

USER GUIDE

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by *life* technologies™

Regal™ Yeast Competent Cells

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Important Information

Shipping and Storage

The Regal™ Yeast Competent Cells kit is shipped on dry ice. Upon receipt, store as detailed below. The kit is guaranteed for 6 months from date of shipment if stored properly.

Item	Storage
Regal™ Yeast Competent Cells	-80°C
PEG/Lithium Acetate Solution and pYES2/CT/ <i>lacZ</i> Control Plasmid	-20°C
Zeocin™	-20°C, protected from light



This product contains Dimethyl Sulfoxide (DMSO), a hazardous material. Review the Material Safety Data Sheet before handling.

Contents

The Regal™ Yeast Competent Cells kit includes the following components. Transformation efficiency is 1×10^3 transformants per μg of control DNA.

Item	Composition	Amount
Regal™ Yeast Competent Cells Containing pRegal	--	11 \times 55 μl
PEG/Lithium Acetate Solution	40% PEG 3350 0.1 M lithium acetate	3 \times 1.5 ml
pYES2/CT/ <i>lacZ</i> Control Plasmid	Lyophilized in TE Buffer, pH 8.0	20 μg
Zeocin™	100 mg/ml in deionized, sterile water	1.25 ml

Genotype

MATa leu2-3 leu2-112 ura3-52 ade1 ile MEL1 cir-0

Product Use

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

Accessory Products

Additional Products

Additional products that may be used with Regal™ Yeast Competent Cells are available from Life Technologies. Ordering information is provided in the table below.

Product	Amount	Catalog no.
Zeocin™	1 g	R250-01
	5 g	R250-05
Yeast Nitrogen Base	1 pouch	Q300-07
	500 g	Q300-09

Galactose-Inducible Expression Vectors

Life Technologies offers a wide selection of yeast vectors containing the *GAL1* promoter and enhancer sequences to facilitate regulated expression of your gene of interest in *Saccharomyces cerevisiae*. Ordering information for some of these vectors is provided below. For more information about the vectors available, refer to our website (www.lifetechnologies.com) or contact Technical Support (page 18).

Product	Amount	Catalog no.
pYES-DEST52	6 µg	12286-019
pYES2.1 TOPO® TA Expression Kit	20 reactions	K4150-01
pYES2	20 µg	V825-20
pYC2/NT A, B, C	20 µg each	V8256-20
pYC2/CT	20 µg	V8255-20

Methods

Overview

Description

Regal™ Yeast Competent Cells allow inducible expression of the gene of interest from *GAL1*-based yeast expression vectors. When transformed with the pRegal vector, Regal™ cells express 20-to 40-fold higher levels of Gal3p, Gal4p, and Gal80p – three proteins that comprise a galactose-responsive regulatory switch for *GAL* promoters (Platt and Reece, 1998; Sil *et al.*, 1999). Overexpression of this switch machinery in Regal™ cells results in higher levels of expression of *GAL1*-based proteins when compared to the same yeast cells not carrying the pRegal vector.

Features

Features of the Regal™ Yeast Competent Cells kit include the following:

- pRegal vector (supplied in the same vial with the cells) for amplified expression of the galactose transcriptional switch machinery
 - Genetically defined haploid yeast strain suitable for genetic studies
 - *cir-0* genotype for optimal propagation of plasmids containing the entire 2 μ plasmid (i.e. pRegal) (Rose and Broach, 1990)
 - Competent cells provided in easy-to-use single format tubes
-

pRegal Vector

pRegal is a 2 μ plasmid containing the wild-type *GAL3*, *GAL4*, and *GAL80* yeast genes. pRegal also contains the Zeocin™ resistance gene and the *LEU2* gene for selection in yeast. Regal™ yeast cells transformed with the pRegal vector will constitutively overexpress Gal3p, Gal4p, and Gal80p while maintaining wild-type stoichiometry between the proteins (Sil *et al.*, 2000). For more information on the Gal3p-Gal4p-Gal80p switch proteins, refer to Platt and Reese, 1998 and Sil *et al.*, 1999. For more information on the pRegal vector, refer to the map on page 15.

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Overview, continued

Note

pRegal is supplied as part of the Regal™ Yeast Competent Cells solution. When you transform Regal™ competent cells, you will be performing a co-transformation with the pRegal vector, already in the cell mix, and your expression construct.

Transforming Regal™ Competent Cells

Introduction

A protocol to transform Regal™ cells is provided in this section. We recommend including the pYES2/CT/*lacZ* control plasmid in your experiments to help you evaluate your results.

Important Guidelines for Transformation

Consider the following before transforming Regal™ cells with your plasmid of interest:

- To select for the pRegal plasmid, you must use selective medium containing Zeocin™. Refer to page 14 for information on storing and handling Zeocin™.
- Transform Regal™ cells with an expression construct carrying an *ADE1*, *ILE*, or *URA3* selectable marker and select for transformants using media deficient for the appropriate amino acid (refer to page v for the genotype of Regal™ yeast cells).

Note: Do not use an expression construct with a leucine selectable marker as the *LEU2* gene is present in the pRegal vector.

Important

Do not use leucine selection to select for the pRegal plasmid. pRegal contains the *leu2-d* allele of the *LEU2* gene which has a truncated but functional promoter. Due to reduced promoter efficiency, newly transformed yeast cells will not express enough Leu2p to survive in media lacking leucine. You must use Zeocin™ selection to select for the pRegal plasmid (see **Important Guidelines for Transformation**, above).

URA3 Expression Vectors

Life Technologies offers a wide selection of *URA3*, *GAL1*-based expression vectors for regulated expression of your gene of interest. For more information about the vectors available, refer to our website (www.lifetechnologies.com) or contact Technical Support (page 18).

Positive Control

pYES2/CT/*lacZ* is provided as a positive control for yeast transformation and contains a *URA3* selectable marker. To use the plasmid, resuspend in 20 µl of sterile water to obtain a final concentration of 1 µg/µl.

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Transforming Regal™ Competent Cells, continued

Required Selective Plates

The table below lists the selective plates needed to select for Regal™ cells containing the pRegal vector and the pYES2/CT/*lacZ* control plasmid. You will need to make the appropriate adjustments if your expression construct contains a selectable marker other than *URA3*.

pRegal	pYES2/CT/ <i>lacZ</i>	Selective Plates
Zeocin™ selection marker	<i>URA3</i> selection marker	Synthetic complete with Zeocin™ minus uracil (SC+Zeo-U)

Note

The list of required materials and the transformation protocol in this section are provided with the assumption that your expression construct contains a *URA3* selectable marker. If your expression construct contains an *ADE1* or *ILE* selectable marker, you will need to have selective plates that contain Zeocin™ and are deficient for the appropriate amino acid (i.e. adenine or isoleucine).

Materials Needed

You should have the following materials on hand before beginning:

- PEG/LiAc solution (supplied with the kit)
 - Regal™ Yeast Competent Cells (supplied with the kit; one vial per transformation)
 - Plasmid DNA containing gene of interest
 - pYES2/CT/*lacZ* control plasmid, optional (resuspended to 1 µg/µl)
 - DMSO
 - YPD liquid medium (see page 13 for a recipe)
 - Synthetic complete plates minus uracil plus 100 µg/ml Zeocin™ (prewarmed to room temperature; see page 12 for a recipe)
 - 30°C water bath
 - 42°C water bath
 - 30°C shaking incubator
-

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Transforming Regal™ Competent Cells, continued

Transformation Protocol

Use this protocol to transform Regal™ cells with your expression construct. Note that this protocol includes a two hour recovery period at 30°C (Step 9) prior to selection on Zeocin™ selective plates.

1. Thaw the PEG/LiAc solution in a beaker of room temperature water.
2. Thaw one vial of Regal™ competent cells for each transformation by placing the vial in a 30°C water bath for no more than 30 seconds. Proceed immediately to Step 3.
3. Add 1 µg of plasmid DNA into a vial of Regal™ cells and mix gently by tapping. **Do not mix by pipetting up and down.** For the pYES2/CT/*lacZ* control plasmid, add 1 µl (1 µg) into a separate vial of Regal™ cells and mix gently.
4. Mix the thawed PEG/LiAc solution by pipetting up and down and transfer 300 µl to each vial of cells. Mix the contents by inverting the vial several times.
5. Incubate the cells for 30 minutes in a 30°C water bath. During this incubation time, invert the vial(s) every 10 minutes to ensure components are resuspended.
6. Add 18 µl of DMSO to each vial. Mix the contents by inverting the vial(s) several times.
7. Heat shock the cells for 20 minutes in a 42°C water bath. Occasionally invert the vial(s) to ensure components are resuspended.
8. Centrifuge vial(s) in a microcentrifuge for 5 seconds at low speed (1800 rpm, 200-400 × g). Carefully discard the supernatant.
9. Add 1 ml YPD medium to the cell pellet and resuspend by gently pipetting. Incubate samples for 2 hours in a 30° shaking incubator.
10. Plate 100 µl of cells on synthetic complete plates minus uracil plus 100 µg/ml Zeocin™. Incubate plates at 30°C for 72 hours.

You should see >100 colonies on a plate for your control transformation with pYES2/CT/*lacZ*. Refer to the **Troubleshooting** section, page 10, if you have problems obtaining transformants.

Expressing Your Protein

Introduction

Once you have obtained a transformant containing both pRegal and your expression construct, you are ready to induce expression of your protein of interest. Guidelines are provided below. Note that you will be using leucine selection and not Zeocin™ selection during the expression studies. For more information about expression in yeast, refer to the *Guide to Yeast Genetics and Molecular Biology* (Guthrie and Fink, 1991).

GAL1 Promoter

In typical *S. cerevisiae* laboratory strains, 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.

Raffinose as a Carbon Source

As an alternative to glucose, 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.

For optimal expression of your protein, we recommend incubating your yeast cells overnight in medium containing raffinose before inducing with galactose. Protein can be detected in as little as 2 hours after galactose induction.

Note

The list of required materials and the protocols in this section are provided with the assumption that your expression construct contains a *URA3* selectable marker. If your expression construct contains an *ADE1* or *ILE* selectable marker, you will need to have selective medium that is deficient for leucine as well as the appropriate amino acid (i.e. adenine or isoleucine).

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Expressing Your Protein, continued

Materials Needed

You should have the following materials on hand before beginning:

- Synthetic complete medium minus leucine, minus uracil (SC-L-U) with 2% glucose (see page 12 for a recipe)
 - Synthetic complete medium minus leucine, minus uracil (SC-L-U) with 2% raffinose (see page 12 for a recipe)
 - Induction medium (SC-L-U with 2% galactose; see page 13 for a recipe)
 - Sterile water
 - 50 ml conical tubes
 - 250 ml culture flasks
 - 15 ml snap-cap tubes
 - 30°C water bath
-

Preparing Yeast Cells for Induction

Use this protocol to grow transformed yeast cells overnight in medium containing raffinose.

1. Inoculate a single transformed colony into 15 ml of SC-L-U medium containing 2% glucose. Grow overnight at 30°C with shaking.
2. Determine the OD₆₀₀ of your overnight culture. Calculate the amount of overnight culture necessary to obtain an OD₆₀₀ of 0.1 in 15 ml of medium.

Example: The OD₆₀₀ of your overnight culture is 3 OD₆₀₀ per ml. The amount of overnight culture needed to inoculate a 15 ml culture to OD₆₀₀ = 0.1 is:

$$\frac{(0.1 \text{ OD/ml}) (15 \text{ ml})}{3 \text{ OD/ml}} = 0.5 \text{ ml}$$

3. Remove the amount of culture determined in Step 2 and pellet the cells at 1500 × g for 5 minutes at +4°C. Discard the supernatant.
 4. Resuspend the cells in 1-2 ml of SC-L-U medium containing 2% raffinose and inoculate into a total volume of 15 ml of SC-L-U medium containing 2% raffinose. Grow overnight at 30°C with shaking. Proceed to **Inducing Yeast Cells with Galactose**, page 8.
-

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Expressing Your Protein, continued

Inducing Yeast Cells with Galactose

If you are assaying expression of your protein for the first time, we recommend that you perform a time course to optimize induction and expression. A standard protocol is provided below. Other protocols are suitable.

1. Determine the OD₆₀₀ of your overnight culture. Calculate the amount of overnight culture necessary to obtain an OD₆₀₀ of 0.4 in 50 ml of medium.
 2. Remove the amount of culture determined in Step 1 and pellet the cells at 1500 × g for 5 minutes at +4°C. Discard the supernatant.
 3. Resuspend the cells in 1-2 ml of induction medium (SC-L-U medium containing 2% galactose) and inoculate into a total volume of 50 ml of induction medium.
 4. For each time point (0, 4, 8, 12, 16, and 24 hours after addition of induction medium), remove 5 ml of culture from the flask and determine the OD₆₀₀ of each sample. You will use this information when assaying your protein (see Step 2 page 9). Perform Steps 5-8 for each time point sample.
 5. Centrifuge the cells at 1500 × g for 5 minutes at +4°C.
 6. Decant the supernatant. Resuspend cells in 500 µl of sterile water.
 7. Transfer cells to a sterile microcentrifuge tube. Centrifuge samples for 30 seconds at top speed in the microcentrifuge.
 8. Decant the supernatant. Store the cell pellets at -80°C until ready to use. Proceed to **Analyzing Samples**, page 9.
-

Analyzing Samples

Introduction

You may detect your protein by Western blot using an antibody to your protein or an antibody to any epitope tag that may be fused to your protein. A general protocol to prepare cell lysates for analysis is provided below. Other protocols are suitable.

Materials Needed

You should have the following materials on hand before beginning:

- Breaking buffer (50 mM sodium phosphate, pH 7.4; 1 mM EDTA; 1 mM PMSF; 5% glycerol)
 - Acid-washed glass beads (0.4-0.6 mm size; Sigma-Aldrich, Catalog no. G8772)
 - 4X SDS-PAGE sample buffer (see page 13)
-

Preparing Cell Lysates

1. Resuspend fresh or frozen cell pellets in 500 μ l of breaking buffer. Centrifuge at 1500 \times g for 5 minutes at +4°C to pellet cells.
 2. Remove supernatant and resuspend the cells in a volume of breaking buffer to obtain an OD₆₀₀ of 50-100. Use the OD₆₀₀ determined in Step 4 of the protocol on the previous page to calculate the appropriate volume of breaking buffer to use.
 3. Add an equal volume of acid-washed glass beads.
 4. Vortex mixture for 30 seconds followed by 30 seconds on ice. Repeat four times for a total of four minutes to lyse the cells.
 5. Centrifuge samples in a microcentrifuge for 10 minutes at maximum speed.
 6. Remove supernatant and transfer to a fresh microcentrifuge tube. Assay the lysate for protein concentration using BSA as a standard.
 7. Add SDS-PAGE sample buffer to a final concentration of 1X and heat the sample for 5 minutes at 70°C.
 8. Load 20 μ g of lysate onto an SDS-PAGE gel and electrophorese.
-

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Analyzing Samples, continued

Polyacrylamide Gel Electrophoresis

To facilitate separation and visualization of your protein by polyacrylamide gel electrophoresis, a wide range of pre-cast NuPAGE® and Novex® Tris-Glycine polyacrylamide gels and electrophoresis apparatus are available from Life Technologies. For more information, refer to our website (www.lifetechnologies.com) or contact Technical Support (page 18).

Detecting β -galactosidase

If you use the pYES2/CT/*lacZ* plasmid as a positive control vector, you may assay for β -galactosidase expression with the β -Gal Assay Kit (Catalog no. K1455-01) or the β -Gal Staining Kit (Catalog no. K1465-01) available from Life Technologies.

You may also detect β -galactosidase expression by Western blot analysis using the β -Gal Antiserum, Anti-V5 antibodies, or Anti-His(C-term) antibodies available from Life Technologies. For more information on these antibodies, refer to our website (www.lifetechnologies.com) or contact Technical Support (page 18).

Troubleshooting

Introduction

The table below lists some potential problems and possible solutions that may help you troubleshoot transformation of Regal™ Yeast Competent Cells.

Problem	Reason	Solution
Few or no colonies obtained from sample reaction and the transformation control gave colonies	Incorrect selective plates used to select for expression construct with <i>ADE1</i> or <i>ILE</i> selectable marker	Use synthetic complete plates containing 100 µg/ml Zeocin™ and lacking either adenine or isoleucine.
Few or no colonies obtained from sample reaction and the transformation control gave no colonies	Transformants selected on plates deficient in leucine	Select for transformants on plates containing both leucine and Zeocin™ (see page 3 for transformation guidelines).
	Zeocin™ recovery step omitted or performed in incorrect media	Allow transformants to recover for 2 hours at 30°C in YPD media before plating (see Step 9 of the Transformation Protocol on page 5).
	Incorrect selective plates used	<ul style="list-style-type: none"> • To select for your expression construct, use synthetic complete plates containing 100 µg/ml Zeocin™ and lacking the appropriate amino acid. • To select for the pYES2/CT/<i>lacZ</i> control, use synthetic complete plates containing 100 µg/ml Zeocin™ and lacking uracil.

Appendix

Recipes

SC Minimal Medium and Plates

- 0.67% yeast nitrogen base (**without** amino acids **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)
 - 0.01% Zeocin™ (for Zeocin™ selection of transformants)
 - 2% agar (for plates)
1. Dissolve the following reagents in 900 ml deionized water (800 ml if preparing medium containing raffinose). **Note:** You may also prepare 100X solutions of each amino acid and add as needed.

**Reminder: Omit uracil to make SC-U selective plates.
Omit uracil and leucine to make SC-U-L selective plates.**

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

2. For plates, add agar after dissolving the reagents above.
3. Autoclave for 20 minutes on liquid cycle.
4. Cool to 50°C and add 100 ml of filter-sterilized 20% glucose or 200 ml of filter-sterilized 10% raffinose.
5. For synthetic complete medium with Zeocin™, add 1 ml of 100 mg/ml Zeocin™.
6. Pour plates and allow to harden. Invert the plates and store at +4°C. Plates without Zeocin™ are stable for 6 months. Plates containing Zeocin™ should be stored in the dark and are stable for 1 month.

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Recipes, continued

Important

If you are making selective plates, add filter-sterilized glucose after the broth has been autoclaved and cooled. Autoclaving agar and glucose together will cause the glucose to caramelize.

Induction Medium

If you are making induction medium, follow Steps 1-3 on the previous page. Cool the medium to 50°C and add 100 ml of filter-sterilized 20% galactose.



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 Broth

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

1. Dissolve 10 g yeast extract, 20 g peptone, and 20 g dextrose in 1000 ml of water.
 2. Autoclave for 20 minutes on liquid cycle.
 3. Store at room temperature. YPD broth is stable for one to two months.
-

4X SDS-PAGE Sample Buffer

1. Combine the following reagents:

0.5 M Tris-HCl, pH 6.8	5 ml
Glycerol (100%)	4 ml
β -mercaptoethanol	0.8 ml
Bromophenol Blue	0.04 g
SDS	0.8 g
 2. Bring the volume to 10 ml with sterile water.
 3. Aliquot and freeze at -20°C until needed.
-

Zeocin™

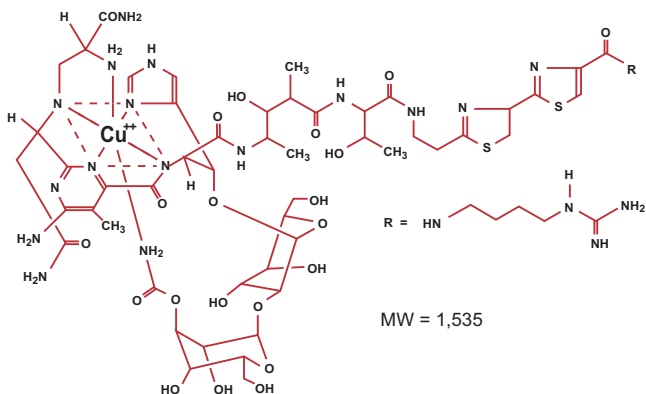
Introduction

Zeocin™ is a member of the bleomycin/phleomycin family of antibiotics isolated from *Streptomyces*. It shows strong toxicity against bacteria, yeast, plants and mammalian cell lines (Calmels *et al.*, 1991; Drocourt *et al.*, 1990; Gatignol *et al.*, 1987; Mulsant *et al.*, 1988; Perez *et al.*, 1989).

A Zeocin™ resistance protein has been isolated and characterized (Calmels *et al.*, 1991; Drocourt *et al.*, 1990). This 13,665 Da protein, the product of the *Sh ble* gene (*Streptoalloteichus hindustanus* bleomycin gene), binds stoichiometrically to Zeocin™ and inhibits its DNA strand cleavage activity. Expression of this protein in eukaryotic and prokaryotic hosts confers resistance to Zeocin™. Review the Material Safety Data Sheet for more information.

Molecular Weight, Formula, and Structure

The formula for Zeocin™ is $C_{60}H_{89}N_{21}O_{21}S_3$ and the molecular weight is 1,535. The structure of Zeocin™ is shown below.



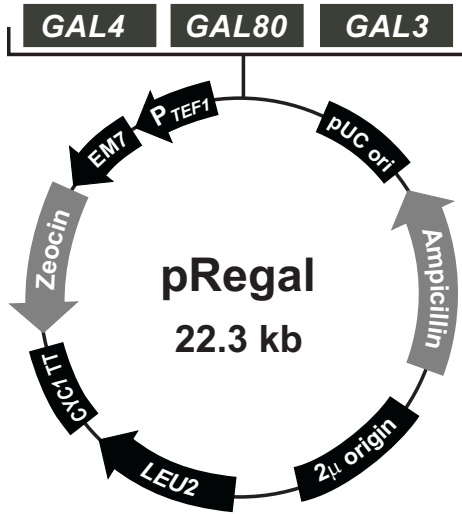
Handling Zeocin™

- Store Zeocin™ at -20°C and thaw on ice before use.
- Zeocin™ is light sensitive. Store the drug and plates or medium containing the drug in the dark.
- Wear gloves, a laboratory coat, and safety glasses when handling Zeocin™-containing solutions.
- Do not ingest or inhale solutions containing the drug.
- Be sure to bandage any cuts on your fingers to avoid exposure to the drug.

Map and Features of pRegal

Map of pRegal

The map below shows the elements of the pRegal vector. pRegal is provided in the same vial with the Regal™ competent cells. The complete sequence of pRegal is available from our website (www.lifetechnologies.com) or by contacting Technical Support (page 18).



Comments for pRegal 22336 nucleotides

GAL4 gene: bases 429-3074

GAL80 gene: bases 5042-6349

GAL3 gene: bases 7153-8715

pUC origin: bases 11321-11994

Ampicillin (*bla*) resistance gene: bases 12139-12999 (complementary strand)

2μ origin: bases 14013-15204

LEU2 gene: bases 15262-16356

CYC1 transcription termination signal: bases 21158-21411

Zeocin™ (*Sh ble*) resistance gene: bases 21476-21850 (complementary strand)

EM7 promoter: bases 21851-21917 (complementary strand)

TEF1 promoter: bases 21921-22331 (complementary strand)

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Map and Features of pRegal, continued

Features of pRegal

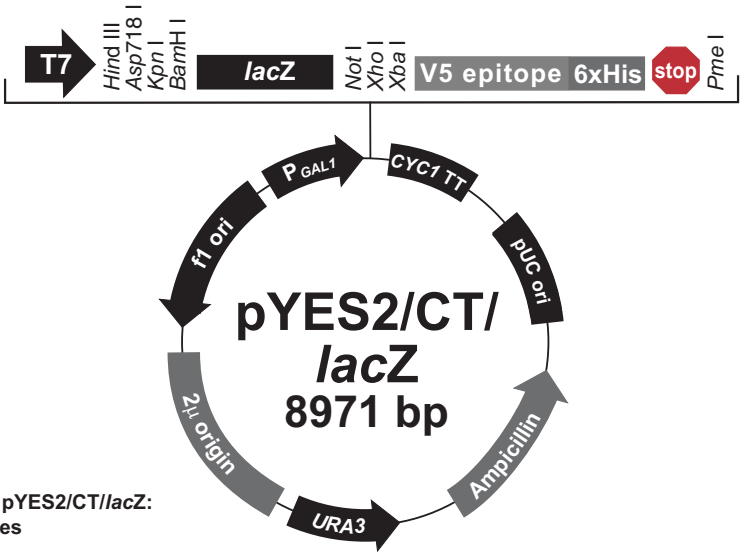
pRegal contains the following elements. All features have been functionally tested.

Feature	Benefit
<i>GAL4</i> , <i>GAL80</i> , and <i>GAL3</i> genes	Encode for transcription switch proteins required for activation of <i>GAL</i> gene promoters (Platt and Reece, 1998; Sil <i>et al.</i> , 1999)
pUC origin	Allows high copy number replication and growth in <i>E. coli</i>
Ampicillin (<i>bla</i>) resistance gene	Allows selection of transformants in <i>E. coli</i>
2 μ origin	Allows maintenance and high copy number replication in yeast
<i>LEU2</i> gene	Allows maintenance of yeast transformants in leucine-deficient medium
<i>CYC1</i> transcription termination signal	Allows efficient termination and stabilization of mRNA
Zeocin™ (<i>Sh ble</i>) resistance gene	Allows selection of yeast transformants in medium containing Zeocin™
EM7 promoter	Allows expression of the Zeocin™ resistance gene in <i>E. coli</i>
<i>TEF1</i> promoter	Allows expression of the Zeocin™ resistance gene in yeast

Map of pYES2/CT/lacZ

Introduction

pYES2/CT/lacZ is a 8971 bp vector containing the gene for β -galactosidase. β -galactosidase is expressed as a fusion protein containing a C-terminal V5 epitope and polyhistidine (6xHis) tag. The molecular weight of the fusion protein is approximately 120 kDa. **The complete sequence of pYES2/CT/lacZ is available from our website (www.lifetechnologies.com) or by contacting Technical Support (page 18).**



Comments for pYES2/CT/lacZ: 8971 nucleotides

GAL1 promoter: bases 1-451

lacZ ORF: bases 528-3583

V5 epitope: bases 3615-3656

Polyhistidine (6xHis) region: bases 3666-3683

CYC1 transcription termination signal: bases 3716-3969

pUC origin: bases 4153-4826

Ampicillin (*bla*) resistance gene: bases 4971-5831 (complementary strand)

URA3 gene: bases 5849-6956 (complementary strand)

2 μ origin: bases 6960-8431

f1 origin: bases 8499-8954 (complementary strand)

Technical Support

Obtaining Support

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

At the website, you can:

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

Safety Data Sheets (SDS)

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

Certificate of Analysis

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

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Technical Support, continued

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**Information for
European
Customers**

Regal™ Yeast Competent Cells and the pRegal vector are supplied together in the same vial. When a transformation procedure is performed, Regal™ cells will carry the pRegal plasmid and will become genetically modified organisms even in the absence of any exogenously introduced plasmid.

References

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