

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

USER GUIDE

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

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

Kit contents and storage

Contents

This manual is supplied with the following kits.

Cat. No.	Contents
V43001	20 µg each of pBAD/His A, B, and C vector in TE Buffer, pH 8.0 ^[1] (40 µl at 0.5 µg/µl) 20 µg each of pBAD/His/lacZ 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
V44001	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/lacZ 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

^[1] 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 “Recipes” on page 34).

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 ().

Detection of recombinant proteins

Expression of your recombinant protein can be detected using an antibody to the appropriate epitope. The table 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™	R91025
		Anti-Xpress™-HRP Antibody	R91125
	N-terminal polyhistidine tag	Penta-His™ Mouse IgG1 Monoclonal Antibody	P21315
pBAD/Myc-His	<i>c-myc</i>	Anti-Myc	R95025
		Anti-Myc-HRP	R95125
	C-terminal polyhistidine	Anti-His(C-term)	R93025
		Anti-His(C-term)-HRP	R93125



(continued)

Vector	Epitope	Antibody	Cat. No.
pBAD/Myc-His	C-terminal polyhistidine	Anti-His (C-term)-AP Antibody	R93225
		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 table for ordering information.

Product	Quantity	Cat. No.
ProBond™ Purification System	6 purifications	K85001
ProBond™ Metal-Binding Resin (precharged resin provided as a 50% slurry in 20% ethanol)	50 ml	R80101
	150 ml	R80115
Ni-NTA Purification System	6 purifications	K95001
Purification Columns (10 ml polypropylene columns)	50	R64050
EKMax™ Enterokinase	250 units	E18001

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 ().

Item	Quantity	Cat. No.
Electrocomp™ TOP10	20 reactions	C66455
	2 × 20 reactions	C66411
One Shot™ TOP10 Competent Cells	20 reactions	C404003



Pre-mixed media

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

Item	Quantity	Cat. No.
imMedia™ Growth Medium, liquid, ampicillin	20 pouches (200 ml medium)	Q60020
imMedia™ Growth Medium, agar, ampicillin	20 pouches (8–10 plates)	Q60120
Ampicillin, sodium salt, irradiated	200 mg	11593027



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 *araBAD* 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.



Experimental outline

The table 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:	“General cloning” on page 17
	pBAD/His A, B, and C	“Cloning into pBAD/His” on page 18
	pBAD/Myc-His A, B, and C	“pBAD/Myc-His multiple cloning sites” on page 22
2	To propagate and maintain the empty vectors and recombinant constructs, transform them into a <i>recA</i> , <i>endA</i> <i>E. coli</i> host (i.e., TOP10).	“General cloning” on page 17
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.	“E. coli transformation” on page 25
4	Sequence your construct to ensure that it is in frame with the N-terminal (pBAD/His) or C-terminal (pBAD/Myc-His) peptide.	“E. coli transformation” on page 25



(continued)

Step	Action	Page
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 “Detection of recombinant proteins” on page 7 for ordering information).	“Introduction” on page 26
6	Optimize expression by varying L-arabinose concentration or the time of induction.	“Optimization of expression” on page 29
7	Purify your recombinant protein by chromatography on a metal-chelating resin (see “Detection of recombinant proteins” on page 7 for ordering information).	“Purification” on page 31

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
<i>araBAD</i> promoter (P_{BAD})	Provides tight, dose-dependent regulation of heterologous gene expression (Guzman <i>et al.</i> , 1995)
Optimized ribosome binding site	Increases efficiency of recombinant fusion protein expression
Initiation ATG	Provides a translational initiation site for the fusion protein



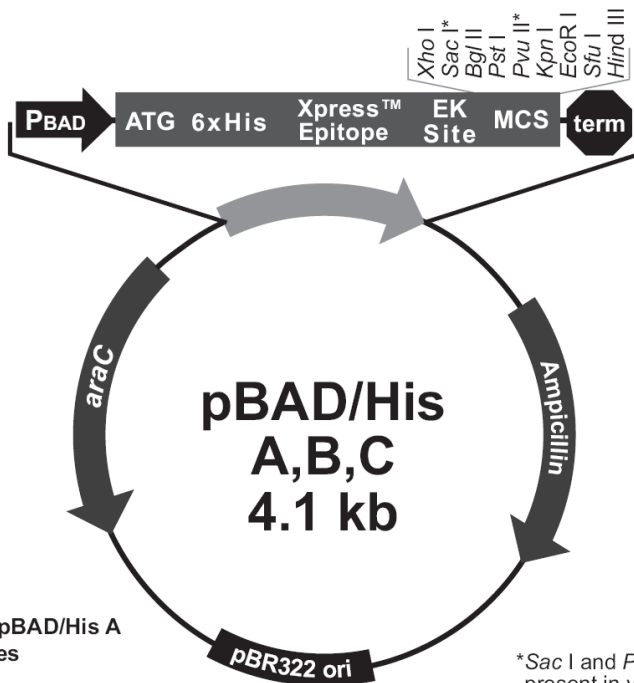
(continued)

Feature	Benefit
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 “Detection of recombinant proteins” on page 7 for ordering information)
Anti-Xpress™ epitope (Asp-Leu-Tyr-Asp-Asp-Asp-Lys)	Permits detection of recombinant fusion protein by appropriate antibodies (see “Detection of recombinant proteins” on page 7 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 “Detection of recombinant proteins” on page 7 for ordering information).
Multiple cloning site	Allows insertion of your gene for expression
<i>rrnB</i> transcription termination region	Strong transcription termination region
Ampicillin resistance gene (β -lactamase)	Allows selection of the plasmid in <i>E. coli</i>
pBR322 origin	Low copy replication and growth in <i>E. coli</i>
<i>araC</i> gene	Encodes the regulatory protein for tight regulation of the P _{BAD} promoter (Lee, 1980; Schleif, 1992)



Map of pBAD/His

The figure 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). Details of each multiple cloning site are shown in “pBAD/His A multiple cloning site” on page 19.



Comments for pBAD/His A 4102 nucleotides

araBAD 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
 rrmB transcription termination region: bases 553-710
 Ampicillin ORF: bases 989-1849
 pBR322 origin: bases 1994-2667
 AraC ORF: bases 4076-3198

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



pBAD/Myc-His vector

Features of pBAD/Myc-His

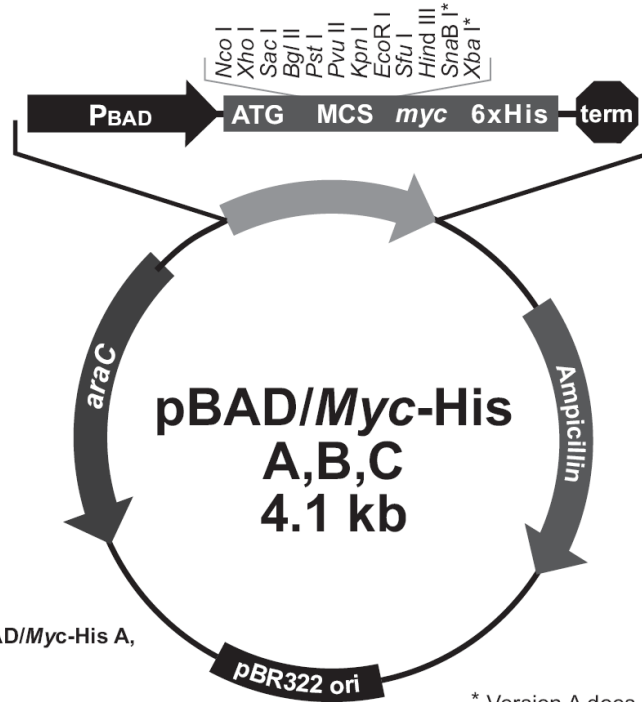
The important elements of pBAD/Myc-His A (4094 bp), pBAD/Myc-His B (4092 bp), and pBAD/Myc-His C (4093 bp) are described in the following table. All features have been functionally tested.

Feature	Benefit
<i>araBAD</i> promoter (P_{BAD})	Provides tight, dose-dependent regulation of heterologous gene expression (Guzman <i>et al.</i> , 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 <i>myc</i> epitope tag (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu)	Allows detection of the fusion protein by the Anti-Myc Antibody (Evans <i>et al.</i> , 1985) (see “Detection of recombinant proteins” on page 7 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 “Detection of recombinant proteins” on page 7 for ordering information)
<i>rrnB</i> transcription termination region	Strong transcription termination region
Ampicillin resistance gene (β -lactamase)	Allows selection of the plasmid in <i>E. coli</i>
pBR322 origin	Low copy replication and growth in <i>E. coli</i>
<i>araC</i> gene	Encodes the regulatory protein for tight regulation of the P_{BAD} promoter (Lee, 1980; Schleif, 1992)



Map of pBAD/Myc-His

The figure 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). Details of each multiple cloning site are shown in “pBAD/His A multiple cloning site” on page 19.



Comments for pBAD/Myc-His A, 4094 nucleotides

araBAD 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
rrnB 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 *SnaB* I or *Xba* I.
 Version B contains *Xba* I only.
 Version C contains *SnaB* I only.



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 *recA*, *endA* strain such as TOP10 (included in the kit, also available separately; see “Competent *E. coli*” on page 8). 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 *araBADC*⁻ and *araEFGH*⁺ (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⁻ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) φ80*lacZ*ΔM15 Δ*lacX74 recA1 araD139* Δ(*araA-leu*)7697 *galU galK rpsL endA1 nupG*.

Note: This strain is *araBADC*⁻. It is deleted for both *araBA* and *araC*, and the gene for *araD* has a point mutation in it, making it inactive.

Genotype of LMG194

F⁻ Δ*lacX74 gal E thi rpsL ΔphoA* (*Pvu* II) Δ*ara714 leu::Tn10*. 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 *recA*, *endA* *E. coli* strain like TOP10F⁺, DH5αTM-T1^R, TOP10, or equivalent. Select transformants on LB plates containing 50–100 µg/ml ampicillin.

Note: Use strains like DH5αTM **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 “Glycerol stock” on page 26).

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 in “pBAD/His A multiple cloning site” on page 19. 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 *araBAD* and *araC* promoters are marked and described as follows. For more information see Lee, 1980; Miyada, *et al.*, 1984; Lee, *et al.*, 1987; and Schleif, 1992.

- O₂ region: Binding site of AraC that represses transcription from P_{BAD}.
- O₁ region: Binding site of AraC that represses transcription of the *araC* promoter (PC) (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.
- I₂ and I₁ regions: Binding sites of AraC that activate transcription from P_{BAD}.
- –10 and –35 regions: Binding sites of RNATM polymerase for transcription of P_{BAD}.

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



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).**

```

1      AAGAAACCAA TTGTCCATAT TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT
      O2 Region

61     TCTCGCTAAC CAAACCGGTA ACCCCGCTTA TAAAAGCAT TCTGTAACAA AGCGGGACCA

121    AAGCCATGAC AAAAAACGCGT AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG
      O1 Region

181    ATTATTTGCA CGGCGTCACA CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG
      CAP binding site      pBAD forward priming site

241    ATCCTACCTG ACGCTTTTTA TCGCAACTCT C TACTGTTTC TCCATACCCG TTTTITGGGC
      -35      -10      I2 and I1 Region

301    TAACAGGAGG AATTAACC ATG GGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT
      RBS      Nco I      Polyhistidine Region
      Met Gly Gly Ser His His His His His His Gly Met Ala

358    AGC ATG ACT GGT GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT GAC GAT AAG
      Ser Met Thr Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys
      Xpress™ Epitope      EK recognition site
      EK cleavage site ▲

412    GAT CGA TGG GGA TCC GAG CTC GAG ATC TGC AGC TGG TAC CAT ATG GGA ATT CGA
      Asp Arg Trp Gly Ser Glu Leu Glu Ile Cys Ser Trp Tyr His Met Gly Ile Arg
      Xho I Sac I Bgl II Pst I Pvu II Kpn I EcoR I Sfu I

466    AGC TTG GCTGTTTTG GCGGATGAGA GAAGATTTTC AGCCTGATAC AGATTAAATC AGAACGCAGA
      Hind III      pBAD reverse priming site
      Ser Leu

531    AGCGGTCTGA TAAAACAGAA TTTGCCTGGC GGCAGTAGCG CGGTGGTCCC ACCTGACCCC

591    ATGCCGAACT CAGAAGTGAA ACGCCGTAGC GCCGATGGTA GTGTGGGGTC TCCCATGCG
      rmb T1 and T2 transcriptional terminator

651    AGAGTAGGGA ACTGCCAGGC ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCTT

711    TCGTTTTAT
  
```



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).**

```

1      AAGAAACCAA TTGTCCATAT TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT

61     TCTCGCTAAC CAAACCGGTA ACCCCGCTTA TAAAAGCAT TCTGTAACAA AGCGGGACCA

121    AAGCCATGAC AAAAACGCGT AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG
                                     O1 Region
181    ATTATTGCA CGGCGTCACA CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG
      CAP binding site      pBAD forward
                             priming site
241    ATCCTACCTG ACGCTTTTTA TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTGGGG
      -35                    -10
      I2 and I1 Region
301    TAACAGGAGG AATTAACC ATG GGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT
      RBS                    Nco I      Polyhistidine Region
      Met Gly Gly Ser His His His His His His Gly Met Ala
358    AGC ATG ACT GGT GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT GAC GAT AAG
      Ser Met Thr Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys
      Xpress™ Epitope      EK recognition site
      EK cleavage site ▲
412    GAT OCG AGC TCG AGA TCT GCA GCT GGT ACC ATA TGG GAA TTC GAA GCT TGG
      Asp Pro Ser Ser Arg Ser Ala Ala Gly Thr Ile Trp Glu Phe Glu Ala Trp
      Xho I Sac I Bgl II Pst I Pvu II Kpn I EcoR I Sfu I Hind III
463    CTGTTTTG GCGGATGAGA GAAGATTTTC AGCCTGATAC AGATTAAATC AGAACGCAGA
      pBAD reverse priming site
521    AGCGGTCTGA TAAACAGAA TTGCCTGGC GGCAGTAGCG CGGTGGTCCC ACCTGACCCC

581    ATGCCGAAC T CAGAAGTGAA ACGCCGTAGC GCCGATGGTA GTGTGGGGTC TCCCATGCG
      rmb T1 and T2 transcriptional terminator
641    AGAGTAGGGA ACTGCCAGGC ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCTT

701    TCGTTTTATC TGTGTTTG
  
```



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).**

```

1      AAGAAACCAA TTGTCCATAT TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT
      O2 Region

61     TCTCGCTAAC CAAACCGGTA ACCCCGCTTA TTAAAAGCAT TCTGTAACAA AGCGGGACCA

121    AAGCCATGAC AAAAACGCGT AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG
      O1 Region

181    ATTATTTGCA CGGCGTCACA CTTTGTATG CCATAGCATT TTTATCCATA AGATTAGCGG
      CAP binding site pBAD forward priming site

241    ATCCTACCTG ACGCTTTTTA TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTTGGGC
      -35 -10 I2 and I1 Region

301    TAACAGGAGG AATTAACC ATG GGG GGT TCT CAT CAT CAT CAT CAT CAT GGT ATG GCT
      RBS Nco I Polyhistidine Region
      Met Gly Gly Ser His His His His His His Gly Met Ala

358    AGC ATG ACT GGT GGA CAG CAA ATG GGT CGG GAT CTG TAC GAC GAT GAC GAT AAG
      Xpress™ Epitope EK recognition site
      Ser Met Thr Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys
      EK cleavage site ▲

412    GAT CGA TGG ATC CGA CTT CGA GAT CTG CAG ATG GTA CCA TAT GGG AAT
      Xho I Bgl II Pst I Kpn I EcoR I
      Asp Arg Trp Ile Arg Pro Arg Asp Leu Gln Met Val Pro Tyr Gly Asn

460    TCG AAG CTT GGCTGTTTTG GCGGATGAGA GAAGATTTTC AGCCTGATAC AGATTAATC
      Sfu I Hind III pBAD reverse priming site
      Ser Lys Leu

519    AGAACGCAGA AGCGGTCTGA TAAACAGAA TTTGCCTGGC GGCAGTAGCG CGGTGGTCCC

579    ACCTGACCCC ATGCCGAAC T CAGAAGTGAA ACGCCGTAGC GCCGATGGTA GTGTGGGGTC
      rrnB T1 and T2 transcriptional terminator

639    TCCCCATGCG AGAGTAGGGA ACTGCCAGGC ATCAAATAAA ACGAAAGGCT CAGTCGAAAG

699    ACTGGGCCTT TCGTTTTATCT
  
```



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 in “pBAD/His A multiple cloning site” on page 19. 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 *araBAD* and *araC* promoters are marked and described as follows. For more information see Lee, 1980; Miyada, *et al.*, 1984; Lee, *et al.*, 1987; and Schleif, 1992.

- O₂ region: Binding site of AraC that represses transcription from P_{BAD}.
- O₁ region: Binding site of AraC that represses transcription of the *araC* 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.
- I2 and I1 regions: Binding sites of AraC that activate transcription from P_{BAD}.
- -10 and -35 regions: Binding sites of RNATM polymerase for transcription of P_{BAD}.

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



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).**

```

1      AAGAAACCAA TTGTCCATAT TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT
      O2 Region

61     TCTCGCTAAC CAAACCGGTA ACCCCGCTTA TTAAAAGCAT TCTGTAACAA AGCGGGACCA

121    AAGCCATGAC AAAAACGCGT AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG
      O1 Region

181    ATTATTTGCA CGGCGTCACA CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG
      CAP binding site  pBAD forward priming site

241    ATCCTACCTG ACGCTTTTTA TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTTGGGC
      -35  -10  I2 and I1 Region

301    TAACAGGAGG AATTAACC ATG GATCCGAGCT CGAGATCTGC AGCTGGTACC ATATG
      RBS  Nco I  Xho I  Sac I  Bgl II  Pst I  Kpn I
      Met

357    GGAATTCGAA GCTTGGGCC GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG AAT AGC
      EcoR I  Sfu I  Hind III  myc epitope
      Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ser

413    GCC GTC GAC CAT CAT CAT CAT CAT CAT TGA GTTTAAACGG TCTCCAGCTT GGCTGTTTGG
      Polyhistidine Region
      Ala Val Asp His His His His His His ***

473    GCGGATGAGA GAAGATTTTC AGCCTGATAC AGATTAAATC AGAACGCAGA AGCGGTCTGA
      pBAD reverse priming site

533    TAAACAGAA TTGCTGGC GGCAGTAGCG CCGTGGTCCC ACCTGACCCC ATGCCGAAT

593    CAGAAGTGAA ACGCCGTAGC GCCGATGGTA GTGTGGGGTC TCCCATGCG AGAGTAGGGA
      rrnB T1 and T2 transcriptional terminators

653    ACTGCCAGGC ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCTT TCGTTTTATC
  
```



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).**

```

1      O2 Region
      AAGAAACCAA TTGTCCATAT TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT

61     TCTCGCTAAC CAAACCGGTA ACCCCGCTTA TTAAAAGCAT TCTGTAACAA AGCGGGACCA

121    AAGCCATGAC AAAAACGCGT AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG
      O1 Region
181    ATTATTTGCA CGGCGTCACA CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG
      CAP binding site      pBAD forward
      priming site
241    ATCCTACCTG ACGCTTTTTA TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTGGGC
      -35      -10      I2 and I1 Region
301    TAACAGGAGG AATTAACC ATG GATCCGAGCT CGAGATCTGC AGCTGGTACC ATATG
      RBS      Nco I      Xho I      Sac I Bgl II      Pst I      Kpn I
      Met
357    GGAATTCGAA GCTTTCTA GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG AAT AGC GCC
      EcoR I      Sfu I      Hind III      Xba I      myc epitope
      Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ser Ala
414    GTC GAC CAT CAT CAT CAT CAT TGA GTTTAAACGG TCTCCAGCTT GGCTGTTTTG
      Polyhistidine Region
      Val Asp His His His His His His ***
471    GCGGATGAGA GAAGATTTTC AGCCTGATAC AGATTAAATC AGAACGCAGA AGCGGTCTGA
      pBAD reverse priming site
531    TAAAACAGAA TTTCCTGGC GGCAGTAGCG CGGTGGTCCC ACCTGACCCC ATGCCGAACT
      mmB T1 and T2 transcriptional terminators
591    CAGAAGTGAA ACGCCGTAGC GCCGATGTA GTGTGGGGTC TCCCCATGCG AGAGTAGGGA
651    ACTGCCAGGC ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCTT TCGTTTTATC

```




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).**

```

1      O2 Region
      AAGAAACCAA TTGTCATAT TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT
61     TCTCGCTAAC CAAACCGGTA ACCCCGCTTA TTAAAAGCAT TCTGTAACAA AGCGGGACCA
121    AAGCCATGAC AAAAACGCGT AACAAAAGTG TCTATAATCA CCGCAGAAAA GTCCACATTG
      O1 Region
181    ATTATTTGCA CGGCGTCACA CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG
      CAP binding site      pBAD forward
      priming site
241    ATCCTACCTG ACGCTTTTTA TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTTGGGC
      I1 and I2 Region
301    TAACAGGAGG AATTAACC ATG GATCCGAGCT CGAGATCTGC AGTGGTACC ATATGGGAAT
      RBS      Nco I      Xho I      Sac I Bgl II      Pst I      Kpn I      EcoR I
      Met
362    TCGAAGCT TA CGTA GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG AAT AGC GCC
      Sfu I Hind III      SnaB I      myc epitope
      Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ser Ala
415    GTC GAC CAT CAT CAT CAT CAT CAT TGA GTTTAAACGG TCTCCAGCTT GGCTGTTTTG
      Polyhistidine Region
      Val Asp His His His His His His ***
472    GCGGATGAGA GAAGATTTTC AGCCTGATAC AGATTAAATC AGAACGCAGA AGCGGTCTGA
      pBAD reverse priming site
532    TAAAACAGAA TTGCTGGC GGCAGTAGCG CGGTGGTCCC ACCTGACCCC ATGCCGAACT
592    CAGAAGTGAA ACGCCGTAGC GCCGATGGTA GTGTGGGGTC TCCCCATGCG AGAGTAGGGA
      mmB T1 and T2 transcriptional terminators
652    ACTGCCAGGC ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCTT TCGTTTTATC

```

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 $\mu\text{g/ml}$ 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/lacZ or pBAD/Myc-His/lacZ are included for use as positive expression controls (see “pBAD/His/lacZ” on page 32). 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 for protocol.
2. To optimize expression of your protein, you may wish to try L-arabinose concentrations spanning the amount determined in Step 1 on page 26. 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 “Recipes” on page 34) 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 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

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” on page 34)
- RM medium containing glucose (see “Recipes” on page 34)
- 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 “Recipes” on page 34)
- Sterile water

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 $OD_{600} = \sim 0.5$ (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 on page 28 and 8 on page 28.

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 for a protocol.

1. When all the samples have been collected from Steps 8 on page 28 and 11 on page 28, 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/lacZ or pBAD/Myc-His/lacZ) 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 "Detection of recombinant proteins" on page 7 for ordering information. For more information, refer to www.invitrogen.com or contact Technical Support ().

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'- ATGCCATAGCATT TTTATCC -3'
pBAD reverse primer	5'- GATTTAATCTGTATCAGG -3'

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 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” on page 34).
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 $+4^{\circ}\text{C}$ to pellet insoluble proteins. Transfer™ supernatant to a fresh tube and store on ice. Store the pellets on ice (see Step 5 on page 30).
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 on page 30 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 *araBAD* 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 “Pilot expression” on page 27, substituting RM Medium + Glucose medium (see “RM medium + glucose” on page 36) 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 “Purification of recombinant proteins” on page 8 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 (“RM medium + glucose” on page 36) 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 $OD_{600} = \sim 0.5$ (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 “Purification of recombinant proteins” on page 8 for other recommended purification products available from Invitrogen™. For more information, refer to www.invitrogen.com or contact Technical Support (see).

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



Appendix

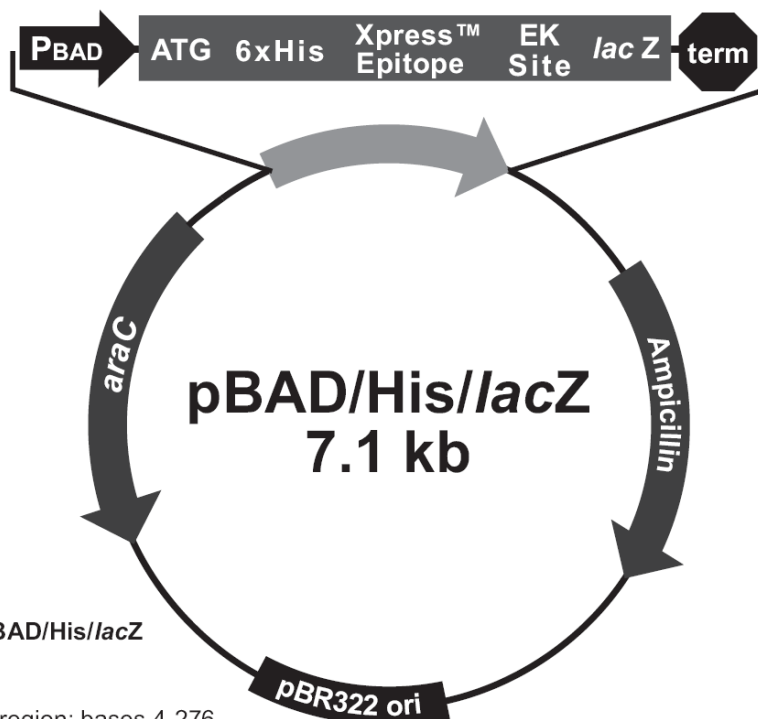
pBAD/His/lacZ

Description

pBAD/His/lacZ 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/lacZ with *Nco* I and *Nsi* I to remove the *lacI^q* gene and the *trc* promoter and replacing with an *Nco* I–*Nsi* I fragment containing the *araC* gene and the *araBAD* promoter. The β -galactosidase portion of the fusion may be released by digestion with *Bam*H I and *Hind* III. The vector expresses a 120 kDa protein.

Map of control vector

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



Comments for pBAD/His/lacZ 7115 nucleotides

araBAD 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
rnnB transcription termination region: bases 3565-3722
 Ampicillin ORF: bases 4002-4862
 pBR322 origin: bases 5007-5680
AraC ORF: bases 7089-6211

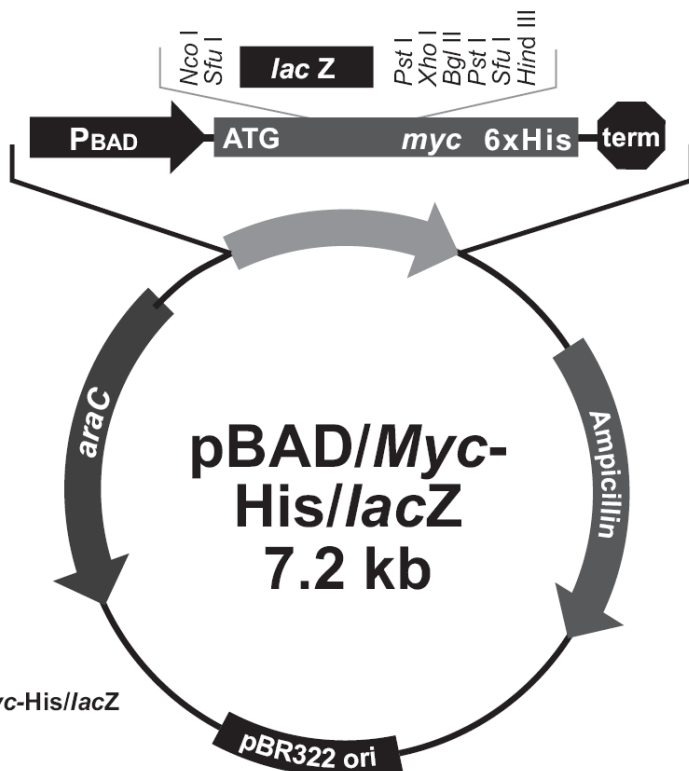
pBAD/Myc-His/lacZ

Description

pBAD/Myc-His/lacZ 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/lacZ with *Nco* I and *Nsi* I to remove the *lacI* gene and the *trc* promoter and replacing with an *Nco* I-*Nsi* I fragment containing the *araC* gene and the *araBAD* 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 summarizes the features of the pBAD/*Myc*-His/*lacZ* vector. **The complete nucleotide sequence for pBAD/*Myc*-His/*lacZ* is available at www.invitrogen.com or by contacting Technical Support (see).**



Comments for pBAD/*Myc*-His/*lacZ* 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
rrnB 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 “Pre-mixed media” on page 9 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.

SOB medium (with ampicillin)

SOB (per liter)

2% Tryptone

0.5% Yeast Extract

0.05% NaCl

2.5 mM KCl

10 mM MgCl₂

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 on page 36.
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 MgCl₂. 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 for recipe for 10X M9 Salts)

2% Casamino Acids

0.2% glucose

1 mM MgCl₂

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 MgCl₂ 1 ml
6. 20% glucose 10 ml



- 100 mg/ml ampicillin 0.5 to 1 ml
- Mix well and store medium containing ampicillin at +4°C.

Medium is good for 1 month at +4°C.

10X M9 salts

For 1 liter:

Na ₂ HPO ₄	60 g
KH ₂ PO ₄	30 g
NaCl	5 g
NH ₄ Cl	10 g
Water	900 ml

- Dissolve reagents in the water and adjust the pH to 7.4 with 10 M NaOH.
- Add water to 1 liter and autoclave for 20 minutes on liquid cycle.
- 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 CaCl₂

- 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 CaCl₂.
- Gently mix and store on ice. Use immediately.

References

- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1994). *Current Protocols in Molecular Biology* (New York: Greene Publishing Associates and Wiley-Interscience).
- Bachmann, B. J. (1990). Linkage map of *Escherichia coli* K-12, edition 8. *Microbiol. Rev* *54*, 130-197.
- Carson, M. J., Barondess, J. J., and Beckwith, J. (1991). The FtsQ Protein of *Escherichia coli*: Membrane Topology, Abundance, and Cell Division Phenotypes Due to Overproduction and Insertion Mutations. *J. Bacteriol.* *173*, 2187-2195.
- Dalbey, R. E., and Wickner, W. (1985). Leader Peptidase Catalyzes the Release of Exported Proteins from the Outer Surface of the *Escherichia coli* Plasma Membrane. *J. Biol. Chem.* *260*, 15925-15931.
- Evans, G. I., Lewis, G. K., Ramsay, G., and Bishop, V. M. (1985). Isolation of Monoclonal Antibodies Specific for *c-myc* Proto-oncogene Product. *Mol. Cell. Biol.* *5*, 3610-3616.
- Guzman, L.-M., Barondess, J. J., and Beckwith, J. (1992). FtsL, an Essential™ Cytoplasmic Membrane Protein Involved in Cell Division in *Escherichia coli*. *J. Bacteriol.* *174*, 7716-7728.
- Guzman, L.-M., Belin, D., Carson, M. J., and Beckwith, J. (1995). Tight Regulation, Modulation, and High-Level Expression by Vectors Containing the Arabinose P_{BAD} Promoter. *J. Bacteriol.* *177*, 4121-4130.
- Kuhn, A., and Wickner, W. (1985). Isolation of Mutants in M13 Coat Protein That Affect its Synthesis, Processing and Assembly into Phage. *J. Biol. Chem.* *260*, 15907-15913.
- Lee, N. (1980) Molecular Aspects of *ara* Regulation. In *The Operon*, J. H. Miller and W. S. Reznikoff, eds. (Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory), pp. 389-410.
- Lee, N., Francklyn, C., and Hamilton, E. P. (1987). Arabinose-Induced Binding of AraC Protein to *araI2* Activates the *araBAD* Operon Promoter. *Proc. Natl. Acad. Sci. USA* *84*, 8814-8818.
- Miyada, C. G., Stoltzfus, L., and Wilcox, G. (1984). Regulation of the *araC* Gene of *Escherichia coli*: Catabolite Repression, Autoregulation, and Effect on *araBAD* Expression. *Proc. Natl. Acad. Sci. USA* *81*, 4120-4124.
- Russell, C. B., Stewart, R. C., and Dahlquist, F. W. (1989). Control of Transducer Methylation Levels in *Escherichia coli*: Investigation of Components Essential™ for Modulation of Methylation and Demethylation Reactions. *J. Bacteriol.* *171*, 3609-3618.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, Second Edition (Plainview, New York: Cold Spring Harbor Laboratory Press).
- San Millan, J. L., Boyd, D., Dalbey, R., Wickner, W., and Beckwith, J. (1989). Use of *phoA* Fusion™s to Study the Topology of the *Escherichia coli* Inner Membrane Protein Leader Peptidase. *J. Bacteriol.* *171*, 5536-5541.



Schleif, R. S. (1992). DNA Looping. *Ann. Rev. Biochem.* 61, 199-223.



Safety



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
 - Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.
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Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
<https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf>
 - World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf
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 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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