invitrogen™

pTrcHis A, B, and C

Vectors for Expression of Recombinant Proteins Containing N-Terminal 6xHis Tags in *E. coli*

Catalog no. V360–20

Version H
20 October 2008
25-0038

User Manual

Table of Contents

Kit Contents and Storage

Shipping and Storage

The vectors included with Catalog no. V360–20 are shipped on wet ice.

Upon receipt, **store vectors at –20°C**.
Upon receipt, **store stabs at 4°C**.

Kit Contents

The following components are included with Catalog no. V360–20. Note that the vectors are supplied in suspension.

Note: For long-term storage of your stab strains, we recommend preparing a glycerol stock immediately upon receipt and storing at –80°C.

Component	Quantity	Composition
pTrcHis A Expression Vector	20 µg	40 µl of 0.5 µg/µl plasmid DNA in 10 mM Tris–HCl, 1 mM EDTA, pH 8.0
pTrcHis B Expression Vector	20 µg	40 µl of 0.5 µg/µl plasmid DNA in 10 mM Tris–HCl, 1 mM EDTA, pH 8.0
pTrcHis C Expression Vector	20 µg	40 µl of 0.5 µg/µl plasmid DNA in 10 mM Tris–HCl, 1 mM EDTA, pH 8.0
TOP10 stab pTrcHis/CAT Positive Control	1 Stab	– –
TOP10 *E. coli* stab	1 Stab	– –

Accessory Products

Additional Products

The following products are available separately from Invitrogen. For more information, refer to our web site at www.invitrogen.com or contact **Technical Support** (page 14).

Product	Quantity	Catalog No.
Anti-Xpress™ Antibody	50 µl	R910–25
Anti-Xpress™-HRP Antibody	50 µl	R911–25
ProBond™ Resin	50 ml	R801–01
	150 ml	R801–50
ProBond™ Purification System	6 purifications	K850–01
EKMax™	250 units	E180–01
One Shot® Top 10 Electrocomp™ *E. coli*	10 × 50 µl	C4040–50
One Shot® Top 10 Chemically Competent *E. coli*	10 × 50 µl	C4040–10

Introduction

Overview

Introduction

The pTrcHis vectors are pBR322-derived expression vectors designed for efficient recombinant protein expression and purification in *E. coli*. High levels of expression are possible using the *trc* (*trp-lac*) promoter (Egon *et al.*, 1983) and the *rrn*B anti-termination region (Li *et al.*, 1984). The *trc* promoter contains the -35 region of the *trp* promoter together with the -10 region of the *lac* promoter (Brosius *et al.*, 1985; Egon *et al.*, 1983; Mulligan *et al.*, 1985). The pTrcHis vectors also contain a copy of the *lacI*^{q} gene which codes for the *lac* repressor protein. This allows for efficient repression of transcription of the cloned insert in *E. coli* regardless of whether the strain is *lacI*$^{q+}$ or *lacI*$^{q-}$.

When expression is desired, the *E. coli* are grown to mid-log phase and IPTG (isopropyl-β-D-thiogalactoside) is added to 1 mM to induce expression (via derepression). Translation is enhanced by the presence of a minicistron that provides highly efficient translational restart into the open reading frame (ORF) of the multiple cloning site (MCS). DNA inserts are positioned downstream and in frame with a sequence that encodes an N-terminal fusion peptide. The N-terminal peptide codes for (5′ to 3′ from the promoter) an ATG translation initiation codon, six histidine residues in series that function as a metal binding domain in the translated protein, the bacteriophage T7 gene 10 translation enhancer, the Xpress™ epitope, and an enterokinase cleavage recognition sequence.

Detection and Purification of Recombinant Protein

Expression of your recombinant protein can be detected using an antibody to the Xpress™ epitope encoded in the N-terminal fusion peptide (i.e. Anti-Xpress™ Antibody). In addition, the metal binding domain of the fusion peptide allows simple, one-step purification of recombinant proteins by Immobilized Metal Affinity Chromatography (IMAC), using Invitrogen's ProBond™ resin (page vi). The enterokinase cleavage recognition site in the fusion peptide between the metal binding domain and the recombinant protein allows for subsequent removal of this N-terminal fusion peptide from the purified recombinant protein.

Methods

Cloning into pTrcHis A, B, and C

General Molecular Biology Techniques

The following information is provided to help you clone your gene of interest into pTrcHis. For help with 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).

Maintenance of pTrcHis

If you wish to propagate and maintain pTrcHis, we recommend using 10 ng of the vector to transform a *rec*A, *end*A *E. coli* strain like TOP10, DH5α, or equivalent. Transformants are selected on LB plates containing 50–100 µg/ml ampicillin.

E. coli Strain

The *E. coli* strain TOP10 is provided for propagation of the pTrcHis vector. *E. coli* strains with comparable genotypes may be substituted. We recommend that you propagate vectors containing inserts in recombination deficient (*rec*A), endonuclease A-deficient (*end*A) *E. coli* strains.

TOP10 contains:

- *recA* for stable replication of high copy number plasmids.
- *endA* for improved yield and quality of miniprep DNA.
- *hsd*RMS to eliminate cleavage of recombinant plasmid by the endogenous *EcoR* restriction system.
- For your convenience, TOP10 is available as both electrocompetent and chemically competent cells from Invitrogen (page vi).

Genotype of TOP10

F^-, *mcr*A Δ (*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 (*ara-leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G

Transformation Method

You may use any method of choice for transformation. Chemical transformation is the most convenient for many researchers. Electroporation is the most efficient and the method of choice for large plasmids. Refer to **Appendix** (page 7) for protocols.

Cloning into the Expression Vector

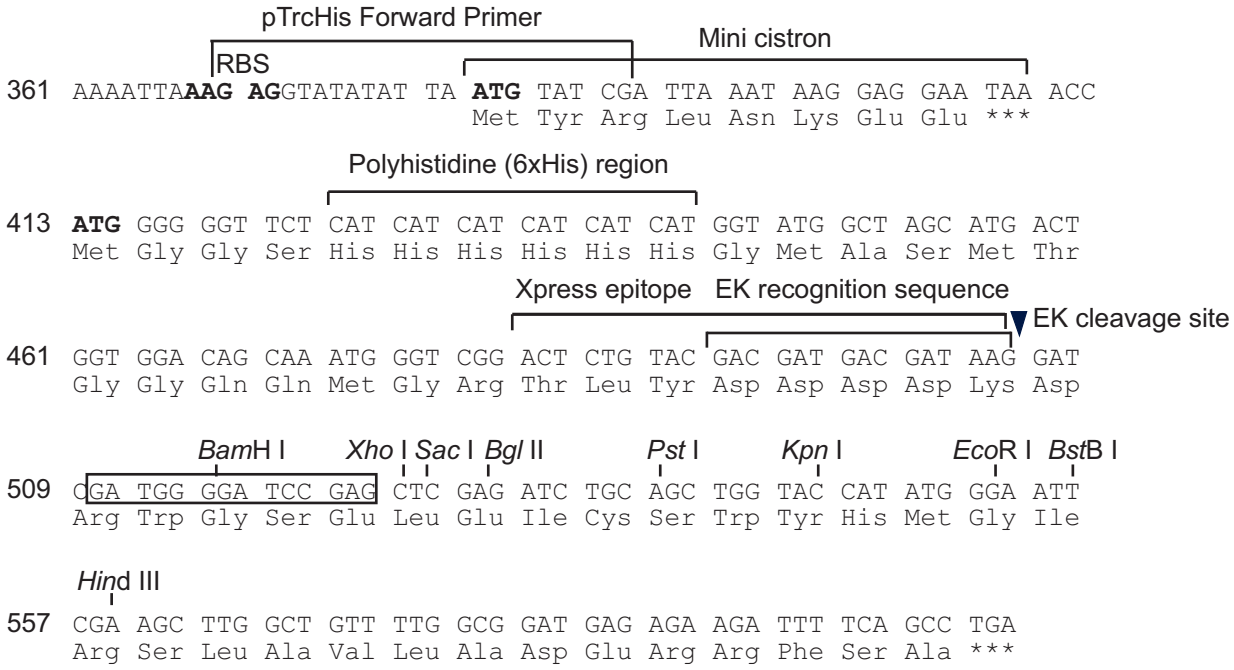
Downstream of the 5′ sequences, there is a multiple cloning site (MCS) that has eight unique restriction sites: *Bam*H I, *Xho* I, *Bgl* II, *Pst* I, *Kpn* I, *Eco*R I, *Bst*B I, and *Hin*d III (refer to pages 3–4 for details). To generate recombinant proteins that include the correct N-terminal fusion peptide, clone the DNA into the MCS in frame with the ATG. To perform in-frame cloning, three different versions of the vector—pTrcHis A, pTrcHis B, and pTrcHis C - that differ only in the spacing between the sequences that code for the N-terminal peptide and the MCS are supplied. For proper expression, first determine which restriction site is appropriate for ligation and then which vector will preserve the reading frame between the 5′ sequences and the insert when ligated into that site. This will vary depending on which restriction site in the MCS is chosen for fragment insertion—not all cloning sites are in the same frame in each vector. The complete sequences for the pTrcHis vectors are available for downloading from our web site at www.invitrogen.com or by contacting **Technical Support** (see page 14).

Continued on next page

Cloning into pTrcHis A, B, and C, Continued

Multiple Cloning Site of pTrcHis A

Below is the multiple cloning site for pTrcHis A. Restriction sites are labeled to indicate the actual cleavage site. Boxed nucleotides indicate the variable region. Sequencing and functional testing have confirmed the multiple cloning site. The complete sequence of pTrcHis A is available for downloading from our web site at www.invitrogen.com or by contacting **Technical Support** (page 14). For a map and description of the features of pTrcHis, refer to pages 11–12.

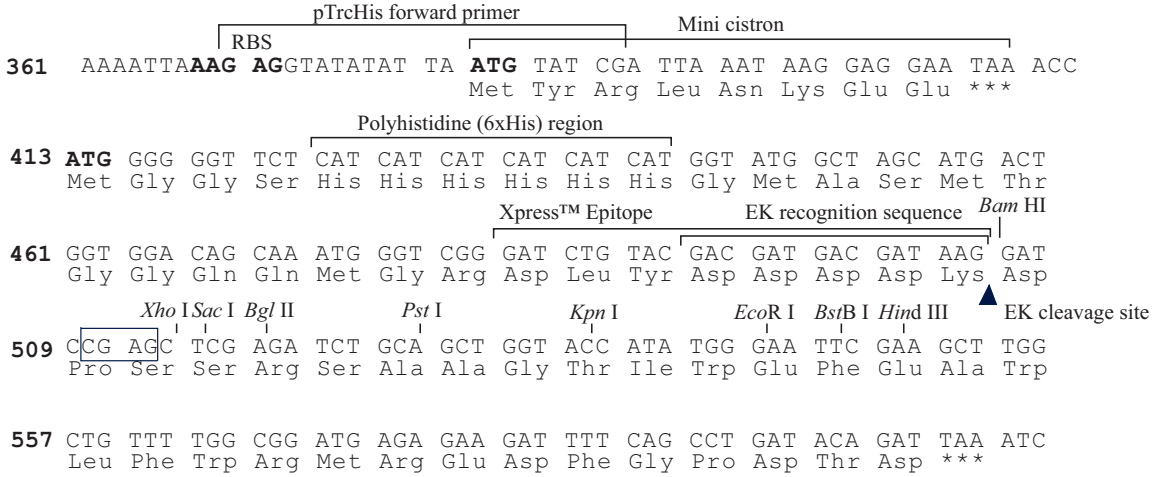


Continued on next page

Cloning into pTrcHis A, B, and C, Continued

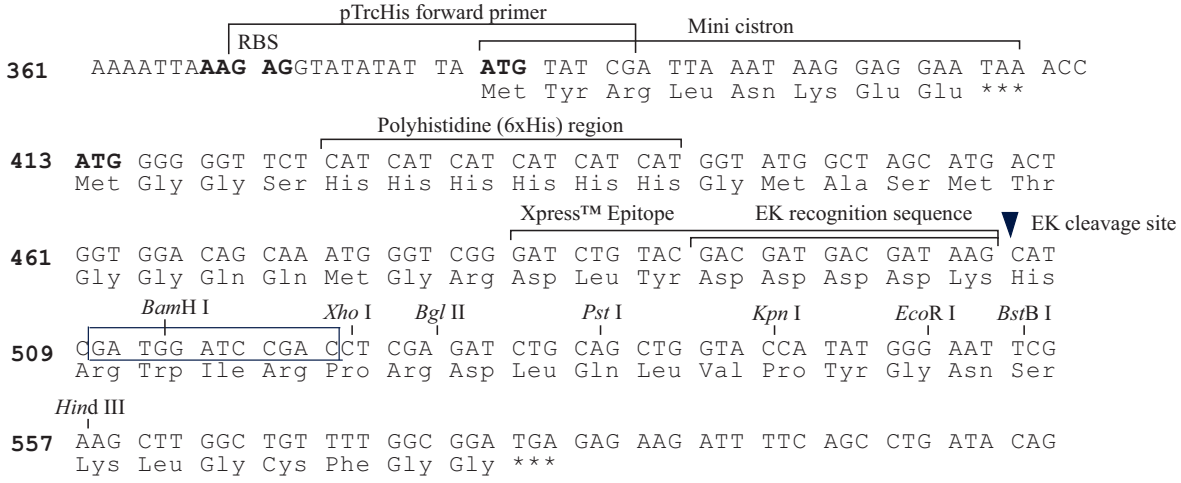
Multiple Cloning Site of pTrcHis B

Below is the multiple cloning site for pTrcHis B. Restriction sites are labeled to indicate the actual cleavage site. Boxed nucleotides indicate the variable region. Sequencing and functional testing have confirmed the multiple cloning site. The complete sequence of pTrcHis B is available for downloading from our web site at www.invitrogen.com or by contacting **Technical Support** (page 14). For a map and description of the features of pTrcHis, refer to pages 11–12.



Multiple Cloning Site of pTrcHis C

Below is the multiple cloning site for pTrcHis C. Restriction sites are labeled to indicate the actual cleavage site. Boxed nucleotides indicate the variable region. Sequencing and functional testing have confirmed the multiple cloning site. The complete sequence of pTrcHis C is available for downloading from our web site at www.invitrogen.com or by contacting **Technical Support** (page 14). For a map and description of the features of pTrcHis, refer to pages 11–12.



Expression

Introduction

Since each recombinant protein has different characteristics that may affect optimal expression parameters, we recommend performing a time course of expression to determine the optimal expression conditions for your particular protein. Be sure to perform mock expression consisting of pTrcHis vector alone in parallel as a negative control. Use the pTrcHisCAT as a positive expression control.

Pilot Expression

1. Inoculate 2 ml of SOB or LB + Ampicillin (50 µg/ml) with a single recombinant *E. coli* colony.
2. Grow overnight at 37°C with shaking.
3. The next day, inoculate 50 ml of SOB or LB + Ampicillin (50 µg/ml) with 0.2 ml of the overnight culture.
4. Grow the culture at 37°C with shaking to an OD600 = 0.6 (the cells should be in mid-log phase).
5. Remove a 1 ml aliquot of cells prior to IPTG induction, centrifuge the sample in a microcentrifuge, aspirate the supernatant. Freeze at –20°C. This will be the time zero sample.
6. Add IPTG to a final concentration of 1 mM (0.5 ml of 100 mM IPTG stock to 50 ml of culture) and grow at 37°C with shaking. Take samples at one hour intervals for 5 hours (or more). Centrifuge each sample and store both the supernatant and the pellet at 4°C. For long term storage (>5 hours), store the samples at –20°C.
7. When all time points are collected, resuspend each pellet in 100 µl of 20 mM phosphate buffer at neutral pH, and freeze in liquid nitrogen or methanol/dry ice (exercise caution when handling liquid nitrogen, it can cause severe burns if it comes in contact with the skin, wear appropriate protective equipment). Thaw the frozen lysate at 42°C. Repeat this freeze-thaw 2–3 additional times and pellet the insoluble protein in a refrigerated microcentrifuge for 10 minutes at maximum speed.
8. Remove the supernatant to a fresh labeled tube. Resuspend the pellet in 100 µl of Laemmli Buffer. To 100 µl of supernatant sample, add an equal volume of 2X Laemmli Buffer.
9. Analyze 10–20 µl of both the supernatant and pellet samples on a 10% SDS-polyacrylamide gel. Stain the gel with Coomassie blue and look for a band of increasing intensity in the expected size range for the protein. Compare it to the negative control time course to distinguish the recombinant proteins from the background proteins.
10. Determine the optimal time post IPTG-induction to harvest the cells.

Note

The N-terminal fusion peptide adds approximately 3 kDa to the size of your protein.

Continued on next page

Positive Control Vector

Included is a stab of *E. coli* strain TOP10 containing pTrcHis/CAT. pTrcHis/CAT contains the chloramphenicol acetyl transferase gene (CAT) for use as a positive control for expression. The CAT protein is approximately 30 kD. It begins to appear at approximately 0.5 hours after IPTG induction and reaches a peak level of expression after approximately 4 hours post induction. At that point, expression levels off and continues for several hours.

Expression Of Recombinant Protein

1. Inoculate 2 ml of SOB + Ampicillin (50 µg/ml) with a single recombinant *E. coli* colony. Grow overnight at 37°C with shaking.
2. The next day, inoculate 50 ml of SOB + Ampicillin (50 µg/ml) with 0.3 ml of the overnight culture.
3. Grow the culture at 37°C with vigorous shaking to an OD_{600} = 0.6.
4. Add IPTG to a final concentration of 1 mM (0.5 ml of 100 mM IPTG stock to 50 ml of culture) and grow at 37°C with vigorous shaking for the optimal time determined as described on the previous page. Proceed with protein purification as detailed in the ProBond™ Purification System Manual. The ProBond™ Purification System Manual is available for downloading from our web site at www.invitrogen.com or by contacting **Technical Support** (see page 14).

Appendix

Transformation Protocol for Competent *E. coli*

Protocol

The following protocol is provided for your convenience. Any comparable protocol may be used.

1. Take the TOP10 stab provided and streak out a small portion of it on an LB media plate (without ampicillin). Incubate at 37°C overnight. Store the stab at 4°C in the dark - it should be viable for several months.

   Important: Prepare a frozen glycerol stock for long-term storage (see below for instructions).

2. Pick a single colony and transfer it into 100 ml of SOB media in a 1 liter flask (see page 9 for recipe). Incubate the flask at 37°C with vigorous shaking (>200 cycles/minute in a rotary shaker).
3. When the OD_{600} reaches approximately 0.5, collect the cells by centrifuging at 2,600 × g for 10 minutes at 4°C.
4. Resuspend the pellet in 10 ml of ice-cold 50 mM $CaCl_2$. Keep the cells on ice for at least 30 minutes.
5. Centrifuge the CaCl2-treated cells in a 4°C rotor (2,600 × g, 4°C, 5 minutes). Gently resuspend the cells in 4 ml of ice-cold 50 mM CaCl2. Keep the cells on ice.
6. Aliquot 100 µl of CaCl2-treated cells for each transformation into a pre-chilled (on ice) Falcon 2059 tube (or equivalent). Add transforming DNA (10–100 ng in 1-10 µl) and incubate on ice for 30 minutes.
7. After 30 minutes on ice, heat shock the cells at 42°C for 45 seconds (in a water bath). After the heat shock, return the tube(s) to ice for an additional 2 minutes.
8. Add 1 ml of SOC media and incubate the cultures for 1 hour at 37°C with vigorous shaking (>200 rpm in a rotary shaking incubator).
9. Plate appropriate amounts of cells onto SOB or LB plates containing ampicillin (50 µg/ml).

Preparing Frozen *E. coli* Glycerol Stocks

1. Grow 1 to 2 ml of the strain to be frozen in rich bacterial media (e.g. SOB, see page 9 for recipe) overnight with antibiotic selection when appropriate.
2. Combine 0.85 ml of the overnight culture with 0.15 ml of sterile glycerol (sterilized by autoclaving).
3. Mix the culture well by vortexing. Transfer to an appropriate freezing vial.
4. Freeze in an ethanol-dry ice bath or liquid nitrogen and then transfer to –80°C for long-term storage.

Bacterial Alkaline Lysis Miniprep

Procedure

1. Grow 2 ml of bacterial culture (LB broth with the appropriate antibiotic) at 37°C overnight in a rotary shaking incubator.
2. Decant 1.5 ml of the culture into a microcentrifuge tube and spin it for 10 seconds. Discard the supernatant, leaving 50–100 µl of medium in the tube. Vortex the tube to completely resuspend the cells.
3. Add 300 µl of TENS solution (10 mM Tris-HCl, pH 7.5; 1 mM EDTA; 0.1 N NaOH; 0.5% SDS) then vortex the tube for 2–5 seconds or until the mixture becomes viscous.
4. Add 150 µl of 3 M sodium acetate, pH 5.2, then vortex the tube for 2–5 seconds to mix completely.
5. Centrifuge the tube for 2 minutes in a microcentrifuge to pellet the cell debris and the chromosomal DNA. Transfer the supernatant to a fresh microcentrifuge tube, add 900 µl of cold 100% ethanol and mix well. Freeze the solution on dry ice.
6. Centrifuge the tube for 5 minutes to pellet the plasmid DNA and the RNA. The pellet should have a white appearance. Discard the supernatant and rinse the pellet twice with 1 ml of 70% ethanol. Remove the residual ethanol after another quick spin.
7. Resuspend the pellet for further analysis in 20–50 µl of TE buffer, pH 8.0 (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0) or sterile water containing RNase A at a concentration of 100 µg/ml.

Recipes

SOB (For 1 Liter)

To 950 ml of deionized water add:

20.0 g Tryptone
5.0 g Yeast Extract
0.5 g NaCl

1. Mix the solution until dissolved.
2. Add 10 ml of a 250 mM solution of KCl.
3. Adjust the pH to 7.0 with 5 N NaOH (approximately 0.2 ml).
4. If making solid media (for plates or top agar), add 15 g of agar after adjusting the pH.
5. Adjust the volume to 1000 ml and sterilize by autoclaving.
6. Once autoclaved, add 5 ml of sterile 2 M $MgCl_2$ (alternatively, use 10 ml of either sterile 1 M $MgCl_2$ or sterile 1 M $MgSO_4$).

SOC (For 1 Liter)

Follow recipe as per SOB. After autoclaving, let cool to about 60°C or less (comfortable to touch with hand) and add 20 ml of a 1 M solution of glucose. Mix the media well.

LB (For 1 Liter)

Component	liquid	plates	top agar
Tryptone	10 g	10 g	10 g
Yeast Extract	5 g	5 g	5 g
NaCl	10 g	10 g	10 g
Agar	------	15 g	7 g

1. Combine the tryptone, yeast extract, and NaCl with 950 ml of deionized water. Mix the solution until dissolved.
2. Adjust the pH to 7.0 with 5 N NaOH (will take about 0.2 ml). For plates, add the appropriate amount of agar after adjusting the pH.
3. Adjust volume to 1 liter with water.
4. Sterilize by autoclaving.

Ampicillin

Prepare a stock solution of 50 mg/ml in deionized water. Filter-sterilize through a 0.22 micron filter. To prepare media containing ampicillin, cool media to ~50°C, add 1 ml of the ampicillin stock per liter of media (both liquid and solid) for a final concentration of 50 µg/ml. Store the stock solution at –20°C.

50 mM $CaCl_2$

For 100 ml of a 50 mM solution:

Dissolve 0.56 g of anhydrous $CaCl_2$ in 100 ml of deionized water. Filter sterilize (0.22 micron filter). Use this solution ice cold for competent cell preparation.

250 mM KCl

For 100 ml:

Dissolve 1.86 grams KCl in deionized water. Bring the final volume to 100 ml and filter sterilize (0.22 micron filter).

Continued on next page

2 M $MgCl_2$

For 100 ml:

Dissolve 19 grams of $MgCl_2$ in deionized water. Bring the final volume to 100 ml and filter sterilize (0.22 micron filter).

1 M glucose

For 100 ml:

Dissolve 18 grams of glucose in 90 ml of deionized water. Bring the final volume to 100 ml and filter sterilize (0.22 micron filter).

100 mM IPTG

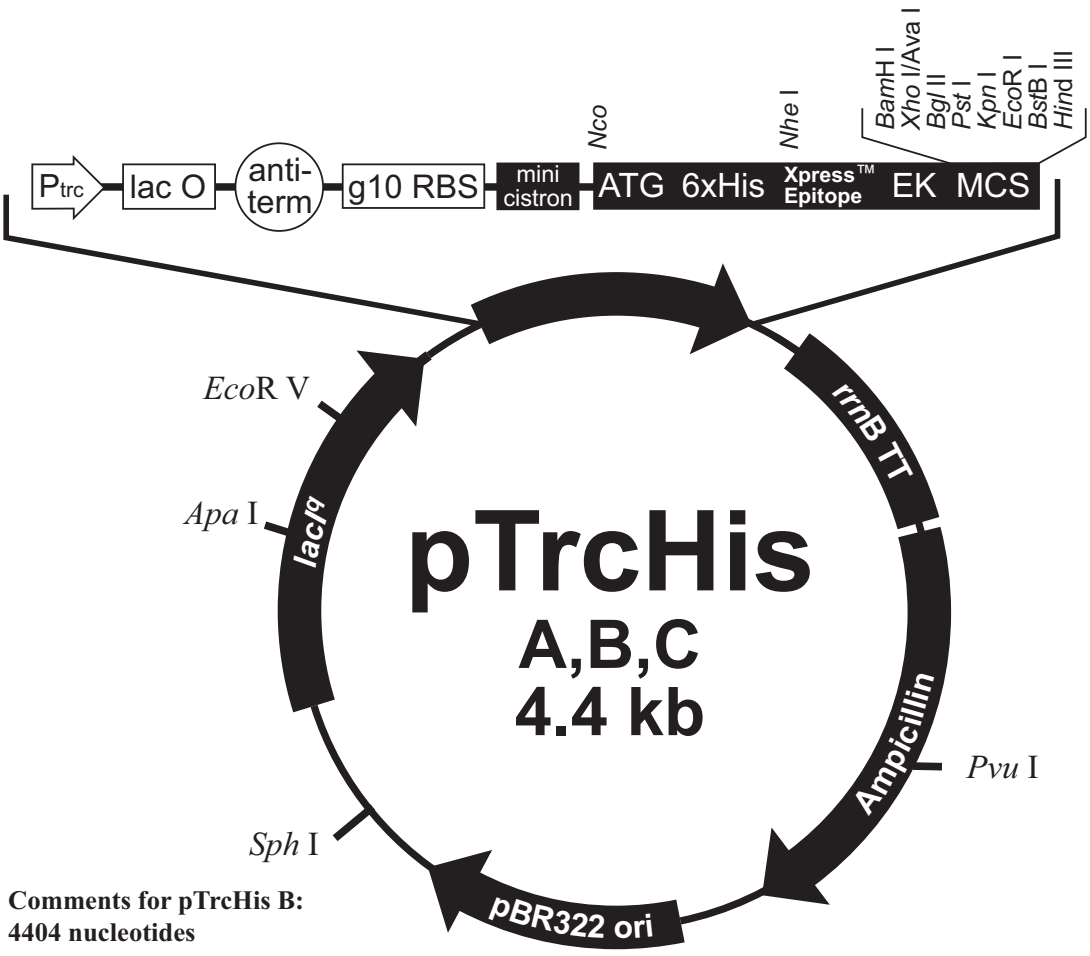
For 10 ml of a 100 mM solution:

Dissolve 0.24 g of IPTG (m.w. = 238.3) in 10 ml of sterile, deionized water. Filter sterilize and store at –20°C.

Map of pTrcHis A, B, and C Vectors

pTrcHis A, B, and C Map

The figure below summarizes the features of the pTrcHis vectors. Details of the multiple cloning site are shown on pages 3–4. The full sequence of pTrcHis is available for downloading from our web site at www.invitrogen.com or by contacting **Technical Support** (see page 14).



Comments for pTrcHis B: 4404 nucleotides

trc promoter: bases 191-221
lac operator: bases 228-248
*rrn*B anti-termination sequences: bases 264-333
T7 gene 10 translational enhancer: bases 346-354
Ribosome binding site: bases 370-374
Mini-cistron: bases 383-409
Polyhistidine and enterokinase cleavage site: bases 425-504
Xpress™ epitope: bases 482-505
Multiple cloning site: bases 515-554
*rrn*B transcriptional termination sequence: bases 637-794
Ampicillin resistance ORF: bases 1074-1934
pBR322 origin: bases 2079-2752
lac I^{q} ORF: bases 3406-4365

Features of pTrcHis Vector

Features of pTrcHis

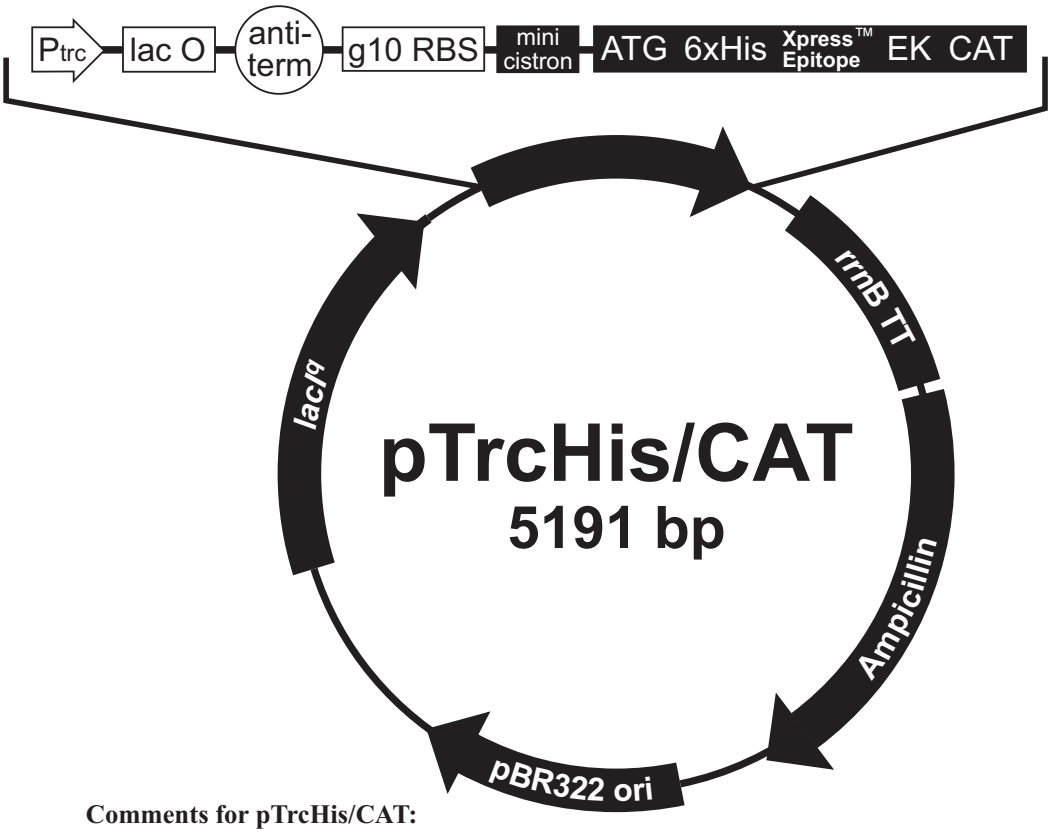
The important elements of pTrcHis A, B, and C are described in the table below. All features have been functionally tested.

Feature	Benefit
trc promoter	-35 (*trp*B) and -10 (*lac*UV5) hybrid promoter for high-level expression of fusion protein (Brosius *et al.*, 1985; Egon *et al.*, 1983; Mulligan *et al.*, 1985).
lac operator	Permits binding of the Lac repressor to repress transcription.
*rrn*B anti-termination sequences	Reduces the level of premature transcription termination(Li *et al.*, 1984).
Bacteriophage gene 10 translational enhancer	Optimizes translation initiation of minicistron (Olins *et al.*, 1988).
Minicistron and reinitiation ribosome binding site	Contains a second ribosome site for efficient reinitiation of translation into the gene of interest (Schoner *et al.*, 1986).
Polyhistidine (6xHis) region	Permits purification of recombinant fusion protein on metal-chelating resins (i.e. ProBond™).
Enterokinase cleavage site	Provides a site for efficient removal of the fusion tag.
Multiple cloning site	Allows insertion of your gene for expression.
*rrn*B transcription terminator	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.*
lacI^{q} gene	Encodes and overproduces the Lac repressor protein.

Map of pTrcHis/CAT

pTrcHis/CAT Map

The figure below summarizes the features of the pTrcHis/CAT vector. The complete nucleotide sequence for pTrcHis2/CAT is available for downloading from our web site at www.invitrogen.com or by contacting **Technical Support** (page 14).



Comments for pTrcHis/CAT:
5191 nucleotides

trc promoter: bases 191-221
lac operator: bases 228-248
*rrn*B antitermination sequences: bases 264-333
T7 gene 10 translational enhancer: bases 346-354
Ribosome binding site: bases 370-374
Mini-cistron: bases 383-409
Initiation ATG: bases 413-415
Polyhistidine (6xHis) region: 425-442
Xpress™ epitope: bases 482-505
Enterokinase (EK) recognition site: bases 491-505
CAT ORF: bases 587-1246
*rrn*B transcriptional termination sequence: bases 1424-1581
Ampicillin resistance ORF: bases 1861-2721
pBR322 origin: bases 2866-3539
lac I^q ORF: bases 4068-5152

Technical Support

Web Resources

Visit the Invitrogen web site at www.invitrogen.com for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our web site (www.invitrogen.com).

Corporate Headquarters:	**Japanese Headquarters:**	**European Headquarters:**
Invitrogen Corporation 5791 Van Allen Way Carlsbad, CA 92008 USA Tel: 1 760 603 7200 Tel (Toll Free): 1 800 955 6288 Fax: 1 760 602 6500 E-mail: tech_support@invitrogen.com	Invitrogen Japan LOOP-X Bldg. 6F 3-9-15, Kaigan Minato-ku, Tokyo 108-0022 Tel: 81 3 5730 6509 Fax: 81 3 5730 6519 E-mail: jpinfo@invitrogen.com	Invitrogen Ltd Inchinnan Business Park 3 Fountain Drive Paisley PA4 9RF, UK Tel: +44 (0) 141 814 6100 Tech Fax: +44 (0) 141 814 6117 E-mail: eurotech@invitrogen.com

MSDS

MSDSs (Material Safety Data Sheets) are available on our web site at www.invitrogen.com/msds.

Certificate of Analysis

Product qualification is described in the Certificate of Analysis (CofA), available on our website by product lot number at www.invitrogen.com/support.

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, contact our Technical Support Representatives.

Invitrogen warrants that all of its products will perform according to specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order.

Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore, Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Support Representatives.

Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.

Purchaser Notification

Limited Use Label License No: 22 Vectors and Clones Encoding Histidine Hexamer

This product is licensed under U.S. Patent Nos. 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.

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*, Greene Publishing Associates and Wiley-Interscience, New York

Brosius, J., Erfle, M., and Storella, J. (1985) Spacing of the -10 and -35 Regions in the *tac* Promoter. J. Biol. Chem *260*, 3539-3541

Egon, A., Brosius, J., and Ptashne, M. (1983) Vectors Bearing a Hybrid *trp-lac* Promoter Useful for Regulated Expression of Cloned Genes in *Escherichia coli*. Gene *25*, 167-178

Li, S. C., Squires, C. L., and Squires, C. (1984) Antitermination of *E. coli* rRNA Transcription is Caused by a Control Region Segment Containing Lambda *nut*-like Sequences. Cell *38*, 851-860

Mulligan, M. E., Brosius, J., and Clure, W. R. (1985) Characterization *in vitro* of the Effect of Spacer Length on the Activity of *Escherichia coli* RNA Polymerase at the *tac* Promoter. J. Biol. Chem. *260*, 3539-3538

Olins, P. O., Devine, C. S., Rangwala, S. H., and Kavka, K. S. (1988) T7 Phage Gene 10 Leader RNA, a Ribosome-binding Site the Dramatically Enhances the Expression of Foreign Genes in *Escherichia coli*. Gene *73*, 227-235

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Second Ed., Cold Spring Harbor Laboratory Press, Plainview, New York

Schoner, B. E., Belagaje, R. M., and Schoner, R. G. (1986) Translation of a Synthetic Two-cistron mRNA in *Escherichia coli*. Proc. Natl. Acad. Sci. USA *83*, 8506-8510

invitrogen™

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

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