invitrogen™

# pYES2/CT, pYES3/CT, and pYC2/CT

## Yeast expression vectors with C-terminal tags and auxotrophic selection markers

**Catalog no. V8251-20, V8253-20, and V8255-20**

**Rev. date: 30 November 2009**

Manual part no. 25-0304

MAN0000128

User Manual

# Table of Contents

# Kit Contents and Storage

## Shipping and Storage

pYES2/CT, pYES3 and pYC2 vectors are shipped on wet ice. Upon receipt, store vectors at -20°C.

## Kit Contents

All vectors are supplied as detailed below. **Store the vectors at –20°C.**

| Cat. no. | Vector | Composition | Amount |
|---|---|---|---|
| V8251-20 | pYES2/CT | 40 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 | 20 µg |
| | pYES2/CT/*lacZ* | 40 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 | 20 µg |
| V8253-20 | pYES3/CT | 40 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 | 20 µg |
| | pYES3/CT/*lacZ* | 40 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 | 20 µg |
| V8255-20 | pYC2/CT | 40 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 | 20 µg |
| | pYC2/CT/*lacZ* | 40 µL of 0.5 µg/µL vector in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 | 20 µg |

**Note**

The INVSc1 Yeast Strain is included with each vector kit.

**Note**: For long-term storage of your stab, we recommend preparing a glycerol stock immediately upon receipt and storing at –80°C.

## Genotype/ Phenotype of INVSc1

The genotype and phenotype of the INVSc1 host strain are provided below.

**Genotype:** MAT**a** *his3Δ1 leu2 trp1-289 ura3-52*/MATα *his3Δ1 leu2 trp1-289 ura3-52*

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

## Preparing INVSc1 Glycerol Stocks

We recommend that you prepare a set of glycerol master stocks within two 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.

## Intended Use

**For research use only.** Not intended for any animal or human therapeutic or diagnostic use.

# Introduction

## Product Overview

### Description of the System

pYES2/CT, pYES3/CT, and pYC2/CT are 6.0 kb, 5.9 kb, and 4.6 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 13–20 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 9 for more information)
- Multiple cloning site (MCS) with 8 or 9 unique sites (plus two *Bst*X I sites) to facilitate in-frame cloning with the C-terminal peptide (see page 7 for more information)
- C-terminal peptide encoding the V5 epitope and a polyhistidine (6xHis) tag for detection and purification of your recombinant fusion protein
- 2µ origin for episomal maintenance and high copy replication (pYES2/CT and pYES3/CT) or CEN6/ARSH4 sequence for non-integrative centromeric maintenance and low copy replication (pYC2/CT)
- *URA3* or *TRP1* auxotrophic marker for selection of yeast transformants (see below)
- Ampicillin resistance gene for selection in *E. coli*

The table below summarizes the specific elements found in each vector.

| Vector | MCS | Auxotrophic Marker | Origin |
|---|---|---|---|
| pYES2/CT | 9 unique sites plus two *Bst*X I sites | *URA3* | 2µ |
| pYES3/CT | 8 unique sites plus two *Bst*X I sites | *TRP1* | 2µ |
| pYC2/CT | 9 unique sites plus two *Bst*X I sites | *URA3* | CEN6/ARSH4 |

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### Experimental Outline

The table below outlines the major steps required to clone and express your gene of interest in pYES2/CT, pYES3/CT, or pYC2/CT.

| Step | Action |
|---|---|
| 1 | Consult the multiple cloning site described on page 7 to determine a strategy to clone your gene in frame with the C-terminal peptide. |
| 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 C-terminal peptide. |
| 5 | Transform your construct into competent INVSc1 cells and select for the appropriate amino acid 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

## Cloning into pYES2/CT, pYES3/CT, or pYC2/CT

### General Molecular Biology Techniques

For help with DNA ligations, *E. coli* transformations, restriction enzyme analysis, 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 pYES2/CT, pYES3/CT, and pYC2/CT. We recommend that you propagate the 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 electrocompetent cells from Invitrogen (page 27).

### 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.

### Propagating and Maintaining Plasmids

To propagate and maintain the pYES2/CT, pYES3/CT, and pYC2/CT vectors, use a small amount of the supplied 0.5 µg/µL stock solution in TE, pH 8.0 to transform a *rec*A, *end*A *E. coli* strain like TOP10F´, DH5α, JM109, or equivalent. Select transformants on LB plates containing 50–100 µg/mL ampicillin. Be sure to prepare a glycerol stock of each plasmid for long term storage (see page 6).

### 2µ Origin

The pYES2/CT and pYES3/CT 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 a heterologous expression plasmid (i.e. pYES2/CT or pYES3/CT), 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).

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### CEN6/ARSH4 Sequence

The pYC2/CT vector contains 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 a heterologous expression plasmid (i.e. pYC2/CT), 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).

### Cloning Considerations

pYES and pYC 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)AATGTC(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.

To express your gene as a recombinant fusion protein, you must clone your gene in frame with the C-terminal peptide containing the V5 epitope and the polyhistidine (6xHis) tag. See the diagram on the next page to develop a cloning strategy. Note that pYES2/CT, pYES3/CT, and pYC2/CT possess the same multiple cloning site.

If you wish to express your protein WITHOUT the C-terminal peptide, be sure to include a stop codon.

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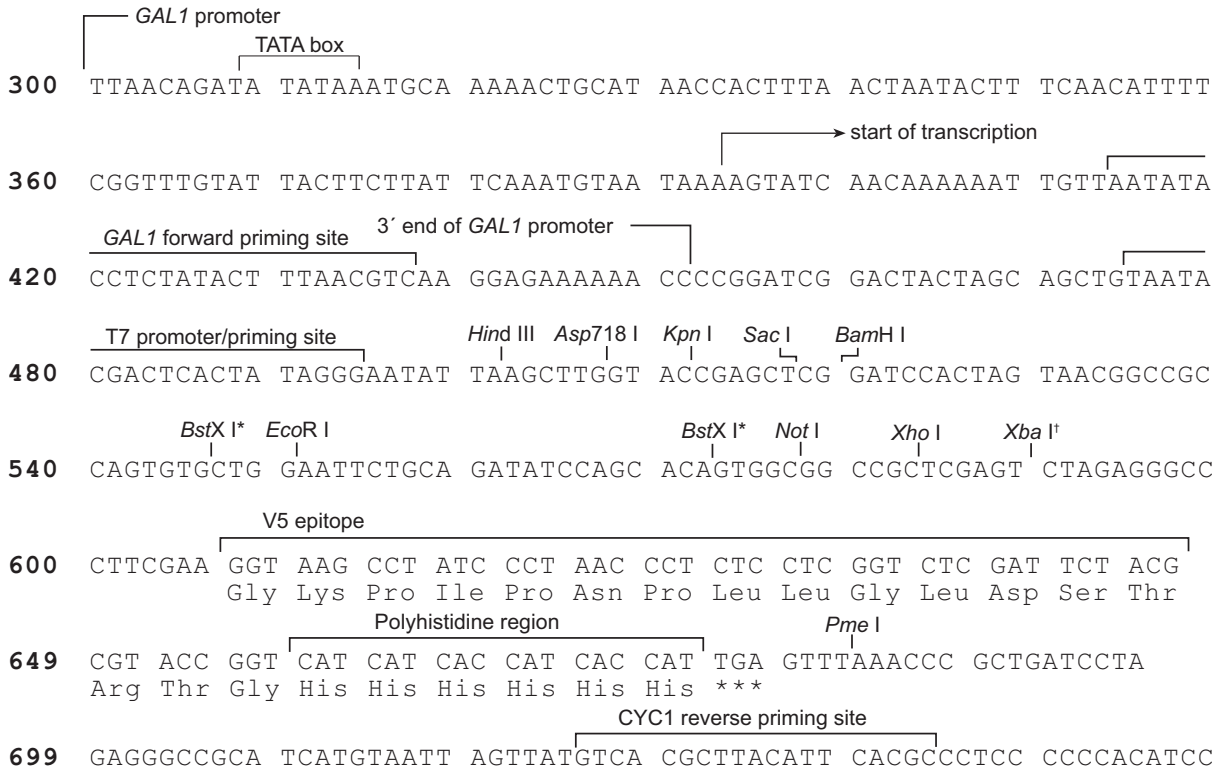
# Cloning into pYES2/CT, pYES3/CT, or pYC2/CT, Continued

## Multiple Cloning Site of pYES2/CT, pYES3/CT, and pYC2/CT

Below is a diagram of the multiple cloning site for pYES2/CT, pYES3/CT, and pYC2/CT. 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 multiple cloning site has been confirmed by sequencing and functional testing. The vector sequences of pYES2/CT, pYES3/CT, and pYC2/CT are available for downloading from www.invitrogen.com or from **Technical Support** (see page 29)**.** For maps and a description of the features of pYES2/CT, pYES3/CT, and pYC2/CT, refer to pages 13–20.




*Please note that there are two *Bst*X I sites in the polylinker.
†The *Xba* I site is not unique in pYES3/CT.

*Continued on next page*

### *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 C-terminal V5 epitope and the polyhistidine (6xHis) tag. To sequence your construct we suggest using either the *GAL1* Forward or the T7 Promoter primer sequences along with the *CYC1* Reverse primer sequences. Refer to the diagram on the previous page for the sequences and location of the priming sites.

### 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.

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.

### 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 Plasmid Miniprep Kit or the PureLink™ HiPure Plasmid Midiprep Kit (see page 27).

# Yeast Transformation

## Introduction

In this section, you will use a small-scale yeast transformation protocol to transform your pYES2/CT, pYES3/CT, or pYC2/CT construct into the INVSc1 yeast host strain included with each vector. After transformation, expression of your recombinant fusion protein from pYES2/CT, pYES3/CT, or pYC2/CT can be induced using galactose.

## Basic Yeast Molecular Biology

To familiarize yourself 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 provided below.

**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**, page 22.

**Important: 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.**

## Initiating INVSc1 Culture

To initiate a culture of INVSc1 from the stab provided with the kit, streak a small amount from the stab on a YPD plate (see **Appendix** for recipe, page 23) and incubate at 30°C. Once 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/CT, pYES3/CT, and pYC2/CT vectors are supplied with a corresponding positive control vector (pYES2/CT/*lacZ*, pYES3/CT/*lacZ*, and pYC2/CT/*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 (see next page).

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# Yeast Transformation, Continued

## Assay for β-galactosidase Activity

You may assay for β-galactosidase expression by activity assay using cell-free lysates (Miller, 1972). Invitrogen offers the β-Gal Assay Kit for fast and easy detection of β-galactosidase expression (see page 27 for ordering).

## Reagents for Yeast Transformation

Many protocols are suitable for the preparation of competent INVSc1 yeast cells. The *S.c.* EasyComp™ Kit provides a quick and easy method for preparing competent yeast cells that can be used immediately or stored frozen for future use (see page 27 for ordering). Transformation efficiency is guaranteed at $>10^3$ transformants per µg DNA.

A small-scale yeast transformation protocol is included in the **Appendix** (see page 25) 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/CT, pYES3/CT, or pYC2/CT 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 minimal media lacking the appropriate amino acids as described below. Transformants should exhibit the appropriate amino acid prototrophy (see table below). See the **Appendix**, page 22 for a recipe to prepare SC minimal media.

| Vector | Selection Medium | Expected Growth Phenotype |
|---|---|---|
| pYES2/CT | SC-U | $Ura^+$ |
| pYES3/CT | SC-W | $Trp^+$ |
| pYC2/CT | SC-U | $Ura^+$ |

Once you have identified a transformant, be sure to purify the colony and make a glycerol stock for long-term storage.

## Maintaining Transformants

Maintain yeast cells containing your pYES2/CT, pYES3/CT, or pYC2/CT construct in the appropriate selective medium (SC-U or SC-W) containing 2% glucose or 2% raffinose (see the next page). See the **Appendix**, page 22 for a recipe for SC minimal medium.

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

# Expression of Recombinant Protein

## *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. Addition of galactose to the medium induces transcription from the *GAL1* promoter even in the presence of raffinose. Induction of 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/CT, pYES3/CT, or pYC2/CT 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, galactose is added 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 below to perform a time course experiment. Other protocols are suitable.

1. Inoculate a single colony of INVSc1 containing your pYES2/CT, pYES3/CT, or pYC2/CT construct into 15 mL of the appropriate SC selective 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 selective 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 1,500 × *g* for 5 minutes at room temperature. Discard the supernatant.
4. Resuspend the cells in 50 mL of *induction medium*. See page 22 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 1,500 × *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 the next page).

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### Detecting Recombinant Fusion Protein

To detect expression of your recombinant fusion protein by western blot (see below), you may use the Anti-V5 antibodies or the Anti-His(C-term) antibodies available from Invitrogen (see page 27 for ordering information) or an antibody to your protein of interest.

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 below for your convenience. Other protocols are suitable. Refer to *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994) for more information. For large-scale preparations (culture volumes over 1 liter), see **Scale-up** on the next page.

**Materials Needed:**

- Breaking buffer

  50 mM sodium phosphate, pH 7.4 (see page 23 for recipe of 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 frozen cells or fresh cells.

   **Reminder:** You will need to know the $OD_{600}$ of your cell sample(s) before beginning (see Step 5, previous page).
2. Resuspend fresh or frozen cell pellets in 500 µL of breaking buffer. Centrifuge at 1,500 × *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, previous page, 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 four 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 C-terminal peptide containing the V5 epitope and the polyhistidine (6xHis) tag will add approximately 5 kDa to the size of your protein.

### Scale-up of Expression for Purification

Once you have determined the optimal induction time necessary to obtain maximal protein expression, you may increase the protein yield by scaling up the procedure described on page 10. If you plan to use ProBond™ resin to purify your recombinant fusion protein, see the **Note** below. 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* (Ausubel *et al.*, 1994) for a suitable protocol to lyse cells with a bead beater.

**Note**

If you are using breaking buffer (see previous page) 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™ Purification System manual.

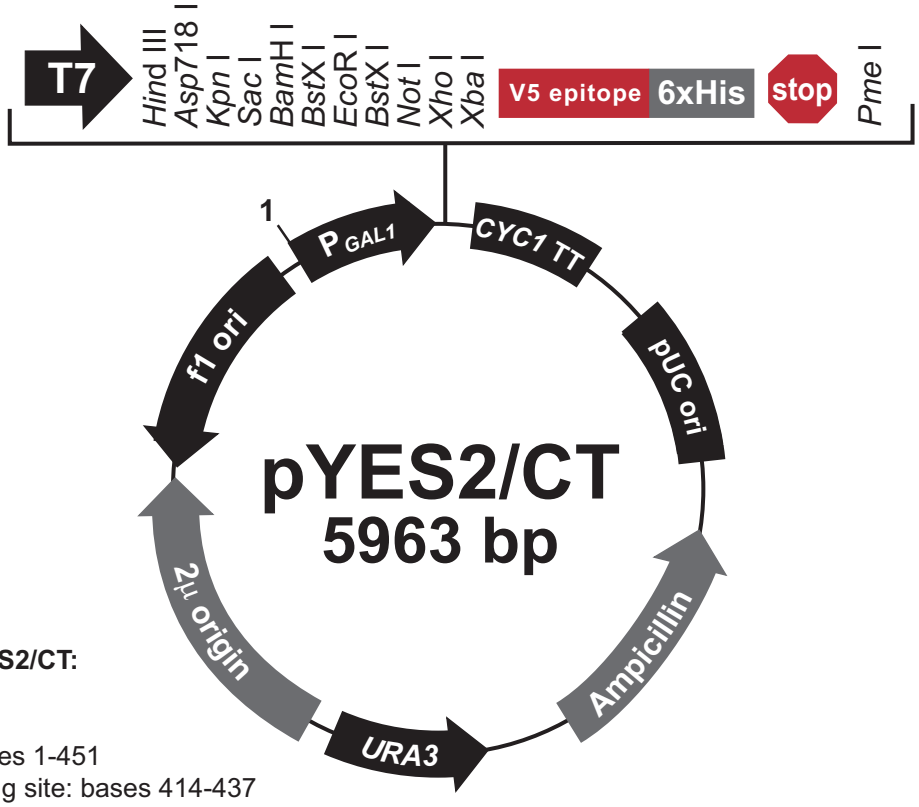
If you are using another type of resin, refer to the manufacturer's recommendations.

# Appendix

## pYES2/CT Vector

### Map of pYES2/CT

The figure below summarizes the features of the pYES2/CT vector. The vector sequence for pYES2/CT is available for downloading from www.invitrogen.com or from **Technical Support** (see page 29).




**Comments for pYES2/CT:**
**5963 nucleotides**

*GAL1* promoter: bases 1-451
*GAL1* forward priming site: bases 414-437
T7 promoter/priming site: bases 475-494
Multiple cloning site: bases 501-594
V5 epitope: bases 607-648
Polyhistidine (6xHis) region: bases 658-675
*CYC1* transcription termination signal: bases 708-961
*CYC1* reverse priming site: bases 725-743
pUC origin: bases 1145-1818
Ampicillin resistance gene: bases 1963-2823 (complementary strand)
*URA3* gene: bases 2841-3948 (complementary strand)
2µ origin: bases 3952-5423
f1 origin: bases 5491-5946 (complementary strand)

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# pYES2/CT Vector, Continued

## Features of pYES2/CT

pYES2/CT (5963 bp) contains the following elements. All features have been functionally tested.

| Feature | Benefit |
|---|---|
| *GAL1* promoter | Permits galactose-inducible expression of genes cloned into pYES2/CT (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. |
| Multiple cloning site with 9 unique sites and two *Bst*X I sites | Allows insertion of your gene and facilitates cloning in frame with the V5 epitope and the polyhistidine tag. |
| V5 epitope | Permits detection of the fusion protein with the Anti-V5 Antibody or the Anti-V5-HRP Antibody (Southern *et al.*, 1991). |
| C-terminal polyhistidine (6xHis) tag | Permits purification of your fusion protein on metal-chelating resin such as ProBond™<br>In addition, the C-terminal polyhistidine tag is the epitope for the Anti-His(C-term) Antibody and the Anti-His(C-term)-HRP Antibody (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 | Permits 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. |

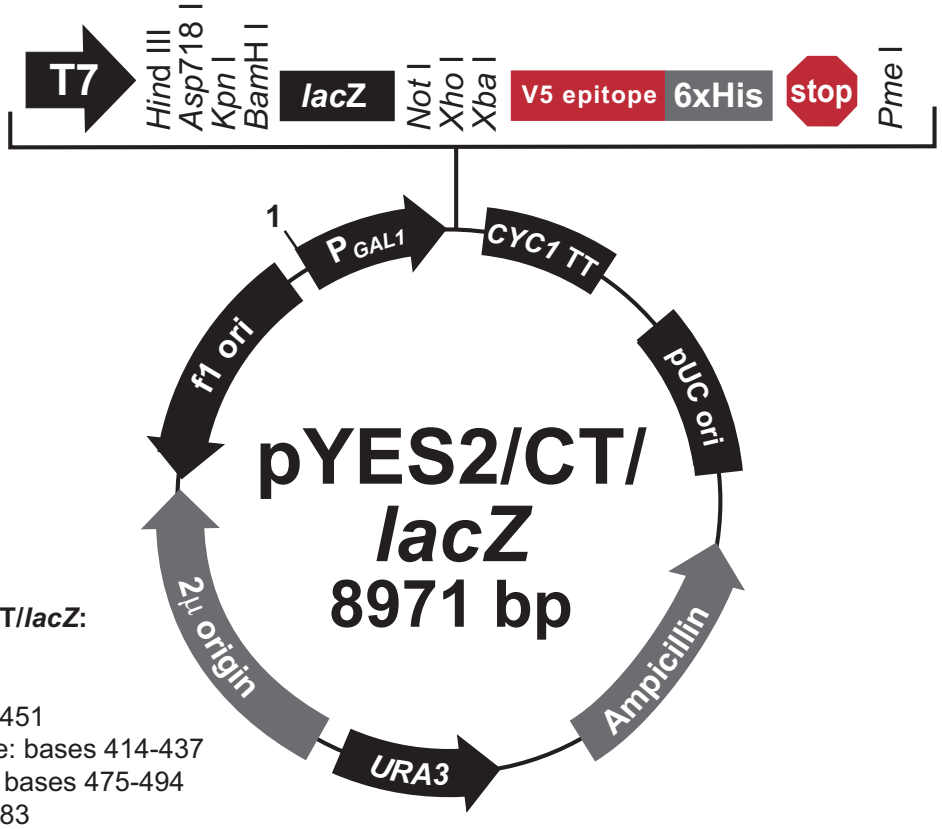
# pYES2/CT/*lacZ*

## Description

pYES2/CT/*lacZ* is a 8971 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 *Bam*H I-*Not* I site of pYES2/CT. Note that β-galactosidase will be expressed as a fusion protein containing the C-terminal V5 epitope and a polyhistidine (6xHis) tag.

## Map of pYES2/CT/*lacZ*

The figure below summarizes the features of the pYES2/CT/*lacZ* vector. The vector nucleotide sequence for pYES2/CT/*lacZ* is available for downloading from www.invitrogen.com or by contacting **Technical Support** (see page 29)



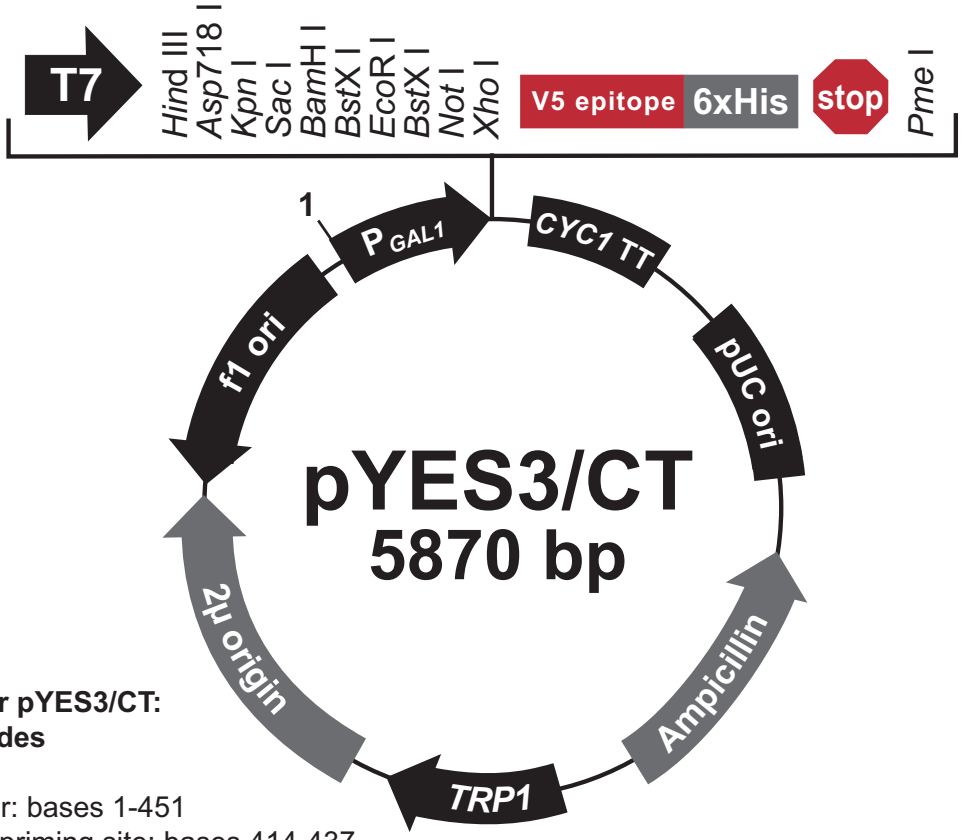

**Comments for pYES2/CT/*lacZ*: 8971 nucleotides**

*GAL1* promoter: bases 1-451
*GAL1* forward priming site: bases 414-437
T7 promoter/priming site: bases 475-494
*LacZ* ORF: bases 528-3583
V5 epitope: bases 3615-3656
Polyhistidine (6xHis) region: bases 3666-3683
*CYC1* transcription termination signal: bases 3716-3969
*CYC1* reverse priming site: bases 3733-3751
pUC origin: bases 4153-4826
Ampicillin resistance gene: bases 4971-5831 (complementary strand)
*URA3* gene: bases 5849-6956 (complementary strand)
2μ origin: bases 6960-8431
f1 origin: bases 8499-8954 (complementary strand)

# pYES3/CT Vector

## Map of pYES3/CT

The figure below summarizes the features of the pYES3/CT/*lacZ* vector. The vector sequence for pYES3/CT/*lacZ* is available for downloading from www.invitrogen.com or from **Technical Support** (see page 29).




**Comments for pYES3/CT:**
**5870 nucleotides**

*GAL1* promoter: bases 1-451
*GAL1* forward priming site: bases 414-437
T7 promoter/priming site: bases 475-494
Multiple cloning site: bases 501-588
V5 epitope: bases 607-648
Polyhistidine (6xHis) region: bases 658-675
*CYC1* transcription termination signal: bases 708-961
*CYC1* reverse priming site: bases 725-743
pUC origin: bases 1145-1818
Ampicillin resistance gene: bases 1963-2823 (complementary strand)
*TRP1* promoter: bases 3031-3132
*TRP1* gene: bases 3133-3807
2µ origin: bases 3859-5330
f1 origin: bases 5398-5853 (complementary strand)

*Continued on next page*

# pYES3/CT Vector, Continued

## Features of pYES3/CT

pYES3/CT (5870 bp) contains the following elements. All features have been functionally tested.

| Feature | Benefit |
|---|---|
| *GAL1* promoter | Permits galactose-inducible expression of genes cloned into pYES3/CT (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. |
| Multiple cloning site with 8 unique sites and two *Bst*X I sites | Allows insertion of your gene and facilitates cloning in frame with the V5 epitope and the polyhistidine tag. |
| V5 epitope | Permits detection of the fusion protein with the Anti-V5 Antibody or the Anti-V5-HRP Antibody (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 and the Anti-His(C-term)-HRP Antibody (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.* |
| *TRP1* promoter | Allows expression of the *TRP1* gene. |
| *TRP1* gene | Permits selection of yeast transformants in tryptophan-deficient medium (Tschumper and Carbon, 1980). |
| 2µ origin | Permits episomal maintenance and high copy replication in yeast. |
| f1 origin | Allows rescue of single-stranded DNA. |

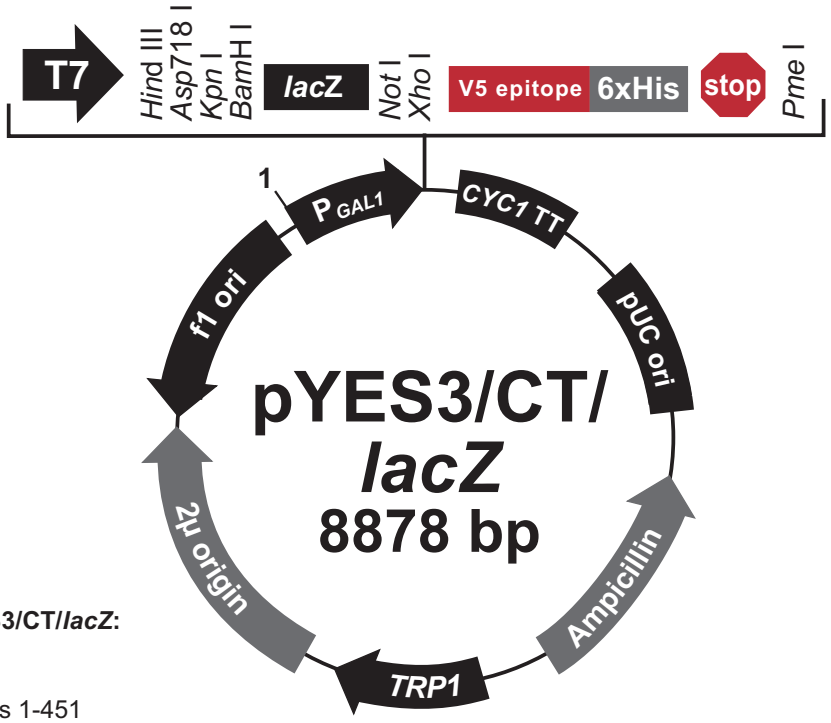
# pYES3/CT/*lacZ*

## Description

pYES3/CT/*lacZ* is a 8878 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 *Bam*H I-*Not* I site of pYES3/CT. Note that β-galactosidase will be expressed as a fusion protein containing the C-terminal V5 epitope and a polyhistidine (6xHis) tag.

## Map of pYES3/CT/*lacZ*

The figure below summarizes the features of the pYES3/CT/*lacZ* vector. The vector nucleotide sequence for pYES3/CT/*lacZ* is available for downloading from www.invitrogen.com or by contacting **Technical Support** (see page 29)



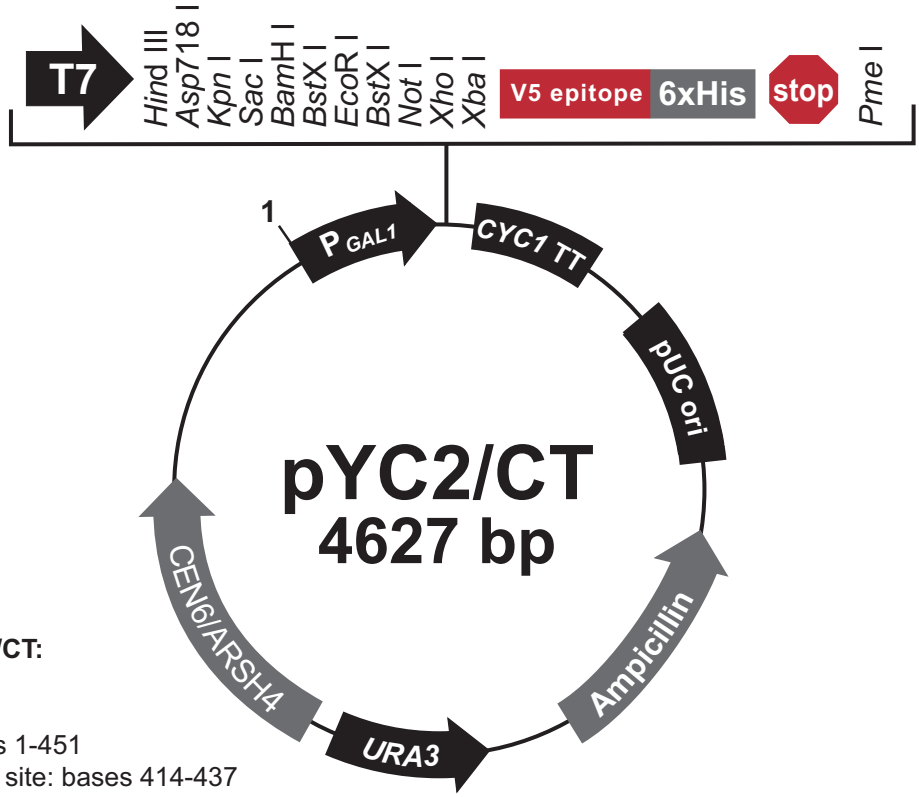

**Comments for pYES3/CT/*lacZ*: 8878 nucleotides**

*GAL1* promoter: bases 1-451
*GAL1* forward priming site: bases 414-437
T7 promoter/priming site: bases 475-494
*LacZ* ORF: bases 528-3583
V5 epitope: bases 3615-3656
Polyhistidine (6xHis) region: bases 3666-3683
*CYC1* transcription termination signal: bases 3716-3969
*CYC1* reverse priming site: bases 3733-3751
pUC origin: bases 4153-4826
Ampicillin resistance gene: bases 4971-5831 (complementary strand)
*TRP1* promoter: bases 6039-6140
*TRP1* gene: bases 6141-6815
2μ origin: bases 6867-8338
f1 origin: bases 8406-8861 (complementary strand)

# pYC2/CT Vector

## Map of pYC2/CT

The figure below summarizes the features of the pYC2/CT vector. The vector sequence for pYC2/CT is available for downloading from www.invitrogen.com or from **Technical Support** (see page 29).




**Comments for pYC2/CT:**
**4627 nucleotides**

*GAL1* promoter: bases 1-451
*GAL1* forward priming site: bases 414-437
T7 promoter/priming site: bases 475-494
Multiple cloning site: bases 501-594
V5 epitope: bases 607-648
V5 C-term reverse priming site: bases 616-636
Polyhistidine (6xHis) region: bases 658-675
*CYC1* transcription termination signal: bases 708-961
*CYC1* reverse priming site: bases 725-743
pUC origin: bases 1145-1818 (complementary strand)
Ampicillin resistance gene: bases 1963-2823 (complementary strand)
*URA3* gene: bases 2841-3948 (complementary strand)
CEN6/ARSH4: bases 3961-4479

*Continued on next page*

### Features of pYC2/CT

pYC2/CT (4627 bp) contains the following elements. All features have been functionally tested.

| Feature | Benefit |
|---|---|
| *GAL1* promoter | Permits galactose-inducible expression of genes cloned into pYC2/CT (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. |
| Multiple cloning site with 9 unique sites and two *Bst*X I sites | Allows insertion of your gene and facilitates cloning in frame with the V5 epitope and the polyhistidine tag. |
| V5 epitope | Permits detection of the fusion protein with the Anti-V5 Antibody or the Anti-V5-HRP Antibody (Southern *et al.*, 1991). |
| C-terminal polyhistidine (6xHis) tag | Permits purification of your fusion protein on metal-chelating resin such as ProBond™<br>In addition, the C-terminal polyhistidine tag is the epitope for the Anti-His(C-term) Antibody and the Anti-His(C-term)-HRP Antibody (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 | Permits 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). |

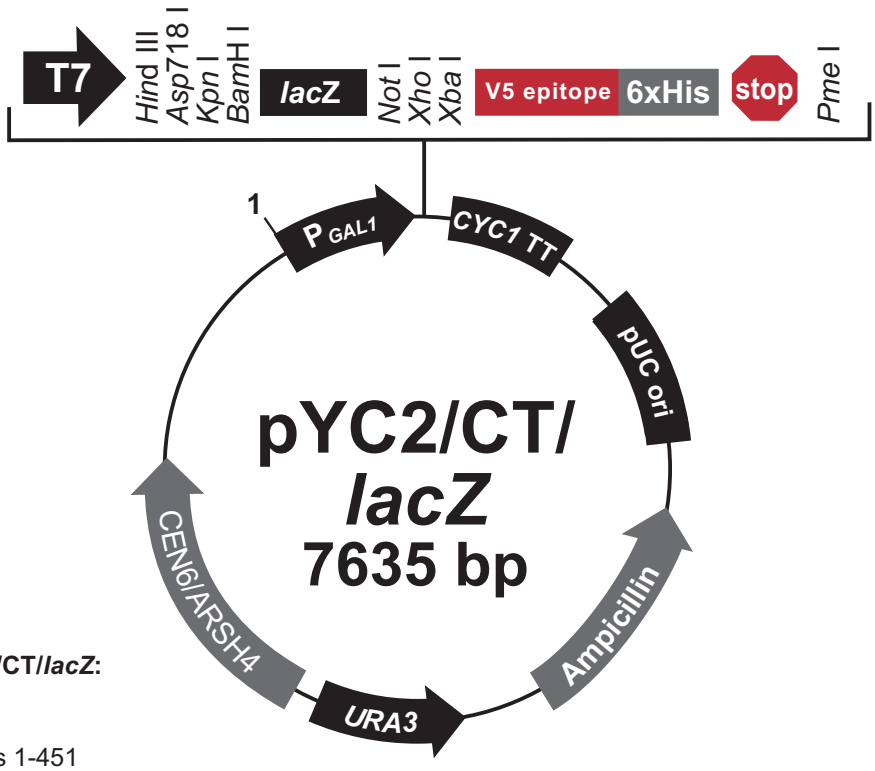
# pYC2/CT/*lacZ*

## Description

pYC2/CT/*lacZ* is a 7635 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 *Bam*H I-*Not* I site of pYC2/CT. Note that β-galactosidase will be expressed as a fusion protein containing the C-terminal V5 epitope and a polyhistidine (6xHis) tag.

## Map of pYC2/CT/*lacZ*

The figure below summarizes the features of the pYC2/CT/*lacZ* vector. The vector nucleotide sequence for pYC2/CT/lacZ is available for downloading from www.invitrogen.com or from **Technical Support** (see page 29).




**Comments for pYC2/CT/*lacZ*: 7635 nucleotides**

*GAL1* promoter: bases 1-451
*GAL1* forward priming site: bases 414-437
T7 promoter/priming site: bases 475-494
*LacZ* ORF: bases 528-3583
V5 epitope: bases 3615-3656
Polyhistidine (6xHis) region: bases 3666-3683
*CYC1* transcription termination signal: bases 3716-3969
*CYC1* reverse priming site: bases 3733-3751
pUC origin: bases 4153-4826
Ampicillin resistance gene: bases 4971-5831 (complementary strand)
*URA3* gene: bases 5849-6956 (complementary strand)
CEN6/ARSH4: bases 6969-7487

# 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, 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 (800 mL if preparing medium containing raffinose). **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 that they need.

**Reminder: Omit uracil to make selective plates for growing pYES2/CT or pYC2/CT transformants. Omit tryptophan to make selective plates for growing pYES3/CT 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 (W) | serine |
| | uracil (U) | tyrosine |
| | | valine |

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 or 200 mL of filter-sterilized 10% raffinose.
5. Pour plates and allow the plates 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 above 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*

**Important**

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

### 0.1 M Sodium Phosphate, pH 7.4

**Materials Needed**

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*

# **Recipes,** Continued

**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.

# Small-Scale Yeast Transformation

## Materials Needed

- YPD liquid medium
- 1X TE (see **Recipe**, page 24)
- 1X LiAc/0.5X TE (see **Recipe**, page 24)
- Denatured salmon sperm DNA (see recipe on the next page)
- pYES2/CT, pYES3/CT, or pYC2/CT vector construct (or other plasmid DNA to be transformed)
- 1X LiAc/40% PEG-3350/1X TE (See **Recipe**, page 24)
- 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 1,500 × *g* and resuspend the pellet in 40 mL 1X TE.
4. Pellet the cells at 1,500 × *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 = ~$2.0 \times 10^7$ yeast cells.

# Preparing Denatured Salmon Sperm DNA

## 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

## 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 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 × *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 following products may be used with the pYES2/CT, pYES3 and pYC2 vectors. For details, visit www.invitrogen.com or contact **Technical Support** (see page29).

| Item | Amount | Catalog no. |
|---|---|---|
| ProBond™ Purification System | 6 × 2 mL precharged, prepacked ProBond™ resin columns and buffers for native and denaturing purification | K850-01 |
| ProBond™ Resin | 50 mL | R801-01 |
| | 150 mL | R801-15 |
| Electrocomp™ TOP10F´ | 5 × 80 µL | C665-55 |
| One Shot® TOP10F´ Chemically Competent *E. coli* | 21 × 50 µL | C3030-03 |
| PureLink™ HiPure Plasmid Miniprep Kit | 100 preps | K2100-03 |
| PureLink™ HiPure Plasmid Midiprep Kit | 25 preps | K2100-04 |
| β-Gal Assay Kit | 80 mL | K1455-01 |
| β-Gal Staining Kit | 1 kit | K1465-01 |
| *S.c.* EasyComp™ Kit | 1 kit | K5050-01 |

## Antibodies

If you do not have an antibody specific to your protein, Invitrogen offers the Anti-V5, or Anti-His(C-term) antibodies to detect your recombinant fusion protein. Horseradish peroxidase (HRP)- and alkaline phosphatase (AP)-conjugated antibodies are available for convenient one-step detection.

| Antibody | Epitope | Catalog no. |
|---|---|---|
| Anti-V5 | Detects a 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-V5-AP | | R962-25 |
| 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 |
| Anti-His(C-term)-AP | | R932-25 |

*Continued on next page*

# Accessory Products, Continued

## Primers

For your convenience, Invitrogen offers a custom primer synthesis service. Visit www.invitrogen.com for more details.

## Other Yeast Expression Vectors

Invitrogen 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.invitrogen.com or call **Technical Support** (see page 29).

# Technical Support

## Web Resources

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, SDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

## Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our website (www.invitrogen.com).

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Tech Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

## SDS

Safety Data Sheets (MSDSs) are available on our website at www.invitrogen.com/sds.

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# Notes

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