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

Version F
120701
25-0187

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 6xHis Tags in *E. coli*

Catalog nos. V430-01, V440-01

www.invitrogen.com
tech_service@invitrogen.com

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Important Information

Contents

This manual is provided with the following kits:

Catalog No.	Contents
V430-01	20 µg each pBAD/His A, B, and C, lyophilized 20 µg pBAD/His/*lac*Z, lyophilized 1 ml sterile, 20% L-arabinose 1 stab LMG194 1 stab TOP10
V440-01	20 µg each pBAD/*Myc*-His A, B, and C, lyophilized 20 µg pBAD/*Myc*-His/*lac*Z, lyophilized 1 ml sterile, 20% L-arabinose 1 stab LMG194 1 stab TOP10

Shipping/Storage

Kits are shipped at room temperature. Upon receipt, store the plasmids and the 20% L-arabinose solution at -20°C. Stabs should be stored at room temperature.

Purchaser Notification

***araB* promoter**

Products containing the *araB* promoter are sold under patent license for **research purposes only** and are non-transferable. Inquiries for any commercial use, including production of material to be sold commercially or used in production or in product development efforts which includes efforts toward regulatory approval, should be made directly to Xoma Corporation, Berkeley, California.

Xoma Corporation
2910 Seventh Street
Berkeley, CA 94710
Tel: 1-510-644-1170
Fax: 1-510-649-7571

Polyhistidine (6xHis) Tag

This product is licensed under U.S. Patents 5,284,933 and 5,310,663 and foreign equivalents from Hoffmann-LaRoche, Inc., Nutley, NJ and/or Hoffmann-LaRoche Ltd., Basel, Switzerland and is provided only for use in research. Information about licenses for commercial use is available from:

QIAGEN GmbH
Max-Volmer-Str. 4
D-40724 Hilden, Germany

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, please refer to Schleif, 1992.

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, please refer to the page(s) indicated.

Step	Action	Page
1	Develop a cloning strategy to ligate your gene of interest into the desired vector. Please 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 next page).	18
6	Optimize expression by varying L-arabinose concentration or the time of induction.	19-20
7	Purify your recombinant protein by chromatography on metal-chelating resin (e.g. ProBond™).	21

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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	Catalog No.
pBAD/His	Anti-Xpress™	Anti-Xpress™	R910-25
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

Purification of Recombinant Protein

The metal binding domain encoded by the polyhistidine tag allows simple, easy purification of your recombinant protein by Immobilized Metal Affinity Chromatography (IMAC) using Invitrogen's ProBond™ Resin (see below). To purify proteins expressed using pBAD/His or pBAD/*Myc*-His, the ProBond™ Purification System or the ProBond™ resin in bulk are available separately. See the table below for ordering information.

Product	Quantity	Catalog 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
Purification Columns (10 ml polypropylene columns)	50	R640-50

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 metal-chelating resin (i.e. ProBond™)
Anti-Xpress™ epitope (Asp-Leu-Tyr-Asp-Asp-Asp-Asp-Lys)	Permits detection of recombinant fusion protein by the Anti-Xpress™ Antibody (Catalog no. R910-25)
Enterokinase cleavage site (Asp-Asp-Asp-Asp-Lys)	Allows removal of the N-terminal peptide by enterokinase (i.e. EKMax™, Catalog no. E180-01) for production of native protein
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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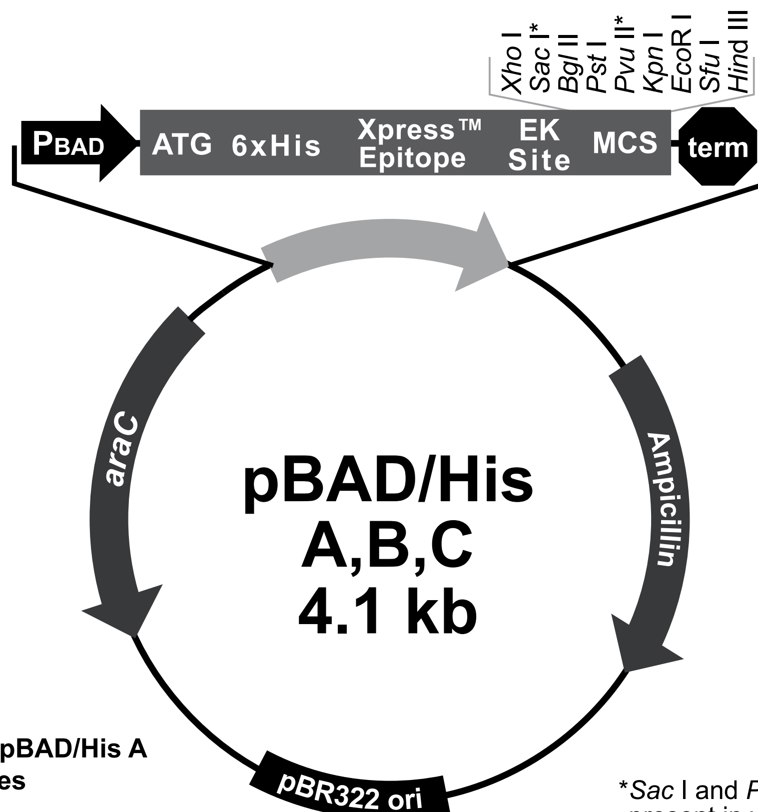
pBAD/His Vectors, continued

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 from our World Wide Web site (www.invitrogen.com) or by contacting Technical Service (see page 27). Details of each multiple cloning site are shown on pages 9-11.



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

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

pBAD/*Myc*-His Vector

Features of pBAD/*Myc*-His

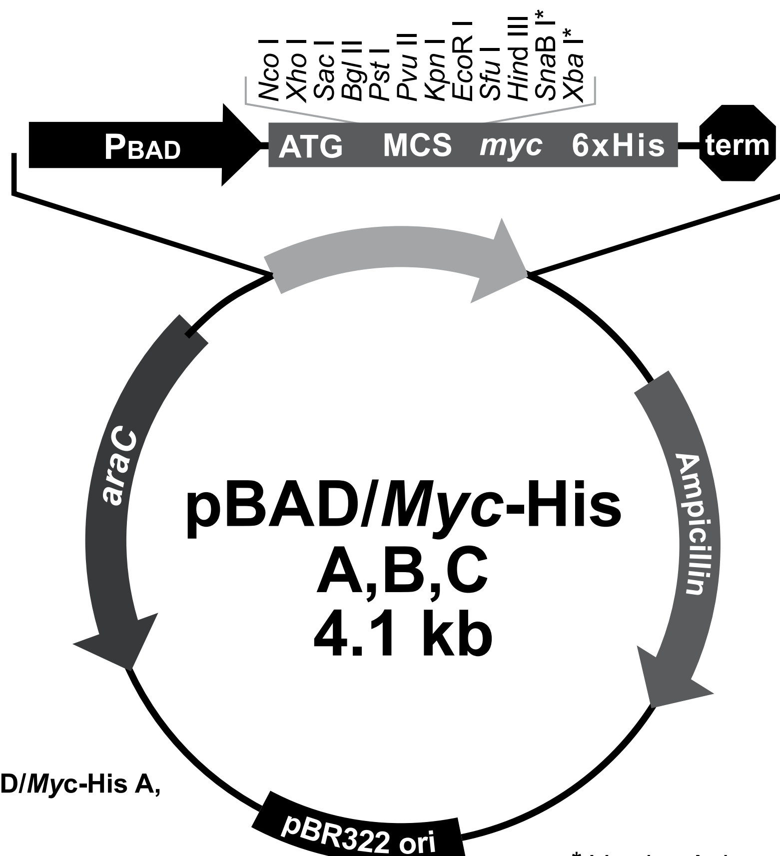
The important elements of pBAD/*Myc*-His A (4094 bp), pBAD/*Myc*-His B (4092 bp), and pBAD/*Myc*-His C (4093 bp) are described in the following table. All features have been functionally tested.

Feature	Benefit
*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 (Catalog no. R950-25) (Evans *et al.*, 1985)
C-terminal polyhistidine region	Forms metal-binding site for affinity purification of recombinant fusion protein on metal-chelating resin (i.e. ProBond™) In addition, it allows detection of the recombinant protein with Anti-His (C-term) Antibody (see page 2)
*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)

continued on next page

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 from our World Wide Web site (www.invitrogen.com) or by contacting Technical Service (see page 27). 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, please 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). 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.**

For your convenience, TOP10 is available as electrocompetent or chemically competent cells in a One Shot® kit format.

Item	Quantity	Catalog no.
Electrocomp™ TOP10	5 x 80 µl	C664-55
	10 x 80 µl	C664-11
	30 x 80 µl	C664-24
One Shot® TOP10 Competent Cells	21 x 50 µl	C4040-03

Genotype of TOP10

F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lac*ZΔM15 Δ*lac*X74 *deo*R *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, we recommend that you resuspend the lyophilized vector in 20 µl sterile water to make a 1 µg/µl stock solution. Store the stock solution at -20°C.

Use this stock solution to transform a *rec*A, *end*A *E. coli* strain like DH5α, TOP10 or equivalent. Transformants are selected 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 please 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

pBAD/His A Multiple Cloning Site

```
              O2 Region
1      AAGAAACCAA TTGTCCATAT TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT

61     TCTCGCTAAC CAAACCGGTA ACCCCGCTTA TTAAAAGCAT TCTGTAACAA AGCGGGACCA

                                                        O1 Region
121    AAGCCATGAC AAAAACGCGT AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG

                            CAP binding site
181    ATTATTTGCA CGGCGTCACA CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG
                                                      I2 and I1 Region
            -35                         -10
241    ATCCTACCTG ACGCTTTTTA TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTTGGGC

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

                                                               EK recognition site
                                                  Xpress™ Epitope
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
                                                                    EK cleavage site

                             Xho I  Sac I  Bgl II     Pst I  Pvu II    Kpn I        EcoR I   Sfu I
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

    Hind III
466    AGC TTG GCTGTTTTG GCGGATGAGA GAAGATTTTC AGCCTGATAC AGATTAAATC AGAACGCAGA
       Ser Leu

531    AGCGGTCTGA TAAAACAGAA TTTGCCTGGC GGCAGTAGCG CGGTGGTCCC ACCTGACCCC

                         rrnB T1 and T2 transcriptional terminator
591    ATGCCGAACT CAGAAGTGAA ACGCCGTAGC GCCGATGGTA GTGTGGGGTC TCCCCATGCG

651    AGAGTAGGGA ACTGCCAGGC ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCTT

711    TCGTTTTAT
```

continued on next page

pBAD/His B Multiple Cloning Site

```
                O2 Region
1      AAGAAACCAA TTGTCCATAT TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT

61     TCTCGCTAAC CAAACCGGTA ACCCCGCTTA TTAAAAGCAT TCTGTAACAA AGCGGGACCA

                                                        O1 Region
121    AAGCCATGAC AAAAACGCGT AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG

                              CAP binding site
181    ATTATTTGCA CGGCGTCACA CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG
                                                  I2 and I1 Region

                 -35                         -10
241    ATCCTACCTG ACGCTTTTTA TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTTGGGC

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

                                                    Xpress™ Epitope  EK recognition site
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
                                                                EK cleavage site ▲

                   Xho I  Sac I  Bgl II   Pst I  Pvu II   Kpn I     EcoR I Sfu I  Hind III
412    GAT CCG 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

463    CTGTTTTG GCGGATGAGA GAAGATTTTC AGCCTGATAC AGATTAAATC AGAACGCAGA

521    AGCGGTCTGA TAAAACAGAA TTTGCCTGGC GGCAGTAGCG CGGTGGTCCC ACCTGACCCC

                         rrnB T1 and T2 transcriptional terminator
581    ATGCCGAACT CAGAAGTGAA ACGCCGTAGC GCCGATGGTA GTGTGGGGTC TCCCCATGCG

641    AGAGTAGGGA ACTGCCAGGC ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCTT

701    TCGTTTTATC TGTTGTTTG
```

pBAD/His C Multiple Cloning Site

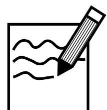
```
             O2 Region
1     AAGAAACCAA TTGTCCATAT TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT

61    TCTCGCTAAC CAAACCGGTA ACCCCGCTTA TTAAAAGCAT TCTGTAACAA AGCGGGACCA

                                                        O1 Region
121   AAGCCATGAC AAAAACGCGT AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG

                               CAP binding site
181   ATTATTTGCA CGGCGTCACA CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG
                                                     I2 and I1 Region
           -35                         -10
241   ATCCTACCTG ACGCTTTTTA TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTTGGGC

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

                                           Xpress™ Epitope    EK recognition site
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
                                                                 EK cleavage site

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

      Sfu I Hind III
460   TCG AAG CTT GGCTGTTTTG GCGGATGAGA GAAGATTTTC AGCCTGATAC AGATTAAATC
      Ser Lys Leu

519   AGAACGCAGA AGCGGTCTGA TAAAACAGAA TTTGCCTGGC GGCAGTAGCG CGGTGGTCCC

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

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 Site

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 please 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

pBAD/*Myc*-His A Multiple Cloning Site

```
          O2 Region
1     AAGAAACCAA TTGTCCATAT TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT

61    TCTCGCTAAC CAAACCGGTA ACCCCGCTTA TTAAAAGCAT TCTGTAACAA AGCGGGACCA

                                                         O1 Region
121   AAGCCATGAC AAAAACGCGT AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG

                             CAP binding site
181   ATTATTTGCA CGGCGTCACA CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG
                                                  I2 and I1 Region

           -35                        -10
241   ATCCTACCTG ACGCTTTTTA TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTTGGGC

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

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

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

473   GCGGATGAGA GAAGATTTTC AGCCTGATAC AGATTAAATC AGAACGCAGA AGCGGTCTGA

533   TAAAACAGAA TTTGCCTGGC GGCAGTAGCG CGGTGGTCCC ACCTGACCCC ATGCCGAACT

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

653   ACTGCCAGGC ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCTT TCGTTTTATC
```


continued on next page

Cloning into pBAD/*Myc*-His, continued

pBAD/*Myc*-His B
Multiple Cloning Site

```
           O2 Region
1     AAGAAACCAA TTGTCCATAT TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT

61    TCTCGCTAAC CAAACCGGTA ACCCCGCTTA TTAAAAGCAT TCTGTAACAA AGCGGGACCA

                                                       O1 Region
121   AAGCCATGAC AAAAACGCGT AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG

                              CAP binding site
181   ATTATTTGCA CGGCGTCACA CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG
                                                   I2 and I1 Region
               -35                       -10
241   ATCCTACCTG ACGCTTTTTA TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTTGGGC

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

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

                     Polyhistidine Region
414   GTC GAC CAT CAT CAT CAT CAT CAT TGA GTTTAAACGG TCTCCAGCTT GGCTGTTTTG
      Val Asp His His His His His His ***

471   GCGGATGAGA GAAGATTTTC AGCCTGATAC AGATTAAATC AGAACGCAGA AGCGGTCTGA

531   TAAAACAGAA TTTGCCTGGC GGCAGTAGCG CGGTGGTCCC ACCTGACCCC ATGCCGAACT

                       rrnB T1 and T2 transcriptional terminators
591   CAGAAGTGAA ACGCCGTAGC GCCGATGGTA GTGTGGGGTC TCCCCATGCG AGAGTAGGGA

651   ACTGCCAGGC ATCAAATAAA ACGAAAGGCT CAGTCGAAAG ACTGGGCCTT TCGTTTTATC
```

pBAD/*Myc*-His C Multiple Cloning Site

```
              O2 Region
1     AAGAAACCAA TTGTCCATAT TGCATCAGAC ATTGCCGTCA CTGCGTCTTT TACTGGCTCT

61    TCTCGCTAAC CAAACCGGTA ACCCCGCTTA TTAAAAGCAT TCTGTAACAA AGCGGGACCA

                                                           O1 Region
121   AAGCCATGAC AAAAACGCGT AACAAAAGTG TCTATAATCA CGGCAGAAAA GTCCACATTG

                              CAP binding site
181   ATTATTTGCA CGGCGTCACA CTTTGCTATG CCATAGCATT TTTATCCATA AGATTAGCGG
                                                      I1 and I2 Region
           -35                           -10
241   ATCCTACCTG ACGCTTTTTA TCGCAACTCT CTACTGTTTC TCCATACCCG TTTTTTGGGC

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

 Sfu I Hind III  SnaB I                    myc epitope
362   TCGAAGCTTA CGTA GAA CAA AAA CTC ATC TCA GAA GAG GAT CTG AAT AGC GCC
                      Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ser Ala

                Polyhistidine Region
415   GTC GAC CAT CAT CAT CAT CAT CAT TGA GTTTAAACGG TCTCCAGCTT GGCTGTTTTG
      Val Asp His His His His His His ***

472   GCGGATGAGA GAAGATTTTC AGCCTGATAC AGATTAAATC AGAACGCAGA AGCGGTCTGA

532   TAAAACAGAA TTTGCCTGGC GGCAGTAGCG CGGTGGTCCC ACCTGACCCC ATGCCGAACT

                     rrnB T1 and T2 transcriptional terminators
592   CAGAAGTGAA ACGCCGTAGC GCCGATGGTA GTGTGGGGTC TCCCCATGCG AGAGTAGGGA

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 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 22-23). TOP10 may be used as a general host for expression. LMG194 should be used if your protein is toxic or essential to *E. coli*.

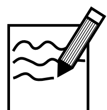
Basic Strategy

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. Please 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

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 24-25)
- 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 26 for recipe)
- 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 containing 50 µg/ml ampicillin with a single recombinant *E. coli* colony. **Note: 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

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, please 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 the Anti-Xpress™ (for proteins expressed using pBAD/His) or Anti-*Myc* (for proteins expressed using pBAD/*Myc*-His) antibodies to detect expression of your protein (see page 2 for ordering information). **Note**: Proteins expressed using pBAD/*Myc*-His may also be detected with the Anti-His (C-term) Antibody (Catalog no. R930-25) which recognizes histidine tags with a free carboxyl group.

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.

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. Please refer to the protocol on the next page to prepare samples.

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

continued on next page

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 26).
2. Place sample on ice and sonicate 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 18, 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

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. See page 2 for ordering information. **Note**: Remember to use RM medium (page 26) with LMG194.

1. Inoculate 2 ml of SOB or LB 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 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 x g for 10 minutes at +4°C).
7. At this point, you may proceed directly to purification (see ProBond™ Purification System manual) or store at -80°C for future use.

Purification

For help with purification of your recombinant protein, please refer to the ProBond™ Purification System manual.

If you are using another type of resin, please refer to the manufacturer's recommendations.

Appendix

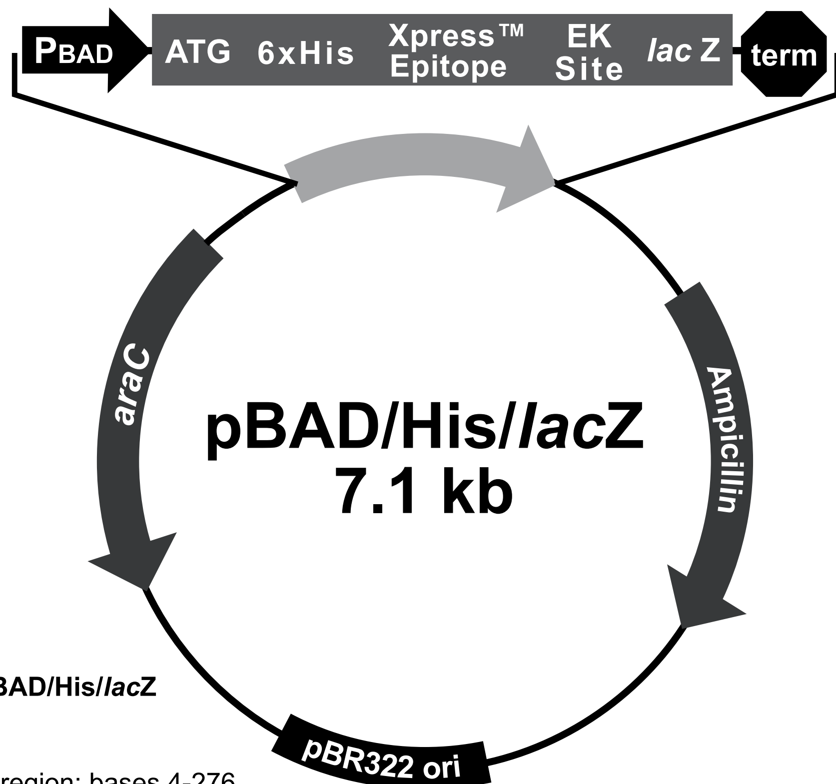
pBAD/His/*lac*Z

Description

pBAD/His/*lac*Z is a 7115 bp control vector containing the gene for β-galactosidase 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 for downloading from our World Wide Web site (www.invitrogen.com) or by contacting Technical Service (see page 27).**



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

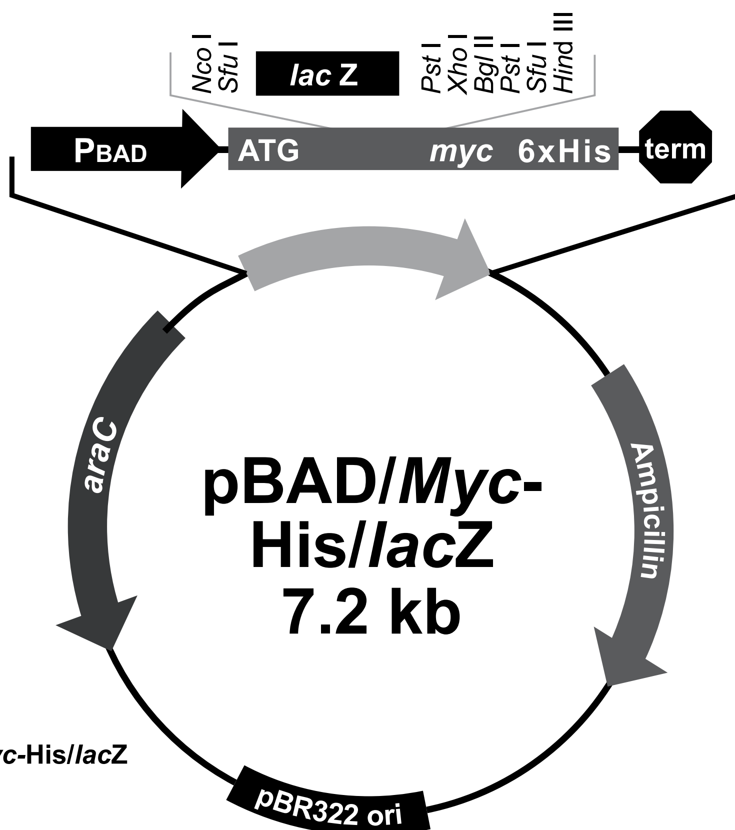
pBAD/*Myc*-His/*lac*Z

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^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 *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 for downloading from our World Wide Web site (www.invitrogen.com) or by contacting Technical Service (see page 27).**



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 in convenient pouches or in bulk. Please see the table below for ordering information.

Item	Amount	Catalog no.
imMedia™ Amp Liquid	20 pouches (200 ml medium)	Q600-20
imMedia™ Amp Agar	20 pouches (8-10 plates)	Q601-20
Ampicillin	20 ml	11593-019

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

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 below 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:

10X M9 Salts	100 ml
1 M $MgCl_2$	1 ml
20% glucose	10 ml
100 mg/ml ampicillin	0.5 to 1 ml

4. 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_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.

continued on next page

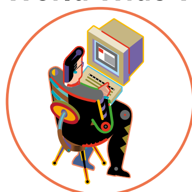
Lysis Buffer

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 Service

World Wide Web



Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

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Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

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United States Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail:
tech_service@invitrogen.com

Japanese Headquarters
Invitrogen Japan K.K.
Nihonbashi Hama-Cho Park Bldg. 4F
2-35-4, Hama-Cho, Nihonbashi
Tel: 81 3 3663 7972
Fax: 81 3 3663 8242
E-mail: jpinfo@invitrogen.com

European Headquarters:
Invitrogen Ltd
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Tel (Free Phone Orders): 0800 269 210
Tel (General Enquiries): 0800 5345 5345
Fax: +44 (0) 141 814 6287
E-mail: eurotech@invitrogen.com

MSDS Requests

To request an MSDS, please visit our Web site (www.invitrogen.com) and follow the instructions below.

1. On the home page, go to the left-hand column under ‘Technical Resources’ and select ‘MSDS Requests’.
2. Follow instructions on the page and fill out all the required fields.
3. To request additional MSDSs, click the ‘Add Another’ button.
4. All requests will be faxed unless another method is selected.
5. When you are finished entering information, click the ‘Submit’ button. Your MSDS will be sent within 24 hours.

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Technical Service, continued

Emergency Information

In the event of an emergency, customers of Invitrogen can call the 3E Company, 24 hours a day, 7 days a week for disposal or spill information. The 3E Company can also connect the customer with poison control or with the University of California at San Diego Medical Center doctors.

3E Company
Voice: 1-760-602-8700

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Product Qualification

Introduction

Invitrogen qualifies the pBAD/His and pBAD/*Myc*-His vectors as described below.

Restriction Digest

pBAD/His A, B, and C, pBAD/His/*lac*Z, pBAD/*Myc*-His A, B, and C, and pBAD/*Myc*-His/*lac*Z are qualified by restriction enzyme digestion. The table below lists the restriction enzymes and the expected fragments.

Vector	Restriction Enzyme	Expected Results
pBAD/His A	*Afl* III *Nhe* I *Sac* I *Sal* I	2.5, 1.6 kb 4.1 kb 4.1 kb --------
pBAD/His B	*Afl* III *Nhe* I *Sac* I *Sal* I	2.5, 1.6 kb 4.1 kb 4.1 kb --------
pBAD/His C	*Afl* III *Nhe* I *Sac* I *Sal* I	2.5, 1.6 kb 4.1 kb -------- --------
pBAD/His/*lac*Z	*Afl* III *Bam*H I/*Hin*D III *Sac* I *Sal* I	2500, 1573, 1540, 780, 425, 252 bp 3877, 3238, 173 bp 7.1 kb --------
pBAD/*Myc*-His A	*Afl* III *Nhe* I *Sac* I *Sal* I *Sna*B I *Xba* I	2.5, 1.6 kb -------- 4.1 kb 4.1 kb -------- --------
pBAD/*Myc*-His B	*Afl* III *Nhe* I *Sac* I *Sal* I *Sna*B I *Xba* I	2.5, 1.6 kb -------- 4.1 kb 4.1 kb -------- 4.1 kb
pBAD/*Myc*-His C	*Afl* III *Nhe* I *Sac* I *Sal* I *Sna*B I *Xba* I	2.5, 1.6 kb -------- 4.1 kb 4.1 kb 4.1 kb --------
pBAD/*Myc*-His/*lac*Z	*Afl* III *Bam*H I/*Hin*D III *Sac* I *Sal* I	2672, 1573, 1585, 780, 425, 252 bp 3273 bp, 3969 bp 7.1 kb 7.1 kb

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