USER GUIDE

invitrogen™
by life technologies™

pYES2/NT A, B, and C
pYC2/NT A, B, and C

Yeast expression vectors with N-terminal tags

Catalog numbers V8252-20 and V8256-20

Revision date 9 July 2012
Publication Part number 25-0303

MAN0000127

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Not for human or animal therapeutic or diagnostic use.

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Contents

Kit Contents and Storage

Kit Contents

This manual is supplied with the vectors listed in the following table.

Vector	Catalog no.
pYES2/NT A, B, and C	V8252-20
pYC2/NT A, B, and C	V8256-20

Contents

The reagents listed below are provided with each vector. Each vector is supplied with the multiple cloning site in three reading frames to facilitate in-frame cloning with the N-terminal peptide. A control vector containing the *lacZ* gene in pYES2/NT or pYC2/NT is provided for use as a positive control for expression.

Reagent	Amount	Comments
pYES2/NT A, B, and C or pYC2/NT A, B, and C	40 µL of 0.5 µg/µL vector in TE, pH 8.0	Yeast expression vector in three reading frames
pYES2/NT/*lacZ* or pYC2/NT/*lacZ*	40 µL of 0.5 µg/µL vector in TE, pH 8.0	Control vector expressing ß-galactosidase
INVSc1 Yeast Strain	1 stab	Yeast expression strain

Shipping and Storage

All reagents are shipped at room temperature.

Upon receipt, store the plasmid DNA at –30°C to –10°C.

Store the INVSc1 stab at 2°C to 8°C.

Preparing INVSc1 Glycerol Stocks

We recommend that you prepare a set of glycerol master stocks within 2 weeks of receiving the INVSc1 yeast cells.

1. Use a sterile loop to inoculate a 50 mL tube containing 5 mL YPD medium with the INVSc1 yeast stab.
2. Incubate the cells at 30ºC with shaking overnight or until the culture is turbid.
3. Add 1 mL sterile 80% glycerol and mix thoroughly.
4. Dispense the stock into cryovials and freeze at –80ºC.
5. Revive the yeast by transferring a small portion of the frozen sample onto an YPD agar plate.

Introduction

About the kit

Introduction

pYES2/NT A, B, and C and pYC2/NT A, B, and C are 6.0 kb and 4.7 kb vectors, respectively, designed for inducible expression of recombinant proteins in *Saccharomyces cerevisiae*. Features of the vectors allow purification and detection of expressed proteins (see pages 21–24 for more information). The vectors contain the following elements:

- Yeast *GAL1* promoter for high level inducible protein expression in yeast by galactose and repression by glucose (Giniger *et al.*, 1985; West *et al.*, 1984) (see page 14 for more information).
- Three reading frames to facilitate in-frame cloning with an N-terminal peptide encoding the Xpress™ epitope and a polyhistidine (6xHis) tag.
- C-terminal peptide encoding the V5 epitope and a polyhistidine tag for purification and detection of a recombinant fusion protein, if desired.
- 2µ origin (pYES2/NT only) for episomal maintenance and high copy replication of the plasmid in yeast (see page 6 for more information).
- CEN6/ARSH4 sequence (pYC2/NT only) for non-integrative centromeric maintenance and low copy replication of the plasmid in yeast (see page 6 for more information).
- *URA3* auxotrophic marker for selection of yeast transformants.
- Ampicillin resistance gene for selection in *E. coli.*

Experimental Outline

The following table outlines the major steps required to clone and express your gene of interest in pYES2/NT or pYC2/NT.

Step	Action
1	Consult the multiple cloning sites described on pages 8–10 to determine which vector (A, B, or C) should be used to clone your gene in frame with the N-terminal peptide. You may also clone your gene in frame with the C-terminal peptide, if desired.
2	Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants on LB plates containing 50 to 100 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 frame with the N-terminal peptide.
5	Transform your construct into competent INVSc1 cells and select for uracil prototrophy.
6	Test for expression of your recombinant protein by Western blot analysis or functional assay.
7	Use metal-chelating resin such as ProBond™ to purify your recombinant protein.

Methods

Clone into pYES2/NT A, B, and C or pYC2/NT A, B, and C

Introduction

Diagrams are provided on pages 8–10 to help you ligate your gene of interest in frame with the N-terminal peptide in pYES2/NT or pYC2/NT (and the C-terminal peptide, if desired). General considerations for cloning and transformation are discussed in this section.

General Molecular Biology Techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, DNA sequencing, and DNA biochemistry, please 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 propagating the pYES2/NT or pYC2/NT vectors including TOP10, DH5 , or equivalent. We recommend that you propagate the pYES2/NT or pYC2/NT vectors in *E. coli* strains that are recombination deficient (*rec*A) and endonuclease deficient (*end*A).

For your convenience, TOP10 *E. coli* are available as chemically competent or electro-competent cells (see page 31 for ordering information).

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.

Propagate and Maintain Plasmids

Transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5α, or equivalent. Select transformants on LB plates containing 50 to 100 µg/mL ampicillin. Be sure to prepare a glycerol stock from a transformant containing each plasmid for long-term storage (see page 11 for a protocol).

2µ Origin

The pYES2/NT vectors contain the 2µ origin for maintenance and replication in yeast. The sequence containing the 2µ origin was originally isolated from the naturally occurring yeast 2µ plasmid (Hartley and Donelson, 1980). When placed in an expression plasmid (i.e. pYES2/NT), the presence of the 2µ origin allows the plasmid to be episomally maintained and replicated at high copy number (generally 10–40 copies per cell).

CEN6/ARSH4 Sequence

The pYC2/NT vectors contain the CEN6/ARSH4 sequence (Sikorski and Hieter, 1989) for maintenance and replication in yeast. The CEN6/ARSH4 sequence is a 518 bp hybrid DNA fragment that contains a yeast centromere sequence (CEN) and an autonomously replicating sequence (ARS) (Sikorski and Hieter, 1989). The CEN6 sequence is derived from the CEN6 locus of yeast chromosome 6 (Panzeri and Philippsen, 1982) while the ARSH4 sequence is derived from the yeast histone H4-associated ARS (Bouton and Smith, 1986). When placed in an expression plasmid (i.e. pYC2/NT), the presence of the CEN6/ARSH4 sequence allows non-integrative centromeric maintenance and low copy number replication of the plasmid (generally 1–2 copies per cell).

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Cloning Considerations

The pYES2/NT and pYC2/NT vectors are fusion vectors. To ensure proper expression of your recombinant protein, you must clone your gene in frame with the ATG at base pairs 511–513. This will create a fusion with the N-terminal polyhistidine (6xHis) tag, Xpress™ epitope, and the enterokinase cleavage site. Both pYES2/NT and pYC2/NT are supplied with the multiple cloning site in three reading frames relative to the N-terminal peptide to facilitate cloning. See pages 8–10 to develop a cloning strategy. Note that pYES2/NT and pYC2/NT possess the same multiple cloning site.

To clone your gene as closely as possible to the enterokinase cleavage site, use the following guidelines:

- Digest pYES2/NT A, B, or C (or pYC2/NT A, B, or C) with *Kpn* I.
- Create blunt ends with T4 DNA polymerase and dNTPs.
- Clone your blunt-ended insert in frame with the lysine codon (AAG) of the enterokinase recognition site.

If you intend to separate your protein of interest from the N-terminal peptide tag, you may use any suitable enterokinase including EnterokinaseMax™ (see page 31 for ordering information). Following enterokinase cleavage, no vector-encoded amino acid residues will be present in your protein.

Yeast Consensus sequence

The pYES2/NT and pYC2/NT vectors do not contain an ATG initiation codon for proper initiation of translation. Be sure to design your insert to contain an ATG initiation sequence. In addition to the initiation codon, you may also include the yeast consensus sequence at the translation initiation site. An example of the yeast consensus sequence is provided below, where the ATG translation initiation codon is shown underlined.

(A/Y)A(A/C)A(A/C)A<u>ATG</u>TC(T/C)

Note that other sequences are also possible. The prevalence of the TCT as the second codon is thought to contribute to stabilization under the N-end rule (Hamilton *et al.*, 1987). Although not as strong as the mammalian Kozak translation initiation sequence, the yeast consensus sequence is thought to have a 2–3-fold effect on the efficiency of translation initiation.

Your insert must also contain a stop codon for proper termination of your mRNA. Note that the *Xba* I site contains an internal stop codon (TC<u>TAG</u>A).

Note

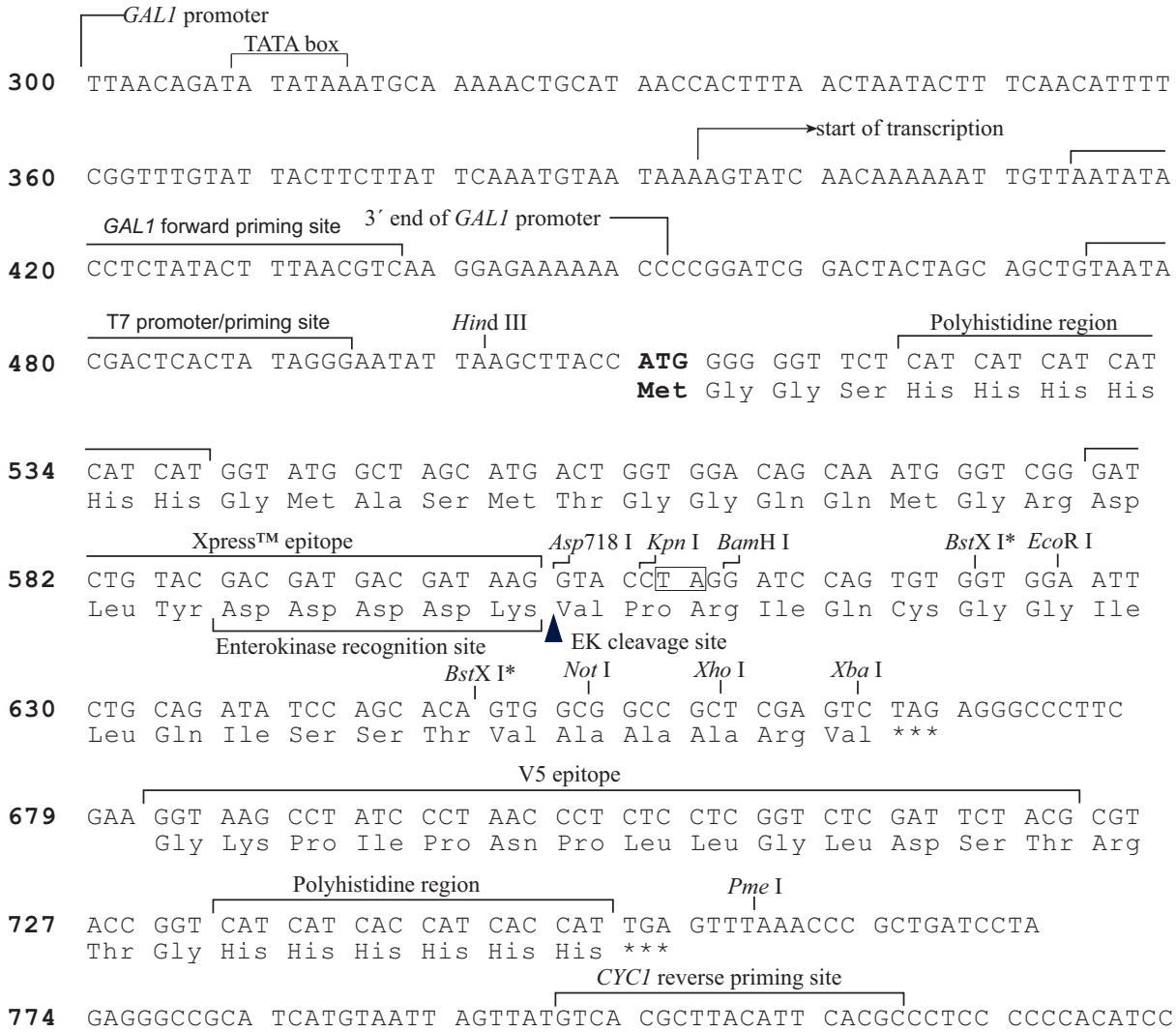
The pYES2/NT and pYC2/NT vectors also contain a C-terminal peptide encoding the V5 epitope and a polyhistidine (6xHis) tag. If you intend to express your recombinant protein *without* the C-terminal peptide, be sure that your insert includes a stop codon.

If you intend to express a recombinant protein that contains both the N-terminal peptide and the C-terminal peptide, make sure that you design your cloning strategy such that your insert is cloned in frame with both the N-terminal and C-terminal peptides.

Continued on next page

Multiple Cloning Site of pYES2/NT A and pYC2/NT A

The following diagram shows the multiple cloning site for pYES2/NT A and pYC2/NT A. Features of the *GAL1* promoter are marked as previously described (Giniger *et al.*, 1985; Johnston and Davis, 1984; Yocum *et al.*, 1984). Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. **Note that there is a stop codon within the *Xba* I site.** The multiple cloning site has been confirmed by sequencing and functional testing. The complete sequences of pYES2/NT A and pYC2/NT A are available from www.lifetechnologies.com or by contacting Technical Support (see page 32). For a map and description of the features of pYES2/NT and pYC2/NT, refer to pages 21–24 in the **Appendix**.



*Note that there are two *Bst*X I sites in the polylinker.

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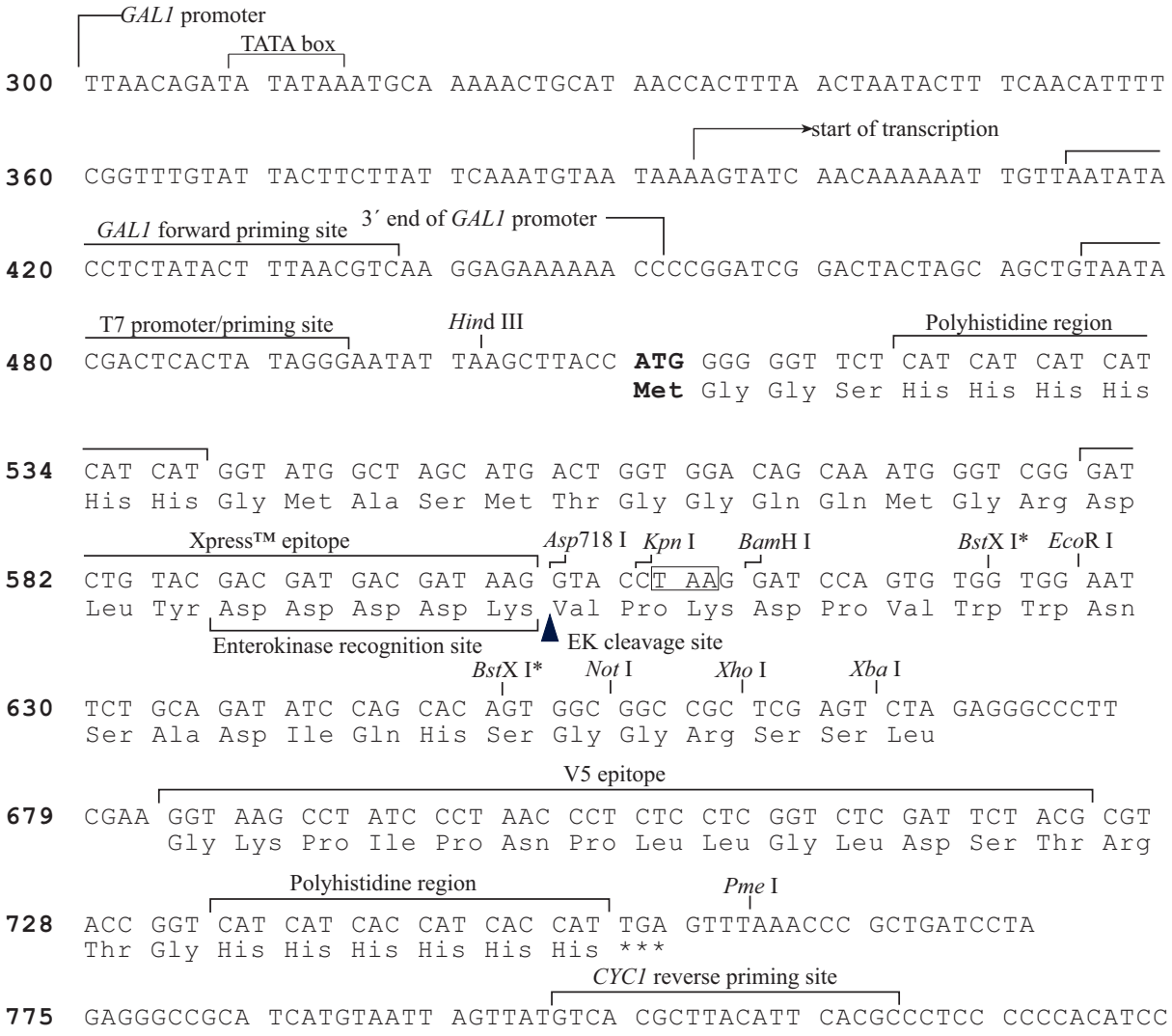
Multiple Cloning Site of pYES2/NT B and pYC2/NT B

The following diagram shows the multiple cloning site for pYES2/NT B and pYC2/NT B. Features of the *GAL1* promoter are marked as previously described (Giniger *et al.*, 1985; Johnston and Davis, 1984; Yocum *et al.*, 1984). Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. The complete sequences of pYES2/NT B and pYC2/NT B are available from **www.lifetechnologies.com** or by contacting Technical Support (see page 32). For a map and description of the features of pYES2/NT and pYC2/NT, refer to pages 21–24 in the **Appendix**.



*Note that there are two *Bst*X I sites in the polylinker.

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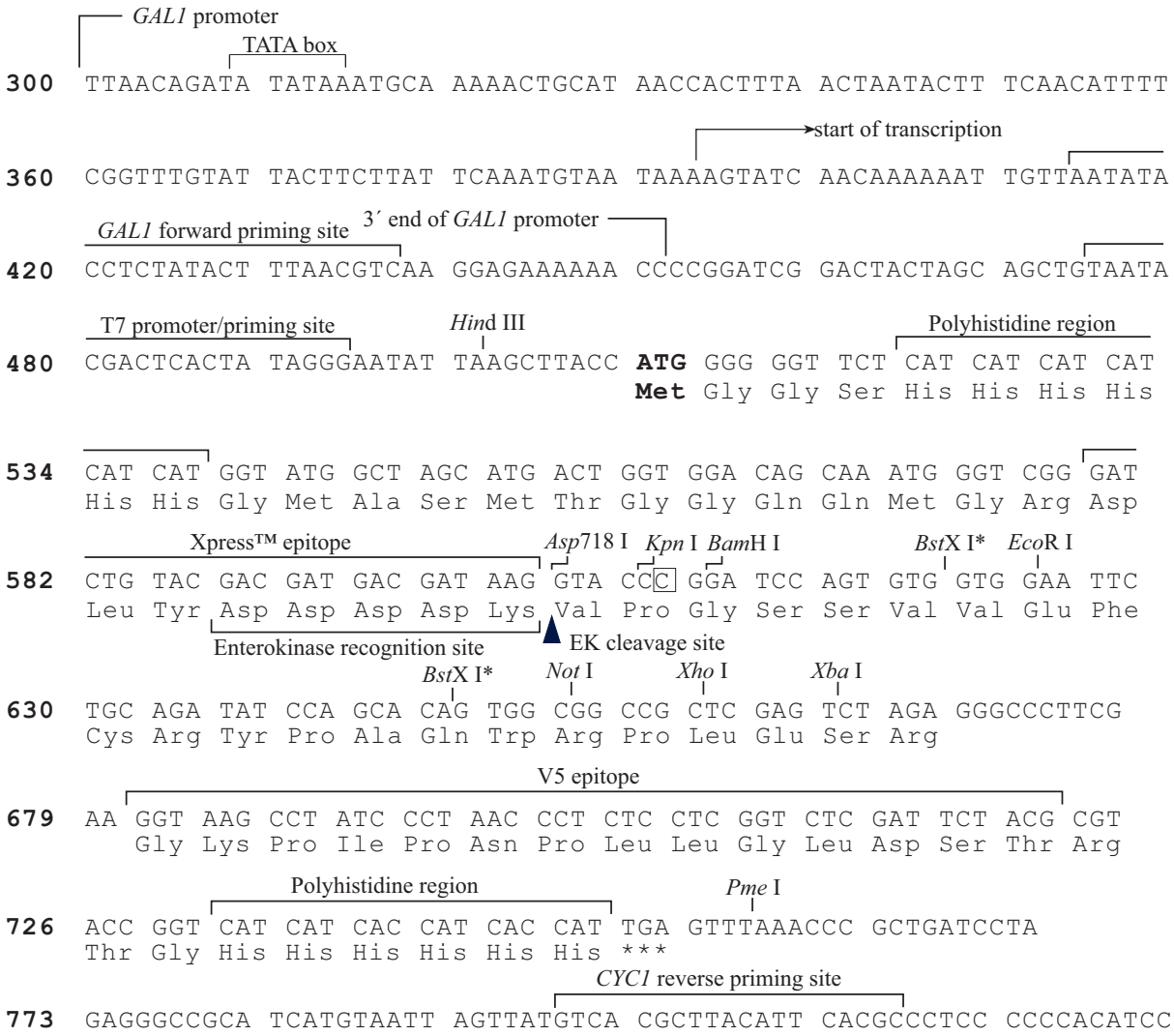
Multiple Cloning Site of pYES2/NT C and pYC2/NT C

The following diagram shows the multiple cloning site for pYES2/NT C and pYC2/NT C. Features of the *GAL1* promoter are marked as previously described (Giniger *et al.*, 1985; Johnston and Davis, 1984; Yocum *et al.*, 1984). Restriction sites are labeled to indicate the cleavage site. The boxed nucleotide indicates the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. The complete sequences of pYES2/NT C and pYC2/NT C are available from **www.lifetechnologies.com** or by contacting Technical Support (see page 32). For a map and description of the features of pYES2/NT and pYC2/NT, refer to pages 21–24 in the **Appendix**.



*Note that there are two *BstX* I sites in the polylinker.

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E. coli Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain of your choice. Select for transformants on LB plates containing 50 to 100 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 N-terminal polyhistidine (6xHis) tag and the Xpress™ epitope. To sequence your construct we suggest using either the *GAL1* Forward or the T7 Promoter primer sequences along with the *CYC1* Reverse primer sequence. Refer to the diagrams on pages 8–10 for the sequences and location of the priming sites.

For your convenience, Life Technologies offers the T7 Promoter primer (see page 31 for ordering information) as well as a custom primer service. For more information, visit **www.lifetechnologies.com** or call Technical Support (see page 32).

Prepare a Glycerol Stock

After identifying 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.

- Streak the original colony out on an LB plate containing 50 µg/mL ampicillin. Incubate the plate at 37°C overnight.
- Isolate a single colony and inoculate into 1–2 mL of LB containing 50 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.

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 PureLink® HiPure Miniprep Kit or the PureLink® HiPure Midiprep Kit (see page 31 for ordering information).

Yeast Transformation

Introduction

In this section, you will use a small-scale yeast transformation protocol to transform your pYES2/NT or pYC2/NT construct into the INVSc1 yeast host strain included with each vector. After transformation, expression of your recombinant fusion protein from pYES2/NT or pYC2/NT can be induced using galactose.

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.

Genotype/ Phenotype of INVSc1

The genotype and phenotype of the INVSc1 host strain are:

Genotype: *his3Δ1/his3Δ1 leu2/leu2 trp1-289/trp1-289 ura3-52/ura3-52*

Phenotype: His^-, Leu^-, Trp^-, Ura^-

Note that INVSc1 is a **diploid** strain that is auxotrophic for histidine, leucine, tryptophan, and uracil. The strain will not grow in SC minimal medium that is deficient in histidine, leucine, tryptophan, or uracil. A recipe for preparation of SC minimal medium is provided in the **Appendix** (see page 18).

The INVSc1 strain is a suitable strain to use for expression purposes, but should not be used for genetic analyses because it does not sporulate well.

Initiate INVSc1 Culture

To initiate a culture of INVSc1 from the stab provided with the kit, streak a small amount from the stab on a Yeast Extract Peptone Dextrose Medium (YPD) plate (see **Appendix** for recipe, page 19) and incubate at 30°C. After growth is established, you may check the phenotype of the strain by streaking a single colony on an SC minimal plate supplemented with the appropriate amino acids. INVSc1 will not grow in SC minimal medium that is deficient in histidine, leucine, tryptophan, or uracil.

Be sure to make glycerol stocks of the strain. Store glycerol stocks at −80°C. If you plan to use the strain directly from plates, be sure that the plates are less than 4 days old.

Positive Control

The pYES2/NT and pYC2/NT vectors are supplied with a corresponding positive control vector (pYES2/NT/*lacZ* and pYC2/NT/*lacZ*, respectively) to help you optimize expression conditions for your protein. The gene encoding β-galactosidase is expressed in yeast cells under the control of the *GAL1* promoter. Successful transformation and galactose induction will result in β-galactosidase expression that can be easily assayed.

Continued on next page

Yeast Transformation, Continued

Assay for β-galactosidase Activity

You may assay for β-galactosidase expression by activity assay using cell-free lysates (Miller, 1972). Life Technologies offers the β-Gal Assay Kit (see page 31 for ordering information) for fast and easy detection of β-galactosidase expression.

Reagents for Yeast Transformation

Many protocols are suitable for the preparation of competent INVSc1 yeast cells. The *S. c.* EasyComp™ Kit (see page 31 for ordering information) provides a quick and easy method for the preparation of 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.

A small-scale yeast transformation protocol is included in the **Appendix** (see page 27) for your convenience. Alternatively, there are published references for other small-scale transformation methods (Gietz *et al.*, 1992; Gietz *et al.*, 1995; Hill *et al.*, 1991; Schiestl and Gietz, 1989).

Yeast Transformation

Use one of the methods described above (or one of your own choosing) to transform your pYES2/NT or pYC2/NT plasmid construct into competent INVSc1. We recommend that you include the appropriate control vector (see the previous page) as a positive control for expression and a sample with no DNA as a negative control for transformation.

Select for transformants on SC-U (SC minimal media lacking uracil) selective plates (see the **Appendix**, page 18 for a recipe). Transformants should exhibit uracil prototrophy. After identifying a transformant, be sure to purify the colony and make a glycerol stock for long-term storage.

Maintain Transformants

Maintain yeast cells containing your pYES2/NT or pYC2/NT construct in SC-U medium containing 2% glucose or 2% raffinose. See the **Appendix**, page 18 for a recipe for SC-U medium.

Note: The growth rate of yeast strains varies with the carbon source. Typically, yeast strains exhibit the fastest growth in medium containing glucose.

Expression of Recombinant Protein

Introduction

After obtaining a transformant containing your pYES2/NT or pYC2/NT construct, you are ready to induce expression of your recombinant fusion protein of interest. This section provides information on how to induce and assay for expression of your protein of interest.

GAL1 Promoter

In typical *S. cerevisiae* laboratory strains (i.e. INVSc1), transcription from the *GAL1* promoter is repressed in the presence of glucose (West *et al.*, 1984). Transcription may be induced by removing glucose and adding galactose as a carbon source (Giniger *et al.*, 1985). Maintaining cells in glucose gives the most complete repression and the lowest basal transcription of the *GAL1* promoter. Transferring cells from glucose- to galactose-containing medium causes the *GAL1* promoter to become de-repressed and allows transcription to be induced.

Alternatively, cells may be maintained in medium containing raffinose as a carbon source. The presence of raffinose does not repress or induce transcription from the *GAL1* promoter. Adding galactose to the medium induces transcription from the *GAL1* promoter even in the presence of raffinose. Inducing the *GAL1* promoter by galactose is more rapid in cells maintained in raffinose when compared to those maintained in glucose.

You may choose to grow cells containing your pYES2/NT or pYC2/NT construct in glucose or raffinose depending on how quickly you want to obtain your expressed protein after induction with galactose and on the toxicity of the expressed protein. For more information about expression in yeast, refer to the *Guide to Yeast Genetics and Molecular Biology* (Guthrie and Fink, 1991).

For a protocol to induce expression of your fusion protein with galactose, proceed to **Time Course of Protein Induction by Galactose** on the next page.

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Time Course of Protein Induction by Galactose

To induce expression of your protein of interest from the *GAL1* promoter, add galactose to the medium. For cells that have been maintained in glucose, recombinant fusion protein can be detected in as little as 4 hours after galactose induction. Recombinant fusion protein can be detected in cells that have been cultured in raffinose by 2 hours after galactose induction.

If you are assaying for expression of your recombinant fusion protein for the first time, we recommend that you perform a time course to optimize expression of your recombinant protein (e.g. 0, 4, 8, 12, 16, 24 hours after galactose induction). A standard protocol is provided to perform a time course experiment. Other protocols are suitable.

1. Inoculate a single colony of INVSc1 containing your pYES2/NT or pYC2/NT construct into 15 mL of SC-U medium containing 2% glucose or 2% raffinose. Grow overnight at 30°C with shaking.
2. Determine the OD_{600} of your overnight culture. Calculate the amount of overnight culture necessary to obtain an OD_{600} of 0.4 in 50 mL of *induction medium* (SC-U medium containing 2% galactose).

   Example: Assume that the OD_{600} of an overnight culture is 3 OD_{600} per mL. Then, the amount of overnight culture needed to inoculate a 50 mL culture to OD_{600} = 0.4 is

   $$\frac{(0.4\ \text{OD/mL})\ (50\ \text{mL})}{3\ \text{OD/mL}} = 6.67\ \text{mL}$$
3. Remove the amount of overnight culture as determined in Step 2 and pellet the cells at 1500 x g for 5 minutes at 4°C. Discard the supernatant.
4. Resuspend the cells in 50 mL of *induction medium*. See the **Appendix**, page 18 for a recipe for *induction medium*. Grow at 30°C with shaking.
5. Harvest an aliquot of cells at 0, 4, 8, 12, 16, and 24 hours after addition of cells to the *induction medium*. For each time point, remove 5 mL of culture from the flask and determine the OD_{600} of each sample. You will use this information when assaying for your recombinant fusion protein (see Step 3 on the next page).
6. Centrifuge the cells at 1500 × *g* for 5 minutes at 4°C.
7. Decant the supernatant. Resuspend cells in 500 µL of sterile water.
8. Transfer cells to a sterile microcentrifuge tube. Centrifuge samples for 30 seconds at top speed in the microcentrifuge.
9. Remove the supernatant.
10. Store the cell pellets at −80°C until ready to use. Proceed to the next section to prepare cell lysates to detect your recombinant protein (see page 16).

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Detect Recombinant Fusion Protein

To detect expression of your recombinant fusion protein by Western blot (see the following protocol), you may use the Anti-Xpress™ antibodies or the Anti-HisG antibodies (see page 31 for ordering information) or an antibody to your protein of interest. If your recombinant protein also contains the C-terminal peptide, you may use the Anti-V5 antibodies or the Anti-His(C-term) antibodies available from Life Technologies for detection (see page 31 for ordering information).

You will also need to prepare a cell lysate from your yeast transformant. A general protocol for small-scale preparation of cell lysates using acid-washed glass beads is provided for your convenience. Other protocols are suitable. Refer to *Current Protocols in Molecular Biology*, Unit 13.13 (Ausubel *et al.*, 1994) for more information. For large-scale preparations (culture volumes over 1 liter), see **Scale-up** on page 17.

Materials Needed:

- Breaking buffer:
  - 50 mM sodium phosphate, pH 7.4, (see page, page 19 for recipe of the stock buffer)
  - 1 mM EDTA (omit EDTA if using this buffer for purification on metal-chelating resins)
  - 5% glycerol
  - 1 mM PMSF
- Acid-washed glass beads (0.4–0.6 mm size; Sigma-Aldrich, Catalog no. G8772)

Protocol:

1. You may prepare cell lysates from either frozen cells or fresh cells.

   Reminder: You will need to know the OD_{600} of your cell sample(s) before beginning (see Step 5, page 15).
2. Resuspend fresh or frozen cell pellets in 500 µL of breaking buffer. Centrifuge at 1500 × g for 5 minutes at 4°C to pellet cells.
3. Remove supernatant and resuspend the cells in a volume of breaking buffer to obtain an OD_{600} of 50–100. Use the OD_{600} determined in Step 5, page 15, to calculate the appropriate volume of breaking buffer to use.
4. Add an equal volume of acid-washed glass beads.
5. Vortex mixture for 30 seconds, followed by 30 seconds on ice. Repeat four times for a total of 4 minutes to lyse the cells. Cells will be lysed by shear force. You can check for the extent of lysis by checking a small aliquot under the microscope.
6. Centrifuge in a microcentrifuge for 10 minutes at maximum speed.
7. Remove supernatant and transfer to a fresh microcentrifuge tube. Assay the lysate for protein concentration using BSA as a standard.
8. Add SDS-PAGE sample buffer to a final concentration of 1X and boil the sample for 5 minutes.
9. Load 20 µg of lysate onto an SDS-PAGE gel and electrophorese. Use the appropriate percentage of acrylamide to resolve your recombinant protein.

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Note

The N-terminal peptide containing the Xpress™ epitope and the polyhistidine (6xHis) tag will add ~3.4 kDa to the size of your protein. If you have also cloned your protein in frame with the C-terminal peptide, the C-terminal peptide containing the V5 epitope and the C-terminal polyhistidine tag will add ~5 kDa to the size of your protein.

Scale-up of Expression for Purification

After determining the optimal induction time necessary to obtain maximal protein expression, you may increase the protein yield by scaling up the procedure described on page 15. If you plan to use ProBond™ resin to purify your recombinant fusion protein, see the following **Note**. To prepare cell lysates from culture volumes over 1 liter, we recommend that you use a bead beater (Biospec Products, Bartlesville, OK) to lyse the cells. Refer to *Current Protocols in Molecular Biology*, Unit 13.13 (Ausubel *et al.*, 1994) for a suitable protocol to lyse cells with a bead beater.

Note

When purifying recombinant protein using ProBond™ resin, note that the largest culture volume that can be used with the 2 mL prepacked columns included in the ProBond™ Protein Purification System is 50 mL of cells. If you need to purify recombinant protein from larger culture volumes, you may need more ProBond™ resin (e.g. bulk ProBond™ resin). See page 31 for ordering information.

If you are using the breaking buffer (see page 16) for purification of your recombinant protein on ProBond™, do not include EDTA in this buffer, as it will interfere with the binding of proteins on ProBond™.

Purification

For help with purification of your recombinant fusion protein, refer to the ProBond™ Protein Purification System manual. This manual is available from **www.lifetechnologies.com/manuals** or by contacting Technical Support (see page 32).

If you are using another type of resin, refer to the manufacturer's recommendations.

Appendix

Recipes

SC Minimal Medium and Plates

SC is synthetic minimal defined medium for yeast.

0.67% yeast nitrogen base (**without** amino acids but **with** ammonium sulfate)

2% carbon source (i.e. glucose or raffinose)

0.01% (adenine, arginine, cysteine, leuine, 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 (800 mL if preparing medium containing raffinose). **Note:** We recommend that you make medium and plates as needed and weigh out each amino acid. Many researchers prepare 100X solutions of each amino acid that they need.

Reminder: Omit uracil to make selective plates for growing pYES2/NT or pYC2/NT transformants.

6.7 g Yeast Nitrogen Base	**0.1 g each**	**0.05 g each**
	adenine	aspartic acid
	arginine	histidine
	cysteine	isoleucine
	leucine	methionine
	lysine	phenylalanine
	threonine	proline
	tryptophan	serine
	uracil (U)	tyrosine
		valine

2. If you are making plates, add the agar after dissolving the reagents listed in the preceding table.
3. Autoclave at 15 psi, 121°C for 20 minutes.
4. Cool to 50°C and add 100 mL of filter-sterilized 20% glucose or 200 mL of filter-sterilized 10% raffinose.
5. Pour plates and allow to harden. Invert the plates and store at 4°C. Plates are stable for 6 months.

Induction Medium

If you are making induction medium, follow Steps 1–3 in the preceding section, except dissolve the reagents in 800 mL of deionized water. Cool the medium to 50°C and add 100 mL of filter-sterilized 20% galactose and 100 mL of filter-sterilized 10% raffinose to the medium.

Continued on next page

When making stock solutions of raffinose, do not autoclave the stock solution. Autoclaving the solution will convert the raffinose to glucose. Filter-sterilize the stock solution.

YPD

Yeast Extract Peptone Dextrose Medium (YPD) (1 L)

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 (900 mL volume) has been autoclaved, add 100 mL of 20% dextrose to the medium.

0.1 M Sodium Phosphate, pH 7.4

Before beginning, have the following reagents on hand.

Sodium phosphate, monobasic ($NaH_2PO_4 \cdot H_2O$; Sigma-Aldrich S9638)
Sodium phosphate, dibasic ($Na_2HPO_4 \cdot 7H_2O$; Sigma-Aldrich S9390)

1. Prepare 100 mL of 1 M $NaH_2PO_4 \cdot H_2O$ by dissolving 13.8 g in 90 mL of deionized water. Bring volume up to 100 mL. Filter-sterilize.
2. Prepare 100 mL of 1 M $Na_2HPO_4 \cdot 7H_2O$ by dissolving 26.81 g in 90 mL of deionized water. Bring volume up to 100 mL. Filter-sterilize.
3. For 1 liter of 0.1 M sodium phosphate, pH 7.4, mix together 22.6 mL of 1 M NaH_2PO_4 and 77.4 mL of 1 M Na_2HPO_4. Bring the volume up to 1 liter with sterile water.
4. Filter-sterilize and store at room temperature.

Continued on next page

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.

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

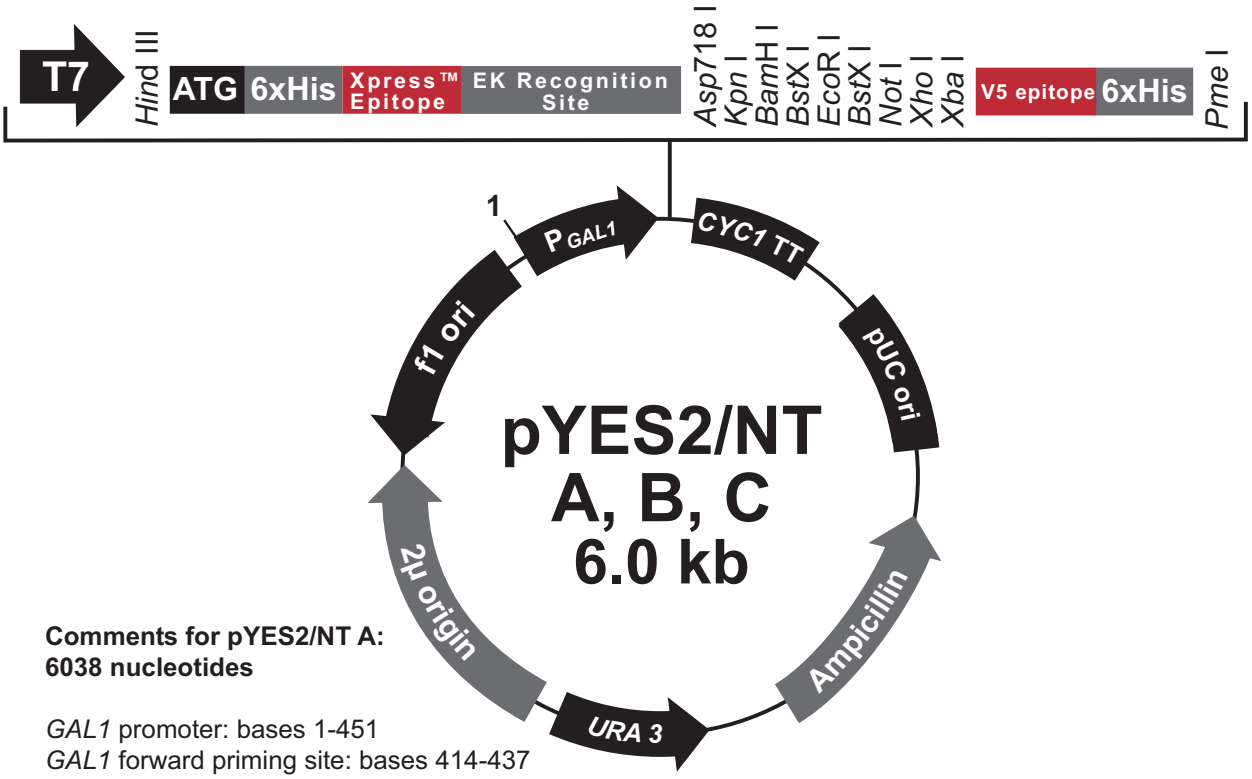
100 mM Lithium Acetate, pH 7.5
40% PEG-3350
10 mM Tris-HCl, pH 7.5
1 mM EDTA

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.

pYES2/NT Vector

Map of pYES2/NT

The following figure summarizes the features of the pYES2/NT vectors. The complete sequences for pYES2/NT A, B, and C are available from **www.lifetechnologies.com** or by contacting Technical Support (see page 32).



Comments for pYES2/NT A:
6038 nucleotides

GAL1 promoter: bases 1-451
GAL1 forward priming site: bases 414-437
T7 promoter/priming site: bases 475-494
ATG initiation codon: bases 510-512
Polyhistidine (6xHis) region: bases 522-539
Xpress™ epitope: bases 579-602
Enterokinase (EK) recognition site: bases 588-602
Multiple cloning site: bases 602-669
V5 epitope: bases 682-723
Polyhistidine (6xHis) region: bases 733-750
CYC1 transcription termination signal: 783-1036
CYC1 reverse priming site: bases 800-818
pUC origin: bases 1220-1893
Ampicillin resistance gene: bases 2038-2898 (complementary strand)
URA3 gene: bases 2916-4023 (complementary strand)
2µ origin: bases 4027-5498
f1 origin: bases 5566-6021 (complementary strand)

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Features of pYES2/NT

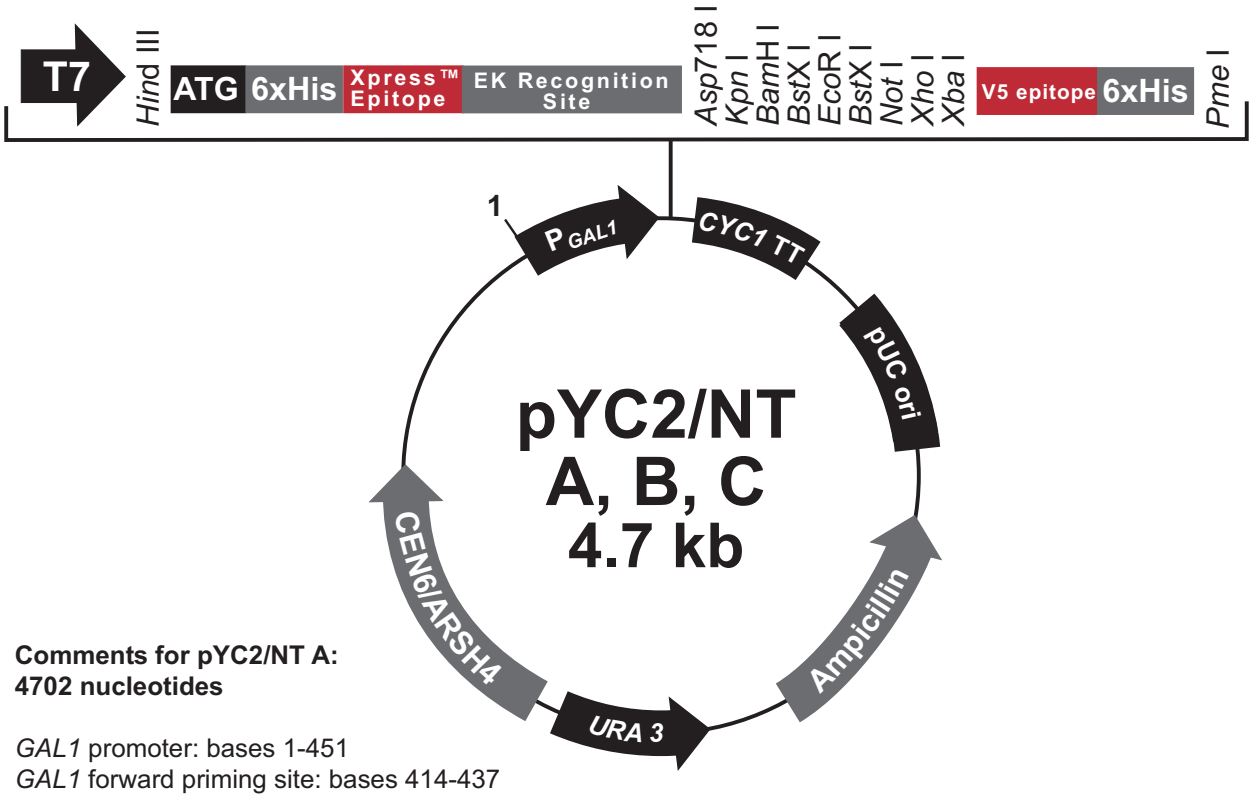
pYES2/NT A (6038 bp), pYES2/NT B (6039 bp), and pYES2/NT C (6037 bp) contain the following elements. All features have been functionally tested.

Feature	Benefit
GAL1 promoter	Permits galactose-inducible expression of genes cloned into pYES2/NT (West *et al.*, 1984).
GAL1 forward priming site	Allows sequencing through the insert.
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation and sequencing through the insert.
N-terminal polyhistidine (6xHis) tag	Permits purification of your recombinant protein on metal-chelating resin such as ProBond™. In addition, the N-terminal polyhistidine tag is the epitope for the Anti-HisG Antibody (Catalog no. R940-25) and the Anti-HisG-HRP Antibody (Catalog no. R941-25).
Xpress™ epitope	Allows detection of your recombinant protein with the Anti-Xpress™ Antibody (Catalog no. R910-25) or the Anti-Xpress™-HRP Antibody (Catalog no. R911-25).
Enterokinase cleavage site	Permits removal of the N-terminal tag from your recombinant protein using an enterokinase such as EnterokinaseMax™ (Catalog no. E180-01).
Multiple cloning site in three reading frames	Allows insertion of your gene and facilitates cloning in frame with the Xpress™ epitope and N-terminal polyhistidine tag
V5 epitope	Permits detection of the fusion protein with the Anti-V5 Antibody (Catalog no. R960-25) or the Anti-V5-HRP Antibody (Catalog no. R961-25) (Southern *et al.*, 1991).
C-terminal polyhistidine (6xHis) tag	Permits purification of your fusion protein on metal-chelating resin such as ProBond™. In addition, the C-terminal polyhistidine tag is the epitope for the Anti-His(C-term) Antibody (Catalog no. R930-25) and the Anti-His(C-term)-HRP Antibody (Catalog no. R931-25) (Lindner *et al.*, 1997).
CYC1 transcription termination signal	Permits efficient termination and stabilization of mRNA.
CYC1 reverse priming site	Allows sequencing through the insert.
pUC origin	Allows maintenance and high copy replication in *E. coli.*
Ampicillin resistance gene	Allows selection of transformants in *E. coli.*
URA3 gene	Allows selection of yeast transformants in uracil-deficient medium.
2µ origin	Permits episomal maintenance and high copy replication in yeast.
f1 origin	Allows rescue of single-stranded DNA.

pYC2/NT Vector

Map of pYC2/NT

The following figure summarizes the features of the pYC2/NT vectors. The complete sequences for pYC2/NT A, B, and C are available from **www.lifetechnologies.com** or by contacting Technical Support (see page 32).



Comments for pYC2/NT A:
4702 nucleotides

GAL1 promoter: bases 1-451
GAL1 forward priming site: bases 414-437
T7 promoter/priming site: bases 475-494
ATG initiation codon: bases 510-512
Polyhistidine (6xHis) region: bases 522-539
Xpress™ epitope: bases 579-602
Enterokinase (EK) recognition site: bases 588-602
Multiple cloning site: bases 602-669
V5 epitope: bases 682-723
Polyhistidine (6xHis) region: bases 733-750
CYC1 transcription termination signal: 783-1036
CYC1 reverse priming site: bases 800-818
pUC origin: bases 1220-1893
Ampicillin resistance gene: bases 2038-2898 (complementary strand)
URA3 gene: bases 2916-4023 (complementary strand)
CEN6/ARSH4: bases 4036-4554

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Features of pYC2/NT

pYC2/NT A (4702 bp), pYC2/NT B (4703 bp), and pYC2/NT C (4701 bp) contain the following elements. All features have been functionally tested.

Feature	Benefit
GAL1 promoter	Permits galactose-inducible expression of genes cloned into pYC2/NT (West *et al.*, 1984).
GAL1 forward priming site	Allows sequencing through the insert.
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation and sequencing through the insert.
N-terminal polyhistidine (6xHis) tag	Permits purification of your recombinant protein on metal-chelating resin such as ProBond™. In addition, the N-terminal polyhistidine tag is the epitope for the Anti-HisG Antibody (Catalog no. R940-25) and the Anti-HisG-HRP Antibody (Catalog no. R941-25).
Xpress™ epitope	Allows detection of your recombinant protein with the Anti-Xpress™ Antibody (Catalog no. R910-25) or the Anti-Xpress™-HRP Antibody (Catalog no. R911-25).
Enterokinase cleavage site	Permits removal of the N-terminal tag from your recombinant protein using an enterokinase such as EnterokinaseMax™ (Catalog no. E180-01).
Multiple cloning site in three reading frames	Allows insertion of your gene and facilitates cloning in frame with the Xpress™ epitope and N-terminal polyhistidine tag.
V5 epitope	Permits detection of the fusion protein with the Anti-V5 Antibody (Catalog no. R960-25) or the Anti-V5-HRP Antibody (Catalog no. R961-25) (Southern *et al.*, 1991).
C-terminal polyhistidine (6xHis) tag	Permits purification of your fusion protein on metal-chelating resin such as ProBond™. In addition, the C-terminal polyhistidine tag is the epitope for the Anti-His(C-term) Antibody (Catalog no. R930-25) and the Anti-His(C-term)-HRP Antibody (Catalog no. R931-25) (Lindner *et al.*, 1997).
CYC1 transcription termination signal	Permits efficient termination and stabilization of mRNA.
CYC1 reverse priming site	Allows sequencing through the insert.
pUC origin	Allows maintenance and high copy replication in *E. coli.*
Ampicillin resistance gene	Permits selection of transformants in *E. coli.*
URA3 gene	Allows selection of yeast transformants in uracil-deficient medium.
CEN6/ARSH4 sequence	Permits non-integrative centromeric maintenance and low copy replication in yeast (Sikorski and Hieter, 1989).

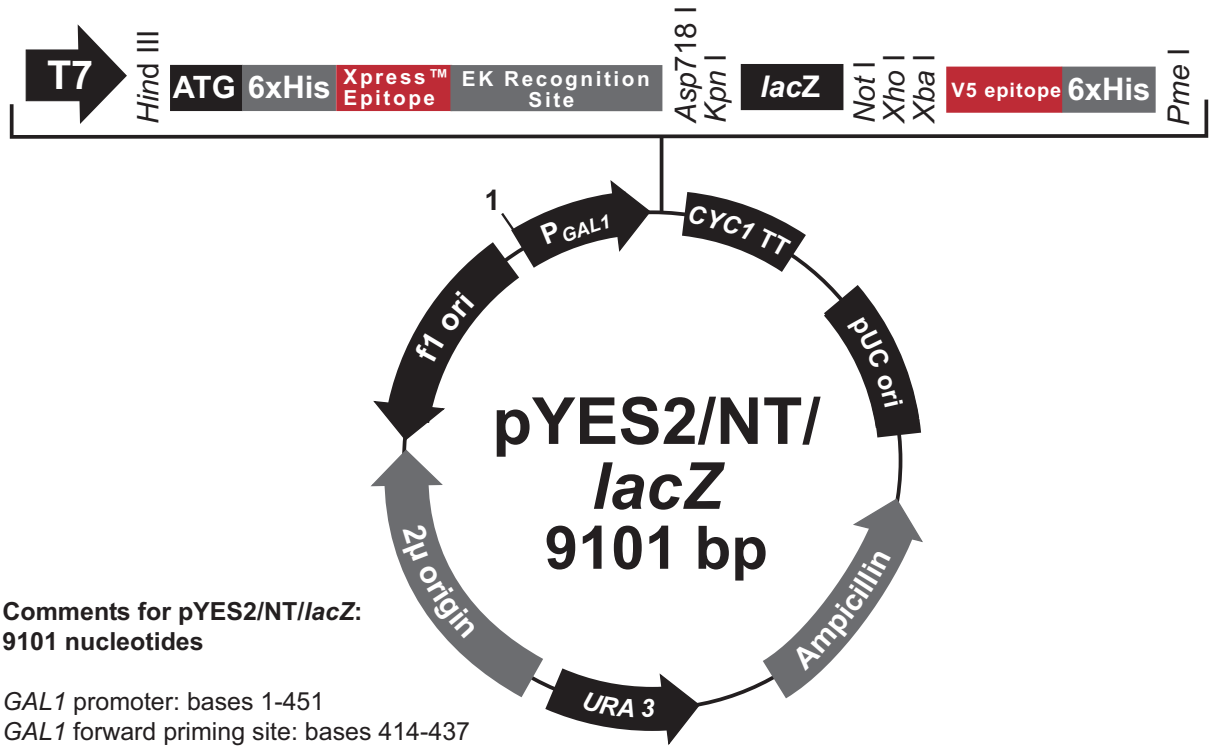
pYES2/NT/*lacZ*

Description

pYES2/NT/*lacZ* is a 9101 bp control vector containing the gene for β-galactosidase. This vector was constructed by ligating a 3.1 kb fragment containing the *lacZ* gene into the *Kpn* I-*Xho* I site of pYES2/NT B. Note that the *lacZ* gene is cloned in frame with the N-terminal fusion tag, but **not** with the C-terminal fusion tag.

Map of pYES2/NT/*lacZ*

The following figure summarizes the features of the pYES2/NT/*lacZ* vector. The complete nucleotide sequence for pYES2/NT/*lacZ* is available from **www.lifetechnologies.com** or by contacting Technical Support (see page 32).



Comments for pYES2/NT/*lacZ*: 9101 nucleotides

GAL1 promoter: bases 1-451
GAL1 forward priming site: bases 414-437
T7 promoter/priming site: bases 475-494
ATG initiation codon: bases 510-512
Polyhistidine (6xHis) region: bases 522-539
Xpress™ epitope: bases 579-602
Enterokinase (EK) recognition site: bases 588-602
LacZ ORF: bases 627-3677
V5 epitope: bases 3745-3786
Polyhistidine (6xHis) region: bases 3796-3813
CYC1 transcription termination signal: bases 3846-4099
CYC1 reverse priming site: bases 3863-3881
pUC origin: bases 4283-4956
Ampicillin resistance gene: bases 5101-5961 (complementary strand)
URA3 gene: bases 5979-7086 (complementary strand)
2µ origin: bases 7090-8561
f1 origin: bases 8629-9084 (complementary strand)

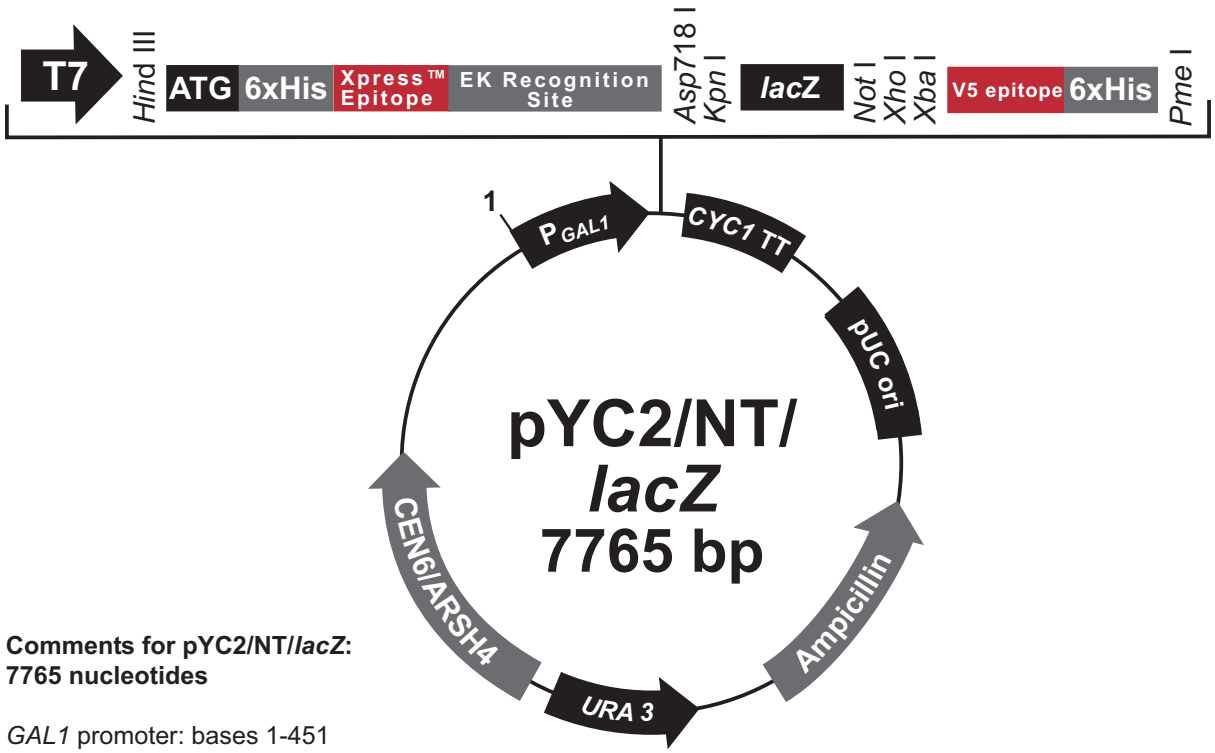
pYC2/NT/*lacZ*

Description

pYC2/NT/*lacZ* is a 7765 bp control vector containing the gene for β-galactosidase. This vector was constructed by ligating a 3.1 kb fragment containing the *lacZ* gene into the *Kpn* I-*Xho* I site of pYC2/NT B. Note that the *lacZ* gene is cloned in frame with the N-terminal fusion tag, but **not** with the C-terminal fusion tag.

Map of pYC2/NT/*lacZ*

The following figure summarizes the features of the pYC2/NT/*lacZ* vector. The complete nucleotide sequence for pYC2/NT/*lacZ* is available from **www.lifetechnologies.com** or by contacting Technical Support (see page 32).



Comments for pYC2/NT/*lacZ*: 7765 nucleotides

GAL1 promoter: bases 1-451
GAL1 forward priming site: bases 414-437
T7 promoter/priming site: bases 475-494
ATG initiation codon: bases 510-512
Polyhistidine (6xHis) region: bases 522-539
Xpress™ epitope: bases 579-602
Enterokinase (EK) recognition site: bases 588-602
LacZ ORF: bases 627-3677
V5 epitope: bases 3745-3786
Polyhistidine (6xHis) region: bases 3796-3813
CYC1 transcription termination signal: bases 3846-4099
CYC1 reverse priming site: bases 3863-3881
pUC origin: bases 4283-4956
Ampicillin resistance gene: bases 5101-5961 (complementary strand)
URA3 gene: bases 5979-7086 (complementary strand)
CEN6/ARSH4: bases 7099-7617

Small-Scale Yeast Transformation

Introduction

A small-scale yeast transformation protocol for routine transformations is provided in the following sections. Other protocols are suitable.

Materials Needed

- YPD liquid medium
- 1X TE (see **Recipe**, page 20)
- 1X LiAc/0.5X TE (see **Recipe**, page 20)
- Denatured salmon sperm DNA (see the recipe page 28)
- pYES2/NT or pYC2/NT vector construct (or other plasmid DNA to be transformed)
- 1X LiAc/40% PEG-3350/1X TE (See **Recipe**, page 20)
- DMSO
- Selective plates

Protocol

1. Inoculate 10 mL of YPD medium with a colony of INVSc1 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 1500 × g and resuspend the pellet in 40 mL 1X TE.
4. Pellet the cells at 1500 × g and resuspend the 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 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 x 10^7 yeast cells.

Prepare 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 is also suitable.

Materials Needed

- 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

Continued on next page

Prepare Denatured Salmon Sperm DNA, Continued

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 pipette to dissolve completely.
2. Incubate overnight at 4°C.
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 × 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 phenol:chloroform:isoamyl alcohol (25:24:1). Centrifuge at 10,000 × 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 × 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 × 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. 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.

Accessory Products

Introduction

The products listed in the following table are designed to help you detect and purify your recombinant fusion protein expressed from pYES2/NT or pYC2/NT. In addition, Life Technologies has a wide variety of yeast expression vectors that can be used with pYES2/NT or pYC2/NT to express and detect multiple proteins in the same cell (see page 31).

Detect Recombinant Protein

Once cloned into pYES2/NT or pYC2/NT, expression of your recombinant protein can be detected using an antibody to the protein itself or to the appropriate epitope. If you do not have an antibody to your protein, Life Technologies offers the Anti-Xpress™ antibodies and the Anti-HisG antibodies to detect your recombinant protein as an N-terminal fusion. In addition, the Anti-V5 antibodies and the Anti-His(C-term) antibodies are available for detecting C-terminal fusion proteins, if needed. Horseradish peroxidase (HRP)-conjugated antibodies allow one-step detection using colorimetric or chemiluminescent detection methods.

Antibody	Epitope	Catalog no.
Anti-Xpress™	Detects 8 amino acid Xpress™ epitope: DLYDDDDK	R910-25
Anti-Xpress™-HRP		R911-25
Anti-HisG	Detects the N-terminal polyhistidine (6xHis) tag followed by glycine: HHHHHHG	R940-25
Anti-HisG-HRP		R941-25
Anti-V5	Detects 14 amino acid epitope derived from the P and V proteins of the paramyxovirus, SV5 (Southern *et al.*, 1991): GKPIPNPLLGLDST	R960-25
Anti-V5-HRP		R961-25
Anti-His(C-term)	Detects the C-terminal polyhistidine tag (requires the free carboxyl group for detection) (Lindner *et al.*, 1997): HHHHHH-COOH	R930-25
Anti-His(C-term)-HRP		R931-25

Continued on next page

Purify Recombinant Protein

The metal binding domain encoded by the polyhistidine tag allows easy purification of your recombinant protein by Immobilized Metal Affinity Chromatography (IMAC) using ProBond™ Resin (see the following table). To purify proteins expressed using pYES2/NT or pYC2/NT, the ProBond™ Protein Purification System is available separately. Additional ProBond™ resin is available in bulk. See the following table for ordering information.

Product	Quantity	Catalog no.
ProBond™ Protein Purification System	6 purifications	K850-01
ProBond™ Protein Purification System with Anti-Xpress™ Antibody	1 Kit	K851-01
ProBond™ Protein Purification System with Anti-V5-HRP Antibody	1 Kit	K854-01
ProBond™ Protein Purification System with Anti-His(C-term)-HRP Antibody	1 Kit	K853-01
ProBond™ Metal-Binding Resin (precharged resin provided as a 50% slurry in 20% ethanol)	50 mL	R801-01
	150 mL	R801-15
Purification Columns (10 ml polypropylene columns)	50 each	R640-50

Additional Products

Additional reagents that may be used with the pYES2/NT and pYC2/NT vectors are available. Ordering information is provided in the following table.

Product	Amount	Catalog no.
S.c. EasyComp™ Kit	20 reactions	K5050-01
T7 Promoter Primer	2 µg	N560-02
PureLink® HiPure Plasmid Miniprep Kit	100 preps	K2100-03
PureLink® HiPure Plasmid Midiprep Kit	25 preps	K2100-04
One Shot® TOP10 (chemically competent cells)	21 × 50 µL	C4040-03
Electrocomp™ TOP10 (electrocompetent cells)	20 reactions	C664-55
EKMax™	250 units	E180-01
ß-Gal Assay Kit	80 mL	K1455-01

Other Yeast Expression Vectors

Life Technologies has a wide variety of yeast expression (YES™) vectors utilizing the *GAL1* promoter. Vectors are available with the Xpress™ (N-terminal) or V5 (C-terminal) epitope for detection, the 2µ origin or CEN6/ARSH4 sequence for high copy or low copy replication, and either dominant or auxotrophic markers for selection in yeast. All vectors contain a polyhistidine tag for purification of recombinant protein using ProBond™ resin. For more information on the YES™ expression vectors available, see **www.lifetechnologies.com** or call Technical Support (see page 32).

Technical Support

Obtaining support

For the latest services and support information for all locations, go to **www.lifetechnologies.com/support**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288
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