pESC Yeast Epitope Tagging Vectors

Instruction Manual

Catalog #217451 (pESC-HIS Vector), #217452 (pESC-LEU Vector), #217453 (pESC-TRP Vector), #217454 (pESC-URA Vector), and #217455 (All four pESC vectors)

Revision B

Research Use Only. Not for Use in Diagnostic Procedures.

217451-12

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pESC Yeast Epitope Tagging Vectors

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pESC Yeast Epitope Tagging Vectors

MATERIALS PROVIDED

Vectors (supercoiled)					Sequencing primers (lyophilized, 4 per kit)	Yeast host strains
Catalog #	pESC-HIS	pESC-LEU	pESC-TRP	pESC-URA		
217451	20 µg	—	—	—	2.5 µg/primer	YPH 499/500/501
217452	—	20 µg	—	—	2.5 µg/primer	YPH 499/500/501
217453	—	—	20 µg	—	2.5 µg/primer	YPH 499/500/501
217454	—	—	—	20 µg	2.5 µg/primer	YPH 499/500/501
217455	20 µg	20 µg	20 µg	20 µg	2.5 µg/primer	YPH 499/500/501

STORAGE CONDITIONS

pESC Vectors: –20°C
Lyophilized Sequencing Primers: Room Temperature
Yeast Strains: –80°C

SEQUENCING PRIMERS AND SEQUENCES

Sequencing primers	Sequence
GALl forward primer	5′ ATTTTCGGTTTGTATTACTTC 3′
GALl reverse primer	5′ GTTCTTAATACTAACATAACT 3′
GALl0 forward primer	5′ GGTGGTAATGCCATGTAATATG 3′
GALl0 reverse primer	5′ GGCAAGGTAGACAAGCCGACAAC 3′

HOST STRAIN GENOTYPES

Host strain	Genotype	Mating type
YPH499 Yeast Strain	*ura3–52 lys2–801*^{amber} *ade2–101*^{ochre} *trp1–Δ63 his3–Δ200 leu2–Δ1*	a
YPH500 Yeast Strain	Same as the YPH499 yeast strain.	α
YPH501 Yeast Strain	Same as the YPH499 yeast strain.	a/α

ADDITIONAL MATERIALS REQUIRED

Tween® 20
Ampicillin
Polyethylene glycol 3350 (PEG 3350)
L-adenine hemisulfate salt
Yeast extract
Bacto® peptone
Bacto® agar
T4 DNA ligase
Yeast nitrogen base without amino acids [e.g., Difco® Yeast Nitrogen Base (Catalog #0919-15-3) or equivalent]

Revision B

NOTICES TO PURCHASER

FLAG® Epitope Tag

Manufactured under license from Sigma-Aldrich Co. under U.S. Patent Nos. 4,703,004, 4,782,137 and 4,851,341. Other patents pending.

Anti-FLAG® Antibody

Purchased from Sigma for resale under license from Sigma-Aldrich Co. under U.S. Patent Nos. 4,703,004, 4,782,137 and 4,851,341. Other patents pending. FLAG® and Anti-FLAG® are ® s of Sigma-Aldrich Co. M1, M2, and M5 are trademarks of Sigma-Aldrich Co. Use of this product is solely for investigational, experimental or research purposes. Not for commercial, diagnostic or therapeutic uses.

INTRODUCTION

The pESC vectors are a series of epitope-tagging vectors designed for expression and functional analysis of eukaryotic genes in the yeast *S. cerevisiae*. These vectors contain the *GAL1* and *GAL10* yeast promoters in opposing orientation. With these vectors one or two cloned genes can be introduced into a yeast host strain under the control of a repressible promoter. When two genes are co-expressed, protein-protein interactions can be confirmed by immunoprecipitation analysis. These vectors feature an extensive polylinker sequence and end-specific RNA transcripts generated from T3 and T7 promoters. Each of the pESC vectors contains one of four different yeast-selectable markers (*HIS3, TRP1, LEU2,* or *URA3*) in the same vector backbone, which allows expression and epitope-tagging analysis of two different genes in a single yeast cell.

The pESC vectors contain DNA sequences coding for epitope peptides that can be specifically recognized by monoclonal antibodies. A sequence for the FLAG® epitope (DYKDDDDK)[1] is located in the multiple cloning site (MCS) downstream of the *GAL10* promoter; a sequence for the c-myc epitope (EQKLISEEDL)[2] is located in the MCS downstream of the *GAL1* promoter. The gene of interest can be inserted in front of the epitope sequence to generate C-terminal tagging or after the epitope sequence for N-terminal tagging. These tags allow the protein of interest to be studied without generating a specific antibody to that protein. The epitope tags can be easily detected in transformed cells using well-characterized, commercially available antibodies.

The pESC vectors are yeast episomal plasmids (YEps) that use the pBluescript II SK(+) phagemid backbone. Each pESC vector contains the following features:

- pUC plasmid origin of replication and the ampicillin-resistance gene (*bla*), which allow for replication and selection in *E. coli*
- the yeast 2μ origin, which enables autonomous replication of the plasmids in *S. cerevisiae*
- an auxotrophic selectable marker gene (*HIS3, TRP1, LEU2,* or *URA3*) to select and maintain the expression vector in yeast cells
- the expression cassette, which contains the *S. cerevisiae GAL1* and *GAL10* promoters in opposing orientation, two MCS sequences, and a transcription termination sequence downstream of each promoter

pESC Vector Maps

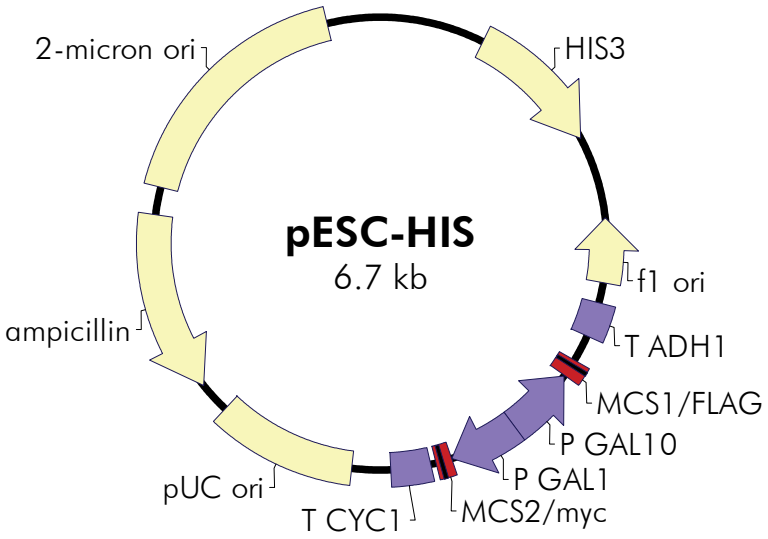
Circular maps and MCS sequences for the pESC vectors are shown in Figures 1–4. The complete sequences for the vectors are available from *www.genomics.agilent.com* and from the GenBank® database (pESC-HIS: Accession #AF063850, pESC-LEU: Accession #AF063849, pESC-TRP: Accession #AF063848, pESC-URA: Accession #AF063585). The table below summarizes the unique restriction sites found in the multiple cloning sites of the pESC vectors.

Unique Polylinker Sites in the pESC Vectors

Enzymes	Vector			
	pESC-HIS	pESC-LEU	pESC-TRP	pESC-URA
Apa I	Yes	Yes	Yes	No
BamH I*	Yes	Yes	Yes	Yes
Bgl II	No	Yes	Yes	Yes
Cla I	Yes	No	Yes	Yes
EcoR I	Yes	No	Yes	Yes
Hind III	No	Yes	No	Yes
Kpn I	No	No	Yes	Yes
Nhe I	No	Yes	Yes	Yes
Not I	Yes	Yes	Yes	Yes
Pac I	Yes	Yes	Yes	Yes
Sac I	Yes	Yes	Yes	Yes
Sal I	Yes	Yes	Yes	Yes
Spe I	Yes	Yes	Yes	Yes
Srf I	Yes	Yes	Yes	Yes
Xho I	Yes	Yes	Yes	Yes

*The BamH I site may be used for directional cloning only, due to the presence of an in-frame stop codon in the MCS2 sequence of each pESC vector. When constructing C-terminal myc-tagged proteins using the BamH I site, use a directional cloning strategy that removes the stop codon by double-digestion of the vector with BamH I plus an enzyme that cleaves downstream of the stop codon.

The pESC-HIS Vector



pESC-HIS Multiple Cloning Site 1 Region
(sequence shown 2255–2338, bottom strand)

```
EcoR I                          Not I       Spe I   Cla I                                   Sac I   Pac I
GAA TTC AAC CCT CAC TAA AGG GCG GCC GCA CTA GTA TCG ATG GAT TAC AAG GAT GAC GAC GAT AAG ATC TGA GCTCTTAATTAA
                                                    M  D  Y  K  D  D  D  D  K  I  STOP
                                                    FLAG epitope
```

pESC-HIS Multiple Cloning Site 2 Region
(sequence shown 3011–3108, top strand)

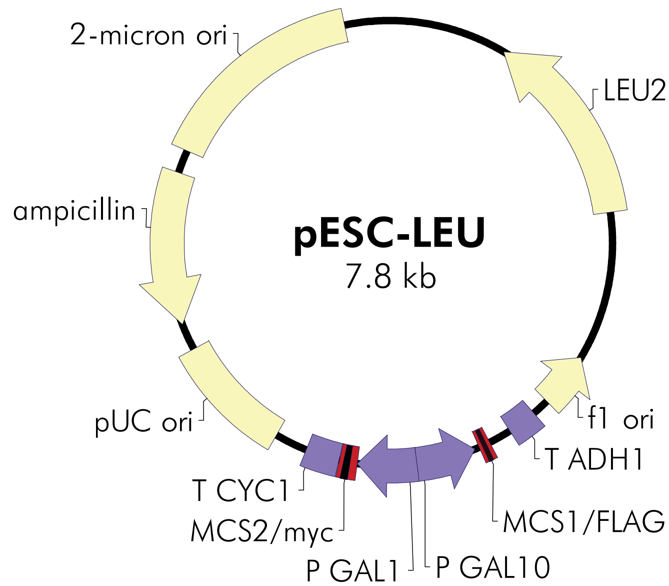
```
BamH I                                  Apa I  Srf I        Sal I
G GAT CCG TAA TAC GAC TCA CTA TAG GGC CCG GGC GTC GAC...
                                (STOP)

                                                        Xho I
...ATG GAA CAG AAG TTG ATT TCC GAA GAA GAC CTC GAG TAA GCTTGGTACCGCGGCTAGC
   M  E  Q  K  L  I  S  E  E  D  L  E  STOP
   myc epitope
```

Feature	Nucleotide Position
yeast *HIS3* selection marker ORF	504–1163
f1 origin of ss-DNA replication	1558–1864
yeast *ADH1* terminator	1960–2124
multiple cloning site 1	2255–2338
FLAG tag	2273–2299
yeast *GAL1/GAL10* divergent promoters	2343–3009
multiple cloning site 2	3011–3086
c-myc tag	3051–3086
yeast *CYC1* terminator	3113–3302
pUC origin of replication	3939–4156
ampicillin resistance (*bla*) ORF	4307–5164
2μ yeast origin of replication	5298–6453

FIGURE 1 The pESC-HIS vector. The complete sequence and list of restriction sites are available at *www.genomics.agilent.com*. Note that the sequence shown for MCS1 corresponds to the bottom strand sequence (the reverse-complement of the sequence available for this vector at *www.genomics.agilent.com*).

The pESC-LEU Vector



pESC-LEU Multiple Cloning Site 1 Region
(sequence shown 3294–3377 bottom strand)

```
                                          Not I          Spe I                                                                        Bgl II       Sac I      Pac I
GAA TTC AAC CCT CAC TAA AGG GCG GCC GCA CTA GTA TCG ATG GAT TAC AAG GAT GAC GAC GAT AAG ATC TGA GCTCTTAATTAA
                                                     M   D   Y   K   D   D   D   D   K   I  STOP
                                                         |_______FLAG epitope________|
```

pESC-LEU Multiple Cloning Site 2 Region
(sequence shown 4050–4147, top strand)

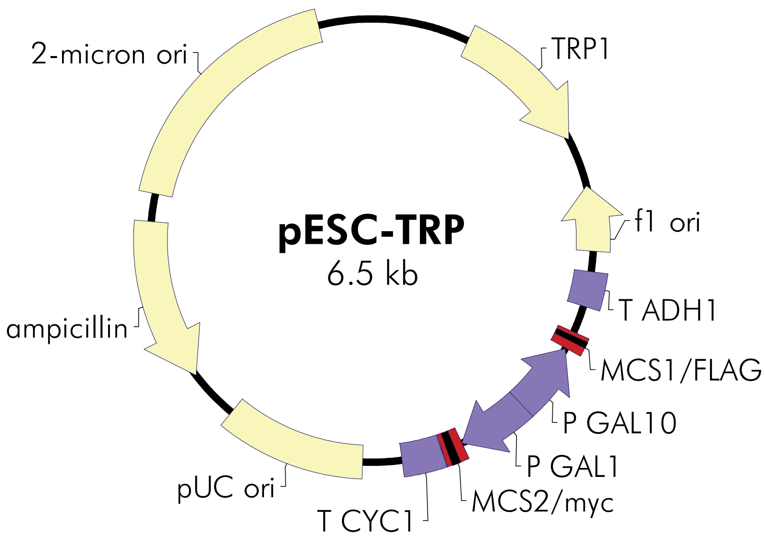
```
BamH I                                     Apa I Srf I         Sal I
G GAT CCG TAA TAC GAC TCA CTA TAG GGC CCG GGC GTC GAC...
                              (STOP)

                                             Xho I     Hind III          Nhe I
...ATG GAA CAG AAG TTG ATT TCC GAA GAA GAC CTC GAG TAA GCTTGGTACCGCGGCTAGC
    M   E   Q   K   L   I   S   E   E   D   L   E  STOP
        |__________myc epitope______________|
```

Feature	Nucleotide Position
yeast *LEU2* selection marker ORF	663–1757
f1 origin of ss-DNA replication	2597–2903
yeast *ADH1* terminator	2999–3163
multiple cloning site 1	3294–3369
FLAG tag	3325–3356
yeast *GAL1*/*GAL10* divergent promoters	3382–4048
multiple cloning site 2	4050–4147
c-myc tag	4090–4125
yeast *CYC1* terminator	4152–4341
pUC origin of replication	4528–5195
ampicillin resistance (*bla*) ORF	5346–6203
2μ yeast origin of replication	6337–7492

FIGURE 2 The pESC-LEU vector. The complete sequence and list of restriction sites are available at *www.genomics.agilent.com*. Note that the sequence shown for MCS1 corresponds to the bottom strand sequence (the reverse-complement of the sequence available for this vector at *www.genomics.agilent.com*).

The pESC-TRP Vector



pESC-TRP Multiple Cloning Site 1 Region
(sequence shown 2071–2154, bottom strand)

```
EcoR I                                        Not I          Spe I        Cla I                                                           Bgl II       Sac I      Pac I
GAA TTC AAC CCT CAC TAA AGG GCG GCC GCA CTA GTA TCG ATG GAT TAC AAG GAT GAC GAC GAT AAG ATC TGA GCTCTTAATTAA
                                                    M   D   Y   K   D   D   D   D   K   I   STOP
                                                        |_______ FLAG epitope _______|
```

pESC-TRP Multiple Cloning Site 2 Region
(sequence shown 2827–2924, top strand)

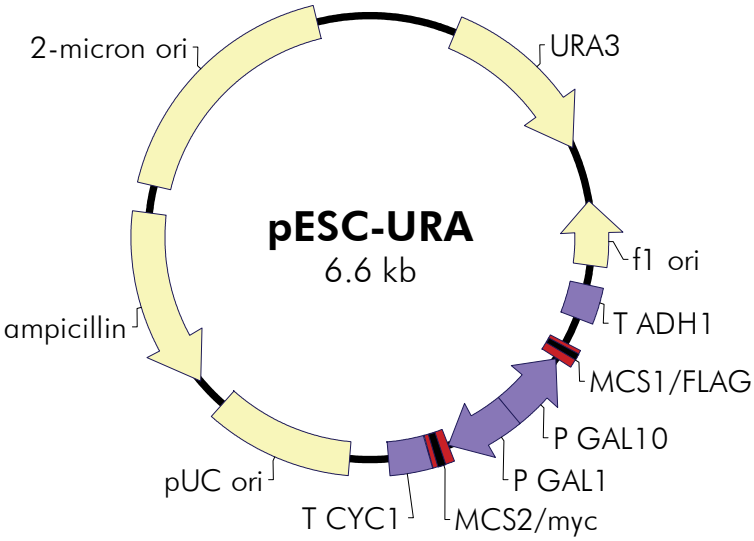
```
BamH I                                  Apa I Srf I        Sal I
G GAT CCG TAA TAC GAC TCA CTA TAG GGC CCG GGC GTC GAC...
                              (STOP)

                                               Xho I               Kpn I        Nhe I
...ATG GAA CAG AAG TTG ATT TCC GAA GAA GAC CTC GAG TAA GCTTGGTACCGCGGCTAGC
    M   E   Q   K   L   I   S   E   E   D   L   E   STOP
       |________ myc epitope ________|
```

Feature	Nucleotide Position
yeast *TRP1* selection marker ORF	469–1140
f1 origin of ss-DNA replication	1374–1680
yeast *ADH1* terminator	1776–1940
multiple cloning site 1	2071–2154
FLAG tag	2089–2115
yeast *GAL1*/*GAL10* divergent promoters	2159–2825
multiple cloning site 2	2827–2924
c-myc tag	2867–2902
yeast *CYC1* terminator	2929–3118
pUC origin of replication	3305–3972
ampicillin resistance (*bla*) ORF	4123–4980
2μ yeast origin of replication	5114–6269

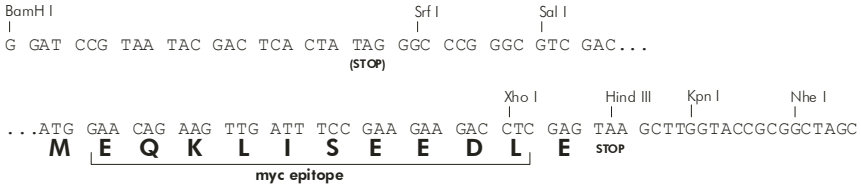
Figure 3 The pESC-TRP vector. The complete sequence and list of restriction sites are available at *www.genomics.agilent.com*. Note that the sequence shown for MCS1 corresponds to the bottom strand sequence (the reverse-complement of the sequence available for this vector at *www.genomics.agilent.com*).

The pESC-URA Vector



pESC-URA Multiple Cloning Site 1 Region
(sequence shown 2180–2263, bottom strand)

pESC-URA Multiple Cloning Site 2 Region
(sequence shown 2936–3033, top strand)



Feature	Nucleotide Position
yeast *URA3* selection marker ORF	417–1217
f1 origin of ss-DNA replication	1483–1789
yeast *ADH1* terminator	1885–2049
multiple cloning site 1	2180–2263
FLAG tag	2198–2224
yeast *GAL1/GAL10* divergent promoters	2268–2934
multiple cloning site 2	2936–3033
c-myc tag	2976–3011
yeast *CYC1* terminator	3038–3227
pUC origin of replication	3414–4081
ampicillin resistance (*bla*) ORF	4232–5089
2μ yeast origin of replication	5223–6378

FIGURE 4 The pESC-URA vector. The complete sequence and list of restriction sites are available at *www.genomics.agilent.com*. Note that the sequence shown for MCS1 corresponds to the bottom strand sequence (the reverse-complement of the sequence available for this vector at *www.genomics.agilent.com*).

YEAST HOST STRAIN

The yeast host strain is provided as a yeast glycerol stock. For the appropriate media, please refer to the following table:

Host strain	Agar plate for yeast streak	Medium for yeast glycerol stock
YPH strain	YPAD[a,b]	YPAD[a,b]

[a] See *Preparation of Media and Reagents*.

[b] Adenine sulfate is added to the medium to reduce the reversion rate of the *ade2-101* mutation thereby reducing the amount of reddish pigment in the yeast colonies.

On arrival, prepare the following from the yeast glycerol stock using the appropriate media as indicated in the previous table:

Note *The yeast host strain should be stored immediately at –80°C. It is also best to avoid repeated thawing of the yeast host strain in order to maintain extended viability.*

1. Take a sample of yeast cells from the frozen glycerol stock by scraping off splinters of solid ice with a sterile wire loop or sterile inoculating stick.

2. Streak the splinters onto a YPAD agar plate.

3. Incubate the plate at 30°C until colonies appear (~2–3 days).

4. Seal the plate with Parafilm® laboratory film and store the plate at 4°C for up to 1 week.

5. Restreak the colonies onto a fresh plate every week.

Stock Preservation

Yeast strains can be stored indefinitely in 15% (v/v) glycerol at ≤–60°C. (Yeast cannot be stored at temperatures above –55°C for prolonged periods.)

Grow the strains on YPAD agar plates. Scrape the yeast cells with a sterile applicator stick and suspend them in 1 ml of sterile 15% (v/v) glycerol in a 2-ml vial (35 × 12 mm). Tighten the cap and shake the vial before freezing. The yeast can be revived by transferring a small portion of the frozen sample to a YPAD agar plate.

PREPARING THE pESC VECTOR

The following are restriction enzymes with compatible ends to cloning sites in the pESC vectors.

Compatible Restriction Enzymes

Enzyme	Compatible enzymes
Bam HI	Bcl I, Bgl II, Mbo I, Sau 3A
Bgl II	Bcl I, Bam HI, Mbo I, Sau 3A
Cla I	Taq I, Bst BI, Acc I (certain sites)
Eco RI	Apo I, Mun I, Tsp 509I
Nhe I	Avr II, Spe I, Xba I
Not I	Bsp 120I, Eae I, Eag I
Sal I	Xho I
Spe I	Avr II, Nhe I, Xba I
Srf I	Blunt ends
Xho I	Sal I

- Cloned genes may be inserted to allow for either N- or C-terminal tagging with the c-myc or FLAG epitopes. Note that both the c-myc and the FLAG epitope coding sequences are flanked by an in-frame stop codon. To produce N-terminal fusions of these tags, the cloning strategy must remove the stop codon. For example, to produce a c-myc N-terminal fusion, insert the gene of interest at the *Xho* I site for bidirectional cloning or use *Xho* I as the upstream restriction site for unidirectional cloning.

- Ensure that the coding sequence of the insert is in the correct reading frame.

- To reduce background, dephosphorylate the digested pESC vector with CIAP prior to ligating with the insert DNA. If more than one restriction enzyme is used, the background can be reduced further by electrophoresing the DNA on an agarose gel and removing the desired plasmid band through electroelution, leaving behind the small fragment that appears between the two restriction enzyme sites.

- After purifying and ethanol precipitating the DNA, resuspend in a volume of TE buffer (see Preparation of Media and Reagents) that will allow the concentration of the plasmid DNA to be the same as the concentration of the insert DNA (~0.1 μg/μl).

LIGATING THE INSERT

The ideal insert-to-vector molar ratio of DNA is variable; however, a reasonable starting point is a 2:1 insert-to-vector ratio. The ratio is calculated using the following equation:

$$X\ \mu g \text{ of insert} = \frac{(\text{Number of base pairs of insert})(0.1\ \mu g \text{ of pESC vector})}{\text{Number of base pairs of pESC vector}}$$

where X is the quantity of insert (in micrograms) required for a 1:1 insert-to-vector molar ratio. Multiply X by 2 to get the quantity of insert required for a 2:1 ratio.

1. Prepare three control and two experimental 10-µl ligation reactions by adding the following components to separate sterile 1.5-ml microcentrifuge tubes:

   Note *For blunt-end ligation, reduce the rATP to 5 mM and incubate the reactions overnight at 12–14°C.*

Ligation reaction components	Control			Experimental	
	1[a]	2[b]	3[c]	4[d]	5[d]
Prepared pESC vector (0.1 µg/µl)	1.0 µl	1.0 µl	0.0 µl	1.0 µl	1.0 µl
Prepared insert (0.1 µg/µl)	0.0 µl	0.0 µl	1.0 µl	*W* µl	*X* µl
rATP [10 mM (pH 7.0)]	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl
Ligase buffer (10×)[e]	1.0 µl	1.0 µl	1.0 µl	1.0 µl	1.0 µl
T4 DNA ligase (4 U/µl)	0.5 µl	0.0 µl	0.5 µl	0.5 µl	0.5 µl
Double-distilled (ddH_2O) to 10 µl	6.5 µl	7.0 µl	6.5 µl	*Y* µl	*Z* µl

[a] This control tests for the effectiveness of the digestion and the CIAP treatment. Expect a low number of transformant colonies if the digestion and CIAP treatment are effective.

[b] This control indicates whether the plasmid is cleaved completely or whether residual uncut plasmid remains. Expect an absence of transformant colonies if the digestion is complete.

[c] This control verifies that the insert is not contaminated with the original plasmid. Expect an absence of transformant colonies if the insert is pure.

[d] These experimental ligation reactions vary the insert-to-vector ratio. Expect a majority of the transformant colonies to represent recombinants.

[e] See *Preparation of Media and Reagents*.

2. Incubate the reactions for 2 hours at room temperature (22°C) or overnight at 4°C.

Transformation

Transform competent bacteria with 1–2 µl of the ligation reaction, and plate the transformed bacteria on LB–ampicillin agar plates (see *Preparation of Media and Reagents*). See reference 3 for a transformation protocol.

VERIFICATION OF INSERT

Individual colonies can be examined to determine the presence of vectors with inserts by PCR directly from the colony or by restriction analysis.

Polymerase Chain Reaction Amplification of DNA from Individual Colonies

GAL1 forward/reverse primers can be used to confirm DNA insertion downstream of the *GAL1* promoter; GAL10 forward/reverse primers can be used to confirm DNA insertion downstream of the *GAL10* promoter. The corresponding positions of each primer in each vector are listed in the following table.

Primer complementary sequences

Primer	Promoter location	Vector positions			
		pESC-HIS	pESC-LEU	pESC-TRP	pESC-URA
GALI forward primer	GALI	2913-2933	3964-3984	2731-2751	2841-2861
GALI reverse primer	CYC1	3200-3180	4251-4231	3018-2998	3128-3108
GALI0 forward primer	GALI0	2416-2395	3467-3446	2234-2213	2344-2323
GALI0 reverse primer	ADH1	2194-2216	3245-3267	2012-2034	2122-2144

1. For each colony to be tested for the presence of an insert-containing pESC plasmid, prepare a PCR amplification reaction containing the following components:

   4.0 µl of 10× *Taq* DNA polymerase buffer††
   0.4 µl of dNTP mix (25 mM each dNTP)
   40.0 ng of forward primer
   40.0 ng of reverse primer
   0.4 µl of 10% (v/v) Tween® 20
   1.0 U of *Taq* DNA polymerase†
   dH_2O to a final volume of 40 µl

2. Stab a transformed colony with a sterile toothpick and swirl cells from the colony into the amplification reaction mixture. Immediately following inoculation into the reaction mixture, remove the toothpick and streak the remaining cells onto antibiotic-containing patch plates for future reference.

†,†† See *Endnotes*.

3. Gently mix each reaction, overlay each reaction with 30 µl of mineral oil and perform PCR using the following cycling parameters:

Number of cycles	Temperature	Length of time
1 cycle	94°C	4 minutes
	50°C	2 minutes
	72°C	2 minutes
30 cycles	94°C	1 minute
	56°C	2 minutes
	72°C	1 minute
1 cycle	72°C	5 minutes

4. Analyze the PCR products for the sizes of the gene inserted into the expression construct using standard 1% (w/v) agarose gel electrophoresis. Because the forward and reverse PCR/sequencing primers are located on both sides of the MCS, **the expected size of the PCR product should be 288 bp plus the size of the insert when GAL1 forward/reverse primers are used; the expected size of the PCR product should be 222 bp plus the size of the insert when GAL10 forward/reverse primers are used.** Additional information can be obtained by further restriction analysis of the PCR products.

PREPARATION OF YEAST COMPETENT CELLS

1. Dilute an overnight yeast culture 1:20 in 50 ml YPAD broth[§] to an OD_{600} of 0.25 (for 10 transformations).

2. Incubate the culture at 30°C for approximately 4–5 hours until it reaches an OD_{600} of 1.0.

3. Centrifuge the cells at 1000 × *g* for 5 minutes. Discard the supernatant.

4. Resuspend the cells in 10 ml of LTE buffer.[§]

5. Centrifuge the suspension at 1000 × *g* for 5 minutes. Discard the supernatant.

6. Resuspend the cells in 0.5 ml of LTE buffer. This cell suspension can be stored at 4°C for up to 3 days.

7. Aliquot 50 µl of the cell suspension into microcentrifuge tubes.

TRANSFORMATION OF WHOLE YEAST CELLS

Note *Prepare the Transformation Mix and amino acid dropout plates prior to beginning* Transformation of Whole Yeast Cells.

1. Add 1-3 µg of the chosen pESC plasmid or its derivatives.

2. Add 300 µl of the Transformation Mix[§] to each tube. Invert to mix.

3. Incubate the tubes at 30°C for 30 minutes.

4. Heat the tubes at 42°C for 15 minutes.

5. Plate 100 µl and 250 µl, respectively, of the transformation reaction onto two appropriate amino acid dropout plates (SD dropout plates[§]) with dextrose (D-glucose) as the carbon source. Incubate the plates at 30°C for 2-3 days.

[§] See *Preparation of Media and Reagents.*

GALACTOSE INDUCTION OF GENE EXPRESSION IN TRANSFORMED YEAST

Note *The* GAL1 *and* GAL10 *promoters are repressed when transformed cells are grown in SD dropout media and are induced when the cells are grown in SG dropout media.*

1. Pick one transformed colony, and streak out on an appropriate SD dropout plate. Incubate at 30°C overnight.

2. Inoculate 5 ml of the appropriate SD or SG drop out media with an isolated colony. Incubate the culture at 30°C for approximately 16 hours.

3. Harvest the cells by centrifugation at 1000 × *g* for 5 minutes. Discard the supernatant. The cells are now ready for further biochemical analysis.

TROUBLESHOOTING

Observations	Suggestions
No yeast transformants	Check to make sure that the appropriate plates and vectors were used in the transformation procedure.
	Use fresh 40% PEG Transformation Mix. Ensure that PEG with an average molecular weight of 3350 is used to prepare the Transformation Mix.
Failure to detect epitope-tagged fusion protein by western blotting	Use galactose-containing media for induction.
	Check whether the gene of interest is cloned in the correct orientation and that it is fused in frame with the epitope-coding sequence.

PREPARATION OF MEDIA AND REAGENTS

Synthetic Dextrose Minimal Medium (SD dropout media) (per Liter)	Synthetic Galactose Minimal Medium (SG dropout media) (per Liter)
6.7 g of yeast nitrogen base without amino acids (0.67% final) 20 g of dextrose (2% final) 1.3 g of amino acid dropout powder (see below for preparation and final concentration) add 20 g of Bacto agar (2% final) for SD dropout agar plates Adjust the total volume to 1 liter with dH_2O Autoclave for 15 minutes at 121°C	6.7 g of yeast nitrogen base without amino acids (0.67% final) 20 g of galactose (2% final) 1.3 g of amino acid dropout powder (see below for preparation and final concentration) add 20 g of Bacto agar (2% final) for SG dropout agar plates Adjust the total volume to 1 liter with dH_2O Autoclave for 15 minutes at 121°C

Preparation of Amino Acid Dropout Powder

The amino acids listed in the following table are to be combined in powder form, mixed, and then added to the growth media. Grind the powder into a homogeneous mixture with a clean, dry mortar and pestle. Store in a clean, dry bottle at room temperature. To select for transformed cells, leave out the amino acid for which the plasmid contains a marker gene essential for biosynthesis of the amino acid. For example, select transformants of the pESC-HIS plasmid on SD plates that do not contain L-histidine.

Amino Acid	Quantity in dropout powder (g)	Final working concentration (mg/liter)
Adenine sulfate	2.5	40
L-arginine (HCl)	1.2	20
L-aspartic acid	6.0	100
L-glutamic acid	6.0	100
L-histidine[a]	1.2	20
L-leucine[a]	3.6	60
L-lysine	1.8	30
L-methionine	1.2	20
L-phenylalanine	3.0	50
L-serine	22.5	375
L-threonine	12	200
L-tryptophan[a]	2.4	40
L-tyrosine	1.8	30
L-valine	9.0	150
Uracil[a]	1.2	20

[a] The omission of histidine, leucine, tryptophan, and/or uracil selects for the plasmid or plasmids that express(es) the respective gene or genes.

LB–Ampicillin Agar (per Liter)

1 liter of LB agar, autoclaved
Cool to 55°C
Add 10 ml of 10-mg/ml filter-sterilized ampicillin
Pour into petri dishes (~25 ml/100-mm plate)

10× Ligase Buffer

500 mM Tris-HCl (pH 7.5)
70 mM $MgCl_2$
10 mM dithiothreitol (DTT)

Note *rATP is added separately in the ligation reaction*

LB Agar (per Liter)

10 g of NaCl
10 g of tryptone
5 g of yeast extract
20 g of agar
Add deionized H_2O to a final volume of 1 liter
Adjust pH to 7.0 with 5 N NaOH
Autoclave
Pour into petri dishes (~25 ml/100-mm plate)

YPAD Agar (30–40 Plates/Liter)

0.0075% l-adenine hemisulfate salt
1% yeast extract
2% Bacto® peptone
2% dextrose
2% Bacto® agar
Autoclave for 15 minutes
Dry the plates at room temperature for 2–3 days
Store the plates in a sealed bag

LTE Buffer

0.1 M LiOAc
10 mM Tris-HCl (pH 7.5)
1 mM EDTA

TE Buffer

10 mM Tris-HCl (pH 7.5)
1 mM EDTA

Transformation Mix

40% polyethylene glycol 3350 (PEG 3350)
0.1 M LiOAc
10 mM Tris-HCl (pH 7.5)
1 mM EDTA

YPAD Broth

0.0075% l-adenine hemisulfate salt
1% yeast extract
2% Bacto® peptone
2% dextrose
Autoclave for 15 minutes

REFERENCES

1. Hopp, T., Prickett, K., Price, V., Libby, R., March, C. *et al.* (1988) *BioTechnology* 6:1204-1210.
2. Evan, G. I., Lewis, G. K., Ramsay, G. and Bishop, J. M. (1985) *Mol Cell Biol* 5(12):3610-6.
3. Hanahan, D. (1983) *J Mol Biol* 166(4):557-80.

ENDNOTES

MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at *http://www.genomics.agilent.com*. MSDS documents are not included with product shipments.