invitrogen™

pBAD/His A, B, and C
pBAD/*Myc*-His A, B, and C

Vectors for Dose-Dependent Expression of Recombinant Proteins Containing N- or C-Terminal 6×His Tags in *E. coli*

Catalog nos. V430-01, V440-01

Version J

29 December 2010
25-0187

User Manual

Table of Contents

Kit Contents and Storage

Contents

This manual is supplied with the following kits.

Cat. No.	Contents
V430-01	20 µg each of pBAD/His A, B, and C vector in TE Buffer, pH 8.0* (40 µl at 0.5 µg/µl) 20 µg each of pBAD/His/*lac*Z vector in TE Buffer, pH 8.0 (40 µl at 0.5 µg/µl) 1 ml sterile, 20% L-arabinose 1 stab LMG194 1 stab TOP10
V440-01	20 µg each of pBAD/*Myc*-His A, B, and C vector in TE Buffer, pH 8.0 (40 µl at 0.5 µg/µl) 20 µg each of pBAD/*Myc*-His/*lac*Z vector in TE Buffer, pH 8.0 (40 µl at 0.5 µg/µl) 1 ml sterile, 20% L-arabinose 1 stab LMG194 1 stab TOP10

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

Shipping/Storage

Kits are shipped on wet ice. Upon receipt, store the plasmids and the 20% L-arabinose solution at –20°C, and the stabs at 4°C.

Long-Term Storage

For long-term storage of *E. coli* strains supplied as stabs with this kit, prepare glycerol stocks as follows:

1. Grow the *E. coli* strain overnight in LB medium overnight with antibiotic selection when appropriate.
2. Combine 0.85 ml of the overnight culture with 0.15 ml of sterile glycerol.
3. Vortex and transfer to a labeled cryovial.
4. Freeze the tube in liquid nitrogen or dry ice/ethanol bath and store at –80°C.

Note: Grow LMG194 strain in RM medium containing M9 salts (see page 26–27).

Additional Products

Accessory Products

Invitrogen offers a variety of products that are suitable for use with the pBAD/His and pBAD/*Myc*-His plasmids. 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 28).

Detection of Recombinant Proteins

Expression of your recombinant protein can be detected using an antibody to the appropriate epitope. The table below describes the antibodies available for use with pBAD/His or pBAD/*Myc*-His. Horseradish peroxidase (HRP)-conjugated antibodies allow one-step detection using colorimetric or chemiluminescent detection methods.

Vector	Epitope	Antibody	Cat. No.
pBAD/His	Anti-Xpress™	Anti-Xpress™	R910-25
		Anti-Xpress™-HRP Antibody	R911-25
	N-terminal polyhistidine tag	Penta-His™ Mouse IgG1 Monoclonal Antibody	P21315
pBAD/*Myc*-His	*c-myc*	Anti-*Myc*	R950-25
		Anti-*Myc*-HRP	R951-25
	C-terminal polyhistidine tag	Anti-His(C-term)	R930-25
		Anti-His(C-term)-HRP	R931-25
		Anti-His (C-term)-AP Antibody	R932-25
		Penta-His™ Mouse IgG1 Monoclonal Antibody	P21315

Purification of Recombinant Proteins

The metal binding domain encoded by the polyhistidine tag allows simple, easy purification of your recombinant protein by Immobilized Metal Affinity Chromatography (IMAC), while EKMax™ Enterokinase allows removal of the N-terminal peptide for production of native protein. See the table below for ordering information.

Product	Quantity	Cat. No.
ProBond™ Purification System	6 purifications	K850-01
ProBond™ Metal-Binding Resin (precharged resin provided as a 50% slurry in 20% ethanol)	50 ml	R801-01
	150 ml	R801-15
Ni-NTA Purification System	6 purifications	K950-01
Purification Columns (10 ml polypropylene columns)	50	R640-50
EKMax™ Enterokinase	250 units	E180-01

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Additional Products, continued

Competent *E. coli*

For your convenience, TOP10 is available as electrocompetent or chemically competent cells in a One Shot® kit format. For more information, refer to www.invitrogen.com or contact Technical Support (page 28).

Item	Quantity	Cat. No.
Electrocomp™ TOP10	20 reactions	C664-55
	2 × 20 reactions	C664-11
	6 × 20 reactions	C664-24
One Shot® TOP10 Competent Cells	20 reactions	C4040-03

Pre-mixed Media

Invitrogen carries pre-mixed growth media in convenient pouches or in bulk. See the table below for ordering information.

Item	Quantity	Cat. No.
imMedia™ Amp Liquid	20 pouches (200 ml medium)	Q600-20
imMedia™ Amp Agar	20 pouches (8–10 plates)	Q601-20
Ampicillin Sodium Salt, irradiated	200 mg	11593-027

Introduction

Overview

Introduction

The pBAD/His and pBAD/*Myc*-His plasmids are pBR322-derived expression vectors designed for regulated, dose-dependent recombinant protein expression and purification in *E. coli*. Optimum levels of soluble, recombinant protein are possible using the *ara*BAD promoter (P_{BAD}) from *E. coli*. The regulatory protein, AraC, is provided on the pBAD/His and pBAD/*Myc*-His vectors allowing regulation of P_{BAD}.

Regulation of Expression by L-arabinose

In the presence of L-arabinose, expression from P_{BAD} is turned on while the absence of L-arabinose produces very low levels of transcription from P_{BAD} (Lee, 1980; Lee *et al.*, 1987). Uninduced levels are repressed even further by growth in the presence of glucose. Glucose reduces the levels of 3´,5´-cyclic AMP, thus lowering expression of the catabolite-repressed P_{BAD} promoter (Miyada *et al.*, 1984). By varying the concentration of L-arabinose, protein expression levels can be optimized to ensure maximum expression of soluble protein. In addition, the tight regulation of P_{BAD} by AraC is useful for expression of potentially toxic or essential genes (Carson *et al.*, 1991; Dalbey and Wickner, 1985; Guzman *et al.*, 1992; Kuhn and Wickner, 1985; Russell *et al.*, 1989; San Millan *et al.*, 1989). For more information on the mechanism of expression and repression of the *ara* regulon, refer to Schleif, 1992.

Continued on next page

Overview, continued

Experimental Outline

The table below describes the basic steps needed to clone and express your protein using pBAD/His or pBAD/*Myc*-His. For more details, refer to the page(s) indicated.

Step	Action	Page
1	Develop a cloning strategy to ligate your gene of interest into the desired vector. Refer to the appropriate pages for the multiple cloning sites of each version of the vector:	7
	pBAD/His A, B, and C	8–11
	pBAD/*Myc*-His A, B, and C	12–15
2	To propagate and maintain the empty vectors and recombinant constructs, transform them into a *rec*A, *end*A *E. coli* host (i.e., TOP10).	7
3	Ligate your gene of interest into pBAD/His or pBAD/*Myc*-His, transform into TOP10 or LMG194, and select on 50–100 µg/ml ampicillin.	16
4	Sequence your construct to ensure that it is in frame with the N-terminal (pBAD/His) or C-terminal (pBAD/*Myc*-His) peptide.	16
5	Perform a 4-hour expression using a 10,000-fold range of L-arabinose concentrations (e.g. 0.00002%, 0.0002%, 0.002%, 0.02%, and 0.2%). Use appropriate controls. Vectors expressing β -galactosidase are available with each kit. Antibodies are available for detection of recombinant proteins (see page vi for ordering information).	17
6	Optimize expression by varying L-arabinose concentration or the time of induction.	21
7	Purify your recombinant protein by chromatography on a metal-chelating resin (see page vi for ordering information).	22

pBAD/His Vector

Features of pBAD/His

The important elements of pBAD/His A (4102bp), pBAD/His B (4092 bp), and pBAD/His C (4100 bp) are described in the following table. All features have been functionally tested.

Feature	Benefit
*ara*BAD promoter (P_{BAD})	Provides tight, dose-dependent regulation of heterologous gene expression (Guzman *et al.*, 1995)
Optimized ribosome binding site	Increases efficiency of recombinant fusion protein expression
Initiation ATG	Provides a translational initiation site for the fusion protein
N-terminal polyhistidine tag	Forms metal-binding site for affinity purification of recombinant fusion protein on a metal-chelating resin. In addition, it allows detection of the recombinant protein with the Penta-His™ Mouse IgG1 Monoclonal Antibody (see page vi for ordering information)
Anti-Xpress™ epitope (Asp-Leu-Tyr-Asp-Asp-Asp-Asp-Lys)	Permits detection of recombinant fusion protein by appropriate antibodies (see page vi for ordering information)
Enterokinase cleavage site (Asp-Asp-Asp-Asp-Lys)	Allows removal of the N-terminal peptide by enterokinase for production of native protein (see page vi for ordering information).
Multiple cloning site	Allows insertion of your gene for expression
*rrn*B transcription termination region	Strong transcription termination region
Ampicillin resistance gene (β-lactamase)	Allows selection of the plasmid in *E. coli*
pBR322 origin	Low copy replication and growth in *E. coli*
*ara*C gene	Encodes the regulatory protein for tight regulation of the P_{BAD} promoter (Lee, 1980; Schleif, 1992)

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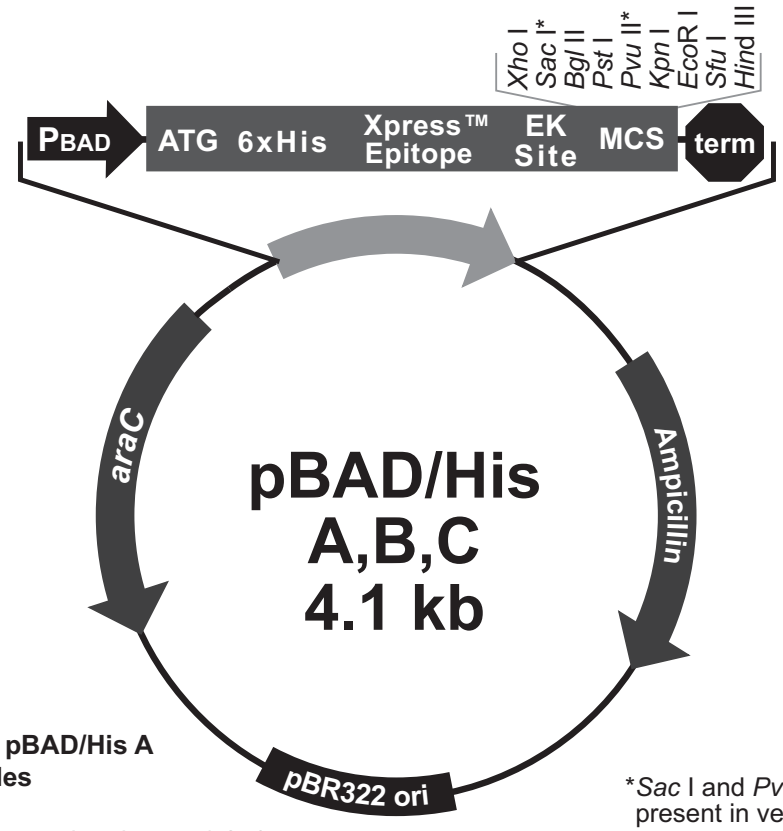
pBAD/His Vector, continued

Map of pBAD/His

The figure below summarizes the features of the pBAD/His vector. Complete sequences for all three pBAD/His vectors are available for downloading at www.invitrogen.com or by contacting Technical Support (see page 28). Details of each multiple cloning site are shown on pages 9–11.



Sac I and *Pvu* II are not present in version C.

Comments for pBAD/His A
4102 nucleotides

*ara*BAD promoter region: bases 4-276
Initiation ATG: bases 319-321
Polyhistidine tag: bases 331-348
Xpress™ epitope: bases 388-411
Enterokinase recognition site: bases 397-411
Multiple cloning site: bases 430-470
*rrn*B transcription termination region: bases 553-710
Ampicillin ORF: bases 989-1849
pBR322 origin: bases 1994-2667
AraC ORF: bases 4076-3198

pBAD/*Myc*-His Vector

Features of pBAD/*Myc*-His

The important elements of pBAD/*Myc*-His A (4094 bp), pBAD/*Myc*-His B (4092 bp), and pBAD/*Myc*-His C (4093 bp) are described in the following table. All features have been functionally tested.

Feature	Benefit
*ara*BAD promoter (P_{BAD})	Provides tight, dose-dependent regulation of heterologous gene expression (Guzman *et al.*, 1995)
Optimized ribosome binding site	Increases efficiency of recombinant fusion protein expression
Initiation ATG	Provides a translational initiation site for the fusion protein
Multiple cloning site	Allows insertion of your gene for expression
C-terminal *myc* epitope tag (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu)	Allows detection of the fusion protein by the Anti-*Myc* Antibody (Evans *et al.*, 1985) (see page vi for ordering information)
C-terminal polyhistidine region	Forms metal-binding site for affinity purification of recombinant fusion protein on metal-chelating resin. In addition, it allows detection of the recombinant protein with Anti-His (C-term) antibodies, and the Penta-His™ Mouse IgG1 Monoclonal Antibody (see page vi for ordering information)
*rrn*B transcription termination region	Strong transcription termination region
Ampicillin resistance gene (β-lactamase)	Allows selection of the plasmid in *E. coli*
pBR322 origin	Low copy replication and growth in *E. coli*
*ara*C gene	Encodes the regulatory protein for tight regulation of the P_{BAD} promoter (Lee, 1980; Schleif, 1992)

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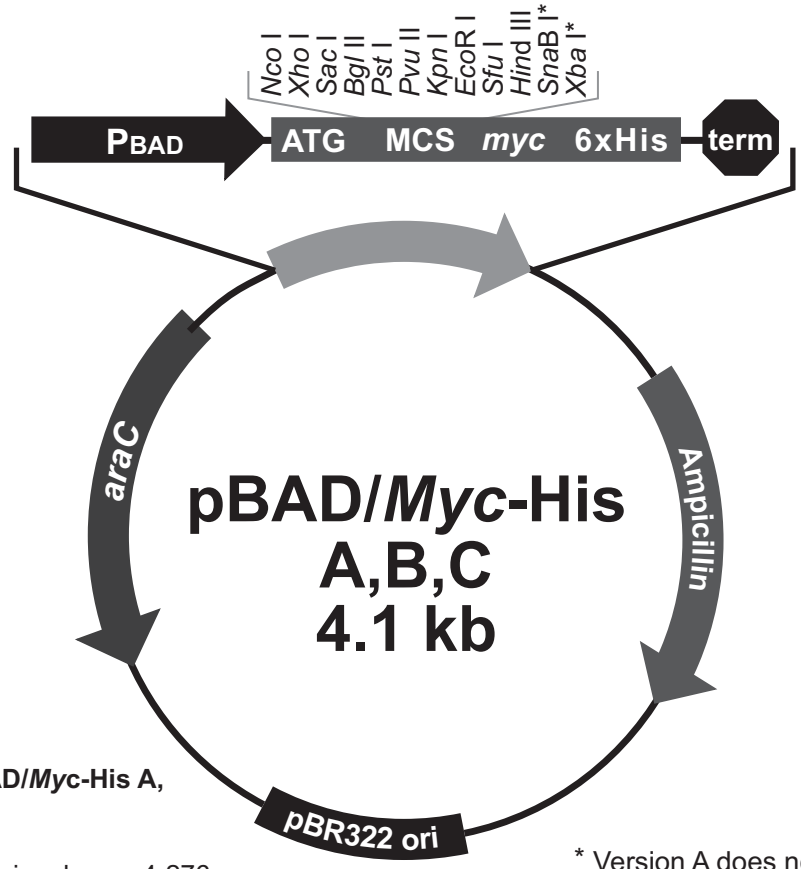
pBAD/*Myc*-His Vector, continued

Map of pBAD/*Myc*-His

The figure below summarizes the features of the pBAD/*Myc*-His vector. Complete sequences for all three pBAD/*Myc*-His vectors are available for downloading at www.invitrogen.com or by contacting Technical Support (see page 28). Details of each multiple cloning site are shown on pages 13–15.



Comments for pBAD/*Myc*-His A, 4094 nucleotides

*ara*BAD promoter region: bases 4-276
Initiation ATG: bases 319-321
Multiple cloning site: bases 317-370
myc epitope: bases 377-406
Polyhistidine tag: bases 422-439
*rrn*B transcription termination region: bases 545-702
Ampicillin ORF: bases 981-1841
pBR322 origin: bases 1986-2659
AraC ORF: bases 4068-3190

* Version A does not contain *Sna*B I or *Xba* I.

Version B contains *Xba* I only.

Version C contains *Sna*B I only.

Methods

General Cloning

Introduction

The following information is provided to help you clone your gene of interest into pBAD/His or pBAD/*Myc*-His. For basic information on DNA ligations, *E. coli* transformations, restriction enzyme analysis, DNA sequencing, and DNA biochemistry, see *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

E. coli Host

For cloning and transformation, we recommend using a *rec*A, *end*A strain such as TOP10 (included in the kit, also available separately; see page vii). This strain is capable of transporting L-arabinose, but not metabolizing it. This is important for expression studies as the level of L-arabinose will be constant inside the cell and not decrease over time. Please note that other strains may be suitable for general use. Be sure to check the genotype of your strain. It should be *ara*BADC^{-} and *ara*EFGH^{+} (Bachmann, 1990).

The *E. coli* strain LMG194 is included in the kit to ensure low basal level expression of toxic genes (Guzman *et al.*, 1995). This strain is capable of growth on minimal medium (RM medium), which allows additional repression of P_{BAD} by glucose. **Once you have determined that you have the correct construct, transform it into LMG194 prior to performing expression experiments.**

Genotype of TOP10

F^{-} *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara*A-*leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G.

Note: This strain is *ara*BADC^{-}. It is deleted for both *ara*BA and *ara*C, and the gene for *ara*D has a point mutation in it, making it inactive.

Genotype of LMG194

F^{-} Δ*lac*X74 *gal* E *thi rps*L Δ*pho*A (*Pvu* II) Δ*ara*714 *leu*::Tn*10*. Please note that this strain is streptomycin and tetracycline resistant.

Maintenance of pBAD/His and pBAD/*Myc*-His

To propagate and maintain pBAD/His or pBAD/*Myc*-His, use the supplied 0.5 µg/µl stock solution in TE, pH 8.0 to transform a *rec*A, *end*A *E. coli* strain like TOP10F', DH5α™-T1^{R}, TOP10, or equivalent. Select transformants on LB plates containing 50–100 µg/ml ampicillin.

Note: Use strains like DH5α™ **only** for propagation of pBAD/His or pBAD/*Myc*-His, but not expression of recombinant proteins (see explanation above). Be sure to prepare a glycerol stock of each plasmid for long-term storage (see page 16).

Cloning into pBAD/His

Important

To generate recombinant proteins that are expressed correctly and contain the N-terminal fusion peptide, it is necessary to clone in frame with the N-terminal peptide. To facilitate cloning, the pBAD/His vector is provided in three different reading frames. They differ only in the spacing between the sequences that code for the N-terminal peptide and the multiple cloning site. For proper expression, first determine which restriction sites are appropriate for ligation and then which vector will preserve the reading frame at the 5´ end. Be sure to include a stop codon to terminate translation of your protein.

pBAD/His Multiple Cloning Sites

The multiple cloning sites of each version of pBAD/His are provided on pages 9–11. Restriction sites are labeled to indicate cleavage site. The boxed sequence is the variable region that facilitates in frame cloning with the N-terminal peptide. This variable region is located between the enterokinase cleavage site and the *Xho* I site.

Features of the *ara*BAD and *ara*C promoters are marked and described as follows. For more information see Lee, 1980; Miyada, *et al.*, 1984; Lee, *et al.*, 1987; and Schleif, 1992.

- O_2 region: Binding site of AraC that represses transcription from P_{BAD}.
- O_1 region: Binding site of AraC that represses transcription of the *ara*C promoter (P_C) (transcribed on the opposite strand; not shown).
- CAP binding site: Site where CAP (cAMP binding protein) binds to help activate transcription from P_{BAD} and P_C.
- I_2 and I_1 regions: Binding sites of AraC that activate transcription from P_{BAD}.
- –10 and –35 regions: Binding sites of RNA polymerase for transcription of P_{BAD}.

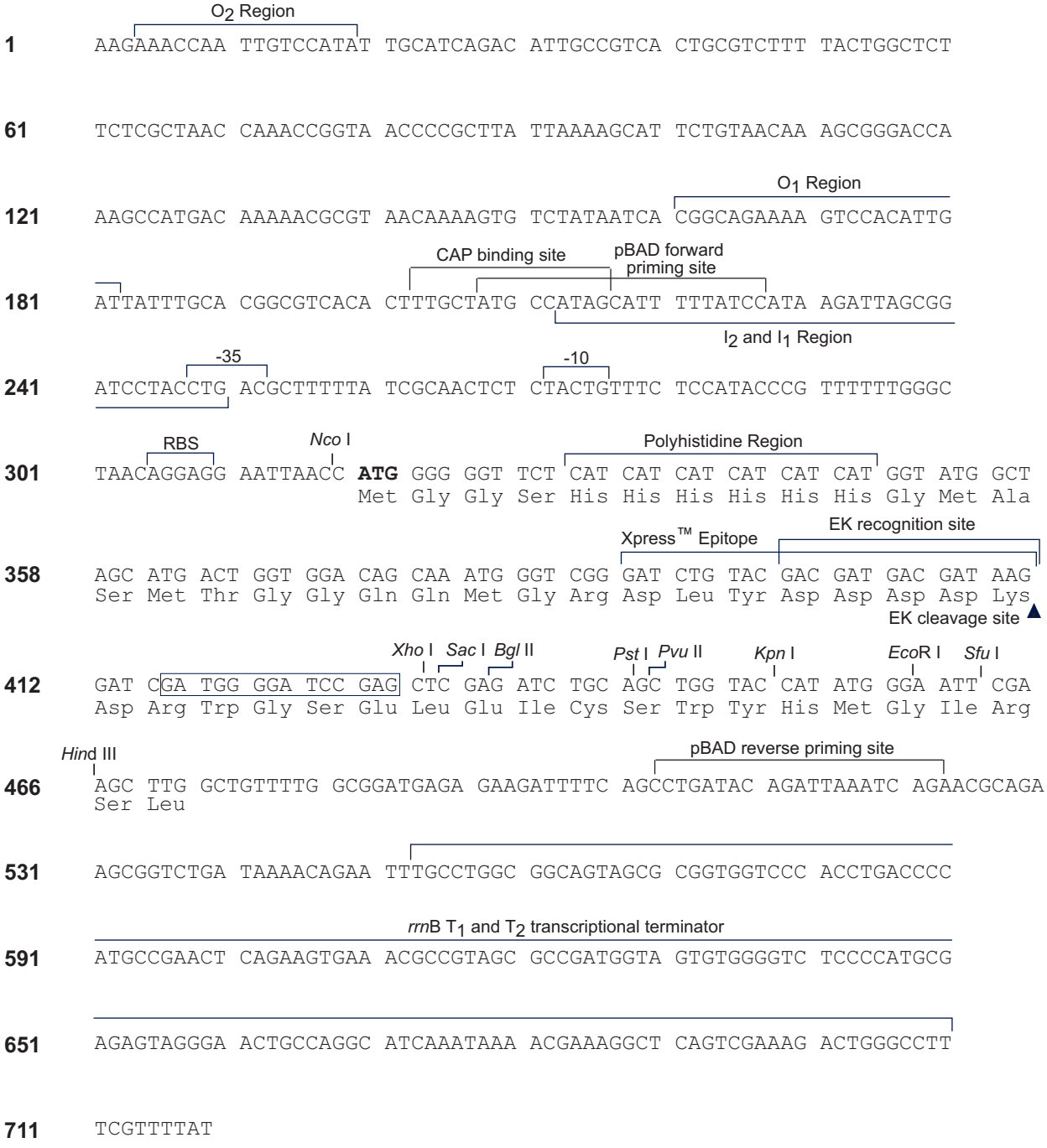
Each multiple cloning site has been confirmed by sequencing and functional testing.

Continued on next page

Cloning into pBAD/His, continued

pBAD/His A Multiple Cloning Site

Below is the multiple cloning site for pBAD/His A. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pBAD/His A is available for downloading at www.invitrogen.com or from Technical Support (see page 28).**

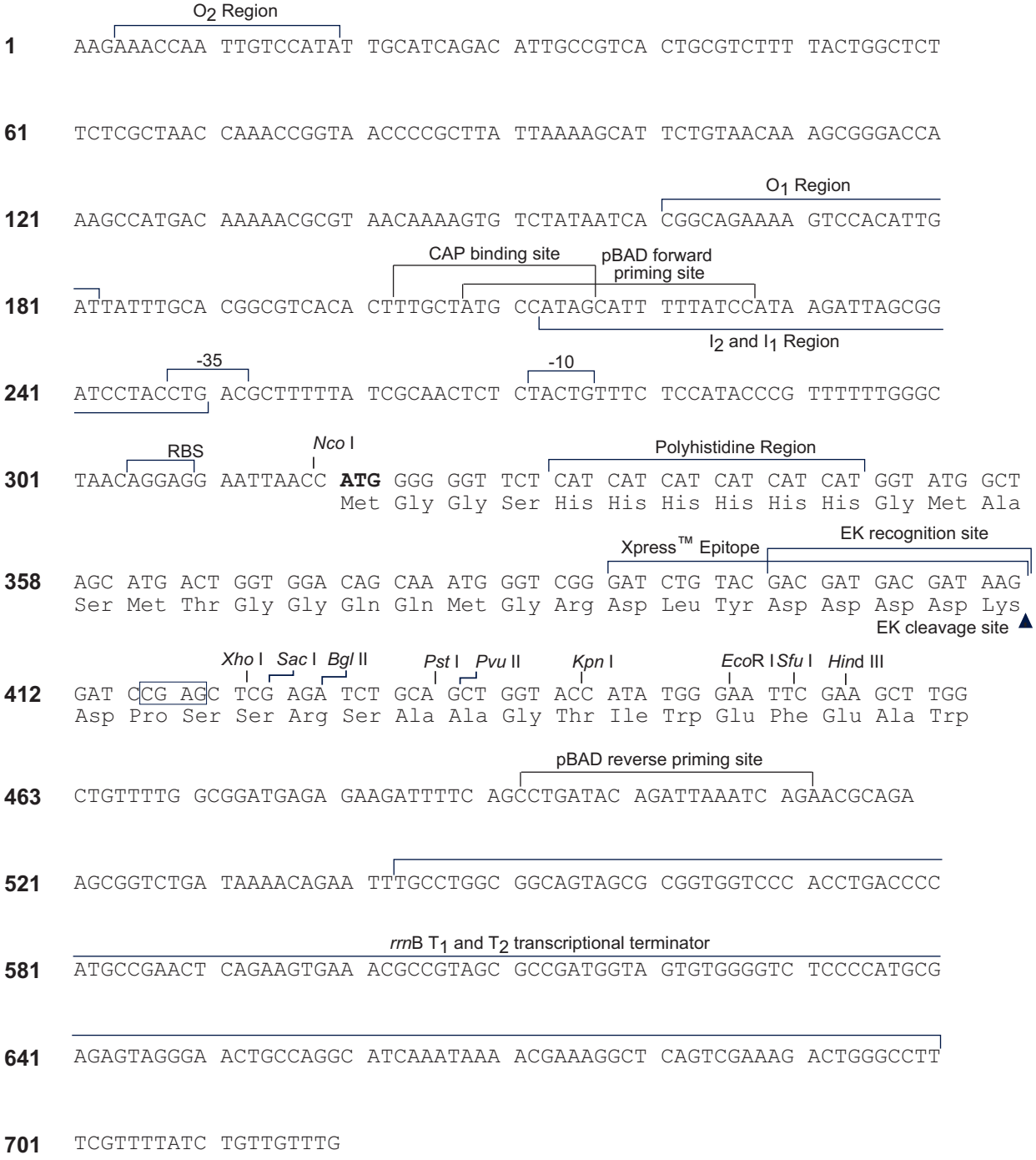


Continued on next page

Cloning into pBAD/His, continued

pBAD/His B Multiple Cloning Site

Below is the multiple cloning site for pBAD/His B. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pBAD/His B is available for downloading at www.invitrogen.com or from Technical Support (see page 28).**

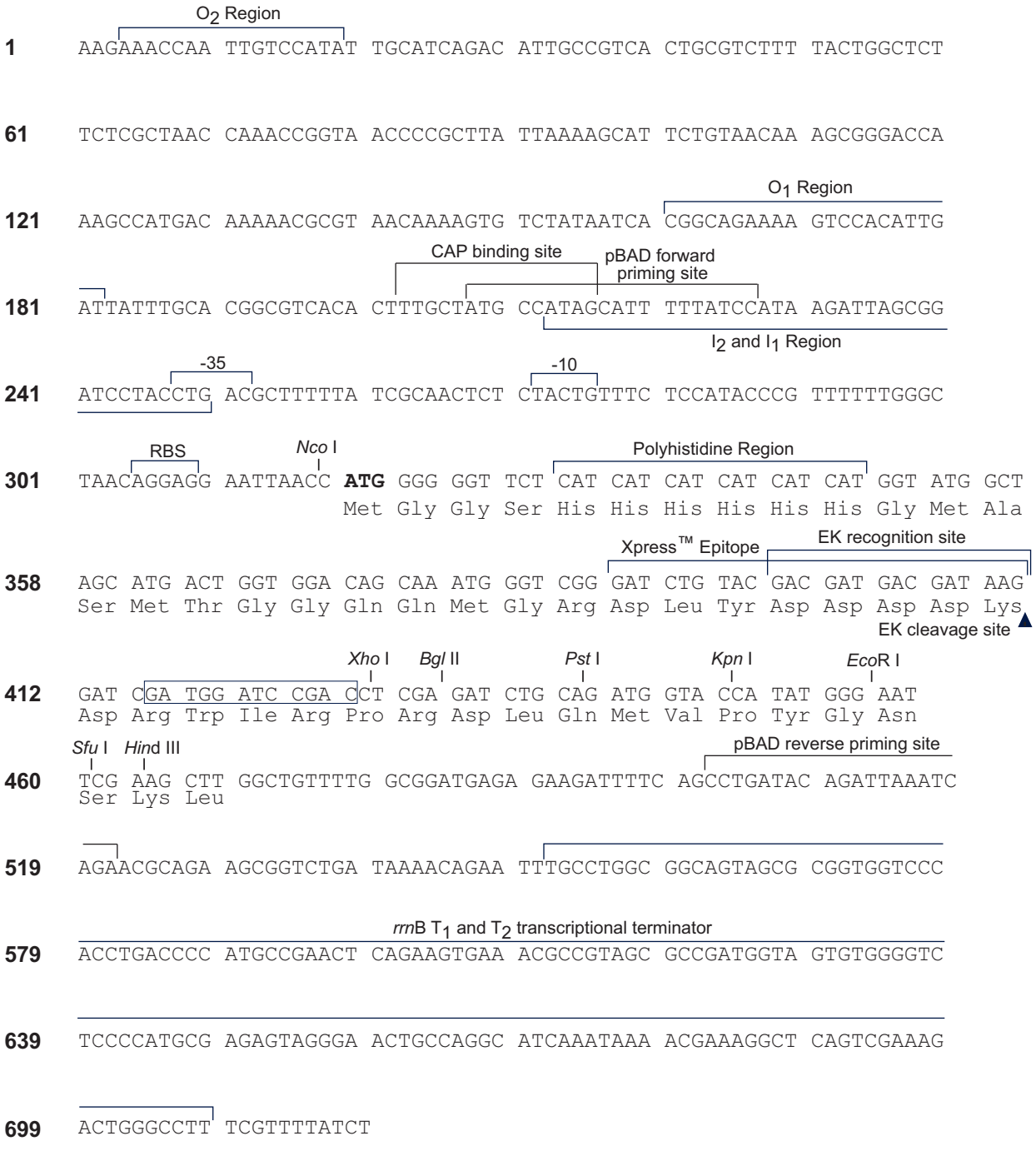


Continued on next page

Cloning into pBAD/His, continued

pBAD/His C Multiple Cloning Site

Below is the multiple cloning site for pBAD/His C. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pBAD/His C is available for downloading at www.invitrogen.com or from Technical Support (see page 28).**



Cloning into pBAD/*Myc*-His

Important

To generate recombinant proteins that are expressed correctly and contain the C-terminal fusion peptide, it is necessary to clone in frame with **BOTH** the initiation ATG (bp 320-322) and the C-terminal peptide. The initiation ATG is correctly spaced from the optimized RBS to ensure optimum translation.

To facilitate cloning, the pBAD/*Myc*-His vector is provided in three different reading frames. They differ only in the spacing between the sequences that code for the multiple cloning site and the C-terminal peptide. For proper expression, first determine which restriction sites are appropriate for ligation and then which vector will preserve the reading frame at **BOTH** the 5´ and the 3´ ends. You may have to use PCR to create a fragment with the appropriate restriction sites to clone in frame at both ends. Be sure that there is no stop codon in the open reading frame of your gene (except as noted below).

Note

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

pBAD/*Myc*-His Multiple Cloning Sites

The multiple cloning sites of each version of pBAD/*Myc*-His are provided on pages 13–15. Restriction sites are labeled to indicate cleavage site. The boxed sequence is the variable region that facilitates in frame cloning with the ATG codon and C-terminal peptide. This variable region is located between the multiple cloning site and the *myc* epitope.

Features of the *ara*BAD and *ara*C promoters are marked and described as follows. For more information see Lee, 1980; Miyada, *et al.*, 1984; Lee, *et al.*, 1987; and Schleif, 1992.

- O_2 region: Binding site of AraC that represses transcription from P_{BAD}.
- O_1 region: Binding site of AraC that represses transcription of the *ara*C promoter (P_C) (transcribed on the opposite strand; not shown).
- CAP binding site: Site where CAP (cAMP binding protein) binds to help activate transcription from P_{BAD} and P_C.
- I_2 and I_1 regions: Binding sites of AraC that activate transcription from P_{BAD}.
- –10 and –35 regions: Binding sites of RNA polymerase for transcription of P_{BAD}.

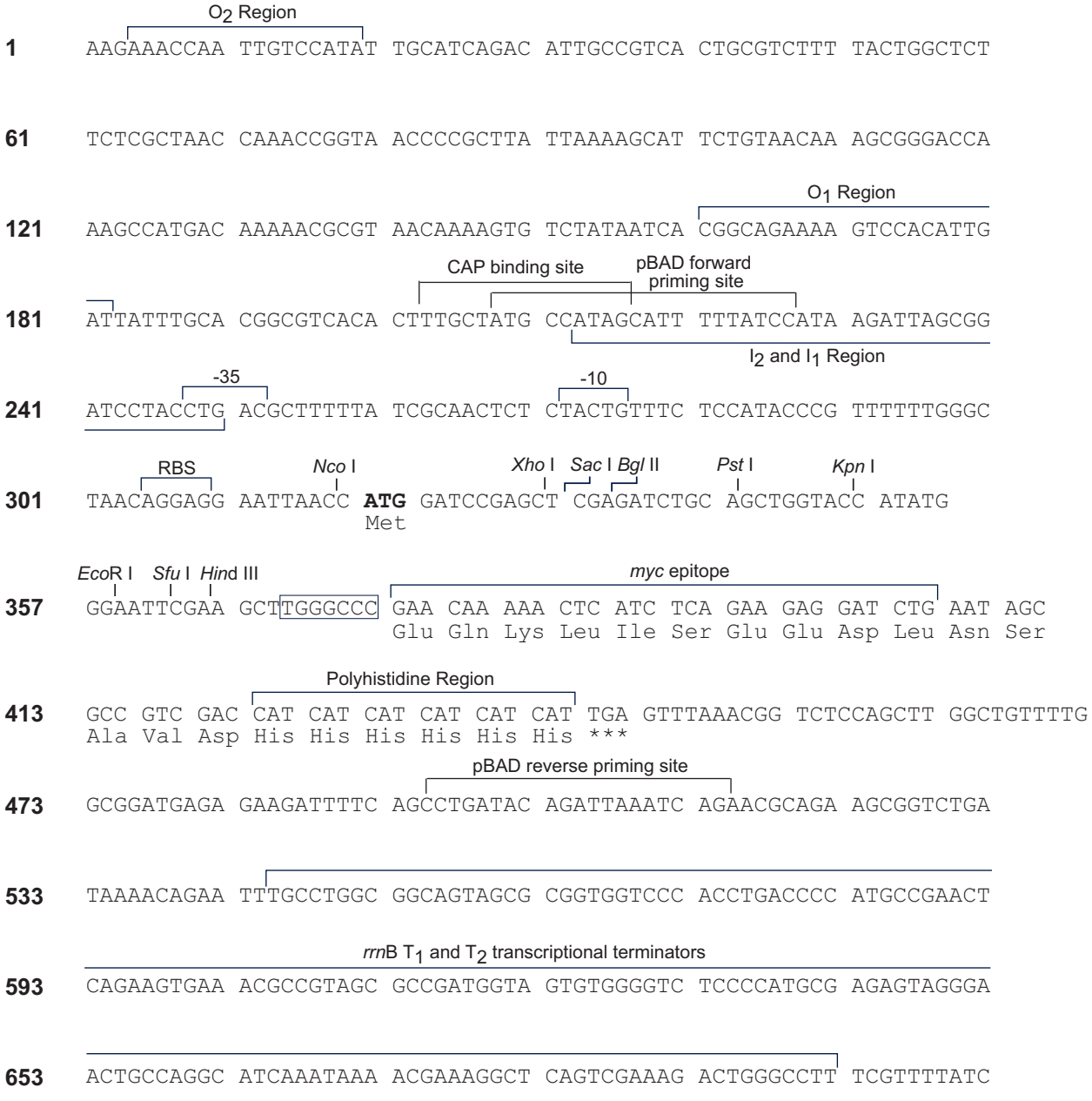
Each multiple cloning site has been confirmed by sequencing and functional testing.

Continued on next page

Cloning into pBAD/*Myc*-His, continued

pBAD/*Myc*-His A Multiple Cloning Site

Below is the multiple cloning site for pBAD/*Myc*-His A. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pBAD/*Myc*-His A is available for downloading at www.invitrogen.com or from Technical Support (see page 28).**



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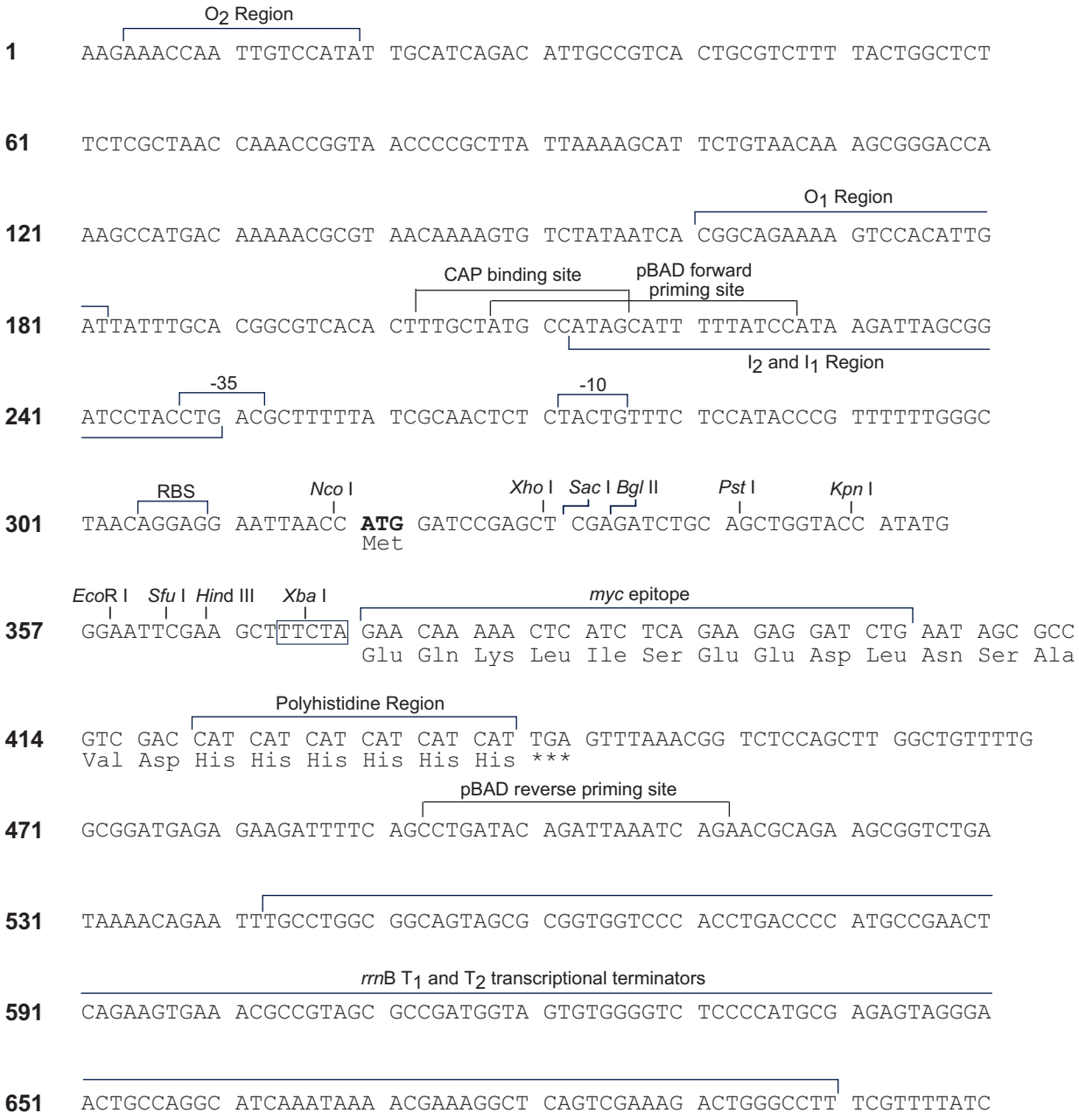
Cloning into pBAD/*Myc*-His, continued

pBAD/*Myc*-His B Multiple Cloning Site

Below is the multiple cloning site for pBAD/*Myc*-His B. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pBAD/*Myc*-His B is available for downloading at www.invitrogen.com or from Technical Support (see page 28).**



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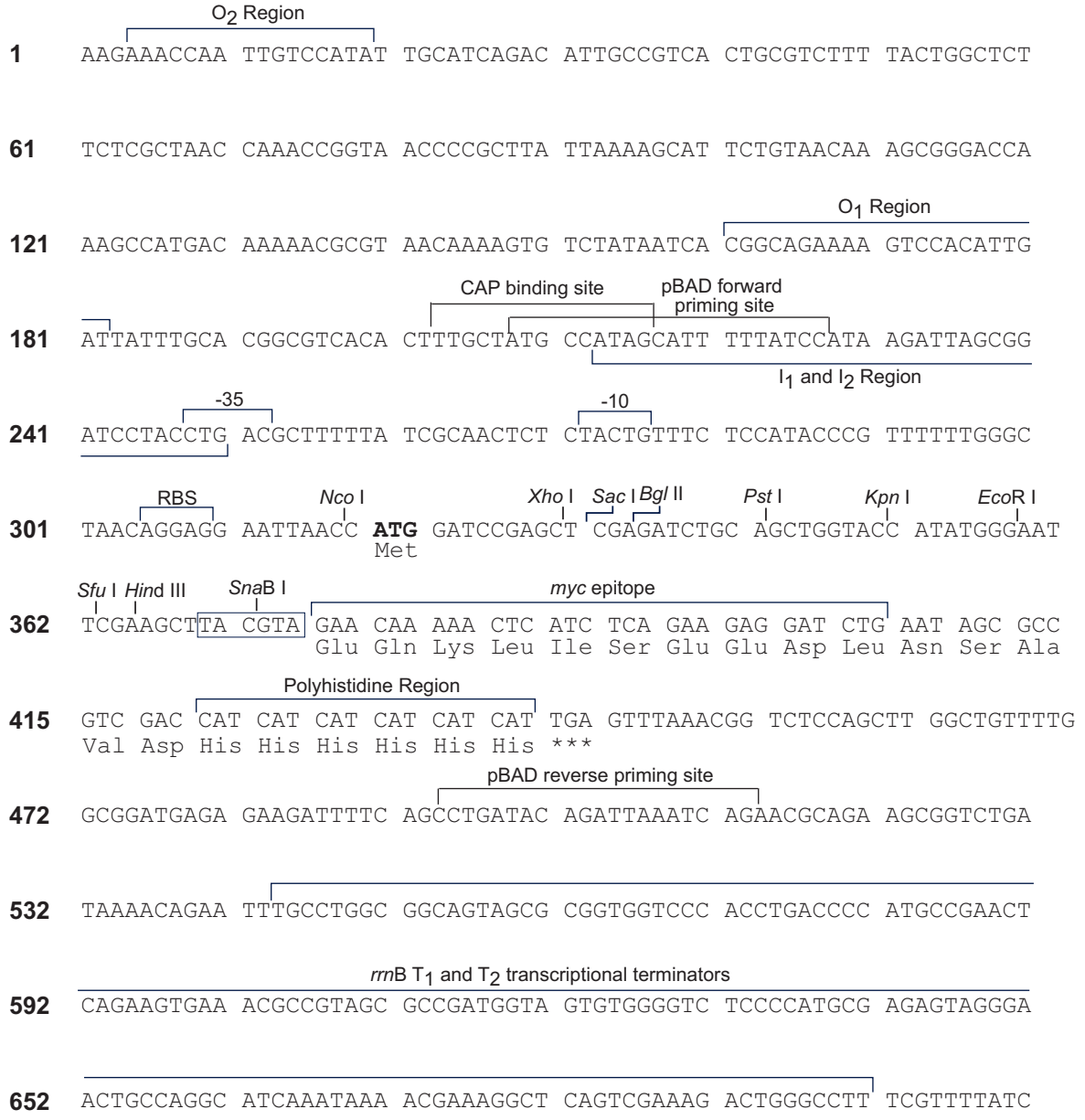
Cloning into pBAD/*Myc*-His, continued

pBAD/*Myc*-His C Multiple Cloning Site

Below is the multiple cloning site for pBAD/*Myc*-His C. Restriction sites are labeled to indicate the cleavage site. The boxed nucleotides indicate the variable region. The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pBAD/*Myc*-His C is available for downloading at www.invitrogen.com or from Technical Support (see page 28).**



E. coli transformation

E. coli Transformation

After ligating your insert into the appropriate vector, transform your ligation mixtures into TOP10 and select on LB plates containing 50–100 µg/ml ampicillin. Select 10–20 clones and analyze for the presence and orientation of your insert.

Glycerol Stock

Once you have obtained your desired construct, we recommend that you store your clone as a glycerol stock.

1. Grow 1 to 2 ml of the strain containing your construct in pBAD/His or pBAD/*Myc*-His to saturation (12–16 hours; OD_{600} = 1–2) in LB containing 50–100 µg/ml ampicillin
2. Combine 0.85 ml of the culture with 0.15 ml of sterile glycerol
3. Mix the solution by vortexing
4. Transfer to an appropriate vial for freezing and cap
5. Freeze in an ethanol/dry ice bath or liquid nitrogen and then transfer to –80°C for long-term storage.

Expression

Introduction

Since each recombinant protein has different characteristics that may affect optimum expression, it is helpful to vary the L-arabinose concentration and/or run a time course of expression to determine the best conditions for optimal expression of your particular protein. A mock expression consisting of the pBAD/His or pBAD/*Myc*-His vector alone should be done as a negative control. pBAD/His/*lac*Z or pBAD/*Myc*-His/*lac*Z are included for use as positive expression controls (see pages 23–24). TOP10 may be used as a general host for expression. LMG194 should be used if your protein is toxic or essential to *E. coli*.

Basic Strategy

Once you have some clones that you wish to characterize, we recommend the following strategy to determine the optimal expression level.

1. **Pilot Expression.** In this expression experiment you will vary the amount of L-arabinose over a 10,000 fold range (0.00002% to 0.2%) to determine the approximate amount of L-arabinose needed for maximum expression of your protein. See next page for protocol.
2. To optimize expression of your protein, you may wish to try L-arabinose concentrations spanning the amount determined in Step 1. Or you may wish to perform a time course.

Note: If your expressed protein is insoluble, remember to analyze the supernatant and the pellet of lysed cells for expression of soluble protein.

Note: If you transformed your pBAD/His or pBAD/*Myc*-His construct into LMG194, be sure to perform your expression experiments in RM medium with glucose (see page 26 for recipe) to ensure low basal levels of your protein.

Note

Expression of your protein with N- or C-terminal tags will increase the size of your protein. Refer to the table below for the approximate size of the N- and C-terminal tags. Be sure and account for any additional amino acids between the tag and your protein.

Vector	Tag	Size
pBAD/His	N-terminal Anti-Xpress™ tag	3 kDa
pBAD/*Myc*-His	C-terminal *Myc*-His tag	2 kDa

Continued on next page

Expression, continued

Before Starting

Be sure to have the following solutions and equipment on hand before starting the experiment:

- SOB or LB containing 50 µg/ml ampicillin (see **Recipes**, pages 25–26)
- RM medium containing glucose (see **Recipes**, page 26)
- 37°C shaking incubator
- 20% L-arabinose (provided)
- 37°C heat block or water bath
- 42°C water bath
- Liquid nitrogen
- 1X and 2X SDS-PAGE sample buffer
- Reagents and apparatus for SDS-PAGE gel
- 70°C water bath
- Lysis Buffer (see page 27 for recipe)
- Sterile water

Continued on next page

Expression, continued

Pilot Expression

Remember to include the appropriate negative and positive controls to evaluate your expression experiment.

1. For each transformant or control, inoculate 2 ml of SOB or LB medium containing 50 µg/ml ampicillin with a single recombinant *E. coli* colony.
   If you are using LMG194 as a host, use RM medium containing glucose and 50–100 µg/ml ampicillin.
2. Grow overnight at 37°C with shaking (225–250 rpm) to OD_{600} = 1–2.
3. The next day, label five tubes 1 through 5 and add 10 ml of SOB or LB containing 50 µg/ml ampicillin.
4. Inoculate each tube with 0.1 ml of the overnight culture.
5. Grow the cultures at 37°C with vigorous shaking to an OD_{600} = ~0.5 (the cells should be in mid-log phase).
6. While the cells are growing, prepare four 10-fold serial dilutions of 20% L-arabinose with sterile water and aseptic (e.g., 2%, 0.2%, 0.02%, and 0.002%).
7. Remove a 1 ml aliquot of cells from each tube, centrifuge at maximum speed in a microcentrifuge for 30 seconds, and aspirate the supernatant.
8. Freeze the cell pellet at –20°C. This is the zero time point sample.
9. Add L-arabinose to the five 10 ml cultures as follows:

Tube	Volume (ml)	Stock Solution	Final Concentration
1	0.1	0.002%	0.00002%
2	0.1	0.02%	0.0002%
3	0.1	0.2%	0.002%
4	0.1	2%	0.02%
5	0.1	20%	0.2%

10. Grow at 37°C with shaking for 4 hours.
11. Take 1 ml samples at 4 hours and treat as in Step 7 and 8.

Continued on next page

Expression, continued

Preparation of Samples

Before starting, prepare SDS-PAGE gels to analyze all the samples you collected.

Note: If you wish to analyze your samples for soluble protein, see the next page for a protocol.

1. When all the samples have been collected from Steps 8 and 11, previous page, resuspend each pellet in 100 µl of 1X SDS-PAGE sample buffer.
2. Boil 5 minutes and centrifuge briefly.
3. Load 5 µl of each sample on an SDS-PAGE gel and electrophorese. Save your samples by storing at –20°C.

Analysis of Samples

1. Stain the gel with Coomassie blue and look for a band of increasing intensity in the expected size range for the recombinant protein.
2. Use a negative control (empty vector) to distinguish recombinant proteins from background proteins.
3. Use the positive control (pBAD/His/*lac*Z or pBAD/*Myc*-His/*lac*Z) to confirm that growth and induction was done properly. The positive control should yield a 120 kDa protein.
4. You should be able to determine the approximate L-arabinose concentration for maximum expression.

Low Expression

If you don't see any expression on a Coomassie-stained gel, re-run your samples on an SDS-PAGE gel and perform a western blot. Use antibody to your protein or one of the recommended antibodies appropriate for your protein. See page vi for ordering information. For more information, refer to www.invitrogen.com or contact Technical Support (page 28).

If you still don't see expression of your protein, sequence your construct and make sure it is in frame with the N- or C-terminal peptide.

pBAD sequencing primers

You may use the pBAD forward and reverse sequencing primers to sequence your insert containing your gene of interest in pBAD/His or pBAD/*Myc*-His vectors to make sure that it is in frame with the N- or C-terminal peptide.

Primer	Sequence
pBAD forward primer	5′- ATGCCATAGCATTTTTATCC -3′
pBAD reverse primer	5′- GATTTAATCTGTATCAGG -3′

Continued on next page

Expression, continued

Optimization of Expression

Once you have detected expression of your protein, you may wish to perform some experiments to further optimize expression. Use the Pilot Expression protocol, but vary the L-arabinose concentration over a smaller range. For example, if you obtained the best expression at 0.002%, try 0.0004%, 0.0008%, 0.001%, 0.004%, and 0.008%.

Also you may perform a time course of induction over a 5 to 6 hour time period, taking time points every hour, to determine if varying the time increases expression.

If your protein is insoluble, you may wish to analyze the supernatant and pellet of lysed cells when you vary the L-arabinose concentration. Refer to the protocol on the next page to prepare samples.

Remember to store your time points at –20°C.

Preparation of Samples for Soluble/Insoluble Protein

After collecting all of your samples, prepare SDS-PAGE gels for analysis.

1. When all the samples have been collected, thaw and resuspend each pellet in 100 µl of Lysis Buffer (see **Recipes**, page 27).
2. Place sample on ice and sonicate the solution for 10 seconds.
3. Centrifuge samples in a microcentrifuge at maximum speed for 1 minute at +4°C to pellet insoluble proteins. Transfer supernatant to a fresh tube and store on ice. Store the pellets on ice (see Step 5).
4. Mix together equal amounts of supernatant and 2X SDS Sample buffer and heat for 5 minutes at 70°C.
5. Add 200 µl of 1X SDS-PAGE sample buffer to pellets from Step 3 and heat for 5 minutes at 70°C.
6. Load 10 µl of the supernatant sample and 10 µl of the pellet sample onto an SDS-PAGE and electrophorese.
7. Analyze for optimal, soluble expression of your protein.

Expression of Toxic Proteins

To ensure low levels of expression, you may find it useful to utilize glucose to repress the *ara*BAD promoter further.

Follow the steps below to express your protein.

- Transform your construct into LMG194. LMG194 can be grown in RM medium, which enables repression of P_{BAD} by glucose.
- Follow the Pilot Expression on page 19, substituting RM Medium + Glucose medium (see page 26) to grow the cells.
- Be sure to monitor the OD_{600} as the cells will grow more slowly in RM medium.
- Induce with various concentrations of L-arabinose as described in the Pilot Expression.
- Monitor OD_{600} over time be sure cells are growing.

Purification

Scale-up of Expression for Purification

We recommend using the ProBond™ Purification System available separately from Invitrogen (see page vi for ordering information). Use the conditions determined in the previous section to grow and induce 50 ml of cells. This is the largest culture volume to use with the 2 ml prepacked columns included in the ProBond™ Purification System. If you need to purify larger amounts of recombinant protein, you may need more ProBond™ resin.

Note: Remember to use RM medium (page 26) with LMG194.

1. Inoculate 2 ml of SOB or LB medium containing 50 µg/ml ampicillin with a single recombinant *E. coli* colony.
2. Grow overnight at 37°C with shaking (225–250 rpm) to OD_{600} = 1–2.
3. The next day, inoculate 50 ml of SOB or LB medium containing 50 µg/ml ampicillin with 1 ml of the overnight culture.
4. Grow the culture at 37°C with vigorous shaking to an OD_{600} = ~0.5 (the cells should be in mid-log phase).
5. Add the optimal amount of L-arabinose to induce expression.
6. Grow at 37°C with shaking until the optimal time point is reached. Harvest the cells by centrifugation (3000 × g for 10 minutes at +4°C).
7. At this point, you may proceed directly to purification or store at –80°C for future use.

Purification

For help with purification of your recombinant protein, refer to the ProBond™ Purification System manual. See page vi for other recommended purification products available from Invitrogen. For more information, refer to www.invitrogen.com or contact Technical Support (page 28).

If you are using another type of resin, follow manufacturer's recommendations.

Appendix

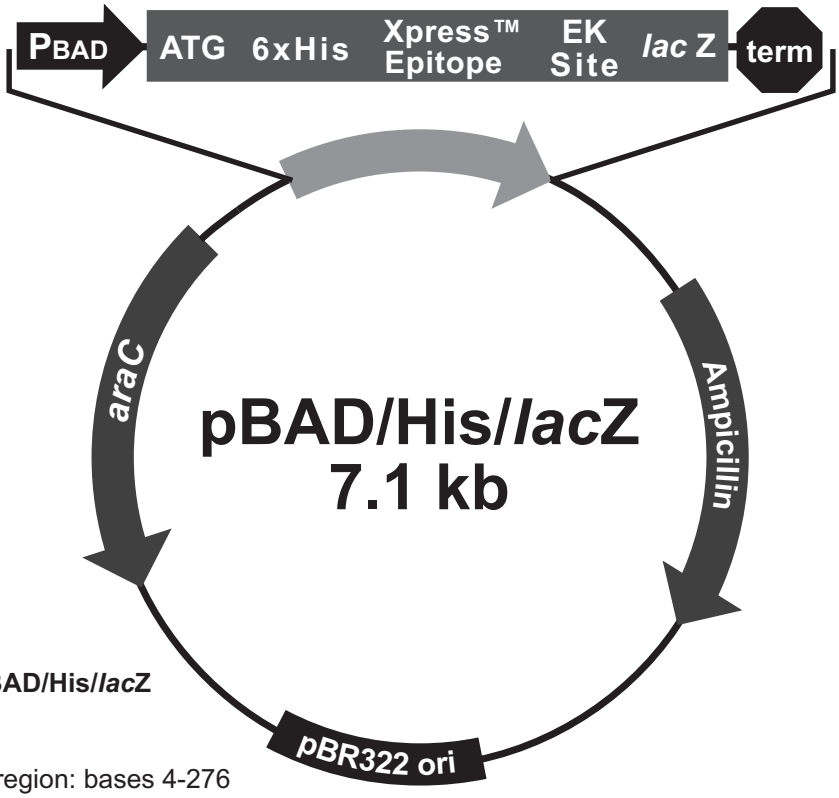
pBAD/His/*lac*Z

Description

pBAD/His/*lac*Z is a 7115 bp control vector containing the β-galactosidase gene fused to the N-terminal peptide. It was constructed by digesting the vector pTrcHis/*lac*Z with *Nco* I and *Nsi* I to remove the *lac*I^q gene and the *trc* promoter and replacing with an *Nco* I–*Nsi* I fragment containing the *ara*C gene and the *ara*BAD promoter. The β-galactosidase portion of the fusion may be released by digestion with *Bam*H I and *Hin*d III. The vector expresses a 120 kDa protein.

Map of Control Vector

The figure below summarizes the features of the pBAD/His/*lac*Z vector. **The complete nucleotide sequence for pBAD/His/*lac*Z is available at www.invitrogen.com or by contacting Technical Support (page 28).**



Comments for pBAD/His/*lac*Z
7115 nucleotides

*ara*BAD promoter region: bases 4-276
Initiation ATG: bases 320-322
Polyhistidine tag: bases 332-349
Xpress™ epitope: bases 389-412
Enterokinase recognition site: bases 398-412
LacZ ORF: bases 419-3475
*rrn*B transcription termination region: bases 3565-3722
Ampicillin ORF: bases 4002-4862
pBR322 origin: bases 5007-5680
AraC ORF: bases 7089-6211

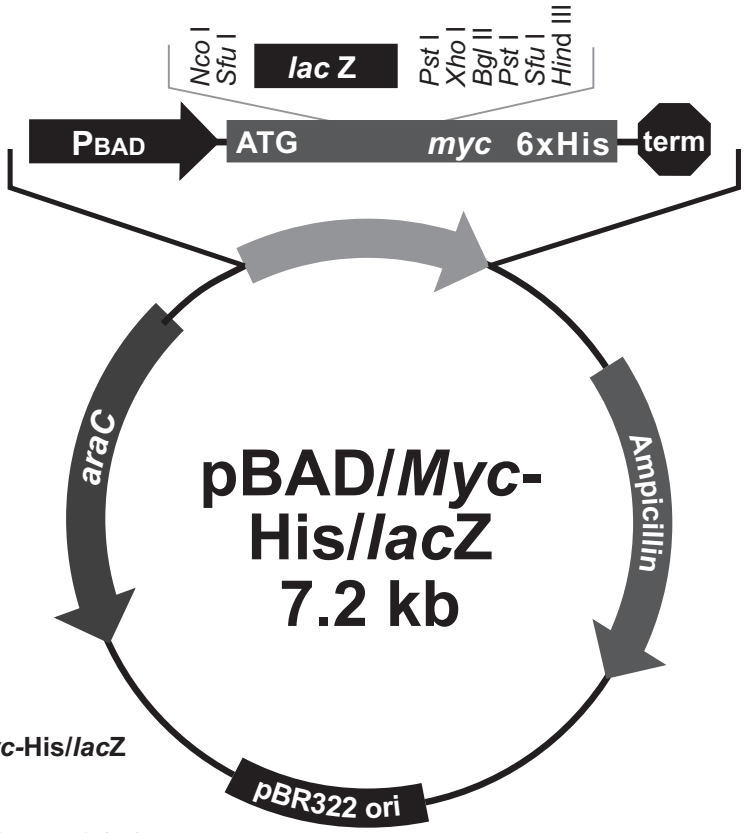
pBAD/*Myc*-His/*lacZ*

Description

pBAD/*Myc*-His/*lac*Z is a 7242 bp control vector containing the gene for β-galactosidase fused to the C-terminal peptide. It was constructed by digesting the vector pTrcHis2/*lac*Z with *Nco* I and *Nsi* I to remove the *lac*I gene and the *trc* promoter and replacing with an *Nco* I-*Nsi* I fragment containing the *ara*C gene and the *ara* BAD promoter. The β-galactosidase portion of the fusion may be released by digestion with *Sfu* I (*Bst*B I). Other cloning options are possible. The vector expresses a 120 kDa protein.

Map of Control Vector

The figure below summarizes the features of the pBAD/*Myc*-His/*lac*Z vector. **The complete nucleotide sequence for pBAD/*Myc*-His/*lac*Z is available at www.invitrogen.com or by contacting Technical Support (see page 28).**



Comments for pBAD/*Myc*-His/*lac*Z
7241 nucleotides

*ara*BAD promoter region: bases 4-276
Initiation ATG: bases 319-321
LacZ ORF: bases 373-3429
myc epitope: bases 3523-3552
Polyhistidine tag: bases 3568-3585
*rrn*B transcription termination region: bases 3691-3848
Ampicillin ORF: bases 4128-4988
pBR322 origin: bases 5133-5806
AraC ORF: bases 7215-6337

Recipes

Pre-mixed Media

Invitrogen carries pre-mixed growth media, such as imMedia™, in convenient pouches or in bulk. imMedia™ is pre-mixed and pre-sterilized for convenient preparation of liquid medium or agar plates for *E. coli* growth, and is available with or without IPTG and X-gal and a choice of three antibiotics: ampicillin, kanamycin, or Zeocin™ selection agent. Refer to page vii for ordering information.

Low Salt LB Medium (with Ampicillin)

LB Medium (per liter)

1% Tryptone

0.5% Yeast Extract

0.5% NaCl

pH 7.0

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 5 g NaCl in 950 ml deionized water.
2. Adjust the pH of the solution to 7.0 with 5 M NaOH and bring the volume to 1 liter.
3. Autoclave for 20 minutes on liquid cycle.
4. Let solution cool to ~55°C. Add ampicillin to a final concentration of 50 µg/ml. Store the medium at +4°C.

Medium is stable for only 1–2 weeks.

Low Salt LB Agar Plates with Ampicillin

LB Medium (per liter)

1% Tryptone

0.5% Yeast Extract

0.5% NaCl

1.5% Agar

pH 7.0

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 5 g NaCl in 950 ml deionized water.
2. Adjust the pH of the solution to 7.0 with 5 M NaOH, add 15 g agar, and bring the volume to 1 liter.
3. Autoclave for 20 minutes on liquid cycle.
4. Let agar cool to ~55°C. Add ampicillin to a final concentration of 50 µg/ml.
5. Pour into 10 cm petri plates. Let the plates harden, then invert and store at +4°C.

Plates containing ampicillin are stable for 1–2 weeks.

Continued on next page

Recipes, continued

SOB Medium (with Ampicillin)

SOB (per liter)

2% Tryptone
0.5% Yeast Extract
0.05% NaCl
2.5 mM KCl
10 mM $MgCl_2$

1. Dissolve 20 g tryptone, 5 g yeast extract, and 0.5 g NaCl in 950 ml deionized water.
2. Make a 250 mM KCl solution by dissolving 1.86 g of KCl in 100 ml of deionized water.
   Add 10 ml of this stock KCl solution to the solution in Step 1.
3. Adjust pH to 7.5 with 5 M NaOH and add deionized water to 1 liter.
4. Autoclave this solution, cool to ~55°C, and add 10 ml of sterile 1 M $MgCl_2$. You may also add ampicillin to 50 µg/ml.
5. Store at +4°C.

Medium is stable for only 1–2 weeks.

RM Medium + Glucose

1X M9 Salts (See next page for recipe for 10X M9 Salts)
2% Casamino Acids
0.2% glucose
1 mM $MgCl_2$
50–100 µg/ml ampicillin

1. For 1 liter of RM medium, mix 20 g Casamino Acids and 890 ml deionized water.
2. Autoclave 20 minutes on liquid cycle.
3. After the autoclaved solution has cooled, add the following sterile solutions aseptically:
4. 10X M9 Salts 100 ml
5. 1 M $MgCl_2$ 1 ml
6. 20% glucose 10 ml
7. 100 mg/ml ampicillin 0.5 to 1 ml
8. Mix well and store medium containing ampicillin at +4°C.

Medium is good for 1 month at +4°C.

Continued on next page

Recipes, continued

10X M9 Salts

For 1 liter:

Na_2HPO_4	60 g
KH_2PO_4	30 g
NaCl	5 g
NH_4Cl	10 g
Water	900 ml

1. Dissolve reagents in the water and adjust the pH to 7.4 with 10 M NaOH.
2. Add water to 1 liter and autoclave for 20 minutes on liquid cycle.
3. Cool and add 1 ml of 1 M thiamine (filter-sterilize). Store at room temperature.

Lysis Buffer

10 mM Tris-HCl, pH 8
1 mM EDTA
0.5 mg/ml lysozyme
0.1 mg/ml DNase I
10 mM $CaCl_2$

1. Prepare just before use. Take 10 ml of TE buffer and add 5 mg of lysozyme, 1 mg of DNase I, and 0.1 ml of 1 M $CaCl_2$.
2. Gently mix and store on ice. Use immediately.

Technical Support

Web Resources

Visit the Invitrogen website 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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Corporate Headquarters:
Invitrogen Corporation
5791 Van Allen Way
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail: tech_support@invitrogen.com

Japanese Headquarters:
Invitrogen Japan
LOOP-X Bldg. 6F
3-9-15, Kaigan
Minato-ku, Tokyo 108-0022
Tel: 81 3 5730 6509
Fax: 81 3 5730 6519
E-mail: jpinfo@invitrogen.com

European Headquarters:
Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

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invitrogen™

Corporate Headquarters
Invitrogen Corporation
5791 Van Allen Way
Carlsbad, CA 92008
T: 1 760 603 7200
F: 1 760 602 6500
E: tech_support@invitrogen.com

For country-specific contact information, visit our web site at **www.invitrogen.com**