pYESTrp2

Expression Vector Designed for use with the Hybrid Hunter™ Yeast Two-Hybrid Systems

Catalog no. V615-20

Version C
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Important Information

Kit Contents

20 µg pYESTrp2, lyophilized in TE, pH 8.0

Shipping/Storage

Lyophilized plasmid is shipped at room temperature and should be stored at -20°C.

Product Qualification

The pYESTrp2 vector is qualified by restriction enzyme digestion with *Hin*d III, *Bam*H I, and *Hin*d III/*Pvu* II. Restriction digests must demonstrate the correct banding pattern when electrophoresed on an agarose gel. The table below lists the restriction enzymes used to digest the vector and the expected fragments.

Vector	Restriction Enzyme	Expected Fragments (bp)
pYESTrp2	*Hin*d III	5822
	*Bam*H I	5822
	*Hin*d III/*Pvu* II	372, 5450

Methods

Overview

Introduction

pYESTrp2 is a 5.8 kb expression vector designed for use with the following Hybrid Hunter™ Systems available from Invitrogen:

Kit	Catalog no.
Hybrid Hunter™ System	K5000-01
Dual Bait Hybrid Hunter™ Yeast Two-Hybrid System	K5200-01

The vector allows expression of a prey protein of interest in *Saccharomyces cerevisiae* as a fusion to the B42 acidic activation domain. The prey may be a known protein or a cDNA library. Transformation of a prey library (in pYESTrp2) into the appropriate *Saccharomyces cerevisiae* bait strain allows identification of novel interactors with a bait (or baits) of interest while transformation of a known prey into the bait strain allows verification of an interaction between the prey and a known bait (or baits). The vector contains the following elements:

- Yeast *GAL1* promoter for galactose-inducible expression of the prey fusion protein in *S. cerevisiae*
- V5 epitope for detection of purified prey fusion protein with Anti-V5 Antibodies
- Nuclear localization signal (NLS) for localization of prey fusion protein to the nucleus
- B42 acidic activation domain for reconstituted transcriptional activation of LexA or cI operator-based reporter genes when brought into proximity with the LexA or cI fusion bait proteins
- A multiple cloning site with 9 unique restriction sites for simplified cloning of the prey protein of interest
- *CYC1* transcriptional terminator for efficient termination of mRNA
- *TRP1* gene for selection of transformants in yeast host strains with a *trp1* genotype
- Ampicillin resistance gene for selection of transformants in *E. coli*

For more information about the various yeast two-hybrid systems available from Invitrogen, refer to the manual for each system, our Web site (www.invitrogen.com), or call Technical Service (see page 20). For more information about the general principles of interaction trap systems, refer to the system manuals, published reviews (Bartel *et al.*, 1993a; Fields and Sternglanz, 1994), or general reference sources (Golemis *et al.*, 1996; Golemis *et al.*, 1998). To order components of the various Hybrid Hunter™ Systems, see pages 3-5.

Compatibility

pYESTrp2 contains the *TRP1* gene for selection of transformants in yeast, therefore, the vector may be used with any yeast host strain with a *trp1* genotype (see page 3 for more information). In principle, pYESTrp2 may be used with any LexA, lambda cI, or GAL4-based bait plasmid in a two-hybrid screen. The prey protein or library expressed from pYESTrp2 will be fused to the B42 activation domain, and should activate transcription of specific reporter genes when an interaction with an appropriate fusion bait has occurred.

Continued on next page

Overview, continued

Experimental Outline

The table below outlines the major steps required to clone and express your prey protein of interest in pYESTrp2.

Step	Action
1	Consult the multiple cloning site diagram on page 8 to design a strategy to clone your prey gene in frame with sequence encoding the V5 epitope-NLS-B42 fusion in pYESTrp2.
2	Ligate your insert into pYESTrp2 and transform into *E. coli*. Select transformants on LB plates containing 50 µg/ml ampicillin.
3	Analyze your transformants for the presence of insert by restriction digestion.
4	Select a transformant with the correct restriction pattern and sequence to confirm that your gene is cloned in the proper orientation and in frame with theV5 epitope-NLS-B42 fusion protein.
5	Use a small-scale transformation protocol to transform your pYESTrp2 prey construct into the appropriate yeast host strain and select for tryptophan prototrophy.
6	Test for expression of your prey fusion protein by Western blot analysis, if desired.
7	Use a small-scale transformation protocol to transform your pYESTrp2 prey construct into the appropriate yeast bait strain and select for tryptophan prototrophy. Test for an interaction between your bait and prey by assaying for the appropriate reporter activity. If you are performing an interactor hunt, use a small-scale or large-scale library transformation protocol to transform your pYESTrp2 library into the appropriate yeast bait strain and select as above.

Accessory Products

Introduction

The products listed below are designed for use with the pYESTrp2 vector and the various Hybrid Hunter™ Systems available from Invitrogen. For more information about the components specific for each Hybrid Hunter™ system, refer to the manual for the particular system.

Yeast Strains

For your convenience, Invitrogen has available many yeast host strains that may be used with the pYESTrp2 vector in an interactor hunt. All of the strains carry the *trp1* genotype to allow selection of pYESTrp2 transformants. The strains have been specially designed for use with a specific Hybrid Hunter™ System. This information as well as the genotype of each strain is listed in the table below. In some cases, a particular yeast strain has been transformed with a LexA and/or cI-based reporter plasmid to facilitate detection of molecular interactions. For more information about a particular yeast strain, refer to the specific system manual or call Technical Service (see page 20).

Each of the yeast strains is supplied as a 20% glycerol stock in a 0.5 ml volume. The pSH18-34 and pLacGUS reporter plasmids may be ordered separately from Invitrogen (see page 5). Other reporter plasmids are also available (see page 5 for ordering information).

Yeast Strain	Genotype	For Use With...	Catalog no.
L40	*MAT**a** his3Δ200 trp1-901 leu2-3112 ade2 LYS2*::(4*lex*Aop-*HIS3*) *URA3*::(8*lex*Aop-*lacZ*) *GAL4*	Hybrid Hunter™	C830-00
EGY48	MATα *ura3 trp1 his3* 6*lex*Aop-*LEU2*	Hybrid Hunter™	C835-00
EGY48/pSH18-34	MATα *ura3 trp1 his3* 6*lex*Aop-*LEU2* *pSH18-34(URA3)*	Hybrid Hunter™	C836-00
EGY191	MATα *ura3 trp1 his3* 2*lex*Aop-*LEU2*	Hybrid Hunter™	C837-00
EGY191/pSH18-34	MATα *ura3 trp1 his3* 2*lex*Aop-*LEU2* *pSH19-34(URA3)*	Hybrid Hunter™	C838-00
SKY48	MATα *ura3 trp1 his3* 6*lex*Aop-*LEU2* 3*cI*-op-*LYS2*	Dual Bait Hybrid Hunter™	C833-00
SKY48/pLacGUS	MATα *ura3 trp1 his3* 6*lex*Aop-*LEU2* 3*cI*-op-*LYS2* *pLacGUS (URA3)*	Dual Bait Hybrid Hunter™	C832-00
SKY191	MATα *ura3 trp1 his3* 2*lex*Aop-*LEU2* 3*cI*-op-*LYS2*	Dual Bait Hybrid Hunter™	C834-00

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Accessory Products, continued

Hybrid Hunter™ Libraries

The following cDNA libraries in pYESTrp and pYESTrp2 are available from Invitrogen. All of the libraries are compatible for use with any of the Hybrid Hunter™ Systems. All libraries are amplified once on plates. For more information, see our Web site (www.invitrogen.com) or call Technical Service (see page 20).

Source	Plasmid	Primary Clones	Size Selection	Catalog no.
Cell Lines				
HeLa cells (Human cervical carcinoma)	pYESTrp	3.66×10^6	0.3 to 1.2 kb	A201-01
BeWo cells (Human fetal placental choriocarcinoma)	pYESTrp	5.35×10^6	0.3 to 0.8 kb	A208-01
BeWo cells (Human fetal placental choriocarcinoma)	pYESTrp	5.5×10^6	0.3 to 1.2 kb	A208-02
Jurkat cells (Human T cell leukemia)	pYESTrp	3.2×10^6	0.3 to 1.2 kb	A209-01
A20 cells (Mouse B cell lymphoma)	pYESTrp	3.11×10^6	0.3 to 1.2 kb	A210-01
Human Adult Tissue				
Bladder	pYESTrp2	17.6×10^6	0.4 to 1.2 kb	A225-01
Brain	pYESTrp2	10.8×10^6	0.4 to 1.2 kb	A204-01
Breast	pYESTrp2	9.00×10^6	0.4 to 1.2 kb	A217-01
Breast Tumor	pYESTrp2	8.84×10^6	0.4 to 1.2 kb	A216-01
Colon Tumor	pYESTrp2	7.98×10^6	0.4 to 1.2 kb	A222-01
Kidney	pYESTrp2	6.96×10^6	0.4 to 1.2 kb	A223-01
Liver	pYESTrp	2.21×10^6	0.3 to 1.2 kb	A203-01
Lung	pYESTrp2	5.95×10^6	0.4 to 1.2 kb	A213-01
Lung Tumor	pYESTrp2	1.85×10^6	0.4 to 1.2 kb	A215-01
Ovary	pYESTrp	4.54×10^6	0.3 to 1.2 kb	A206-01
Placenta	pYESTrp	4.75×10^6	0.3 to 1.2 kb	A207-01
Prostate	pYESTrp2	5.46×10^6	0.4 to 1.2 kb	A218-01
Spleen	pYESTrp2	11.4×10^6	0.4 to 1.2 kb	A214-01
Testes	pYESTrp	6.4×10^6	0.3 to 1.2 kb	A205-01
Human Fetal Tissue				
Fetal Liver	pYESTrp	2.37×10^6	0.3 to 1.2 kb	A202-01

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Accessory Products, continued

Additional Reagents

Additional reagents that may be used with pYESTrp2 and the various Hybrid Hunter™ Systems are available from Invitrogen. Ordering information is provided below. The quantity of antibody supplied is sufficient for 25 Westerns.

Item	Amount	Catalog no.
S.c. EasyComp™ Kit	20 reactions	K5050-01
pHybLex/Zeo (Bait Vector)	20 µg	V610-20
pHybcI/HK (Bait Vector)	20 µg	V614-20
pSH18-34 Reporter Plasmid	20 µg	V611-20
pRB1840 Reporter Plasmid	20 µg	V612-20
pJK103 Reporter Plasmid	20 µg	V613-20
pLacGUS Reporter Plasmid	20 µg	V616-20
Anti-V5 Antibody	50 µl	R960-25
Anti-V5-HRP Antibody	50 µl	R961-25

Cloning into pYESTrp2

Introduction

The pYESTrp2 vector (5822 bp) can be used to make two-hybrid cDNA libraries or to clone genes encoding known proteins. Use the diagram on page 8 to help you clone your gene of interest into pYESTrp2. General considerations for cloning and transformation are discussed below.

General Molecular Biology Techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, purification of single-stranded DNA, DNA sequencing, and DNA biochemistry, refer to *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

E. coli Strain

Many *E. coli* strains are suitable for the propagation of the pYESTrp2 vector including TOP10, TOP10F´, DH5α or equivalent. We recommend that you propagate the vector in *E. coli* strains that are recombination deficient (*rec*A) and endonuclease A deficient (*end*A).

For your convenience, TOP10 *E. coli* are available as chemically competent or electrocompetent cells from Invitrogen.

Item	Quantity	Catalog no.
One Shot™ TOP10 (chemically competent cells)	21 x 50 µl	C4040-03
Electrocomp™ TOP10 (electrocompetent cells)	5 x 80 µl	C664-55

Transformation Method

You may use any method of your choice for transformation. Chemical transformation is the most convenient for most researchers. Electroporation is the most efficient and the method of choice for large plasmids.

Maintenance of pYESTrp2

To propagate and maintain the pYESTrp2 vector, we recommend resuspending the vector in 20 µl sterile water to prepare a 1 µg/µl stock solution. Store the stock solution at -20°C.

Use this stock solution to transform a *rec*A, *end*A *E. coli* strain like TOP10, TOP10F´ or equivalent. Select transformants on LB plates containing 50 µg/ml ampicillin. Be sure to prepare a glycerol stock of the plasmid for long-term storage (see page 9 for a protocol).

B42 Activation Domain

Your prey protein of interest will be fused to the *E.coli* B42 acidic activation domain in pYESTrp2 (Ruden *et al.*, 1991). The B42 activation domain acts as a transcriptional activator when brought into proximity with a DNA binding protein such as LexA (Horii *et al.*, 1981; Markham *et al.*, 1981) or cI (Nilsson *et al.*, 1983). Transcriptional activation then occurs via binding of the LexA or cI protein to LexA or cI operators upstream of heterologous genes (i.e. reporter genes).

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Cloning into pYESTrp2, continued

Cloning Considerations

When designing your cloning strategy, remember that you must clone your gene in frame with the sequence encoding the V5 epitope-NLS-B42 fusion protein in order to create a prey fusion protein with a nuclear localization signal, activation domain, and an epitope for detection.

Note

The N-terminal peptide contains a V5 epitope to allow detection of your expressed prey fusion protein by immunoblot (Western analysis). Anti-V5 antibodies are available from Invitrogen to facilitate detection (see page 12 for more information).

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Cloning into pYESTrp2, continued

Multiple Cloning Site of pYESTrp2

Below is the multiple cloning site for pYESTrp2. Restriction sites are labeled to indicate the cleavage site. The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pYESTrp2 is available for downloading from our Web site (www.invitrogen.com) or from Technical Service (see page 20).** For a map and a description of the features of pYESTrp2, see pages 16-17.

```
                5′ end of GAL1 promoter
   1 CGCGCTTAAT GGGGCGCTAC AGGGCGCGTG GGGATGATCC ACTAGTACGG ATTAGAAGCC

                      GAL4 binding site       GAL4 binding site
  61 GCCGAGCGGG TGACAGCCCT CCGAAGGAAG ACTCTCCTCC GTGCGTCCTC GTCTTCACCG

 121 GTCGCGTTCC TGAAACGCAG ATGTGCCTCG CGCCGCACTG CTCCGAACAA TAAAGATTCT

 181 ACAATACTAG CTTTTATGGT TATGAAGAGG AAAAATTGGC AGTAACCTGG CCCCACAAAC

 241 CTTCAAATGA ACGAATCAAA TTAACAACCA TAGGATGATA ATGCGATTAG TTTTTTAGCC

                                                                TATA Box
 301 TTATTTCTGG GGTAATTAAT CAGCGAAGCG ATGATTTTTG ATCTATTAAC AGATATATAA

 361 ATGCAAAAAC TGCATAACCA CTTTAACTAA TACTTTCAAC ATTTTCGGTT TGTATTACTT

                          transcriptional start
 421 CTTATTCAAA TGTAATAAAA GTATCAACAA AAAATTGTTA ATATACCTCT ATACTTTAAC

                                                 T7 promoter/priming site
 481 GTCAAGGAGA AAAAACCCCG GATCGGACTA CTAGCAGCTG TAATACGACT CACTATAGGG

                                                 V5 epitope
 541 AATATTAAGC TCACC ATG GGT AAG CCT ATC CCT AAC CCT CTC CTC GGT CTC
                      Met Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu
                                                 SV40 NLS
 592 GAT TCT ACA CAA GCT ATG GGT GCT CCT CCA AAA AAG AAG AGA AAG GTA GCT
     Asp Ser Thr Gln Ala Met Gly Ala Pro Pro Lys Lys Lys Arg Lys Val Ala

 643 GGT ATC AAT AAA GAT ATC GAG GAG TGC AAT GCC ATC ATT GAG CAG TTT ATC
     Gly Ile Asn Lys Asp Ile Glu Glu Cys Asn Ala Ile Ile Glu Gln Phe Ile

 694 GAC TAC CTG CGC ACC GGA CAG GAG ATG CCG ATG GAA ATG GCG GAT CAG GCG
     Asp Tyr Leu Arg Thr Gly Gln Glu Met Pro Met Glu Met Ala Asp Gln Ala
                                                 B42 activation domain
 745 ATT AAC GTG GTG CCG GGC ATG ACG CCG AAA ACC ATT CTT CAC GCC GGG CCG
     Ile Asn Val Val Pro Gly Met Thr Pro Lys Thr Ile Leu His Ala Gly Pro

 796 CCG ATC CAG CCT GAC TGG CTG AAA TCG AAT GGT TTT CAT GAA ATT GAA GCG
     Pro Ile Gln Pro Asp Trp Leu Lys Ser Asn Gly Phe His Glu Ile Glu Ala
                                                                 Hind III
 847 GAT GTT AAC GAT ACC AGC CTC TTG CTG AGT GGA GAT GCC TCC AAG CTT GGT
     Asp Val Asn Asp Thr Ser Leu Leu Leu Ser Gly Asp Ala Ser Lys Leu Gly
          Kpn I     Sac I  BamH I                                BstX I*  EcoR I
 898 ACC GAG CTC GGA TCC ACT AGT AAC GGC CGC CAG TGT GCT GGA ATT CTG CAG
     Thr Glu Leu Gly Ser Thr Ser Asn Gly Arg Gln Cys Ala Gly Ile Leu Gln
                    BstX I*  Not I      Xho I              Sph I
 949 ATA TCC ATC ACA CTG GCG GCC GCT CGA GGC ATG CAT CTA GAG GGC CGC ATC
     Ile Ser Ile Thr Leu Ala Ala Ala Arg Gly Met His Leu Glu Gly Arg Ile

1000 ATG TAA TTAGTTA TGTCACGCTT ACATTCACGC CCTCCCCCCA
     Met ***
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*Please note that there are two *Bst*X I sites in the polylinker.

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Cloning into pYESTrp2, continued

E. coli Transformation

Once you have completed your ligation reaction, transform your ligation mixture into a competent *rec*A, *end*A *E. coli* strain (e.g. TOP10, TOP10F´). Select for transformants on LB plates containing 50 µg/ml ampicillin. Select 10-20 clones and analyze by restriction digest or sequencing for the presence and orientation of your insert.

We recommend that you sequence your construct to confirm that your gene is fused in frame with the V5 epitope, nuclear localization signal, and B42 activation domain. For your convenience, Invitrogen offers a Custom Primer service and can construct primers according to your specifications. For more information on this service, visit our Web site (www.invitrogen.com) or call Technical Service (see page 20).

Preparing a Glycerol Stock

Once you have identified the correct clone, be sure to purify the colony and make a glycerol stock for long-term storage. It is also a good idea to keep a DNA stock of your plasmid at -20°C in case you lose the glycerol stock.

1. Streak the original colony out on an LB plate containing 50 µg/ml ampicillin. Incubate the plate at 37°C overnight.
2. Isolate a single colony and inoculate into 1-2 ml of LB containing 50 µg/ml ampicillin.
3. Grow the culture to mid-log phase (OD_{600} = 0.5-0.7).
4. Mix 0.85 ml of culture with 0.15 ml of sterile glycerol and transfer to a cryovial.
5. Store at -80°C.

Constructing a cDNA Library

You may use pYESTrp2 to construct your own two-hybrid cDNA library. General guidelines are provided below to generate a unidirectional cDNA library in pYESTrp2. Refer to *Current Protocols in Molecular Biology*, Unit 5 (Ausubel *et al.*, 1994) for details on cDNA library construction.

1. Isolate mRNA from the source of interest.
2. Prepare first strand cDNA using random primers, Oligo dT(*Not* I) primer (Catalog no. N430-01), or an Oligo dT(*Xho* I) primer.

   Alternatively, the Copy™ Kit (Catalog no. L1311-03) is available from Invitrogen for efficient production of double-stranded blunt-ended cDNA for either bidirectional or unidirectional cloning. Call Technical Service (see page 20 for more information).
3. After second strand synthesis, be sure the ends are blunt prior to adding *Bst*X I/*Eco*R I adaptors. *Bst*X I/*Eco*R I adaptors (Catalog no. N418-18) are available from Invitrogen.
4. Digest with *Not* I or *Xho* I and electrophorese on an agarose gel for size selection.
5. Isolate cDNA for ligation into pYESTrp2.
6. Digest pYESTrp2 with either *Bst*X I (or *Eco*R I) and *Not* I (or *Xho* I) to complement the ends on the cDNA.
7. Ligate cDNA into digested vector and transform into *E. coli*.
8. Determine the number of primary recombinants. You may wish to amplify the library prior to large-scale isolation of plasmid DNA for the library screen.

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Cloning into pYESTrp2, continued

Small-Scale Plasmid Preparation

You may use any method of your choice to prepare purified plasmid DNA for small-scale yeast transformation. Standard protocols can be found in *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994) or *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989).

If you need ultrapure DNA for sequencing, we recommend isolating plasmid DNA using the S.N.A.P.™ MiniPrep Kit (10-15 µg DNA, Catalog no. K1900-01) or the S.N.A.P.™ MidiPrep Kit (10-200 µg DNA, Catalog no. K1910-01).

Large-Scale Plasmid Preparation

If you have constructed a cDNA library in pYESTrp2, you will need to isolate purified plasmid DNA before you can proceed with library transformation. You will need 30 µg of library plasmid DNA to perform a small-scale library transformation and 500 µg of library plasmid DNA to perform a large-scale library transformation. To isolate plasmid DNA, follow the procedure below. Other methods are suitable. If you are using a Hybrid Hunter™ Premade Library, follow the directions supplied with your library.

1. Inoculate 1-2 liters of LB medium containing 50 µg/ml ampicillin with sufficient bacterial library stock to insure 2-3 times the number of independent clones in the library.
2. Incubate at 37°C overnight with shaking.
3. After incubation, pellet the cells and proceed with large- or mega-scale isolation of plasmid DNA. Any standard method is suitable. You may have to adjust the plasmid preparation protocol to account for the density of the culture.
4. Store the plasmid at -20°C until ready for use.

Expressing the Prey Protein

Introduction

If you wish to test for expression of your prey fusion protein before proceeding with an interactor hunt, you may use a small-scale transformation protocol to transform your pYESTrp2 prey construct into a suitable yeast host strain. Once you have selected transformants for tryptophan prototrophy, expression of the prey fusion protein may be induced by addition of galactose to the medium. A procedure to prepare lysates for immunoblot (Western) analysis is provided below.

Basic Yeast Molecular Biology

The user should be familiar with basic yeast molecular biology and microbiological techniques. Refer to *Current Protocols in Molecular Biology* (1996) *Saccharomyces cerevisiae*, pp. 13.01 to 13.2.12 for information on preparing yeast media and handling yeast.

Reagents for Yeast Transformation

The *S. c.* EasyComp™ Kit (Catalog no. K5050-01) provides a quick and easy method to prepare competent yeast cells that can be used immediately or stored frozen for future use. Transformation efficiency is guaranteed at $>10^3$ transformants per µg DNA.

For your convenience, a small-scale transformation protocol is included in the **Appendix**, page 18. Alternatively, there are published references for other small-scale transformation methods (Gietz *et al.*, 1992; Gietz and Schiestl, 1996; Hill *et al.*, 1991; Schiestl and Gietz, 1989).

Materials Required

To assay for expression of your prey fusion protein by immunoblot (Western blot) analysis, be sure to have the following reagents and equipment on hand before proceeding:

- 30°C incubator and shaking incubator
- 60°C and 70°C water baths or temperature blocks and a boiling water bath
- Clinical centrifuge and low-speed centrifuge
- Selective medium and plates
- Cracking buffer (see page 15 for a recipe), prewarmed to 60°C
- Acid washed glass beads (Sigma-Aldrich G-8772, 425-600 microns)
- Reagents for SDS-PAGE and immunoblotting
- Antibody to your prey protein or Anti-V5 Antibody (see the next page)

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Expressing the Prey Protein, continued

Expression of the Prey Fusion Protein

Use the protocol below to prepare lysates from your Trp^+ transformants and untransformed yeast cells for Western blot analysis. Test several transformants in case of heterogeneity in prey expression levels.

1. Inoculate 10 ml of the appropriate selective medium with a single colony of your prey strain (above) and inoculate 10 ml of the appropriately-supplemented YC medium with your untransformed yeast strain as a negative control. Remember that the medium needs to contain galactose to induce expression of the prey protein. Grow overnight with shaking at 30°C.
2. Pellet the cells in Step 1 by centrifuging at 2500 rpm for 5 minutes at room temperature. Decant the medium.
3. Transfer the cell pellets to a -80°C freezer for 10 minutes.
4. Thaw cell pellet in 100 µl of prewarmed (60°C) cracking buffer and resuspend by pipetting the cell pellet in the buffer.
5. Transfer cell suspension to a 1.5 ml microcentrifuge tube containing 100 µl of glass beads.
6. Incubate the solution at 70°C for 10 minutes.
7. Vortex solution for 1 minute.
8. Centrifuge in a microcentrifuge at 14,000 rpm for 5 minutes at room temperature and transfer supernatant to a new tube.
9. Add SDS-PAGE sample buffer and boil sample for 5 minutes. Use 30 to 50 µl for immunoblot analysis. Detect the prey fusion protein using an antibody to your prey of interest or Anti-V5 antibodies available from Invitrogen (see below).

Antibodies for Detection

The prey fusion protein contains the N-terminal V5 epitope to allow detection of the expressed prey protein by Western blot analysis. The Anti-V5 Antibody (Catalog no. R960-25) and the Anti-V5-HRP Antibody (Catalog no. R961-25) are available from Invitrogen for detection of the expressed prey protein (see page 5 for ordering information). For the sequence of the V5 epitope, refer to the diagram on page 8.

Note

Note that the N-terminal peptide containing the V5 epitope, nuclear localization signal, and B42 activation domain will add approximately 12 kDa to the size of your prey protein.

Performing a Two-Hybrid Screen

Once you have verified expression of your prey fusion protein in yeast, you may proceed to assay for an interaction between your prey protein and the bait (or baits) of interest or to identify novel interactors for your bait of interest. For details, refer to the manual for the appropriate Hybrid Hunter™ System.

Retrieving Putative Interactors

There are several methods available to retrieve the prey plasmid or the gene encoding a putative interactor. We generally use PCR to amplify the prey gene of interest and clone it into a PCR cloning vector. Other methods allow retrieval of the original prey plasmid by isolation of yeast plasmid DNA or by plasmid segregation. Further details and protocols for each of these methods are provided in each Hybrid Hunter™ System manual.

Appendix

Recipes

YC Medium and Plates

YC is minimal defined medium for yeast.

0.12% yeast nitrogen base (**without either** amino acids or ammonium sulfate)
0.5% ammonium sulfate
1% succinic acid
0.6% NaOH
2% glucose
0.01% (adenine, arginine, cysteine, leucine, lysine, threonine, tryptophan, uracil)
0.005% (aspartic acid, histidine, isoleucine, methionine, phenylalanine, proline, serine, tyrosine, valine)
2% agar (for plates)

1. Dissolve the following reagents in 900 ml deionized water. **Note**: We make medium and plates as we need them and weigh out each amino acid. Many researchers prepare 100X solutions of each amino acid mix that they need.

1.2 g Yeast Nitrogen Base	**0.1 g each**	**0.05 g each**
5 g Ammonium sulfate	adenine	aspartic acid
10 g Succinic acid	arginine	histidine
6 g NaOH	cysteine	isoleucine
	leucine	methionine
	lysine	phenylalanine
	threonine	proline
	tryptophan (W)	serine
	uracil	tyrosine
		valine

   Note: The amino acids with the one letter code are those you need to omit to make selective plates, depending on the genotype of the host, plasmid markers, and reporters.
2. If you are making plates, add the agar after dissolving the reagents above.
3. Autoclave at 15 psi, 121°C for 20 minutes.
4. Cool to 50°C and add 100 ml of filter-sterilized 20% glucose. **Note**: You may add the sugar before autoclaving; however, the medium will be darker in color because of heating the glucose.

   For plates that contain galactose and raffinose, add 100 ml 20% galactose and 50 ml 20% raffinose instead of glucose.
5. Pour plates and allow to harden. Invert the plates and store at +4°C. Plates are stable for up to 6 months.

Note

The recipe for YC medium has been optimized for use with the Hybrid Hunter™ Systems and its components. Other recipes may be suitable, but should be tested with the host strain, plasmid markers, and reporters.

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Recipes, continued

YPD

Yeast Extract Peptone Dextrose Medium (1 liter)

1% yeast extract
2% peptone
2% dextrose (D-glucose)

1. Dissolve the following in 1000 ml of water:

   10 g yeast extract
   20 g peptone
   20 g dextrose (see note below if making plates)

2. Optional: Add 20 g agar, if making plates.
3. Autoclave for 20 minutes on liquid cycle.
4. Store medium at room temperature or cool the medium and pour plates. The shelf life is approximately one to two months.

Note: If making plates, omit dextrose from Step 1. Autoclaving agar and dextrose together will cause the dextrose to caramelize. Prepare a separate stock solution of 20% dextrose and autoclave or filter-sterilize. After the YPD broth has been autoclaved, add 100 ml of 20% dextrose to the medium.

10X TE

100 mM Tris, pH 7.5
10 mM EDTA

1. For 100 ml, dissolve 1.21 g of Tris base and 0.37 g of EDTA in 90 ml of deionized water.
2. Adjust the pH to 7.5 with concentrated HCl and bring the volume up to 100 ml.
3. Filter sterilize and store at room temperature.

Alternatively, you can make the solution using 1 M Tris-HCl, pH 7.5 and 0.5 M EDTA, pH 8.0.

1X TE

10 mM Tris, pH 7.5
1 mM EDTA

Dilute 10X TE 10-fold with sterile water.

10X LiAc

1 M Lithium Acetate, pH 7.5

1. For 100 ml, dissolve 10.2 g of lithium acetate in 90 ml of deionized water.
2. Adjust pH to 7.5 with dilute glacial acetic acid and bring up the volume to 100 ml.
3. Filter sterilize and store at room temperature.

1X LiAc

100 mM Lithium Acetate, pH 7.5

Dilute 10X LiAc solution 10-fold with sterile, deionized water.

Continued on next page

Recipes, continued

1X LiAc/0.5X TE

100 mM Lithium Acetate, pH 7.5
5 mM Tris-HCl, pH 7.5
0.5 mM EDTA

1. For 100 ml, mix together 10 ml of 10X LiAc and 5 ml of 10X TE.
2. Add deionized water to 100 ml.
3. Filter-sterilize and store at room temperature.

1X LiAc/40% PEG-3350/1X TE

100 mM Lithium Acetate, pH 7.5
40% PEG-3350
10 mM Tris-HCl, pH 7.5
1 mM EDTA

1. Prepare solution immediately prior to use. For 100 ml, mix together 10 ml of 10X LiAc, 10 ml of 10X TE, and 40 g of PEG-3350.
2. Add deionized water to 100 ml and dissolve the PEG. You may have to heat the solution to fully dissolve the PEG.
3. Autoclave at 121°C, 15 psi for 20 minutes. Store at room temperature.

Cracking Buffer

8 M urea
5% SDS
40 mM Tris-HCl pH 6.8
0.1 mM EDTA
1% β-mercaptoethanol
0.4 mg/ml bromophenol blue

1. Prepare a 1 M Tris-HCl, pH 6.8 stock. (12.11 g in 90 ml deionized water and adjust pH to 6.8. Bring the volume to 100 ml).
2. Mix together the following reagents:

Urea	48.0 g
SDS	5 g
1 M Tris-HCl, pH 6.8	4 ml
EDTA	3.72 mg (or 20 µl of a 0.5 M stock)
β-mercaptoethanol	1 ml
Bromophenol blue	40 mg

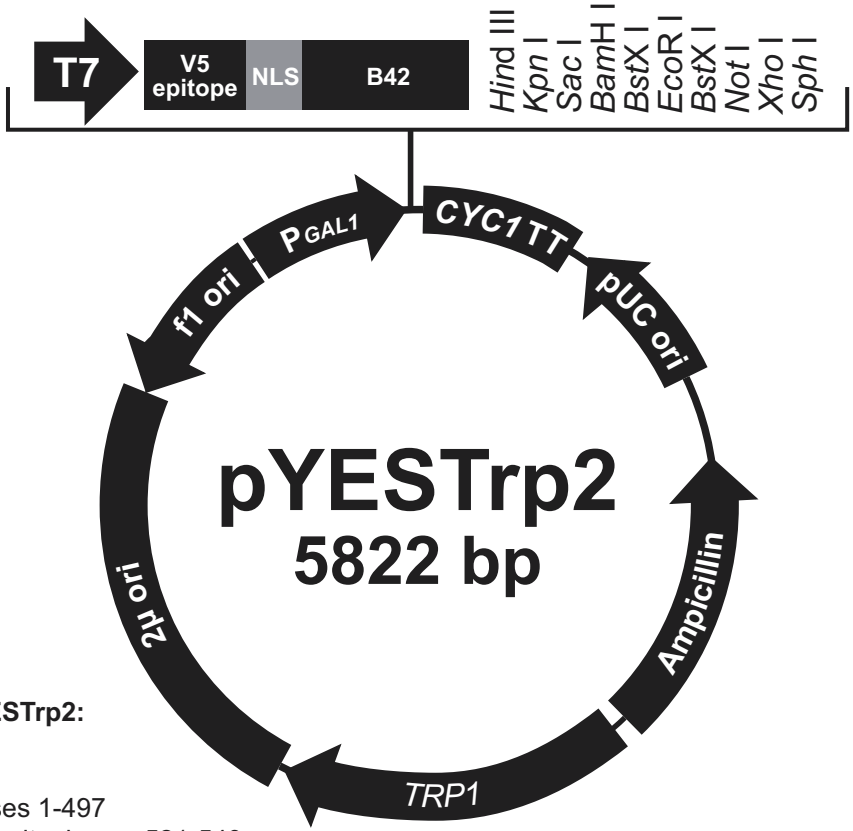
   Bring up in 100 ml deionized water and dissolve reagents.

3. Store at +4°C or -20°C.

pYESTrp2 Vector

Map of pYESTrp2

pYESTrp2 is a 5822 bp prey vector that can be used to make two-hybrid cDNA libraries or to clone genes encoding known proteins. The figure below summarizes the features of the pYESTrp2 vector. **The complete nucleotide sequence for pYESTrp2 is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 20).**



Comments for pYESTrp2:
5822 nucleotides

GAL1 promoter: bases 1-497
T7 promoter/priming site: bases 521-540
Initiation ATG: bases 556-558
V5 epitope: bases 559-600
Nuclear localization signal (NLS): bases 616-642
B42 activation domain: bases 646-883
Multiple cloning site: bases 889-982
CYC1 transcription termination region: bases 997-1245
pUC origin: bases 1427-2100 (complementary strand)
Ampicillin (*bla*) resistance gene: bases 2219-3105 (complementary strand)
bla promoter: bases 3106-3204 (complementary strand)
TRP1 promoter: bases 3313-3414
TRP1 gene: bases 3415-4089
2µ origin: bases 4493-5327
f1 origin: bases 5396-5768 (complementary strand)

Continued on next page

pYESTrp2 Vector, continued

Features of pYESTrp2

The table below describes the features of pYESTrp2. All elements have been functionally tested.

Feature	Benefit
GAL1 promoter	Allows galactose-inducible expression of prey genes cloned into pYESTrp2
T7 promoter/priming site	Permits sequencing of insert or *in vitro* transcription of sense strand
V5 epitope	Allows detection of fusion protein(s) using the Anti-V5 Antibody (Catalog no. R960-25) or Anti-V5-HRP Antibody (Catalog no. R961-25) (Southern *et al.*, 1991)
SV40 large T antigen nuclear localization sequence (NLS)	Localizes prey fusions to the nucleus for potential interaction with bait fusions
B42 activation domain (AD) ORF	Transcriptional activation domain that allows expression of reporter genes when brought into proximity with an appropriate DNA binding domain (DBD) (i.e. LexA or lambda cI) by two interacting proteins (Ma and Ptashne, 1987)
Multiple cloning site with 8 unique sites, plus two *Bst*X I sites.	Allows in-frame cloning of a cDNA library or a single gene with the B42 activation domain
CYC1 transcription termination signal	Permits efficient termination and stabilization of mRNA
pUC origin	Maintenance and high-copy replication in *E. coli*
bla promoter	Allows expression of the *bla* resistance gene
Ampicillin (*bla*) resistance gene	Allows selection of transformants in *E. coli*
TRP1 promoter	Allows expression of the *TRP1* gene
TRP1 gene	Permits auxotrophic selection of the plasmid in Trp^- yeast hosts (Tschumper and Carbon, 1980)
2µ origin	Permits maintenance and high-copy replication in yeast
f1 origin	Allows rescue of single-stranded DNA

Small-Scale Yeast Transformation

Introduction

A small-scale yeast transformation protocol for routine transformations is provided below. Other protocols are suitable.

Materials Needed

Be sure to have the following reagents on hand before starting.

- YPD liquid medium (see recipe, page 14)
- 1X TE (see recipe, page 14)
- 1X LiAc/0.5X TE (see recipe, page 15)
- Denatured salmon sperm DNA (see recipe, next page)
- Plasmid DNA to be transformed
- 1X LiAc/40% PEG-3350/1X TE (see recipe, page 15)
- DMSO
- Selective plates

Protocol

1. Inoculate 10 ml of YPD medium with a colony of the appropriate yeast strain and shake overnight at 30°C.
2. Determine the OD_{600} of your overnight culture. Dilute culture to an OD_{600} of 0.4 in 50 ml of YPD medium and grow an additional 2-4 hours.
3. Pellet the cells at 2500 rpm and resuspend the pellet in 40 ml 1X TE.
4. Pellet the cells at 2500 rpm and resuspend the pellet in 2 ml of 1X LiAc/0.5X TE.
5. Incubate the cells at room temperature for 10 minutes with shaking.
6. For each transformation, mix together 1 µg plasmid DNA and 100 µg denatured sheared salmon sperm DNA with 100 µl of the yeast suspension from Step 5.
7. Add 700 µl of 1X LiAc/40% PEG-3350/1X TE and mix well.
8. Incubate solution at 30°C for 30 minutes with shaking.
9. Add 88 µl DMSO, mix well, and heat shock at 42°C for 7 minutes.
10. Centrifuge in a microcentrifuge for 10 seconds and remove supernatant.
11. Resuspend the cell pellet in 1 ml 1X TE and re-pellet.
12. Resuspend the cell pellet in 50-100 µl 1X TE and plate on a selective plate.

Note

To calculate the number of yeast cells, assume that 1 OD_{600} unit = ~2.0×10^7 yeast cells.

Preparation of Denatured Salmon Sperm DNA

Introduction

A convenient protocol to make denatured salmon sperm DNA (Schiestl and Gietz, 1989) is provided for your convenience. You may also purchase denatured salmon sperm DNA from Sigma-Aldrich (Catalog no. D9156). Alternatively, some researchers have found that using yeast transfer RNA (Sigma-Aldrich, Catalog no. R9001) as a carrier results in a cleaner transformation although there are fewer total colonies.

Materials Needed

Prepare or have the following reagents on hand before starting.

- Salmon Sperm DNA (Sigma-Aldrich, Catalog no. D1626)
- 1X TE
- Sonicator
- 50 ml conical centrifuge tubes
- TE-saturated phenol
- TE-saturated phenol:chloroform:isoamyl alcohol (25:24:1)
- Chloroform
- Low-speed centrifuge
- 3 M sodium acetate, pH 6.0
- 95% ethanol (-20°C)
- 250 ml centrifuge bottle
- Boiling water bath

Protocol

1. In a 250 ml flask, dissolve 1 g salmon sperm DNA into 100 ml of TE (10 mg/ml). Pipet up and down with a 10 ml pipet to dissolve completely.
2. Incubate overnight at +4°C on a rotating wheel.
3. Using a sonicator with a large probe, sonicate the DNA twice for 30 seconds at 3/4 power. The resulting DNA will have an average size of 7 kb. You may verify the size of the DNA on a gel.
4. Aliquot the sonicated DNA into four 50 ml conical centrifuge tubes (25 ml per tube).
5. Extract with 25 ml of TE-saturated phenol. Centrifuge at 10,000 x g for 5 minutes at +4°C. Transfer the DNA (upper layer) to a fresh 50 ml conical centrifuge tube.
6. Extract with 25 ml of TE-saturated pheno:chloroform:isoamyl alcohol (25:24:1). Centrifuge at 10,000 x g for 5 minutes at +4°C. Transfer the DNA (upper layer) to a fresh 50 ml conical centrifuge tube.
7. Extract with 25 ml of chloroform. Centrifuge at 10,000 x g for 5 minutes at +4°C. Transfer the DNA (upper layer) to a 250 ml centrifuge bottle.
8. Add 5 ml of 3 M sodium acetate, pH 6.0 (1/10 volume) and 125 ml ice-cold (-20°C) 95% ethanol (2.5 volume) to precipitate DNA.
9. Pellet the DNA at 12,000 x g for 15 minutes at +4°C.
10. Wash the DNA once with 200 ml 70% ethanol and centrifuge as described in step 9.
11. Partially dry DNA by air or in a Speed-Vac (cover tubes with parafilm and poke holes in top) for 20 minutes.
12. Transfer DNA to a 250 ml sterile flask and dissolve DNA in 100 ml sterile TE (10 mg/ml).
13. Boil for 20 minutes to denature DNA. Immediately place on ice, aliquot in 1 ml samples, and freeze at -20°C.

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