Invitrogen™
life technologies

Instruction Manual

pYES-DEST52 Gateway™ Vector

A destination vector for cloning and inducible expression in *Saccharomyces cerevisiae*

Catalog no. 12286-019

Version D
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Important Information

Shipping and Storage	pYES-DEST52 is shipped at room temperature. Upon receipt, store at -20°C. Products are guaranteed for six months from date of shipment when stored properly.
Contents	6 µg pYES-DEST52, lyophilized in TE, pH 8.0
Quality Control	pYES-DEST52 is qualified by restriction endonuclease digestion. pYES-DEST52 is further qualified in a recombination assay using Gateway® LR Clonase™ enzyme mix. The *ccd*B gene is assayed by transformation using an appropriate *E. coli* strain.

Accessory Products

Additional Products

Additional products that may be used with pYES-DEST52 are available from Invitrogen. Ordering information is provided below.

Product	Amount	Catalog no.
Gateway® LR Clonase™ Enzyme Mix	20 reactions	11791-019
One Shot® TOP10 Chemically Competent Cells	10 reactions	C4040-10
	20 reactions	C4040-03
One Shot® TOP10 Electrocompetent Cells	10 reactions	C4040-50
	20 reactions	C4040-52
INV*Sc*1	1 stab	C810-00
S.c. EasyComp™ Kit	20 reactions	K5050-01

Detection of Recombinant Proteins

Expression of your recombinant fusion protein can be detected using an antibody to the appropriate epitope. Horseradish peroxidase (HRP) or alkaline phosphatase (AP)-conjugated antibodies allow one-step detection using colorimetric or chemiluminescent detection methods. The amount of antibody supplied is sufficient for 25 Westerns.

Product	Epitope	Catalog no.
Anti-V5 Antibody	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 Antibody		R961-25
Anti-V5-AP Antibody		R962-25
Anti-His (C-term) Antibody	Detects the C-terminal polyhistidine (6xHis) tag (requires the free carboxyl group for detection (Lindner *et al.*, 1997) HHHHHH-COOH	R930-25
Anti-His(C-term)-HRP Antibody		R931-25
Anti-His(C-term)-AP Antibody		R932-25

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Accessory Products, continued

Purification of Recombinant Fusion Protein

If your gene of interest is in frame with the C-terminal peptide containing the V5 epitope and the polyhistidine (6xHis) tag, you may use Immobilized Metal Affinity Chromatography (IMAC) to purify your recombinant fusion protein. The ProBond™ Purification System or bulk ProBond™ resin are available separately from Invitrogen. See the table below for ordering information.

Product	Quantity	Catalog no.
ProBond™ Nickel-chelating Resin	50 ml	R801-01
	150 ml	R801-15
ProBond™ Purification System	6 purifications	K850-01
ProBond™ Purification System with Anti-His(C-term)-HRP Antibody	1 kit	K853-01
ProBond™ Purification System with Anti-V5-HRP Antibody	1 kit	K854-01
Purification Columns (10 ml polypropylene columns)	50	R640-50

Methods

Overview

Description

pYES-DEST52 is a 7.6 kb vector derived from pYES2/CT and adapted for use with the Gateway® Cloning Technology. It is designed for high-level, galactose-inducible expression in *Saccharomyces cerevisiae*. For more information on Gateway® Cloning, see the next page.

Features

pYES-DEST52 contains the following elements:

- Yeast *GAL1* promoter for high-level, galactose-inducible protein expression in *Saccharomyces cerevisiae*
- Two recombination sites, *att*R1 and *att*R2, downstream of the *GAL1* promoter for recombinational cloning of the gene of interest from an entry clone
- Chloramphenicol resistance gene located between the two *att*R sites for counterselection
- The *ccd*B gene located between the two *att*R sites for negative selection
- The V5 epitope and 6xHis tag for detection and purification (optional)
- Cytochrome c (*CYC1*) polyadenylation sequence for proper termination and processing of the recombinant transcript
- 2µ origin for episomal maintenance and high copy replication
- *URA3* auxotrophic marker for selection of yeast transformants
- The pUC origin for high copy replication and maintenance of the plasmid in *E. coli*
- The ampicillin (*bla*) resistance gene for selection in *E. coli*
- f1 intergenic region for production of single-strand DNA in F plasmid-containing *E. coli*

For a map of pYES-DEST52, see page 22.

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Overview, continued

The Gateway® Technology

Gateway® is a universal cloning technology that takes advantage of the site-specific recombination properties of bacteriophage lambda (Landy, 1989) to provide a rapid and highly efficient way to move your gene of interest into multiple vector systems. To express your gene of interest using Gateway® Technology, simply:

1. Clone your gene of interest into a Gateway® entry vector to create an entry clone.
2. Generate an expression clone by performing an LR recombination reaction between the entry clone and a Gateway® destination vector (*e.g.* pYES-DEST52).
3. Transform your expression clone into *Saccharomyces cerevisiae* and assay for expression of your gene of interest.

For more information on the Gateway® System, refer to the Gateway® Technology Manual. This manual is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (page 24).

Using pYES-DEST52

Important

The pYES-DEST52 vector is supplied as a supercoiled plasmid. Although Invitrogen has previously recommended using a linearized destination vector for more efficient recombination, further testing has found that linearization of this vector is **NOT** required to obtain optimal results for any downstream application.

Propagating pYES-DEST52

If you wish to propagate and maintain pYES-DEST52, we recommend using Library Efficiency® DB3.1™ Competent Cells (Catalog no. 11782-018) from Invitrogen for transformation. The DB3.1™ *E. coli* strain is resistant to CcdB effects and can support the propagation of plasmids containing the *ccd*B gene.

Note: DO NOT use general *E. coli* cloning strains including TOP10 or DH5α for propagation and maintenance as these strains are sensitive to CcdB effects.

Entry Clone

To recombine your gene of interest into pYES-DEST52, you should have an entry clone containing your gene of interest. For your convenience, Invitrogen offers the pENTR Directional TOPO® Cloning Kit (Catalog no. K2400-20) for highly efficient, 5-minute cloning of your gene of interest into an entry vector. For more information on entry vectors available from Invitrogen, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 24).

For detailed information on constructing an entry clone, refer to the specific entry vector manual. For detailed information on performing the LR recombination reaction, refer to the Gateway® Technology Manual.

Resuspending pYES-DEST52

Before you perform the LR Clonase™ reaction, resuspend pYES-DEST52 to 50-150 ng/µl in sterile water.

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Using pYES-DEST52, continued

Points to Consider Before Recombining

Your insert should contain a yeast consensus sequence for proper initiation of translation, although this requirement is not as stringent in yeast (Cigan and Donahue, 1987; Romanos *et al.*, 1992). An example of a yeast consensus sequence is provided below (Romanos *et al.*, 1992). Note that a "U" at position –3 decreases expression 2-fold. The AUG initiation codon is shown underlined.

(A/Y) A (A/U) A AUGUCU

If you wish to include the V5 epitope and 6xHis tag, your gene in the entry clone **should not** contain a stop codon. In addition, the gene should be designed to be in frame with the C-terminal epitope tag after recombination. Refer to the **Recombination Region** on the next page.

If you do NOT wish to include the V5 epitope and 6xHis tag, be sure that your gene contains a stop codon in the entry clone.

Recombining Your Gene of Interest

Each entry clone contains *att*L sites flanking the gene of interest. Genes in an entry clone are transferred to the destination vector backbone by mixing the DNAs with the Gateway® LR Clonase™ enzyme mix (see page vi for ordering information). The resulting recombination reaction is then transformed into *E. coli* and the expression clone selected. Recombination between the *att*R sites on the destination vector and the *att*L sites on the entry clone replaces the *ccd*B gene and the chloramphenicol (Cm^R) gene with the gene of interest and results in the formation of *att*B sites in the expression clone.

Follow the instructions in the Gateway® Technology Manual to set up the LR Clonase™ reaction, transform *E. coli*, and select for the expression clone.

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Using pYES-DEST52, continued

Confirming the Expression Clone

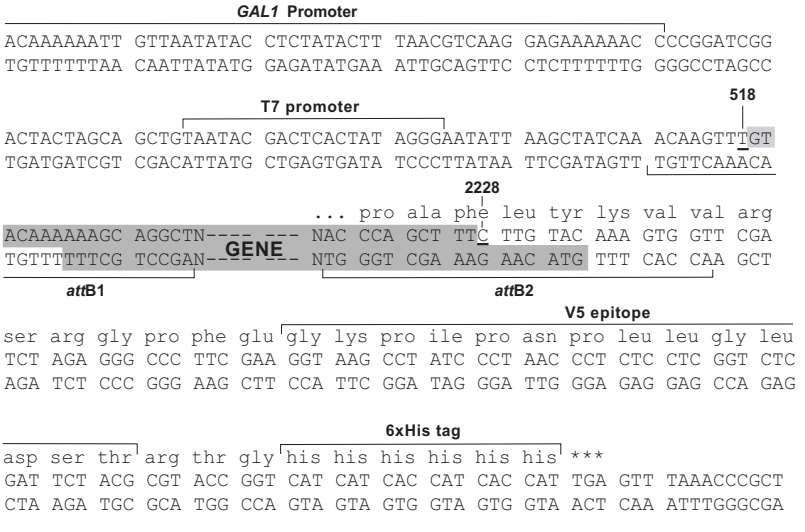
The *ccd*B gene mutates at a very low frequency, resulting in a very low number of false positives. True expression clones will be ampicillin-resistant and chloramphenicol-sensitive. Transformants containing a plasmid with a mutated *ccd*B gene will be both ampicillin- and chloramphenicol-resistant. To check your putative expression clone, test for growth on LB plates containing 30 µg/ml chloramphenicol. A true expression clone will not grow in the presence of chloramphenicol.

Recombination Region

The recombination region of the expression clone resulting from pYES-DEST52 × entry clone is shown below.

Features of the Recombination Region:

- Shaded regions correspond to those DNA sequences transferred from the entry clone into pYES-DEST52 by recombination. Non-shaded regions are derived from the pYES-DEST52 vector.
- The underlined nucleotides flanking the shaded region correspond to bases 518 and 2228, respectively, of the pYES-DEST52 vector sequence.



Yeast Transformation

Introduction

Once you have selected your expression clone, you are ready to transform it into yeast and test for expression of your gene. We recommend that you include a negative control in your experiments to evaluate your results.

Plasmid Preparation

You may use any method of your choice to prepare purified plasmid DNA for small-scale yeast transformation. Standard protocols may be found in *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994) or *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989). For your convenience, the S.N.A.P.™ MidiPrep Kit (Catalog no. K1910-01) is available from Invitrogen for isolation of plasmid DNA from 10-100 ml of bacterial culture.

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.

Yeast Host Strain

To successfully transform your pYES-DEST52 construct into yeast, the strain must contain the *ura3* genotype (Ura^-). For expression, we recommend the diploid strain INVSc1. (see page vi for ordering information). **Note that INV*Sc*1 does not sporulate well and is therefore not suitable for yeast genetic studies.**

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Yeast Transformation, continued

Yeast Transformation

Use any method of your choice to transform your pYES-DEST52 construct into the appropriate yeast host strain. A small-scale yeast transformation protocol is included in the **Appendix** (page 19) for your convenience. Refer to general reference sources (Ausubel *et al.*, 1994) or published references (Gietz *et al.*, 1992; Gietz *et al.*, 1995; Hill *et al.*, 1991; Schiestl and Gietz, 1989) for other protocols.

The *S. c.* EasyComp™ Kit from Invitrogen (Catalog no. K5050-01) 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.

Select for transformants on SC-U (SC minimal media lacking uracil) selective plates (see the **Appendix**, page 15 for a recipe). Transformants should exhibit uracil prototrophy. Once you have identified a transformant, be sure to prepare a glycerol stock for long-term storage.

Maintenance of Transformants

Maintain yeast cells containing your pYES-DEST52 construct in SC-U medium containing 2% glucose or 2% raffinose. See the **Appendix**, page 15, for a recipe for SC-U medium.

Expressing the Gene of Interest

Introduction

Once you have obtained a transformant containing your pYES-DEST52 construct, you are ready to induce expression of your recombinant protein of interest. This section provides guidelines on how to induce and assay for expression of your protein of interest.

GAL1 Promoter

In typical *S. cerevisiae* laboratory strains (*e.g.* INVSc1), transcription from the *GAL1* promoter is repressed in the presence of glucose (West *et al.*, 1984). Removing glucose and adding galactose as a carbon source induces transcription (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 derepressed and 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 pYES-DEST52 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 protein with galactose, proceed to **Time Course of Protein Induction by Galactose** on the next page.

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Expressing the Gene of Interest, continued

Before Starting

Be sure you have the following reagents and equipment on hand before starting.

- SC-U medium containing 2% raffinose or 2% glucose (see Recipes, page 15)
- 50 ml conical tubes
- SC-U medium containing 2% galactose (see Recipes, page 16)
- 250 ml culture flasks
- Table-top centrifuge
- 15 ml snap-cap, sterile plastic tubes
- Sterile water
- Sterile microcentrifuge tubes
- 30°C water bath

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 protein can be detected in as little as 4 hours after galactose induction. Recombinant 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 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.

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Time Course of Protein Induction by Galactose, continued

1. Inoculate a single colony containing your pYES-DEST52 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 (SC-U medium containing 2% galactose).

   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 1500 x g for 5 minutes at +4°C. Discard the supernatant.
4. Resuspend the cells in 1-2 ml of induction medium and inoculate into 50 ml of induction medium. See the **Appendix**, page 16 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 protein (see Step 3 on page 12).
6. Centrifuge the cells at 1500 x 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 page the next page to prepare cell lysates to detect your recombinant protein.

Analyzing Samples

Detecting Recombinant Protein

You may use an enzymatic assay to detect your protein or Western blot. To detect the recombinant 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 vi for ordering information) or an antibody to your protein.

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 (Ausubel *et al.*, 1994) for more information. For large-scale preparations (culture volumes over 1 liter), see **Scale-up** on page 13.

Materials Needed:

- Breaking buffer:
  50 mM sodium phosphate, pH 7.4, (see page 17 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)

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Analyzing Samples, continued

Detecting Recombinant Protein, continued

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 10).

2. Resuspend fresh or frozen cell pellets in 500 µl of breaking buffer. Centrifuge at 1500 x 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 10, 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 your sample(s) containing glass beads 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 heat the sample for 5 minutes at 70°C.
9. Load 20 µg of lysate onto an SDS-PAGE gel and electrophorese. Use the appropriate percentage of acrylamide to resolve your recombinant protein.

Note

The C-terminal peptide containing the V5 epitope and the polyhistidine tag will add approximately 4 kDa to the size of your protein

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Purification

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. 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.

Purification of Recombinant Fusion Proteins

The presence of the C-terminal polyhistidine (6xHis) tag in your recombinant fusion protein allows use of a metal-chelating resin such as ProBond™ to purify your fusion protein. The ProBond™ Purification System and bulk ProBond™ resin are available from Invitrogen (see page vii for ordering information). Refer to the ProBond™ Purification System manual for protocols to purify your fusion protein. Invitrogen also offers Ni-NTA Agarose (Catalog no. R901-01) for purification of proteins containing a polyhistidine (6xHis) tag**. Note:** Other metal-chelating resins and purification methods are suitable.

Note

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

Appendix

Recipes

LB (Luria-Bertani) Medium and Plates

Composition:

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

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 950 ml deionized water.
2. Adjust the pH of the solution to 7.0 with NaOH and bring the volume up to 1 liter.
3. Autoclave on liquid cycle for 20 minutes at 15 psi. Allow solution to cool to 55°C and add antibiotic if needed (50-100 µg/ml ampicillin).
4. Store at room temperature or at +4°C.

LB agar plates

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

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Recipes, continued

SC Minimal Medium and Plates

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

2% carbon source (*e.g.* 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 pYES-DEST52 expression clone 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.

Recipes, continued

Induction Medium

If you are making induction medium, follow Steps 1-3 on the previous page 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.

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 10 g yeast extract, 20 g peptone, and 20 g dextrose (see note below if making plates) in 1000 ml of water.
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.

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Recipes, continued

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 1L 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 up the volume to 1 L 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 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.

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Recipes, continued

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.

50% PEG-3350

1. For 100 ml, dissolve 50 g of PEG-3350 in 90 ml of deionized water. You may have to heat the solution to fully dissolve the PEG.
2. Bring up the volume to 100 ml with deionized water.
3. Autoclave at 121°C, 15 psi for 20 minutes. 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.

Small-Scale Yeast Transformation

Introduction

A small-scale yeast transformation protocol for routine transformations is provided below. Other protocols are suitable. The *S.c.* EasyComp™ Transformation Kit (see page vi for ordering information) is available from Invitrogen for rapid preparation of transformation-competent yeast cells. For more information, refer to our Web site (www.invitrogen.com) or contact Technical Service (page 24).

Materials Needed

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

- YPD liquid medium (see **Recipe**, page 16)
- 1X TE (see **Recipe**, page 17)
- 1X LiAc/0.5X TE (see **Recipe**, page 18)
- Denatured salmon sperm DNA (see recipe on page 21)
- pYES-DEST52 construct
- 1X LiAc/40% PEG-3350/1X TE (see **Recipe**, page 18)
- DMSO
- Selective plates

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Small-Scale Yeast Transformation, continued

Protocol

1. Inoculate 10 ml of YPD medium with a colony of your yeast strain and shake overnight at 30°C.
2. Determine the OD_{600} of your overnight culture. Dilute culture to an OD_{600} of 0.4 in 50 ml of YPD medium and grow an additional 2-4 hours.
3. Pellet the cells at 2500 rpm. Resuspend the pellet in 40 ml 1X TE.
4. Pellet the cells at 2500 rpm. 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/ml.

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 (Catalog no. D9156).

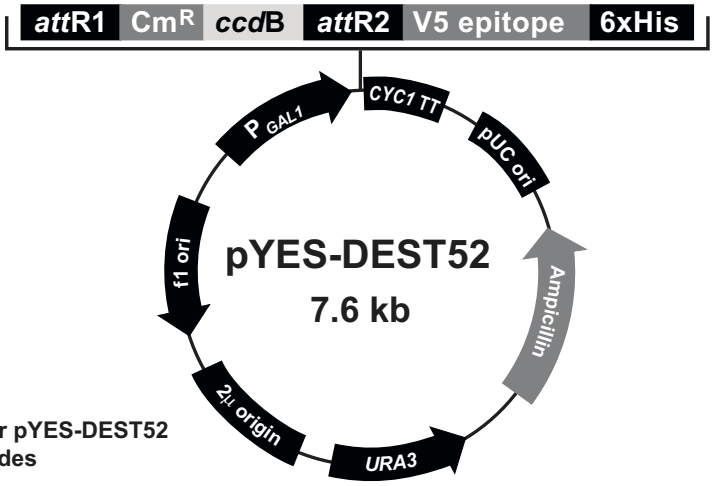
Protocol

1. In a 250 ml flask, dissolve 1 g salmon sperm DNA (Sigma-Aldrich, Catalog no. D1626) into 100 ml of TE (10 mg/ml). Pipet up and down with a 10 ml pipet to dissolve completely.
2. Incubate overnight at +4°C.
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 sonicated DNA into four 50 ml conical centrifuge tubes (25 ml per tube).
5. Extract with 25 ml of TE-saturated phenol. Centrifuge at 10,000 x g for 5 minutes at +4°C. Transfer the DNA (upper layer) to a fresh 50 ml conical centrifuge tube.
6. Extract with 25 ml of TE-saturated phenol:chloroform:isoamyl alcohol (25:24:1). Centrifuge at 10,000 x g for 5 minutes at +4°C. Transfer the DNA (upper layer) to a fresh 50 ml conical centrifuge tube.
7. Extract with 25 ml of chloroform. Centrifuge at 10,000 x g for 5 minutes at +4°C. Transfer the DNA (upper layer) to a 250 ml centrifuge bottle.
8. Add 5 ml of 3 M sodium acetate, pH 6.0 (1/10 volume) and 125 ml ice-cold (-20°C) 95% ethanol (2.5 volume) to precipitate DNA.
9. Pellet the DNA at 12,000 x g for 15 minutes at +4°C.
10. Wash the DNA once with 200 ml 70% ethanol and centrifuge as described in step 9.
11. Partially dry DNA by air or in a Speed-Vac (cover tubes with Parafilm® and poke holes in top) for 20 minutes.
12. Transfer DNA to a 250 ml sterile flask and dissolve DNA in 100 ml sterile TE (10 mg/ml).
13. Boil for 20 minutes to denature DNA. Immediately place on ice, aliquot in 1 ml samples, and freeze at -20°C.

Map and Features of pYES-DEST52

Map of pYES-DEST52

The map below shows the elements of pYES-DEST52. DNA from the entry clone replaces the region between bases 518 and 2228. **The complete sequence of pYES-DEST52 is available from our Web site (www.invitrogen.com) or by contacting Technical Service (page 24).**



Comments for pYES-DEST52
7621 nucleotides

GAL1 promoter: bases 1-451
T7 promoter: bases 475-494
*att*R1 recombination site: bases 511-635
Chloramphenicol resistance gene: bases 771-1430
*ccd*B gene: bases 1772-2077
*att*R2 recombination site: bases 2118-2242
V5 epitope: bases 2265-2306
Polyhistidine (6xHis) region: bases 2316-2333
CYC1 transcription termination signal: bases 2366-2619
pUC origin: bases 2803-3476
Ampicillin (*bla*) resistance gene: bases 3321-4481 (complementary strand)
*URA*3 gene: bases 4499-5606 (complementary strand)
2µ origin: bases 5610-7081
f1 origin: bases 7149-7604 (complementary strand)

continued on next page

Map and Features of pYES-DEST52, continued

Features of pYES-DEST52

pYES-DEST52 (7621 bp) contains the following elements. All features have been functionally tested.

Feature	Benefit
GAL1 promoter	Allows inducible expression of genes cloned into pYES-DEST52 (West *et al.*, 1984)
T7 promoter	Allows *in vitro* transcription in the sense orientation
*att*R1 and *att*R2 sites	Allows recombinational cloning of the gene of interest from an entry clone
Chloramphenicol resistance gene	Allows counterselection of expression clones
*ccd*B gene	Allows negative selection of expression clones
V5 epitope (Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr)	Allows detection of the fusion protein by the Anti-V5 antibodies (Southern *et al.*, 1991)
C-terminal polyhistidine tag	Allows purification of your fusion protein on metal-chelating resin (*e.g.* ProBond™). Allows detection of the recombinant protein by the Anti-His(C-term) antibodies (Lindner *et al.*, 1997)
CYC1 transcription termination signal	Allows efficient termination and stabilization of mRNA
pUC origin	Allows high copy number replication and growth in *E. coli*
Ampicillin (*bla*) resistance gene	Allows selection of transformants in *E. coli*
URA3 gene	Allows selection of yeast transformants in uracil-deficient medium
2µ origin	Allows maintenance and high copy replication in yeast
f1 origin	Allows rescue of single-stranded DNA

Technical Service

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Technical Service, continued

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