pYD1 Yeast Display Vector Kit

Catalog no. V835-01

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Important Information

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20 µg of pYD1, lyophilized in TE, pH 8
EBY100 glycerol stock, 0.5 ml
2 µg pYD1 Forward sequencing primer, lyophilized
2 µg pYD1 Reverse sequencing primer, lyophilized

Shipping/Storage

The pYD1 Vector Kit is shipped on dry ice. Upon receipt:

- Store the glycerol stock at -80°C
- Store the lyophilized plasmid and primers at -20°C

Information for European Customers

The EGY100 yeast strain is genetically modified and contains the plasmid, pIU211 stably integrated into the genome (Boder and Wittrup, 1997). As a condition of sale, this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

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Introduction

Overview

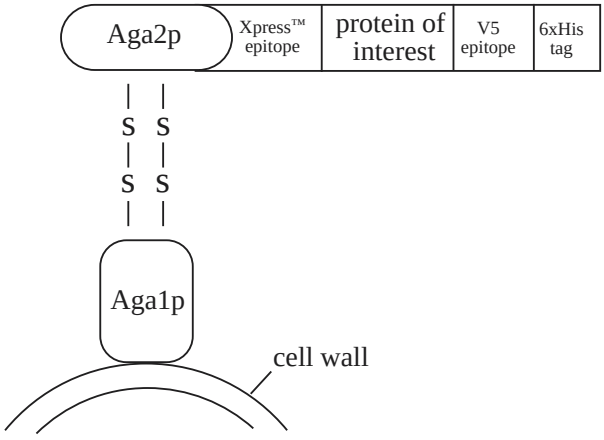
Introduction

pYD1 is a 5.0 kb expression vector designed for expression, secretion, and display of proteins on the extracellular surface of *Saccharomyces cerevisiae* cells. Features of this vector allow regulated expression, secretion, and detection of expressed proteins (see pages 15-16 for more information). The vector contains the following elements:

- *AGA2* gene from *Saccharomyces cerevisiae*. This gene encodes one of the subunits of the **a**-agglutinin receptor. Fusion of the gene of interest to *AGA2* allows secretion and display of the protein of interest.
- *GAL1* promoter for regulated expression of the *AGA2* gene fusion.
- Xpress™ epitope and V5 epitope for detection of the displayed protein.
- Polyhistidine (6xHis) tag for detection and possible purification on metal chelating resin.
- *TRP1* gene for selection in *Saccharomyces cerevisiae*.
- *CEN6*/*ARS4* for stable, episomal replication in yeast.
- Ampicillin resistance gene and the pUC origin for selection and replication in *E. coli*.

Agglutinin Receptor

The native **a** and α agglutinin receptors are believed to act as adhesion molecules to stabilize cell-cell interactions during mating and to facilitate fusion between the **a** and α haploid yeast cells. The Yeast Display System uses the **a**-agglutinin receptor of *S. cerevisiae* to display foreign proteins on the cell surface (Boder and Wittrup, 1997). The **a**-agglutinin receptor consists of two subunits encoded by the *AGA*1 and *AGA*2 genes (see diagram below). The Aga1 protein (Aga1p, 725 amino acids) is secreted from the cell and becomes covalently attached to β-glucan in the extracellular matrix of the yeast cell wall (Lu *et al.*, 1995). The Aga2 protein (Aga2p, 69 amino acids) binds to Aga1p through two disulfide bonds and after secretion remains attached to the cell through its contact with Aga1p (Cappellaro *et al.*, 1994; Cappellaro *et al.*, 1991; Roy *et al.*, 1991). The N-terminal portion of Aga2p is required for attachment to Aga1p, while proteins and peptides can be fused to the C-terminus for presentation on the yeast cell surface (Boder and Wittrup, 1997).



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Overview, continued

Experimental Outline

Use the following outline to clone and express your gene of interest in pYD1

- Consult the multiple cloning site on page 5 to clone your gene in frame with the N-terminal leader peptide encoding Aga2p, a Gly-Ser linker, and the Xpress™ epitope and the C-terminal peptide encoding the V5 epitope and the 6xHis tag (optional).
- Ligate your insert and transform into *E. coli*. Select transformants on LB containing 50-100 µg/ml ampicillin.
- Analyze your transformants for the presence of insert by restriction digestion.
- Select a transformant with the correct restriction pattern and sequence to confirm that your gene is cloned in frame with the N-terminal and C-terminal peptides.
- Transform your construct into the yeast strain EBY100 and select transformants on minimal medium without tryptophan.
- Select a transformant and grow overnight in glucose minimal medium.
- Induce expression with galactose medium and test for display of your protein.

Applications

Yeast display vectors offer an alternative to other display technologies such as phage display, bacterial surface display, and the yeast two-hybrid method. Recently, a yeast display vector has been used to apply library methods for *in vitro* antibody affinity maturation (Boder and Wittrup, 1998; Boder and Wittrup, 1997; Kieke *et al.*, 1997). A yeast display system is particularly useful for mammalian cell surface and secreted proteins (e.g., receptors, cytokines) that require endoplasmic reticulum-specific posttranslational processing for efficient folding and activity. In addition to antibody affinity maturation, other potential applications include:

- Isolation of binding domains from cDNA expression libraries
- Isolation of mutant receptors or ligands

Methods

Cloning into pYD1

Introduction

A diagram is provided on page 5 to help you ligate your gene of interest in frame with the N-terminal and the C-terminal peptide (optional). General considerations for cloning and transformation are listed 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, see *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 growth of this vector including TOP10 and DH5α. We recommend that you propagate vectors containing inserts in *E. coli* strains that are recombination deficient (*rec*A) and endonuclease A deficient (*end*A).

For your convenience, TOP10 is available as chemically competent or electrocompetent cells from Invitrogen.

Item	Quantity	Catalog no.
One Shot™ TOP10 Chemically Competent *E. coli*	21 x 50 µl	C4040-03
Electrocomp™ TOP10	5 x 80 µl	C664-55

Transformation Method

Use your method of choice to transform pYD1 containing your insert into *E. coli*. Chemical transformation is the most convenient method for most researchers. Electroporation is the most efficient and the method of choice for large plasmids (>10 kb).

Maintenance of pYD1

In order to propagate and maintain pYD1, we recommend that you transform the plasmids into *E. coli* and prepare glycerol stocks for long-term storage. To transform plasmids into *E. coli*:

- Resuspend the lyophilized vector in 20 µl sterile water to make a 1 µg/µl stock solution. Store the stock solution at -20°C.
- Use this stock solution (10-100 ng) to transform a *rec*A, *end*A *E. coli* strain like TOP10 or DH5α.
- Select transformants on LB plates containing 50-100 µg/ml ampicillin.

To prepare glycerol stocks, see page 6.

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Cloning into pYD1, continued

Detection of Recombinant Proteins

Before you clone your gene of interest into pYD1, it is best to consider how you will detect your recombinant fusion on the surface of yeast cells. You may detect your protein by activity assay or by fluorescent staining. Usually fluorescent staining with the appropriate antibody is used. If you do not have an antibody to your protein, there are several antibodies from Invitrogen which you can utilize. Ordering information is provided below.

Antibody	Epitope	Catalog no.
Anti-Xpress™ Antibody	Detects 8 amino acid epitope DLYDDDDK	R910-25
Anti-Xpress™-HRP Antibody		R911-25
Anti-Xpress™-FITC Antibody		R913-25
Anti-V5 Antibody	Detects 14 amino acid epitope derived from SV5 (Southern *et al.*, 1991) GKPIPNPLLGLDST	R960-25
Anti-V5-HRP Antibody		R961-25
Anti-V5-AP Antibody		R962-25
Anti-V5-FITC Antibody		R963-25
Anti-His(C-term) Antibody	Detects the C-terminal polyhistidine tag (requires free carboxyl group for detection) (Lindner *et al.*, 1997) HHHHHH-COOH	R930-25
Anti-His(C-term)-HRP Antibody		R931-25
Anti-His(C-term)-AP Antibody		R932-25
Anti-His(C-term)-FITC Antibody		R933-25

Cloning into pYD1

pYD1 is a fusion vector requiring that you clone your gene of interest in frame with the *AGA2* gene and the C-terminal V5 epitope/polyhistidine tag (optional). For proper expression, first determine if any restriction sites are appropriate to preserve the reading frame at **BOTH** the 5´ and the 3´ ends. It may be necessary to PCR your gene product to create a fragment with the appropriate restriction sites to clone in frame at both ends. Carefully inspect your gene and the multiple cloning site before cloning your gene of interest. See the next page for details of the multiple cloning site.

Note

If you wish to express and display your protein without the C-terminal tag, include a termination codon in your gene of interest. Note that if you include a termination codon, you will be unable to detect the fusion protein with the Anti-V5 Antibody or Anti-His (C-term) Antibody.

Other Cloning Strategies

If you wish to clone your gene closer to the *AGA2* gene, the *Hin*d III or the *Pst* I sites between the *AGA2* gene and the DNA encoding the Gly-Ser linker may be used. There is also an *Nhe* I site between the Gly-Ser linker and the Xpress™ epitope which can be used. Several different cloning strategies may be tested to ensure that your protein is displayed properly for your particular application.

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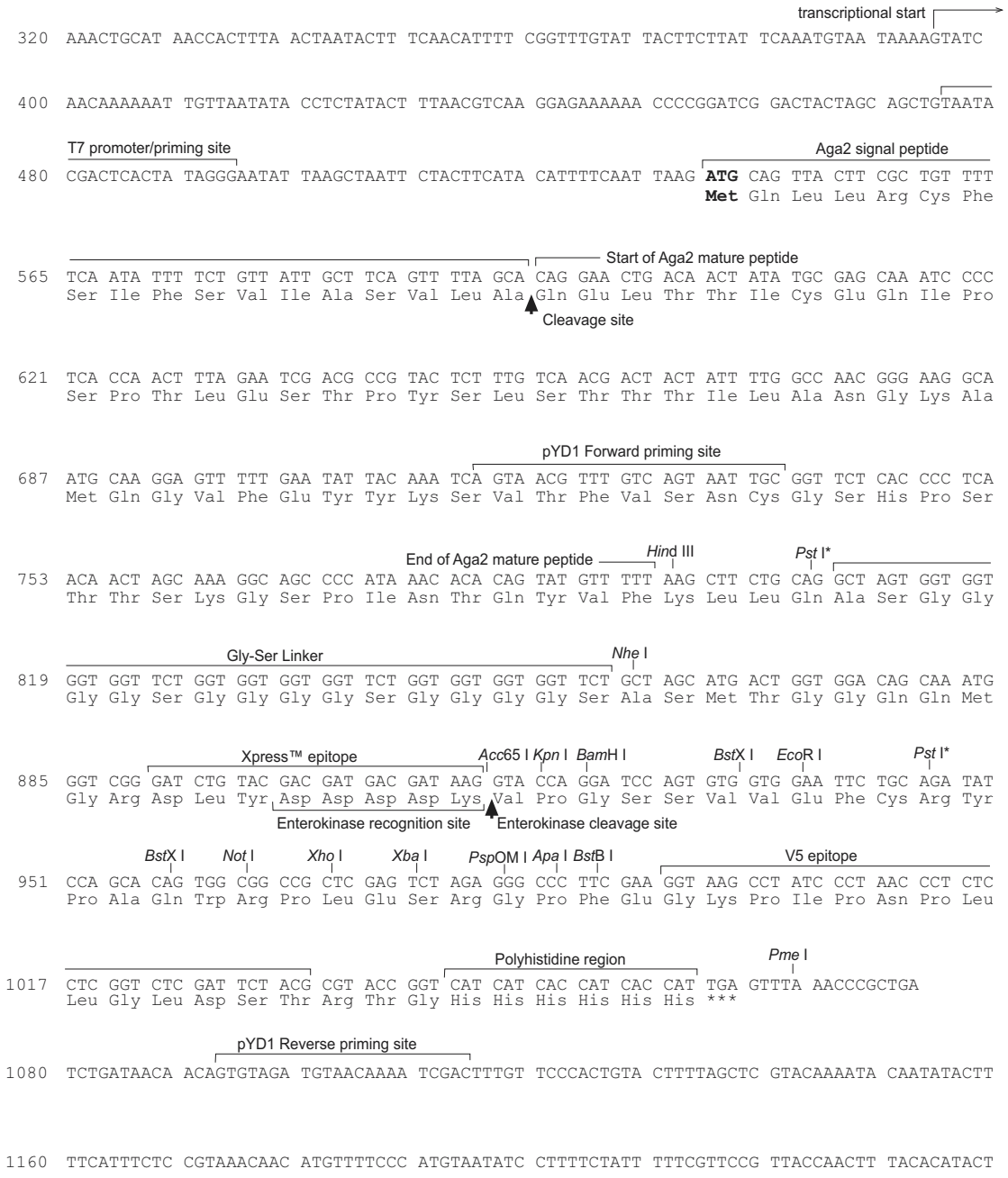
Cloning into pYD1, continued

Multiple Cloning Site of pYD1

Below is the multiple cloning site for pYD1. Restriction sites are labeled to indicate the cleavage site. The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence is available by downloading it from our Web site (www.invitrogen.com) or by calling Technical Service (page 18).**



*The two *Pst* I sites are unique to the multiple cloning site.

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Cloning into pYD1, continued

E. coli Transformation

Transform your ligation mixtures into a competent *recA*, *endA E. coli* strain and select on LB plates containing 50-100 µg/ml ampicillin. Select 10 colonies and analyze for the presence and orientation of your insert.

We recommend that you sequence your construct with the pYD1 Forward and Reverse primers included in the kit to confirm that your gene is in frame with the N-terminal peptide and the C-terminal peptide.

Sequence of Primers

The table below provides the sequences and number of pmoles supplied for the pYD1 Forward and pYD1 Reverse sequencing primers. If you need to purchase more primer, ordering information is also provided.

Primer	Sequence	Pmoles	Catalog no.
pYD1 Forward	5´-AGTAACGTTTGTCAGTAATTGC-3´	297	N842-02
pYD1 Reverse	5´-GTCGATTTTGTTACATCTACAC-3´	301	N843-02

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 as well.

- Streak the original colony out on an LB plate containing 50-100 µg/ml ampicillin. Incubate the plate at 37°C overnight.
- Isolate a single colony and inoculate into 1-2 ml of LB containing 50-100 µg/ml ampicillin.
- Grow the culture to mid-log phase (OD_{600} = 0.5-0.7).
- Mix 0.85 ml of culture with 0.15 ml of sterile glycerol and transfer to a cryovial.
- Store at -80°C.

Yeast Transformation

Introduction

Once you have confirmed that your construct is in the correct orientation and fused in frame with the N-terminal peptide and the C-terminal peptide (if desired), you are ready to transform the yeast strain EBY100.

Basic Yeast Molecular Biology

The user should be familiar with basic yeast molecular biology and microbiological techniques. Refer to *Current Protocols in Molecular Biology*, Unit 13 (Ausubel *et al.*, 1994) and the *Guide to Yeast Genetics and Molecular Biology* (Guthrie and Fink, 1991) for information on preparing yeast media and handling yeast.

Positive Control

We recommend that you transform the empty vector (pYD1) into EBY100 for use as a positive control. pYD1 expresses Aga2p fused to the Xpress™, V5, and polyhistidine epitopes. Expression and detection of this fusion protein by fluorescent staining will demonstrate that the system is working.

EBY100

EBY100 expresses the *AGA1* gene under control of the *GAL1* promoter. It was created by integrating the vector pIU211 into the *AGA1* locus of the *Saccharomyces cerevisiae* strain BJ5465 (MAT**a** *ura* 3-52 *trp* 1 *leu*2Δ1 *his*3Δ200 *pep*4:*HIS3 prb*1Δ1.6R *can*1 *GAL*) (Boder and Wittrup, 1997). pIU211 contains the *AGA1* gene regulated by the *GAL* promoter and a *URA3* selectable marker. The vector was linearized for integration at *AGA1* using *Bsi*W I. *Bsi*W I cuts in the *AGA1* gene, ensuring that the construct integrates at the *AGA1* locus.

EBY100 (Catalog no. C839-00) is available separately.

Transformation Method

You may use your own method of choice to transform EBY100.

If you do not have a method for transformation, we recommend the *S.c.* EasyComp™ Kit (Catalog no. K5050-01). This kit rapidly prepares competent *Saccharomyces cerevisiae* in less than 30 minutes. In addition, the cells can be frozen for future use.

Alternatively, we have provided a small-scale transformation protocol utilizing lithium acetate in the Appendix (page 17).

Before Starting

To transform EBY100 you will need the following reagents. For media recipes, refer to page 13.

- Minimal dextrose plates containing leucine and tryptophan
- YPD medium
- Minimal dextrose plates containing leucine
- 20% Glucose
- 0.5-5 µg plasmid DNA (DNA may be isolated from *E. coli* using your method of choice)

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Yeast Transformation, continued

Transformation of EBY100

Follow the general steps below to transform EBY100.

1. Take the glycerol stock of EBY100 and streak out on a minimal dextrose plate containing leucine and tryptophan. Incubate at 30°C until colonies appear (1-2 days).
2. Use your method of choice to prepare competent cells and transform with pYD1 or pYD1 containing your gene of interest.
3. Plate the transformation reaction (50-150 µl) on minimal dextrose plates containing leucine. Incubate the plates at 30°C for 2 to 4 days until single colonies appear.

Once you have transformants, you are ready to test for display of your fusion protein. Proceed to the next page to induce expression of your fusion protein.

Expression and Display of Protein Fusion

Introduction

To express your fusion protein in EBY100, transformants are grown in glucose-containing medium overnight, then switched to medium containing galactose for induction of expression. Cells are grown in galactose until display of the desired protein fusion is detected by antibody staining. This is usually a function of the protein fusion and is determined empirically. Follow the instructions below to express and detect your protein fusion.

Controls

We recommend including the following controls to evaluate your experiment.

Control	Type of Control	Purpose
EBY100/pYD1	Positive	Expression and detection of the Aga2 fusion protein demonstrates that the system is working properly.
EBY100	Negative	Controls for background resulting from the cells
EBY100/pYD1 + gene of interest (uninduced)	Negative	Controls for leaky expression. (This will be the zero time point for the induction time course on page 10)

Before Starting

Be sure to have following reagents and equipment on hand. Refer to page 13 for media recipes.

- Shaking incubator (adjustable from 20°C to 30°C) **Note**: A shaking incubator with a cooling apparatus may be required to maintain 20°C consistently
- 50 ml conical centrifuge tube or 25-50 ml culture flask
- Spectrophotometer
- YNB-CAA medium
- 20% glucose
- 20% galactose
- 1X Phosphate Buffered Saline (PBS)
- 1X PBS, 1 mg/ml BSA
- Anti-mouse IgG conjugated with FITC (if you are using a polyclonal antibody, use anti-rabbit IgG conjugated with FITC) **Note**: Other fluorophores may be suitable.

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Expression and Display of Protein Fusion, continued

Induction

Grow untransformed EBY100, EBY100/pYD1, and EBY100/pYD1 containing your gene of interest as follows.

1. Inoculate a single yeast colony into 10 ml YNB-CAA containing 2% glucose and grow overnight at 30°C with shaking.
2. Read the absorbance of the cell culture at 600 nm. The OD_{600} should be between 2 and 5. If the OD_{600} is below 2 or over 5, refer to the table below.

If the OD_{600} is....	Then you may...
below 2,	either continue growing the cells until the OD_{600} reaches 2 or proceed to Step 3.
over 5,	proceed to Step 3.

Note: In general, OD_{600} readings less than 2 will decrease the number of cells displaying fusion proteins. OD_{600} readings greater than 5 will delay induction of expression of the displayed protein.

3. Centrifuge the cell culture at 3000-5000 x g for 5-10 minutes at room temperature.
4. Resuspend the cell pellet in YNB-CAA medium containing 2% galactose to an OD_{600} of 0.5 to 1. This is to ensure that the cells continue to grow in log-phase. For example, if the OD_{600} is 2 from Step 2, resuspend the cells in 20 to 40 ml of medium.
5. Immediately remove a volume of cells equivalent to 2 OD_{600} units. For an OD_{600} of 0.5, remove 4 ml and place on ice. This is your zero time point.
6. Incubate the cell culture at 20-25°C with shaking. **Note**: In general, more cells will display the protein fusion at 20°C. However, if you do not have an incubator that maintains 20°C, try 25°C.
7. Assay the cell culture over a 48-hour time period (i.e. 0, 12, 24, 36, 48 hours) to determine the optimal induction time for maximum display. For each time point, read the OD_{600} and remove a volume of cells that is equivalent to 2 OD_{600} units (see Step 5, above). Proceed to **Staining of Displayed Proteins**, below.

Staining of Displayed Proteins

For each time point, assay untransformed EBY100, EBY100/pYD1, and EBY100/pYD1 containing your gene of interest. Time points may be processed as they are collected or placed on ice and stored at +4°C until all time points are collected. **Do not freeze cells.**

1. Take your time points from Steps 4 and 6, above, and centrifuge at 3000-5000 x g for 5-10 minutes at +4°C.
2. Resuspend the cells in 1X PBS and centrifuge as in Step 1.
3. Remove the PBS and resuspend the cell pellet in 250 µl of 1X PBS, 1 mg/ml BSA, and 1 µg antibody.
4. Incubate on ice for 30 minutes with occasional mixing.
5. Centrifuge the cells at 3000-5000 x g for 5-10 minutes at +4°C.
6. Wash the cells once with 1X PBS.
7. Resuspend the cells in 1X PBS, 1 mg/ml BSA, and 1 µg anti-mouse IgG conjugated with fluorescein isothiocyanate (FITC) or any other suitable fluorophore.

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Expression and Display of Protein Fusion, continued

Staining of Displayed Proteins, continued

8. Incubate on ice for 30 minutes in the dark and invert the tube occasionally.
9. Wash the cells two times with 1 ml of 1X PBS (no BSA).
10. Resuspend the cells in 40 µl 1X PBS and spot 5-10 µl on a slide and add a coverslip for microscopic observation under UV light. Record your observations or take a picture.

Evaluating Your Results

The Aga2p fusion can be detected as early as 4 hours after the switch to galactose and is still detectable after 72 hours in galactose medium. Optimal detection occurs between 12 and 48 hours.

You should see little or no fluorescence with untransformed EBY100, uninduced EBY100/pYD1 (zero time point), or uninduced EBY100/pYD1 containing your gene of interest (zero time point).

If you have trouble expressing and displaying your protein, see the next page.

Examples of Displayed Proteins

Growth conditions (i.e. density of cells, temperature) and the type of protein are the major variables affecting expression and display. Displayed proteins can be detected as early as 4 hours and as late as 72 hours after the shift from glucose to galactose. Detection of an Aga2-GFP fusion using the V5 antibody is summarized below.

Time (hours)	Fluorescence
2	Barely detectable above background
4	Detectable, but faint
8	Much better
20-30	Optimal fluorescence

Other proteins, such as an Aga2-T-cell receptor fusion, required 36-48 hours of galactose induction for optimal display (K. D. Wittrup, personal communication).

Percentage of Cells Displaying Protein

Under the conditions described above, 10% to 50% of the cells are easily detectable as displaying the fusion protein. If Fluorescence Activated Cell Sorting (FACS) is used to detect fluorescence, up to 70% of the total cells can be detected as displaying the fusion protein in question (Boder and Wittrup, 1997). Note that this is because of the increased sensitivity of FACS, and the fact that induction of expression was done at 20°C rather than 25°C.

Protein Purification

At this time, we have not tried to purify displayed proteins utilizing the polyhistidine tag and metal-chelating resin. Displayed proteins tend to be glycosylated which may or may not interfere with purification. It is possible to release the Aga2p protein fusion from the cell wall by treatment with 100 µM dithiothreitol (DTT) in buffer for two hours. DTT reduces the disulfide bond holding Aga2p to Aga1p. It is possible that this could be exploited to purify the protein of interest.

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Troubleshooting

Use the table below to troubleshoot your experiment.

Problem	Reason	Solution
No display detected with construct or positive control	Induction not performed properly	Review the protocol on page 10
	Cells not growing	Test for growth on minimal medium without uracil or tryptophan. EBY100 transformed with pYD1 should be able to grow without uracil or tryptophan. Review media recipes and re-make if necessary.
No display detected with construct, but display is detected with the positive control	Gene is not fused correctly to the AGA2 gene	Sequence construct to confirm that gene is cloned in frame with *AGA2*. Re-make construct if necessary.
Construct expresses and displays protein fusion, but percentage of cells is low	Cells not grown to a high enough density before induction	Grow culture on glucose to a density > 2 before the shift to galactose
	Induction temperature is too high	Try inducing your construct at 20°C. Incubation at higher temperatures can lead to poor surface expression. This may be because of inefficient protein folding and retention in the endoplasmic reticulum (ER)
Construct expresses and displays protein fusion, but it takes over 48 hours	Cell density was greater than 5 prior to the shift to galactose	Do not grow cells as long on glucose

Appendix

Recipes

LB (Luria-Bertani) Medium and Plates

Composition:

1.0% Tryptone
0.5% Yeast Extract
1.0% NaCl
pH 7.0

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 950 ml deionized water.
2. Adjust the pH of the solution to 7.0 with NaOH and bring the volume up to 1 liter.
3. Autoclave on liquid cycle for 20 minutes. Allow solution to cool to 55°C and add antibiotic if needed.
4. Store at room temperature or at +4°C.

LB agar plates

1. Prepare LB medium as above, but add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle for 20 minutes at 15 psi.
3. After autoclaving, cool to ~55°C, add antibiotic (50-100 µg/ml of ampicillin), and pour into 10 cm plates.
4. Let harden, then invert and store at +4°C, in the dark. Plates are stable for about 2 weeks with antibiotic.

YPD

Yeast Extract Peptone Dextrose Medium (1 liter)

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

1. Dissolve the following in 900 ml of water:

   10 g yeast extract
   20 g of peptone
2. Optional: Add 20 g agar, if making plates.
3. Autoclave for 20 minutes on liquid cycle and cool to ~55-60°C.
4. Add 100 ml of 20% dextrose (filter-sterilized).

Store medium at room temperature. The shelf life is approximately one to two months.

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Minimal Dextrose Plates (with Amino Acids)

0.67% YNB (with ammonium sulfate, **without** amino acids)
2% glucose
0.01% leucine
0.01% tryptophan (optional)
1.5% agar

Note: Plates without tryptophan are required for selection of pYD1 transformants.

1. For 1 liter, dissolve 6.7 g YNB and 15 g agar in 900 ml deionized water.
2. Autoclave on liquid cycle for 20 minutes.
3. After autoclaving, allow the medium to cool to ~55-60°C and add 100 ml of 20% glucose (filter-sterilized).
4. Add 10 ml of 10 mg/ml leucine (filter-sterilized) and/or 10 ml of 10 mg/ml tryptophan (filter-sterilized). Alternatively, plates can be made ahead of time and amino acids spread onto the plate prior to use. Spread 100 µl of a 10 mg/ml amino acid solution onto the plate and let the solution soak into the plate before plating cells.
5. Store at +4°C. Plates are stable for 1-2 months.

YNB-CAA Medium

0.67% YNB (with ammonium sulfate, **without** amino acids)
0.5% Casamino acids
2% glucose or galactose

1. For 1 liter, dissolve 6.7 g YNB and 5 g Casamino acids in 900 ml deionized water.
2. Autoclave on liquid cycle for 20 minutes. **Note**: Casamino acids contain trace amounts of tryptophan that is destroyed upon autoclaving. Lack of tryptophan helps maintain pYD1 during induction.
3. After autoclaving, cool to 55-60°C and add 100 ml of 20% glucose or galactose.
4. Store medium at room temperature or +4°C. Medium is stable for about 1 month.

Alternatively, you may use prepared drop-out medium (i.e. Bio101 HSM-trp-ura) instead of Casamino acids. Omitting uracil helps to maintain the *AGA1* cassette integrated in EBY100.

1X PBS

137 mM NaCl
2.7 mM KCl
10 mM Na_2HPO_4
1.8 mM KH_2PO_4
pH 7.4

1. Dissolve:
   8 g NaCl
   0.2 g KCl
   1.44 g N_a2HP_{O4}
   0.24 g KH_2PO_4
   in 800 ml deionized water.
2. Adjust pH to 7.4 with concentrated HCl.
3. Bring the volume to 1 liter. You may autoclave the solution to increase shelf life.

pYD1 Vector

Features of pYD1

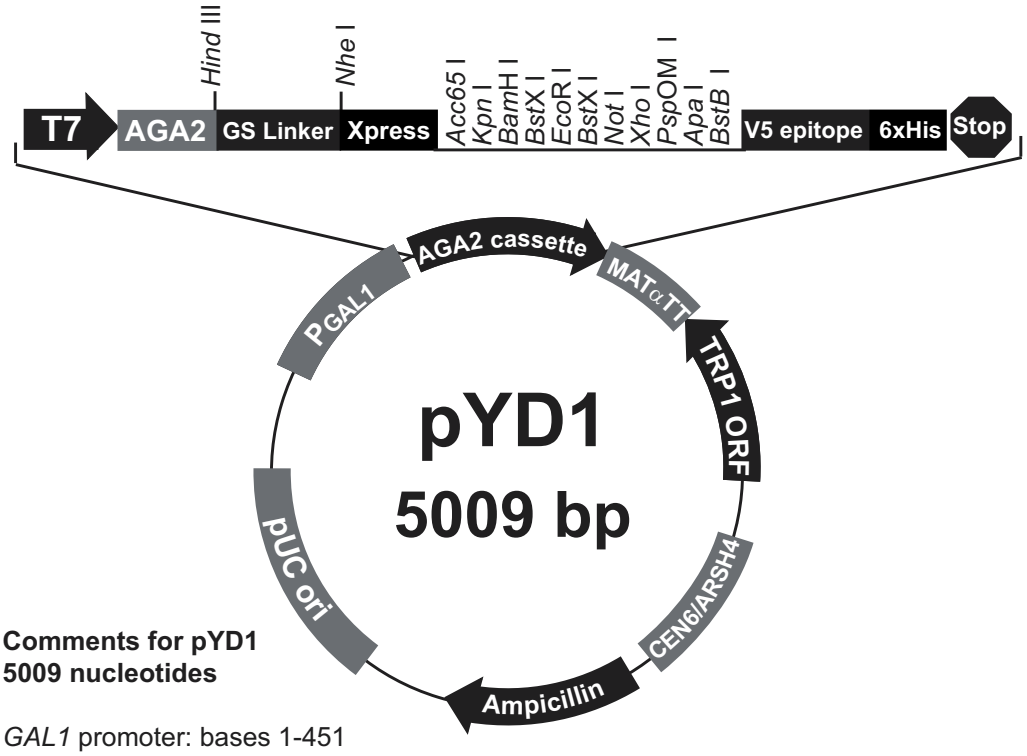
pYD1 (5009 bp) contains the following elements. All features have been functionally tested.

Feature	Benefit
GAL1 promoter	Permits regulated expression of your recombinant protein (Giniger *et al.*, 1985; West *et al.*, 1984)
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation
AGA2 gene	Allows secretion of the fusion protein
pYD1 Forward priming site	Sequencing of insert
Gly-Ser Linker	Permits spacing of protein of interest from Aga2p
Xpress™ epitope (Asp-Leu-Tyr-Asp-Asp-Asp-Asp-Lys)	Allows detection of the fusion protein with the Anti-Xpress™ Antibodies
Multiple cloning site	Allows insertion of your gene of interest
V5 epitope	Allows detection of the fusion protein with the Anti-V5 Antibodies
Polyhistidine epitope (6xHis tag)	Permits detection of pYD1 fusion proteins with the Anti-His(C-term) Antibodies (Lindner *et al.*, 1997) In addition, it may allow purification of pYD1 fusion proteins by chromatography on metal-chelating resins such as ProBond™
pYD1 Reverse priming site	Permits sequencing through the insert
MATα transcription termination region	Efficient transcription termination and polyadenylation of mRNA
TRP1 gene	Allows selection of yeast transformants
CEN6/*ARS4*	Allows stable, episomal replication and partitioning into daughter cells in yeast
Ampicillin resistance gene (β-lactamase)	Selection in *E. coli*
pUC origin	High-copy number replication and growth in *E. coli*

continued on next page

Map of pYD1

The figure below shows the location of features in pYD1. **The complete nucleotide sequence for pYD1 is available for downloading from our World Wide Web site (http://www.invitrogen.com) or from Technical Service (page 18).**



Comments for pYD1
5009 nucleotides

GAL1 promoter: bases 1-451
T7 promoter priming site: bases 475-494
AGA2 ORF: bases 534-794
pYD1 Forward priming site: bases 751-770
Gly/Ser Linker: bases 807-857
Xpress™ epitope: bases 891-914
Multiple cloning site: bases 914-992
V5 epitope: bases 993-1034
Polyhistidine region: bases 1044-1061
pYD1 Reverse priming site: bases 1114-1093 (C)
MATα transcription termination region: bases 1385-1098 (C)
TRP1 ORF: bases 2107-1433 (C)
CEN6/*ARS4*: bases 2797-2282 (C)
Ampicillin resistance gene (ORF): bases 2931-3791
pUC origin: bases 3936-4609

Small-Scale Yeast Transformation

Introduction

A small-scale yeast transformation protocol is provided below.

Materials Needed

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

- YPD
- 1X TE
- 1X LiAc/0.5X TE
- Denatured sheared salmon sperm DNA
- Plasmid DNA to be transformed
- 1X LiAc/40% PEG-3350/1X TE
- DMSO
- Selective plates

Protocol

1. Inoculate 10 ml of YPD with a yeast colony 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 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 pellet in 2 ml of 1X LiAc/0.5X TE.
5. Incubate the cells at room temperature for 10 minutes.
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.
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 pellet in 50-100 µl TE and plate on a selective plate.

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Technical Service, continued

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