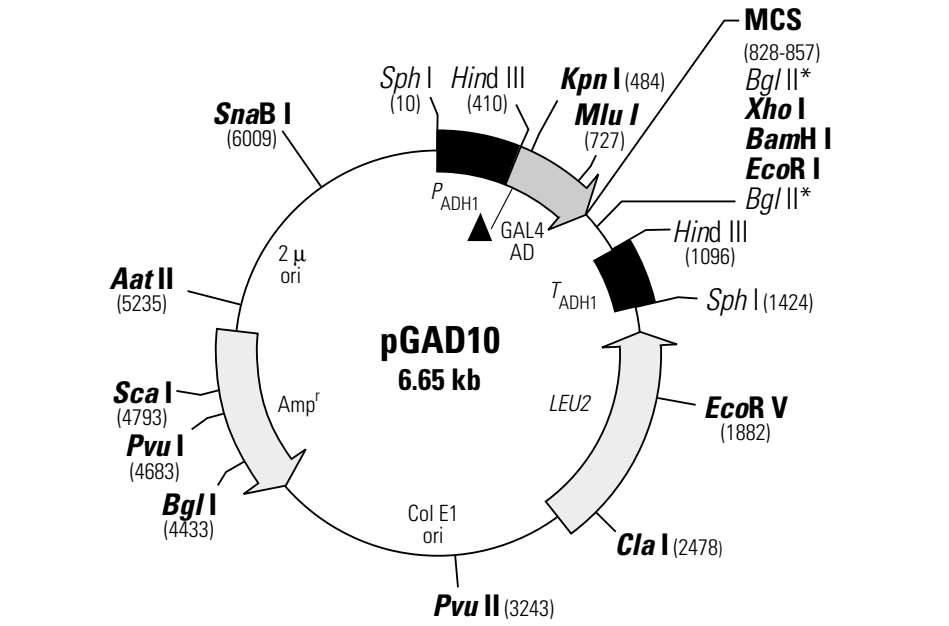


Figure 3. pACT2 map and MCS. Unique sites are in bold. pACT2 (Li *et al*., 1994) is used to generate a hybrid containing the GAL4 AD (a.a. 768–881), an HA epitope tag (Durfee *et al.,* 1993), and another protein of interest (or a protein encoded by a cDNA in a fusion library). The hybrid protein is expressed at medium levels in yeast host cells from an enhanced, truncated *ADH1* promoter and is targeted to the yeast nucleus by the SV40 T-antigen nuclear localization sequence (▲ ; Chien *et al.,* 1991). pACT2 contains the *LEU2* gene for selection in Leu⁻ auxotrophic yeast strains. The *Bgl* II (*) sites can be used as a unique cloning site, but this will remove the HA epitope. pACT2 libraries from CLONTECH are constructed in λACT2; recombinant plasmids are released by Cre-*lox* recombination (see Figure 2). GenBank Accession: #U29899.

II. GAL4 Activation Domain (AD) Cloning Vectors *continued*



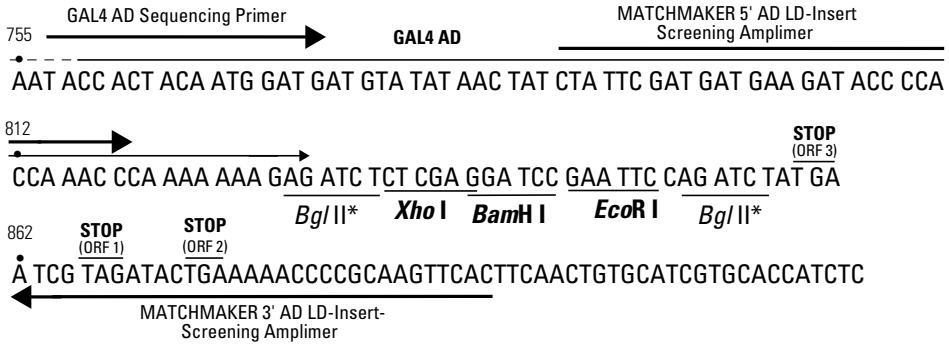


Figure 4. pGAD10 map and MCS. Unique sites are in bold. pGAD10 (Bartel *et al.*, 1993) is used to generate a hybrid containing the GAL4 AD (a.a. 768–881) and another protein of interest (or a protein encoded by a cDNA in a fusion library). The hybrid protein is expressed at low levels in yeast host cells from a truncated *ADH1* promoter and is targeted to the yeast nucleus by the SV40 T-antigen nuclear localization sequence (▲ ; Chien *et al.,* 1991). pGAD10 contains the *LEU2* gene for selection in Leu⁻ auxotrophic yeast strains.
GenBank Accession: #U13188.

II. GAL4 Activation Domain (AD) Cloning Vectors *continued*

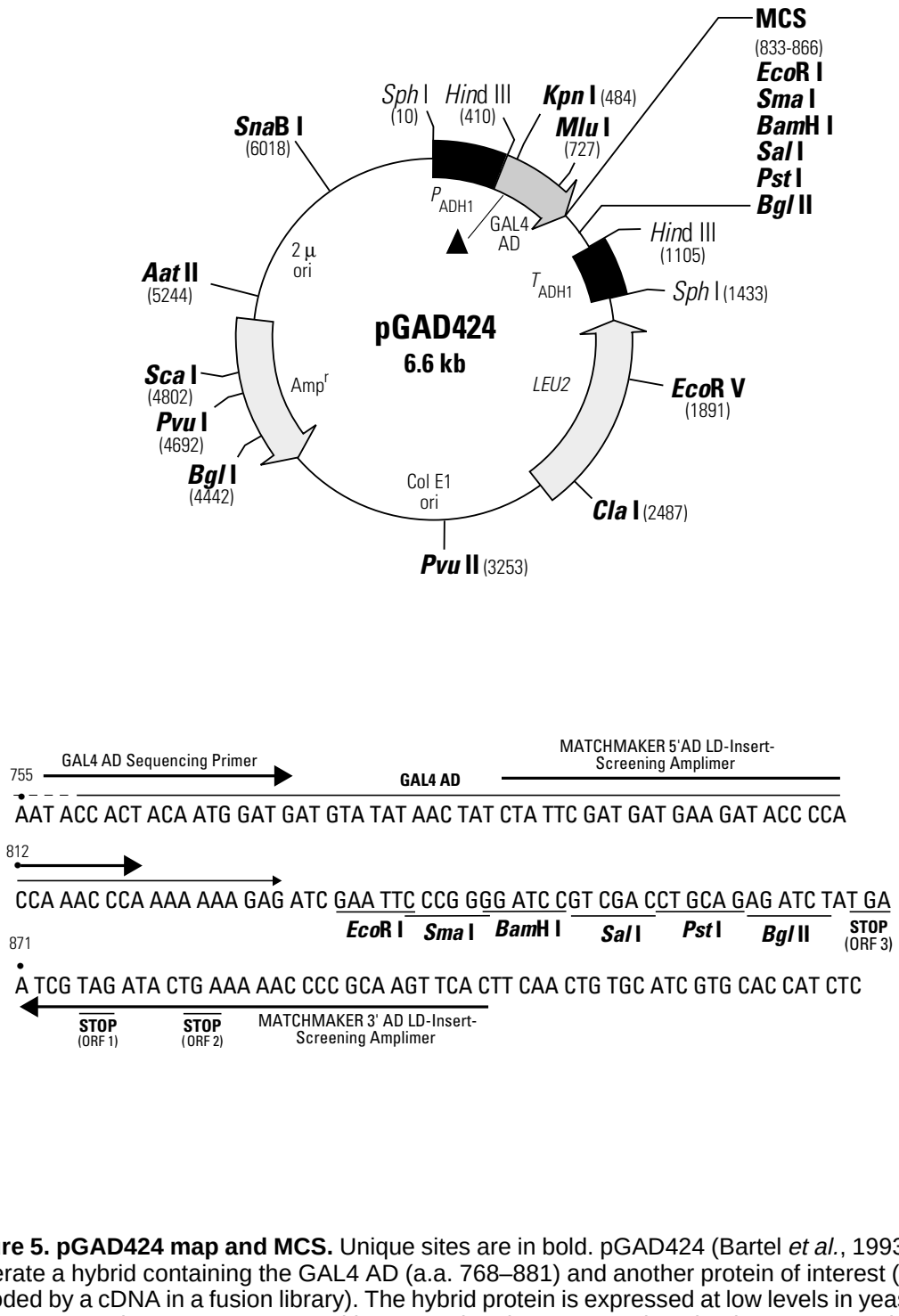


Figure 5. pGAD424 map and MCS. Unique sites are in bold. pGAD424 (Bartel *et al.*, 1993) is used to generate a hybrid containing the GAL4 AD (a.a. 768–881) and another protein of interest (or a protein encoded by a cDNA in a fusion library). The hybrid protein is expressed at low levels in yeast host cells from a truncated *ADH1* promoter and is targeted to the yeast nucleus by the SV40 T-antigen nuclear localization sequence (▲ ; Chien *et al.,* 1991). pGAD424 contains the *LEU2* gene for selection in Leu⁻ auxotrophic yeast strains.
GenBank Accession: #U07647.

II. GAL4 Activation Domain (AD) Cloning Vectors *continued*

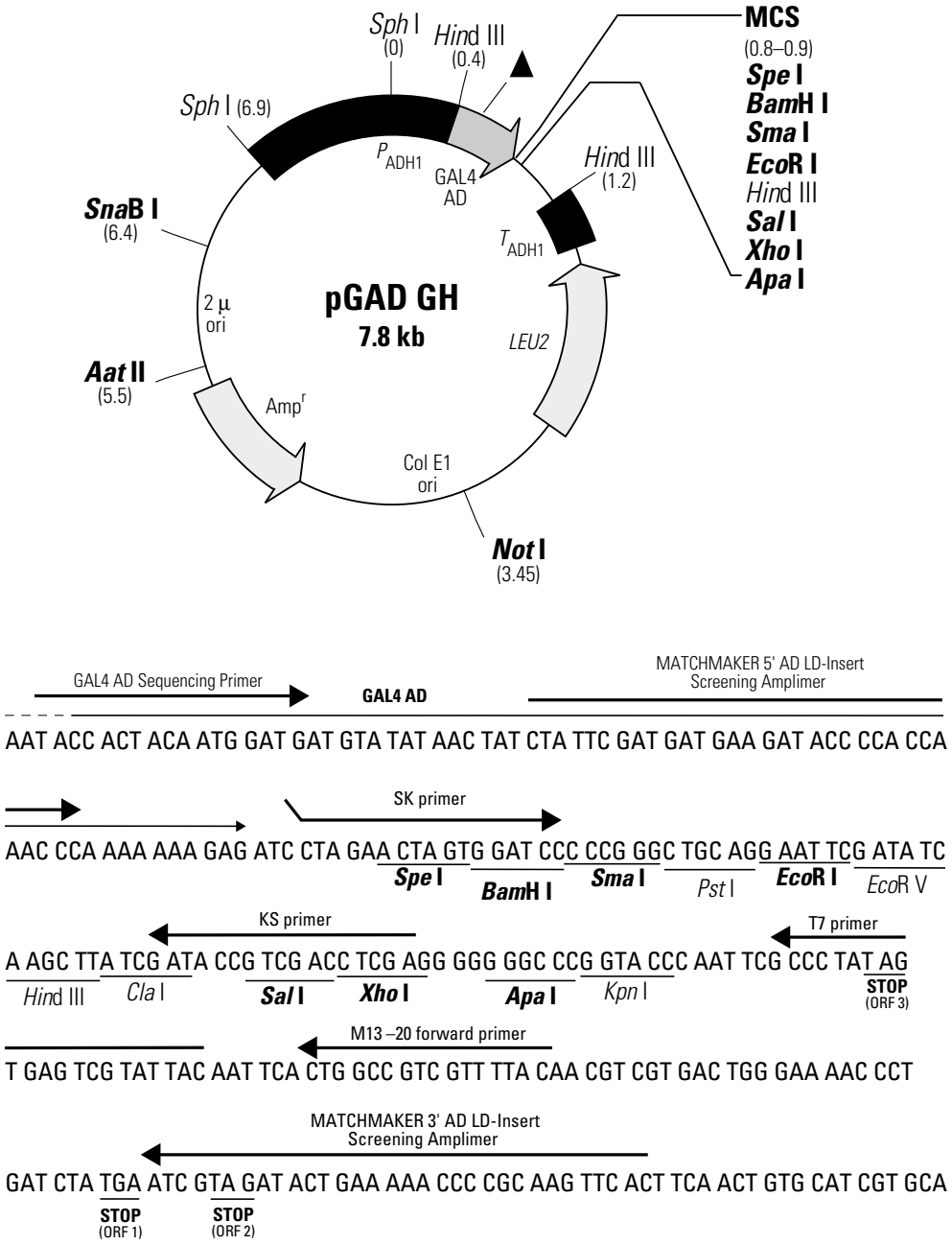


Figure 6. pGAD GH map and MCS. Unique sites are in bold. pGAD GH (van Aelst *et al.*, 1993) is used to generate a hybrid containing the GAL4 AD (a.a. 768–881) and another protein of interest (or a protein encoded by a cDNA in a fusion library). The hybrid protein is expressed at high levels in yeast host cells from the full-length *ADH1* promoter and is targeted to the yeast nucleus by the SV40 T-antigen nuclear localization sequence (▲ ; Chien *et al.,* 1991). pGAD GH contains the *LEU2* gene for selection in Leu⁻ auxotrophic yeast strains. pGAD GH was derived from pGAD GL (Figure 7) and is nearly identical to it, except in the promoter region. Also, an *Sph* I site (at bp 1600) in pGAD GL was mutagenized when pGAD GH was constructed. Indicated bp's are approximate. No further pGAD GH sequence information is available.

II. GAL4 Activation Domain (AD) Cloning Vectors *continued*

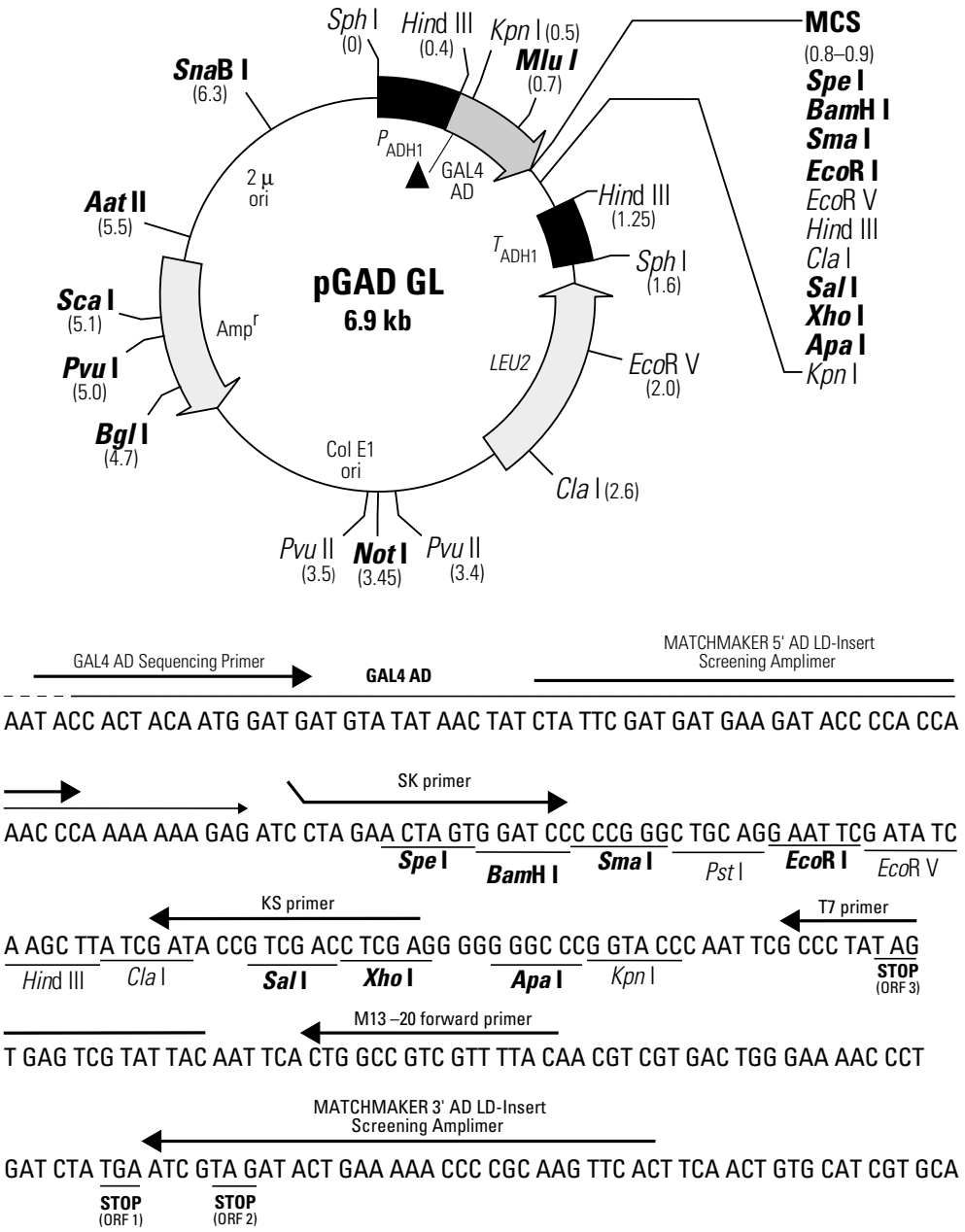
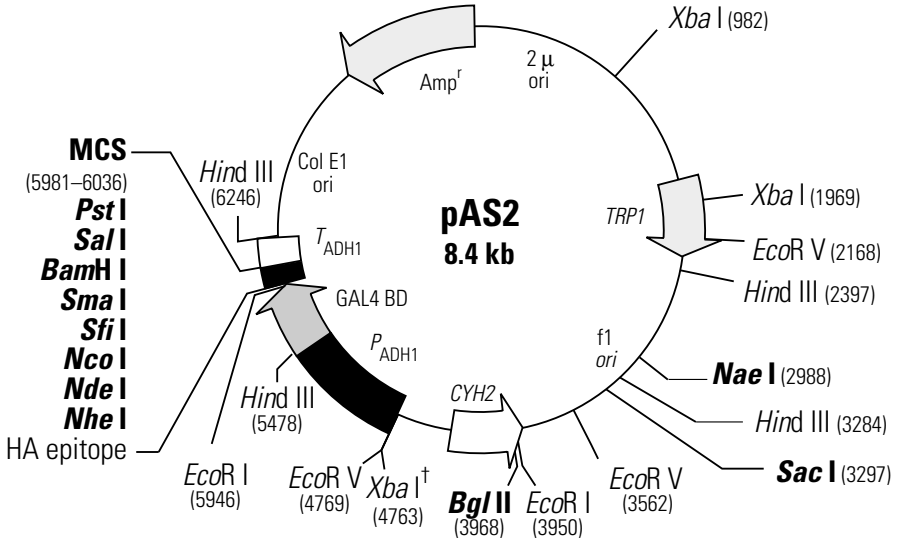


Figure 7. pGAD GL map and MCS. Unique sites are in bold. pGAD GL (van Aelst *et al.*, 1993) is used to generate a hybrid containing the GAL4 AD (a.a. 768–881) and another protein of interest (or a protein encoded by a cDNA in a fusion library). The hybrid protein is expressed at low levels in yeast host cells from a truncated *ADH1* promoter and is targeted to the yeast nucleus by the SV40 T-antigen nuclear localization sequence (▲ ; Chien *et al.*, 1991). pGAD GL contains the *LEU2* gene for selection in Leu⁻ auxotrophic yeast strains. Indicated bp's are approximate. No further pGAD GL sequence information is available.

III. GAL4 DNA-Binding Domain (DNA-BD) Cloning Vectors



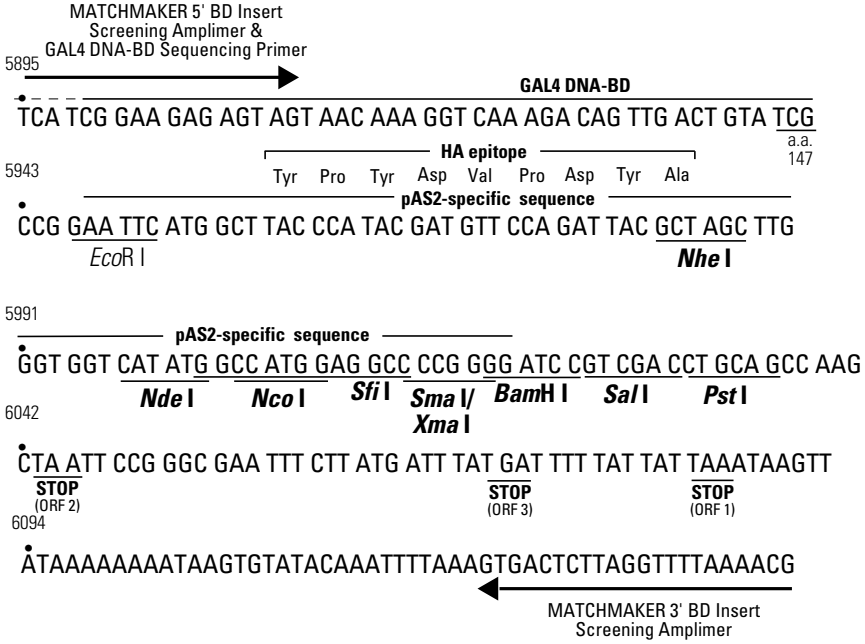
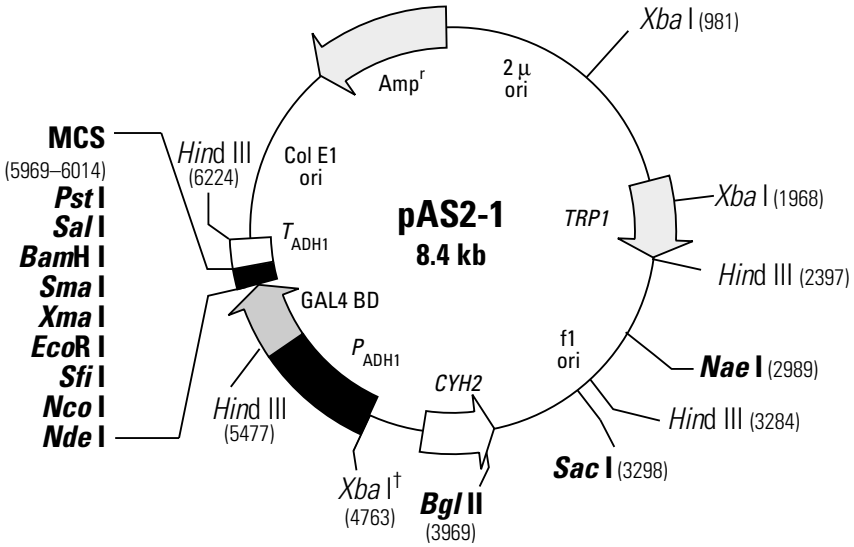


Figure 8. pAS2 map and MCS. Unique sites are in bold. pAS2 is a cloning vector used to generate fusions of a bait protein with the GAL4 DNA-BD (a.a. 1–147). pAS2 is synonomous with $pAS1_{CHY2}$ (Harper *et al.*, 1993) and carries the wild-type yeast *CYH*^{S}*2* gene, which confers sensitivity to cycloheximide in transformed yeast cells. The hybrid protein is expressed at high levels in yeast host cells from the full-length *ADH1* promoter. The hybrid protein is targeted to the yeast nucleus by nuclear localization sequences (Silver *et al.*, 1984). pAS2 contains the *TRP1* gene for selection in Trp^{-} auxotropic yeast strains. The *Xba* I site at bp 4763 (†) is methylation sensitive. The acidic amino acids of the HA epitope tag fused to the GAL4 DNA-BD causes high backgrounds in the two-hybrid assay when pAS2 is used as a negative control. The HA epitope also leads to high backgrounds in some plasmid constructs based on pAS2. pAS2 has been discontinued and replaced by its derivative pAS2-1 (Figure 9), which does not contain an HA epitope.

GenBank Accession: #U30496.

III. GAL4 DNA-Binding Domain (DNA-BD) Cloning Vectors *continued*



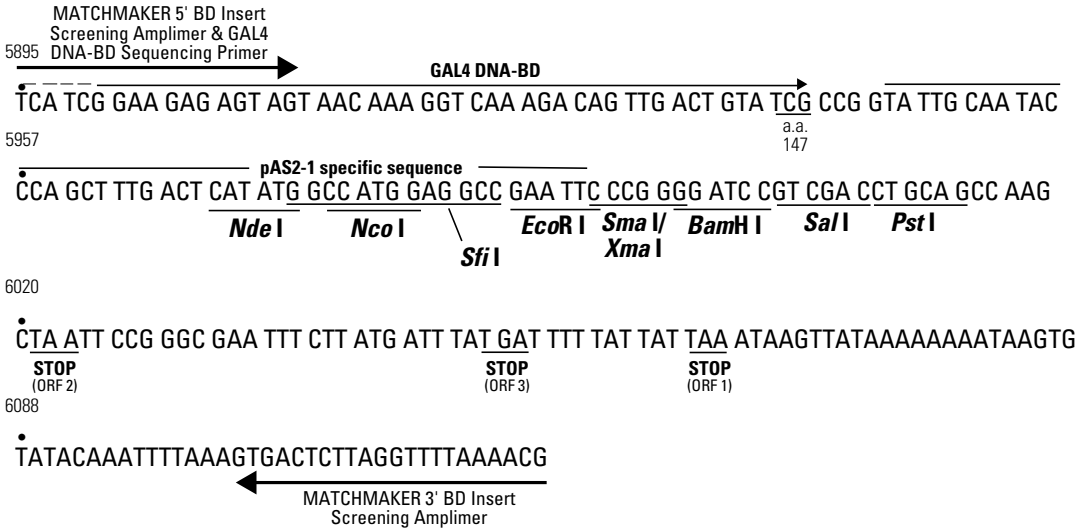


Figure 9. pAS2-1 map and MCS. Unique sites are in bold. pAS2-1 is a cloning vector used to generate fusions of a bait protein with the GAL4 DNA-BD (a.a. 1–147). pAS2-1 is derived from pAS2 (Figure 8) and hence from $pAS1_{CHY2}$ (Harper *et al.*, 1993) and carries the CYH^S2 gene for cycloheximide sensitivity. The hybrid protein is expressed at high levels in yeast host cells from the full-length *ADH1* promoter (P_{ADH1}). The hybrid protein is targeted to the yeast nucleus by nuclear localization sequences (Silver *et al.,* 1984). The *Xba* I site at bp 4763 (†) is methylation sensitive. pAS2-1 contains the *TRP1* gene for selection in Trp^- auxotrophic yeast strains. Plasmid modification was performed at CLONTECH. GenBank Accession: #U30497.

Compared with pAS2, pAS2-1 contains a neutral, short peptide instead of an HA epitope tag. Removing the HA epitope tag and converting a.a.149 from Glu to Val completely eliminates the autonomous activation activity of pAS2 (assayed in Y187 using the *lacZ* reporter; Holtz & Zhu, 1995). pAS2-1 has a different MCS than pAS2; however, the cloning sites that they have in common are in the same reading frame. Furthermore, the *Eco*R I, *Xma* I/*Sma* I, *Bam*H I, Sal I, and Pst I sites in the pAS2-1 MCS are in the same reading frames as those in pGBT9. Thus, inserts from pAS2 or pGBT9 can be excised and moved to pAS2-1 without changing the reading frame. The *Eco*R I site in the pAS2-1 MCS is unique because the *Eco*R I site in the CYH^S2 gene in pAS2 was eliminated by site-directed mutagenesis.

III. GAL4 DNA-Binding Domain (DNA-BD) Cloning Vectors *continued*

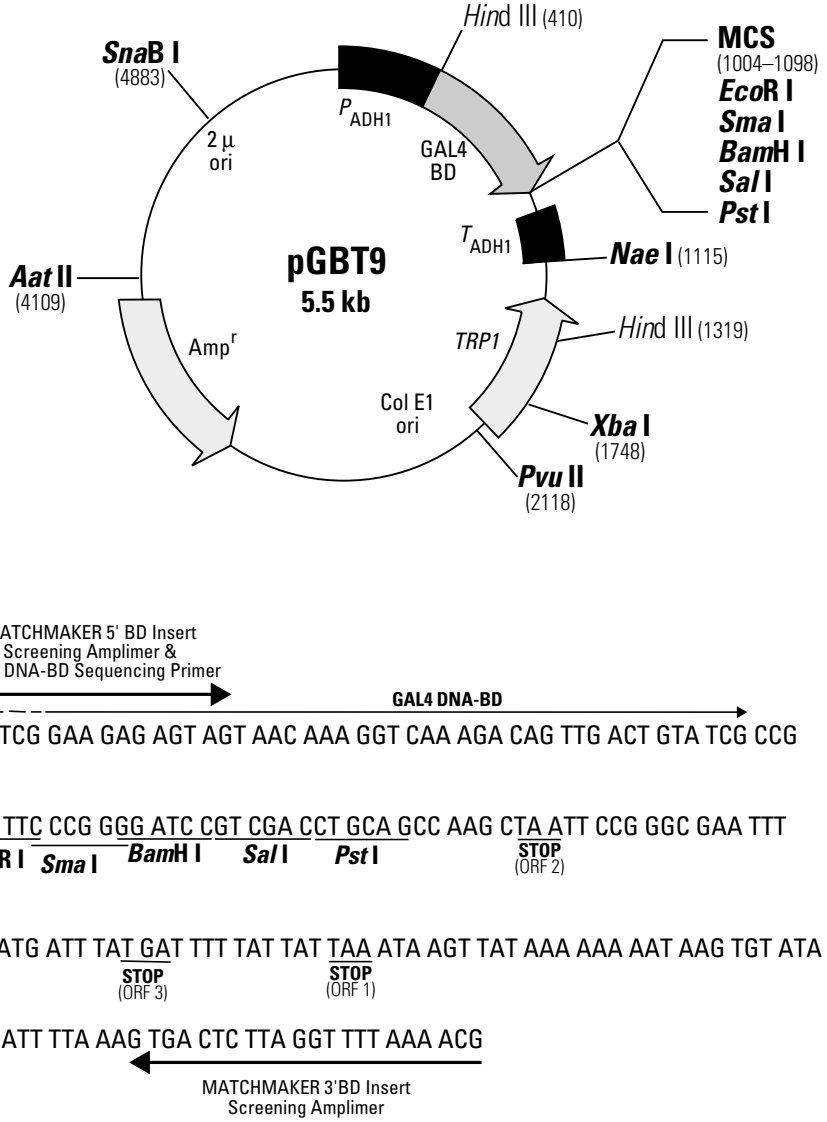


Figure 10. pGBT9 map and MCS. Unique sites are in bold. pGBT9 (Bartel *et al.*, 1993) is a cloning vector used to generate fusions of a bait protein with the GAL4 DNA-BD (a.a. 1–147). The hybrid protein is expressed at low levels in yeast host cells from a truncated ADH1 promoter. The hybrid protein is targeted to the yeast nucleus by nuclear localization sequences (Silver *et al.*, 1984). pGBT9 contains the *TRP1* gene for selection in Trp$^-$ auxotropic yeast strains.
GenBank Accession: #U07646.

IV. Contol Plasmids

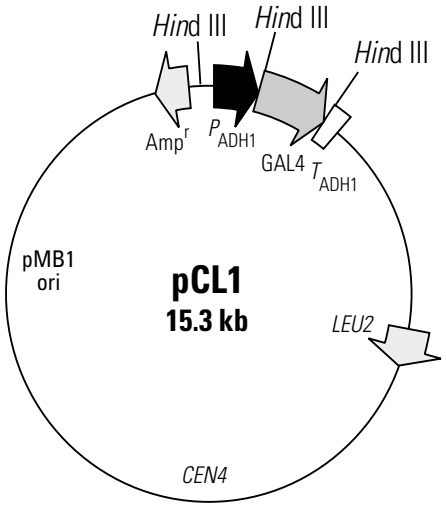


Figure 11. pCL1 map. pCL1 (Fields & Song, 1989) is a positive control plasmid that encodes the full-length, wild-type GAL4 protein. The GAL4 protein activates reporter genes under the control of a GAL4-responsive element (UAS_G). Thus, pCL1 is used as a positive control for the transcription assay in GAL4-based MATCHMAKER Two-Hybrid Systems. This vector is a derivative of YCp50 referenced in Fields & Song, 1989. pCL1 has not been sequenced.

IV. Contol Plasmids *continued*

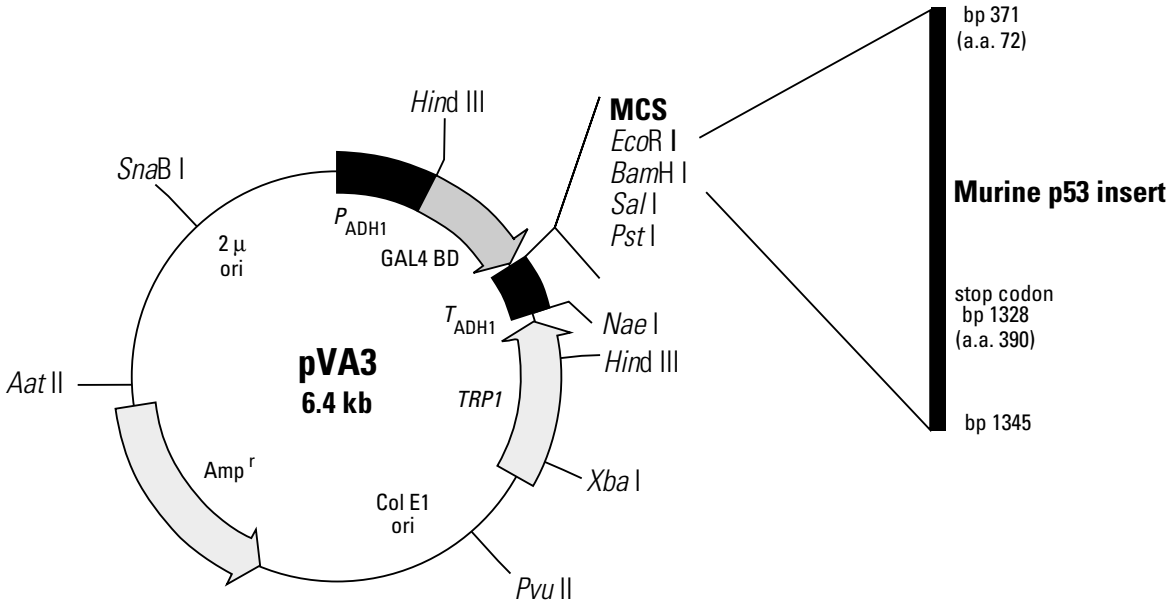


Figure 12. pVA3 map. pVA3 (Iwabuchi *et al*., 1993) is a positive control plasmid that encodes a fusion of the murine p53 protein (a.a. 72–390) and the GAL4 DNA-BD (a.a. 1–147). The murine p53 cDNA (GenBank Accession #K01700) was cloned into pGBT9 (Figure 10). pVA3 has not been sequenced and it is not known whether any of the sites are unique.

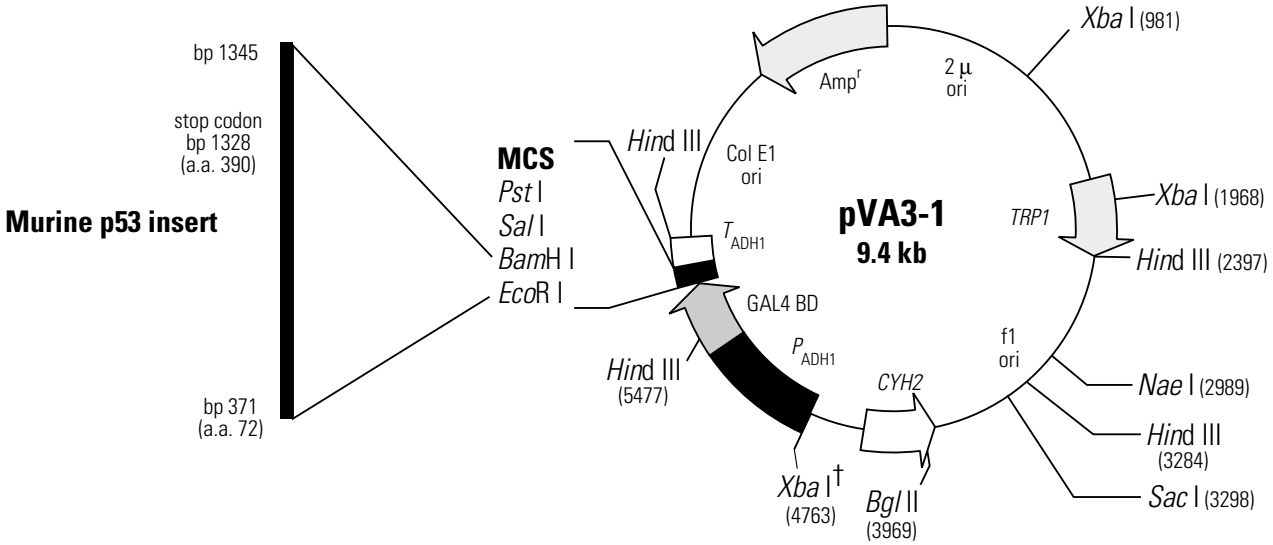


Figure 13. pVA3-1 map. pVA3-1 is a positive control plasmid that encodes a fusion of the murine p53 protein (a.a. 72–390) and the GAL4 DNA-BD (a.a. 1–147). The murine p53 cDNA (GenBank Accession #K01700) was cloned into $pAS1_{CYH2}$ (a precursor of pAS2-1 [Figure 9]; Harper *et al.,* 1993). The p53 insert was derived from the plasmid described in Iwabuchi *et al.* (1993); plasmid modification was performed at CLONTECH. The *Xba* I site at bp 4763 (†) is methylation sensitive. pVA3-1 has not been sequenced and it is not known whether any of the sites are unique.

IV. Contol Plasmids *continued*

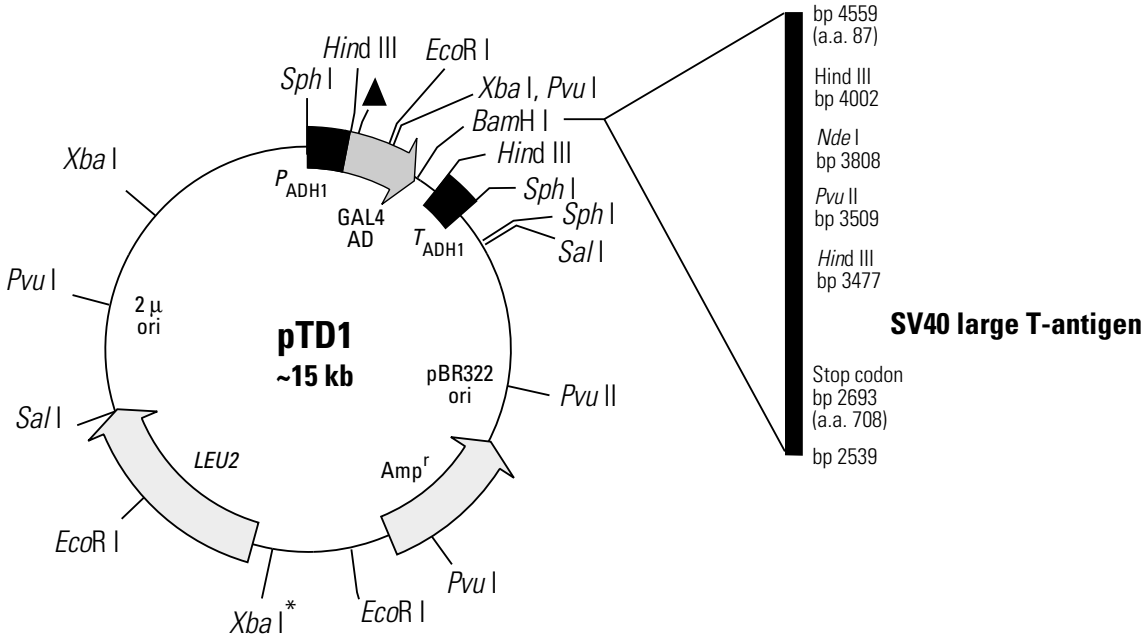


Figure 14. pTD1 map. pTD1 (Li & Fields, 1993) is a positive control plasmid that encodes a fusion of the SV40 large T-antigen (a.a. 86–708) and the GAL4 AD (a.a. 768–881). The SV40 large T-antigen cDNA was cloned into pGAD3F (Chien *et al.*, 1991). pTD1 has not been sequenced and it is not known whether any of the sites are unique.

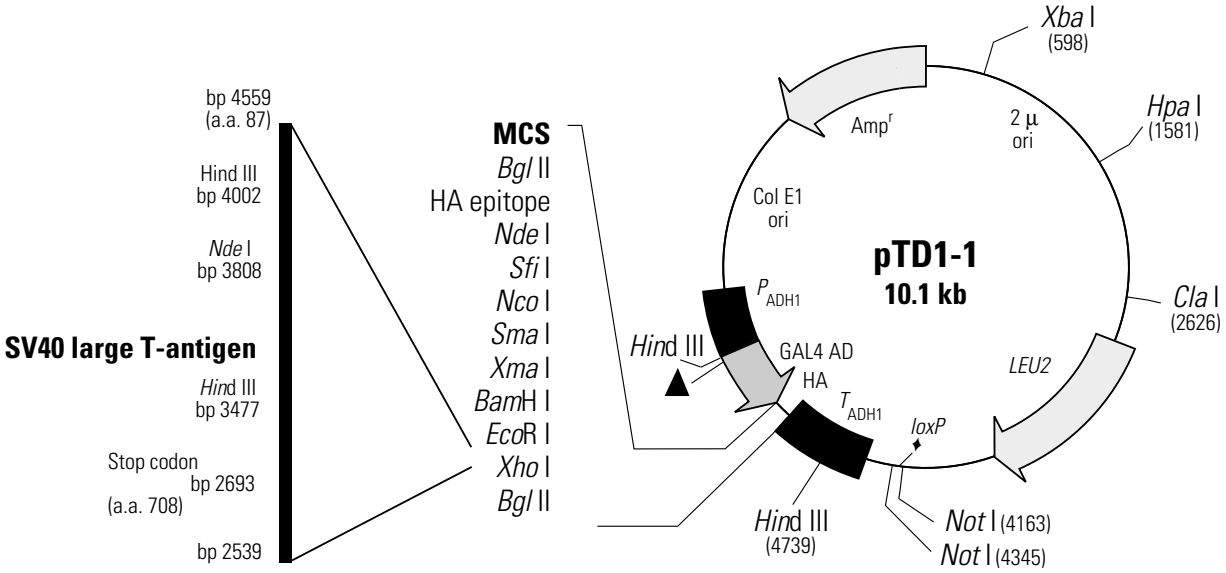


Figure 15. pTD1-1 map. pTD1-1 is a positive control plasmid that encodes a fusion of the SV40 large T-antigen (a.a. 87–708) and the GAL4 AD (a.a. 768–881). The SV40 large T-antigen cDNA (GenBank Locus SV4CG) was cloned into pACT2 (Figure 3). The SV40 T-antigen insert was derived from the plasmid referenced in Li & Fields (1993); plasmid modification was performed at CLONTECH. pTD1-1 has not been sequenced and it is not known whether any of the sites are unique.

IV. Contol Plasmids *continued*

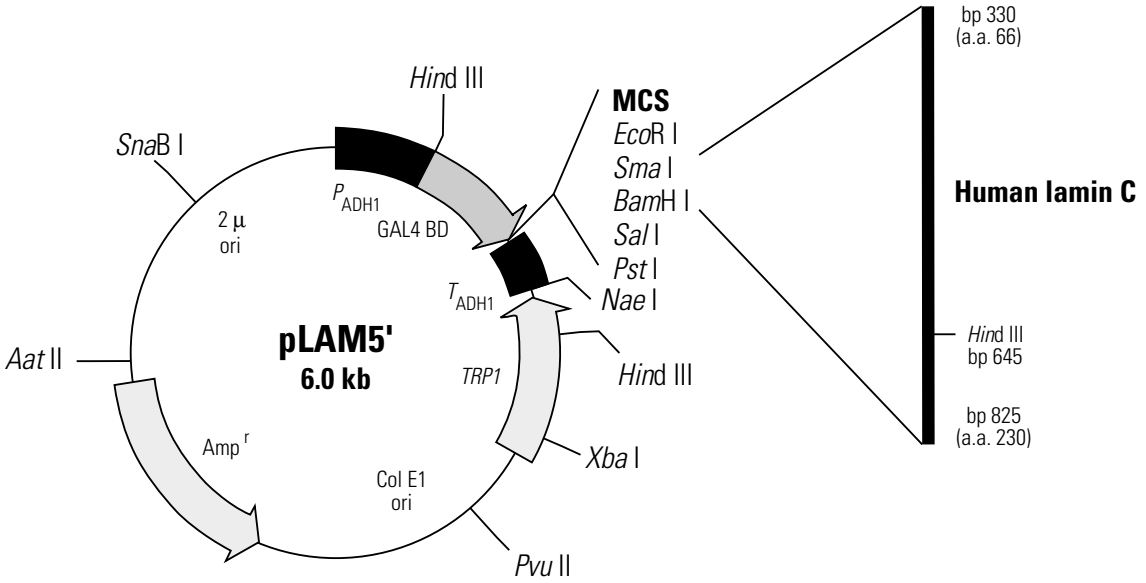


Figure 16. pLAM 5' map. pLAM5' is a false-positive detection plasmid that encodes a fusion of the human lamin C protein (a.a. 66–230) and the GAL4 DNA-BD (a.a. 1–147). The lamin C cDNA insert (GenBank Accession #M13451) was derived from the plasmid referenced in Bartel *et al.* (1993), and was cloned into pGBT9 (Figure 10). Plasmid modification was performed at CLONTECH. pLam5' has not been sequenced and it is not known whether any of the sites are unique.

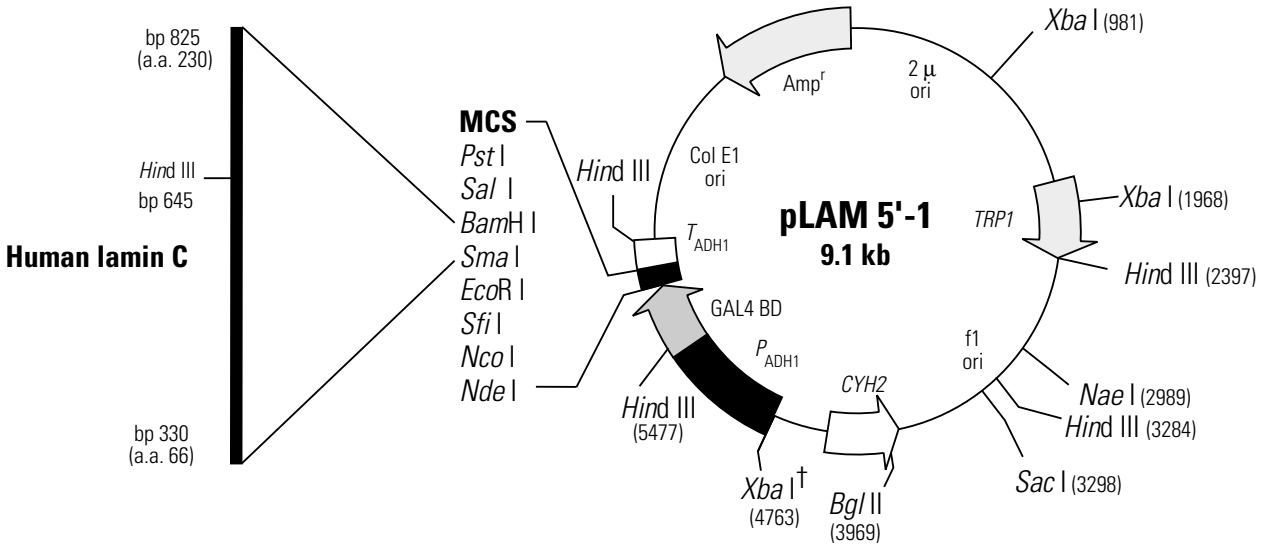


Figure 17. pLAM 5'-1 map. pLAM5'-1 is a false-positive detection plasmid that encodes a fusion of the human lamin C protein (a.a. 66–230) and the GAL4 DNA-BD (a.a. 1–147). The lamin C cDNA insert (GenBank Accession #M13451) was derived from the plasmid referenced in Bartel *et al.* (1993), and was cloned into pAS2-1 (Figure 9). Plasmid modification was performed at CLONTECH. pLAM5'-1 has not been sequenced and it is not known whether any of the sites are unique.

V. References

Bartel, P. L., Chien, C.-T., Sternglanz, R. & Fields, S. (1993) Using the two-hybrid system to detect protein-protein interactions. In *Cellular Interactions in Development: A Practical Approach*., ed. Hartley, D.A. (Oxford University Press, Oxford) pp. 153–179.

Chien, C. T., Bartel, P. L., Sternglanz, R. & Fields, S. (1991) The two-hybrid system: A method to identify and clone genes for proteins that interact with a protein of interest. *Proc. Nat. Acad. Sci. USA* **88**:9578–9582.

Durfee, T., Becherer, K., Chen, P. L., Yeh, S. H., Yang, Y., Kilbburn, A. E., Lee, W. H. & Elledge, S. J. (1993) The retinoblastoma protein associates with the protein phosphatase type 1 catalytic subunit. *Genes Devel.* **7**:555–569.

Fields, S. & Song, O**.** (1989) A novel genetic system to detect protein-protein interactions. *Nature* **340**:245–247.

Harper, J. W., Adami, G. R., Wei, N., Keyomarsi, K. & Elledge, S. J. (1993) The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* **75**:805–816.

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VI. Plasmid and System Ordering Information

Product	Size	Cat.#
MATCHMAKER Two-Hybrid System	each	K1605-1
MATCHMAKER Two-Hybrid System 2	each	K1604-1
Two-Hybrid cDNA Library Construction Kit	each	K1607-1
MATCHMAKER cDNA Libraries		many
MATCHMAKER DNA-BD Insert Screening Amplimer Set	100 rxn	5417-1
MATCHMAKER AD LD-Insert Screening Amplimer Set	100 rxn	9103-1
GAL4 Activation Domain Sequencing Primer	2.5 µg	6473-1
GAL4 Binding Domain Sequencing Primer	2.5 µg	6474-1
GAL4 AD mAb	20 µg	5398-1
GAL4 DNA-BD mAb	20 µg	5399-1
Vectors available separately		
pACT2	40 µg	K1604-A
pGAD10	40 µg	6180-1
pGAD424	40 µg	K1605-B
pGAD GH	40 µg	6182-1
pGAD GL	40 µg	6181-1
pAS2-1	40 µg	K1604-B
pGBT9	40 µg	K1605-A

APPENDIX. Additional MATCHMAKER Plasmid Information

TABLE III. MATCHMAKER GAL4 TWO-HYBRID SYSTEM CLONING VECTORS

Vector	System[a]	Description	Selection on SD Medium	Size (kb)	Diagnostic R.E. Sites (kb)	GenBank Accession #	References
pACT	MM Libraries	*GAL4* AD, *LEU2*, Amp^r, HA epitope tag	–Leu	7.65	*Eco*R I (3.0, 3.05, 1.6)	not available	Durfee *et al.*, 1993; Elledge, pers. comm.
pACT2	GAL4 2H-2 (#K1604-1) & MM Libraries	*GAL4* AD, *LEU2*, Amp^r, HA epitope tag	–Leu	8.1	*Hin*d III (7.3, 0.8)	U29899	Li *et al.*, 1994; Elledge, pers. comm.
pAS2	(#K1605-D)[b]	*GAL4* DNA-BD, *TRP1*, Amp^r	–Trp	8.4	*Hin*d III (4.6, 2.2, 0.9, 0.7)[d]	U30496	Durfee *et al.*, 1993; Harper *et al.*, 1993
pAS2-1[c]	GAL4 2H-2 (#K1604-1)	*GAL4* DNA-BD, *TRP1*, Amp^r, *CYH^{s}2*	–Trp	8.4	*Hin*d III (4.6, 2.2, 0.9, 0.7)[d]	U30497	Harper *et al.*, 1993
MM Libraries	*GAL4* AD,	–Leu *LEU2*, Amp^r	6.6	*Hin*d III	U13188 (5.9, 0.7)[e]	Bartel *et al.*, 1993	**pGAD10**
pGAD424	GAL4 2H (#K1605-1)	*GAL4* AD, *LEU2*, Amp^r	–Leu	6.6	*Hin*d III (5.9, 0.7)[e]	U07647	Bartel *et al.*, 1993
pGAD GH	MM Libraries	*GAL4* AD, *LEU2*, Amp^r	–Leu	7.9	*Hin*d III (7.1, 0.5, 0.3)	not available	van Aelst *et al.*, 1993
pGAD GL	MM Library	*GAL4* AD, *LEU2*, Amp^r	–Leu	6.9	*Hin*d III (6.1, 0.5, 0.3)	not available	van Aelst *et al.*, 1993
pGBT9	GAL4 2H (#K1605-1)	*GAL4* DNA-BD, *TRP1*, Amp^r	–Trp	5.5	*Hin*d III (4.6, 0.9)	U07646	Bartel *et al.*, 1993

[a] Key to system abbreviations: GAL4 2H-2 = MATCHMAKER Two-Hybrid System 2 (#K1604-1); GAL4 2H = MATCHMAKER Two-Hybrid System (#K1605-1). Some plasmids are also available separately (see Section VI).

[b] The MATCHMAKER Supplement Kit (#K1605-D) was replaced by the MATCHMAKER Two-Hybrid System 2 (#K1604-1).

[c] pAS2-1 is a derivative of the plasmid described in this reference; the plasmid was modified at CLONTECH.

[d] The *Eco*R I site is unique in pAS2-1, but not in pAS2.

[e] pGAD424 is linearized by digestion with *Sal* I; pGAD10 does not contain a *Sal* I site.

APPENDIX. Additional MATCHMAKER Plasmid Information *continued*

TABLE IV. MATCHMAKER GAL4 TWO-HYBRID SYSTEM CONTROL PLASMIDS

Vector[a]	System	Description	Selection on SD Medium	Size (kb)	Diagnostic R.E. Sites (kb)	References
pCL1	GAL4 2H & 2H-2 (#K1605-1 & #K1604-1)	wild-type full-length *GAL4* gene in a YCp50 derivative, *LEU2*, Amp^r	–Leu	~15.3	*Hin*d III (~11.2, 2.8, 1.8)	Fields & Song, 1989
pLAM5'	GAL4 2H (#K1605-1)	Human lamin C$_{(66–230)}$ in pGBT9, *TRP1*, Amp^r	–Trp	6.0	*Hin*d III (4.6, 0.8, 0.6)	Bartel *et al.*, 1993
pLAM5'-1	GAL4 2H-2 (#K1604-1)	Human lamin C$_{(66–230)}$ in pAS2-1 *TRP1*, Amp^r	–Trp	~9.0	*Hin*d III (4.6, 2.2, 0.9, 0.85, 0.4)	Bartel *et al.*, 1993
pTD1	GAL4 2H (#K1605-1)	SV40 large T-antigen $_{(84–708)}$ in pGAD3F, *LEU2*, Amp^r	–Leu	~15.0	*Hin*d III (12, 1.3, 1.2, 0.5)	Li & Fields, 1993; Chien *et al.*, 1991
pTD1-1	GAL4 2H-2 (#K1604-1)	SV40 large T-antigen $_{(84–708)}$ in pACT2, *LEU2*, Amp^r	–Leu	~10.0	*Hin*d III (7.3, 1.2, 1.0, 0.5)	Li & Fields, 1993;
pVA3	GAL4 2H (#K1605-1)	murine p53 $_{(72–390)}$ in pGBT9 *TRP1*, Amp^r	–Trp	6.4	*Hin*d III (4.6, 1.8)	Iwabuchi *et al.*, 1993
pVA3-1	GAL4 2H-2 (#K1604-1)	murine p53 $_{(72–390)}$ in pAS2-1 *TRP1*, Amp^r	–Trp	9.4	*Hin*d III (4.6, 2.2, 1.7, 0.9)	Iwabuchi *et al.*, 1993 Chien *et al.*, 1991

[a] pLAM5', pLAM5'-1, pTD1-1, and pVA3-1 are derivatives of the plasmids described in the indicated references; plasmids were modified at CLONTECH.

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