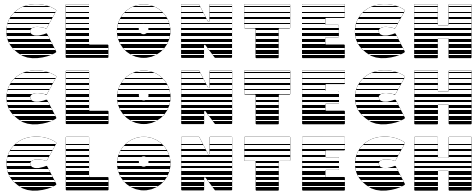


MATCHMAKER One-Hybrid System User Manual

(PT1031-1)

Catalog # K1603-1

See List of Components for storage conditions.

FOR RESEARCH USE ONLY

(PR71132)

Table of Contents

I. Introduction

The MATCHMAKER One-Hybrid System provides the basic tools for conducting a one-hybrid assay—an *in vivo* genetic assay used for isolating novel genes encoding proteins that bind to a target, *cis*-acting regulatory element (or any other short, DNA-binding site; Figure 1). The system may also be used to map the DNA-binding domain of previously known, or newly identified, DNA-binding proteins (DNA-BP). For examples of recent publications using the MATCHMAKER One-Hybrid System, see papers by Kumar *et al.* (1966) and Chen *et al.* (1996).

Cloning genes that encode DNA-BPs has been a difficult part of dissecting transcriptional activation systems. In 1993, Wang & Reed first used the one-hybrid assay to clone the gene encoding the transcription factor OLF-1. The one-hybrid assay was then used to obtain genes encoding several other transcription factors, including REST and ORC-6 (Gstaiger *et al.*, 1995; Lehming *et al.*, 1994; Li & Herskowitz 1993; Luo *et al.*, 1996; and Strubin *et al.*, 1995). Now the MATCHMAKER One-Hybrid System allows you to readily obtain the genes encoding DNA-BPs of interest. The one-hybrid assay offers maximal sensitivity because detection of the DNA-protein interaction occurs while proteins are in their native configurations. In addition, the gene encoding the DNA-BP of interest is immediately available after a library screening.

The one-hybrid assay is based on the finding that many eukaryotic transcriptional activators are composed of physically and functionally independent DNA-binding domains and activation domains (AD). This fact allows researchers to construct various gene fusions that, when expressed as fusion proteins in yeast, can simultaneously bind to a target sequence and activate transcription. Theoretically, in the one-hybrid assay, any target element can be used to trap a protein having a binding-domain specific for that element.

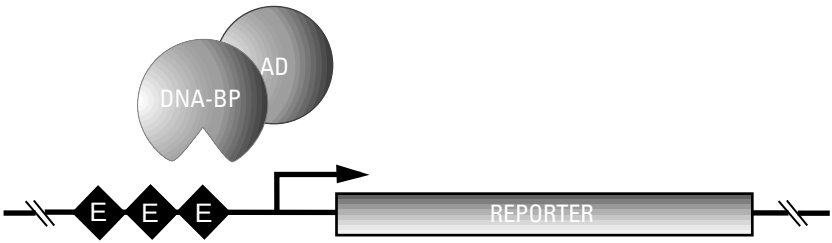


Figure 1. Detection of DNA-binding proteins using the MATCHMAKER One-Hybrid System.

I. Introduction *continued*

- Prepare competent YM4271 cells. **(1 day)**
- Separately transform competent YM4271 with each target-reporter vector, linearized (integration) and circular (control). **(1 day)**
- Select for recombinants on the appropriate minimal selection medium. **(4–6 days)**
- Restreak large, well-isolated colonies from cells transformed with linearized plasmid on selection medium. **(3 days)**
- For yeast transformed with *HIS3* reporter constructs, determine the optimal [3-AT] for inhibiting leaky *HIS3* expression. **(3 days)**
- If applicable, integrate *lacZ* reporter construct into the *HIS3* reporter strain. **(3–5 days)**
- Transform reporter strain with AD fusion library; select on SD/–His/–Leu/+optimal [3-AT]. **(4–8 days)**
- If applicable, test for β-galactosidase activity. **(1 day)**
- His^+; $LacZ^+$ clones are candidates for expressing AD/library proteins that bind to your target element.

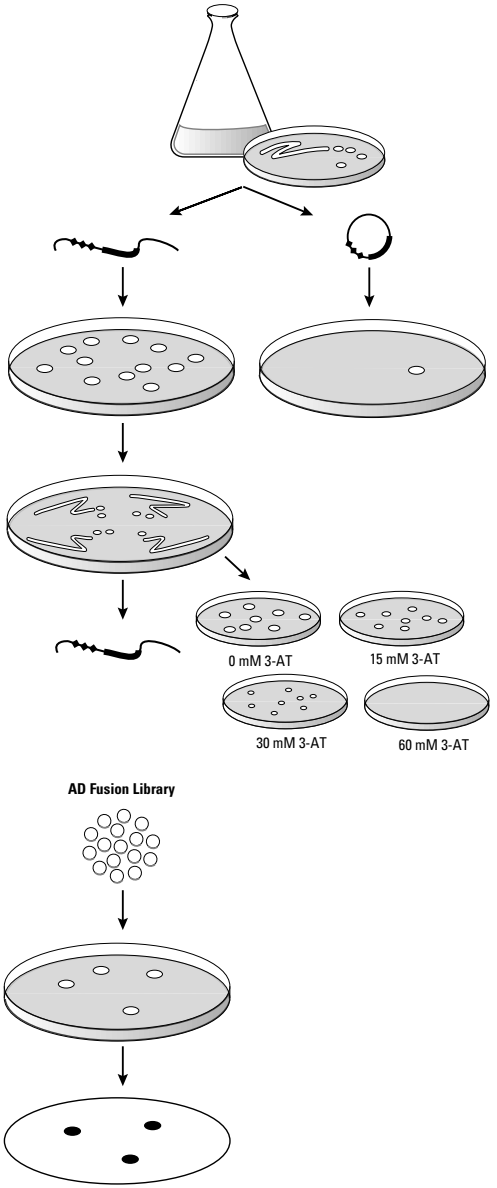


Figure 2. Using a one-hybrid assay to identify cDNAs that encode novel DNA-binding proteins.

I. Introduction *continued*

To conduct the assay, the user must prepare new yeast reporter strains having the sequence of a specific target element upstream of the reporter gene. To prepare each target-reporter strain, one first makes at least three tandem copies of a known target element. These are then inserted upstream of the reporter gene promoter. Next, the user transforms the target-reporter construct into yeast cells and, by marker gene selection, obtains recombinants with genomically integrated reporters to make a new target-reporter strain. Integration is straightforward because the reporter vectors provided in the kit (Appendix A) permit high-frequency, site-specific recombination. In many cases, dual reporter genes, useful for more stringent library screening, may be generated by sequentially integrating the *HIS3* and *lacZ* reporters into the same yeast genome at different loci (*HIS3* and *URA3,* respectively). (See Section VI for details.)

To screen a library for a gene encoding a DNA-BP of interest, the user transforms the target-reporter strain with an AD library of fusions between the target-independent AD and potentially target-specific DNA-BPs. Transformants are then plated on selective medium. If an AD/library hybrid protein interacts with the user's target element, *HIS3* reporter gene expression is activated, allowing colony growth on minimal medium lacking histidine, but containing the concentration of 3-AT needed to inhibit background *HIS3* expression. If a *HIS3*, *lacZ* reporter strain is used, a β-galactosidase assay is performed to verify the DNA-protein interaction and help eliminate any false positive clones. (However, using *lacZ* may not be necessary, because in our experience, the one-hybrid system has a low incidence of false positives.) Next, AD/library plasmids are isolated from the His^+ transformants. Finally, DNA binding should be confirmed by independent methods. Refer to Section VII for detailed instructions for screening an AD fusion library using the one-hybrid assay.

If you wish to use the MATCHMAKER One-Hybrid System to map the DNA-binding domain of a known DNA-BP, the procedure is similar to that described above. The main difference is that instead of transforming your reporter strain with an AD fusion library, you should make AD fusions with gene segments that correspond to specific domains of the known DNA-BP. Then transform these constructs into a target-*lacZ* reporter strain. Only the *lacZ* reporter is used because growth selection is not required, and β-galactosidase activity can be quantified using a liquid assay.

The accompanying CLONTECH Yeast Protocols Handbook (YPH; PT3024-1) contains background information, recipes, and support protocols for use with this User Manual.

I. Introduction *continued*

TABLE I. LIST OF ABBREVIATIONS

MATCHMAKER One-Hybrid Terminology	
AD	GAL4 activation domain (a.a. 768–881)
AD fusion library [or AD library]	A cDNA (or genomic) library constructed in an AD vector such that the proteins encoded by the inserts are fused to the GAL4 AD
AD/library plasmid	Plasmid encoding a fusion of the GAL4 AD and a library insert
AD/library protein	A hybrid comprised of the GAL4 AD fused to a protein encoded by a library insert
AD vector	Plasmid encoding the GAL4 activation domain (AD)
DNA-BP	DNA-binding protein; a protein that binds specifically to your target elements
Target element	The short DNA sequence you wish to use as a potential protein-binding site upstream of a reporter gene
Target-pLacZi, Target-pHISi, Target-pHISi-1	Customized, target reporter vectors: pLacZi, pHISi, or pHISi-1 reporter vector with the target element inserted upstream of the minimal promoter of the *lacZ* or *HIS3* coding region
YM4271[p53HIS], YM4271[p53BLUE]	Yeast strain YM4271 transformed with an integrated p53HIS or p53BLUE vector
Yeast Phenotypes	
His^-, or Leu^-, Trp^-, or Ura^-	Requires histidine (His), or leucine (Leu), or tryptophan (Trp), or uracil (Ura) in the medium to grow; is auxotrophic for one (or more) of these specific nutrients
$LacZ^+$	Expresses the *lacZ* reporter gene; i.e., is positive for β-galactosidase activity
His^+	Expresses the *HIS3* reporter gene; i.e., does not require His in the medium to grow
Miscellaneous	
3-AT	3-amino-1,2,4-triazole; a competitive inhibitor of the *HIS3* gene product (His3p)
DO	Dropout (supplement or solution); a mixture of specific amino acids and nucleosides used to supplement SD base to make SD medium; DO solutions are missing one or more of the nutrients required by untransformed yeast to grow on SD medium
SD medium	Minimal synthetic dropout medium; is comprised of a nitrogen base, a carbon source (glucose unless stated otherwise), and a DO supplement

II. Product Information

A. List of Components

Reporter Vectors (Store plasmids at –20°C.)

Refer to Appendix A for detailed information and maps and to Sections V.A and VI.D for information on choosing vectors.

- **pHISi** (50 µl, 0.1 µg/µl): 6.8-kb vector for integration at the *HIS3* locus; carries the *HIS3* reporter gene
- **pHISi-1** (50 µl, 0.1 µg/µl): 5.4-kb vector for integration at the *HIS3* locus; carries the *HIS3* reporter gene
- **pLacZi** (50 µl, 0.1 µg/µl): 6.9-kb vector for integration at the *URA3* locus; carries the *lacZ* reporter gene

Control Plasmids

See Section III for further information on the control plasmids.

- **p53HIS** (50 µl, 0.1 µg/µl): 6.6-kb positive control plasmid. Three tandem copies of the consensus p53 binding site were inserted into the *Eco*R I/ *Xba* I sites in the MCS of pHISi.
- **p53BLUE** (50 µl, 0.1 µg/µl): 6.7-kb positive control plasmid. Three tandem copies of the consensus p53 binding site were inserted into the *Eco*R I/ S*al* I site in the MCS of pLacZi.
- **pGAD53m** (50 µl, 0.1 µg/µl): 7.8-kb positive control plasmid. Contains mouse p53 gene in frame with the GAL4 AD.
- **pGAD424** (50 µl, 0.1 µg/µl): 6.6-kb negative control plasmid. For expressing the GAL4 AD. (It can also be used as an AD cloning vector, see Figure 8.)

Yeast Strain (Store the yeast strain at –70°C.)

- **YM4271** (0.5 ml); used for reporter vector integration. Genotype is *MATa, ura3-52, his3-200, ade2-101, lys2-801, leu2-3, 112, trp1-901, tyr1-501, gal4-Δ512, gal80-Δ538, ade5::hisG* (Liu *et al*., 1993; Wilson *et al*., 1991).

B. Yeast Strain Information

1. The YM4271 stock is provided frozen in medium containing 25% glycerol and can be stored indefinitely at –70°C.
2. To recover YM4271 from the frozen glycerol stock, scrape a small amount of cells from the frozen stock with a sterile loop or wooden stick and streak them onto a YPD plate. Incubate plate at 30°C for 1–3 days until colonies appear. Seal this working stock plate with Parafilm and store at 4°C. Propagate additional cultures only from isolated colonies on this plate.

   Note: If you cannot recover the strain by scraping the frozen stock, the cells may have settled to the bottom of the tube before the stock was frozen. If this happens, thaw the frozen culture on ice and vortex it before restreaking. The stock may be refrozen.

II. Product Information *continued*

For additional information on the growth and maintenance of yeast, see the YPH, Chapter III. We also recommend Guthrie & Fink's (1991) *Guide to Yeast Genetics and Molecular Biology* (#V2010-1).

3. Healthy yeast colonies grow to >2 mm in diameter. However, small white colonies (<1mm) will form at a rate of 1–2% due to spontaneous mutations that eliminate mitochondrial function (Holm, 1993). Avoid these small colonies when inoculating cultures.
4. 3-AT (3-amino-1,2,4-triazole) is a competitive inhibitor of the yeast *HIS3* protein (His3p). 3-AT is used to inhibit low levels of His3p expressed in a leaky manner and thus to suppress background growth on SD/–His medium (Fields, 1993; Durfee *et al.*, 1993).

III. MATCHMAKER One-Hybrid Positive Control Experiment

To familiarize yourself with the procedures and expected results of a one-hybrid assay, first construct two control yeast reporter strains by integrating the p53HIS and p53BLUE positive control plasmids into YM4271. Both of these vectors have three tandem copies of the consensus p53 binding site inserted upstream of the minimal promoter of the reporter gene (*HIS3* and *lacZ*, respectively). Then perform a control one-hybrid experiment, by simply transforming the YM4271[p53HIS] and YM4271[p53BLUE] reporter strains with pGAD53m, which encodes a p53/AD hybrid. Interaction of the p53/AD hybrid with the p53 binding sites transcriptionally activates the reporter genes, giving a strong positive result in the assay. Transforming the reporter strains with pGAD424, which encodes the GAL4 AD only, should not activate the reporter genes.

From our experience with the YM4271[p53HIS], we know that 45 mM 3-AT is sufficient to completely suppress background growth due to leaky *HIS3* expression in this reporter strain. However, the optimal 3-AT concentration for your customized *HIS3* reporter strain may be different and, therefore, should be experimentally determined before you use it in an AD library screening. If you wish to perform a 3-AT titration on YM4271[p53HIS] for practice, follow the instructions given in Figure 4 for the experimental target *HIS* reporter strains. Note that as the 3-AT concentration increases from 0 to 60 mM, the size of the colonies should get progressively smaller. The 3-AT concentration at which colonies disappear completely is sufficient to permit stringent selection of His^+ transformants on medium lacking His.

1. Separately linearize 1 µg of each positive control plasmid in a total volume of 20 µl using the indicated restriction enzyme. Incubate at 37°C for 2 hr, or as directed by the enzyme manufacturer.
   - p53BLUE: *Nco* I
   - p53HIS: *Xho* I

   Electrophorese a 2-µl sample of the digest on a 1% agarose gel to confirm that the plasmid has been efficiently linearized.
2. Separately transform the linearized reporter plasmids and the same uncut plasmids (as negative controls) into yeast YM4271 as described in Section VI.
   - Plate the **p53HIS** transformation mixture on SD/–His medium. Leaky *HIS3* expression from p53HIS is sufficiently high to permit selection of transformants on medium lacking His.
   - Plate the **p53BLUE** transformation mixture on SD/–Ura medium.

   Expected results for **p53HIS** and **p53BLUE** transformations: Colonies resulting from integration of linearized vectors should be 2–3 mm in diameter, while colonies without integrated vectors should be <0.5 mm. The latter type should not grow larger even after a longer incubation time**.** One or two large colonies appearing on negative control plates may be from the rare integration and expression of nicked plasmid.

III. MATCHMAKER One-Hybrid Positive Control *Continued*

3. Transform YM4271[p53HIS] and YM4271[p53BLUE] separately with pGAD53m (positive control AD/p53 plasmid) and pGAD424 (negative control AD plasmid). Use the small-scale yeast transformation protocol (YPH, Chapter V). **Do not linearize the plasmids before transformation.**
   a. Plate transformation mixtures as described, using the SD selection media indicated in Table II.
   b. Incubate plates at 30°C for 4–6 days. Take note of growth after 3 days and every day thereafter up to 6 days. Perform β-galactosidase colony-lift filters assays on the transformants obtained using YM4271[p53BLUE] as the host strain (YPH, Chapter VI). Compare your results with those shown Table II.
   c. Pick a representative colony from the control transformations shown on lines 1, 2, 4, and 5 of Table II and streak each onto SD/–Leu to maintain the transforming plasmid. After colonies have grown, seal plates with Parafilm and store them at 4°C. Restreak fresh plates at 3–4-week intervals. These transformants are useful as reference strains when you wish to check a new batch of SD selection medium, or when you perform 3-AT titrations or perform β-galactosidase assays on your experimental constructs.
4. Proceed to Section V to prepare your own experimental target-reporter plasmids and host strains.

TABLE II. CONTROL ONE-HYBRID EXPERIMENTS

Expt. #	Integrated Reporter Plasmid	Transforming Plasmid	SD Selection Medium	Expected Results
1.	p53HIS	pGAD53m	–Leu/–His/+45 mM 3-AT	Many large colonies[a]
2.	p53BLUE	pGAD53m	–Leu	Large, β-galactosidase-positive colonies
3.	p53HIS	pGAD424	–Leu/–His/+45 mM 3-AT	No growth or tiny colonies only[b]
4.	p53HIS	pGAD424	–Leu/–His	Large colonies
5.	p53BLUE	pGAD424	–Leu	Large β-galactosidase-negative colonies

[a] If you obtain only small (<1mm) colonies on the 3-AT plates, you may need to perform a 3-AT titration on the reporter strain, as explained in Figure 4, and perhaps use less 3-AT in the medium.

[b] The appearance of tiny colonies only, after 3–4 days incubation, may be due to leaky *HIS3* expression.

IV. MATCHMAKER One-Hybrid System Overview

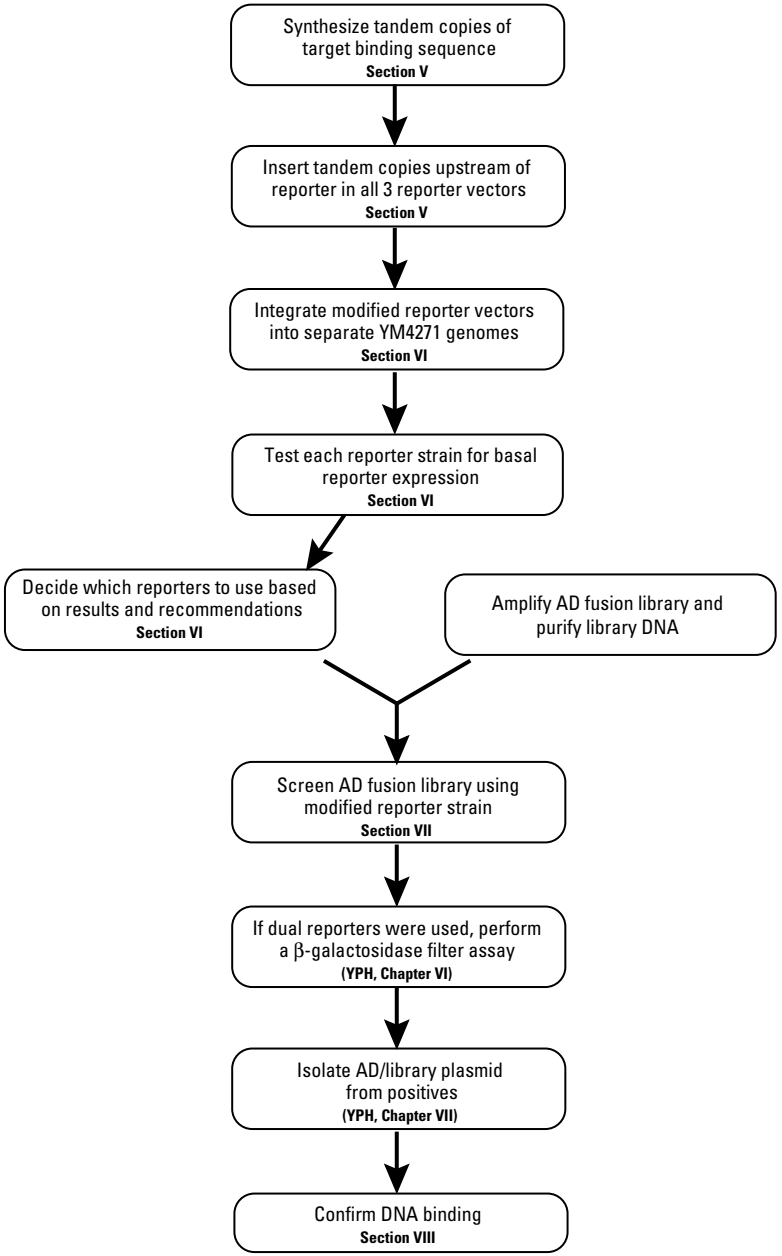


Figure 3. Guide to using the MATCHMAKER One-Hybrid System Protocols to screen an AD fusion library.

V. Preparing Your Target-Reporter Constructs

PLEASE READ THE ENTIRE PROTOCOL BEFORE BEGINNING.

A. Target and Reporter Vector Information

To use the MATCHMAKER One-Hybrid System to screen a cDNA or genomic library for DNA-BPs, you must have identified a true or putative target element. It must be precisely defined using, for example, deletion and/or point mutation analysis. A construct composed of three or more tandem copies of your target regulatory element bordered by restriction sites is then prepared and inserted upstream of the reporter gene in the plasmid's multiple cloning site.

Inserting your target element may alter the level of background *HIS3* and *lacZ* expression. Therefore, constructs should be prepared with your target element in all three reporter vectors provided—pHISi, pHISi-1, and pLacZi (Table III; vector maps in Appendix A). Then follow the guidelines in Section VI.D to determine whether pLacZi can be used and whether to use pHISi or pHISi-1 to provide the most stringent library screening. Although we recommend using dual *HIS3–lacZ* reporters whenever possible, we have found that sometimes using only a *HIS3* reporter works equally well.

The *HIS3* reporter gene is used in two different ways in the MATCHMAKER One-Hybrid System. For vector integration, leaky *HIS3* expression from pHISi and pHISi-1 allows just enough colony growth on SD/–His medium (without 3-AT) to permit its use as a selectable marker. Then, in a library screening, background growth due to leaky *HIS3* expression is controlled by 3-AT in the medium, and the *HIS3* reporter gene is used to detect interaction of an AD/library protein with the target element.

TABLE III. COMPARISON OF REPORTER VECTORS PROVIDED FOR INTEGRATION

	Vector		
Feature	pHISi	pHISi-1	pLacZi
Reporter gene	*HIS3*	*HIS3*	*lacZ*
Background expression	high[a]	low[a]	high
Integration locus	*his*[b]	*his*	*ura*
Restriction site	*Xho* I or *Afl* II	*Xho* I or *Afl* II	*Nco* I or *Apa* I
Yeast selection medium	SD/–His	SD/–His	SD/–Ura

[a] During selection, high background *HIS3* expression levels will result in larger colonies. Background growth should be controlled by 3-AT after reporter integration. The higher leaky *HIS3* expression observed in pHISi is presumably due to a weak UAS in the flanking vector sequence. (pHISi and pHISi-1 were constructed using different vector backbones.)

[b] pHISi also can be integrated into the *URA3* locus (using the *Apa* I restriction site), but do not attempt to integrate pHISi and pLacZi into the same locus in one strain.

V. Preparing Your Target-Reporter Constructs *continued*

B. Synthesizing Tandem Copies of Your Target Element

We recommend that each target-reporter construct has at least three tandem copies of the target element inserted upstream of the reporter gene. (For information about target copy number, see Ghosh *et al.,* 1993.) Although tandem copies may be generated by various methods, we have found the most convenient and reliable method for generating them to be oligonucleotide synthesis. It works nicely because well-defined regulatory elements are usually ≤20 bp. (See Liaw, 1994 for an alternative method.)

1. Design two antiparallel oligonucleotides, one representing the sense strand and the other its antisense complement.

   The sense strand should consist of at least three tandem copies of the target element with a different restriction site on each end. When the two strands are annealed, the resulting double-stranded DNA will have a different overhang at each end for directional cloning into the reporter plasmid's multiple cloning site. (See Step C.3 below for recommended enzyme pairs.)
2. Synthesize both strands without 5' phosphates (according to the protocol of the synthesizer manufacturer).

C. Inserting Tandem Copies of Target Upstream of Reporter Genes

Reagents and materials required:

- Target element: Sense- and antisense-strand oligo (from Step V.B.2)
- Competent *E. coli* DH5α cells (Sambrook *et al.*, 1989; also available from CLONTECH, #C2022-1, -2 or #C2007-1)
- T4 DNA ligase (Available from CLONTECH, #8406-1, -2)
- 10X T4 ligation buffer (Sambrook *et al.*, 1989; or the buffer provided with the commerical enzyme)
- *Nco* I, *Xho* I, and other restriction enzymes (see Step C.3)
- LB/amp plates (YPH, Appendix C)
- 50 mM NaCl
- pHISi, pHISi-1, and pLacZi (provided in the kit)
- Materials for purifying plasmid from *E. coli* transformants

1. For each construct planned, mix 0.1 µg of sense-strand and 0.1 µg of antisense-strand oligonucleotide in 10 µl of 50 mM NaCl.
2. Anneal the oligonucleotides by heating at 70°C for 5 min and then slowly cooling to room temperature (~30 min).
3. **Completely** digest 0.1 µg of each reporter plasmid in a 20-µl double digest using an appropriate pair of restriction enzymes such as those recommended below. Incubate at 37°C for 2 hr, or as directed by the enzyme manufacturer.

V. Preparing Your Target-Reporter Constructs *continued*

- For pHISi and pHISi-1: *Eco*R I and *Xba* I; or *Eco*R I and *Mlu* I.
- For pLacZi: *Eco*R I and *Sa*l I.

Electrophorese a 2-µl sample of the digest on a 1% agarose gel to confirm that the plasmid has been efficiently linearized.

4. Mix 5 µl of digested plasmid, 1 µl of annealed oligo, and 4 µl of H_2O.
5. Add 1.2 µl of 10X T4 ligation buffer and 0.8 µl (at least 0.8 units) of T4 DNA ligase, and incubate at room temperature for 4 hr.

   Note: Since the molar ratio of oligonucleotide to vector is 100:1 or greater, no gel purification to remove the stuffer fragment is required.
6. Separately transform competent DH5α with each construct using a standard method (Sambrook *et al.,* 1989)**.**
7. Plate transformants on LB/amp plates, and incubate at 37°C overnight.
8. Prepare plasmid using any standard method that yields highly pure DNA (Sambrook *et al*., 1989). Check for inserts by electrophoresing on a 2% agarose gel and sequencing across the junctions.
9. Proceed to Section VI to integrate the newly constructed reporter vectors (target-pHISi, target-pHISi-1, and target-pLacZi) into the yeast genome.

VI. Integrating Target-Reporter Constructs Into the Genome

Although nonintegrated reporters were used in the original one-hybrid library screening method described by Wang & Reed (1993), we have found that using integrated reporter genes is preferrable because integration controls reporter gene copy number and hence expression level. Consequently, detection of the desired DNA-protein interactions is highly reproducible.

The reporter vectors provided in this kit, pHISi, pHISi-1, and pLacZi, were designed to be integrated into the yeast genome; in fact, if they do not integrate, they will be lost because these vectors do not carry a yeast replication origin. Linearization in the 3' untranslated region immediately following the *HIS3* marker (in pHISi and pHISi-1) or within the *URA3* marker (of pLacZi and pHISi), significantly increases the efficiency of homologous recombination at the corresponding locus in the yeast genome. Furthermore, integration into the mutated *ura3* (or *his3*) locus of YM4271 confers a Ura^+ (or His^+) phenotype on the transformants, so they can be selected on the appropriate medium. If you plan to use both reporter plasmids in the same strain, it is necessary to integrate them into different genomic loci (i.e., *ura3*, then *his3*), in two consecutive transformations. This is usually accomplished by integrating the target-LacZi reporter into YM4271 previously transformed with (an integrated) target-*HIS3* construct, as described below and in Figure 4.

A. Linearizing Target-Reporter Vectors

Separately digest 1 µg of each target construct in a total volume of 20 µl, using the indicated restriction enzyme. Incubate at 37°C for 2 hr, or as directed by the enzyme manufacturer.

- *Xho* I or *Afl* II for target-pHISi and target-pHISi-1;
- *Nco* I or *Apa* I for target-pLacZi.

Electrophorese a 2-µl sample of the digest on a 1% agarose gel to confirm that the plasmid has been efficiently linearized.

B. Transforming Competent Cells

As discussed in Section V.A, you should initially prepare three customized yeast reporter strains using your three target-reporter vectors (target-pHISi, target-pHISi-1, and target-pLacZi). Be sure to completely linearize the vectors before using them to transform YM4271. Follow the protocol in the YPH (Section V.D-E), using **1** µg of digested reporter plasmid. As a negative control, perform the transformation with 1 µg of the same *uncut* reporter plasmid.

C. Plating the Transformation Mixture

1. Plate the entire target-pHISi and target-pHISi-1 transformation mixtures on SD/–His plates and the target-pLacZi transformation mixtures on SD/–Ura plates to select for colonies with an integrated reporter gene.

   Note: These colonies are easily distinguished from colonies without an integrated functional reporter gene by their larger size (>2 mm).

VI. Integrating Target-Reporter Constructs *continued*

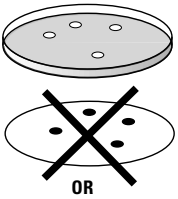
2. Incubate plates up-side-down at 30°C for 4–6 days.
   - Usually for linearized reporter transformants, >100 (2–3-mm) colonies will grow per plate. For uncut reporter transformants, one or two colonies will grow per plate. In addition, more pLacZi than pHISi or pHISi-1 transformants will typically be seen. (This may be a result of the differing lengths of the homologous ends after digestion, or due to differences in the genetic "susceptibility" of the genomic *HIS3* and *URA3* loci to integration.) Ignore tiny background colonies.
   - The integration of a linearized plasmid is a relatively rare event compared to plasmid transformation alone. While autonomously replicating plasmids may yield 10^5 transformants per µg of DNA, linearized plasmid DNA yields only ~10–100 transformants per µg.
3. Restreak colonies that arose from transformation using linearized plasmid on the same selection medium used in Step 1.
4. Incubate plates at 30°C for 4–6 days.
5. These are your master plates. Seal them with Parafilm and store them at 4°C for up to 3–4 weeks.

D. Testing New Reporter Strains for Background Expression

Carefully follow the procedures described in Figure 4 to decide which reporter(s) to use in your library screening experiment and the optimum amount of 3-AT to use in the selection medium.

VI. Integrating Target-Reporter Constructs *continued*

1. Test yeast colonies with integrated target-pLacZi construct in a β-galactosidase filter assay (see YPH, Section VI).

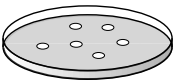


• Analyze results.

→ If colony lift turns blue in <15 min, then background *lacZ* expression is *high*. Do not use pLacZi reporter strain.

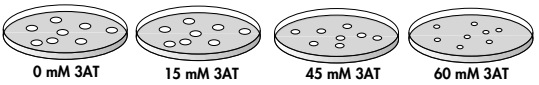
→ If colony lift turns blue in >15 min, then background *lacZ* expression is *low*. Use your pLacZi reporter strain to construct a dual reporter as described below.

2. Next, test yeast colonies with integrated target-pHISi or target-pHISi-1 for background expression. Pick a single colony and suspend it in 1 ml of TE buffer. Plate 5 µl* of the suspension on SD/–His + 0, 15, 30, 45, and 60 mM 3-AT.

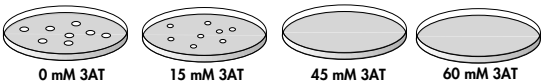


• Analyze results.

→ If yeast grows on SD/–His + >45 mM 3-AT, then background *HIS3* expression is *high* (very leaky).



→ If yeast *only* grows on SD/–His and <45 mM 3-AT, then background *HIS3* expression is *low*.



• Compare results for yeast colonies with integrated target-pHISi or target-pHISi-1.

→ Identify the *HIS3* reporter strain with the lower background level of *HIS3* expression for use in the one-hybrid library screening.

3. Recommendations:

→ If β-galactosidase assay indicated that background *lacZ* expression is *low* in your modified *lacZ* reporter strain, make a dual reporter strain for the library screening by integrating the target-HIS3 construct with lower background *HIS3* expression into the target-pLacZi strain. (Go to Section VI.)

→ If β-galactosidase assay indicated that background *lacZ* expression is *high* in your modified *lacZ* reporter strain, perform the one-hybrid library screening using only the target-HIS3 reporter strain with the lowest level of background *HIS3* expression. (Go to Section VII.)

* Plating too many yeast cells on one plate will result in colonies that are too small.

Figure 4. Testing reporter strains to determine which vector(s) to use with your target.

VII. Screening an AD Fusion Library for DNA-BP Genes

A. Reagents and Materials Required

- YPD (YPH, Appendix C)
- Appropriate sterile tubes and flasks
- Appropriate SD agar plates (YPH, Appendix C)

  Notes:

  - Prepare the selection media, and pour the required number of agar plates in advance. You will need 15 150-mm SD/–Leu/–His plates containing the optimal selection concentration of 3-AT (from Section VI.D) and one 100-mm SD/–Leu plate at 37°C for the transformation control.
  - Allow SD agar plates to dry (unsleeved) at room temperature for 2–3 days or at 30°C for 3 hr prior to plating transformation mixtures. The presence of moisture droplets on the agar surface can lead to uneven spreading of cells and localized variations in 3-AT concentration that can result in false positives.
- Customized yeast reporter strain (from Section VI

.D)

- AD library plasmid DNA in solution. Your AD fusion library should have at least 10^6 clones. Premade MATCHMAKER cDNA and genomic libraries representing many species and tissues are available from CLONTECH. If you have purchased one, see the MATCHMAKER library protocol for more information on amplification. The library may need amplification to provide enough plasmid for the yeast transformation. Alternatively, the Two-Hybrid cDNA Library Construction Kit (#K1607-1) may be used for constructing compatible cDNA libraries. For more information on constructing your own AD libraries, see Vojtek, 1993; Durfee *et al.*, 1993; and Dalton & Triesman, 1992.
- Herring testes carrier DNA (YPH, Appendix D.B; also available from CLONTECH #K1606-A)
- Sterile 1X PEG/LiAc solution (Prepare immediately prior to use from 10X stocks; YPH, Appendix D.B)
- 100% DMSO (Dimethyl sulfoxide; Sigma #D-8779)
- 1X TE buffer (Prepare from 10X TE buffer; YPH, Appendix D.B)
- Sterile glass rod, bent pasteur pipette, or 5-mm glass beads for spreading transformation mixtures on plates.

  Note: The YEASTMAKER™ Yeast Transformation System (#K1606-1) contains all the solutions (except media, H_2O, and DMSO) required for yeast transformation.

VII. Screening an AD Fusion Library *continued*

YEASTMAKER reagents have been optimized for use in the MATCHMAKER Two-Hybrid Systems.

B. Tips for a successful transformation

- Fresh (one- to three-week-old) colonies will give best results for liquid culture inoculation. A single colony may be used for the inoculum if it is 2–3 mm in diameter. Scrape the entire colony into the medium. If colonies on the stock plate are smaller than 2 mm, scrape several colonies into the medium.
- If the overnight or 3-hr cultures are visibly clumped, disperse the clumps with vigorous vortexing before using them in the next step.
- When you are collecting cells by centrifugation, a swinging bucket rotor results in better recovery of the cell pellet.
- **For the highest transformation efficiency (as is necessary for library screening), use competent cells within 1 hr of their preparation.** If necessary, competent cells can be stored (after Step 11) at room temperature for several hours with a minor reduction in competency.
- To obtain an even growth of colonies after plating, continue to spread the transformation mixtures over the agar surface until all liquid has been absorbed. Alternatively, use 5-mm sterile glass beads (5–7 beads per 100-mm plate; 7–9 beads per 150-mm diameter plate) to promote even spreading of the cells.

C. Large-Scale Yeast Transformation

This protocol is scaled for screening >1 x 10^6 independent clones. It is equivalent to a large-scale transformation in the YEASTMAKER Yeast Transformation System (#K1606-1).

1. Inoculate several colonies of the appropriate reporter yeast strain (Section VI.D), 2–3 mm in diameter, into 1 ml of YPD.
2. Vortex vigorously for 2 min to disperse any clumps.
3. Transfer this cell suspension into a flask containing 50 ml of YPD.
4. Incubate at 30°C for 16–18 hr with shaking at 250 rpm to stationary phase (OD_{600}>1.5).
5. Transfer enough overnight culture to produce an OD_{600} = 0.2–0.3 into 300 ml of YPD.
6. Incubate at 30°C for 3 hr with shaking at 230 rpm. The OD_{600} will be 0.5 ± 0.1.
7. Centrifuge the culture in 50-ml tubes at 1,000 x g for 5 min at room temperature.
8. Discard the supernatant and vortex to resuspend each cell pellet in 25 ml of TE buffer.

VII. Screening an AD Fusion Library *continued*

9. Pool the cells into one tube.
10. Centrifuge the cells again at 1,000 x g for 5 min at room temperature.
11. Discard the supernatant and resuspend the cell pellet in 1.5 ml of freshly prepared, sterile 1X TE/LiAc. Mix well by vortexing.
12. In a sterile 50-ml tube, add 20 µg of AD library plasmid and 2 mg of carrier DNA, and mix well.
13. Add 1.0 ml of competent cells to the Step 12 DNA mixture, and mix well by vortexing.

    Note: If cells are not mixed well, transformation efficiency may decline.
14. Add 6 ml of sterile PEG/LiAc to the transformation mixture.
15. Vortex at high speed for 10 sec to mix well.
16. Incubate at 30°C for 30 min with shaking at 200 rpm.
17. Add 700 µl of DMSO and mix well by gentle inversion. Do not vortex.
18. Heat shock for 15 min in a 42°C water bath. Swirl occasionally to mix.
19. Chill on ice for 2 min.
20. Centrifuge at 1,000 x g for 5 min at room temperature, and remove supernatant.
21. Resuspend cells in ~7 ml of TE buffer (for a final volume of ~7.5 ml).

D. Plating the Transformation Mixture

1. Dilute 10 µl of the transformation mixture in 1 ml of TE buffer, and plate 200 µl of the dilution on the 100-mm SD/–Leu plate to determine the transformation efficiency (see the YPH, Section V.E.23).
2. Plate 500 µl of the transformation mixture on each 150-mm plate (15 plates total) containing SD/–His/–Leu/+ optimal [3-AT] (Section VI.D). Spread the cells immediately after pipetting them onto the plate to avoid localized dilutions in the 3-AT concentration.
3. Incubate at 30°C for 4–6 days.
4. Pick the largest colonies and restreak them on SD/–Leu/–His + optimal [3-AT].
   - Colonies resulting from *HIS3* activation should be significantly larger than small colonies resulting from growth due to leaky *HIS3* expression.
   - If a dual reporter strain was used, streak the colonies onto duplicate SD/–Leu/–His/+ optimal [3-AT] plates, and use one set of plates for a β-galactosidase colony-lift filter assay (YPH, Chapter VI). Typically, it takes colonies producing β-galactosidase 0.5–8 hr to turn blue. However, certain strains will turn blue within 20–30 min. Incubation >8 hr often gives false positives.

VIII. Confirming DNA-Binding Activity

We recommend that you confirm the identity of your selected clones with independent methods. First, transform *E. coli* with plasmid isolated from yeast (see YPH, Chapter VII), and then isolate plasmid from *E. coli* using any method that produces highly pure DNA (Sambrook *et al.*, 1989). Although none of the tests suggested below is independently conclusive, the results should provide enough convincing evidence together to support whether the AD library plasmid encodes a DNA-BP.

Note: A protocol for isolation of plasmids from yeast is provided in the YPH, Chapter VII. The YEASTMAKER Yeast Plasmid Isolation Kit (#K1611-1) provides the reagents and a simple protocol for isolating plasmid from yeast. These procedures will provide plasmid DNA suitable for PCR and *E. coli* transformations. A protocol for transforming *E. coli* with plasmid isolated from yeast is also provided in the YPH, Chapter VII. Alternatively, you may wish to try the direct transfer of plasmid DNA from yeast to *E. coli* by electroporation (Marcil & Higgins, 1992).

1. If you have not already done so, consider using the *lacZ* reporter strain to test for transcriptional activation. It is a good independent test for transcriptional activation because it does not rely on *HIS3* growth selection, which can be leaky.
2. Sequence the positive library clones and compare the sequence with that of other DNA-BPs in GenBank, EMBL, or other databases.
   a. If your sequencing results reveal a very short (<10-amino acid) peptide fused to the AD—or no fusion peptide at all—keep sequencing beyond the stop codon. You may find another (larger) open reading frame (ORF) for a peptide that interacts with the target elements in your reporter strain and functions as a transcriptional activator. Nontranslated gaps upstream of ORF inserts are most commonly found in yeast genomic libraries, where intercistronic regions are very short. Such gaps can also occur in cDNA libraries, due to the cloning of a portion of the 5' untranslated region of the mRNA along with the coding region in the cDNA. If the library was bulit in a high-level expression vector (such as pGAD GH or pACT2), a western blot analysis will reveal the presence (and size) of an AD fusion protein.
   b. In some cases, two different ORFs may be expressed as a fusion with the AD even though a nontranslated gap comes between them due, for example, to occasional translational read-through.
   c. If your sequencing results fail to reveal any ORF in frame with the AD coding region, it could be that the positive library clone is transcribed in the reverse orientation from a cryptic promoter within the ADH1 terminator (Chien *et al.*, 1991). Such proteins apparently function as transcriptional activators as well as interact with the target elements.

VIII. Confirming DNA-Binding Activity *continued*

3. If a nonbinding mutant of your target element is available, consider using it in a one-hybrid assay. In this case, first prepare a mutant-type construct otherwise identical to your original target-reporter construct. Then integrate the construct into YM4271, and transform the new reporter strain with the candidate AD/library plasmid. Colonies should result from transcriptional activation using the wild-type but not mutant-type target, indicating that you have identified an interacting DNA-BP. (For an example, see Li & Herskowitz 1993.)
4. Perform *in vitro* translation and a DNA-binding assay (Wu *et al.,* 1994).
5. If you have a library clone that you believe encodes a transcriptional activator, transfer the insert to an expression vector that will generate a fusion of the protein with a cellular localization tag, such as the green fluorescent protein (GFP vectors available from CLONTECH). Nuclear localization is characteristic of many DNA-binding transcriptional activators.
6. Prepare protein extracts and perform a gel-shift DNA-binding assay. When preparing yeast protein extracts for use in an electrophoretic mobility shift assay (EMSA), use a procedure that will yield native proteins. (The protein extraction protocols in the YPH are not suitable for this application because the resulting proteins are denatured.) The following procedure is summarized from Arndt *et al.*, (1987):
   a. Prepare an overnight culture of the yeast transformant in SD/–Leu (to keep selection on the AD/library plasmid). The OD_{600} should be ~1.0.
   b. Centrifuge 100 ml of the culture. Discard the supernatant and resuspend the pellet in 400 µl of extraction buffer: 0.1 M Tris-HCl (pH 7.5), 0.2 M NaCl, 0.01 M β-mercaptoethanol, 20% glycerol, 5 mM EDTA, and 1 mM PMSF.
   c. Transfer cell suspension to a prechilled glass tube and add glass beads. Place sample on ice and vortex vigorously for ~10 min (not including pause times to allow for sample cooling).
   d. Allow glass beads to settle, then transfer all available liquid to another prechilled glass tube.
   e. Add 200 µl of extraction buffer to the liquid and vortex again as described above.
   f. Separate the liquid from the glass beads by centrifugation.

      Note: One way to do this is to punch a pinhole in the microcentrifuge tube and nest this tube inside another tube before adding the sample. Upon centrifuging, the liquid will flow through to the collection tube, leaving the beads behind.

   g. Freeze the liquid quickly in liquid nitrogen and store it at –70°C.
   h. The protein yield is typically 10–20 mg/ml. Use 2–5 µl in the EMSA.

IX. Troubleshooting Guide

A. Excessive Background Growth on Library Screening Medium

Solution 1: Check to make sure that you have prepared the selection medium correctly (YPH, Appendix C). Make sure you have added the appropriate amount of 3-AT to the selection medium (Section VI.D). Perform a 3-AT titration on the target-reporter strain if you haven't already done so.

Solution 2: If your target-pHISi or target-pHISi-1 reporter grows on SD/–His medium even in the presence of ≥60mM 3-AT, the inserted target element may be interacting with yeast endogenous transcriptional activators, or may not require trans-acting factors to activate the *HIS3* reporter. It may be necessary to redesign the target element and construct new reporter strains.

B. Low Transformation Efficiency When Screening an AD Fusion Library

The transformation efficiency is determined by the number of colonies growing on the control SD/–Leu plate (Section VII.E). The transformation efficiency should be at least 10^4 cfu/µg for the library transformation. If your library transformation efficiency is lower than this, try one or more of the following suggestions.

Solution 1. Repeat the experiment using more of the AD/library plasmid (maximum ~50 µg). Check the purity of the DNA and, if necessary, repurify it by ethanol precipitation before using it again. If you are not already doing so, we strongly recommend using the pretested and optimized YEASTMAKER Carrier DNA, which is available separately (#K1606-A) or as part of the YEASTMAKER Yeast Transformation System (#K1606-1).

Solution 2. Repeat the transformation, this time including a “recovery” period after the heat shock. To provide a recovery period, perform the transformation as described (Section VII.D), but add the following steps after Step D.20:

1. Resuspend cells in 50 ml of SD/ –His liquid medium. Divide cell suspension into two 50-ml tubes.
2. Incubate cells at 30°C for 1 hr with shaking at 230 rpm.
3. Centrifuge at 1,000 x g for 5 min at room temperature. Remove supernatant.
4. Continue protocol from Step D.21.

IX. Troubleshooting Guide *continued*

C. Failure To detect an Interaction Between the Target Element and a Protein That Normally Interacts *in vivo* (i.e., False Negative Results)

Solution 1. If expression of the AD hybrid protein is toxic to the cell, transformants will not grow or will grow very slowly on the selection plate. Sometimes truncation of the AD hybrid protein will alleviate the toxicity and still allow the interaction to occur.

Solution 2. If the transformation efficiency is too low (as determined from the control in Section VII.E), you may not be screening a sufficient number of library cotransformants. This can be critical, especially if the interacting protein of interest is encoded by a rare transcript in the source tissue. See Section IX.B above for tips on improving transformation efficiency.

Solution 3. If one of the following situations is occurring, it may interfere with the ability of the AD hybrid proteins to interact with the target element: (1) the hybrid proteins are not stably expressed in the host cell; (2) the fused GAL4 AD occludes the site of interaction; (3) the hybrid protein folds improperly; or (4) the hybrid protein cannot be localized to the yeast nucleus. (See van Aelst *et al.* [1993] for one example.) In these cases, it may help to construct hybrids containing different domains of the DNA-binding protein. For example, to study proteins that normally do not localize to the nucleus, it may be necessary to generate mutant forms of the protein that can be transported across the nuclear membrane.

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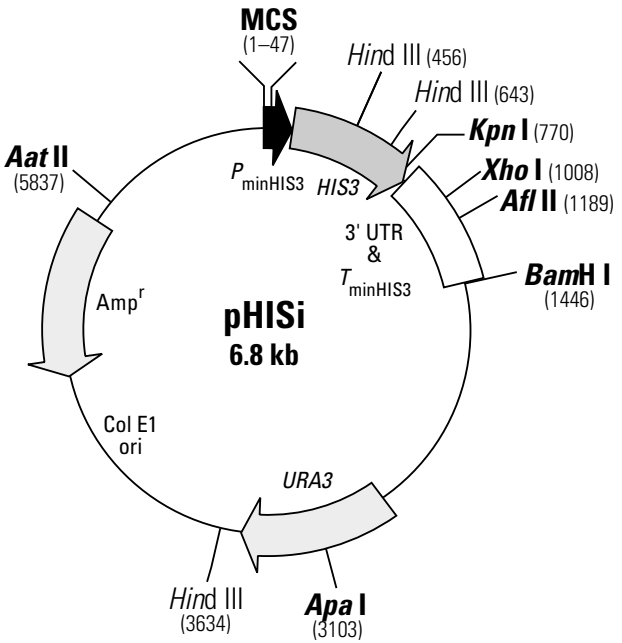
XI. Related Products

Product	Cat. #
MATCHMAKER Two-Hybrid Systems and related products:	
• Mammalian MATCHMAKER Two-Hybrid Assay Kit	K1602-1
• MATCHMAKER Two-Hybrid System	K1605-1
• MATCHMAKER Two-Hybrid System 2	K1604-1
• Two-Hybrid cDNA Library Construction Kit	K1607-1
• MATCHMAKER cDNA and Genomic Libraries	many
• MATCHMAKER Random Peptide Library	NL4000AA
• GAL4 AD Monoclonal Antibody	5398-1
• MATCHMAKER AD LD-Insert Screening Amplimer Set	9103-1
• MATCHMAKER LexA Two-Hybrid System	K1609-1
• MATCHMAKER LexA Libraries	many
General reagents for work with yeast:	
• YEASTMAKER™ Yeast Transformation Kit	K1606-1
• YEASTMAKER Carrier DNA	K1606-A
• YEASTMAKER Yeast Plasmid Isolation Kit	K1611-1
• KC8 Chemically Competent Cells	C2004-1
• KC8 Electrocompetent Cells	C2023-1
• DH5α Chemically Competent Cells	C2007-1
• DH5α Electrocompetent Cells	C2022-1, -2
• YPD Medium	8600-1
• YPD Agar Medium	8601-1
• Minimal SD Base (contains glucose)	8602-1
• Minimal SD Agar Base (contains glucose)	8603-1
• –Trp DO Supplement	#8604-1
• –Leu DO Supplement	#8605-1
• –His DO Supplement	#8606-1
• –Ura DO Supplement	#8607-1
• –His/–Leu DO Supplement	#8609-1
• YEXpress™ Yeast Inducible Expression Systems	many
• YEXpress Secretion Yeast Expression System	6200-1
General Cloning Reagents	
• DH5α Chemically Competent Cells	C2007-1
• DH5α Electrocompetent Cells	C2022-1

Appendix A. MATCHMAKER One-Hybrid Vectors



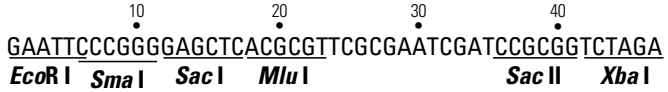
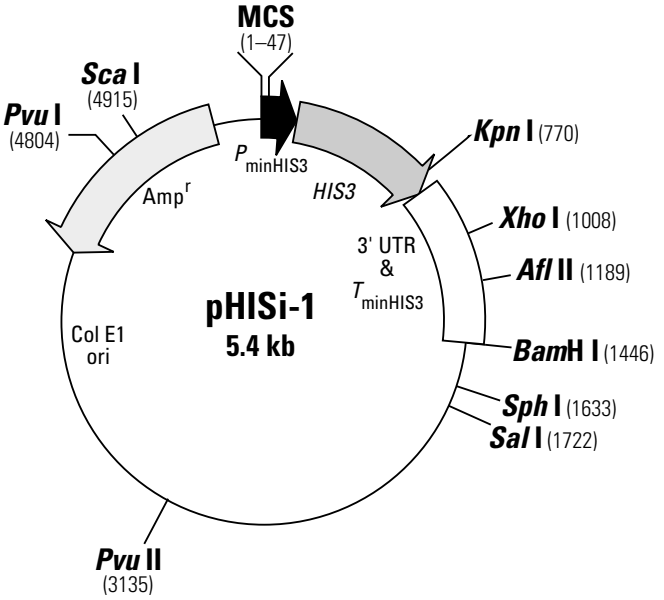


Figure 5. Map and multiple cloning site (MCS) of pHISi. pHISi is a yeast integration and reporter vector for use with the MATCHMAKER One-Hybrid System. pHISi contains the yeast *HIS3* gene downstream of the MCS and the minimal promoter of the *HIS3* locus (P_{minHIS}). *Cis*-acting sequences of interest (i.e., target elements) can be inserted into the MCS. Without activation by a target element, constitutive *HIS3* expression from P_{minHIS} is very low in yeast, but allows enough growth to select for integration when constructing *HIS3* reporter strains. During library screening, the leaky expression of *HIS3* is controlled by adding 3-AT to the medium.

The yeast *URA3* and *HIS3* genes of pHISi can be used as selectable markers for integration into the nonfunctional *ura3* and *his3* loci, respectively, of the YM4271 host strain. Before integrating, the vector is linearized at the *Xho* I or *Afl* II sites (*his3* locus) or at the *Apa* I site (*ura3* locus). The *Kpn* I site cannot be used for integration because it cuts within the coding region of the *HIS3* gene, and that region is deleted in YM4271. pHISi cannot replicate autonomously in yeast. The plasmid contains a bacterial Col E1 origin (ori) and the ampicillin resistance gene (Amp^r) for propagation and selection in *E. coli.* Unique restriction sites are in bold.

Appendix A. MATCHMAKER One-Hybrid Vectors *continued*



GAATTCCCGGGGAGCTCACGCGTTCGCGAATCGATCCGCGGTCTAGA

*Eco*R I *Sma* I/ *Xma* I *Sac* I *Mlu* I *Sac* II *Xba* I

Figure 6. Map and multiple cloning site (MCS) of pHISi-1. pHISi-1 is a yeast integration and reporter vector for use with the MATCHMAKER One-Hybrid System. pHISi-1 contains the yeast *HIS3* gene downstream of the MCS and the minimal promoter of the *HIS3* locus (P_{minHIS}). *Cis*-acting sequences of interest (i.e., target elements) can be inserted into the MCS. Without activation by a target element, constitutive *HIS3* expression from P_{minHIS} is very low in yeast, but allows enough growth to select for integration when constructing *HIS3* reporter strains. During library screening, the leaky expression of *HIS3* is controlled by adding 3-AT to the medium. pHISi-1 was constructed by transferring the *HIS* reporter gene from pHISi to the *Eco*R I/*Bam*H I sites of pBR322. Leaky *HIS3* expression in pHISi-1 is generally lower than that in pHISi, presumably due to differences in the flanking vector sequence.

The yeast *HIS3* gene is used as a selectable marker for integration into the nonfunctional *his3* locus of the YM4271 host strain after linearizing the vector at the *Xho* I or *Afl* II sites. The *Kpn* I site cannot be used for integration because it cuts within the coding region of the *HIS3* gene, and that region is deleted in YM4271. Because it does not carry the *URA3* marker, pHISi-1 can be used (together with pLacZi) to construct a dual *HIS3/lacZ* reporter strain. pHISi-1 cannot replicate autonomously in yeast. pHISi-1 contains a bacterial Col E1 origin (ori) and the ampicillin resistance gene (Amp^r) for propagation and selection in *E. coli*. Unique restriction sites are in bold.

Appendix A. MATCHMAKER One-Hybrid Vectors *continued*

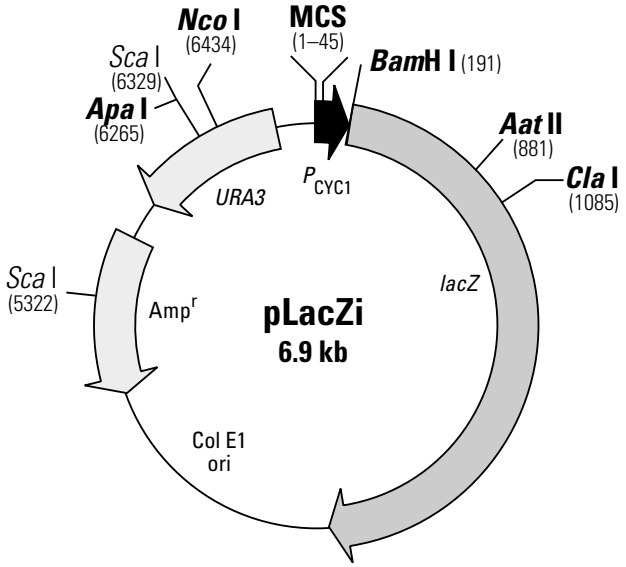


Figure 7. Map and multiple cloning site (MCS) of pLacZi. pLacZi is a yeast integration and reporter vector for use with the MATCHMAKER One-Hybrid System. This plasmid contains the bacterial *lacZ* gene downstream of the minimal promoter of the yeast iso-1-cytochrome C gene (P_{CYC1}). Target elements can be inserted into the MCS upstream of the P_{CYC1}-*lacZ* reporter. Without activation from a *cis*-regulatory element, *lacZ* expression is very low when the vector is integrated into the yeast genome. The yeast *URA3* gene is used as a selectable marker for integration into the nonfunctional *ura* locus of the YM4271 host strain after linearizing the vector at the *Nco* I or *Apa* I site. pLacZi cannot replicate autonomously in yeast. This plasmid contains the ampicillin resistance gene (Amp^r) and the Col E1 origin for selection and propagation in *E. coli*. Unique restriction sites are in bold.

Appendix A. MATCHMAKER One-Hybrid Vectors *continued*

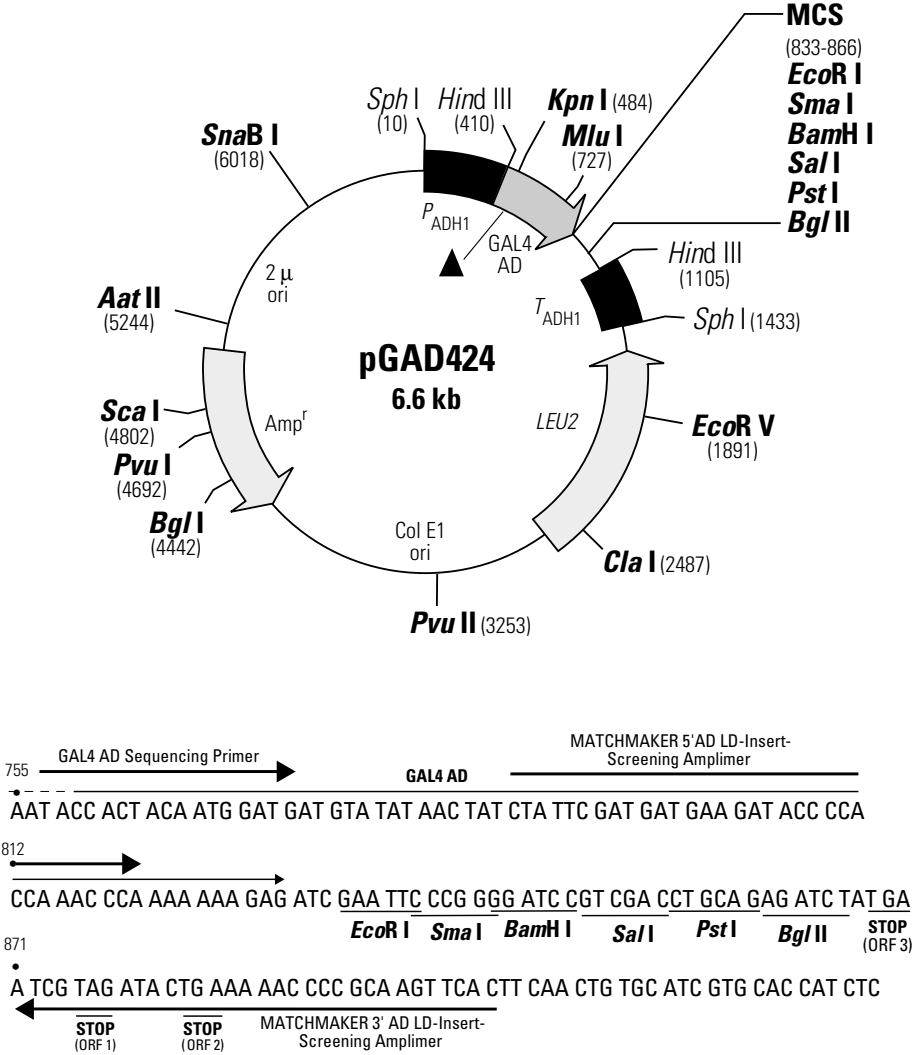


Figure 8. Map and multiple cloning site (MCS) of pGAD424. pGAD424 (Bartel *et al.*, 1993) encodes the activation domain (AD; a.a. 768–881) of the yeast GAL4 transcriptional activator. The AD is expressed at low levels inyeast host cells from a truncated *ADH1* promoter and is targeted to the yeast nucleus by the SV40 T-antigen nuclear localization sequence (▲; Chien *et al.*, 1991). pGAD424 carries the *LEU2* gene for selection in Leu⁻ auxotrophic yeast strains. pGAD424 can also be used to generate GAL4 AD fusion proteins by inserting the cDNA for a protein of interest (or a cDNA library) into the MCS. Unique sites are in bold. GenBank Accession: #U07647.

Appendix B: Yeast Media

CLONTECH carries a full line of yeast media including YPD and SD (with glucose; with or without agar) and Dropout (DO) Supplements ideal for use with the MATCHMAKER One-Hybrid System. Please see Section XI for ordering information. If you purchase yeast media from CLONTECH, follow the directions provided with the product. Alternatively, you can prepare your own media and DO Supplements using the detailed recipes provided in the YPH, Appendix C.

- **YPD medium**
- **SD media**

  Depending on which reporter vectors you use, and which control transformations you choose to perform, you will need all or some of the following minimal SD selection media. SD medium contains 2% glucose as the carbon source for optimal growth and a DO Supplement lacking the appropriate nutrient for plasmid selection in yeast.

  For 3-AT-containing medium: the concentration of 3-AT used in the medium depends on the specific requirements of the customized *HIS3* reporter strains (Section VI.D). In any case, the 3-AT should be added after the SD medium is autoclaved and cooled to ~55°C because 3-AT is heat-labile.

  SD/–His, SD/–His + varying amounts of 3-AT

  SD/–Leu

  SD/–Ura

  SD/–Leu/–Ura

  SD/–His/–Leu/+ optimal [3-AT]; for screening an AD fusion library

Notes: