invitrogen™

pYES2

Cat. no. V825–20

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User Manual

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Important Information

Kit Contents

The following reagents are supplied with the pYES2 vector:

20 µg of pYes2 plasmid in TE buffer, pH 8.0* (40 µl at 0.5 µg/µl)

TOP10F′ *E. coli* cells, 1 stab

INVSc1 yeast host strain, 1 stab

***TE Buffer:** 10 mM Tris-HCl, 1 mM EDTA, pH 8.0

Shipping/Storage

All reagents are shipped at room temperature. Upon receipt, store the plasmid DNA at –20°C. Store the TOP10F′ and INVSc1 stabs at 4°C.

Genotype of TOP10F´

The genotype of TOP10F′ is provided below.

F´ {*lac*I^{q} *Tn10* (Tet^{R})} *mcr*A, Δ(*mrr-hsd*RMS-*mcr*BC), Φ80*lac*ZΔM15, Δ*lac*X74, *end*A1, *rec*A1, *ara*D139, Δ(ara,leu)7697, *gal*U, *gal*K, *nup*G, *rps*L (Str^{R})

Preparing TOP10F´ Glycerol Stocks

We recommend that you prepare a set of TOP10F′ *E. coli* glycerol master stocks within two weeks of receiving the kit.

To prepare 5–10 glycerol master stocks for long-term storage:

1. Streak a small portion of the TOP10F′ cells that you have received as a stab on an LB plate.
2. Invert the plate and incubate at 37°C overnight.
3. Isolate a single colony and inoculate into 5–10 ml of LB medium.
4. Grow the culture to stationary phase (OD_{600} = 1–2).
5. Mix 0.8 ml of culture with 0.2 ml of sterile glycerol and transfer to a cryovial.
6. Store at –80°C. Use one master stock to create working stocks for regular use.

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.

Accessory Products

Additional Products

Invitrogen offers a variety of products that are suitable for use with the pYes2 plasmid. Ordering information is provided below. For detailed instructions on how to use any of the accessory products, refer to the manual provided with each product. For more information, refer to www.invitrogen.com or contact Technical Support (page 17).

Item	Amount	Cat. no.
PureLink™ HiPure Plasmid Miniprep Kit	25 preps 100 preps	K2100–02 K2100–03
PureLink™ HiPure Plasmid Midiprep Kit	25 preps 50 preps	K2100–04 K2100–05
Ampicillin Sodium Salt, irradiated	200 mg	11593-027
One Shot® TOP10 Chemically Competent *E. coli*	10 reactions 20 reactions	C4040-10 C4040-03
Subcloning Efficiency™ DH5α™ Chemically Competent *E. coli*	40 reactions	18265-017
T7 Forward primer	2 µg	N560-02
S. c. EasyComp™ Kit	1 kit	K5050-01
UltraPure™ Salmon Sperm DNA Solution (10 mg/ml)	5 × 1 ml	15632-011

Introduction

Overview

Introduction

pYES2 is a 5.9 kb vector designed for inducible expression of recombinant proteins in *Saccharomyces cerevisiae*. Features of the vector allow easy cloning of your gene of interest and selection of transformants by uracil prototrophy (see pages 15–16). The vector contains 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 7 for more information)
- A versatile multiple cloning site for simplified cloning
- *CYC1* transcriptional terminator for efficient termination of mRNA
- *URA3* gene for selection of transformants in yeast host strains with a *ura3* genotype
- Ampicillin resistance gene for selection in *E. coli*

Experimental Outline

Use the following outline to clone and express your gene of interest in pYES2.

1. Consult the multiple cloning site described on page 3 to design a strategy to clone your gene in pYES2.
2. Ligate your insert into pYES2 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 use sequencing to confirm that your gene is cloned in the proper orientation.
5. Transform your construct into competent INVSc1 cells and select for uracil prototrophy.
6. Test for expression of your recombinant gene by western blot analysis or functional assay.

Methods

Cloning into pYES2

Introduction

A diagram is provided on the next page to help you ligate your gene of interest into pYES2. General considerations for cloning and transformation are discussed below.

General Molecular Biology Techniques

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

E. coli Strain

We recommend that you propagate the pYES2 vector in the TOP10F′ *E. coli* strain. A stab of TOP10F′ is provided for your convenience. Other *E. coli* strains that are recombination deficient (*rec*A) and endonuclease deficient (*end*A) are also suitable for the growth of this vector.

To initiate a culture of TOP10F′ from the supplied stab, streak a small amount of stock from the stab on an LB plate containing 10 µg/ml tetracycline.

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.

Maintaining pYES2

To propagate and maintain the pYES2 vector, use 10 ng of the vector to transform a *rec*A, *end*A *E. coli* strain like TOP10F′, DH5α™, JM109, or equivalent. Select transformants on LB plates containing 50 to 100 µg/ml ampicillin. Be sure to prepare a glycerol stock of the plasmid for long-term storage (see page 4 for a protocol).

Cloning Considerations

pYES2 vector does 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.

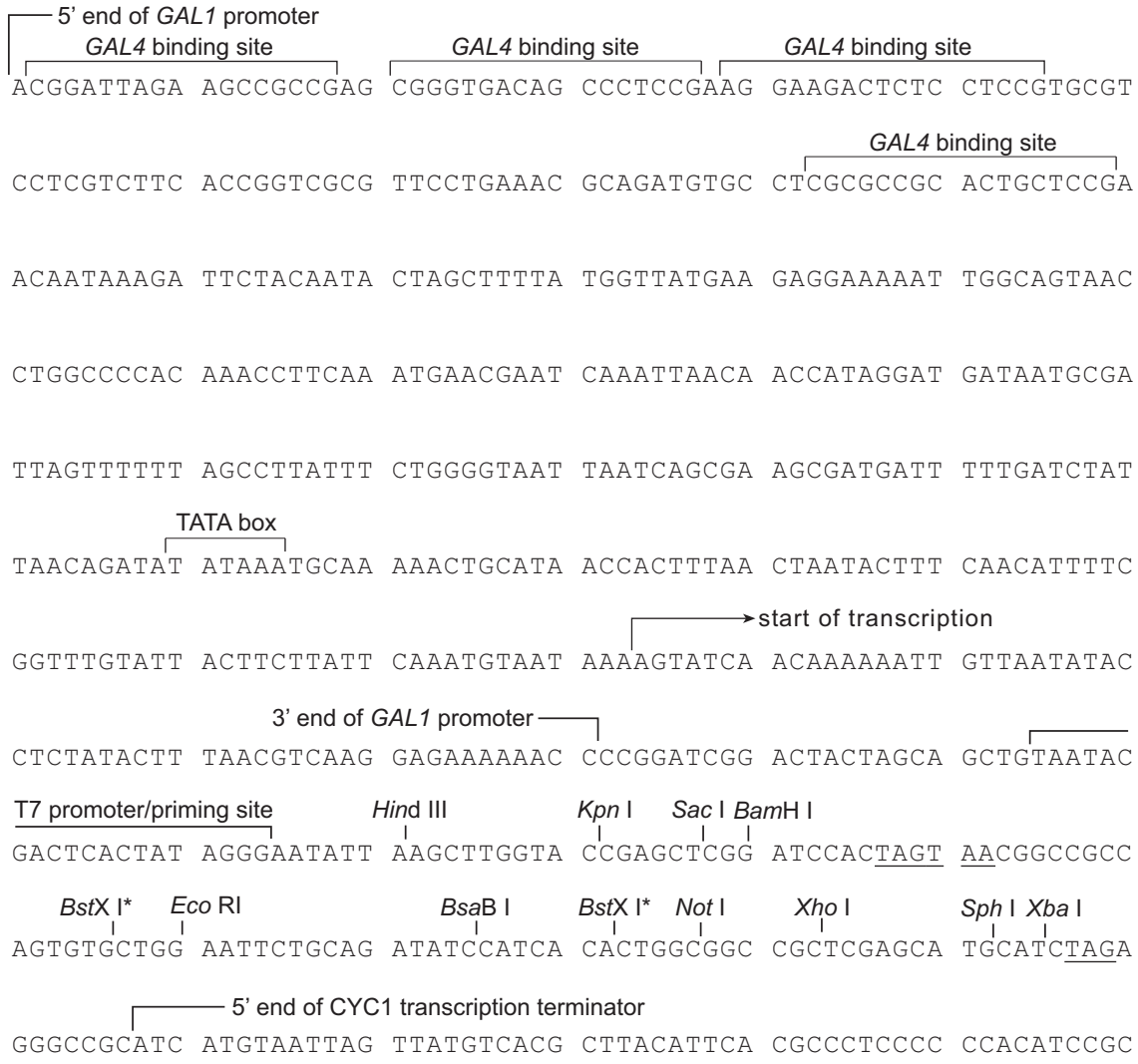
Your insert must also contain a stop codon for proper termination of your mRNA. Note that the *Xba* I site contains an internal stop codon (TCTAGA).

Continued on next page

Cloning into pYES2, continued

Multiple Cloning Site of pYES2

Below is a diagram of the *GAL1* promoter and the multiple cloning site for pYES2. Features of the *GAL1* promoter are marked as per Giniger *et al.*, 1985, Johnston and Davis, 1984, and Yocum *et al.*, 1984. Restriction sites are labeled to indicate the cleavage site. Potential stop codons are underlined. The multiple cloning site has been confirmed by sequencing and functional testing. The vector sequence of pYES2 is available for downloading from our website (www.invitrogen.com) or from Technical Support (see page 17).



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

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Cloning into pYES2, continued

E. coli Transformation

Transform your ligation mixtures into competent TOP10F' *E. coli* or any other *rec*A, *end*A *E. coli* strain of your choice. Select for transformants on LB plates containing 50–100 µg/ml ampicillin. Select 10–20 clones and analyze by restriction digest or sequencing for the presence and orientation of your insert.

Sequencing Inserts in pYES2

The T7 Forward primer is available from Invitrogen (see page v) to allow sequencing from the T7 promoter/priming site (see diagram on page 3 for the location of the priming site). Alternatively, you may wish to design primers that flank your gene of interest to verify the orientation of your insert in pYES2.

Preparing a Glycerol Stock

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

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

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).

We recommend the PureLink™ HiPure Plasmid Miniprep Kit or the PureLink™ HiPure Plasmid Midiprep Kit for preparing plasmid DNA for routine yeast transformations (see page v for ordering information). Refer to our website at www.invitrogen.com or contact Technical Support for more information on a large selection of plasmid purification columns.

Yeast Transformation

Introduction

In this section, you will use a small-scale yeast transformation protocol to transform your construct into the INVSc1 yeast host strain included with the vector.

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 provided below.

Genotype: MAT**a** *his3Δ1 leu2 trp1-289 ura3-52*/MATα *his3Δ1 leu2 trp1-289 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, and uracil. A recipe for preparation of SC minimal medium is provided in the **Appendix** (see page 10).

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 11) and incubate at 30°C. Once growth is established, you may check the phenotype of the strain by streaking a single colony on a 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.

Continued on next page

Yeast transformation, continued

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 v for ordering information). Transformation efficiency is guaranteed at $>10^3$ transformants per µg DNA.

A small-scale yeast transformation protocol is included in the **Appendix** (see page 13) 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 plasmid construct into competent INVSc1. We recommend that you include the pYES2 parental vector as a negative control to evaluate your results.

Select for transformants on SC-U selective plates. Transformants should exhibit uracil prototrophy. Once you have identified a transformant, be sure to purify the colony and make a glycerol stock for long-term storage.

Maintain cells containing your pYES2 construct in SC-U medium containing 2% raffinose or 2% glucose. See the **Appendix**, page 10 for a recipe for SC-U medium.

Expression of Recombinant Protein

Introduction

Once you have obtained a transformant containing your pYES2 construct, you are ready to induce expression of your recombinant 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. 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 construct in glucose or raffinose depending on how quickly you want to obtain your expressed protein after induction with galactose. For more information about expression in yeast, refer to the *Guide to Yeast Genetics and Molecular Biology* (Guthrie and Fink, 1991).

Continued on next page

Expression of Recombinant Protein, continued

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 raffinose, recombinant protein can be detected in as little as 2 hours after galactose induction. Recombinant protein can be detected in cells that have been cultured in glucose by 4 hours after galactose induction.

If you are assaying for expression of your recombinant protein for the first time, we recommend that you perform a time course to optimize expression of your recombinant protein (*e.g.*, 0, 2, 4, 6, 8, 10 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 construct into 15 ml of SC-U medium containing 2% raffinose or 2% glucose. 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.**

   Example: Assume that the OD_{600} of your 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 4°C.
4. Resuspend the cells in 1–2 ml of **induction medium** (SC-U medium containing 2% galactose) and inoculate into 50 ml of **induction medium**. See the **Appendix**, page 10 for a recipe for **induction medium**. Grow at 30°C with shaking.
5. Harvest an aliquot of cells at 0, 2, 4, 6, 8, and 10 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.
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 next page).

Continued on next page

Expression of Recombinant Protein, continued

Detecting Recombinant Protein

You may use any method of your choice to detect expression of your recombinant protein from pYES2. If you wish to use western blot analysis to assay for protein expression, you will need to have an antibody to your protein of interest.

To detect the recombinant protein by western blot, you 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*, Unit 13.13 (Ausubel *et al.*, 1994) for more information. For large-scale preparations (culture volumes over 1 liter), see **Scale-up** below.

Materials Needed:

Breaking buffer (50 mM sodium phosphate, pH 7.4, 1 mM EDTA, 5% glycerol, 1 mM PMSF) (refer to **Appendix**, page 11 for instructions to prepare the sodium phosphate stock buffer)

Glass beads, acid-washed 425–600 µm size (30–40 U.S. sieve) (*e.g.*, Sigma-Aldrich, Cat. 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, 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.

Scale-up

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 8. 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.

Appendix

Recipes

SC Minimal Medium and Plates

SC is synthetic minimal defined medium for yeast.

0.67% yeast nitrogen base (**without** amino acids)
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 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 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 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 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.

Note

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.

Continued on next page

Recipes, continued

YPD

Yeast Extract Peptone Dextrose Medium (1 liter)

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

1. Dissolve the following in 1000 ml of water:

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

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

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

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)

Protocol:

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 deionized water.
4. Filter-sterilize and store at room temperature.

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.

Continued on next page

Recipes, continued

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 80 ml of 50% PEG-3350.
2. Filter-sterilize and store at room temperature.

Small-Scale Yeast Transformation

Introduction

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

Materials Needed

- YPD liquid medium
- 1X TE (see **Recipe**, page 12)
- 1X LiAc/0.5X TE (see **Recipe**, page 12)
- Denatured salmon sperm DNA (see recipe on the next page)
- pYES2 vector construct (or other plasmid DNA to be transformed)
- 1X LiAc/40% PEG-3350/1X TE (See **Recipe**, page 12)
- 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 2500 rpm and resuspend the pellet in 40 ml 1X TE.
4. Pellet the cells at 2500 rpm and resuspend the pellet in 2 ml of 1X LiAc/0.5X TE.
5. Incubate the cells at room temperature for 10 minutes.
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 = ~2.0×10^7 yeast cells.

Preparing 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 (Cat. no. D9156). Alternatively, some researchers have found that using yeast transfer RNA (Sigma-Aldrich, Cat. no. R9001) as a carrier results in a cleaner transformation although there are fewer total colonies.

Materials Needed

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

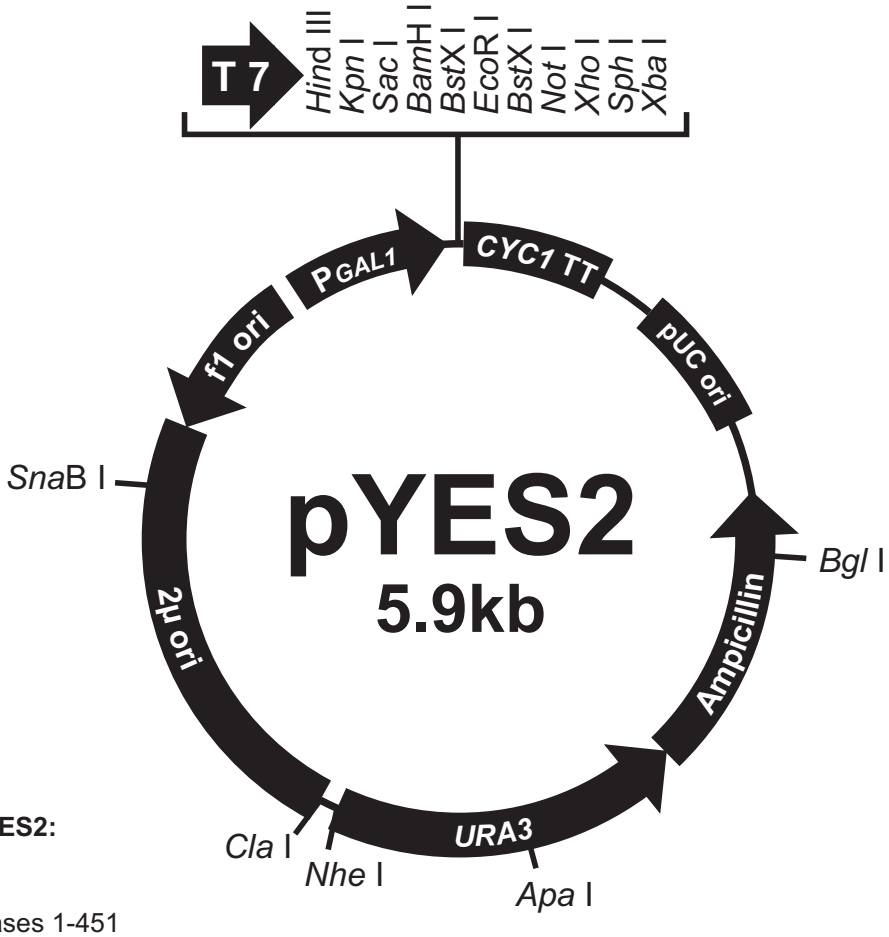
Protocol

1. In a 250 ml flask, dissolve 1 g salmon sperm DNA into 100 ml of TE (10 mg/ml). Pipette 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 pheno: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 x g for 15 minutes at 4°C.
10. Wash the DNA once with 200 ml 70% ethanol and centrifuge as described in step 9.
11. Partially dry DNA by air or in a Speed-Vac (cover tubes with parafilm and poke holes in top) for 20 minutes.
12. Transfer DNA to a 250 ml sterile flask and dissolve DNA in 100 ml sterile TE (10 mg/ml).
13. Boil for 20 minutes to denature DNA. Immediately place on ice, aliquot in 1 ml samples, and freeze at –20°C.

pYES2 Vector

Map of pYES2

The figure below summarizes the features of the pYES2 vector. **The vector sequence of pYES2 is available for downloading from our website (www.invitrogen.com) or from Technical Support (see page 17).**



Comments for pYES2:
5856 nucleotides

GAL1 promoter: bases 1-451
T7 promoter/priming site: bases 475-494
Multiple cloning site: bases 501-600
CYC1 transcription terminator: bases 608-856
pUC origin: bases 1038-1711
Ampicillin resistance gene: bases 1856-2716 (C)
URA3 gene: bases 2734-3841 (C)
2 micron (µ) origin: bases 3845-5316
f1 origin: bases 5384-5839 (C)
(C) = complementary strand

Continued on next page

pYES2 Vector, continued

Features of pYES2

pYES2 (5856 bp) contains the following elements. All features have been functionally tested.

Feature	Benefit
GAL1 promoter	Permits inducible expression of genes cloned into pYES2 (West *et al.*, 1984)
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, plus two *Bst*X I sites	Allows insertion of your gene into pYES2
CYC1 transcription termination signal	Permits efficient termination and stabilization of mRNA
pUC origin	Maintenance and high copy replication in *E. coli*
Ampicillin resistance gene	Selection of transformants in *E. coli*
URA3 gene	Selection of yeast transformants in uracil-deficient medium
2µ origin	Maintenance and high copy replication in yeast
f1 origin	Rescue of single-stranded DNA

Technical Support

Web Resources

Visit the Invitrogen web site at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
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