# pTrcHis and pTrcHis2 TOPO® TA Expression Kits

## Five-minute cloning of *Taq* polymerase-amplified PCR products for expression in *E. coli*

**Catalog no. K4410-01 (pTrcHis TOPO®)**

**Catalog nos. K4400-01 and K4400-40 (pTrcHis2 TOPO®)**

**Version J**
23 February 2006
*25-0209*



**User Manual**

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# Kit Contents and Storage

## Shipping and Storage

The pTrcHis and pTrcHis2 TOPO® TA Expression Kits are shipped on dry ice. Each kit contains a box with TOPO TA Cloning® reagents (Box 1) and a box with TOP10 One Shot® competent cells (Box 2).

**Store Box 1 at -20°C and Box 2 at -80°C.**

## pTrcHis and pTrcHis2 TOPO® TA Expression Kits

The pTrcHis TOPO® TA Expression Kit contains the pTrcHis-TOPO® vector, which allows you to clone in frame with an N-terminal tag. The pTrcHis2 TOPO® TA Expression Kit contains the pTrcHis2-TOPO® expression vector, which allows you to clone in frame with a C-terminal tag. See the table below for ordering information.

| Product | Pack Size | Catalog no. |
|---|---|---|
| pTrcHis TOPO® TA Expression Kit (containing pTrcHis-TOPO® vector) | 20 | K4410-01 |
| pTrcHis2 TOPO® TA Expression Kit (containing the pTrcHis2-TOPO® vector) | 20 | K4400-01 |
| | 40 | K4400-40 |

## TOPO TA Cloning® Reagents

The TOPO TA Cloning® reagents (Box 1) for both kits are listed below. **Please note that the user must supply *Taq* polymerase.** Store Box 1 at -20°C.

| Item | Concentration | Amount |
|---|---|---|
| pTrcHis-TOPO® **OR** pTrcHis2-TOPO® vector | 10 ng/µl plasmid DNA in:<br>50% glycerol<br>50 mM Tris-HCl, pH 7.4 (at 25°C)<br>1 mM EDTA<br>2 mM DTT<br>0.1% Triton X-100<br>100 µg/ml BSA<br>phenol red | 1 tube of 25 µl |
| 10X PCR Buffer | 100 mM Tris-HCl, pH 8.3 (at 42°C)<br>500 mM KCl<br>25 mM $MgCl_2$<br>0.01% gelatin | 100 µl |
| dNTP Mix | 12.5 mM dATP; 12.5 mM dCTP; 12.5 mM dGTP; 12.5 mM dTTP<br>neutralized at pH 8.0 in water | 10 µl |

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# Kit Contents and Storage, continued

## pTrcHis TOPO TA Cloning® Reagents, continued

| Item | Concentration | Amount |
|---|---|---|
| 1 M IPTG | 1 M IPTG in sterile water | 1 ml |
| Xpress™ Forward Sequencing Primer (for pTrcHis-TOPO®) | 0.1 µg/µl in TE Buffer | 20 µl |
| pTrcHis Forward Sequencing Primer (for pTrcHis2-TOPO®) | 0.1 µg/µl in TE Buffer | 20 µl |
| pTrcHis Reverse Sequencing Primer (for both vectors) | 0.1 µg/µl in TE Buffer | 20 µl |
| Control PCR Primers | 0.1 µg/µl each in TE Buffer | 10 µl |
| Control PCR Template | 0.1 µg/µl in TE Buffer | 10 µl |
| Sterile Water | -- | 1 ml |
| Expression Control Plasmid (pTrcHis-TOPO®/*lacZ* or pTrcHis2-TOPO®/*lacZ*) | 0.5 µg/µl in TE Buffer | 10 µl |

### Sequences of Primers

The table below provides the sequences and pmoles supplied of the Forward and Reverse sequencing primers. Two micrograms of each primer are supplied.

| Primer | Sequence | pMoles Supplied |
|---|---|---|
| Xpress™ Forward | 5´-TATGGCTAGCATGACTGGT-3´ | 342 |
| pTrcHis Forward | 5´-GAGGTATATATTAATGTATCG-3´ | 309 |
| pTrcHis Reverse | 5´-GATTTAATCTGTATCAGG-3´ | 363 |

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# Kit Contents and Storage, continued

## One Shot® Reagents

The table below describes the items included in the One Shot® competent cell kit. **Store at -80°C.**

| Item | Composition | Amount |
|---|---|---|
| SOC Medium<br>(may be stored at room temperature or +4°C) | 2% Tryptone<br>0.5% Yeast Extract<br>10 mM NaCl<br>2.5 mM KCl<br>10 mM $MgCl_2$<br>10 mM $MgSO_4$<br>20 mM glucose | 6 ml |
| TOP10 cells | -- | 21 x 50 µl |
| pUC19 Control DNA | 10 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8.0 | 50 µl |

## Genotype

**TOP10**: Use this strain for general cloning and expression of PCR products in pTrcHis-TOPO® or pTrcHis2-TOPO®. Please note that this strain cannot be used for single-strand rescue of DNA.

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

# Methods

## Overview

### Introduction

The pTrcHis and pTrcHis2 TOPO® TA Expression Kits provide a highly efficient, rapid cloning strategy ("TOPO® Cloning") for direct insertion of *Taq* polymerase-amplified PCR products into a plasmid vector for expression in *E. coli*. No ligase, post-PCR procedures, or PCR primers containing specific sequences are required. Once the PCR product of interest is TOPO® Cloned into either pTrcHis-TOPO® or pTrcHis2-TOPO®, the construct is transformed into *E. coli* and expression induced with isopropyl β-thiogalactoside (IPTG).

### Description of the Vectors

pTrcHis-TOPO® and pTrcHis2-TOPO® are designed to facilitate expression of eukaryotic proteins in *E. coli*. Both vectors contain the following:

- The *trc* promoter, a hybrid promoter containing the –35 region from the *trpB* promoter and the –10 region from the *lacUV5* promoter for high-level expression in *E. coli* (Brosius *et al.*, 1985; Egon *et al.*, 1983; Mulligan *et al.*, 1985).
- The *lacO* sequence for binding the Lac repressor encoded by the *lacI*$^q$ gene. In the absence of IPTG, Lac repressor binds to the *lacO* sequence, repressing transcription. Upon addition of IPTG, expression is induced (Jacob and Monod, 1961; Müller-Hill *et al.*, 1968).
- *rrnB* antitermination sequence that reduces premature transcription termination (Li *et al.*, 1984).
- T7 gene 10 translational enhancer sequence for more efficient translational initiation (Olins *et al.*, 1988).
- A minicistron containing nucleotides that are efficiently translated in prokaryotic cells for enhanced translational efficiency (Schoner *et al.*, 1986).

### pTrcHis-TOPO®

In addition to the features above, pTrcHis-TOPO® contains the following additional elements:

- An N-terminal peptide containing the HisG epitope, the Xpress™ epitope and a 6xHis tag for detection and purification of the recombinant protein.
- An enterokinase recognition site for removal of the N-terminal peptide

### pTrcHis2-TOPO®

In addition to the features listed in **Description of Vectors**, above, pTrcHis2-TOPO® encodes a C-terminal peptide containing the *c-myc* epitope and a 6xHis tag for detection and purification of the recombinant protein.

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# Overview, continued

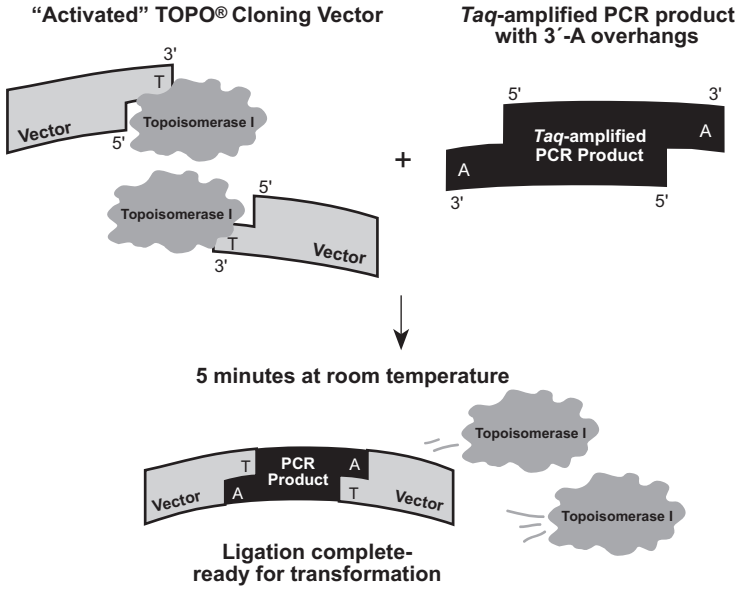
## TOPO® Cloning

Both pTrcHis-TOPO® and pTrcHis2-TOPO® are supplied linearized with:

- Single 3´-thymidine (T) overhangs for TA Cloning®
- Topoisomerase I covalently bound to the vector (this is referred to as "activated" vector)

*Taq* polymerase has a nontemplate-dependent terminal transferase activity that adds a single deoxyadenosine (A) to the 3´ ends of PCR products. The linearized vector supplied in this kit has single, overhanging 3´ deoxythymidine (T) residues. This allows PCR inserts to ligate efficiently with the vector.

TOPO® Cloning exploits the ligation activity of topoisomerase by providing an "activated", linearized TA vector using proprietary technology (Shuman, 1994). Ligation of the vector with a PCR product containing 3´ A-overhangs is very efficient and occurs spontaneously within 5 minutes at room temperature. The TOPO® Cloning reaction can be transformed into chemically competent cells (provided) or electroporated directly into electrocompetent cells.




## Induction of Expression

The strong *trc* promoter regulates expression in *E. coli*. The product of the *lacI*$^{q}$ gene encoded in both vectors represses this promoter. To induce expression, IPTG is added to a final concentration of 1 mM, and the culture monitored for expression of the protein of interest.

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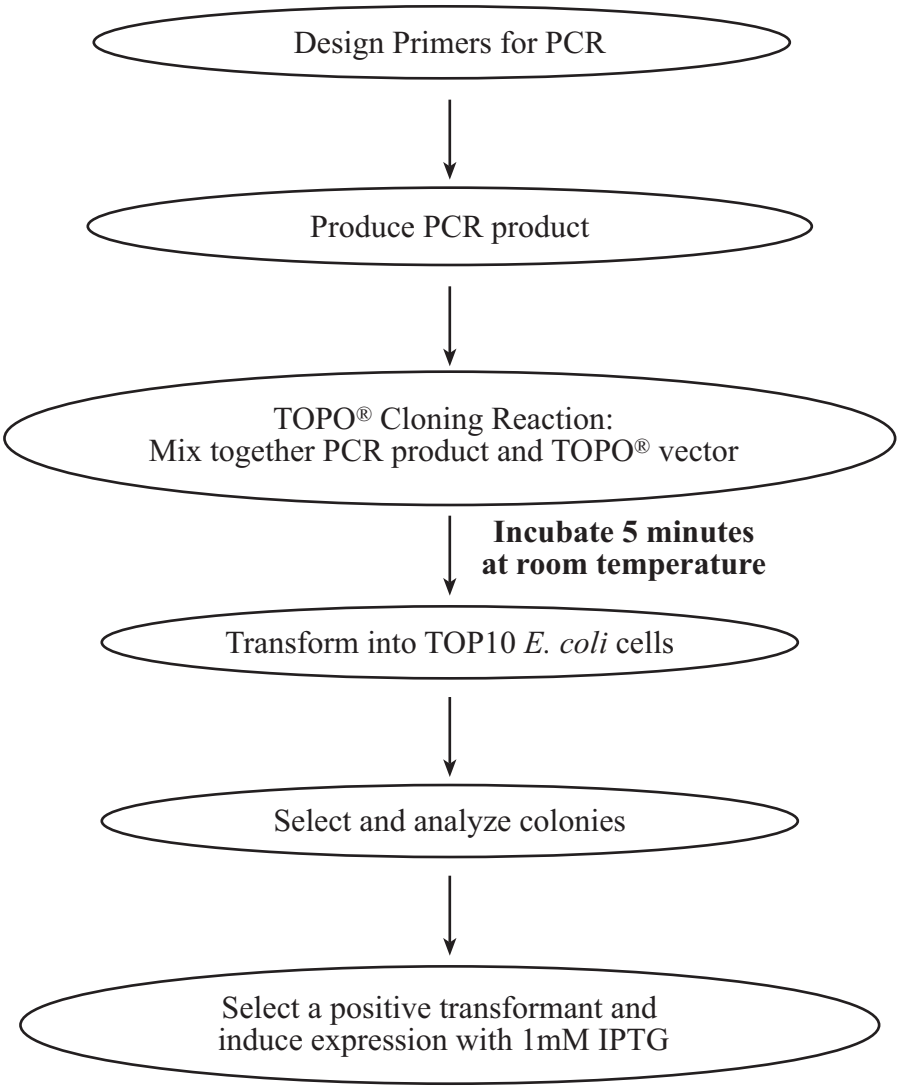
# Overview, continued

## Experimental Outline

The flow chart below outlines the general steps needed to express your protein.

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# Overview, continued

## Detection of Recombinant Proteins

Expression of your recombinant protein can be detected using an antibody to the protein itself or to the appropriate epitope. The table below describes the antibodies available for use with pTrcHis-TOPO® or pTrcHis2-TOPO®. Horseradish peroxidase (HRP)-conjugated antibodies allow one-step detection using colorimetric or chemiluminescent detection methods.

| Vector | Epitope | Antibody | Catalog No. |
|---|---|---|---|
| pTrcHis-TOPO® | Xpress™ | Anti-Xpress™ | R910-25 |
| | | Anti-Xpress™-HRP | R911-25 |
| | HisG | Anti-HisG | R940-25 |
| | | Anti-HisG-HRP | R941-25 |
| pTrcHis2-TOPO® | *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 6xHis 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 pTrcHis-TOPO® or pTrcHis2-TOPO®, the ProBond™ Purification System is available separately. Additional ProBond™ resin is available in bulk. See the table below for ordering information.

| Product | Quantity | Catalog no. |
|---|---|---|
| ProBond™ Metal-Binding Resin<br>(precharged resin provided as a 50% slurry in 20% ethanol) | 50 ml | R801-01 |
| | 150 ml | R801-15 |
| Purification Columns<br>(10 ml polypropylene columns) | 50 | R640-50 |
| ProBond™ Purification System<br>(includes six 2 ml precharged, prepacked ProBond™ resin columns and buffers for native and denaturing purification) | 6 purifications | K850-01 |

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# Overview, continued

## Reagents Available Separately

Some of the reagents in this kit are available separately. Use the table below for ordering information.

| Product | Amount | Catalog no. |
|---|---|---|
| 10 mM dNTPs | 1 ml, 2.5 mM of each dNTP | R725-01 |
| Xpress™ Forward Primer | 2 µg, lyophilized | N576-02 |

# Designing PCR Primers for pTrcHis-TOPO®

## Introduction

It is very important to design your PCR primers to ensure you obtain the recombinant protein you need for your studies. Please use the information below and the diagram on the next page to design your PCR primers.

## Special Considerations

pTrcHis-TOPO® is designed with the initiation ATG correctly spaced from an optimized ribosome binding site to ensure maximum translation. This ATG is located at bp 413-415 and is contained in the unique *Nco* I site.

The N-terminal peptide can be cleaved off from partially purified or purified recombinant protein using enterokinase. Please note that you will have at least four extra amino acids at the N-terminus of your protein (Asp-Pro-Thr-Leu-). The exact number of additional amino acids will depend on your PCR product.

## Primer Design

Suggestions for primer design are provided in the table below.

| If you wish to.... | Then... |
|---|---|
| clone in frame with the DNA encoding the N-terminal peptide…. | the forward PCR primer must be designed to ensure that your ORF is cloned in frame with the DNA encoding the N-terminal peptide. |
| remove the N-terminal leader (for expression of truly native protein)<br>**Note**: Proteins with N-terminal leaders tend to express better in *E. coli*. You may wish to prepare constructs with and without the leader and compare expression. | the forward PCR primer can be designed to include a unique *Nco* I site which contains the first ATG of your protein.<br>Ex. 1. 5´-ACC ATG G....<br>After TOPO® Cloning your PCR product, the vector can be digested with *Nco* I and religated, assuming there are no internal *Nco* I sites in your PCR product. |
| include the native stop codon for your protein | include the native sequence containing the stop codon in the reverse primer or make sure the stop codon is upstream from the reverse PCR primer binding site. |

**Note**

Do not add 5´ phosphates to your primers for PCR. This will prevent ligation into pTrcHis-TOPO®.

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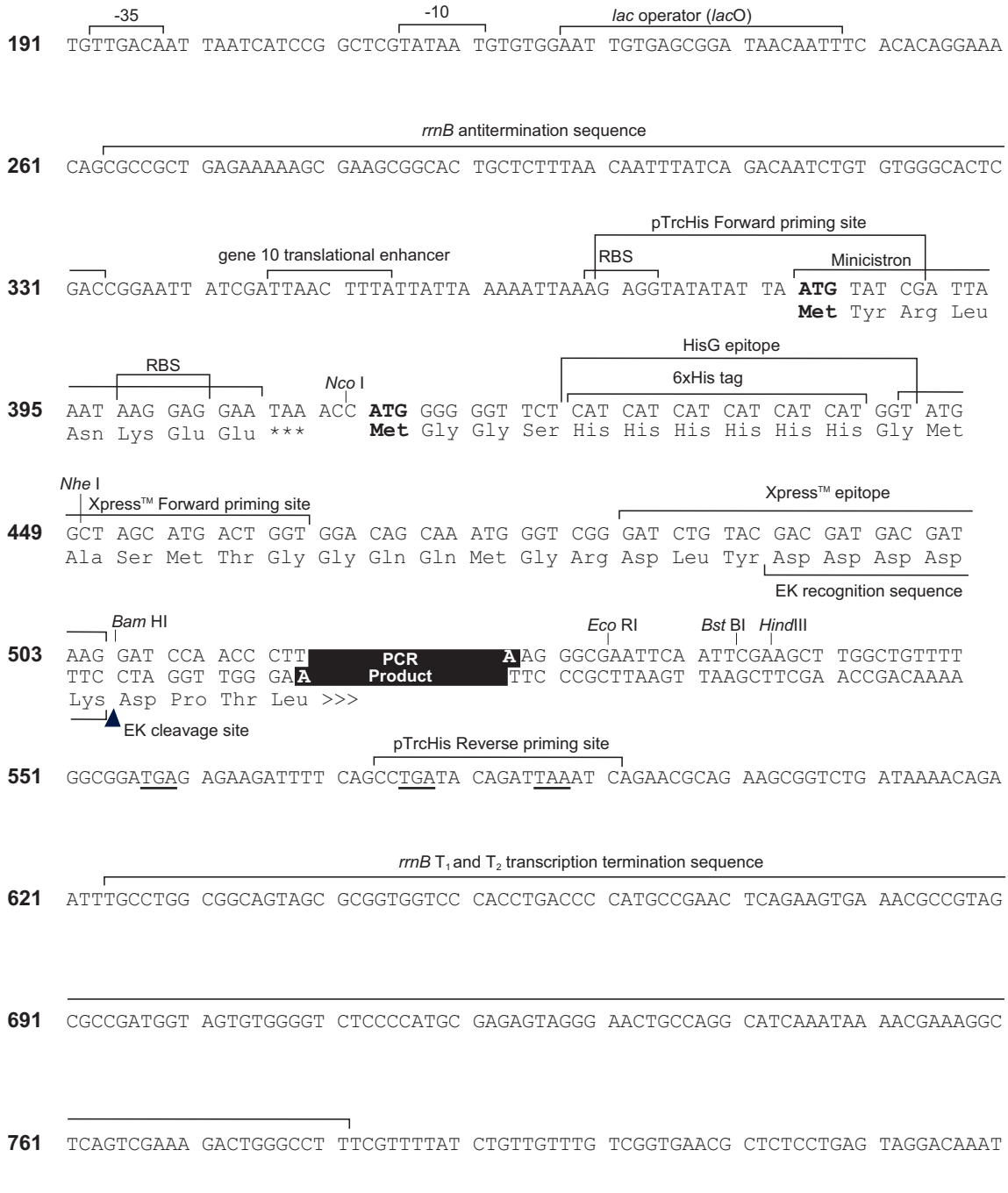
# Designing PCR Primers for pTrcHis-TOPO®, continued

## pTrcHis-TOPO® Cloning Site

The diagram below is supplied to help you design appropriate PCR primers to correctly clone and express your PCR product. Restriction sites are labeled to indicate the actual cleavage site. **The complete sequence is available by downloading from our Web site (www.invitrogen.com) or by calling Technical Service (see page 33).**



# Designing PCR Primers for pTrcHis2-TOPO®

## Introduction

It is very important to design your PCR primers to ensure you obtain the recombinant protein you need for your studies. Please use the information below and the diagram on the next page to design your PCR primers.

## Special Considerations

pTrcHis2-TOPO® is designed with the initiation ATG correctly spaced from the optimized ribosome binding site to ensure optimum translation. This ATG is located at bp 413-415 and is contained in the unique *Nco* I site. Please note that there are two more amino acids encoded in the DNA between the initiation codon and the TOPO® Cloning site.

Please note that the C-terminal tag cannot be cleaved off. If you wish to express your protein without the C-terminal tag, see the table below.

## Primer Design

Suggestions for primer design are provided in the table below.

| If you wish to.... | Then... |
|---|---|
| include the *c-myc* epitope and polyhistidine region | the reverse PCR primer must be designed to remove the native stop codon in the gene of interest and preserve the reading frame through the C-terminal tag. |
| **NOT** include the *c-myc* epitope and polyhistidine region | include the native sequence containing the stop codon in the reverse primer or make sure the stop codon is upstream from the reverse PCR primer binding site. |
| clone in frame with the initiation codon | the forward PCR primer must be designed to ensure that your ORF is in frame with the initiation codon. |
| remove the small N-terminal leader (for expression of truly native protein)<br>**Note**: Proteins with N-terminal leaders tend to express better in *E. coli*. You may wish to prepare constructs with and without the leader and compare expression. | the forward PCR primer can be designed to include a unique *Nco* I site which contains the first ATG of the protein.<br>Ex. 1. 5´-ACC <u>ATG</u> G....<br>After TOPO® Cloning your PCR product, the vector can be digested with *Nco* I and religated, assuming there are no internal *Nco* I sites in your PCR product. |

**Note**

Do not add 5´ phosphates to your primers for PCR. This will prevent ligation into pTrcHis2-TOPO®.

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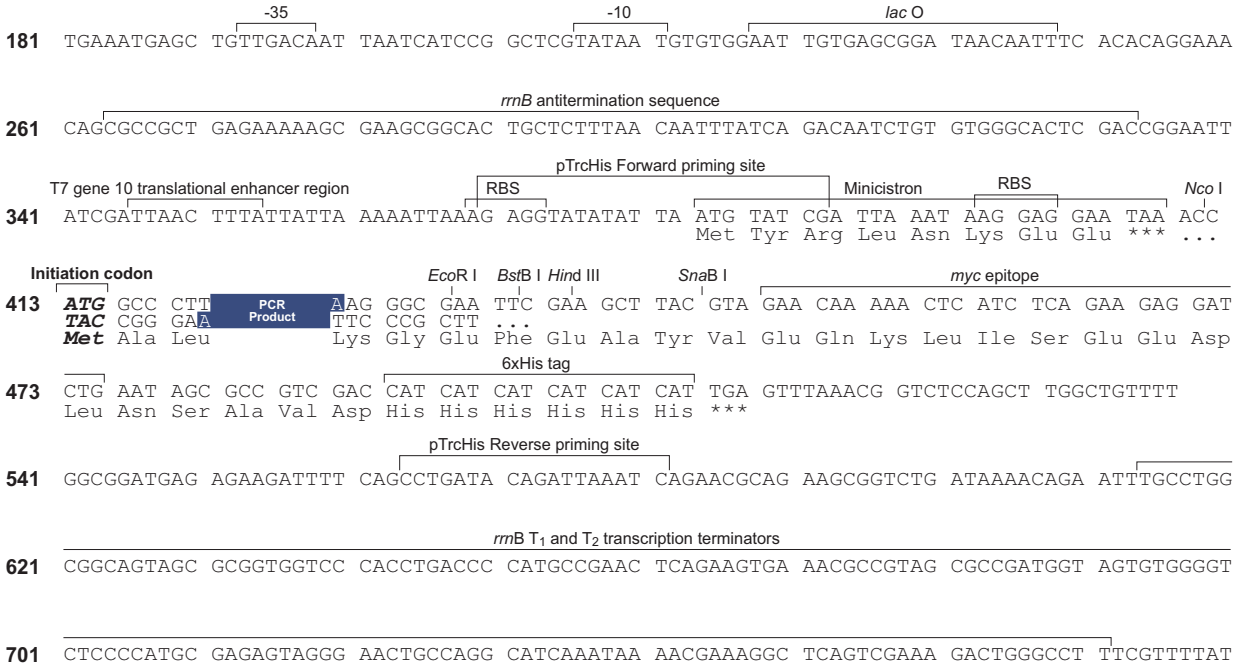
# Designing PCR Primers for pTrcHis2-TOPO®, continued

## pTrcHis2 TOPO® Cloning Site

The diagram below is supplied to help you design appropriate PCR primers to correctly clone and express your PCR product. Restriction sites are labeled to indicate the actual cleavage site. **The complete sequence is available by downloading from our Web site (www.invitrogen.com) or by calling Technical Service (see page 33).**



# Producing PCR Products

## Introduction

This section describes a procedure for PCR using the primers you designed. Please note that other procedures are suitable.

## Materials Supplied by the User

You will need the following reagents and equipment.

- *Taq* polymerase
- Thermocycler
- DNA template
- Primers for PCR product

## Producing PCR Products

1. Set up the following 50 µl PCR reaction. Use the cycling parameters suitable for your primers and template. Be sure to include a 7 to 30 minute extension at 72°C to ensure that all PCR products are full length and 3´ adenylated. Use 10 ng template for plasmids and 100 ng template for genomic DNA.

| | |
|---|---|
| DNA Template | 10-100 ng |
| 10X PCR Buffer | 5 µl |
| 50 mM dNTPs | 0.5 µl |
| Primers (100-200 ng/µl each) | 1 µl each |
| Sterile water | add to a final volume of 49 µl |
| *Taq* Polymerase (1 unit/µl) | 1 µl |
| Total Volume | 50 µl |

2. Check the PCR product by agarose gel electrophoresis. You should see a single, discrete band. If not, see the **Note**, below.
3. Use the PCR product immediately in a TOPO® Cloning reaction (next page) or store the product at -20°C until ready for use. PCR products may be stored at -20°C for about 1 week. Long-term storage may result in removal of the 3´ A-overhangs from your PCR product. This will decrease cloning efficiency in the TOPO® Cloning reaction.

**Note**

If you do not see a single, discrete band from your PCR, use one of the options below to ensure a single PCR species in your TOPO® Cloning reaction. Please note that small PCR products will clone preferentially over larger ones.

- Gel-purify your fragment before using either the pTrcHis or pTrcHis2 TOPO TA Cloning® Kit (see page 21). Take special care to avoid sources of nuclease contamination.
- Optimize your PCR to eliminate multiple bands and smearing (Innis *et al.*, 1990). The PCR Optimizer™ Kit (Catalog no. K1220-01) from Invitrogen can help you optimize your PCR. Please call Technical Service for more information (page 33).

# TOPO® Cloning and Transformation

## Introduction

TOPO® Cloning technology allows you to produce your PCR products, ligate them into the appropriate TOPO® vector, and transform the recombinant vector into *E. coli* all in one day. It is important to have everything you need set up and ready to use to ensure the best possible results. If this is the first time you have TOPO® Cloned, you may wish to perform the control reactions on pages 24-26 in parallel with your samples.

## Materials Supplied by the User

In addition to microbiological supplies (*i.e.* plates and spreaders), you will need the following reagents and equipment.

- 42°C water bath
- 37°C shaking and non-shaking incubator
- Two LB plates containing 50 µg/ml ampicillin and 0.5% glucose per transformation (see page 19 for recipe)

**Tip**: If you already have LB plates containing 50 µg/ml ampicillin only, you may spread 20 µl of a 2 M (or a 50%) glucose solution onto the plate. Please note that the concentration of glucose does not have to be exact.


We recommend that you include glucose (25 mM, 0.5%) in the selection medium to ensure stability of your insert. Promoters based on the *lac* promoter (*i.e. trc*) tend to have higher basal levels of transcription. If your insert is toxic to *E. coli*, DNA rearrangement may occur. Glucose represses basal level transcription to stabilize your construct.

## Mechanism of Glucose Repression

A transcriptional activator protein called CAP (catabolite activator protein) normally binds upstream of the *trc* promoter and activates transcription. This protein requires cAMP to bind to the DNA. Adding glucose to the medium can reduce intracellular cAMP levels. Supplementing LB medium and agar plates with glucose will repress basal level transcription from the *trc* promoter.

## Preparation

For each transformation, you will need one vial of competent cells and one or two selective plates.

- Equilibrate a water bath to 42°C.
- Thaw the vial of SOC medium from Box 2 and bring to room temperature.
- Warm LB plates containing 50 µg/ml ampicillin and 0.5% glucose at 37°C for 30 minutes.
- Thaw **on ice** 1 vial of One Shot® cells for each transformation.

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# TOPO® Cloning and Transformation, continued

## TOPO® Cloning Reaction

In general, 0.5 to 4 µl of a typical PCR sample (10-20 ng/µl) with an average insert length of 400 to 1000 bp will give the proper insert:vector ratio for TOPO® Cloning.

1. Set up the following 5 µl TOPO® Cloning reaction.

| | |
|---|---|
| Fresh PCR product | 0.5 to 4 µl |
| Sterile Water | add to a final volume of 4 µl |
| TOPO® vector | 1 µl |
| Final Volume | 5 µl |

2. Mix gently and incubate for **5 minutes** at room temperature (~25°C). **For the best possible results, do not leave for more than 5 minutes or the transformation efficiencies may decrease.**
3. If needed, the TOPO® Cloning reaction may be stored on ice or frozen at -20°C for up to 24 hours. You may see a decrease in the transformation efficiency, but the cloning efficiency should remain high. We recommend that you proceed immediately to **Transformation**, below.

## One Shot® Transformation Reaction

1. Add 2 µl of the TOPO® Cloning reaction into a vial of One Shot® cells and mix gently. **Do not mix by pipetting up and down.**
2. Incubate on ice for 30 minutes.
3. Heat shock the cells for 30 seconds at 42°C without shaking.
4. Immediately transfer the tubes to ice.
5. Add 250 µl of room temperature SOC medium.
6. Cap the tube tightly and shake the tube horizontally at 37°C for 30 minutes.
7. Spread 10-50 µl from each transformation on a prewarmed selection plate and incubate overnight at 37°C. We recommend that you plate two different volumes to ensure well-spaced colonies. For plating smaller volumes, add 20 µl of SOC to ensure even spreading.
8. An efficient TOPO® Cloning reaction will produce hundreds of colonies. Pick ~10 colonies for analysis.

## Transformation by Electroporation

**Use ONLY electrocompetent cells for electroporation to avoid arcing. Do not use the TOP10 One Shot® chemically competent cells for electroporation.**

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## Analysis of Positive Clones

1. Take the 10 colonies and culture them overnight in LB medium containing 50 µg/ml ampicillin and 0.5% glucose. **Note**: If you use a rich broth like SOC or Terrific Broth, grow the cells for 4 hours before performing a miniprep; do not grow overnight. We obtain less DNA with overnight growth.
2. Isolate plasmid DNA using your method of choice. If you need ultra-pure plasmid DNA for automated or manual sequencing, we recommend the S.N.A.P.™ MiniPrep Kit (Catalog no. K1900-01) or the S.N.A.P.™ MidiPrep Kit (K1910-01).
3. Analyze the plasmids by restriction analysis or by sequencing. For pTrcHis-TOPO® use the Xpress™ Forward and the pTrcHis Reverse sequencing primers for sequencing. For pTrcHis2-TOPO® use the pTrcHis Forward and Reverse sequencing primers to sequence your insert. For the sequence surrounding the TOPO® Cloning site, please refer to the diagram on page 7 for pTrcHis-TOPO® or page 9 for pTrcHis2-TOPO®.

   If you need help with setting up restriction enzyme digests or DNA sequencing, please refer to general molecular biology texts (Ausubel *et al.*, 1994; Sambrook *et al.*, 1989).

## Alternative Method of Analysis

You may wish to use PCR to directly analyze positive transformants. Use either the Forward or Reverse sequencing primer and a primer that hybridizes to your insert as PCR primers. If this is the first time you have used this technique, we recommend that you perform restriction analysis in parallel to confirm that PCR gives you the correct result. False positive and false negative results can be obtained because of mispriming or contaminating template. The following protocol is provided for your convenience. Other protocols are suitable.

**Note**: Additional primers and nucleotides are available separately. See page 5 for ordering information.

1. Prepare a PCR cocktail consisting of PCR buffer, dNTPs, primers, and *Taq* polymerase. Use a 20 µl reaction volume. Multiply by the number of colonies to be analyzed (*e.g.* 10).
2. Pick 10 colonies and resuspend them individually in 20 µl of the PCR cocktail. Remember to patch colonies to a separate plate to preserve the colonies.
3. Incubate the reaction for 10 minutes at 94°C to lyse the cells and inactivate nucleases.
4. Amplify for 20 to 30 cycles (94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute).
5. For the final extension, incubate at 72°C for 10 minutes. Hold at +4°C.
6. Visualize by agarose gel electrophoresis.

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# TOPO® Cloning and Transformation, continued

**Important**

If you have problems obtaining transformants or the correct insert, please see pages 24-26. Control reactions are described using reagents supplied in the kit. This will help you troubleshoot your experiment. Please perform the control reactions before calling Technical Service.

## Long-Term Storage

Once you have identified the correct clone, be sure to purify the colony and make a glycerol stock for long term storage.

1. Streak the original colony out for single colonies on LB plates containing 50 µg/ml ampicillin and 0.5% glucose.
2. Isolate a single colony and inoculate into 1-2 ml of LB containing 50 µg/ml ampicillin and 0.5% glucose. Grow until culture reaches mid-log ($OD_{600}$ = 0.5-0.7)
3. Mix 0.85 ml of culture with 0.15 ml of sterile glycerol, transfer to a cryovial, and store at -80°C.

# Expression of the PCR Product

## Introduction

Since each recombinant protein has different characteristics that may affect optimum expression, it is helpful to run a time course of expression to determine the best conditions for maximum expression of your particular protein. Use the positive control vector included in each kit as an expression control (see pages 31-32). TOP10 cells may be used as a general host for expression.

**Note**

Remember that inclusion of the N- or C-terminal tag will increase the size of your recombinant protein by 3 to 4 kDa.

## Before Starting

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

- Positive control (TOP10 cells containing pTrcHis-TOPO®/*lacZ* or pTrcHis2-TOPO®/*lac*Z, see below)
- Negative control (TOP10 cells only, see below)
- SOB or LB containing 50 µg/ml ampicillin (see **Recipes**, page 19) **Note**: For expression, you generally do not need to include glucose (see the next page for more information)
- 37°C shaking incubator
- Thaw 1 M IPTG stock
- 1X SDS-PAGE sample buffer
- Reagents and apparatus for SDS-PAGE gel

## Positive and Negative Controls

Details of each positive control vector are provided on pages 31-32. Transform the plasmid into TOP10 One Shot® cells as you did for your construct. TOP10 cells that do not contain any vector are used as a negative control.

## Pilot Expression

1. For each strain, 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).
3. The next day, inoculate 10 ml of SOB or LB containing 50 µg/ml ampicillin with 0.2 ml of the overnight culture.
4. Grow the culture at 37°C with vigorous shaking to an $OD_{600}$ = 0.6 (the cells should be in mid-log phase).
5. Remove a 1 ml aliquot of cells, centrifuge at maximum speed in a microcentrifuge for 30 seconds, and aspirate the supernatant. Freeze the cell pellet at -20°C. This is the zero time point sample.
6. Add IPTG to a final concentration of 1 mM (9 µl of a 1 M IPTG stock to 9 ml) and grow at 37°C with shaking.
7. Take 1 ml samples every hour for 5 hours (or more) and treat as described in Step 5. Label each tube to correspond to the number of hours postinduction.

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# Expression of the PCR Product, continued

## Preparation of Time Point Samples

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

1. When all the time points have been collected, resuspend each pellet in 100 µl of 1X SDS-PAGE sample buffer.
2. Boil 5 minutes and centrifuge briefly. If solution is viscous, sonicate briefly and centrifuge again.
3. Analyze 5 µl of each sample on an SDS-PAGE gel.

## Analysis of Time Point 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.

   **Note**: The tags contribute ~3 to 4 kDa to your protein.
2. Use the negative control to distinguish recombinant proteins from background proteins.
3. Use the positive control to confirm that growth and induction was done properly. pTrcHis-TOPO®/*lacZ* should yield a 40 kDa protein and pTrc-His2-TOPO®/*lacZ* should yield a 120 kDa protein with maximum expression occurring between 3-4 hours.
4. You should be able to determine the optimal time point for maximum expression. If you do not see your protein of interest, please see the **Troubleshooting** section, page 18.

## Reducing Basal Levels of Recombinant Protein

You may find that your recombinant protein may be slightly toxic or unstable because of high levels of basal transcription. Use glucose to further repress transcription (see page 11). Supplementing LB medium with 25 mM glucose (0.5% w/v) prior to induction will repress basal level transcription from the *trc* promoter. To induce expression, pellet the cells, and resuspend them in LB without glucose and add IPTG to induce.

## Scale-Up of Expression

Use the conditions determined previously 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 4 for ordering information.

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).
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 $OD_{600}$ = 0.6 (the cells should be in mid-log phase).
5. Add IPTG to a final concentration of 1 mM (50 µl of 1 M IPTG stock to 50 ml).
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 (please refer to the ProBond™ Purification System manual) or store the cell pellet at -80°C for future use.

*continued on next page*

# Expression of the PCR Product, continued

## Enterokinase Cleavage

For recombinant proteins expressed from pTrcHis-TOPO®, you may wish to remove the N-terminal tag from your partially pure or purified protein. We recommend using EnterokinaseMax™ (Catalog no. E180-01), a recombinant form of bovine enterokinase. For more information, please see our Web site (www.invitrogen.com) or contact Technical Service (page 33).

# Troubleshooting

## Troubleshooting Table

If you have trouble expressing your protein, try some of the suggestions listed below. Please be sure to include the positive and negative controls when testing for expression of your protein to ensure that the cells were grown and induced correctly. If you find that the positive control did not express, it may be that the IPTG solution is too old. Prepare fresh IPTG solution (see page 20).

| Problem | Possible Cause | Solution |
|---|---|---|
| Recombinant protein is not detected on a Coomassie stained gel. | Low expression of recombinant protein. | Use western blot analysis to detect recombinant protein expression. You may use antibody to your own protein or the appropriate antibody listed on page 4. |
| Low expression of recombinant protein. | Recombinant plasmid is unstable or protein is slightly toxic | Include glucose in the growth medium to reduce basal levels of transcription. Be sure to check plasmid to ensure that no rearrange-ments have occurred. See page 16 for details. |
| | *Taq* polymerase may introduce mutations. | Sequence your construct. If you find mutations, redo your PCR using a proofreading polymerase and add 3´ A overhangs using the method on page 23. |
| No expression/detection of protein | PCR product is out of frame with the N-terminal peptide or the initiation codon and/or C-terminal peptide. | Sequence your construct to confirm the protein is in frame with the N-terminal peptide or the initiation codon and the C-terminal tag (if the tag is desired). |
| | *Taq* polymerase may introduce mutations. | Sequence your construct. If you find mutations, redo your PCR using a proofreading polymerase and add 3´ A overhangs using the method on page 23. |

# Appendix

## Recipes

### LB (Luria-Bertani) Medium and Plates

**Composition:**

1.0% Tryptone
0.5% Yeast Extract
1.0% NaCl
+/- 0.5% glucose (dextrose)
pH 7.0

1. For 1 liter, dissolve 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 950 ml deionized water.
2. Adjust the pH of the solution to 7.0 with NaOH and bring the volume up to 1 liter.
3. Prepare a 50% solution of glucose (dextrose). Filter-sterilize or autoclave as described below. **Note**: Solution may turn yellowish. This is normal.
4. Autoclave on liquid cycle for 20 minutes at 15 psi. Allow solutions to cool to 55°C. Add antibiotic to the medium if needed. Add glucose to a final concentration of 0.5%.
5. Store at room temperature or at +4°C. Shelf life with ampicillin is 1-2 weeks.

**LB agar plates**

1. Prepare LB medium as above, but add 15 g/L agar before autoclaving.
2. Autoclave on liquid cycle for 20 minutes at 15 psi.
3. After autoclaving, cool to ~55°C, add antibiotic (50 µg/ml of ampicillin) and glucose to 0.5%, if desired. Pour into 10 cm plates.
4. Let harden, then invert and store at +4°C, in the dark. Shelf life with ampicillin is 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 $MgCl_2$

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.**

*continued on next page*

# Recipes, continued

**1 M IPTG**

1. To prepare a 1 M stock solution, dissolve 2.38 g of IPTG in 10 ml of deionized water.
2. Filter-sterilize and store in 1 ml aliquots at -20°C.

# Purifying PCR Products

## Introduction

Smearing, multiple banding, primer-dimer artifacts, or large PCR products (>1 kb) may necessitate gel purification. If you intend to purify your PCR product, be extremely careful to remove all sources of nuclease contamination. There are many protocols to isolate DNA fragments or remove oligonucleotides. Please refer to Current Protocols in Molecular Biology, Unit 2.6 for the most common protocols (Ausubel *et al.*, 1994). Two simple protocols are provided below that work for most people.

**Note**

Please note that cloning efficiency may decrease with purification of the PCR product. You may wish to optimize your PCR to produce a single band (see **Producing PCR Products**, page 10).

## Using the S.N.A.P.™ MiniPrep Kit

The S.N.A.P.™ MiniPrep Kit (Catalog no. K1900-01) allows you to rapidly purify PCR products from regular agarose gels. You will need to prepare a 6 M sodium iodide, 10 mM sodium sulfite solution in sterile water before starting.

1. Electrophorese amplification reaction on a 1 to 5% regular TAE agarose gel.

   **Note**: Do not use TBE to prepare agarose gels. Borate interferes with the sodium iodide step, below.
2. Cut out the gel slice containing the PCR product and melt it at 65°C in 2 volumes of 6 M NaI.
3. Add 1.5 volumes Binding Buffer (provided in the S.N.A.P.™ MiniPrep Kit).
4. Load solution (no more than 1 ml at a time) from Step 3 onto a S.N.A.P.™ column. Centrifuge 1 minute at 3000 x g in a microcentrifuge and discard the supernatant.
5. If you have solution remaining from Step 3, repeat Step 4.
6. Add 900 µl of the Final Wash Buffer (provided in the S.N.A.P.™ MiniPrep Kit).
7. Centrifuge 1 minute at 3000 x g in a microcentrifuge and discard the supernatant.
8. Centrifuge again at maximum speed for 1 minute to fully dry the resin.
9. Elute the purified PCR product in 40 µl of TE or sterile water. Use 4 µl for the TOPO® Cloning reaction and proceed as described on page 8.

## Quick S.N.A.P.™ Method

An even easier method is to simply cut out the gel slice containing your PCR product, place it on top of the S.N.A.P.™ column bed, and centrifuge at full speed for 10 seconds. Use 1-2 µl of the flow-through in the TOPO® Cloning reaction (page 12). Be sure to make the gel slice as small as possible for best results.

*continued on next page*

# Purifying PCR Products, continued

## Low-Melt Agarose Method

If you gel-purify your PCR product in low-melt agarose, use the procedure below. Please note that gel purification will result in a dilution of your PCR product and decreased cloning efficiencies.

1. Electrophorese as much as possible of your PCR reaction on a low-melt agarose gel (0.8 to 1.2%) in TAE buffer.
2. Visualize the band of interest and excise the band.
3. Place the gel slice in a microcentrifuge tube and incubate the tube at 65°C until the gel slice melts.
4. Place the tube at 37°C to keep the agarose melted.
5. In a fresh tube, mix together 4 µl of the melted agarose containing your PCR product and 1 µl of TOPO® vector.
6. Incubate at 37°C for 5 to 10 minutes.
7. Transform 2 to 4 µl directly into TOP10 One Shot® cells using the method on page 12.

# Addition of 3´ A-Overhangs Post-Amplification

## Introduction

Direct cloning of DNA amplified by *Vent*® or *Pfu* polymerases into TOPO TA Cloning® vectors is often difficult because of very low cloning efficiencies. Proofreading polymerases lack the terminal transferase activity that adds the 3´ A-overhangs necessary for TA Cloning®. Invitrogen has developed a simple method to clone these blunt-ended fragments.

## Before Starting

You will need the following items:

- *Taq* polymerase
- A heat block equilibrated to 72°C
- Phenol-chloroform
- 3 M sodium acetate
- 100% ethanol
- 80% ethanol
- TE buffer

## Procedure

This is just one method for adding 3´ adenines. Other protocols may be suitable.

1. After amplification with *Vent*® or *Pfu* polymerase, place vials on ice and add 0.7-1 unit of *Taq* polymerase per tube. Mix well. It is not necessary to change the buffer.
2. Incubate at 72°C for 8-10 minutes (do not cycle).
3. Place the vials on ice. Use immediately in a TOPO® Cloning reaction.

**Note:** If you plan to store your sample(s) overnight before proceeding with TOPO® Cloning, you may want to extract your sample(s) with phenol-chloroform to remove the polymerases. After phenol-chloroform extraction, precipitate the DNA with ethanol and resuspend the DNA in TE buffer to the starting volume of the amplification reaction.

**Note**

You may also gel-purify your PCR product after amplification with *Vent*® or *Pfu* (see previous page). After purification, add *Taq* polymerase buffer, dATP, and 0.5 unit of *Taq* polymerase and incubate 10-15 minutes at 72°C. Use 4 µl in the TOPO® Cloning reaction.

*Vent*® is a registered trademark of New England Biolabs.

# TOPO TA Cloning® Control Reactions

## Introduction

If you have trouble obtaining transformants or vector containing insert, please perform the following control reactions to help troubleshoot your experiment. Performing the control reactions involves producing a 750 bp control PCR product and TOPO® Cloning it using the reagents included in the kit. Successful TOPO® Cloning of the control PCR product will yield >85% recombinants.

## Before Starting

Be sure to prepare LB plates containing 50 µg/ml ampicillin and 0.5% glucose before performing the control reaction. Use two plates per transformation.

**Tip**: If you already have LB plates containing 50 µg/ml ampicillin only, you may spread 20 µl of a 2 M (or a 50%) glucose solution onto the plate. Incubate the plate at 37°C for 30 minutes to allow the glucose to diffuse into the plate. Please note that the concentration of glucose does not have to be exact.

## Producing Control PCR Product

1. To produce the 750 bp control PCR product, set up the following 50 µl PCR:

| | |
|---|---|
| Control DNA Template (100 ng) | 1 µl |
| 10X PCR Buffer | 5 µl |
| 50 mM dNTPs | 0.5 µl |
| Control PCR Primers (0.1 µg/µl each) | 1 µl |
| Sterile Water | 41.5 µl |
| *Taq* Polymerase (1 unit/µl) | 1 µl |
| Total Volume | 50 µl |

2. Overlay with 70 µl (1 drop) of mineral oil.
3. Amplify using the following cycling parameters:

| Step | Time | Temperature | Cycles |
|---|---|---|---|
| Initial Denaturation | 2 minutes | 94°C | 1X |
| Denaturation | 1 minute | 94°C | 25X |
| Annealing | 1 minute | 55°C | |
| Extension | 1 minute | 72°C | |
| Final Extension | 7 minutes | 72°C | 1X |

4. Remove 10 µl from the reaction and analyze by agarose gel electrophoresis. A discrete 750 bp band should be visible. Proceed to the **Control TOPO® Cloning Reactions**, next page.

*continued on next page*

# TOPO TA Cloning® Control Reactions, continued

## Control TOPO® Cloning Reactions

Using the control PCR product produced on the previous page and the pTrcHis-TOPO® or pTrcHis2-TOPO® vectors, set up two 5 µl TOPO® Cloning reactions as described below.

1. Set up control TOPO® Cloning reactions:

| Reagent | "Vector Only" | "Vector + PCR Insert" |
|---|---|---|
| Control PCR Product | -- | 1 µl |
| Sterile Water | 4 µl | 3 µl |
| TOPO® vector | 1 µl | 1 µl |

2. Incubate at 25°C (room temperature) for **5 minutes and place on ice. Do not incubate longer than 5 minutes at room temperature.**
3. Transform 2 µl of each reaction into separate vials of TOP10 One Shot® cells (page 12).
4. Spread 10-50 µl of each transformation mix onto LB plates containing 50 µg/ml ampicillin and 0.5% glucose. We recommend that you plate two different volumes of the transformation reaction to ensure well-spaced colonies. For plating small volumes, add 20 µl SOC to ensure even spreading.
5. Incubate overnight at 37°C.

## Analysis of Results

Hundreds of colonies from the "Vector + PCR Insert" reaction should be produced. Select 10 colonies and culture in LB containing 50 µg/ml ampicillin and 0.5% glucose. Isolate plasmid DNA and analyze by restriction enzyme digestion.

For pTrcHis-TOPO®, use *Eco*R I and *Bam*H I.

For pTrcHis2-TOPO®, use *Nco* I and *Eco*R I.

Digestion of each recombinant vector will yield 3 fragments: 100 bp, 650 bp, and 4.4 kb (vector backbone). Greater than 85% of the transformants will yield this digestion pattern.

The "Vector Only" plate should contain very few colonies (<10% of the number of colonies on the "Vector + PCR Insert" plate).

## Transformation Control

pUC19 plasmid is included to check the transformation efficiency of the One Shot® competent cells. Transform with 10 pg per 50 µl of cells using the protocol on page 12. Plate 10 µl of the transformation reaction plus 20 µl SOC on an LB plate containing 50 µg/ml ampicillin. The transformation efficiency should be ~1 x $10^9$ cfu/µg DNA.

*continued on next page*

# TOPO TA Cloning® Control Reactions, continued

## Factors Affecting Cloning Efficiency

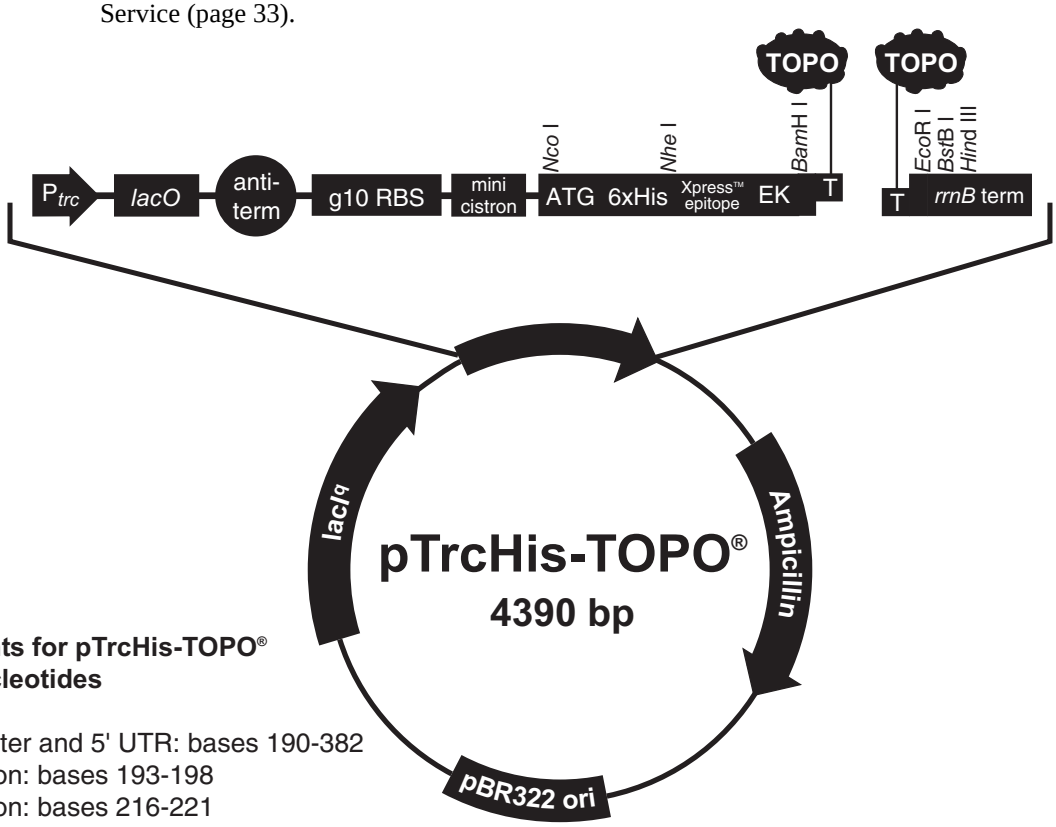
Please note that lower cloning efficiencies will result from the following variables. Most of these are easily correctable, but if you are cloning large inserts, you may not obtain the expected 90% (or more) cloning efficiency.

| Variable | Solution |
|---|---|
| TOPO® Cloning reactions longer than 5 minutes at room temperature | Be sure to incubate for only 5 minutes. Incubations longer than 5 minutes will decrease transformation efficiency. |
| pH>9 in PCR amplification reaction | Check the pH of the PCR amplification reaction and adjust with 1 M Tris-HCl, pH 8. |
| Incomplete extension during PCR | Be sure to include a final extension step of 7 to 30 minutes during PCR. Longer PCR products will need a longer extension time. |
| Cloning large inserts (>1 kb) | Increase amount of insert. Or gel-purify as described on page 21. For cloning large inserts (3 to 10 kb), try the TOPO XL PCR Cloning™ Kit (Catalog no. K4700-01). |
| Excess (or overly dilute) PCR product | Reduce (or concentrate) the amount of PCR product. |
| Cloning blunt-end PCR products | Add 3´ A-overhangs by incubating with *Taq* polymerase (page 23). |
| PCR cloning artifacts ("false positives") | TOPO® Cloning is very efficient for small fragments (< 100 bp) present in certain PCR reactions. Gel-purify your PCR product (page 21) or optimize your PCR. |
| PCR product does not contain sufficient 3´ A-overhangs even though you used *Taq* polymerase | *Taq* polymerase is less efficient at adding a nontemplate 3´ A next to another A. *Taq* is most efficient at adding a nontemplate 3´ A next to a C. You may have to redesign your primers so that they contain a 5´ G instead of a 5´ T (Brownstein *et al.*, 1996). |

# Map and Features of pTrcHis-TOPO®

## pTrcHis-TOPO® Map

The map below shows the features of pTrcHis-TOPO®. For the full sequence of the vector, you may download it from our Web site (www.invitrogen.com) or call Technical Service (page 33).




**Comments for pTrcHis-TOPO®**
**4390 nucleotides**

*trc* promoter and 5' UTR: bases 190-382
-35 region: bases 193-198
-10 region: bases 216-221
*lac* operator site: bases 228-248
*rrnB* anti-termination sequence: bases 264-333
T7 gene 10 translational enhancer: bases 346-354
Ribosome binding site: 369-373
pTrcHis forward priming site: bases 370-390
Minicistron: bases 383-409
Reinitiation RBS: bases 398-403
Initiation ATG: bases 413-415
6xHis tag: bases 425-442
Xpress™ epitope: bases 482-505
Xpress™ forward priming site: bases 445-463
Enterokinase cleavage site: bases 491-505
TOPO® Cloning site: bases 517-518
pTrcHis reverse priming site: bases 574-591
*rrnB* $T_1$ and $T_2$ transcription termination sequence: bases 624-781
*bla* promoter: bases 1002-1059
Ampicillin resistance gene (*bla*): bases 1060-1920
pBR322-derived origin: bases 2065-2738
Lac Repressor (*lacI*$^q$) : bases 3392-4351

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# Map and Features of pTrcHis-TOPO®, continued

## Features of pTrcHis-TOPO®

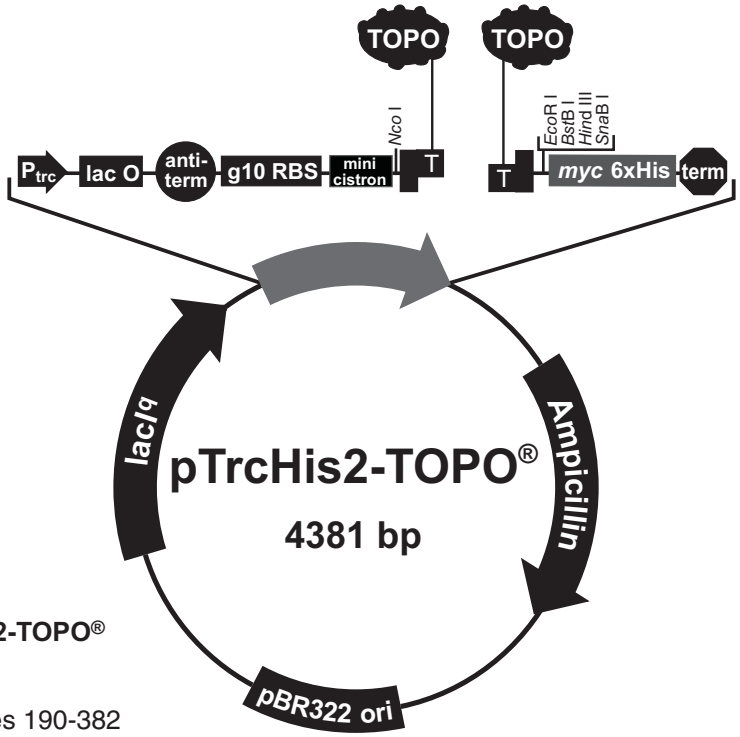
The important elements of pTrcHis-TOPO® (4390 bp) are described in the following table. All features have been functionally tested.

| Feature | Benefit |
|---|---|
| *trc* promoter region | Provides high-level, inducible expression of recombinant proteins in *E. coli*. It is a hybrid promoter consisting of the -35 region from *trpB* and the -10 region from the *lacUV5* promoter (Egon *et al.*, 1983). |
| *lac* operator (*lacO*) | Binding site of the *lac* repressor to provide regulated expression of the *trc* promoter (Jacob and Monod, 1961). |
| *rrnB* antitermination signal | Sequence from the *rrnB* gene that reduces the level of premature transcription termination (Li *et al.*, 1984). |
| T7 gene 10 translational enhancer | Sequence from bacteriophage T7 gene 10 that optimizes translation initiation (Olins *et al.*, 1988). |
| Minicistron, RBS, and Initiation ATG | A short open reading frame containing nucleotide sequences that is efficiently translated in prokaryotic cells. A ribosome binding site (RBS) is present within the coding sequence 5´ to the translation termination codon. This RBS and termination codon are positioned in frame and three nucleotides upstream from the translation initiation codon used to express the fusion protein of interest. Following translation of the open reading frame of the minicistron, ribosomes efficiently reinitiate translation at the second initiation site (Schoner *et al.*, 1986). |
| 6xHis tag | Forms metal-binding site for affinity purification of recombinant fusion protein on metal-chelating resin (*i.e.* ProBond™). |
| HisG epitope | Allows detection of the fusion protein by the Anti-HisG Antibodies (see page 4). |
| Xpress™ Forward priming site | Permits sequencing of your insert from the 5´ end. |
| Xpress™ epitope | Allows detection of the fusion protein by the Anti-Xpress™ Antibodies (see page 4). |
| Enterokinase recognition site | Encodes the binding site for bovine enterokinase to permit removal of the N-terminal peptide from your protein. |
| TOPO® Cloning site | Allows fast insertion of your PCR product for expression. |
| pTrcHis Reverse priming site | Permits sequencing of your insert from the 3´ end. |
| *rrn*B transcription termination region | Strong transcription termination region (Orosz *et al.*, 1991). |
| Ampicillin resistance gene (β-lactamase) | Allows selection of the plasmid in *E. coli*. |
| pBR322-derived origin | Medium copy replication and growth in *E. coli*. |
| *lacI*$^{q}$ gene | Encodes the *lac* repressor for regulation of the *trc* promoter (Müller-Hill *et al.*, 1968). |

# Map and Features of pTrcHis2-TOPO®

## pTrcHis2-TOPO® Map

The map below shows the features of pTrcHis2-TOPO®. For the full sequence of the vector, you may download it from our Web site (www.invitrogen.com) or call Technical Service (page 33).




**Comments for pTrcHis2-TOPO®**
**4381 nucleotides**

*trc* promoter and 5': bases 190-382
  -35 region: bases 193-198
  -10 region: bases 216-221
  *lac* operator (*lacO*): bases 228-248
  *rrn*B antitermination signal: bases 264-333
  gene 10 region: bases 346-354
  Ribosome binding site: bases 369-373
  pTrcHis Forward priming site: bases 370-390
Minicistron ORF: bases 383-409
Reinitiation RBS: bases 398-403
Initiation ATG: bases 413-415
TOPO® Cloning site: bases 421-422
*myc* epitope: bases 446-475
Polyhistidine region: bases 491-508
pTrcHis2 Reverse priming site: bases 564-581
*rrn*B T1 and T2 transcriptional terminators: bases 614-771
*bla* promoter: bases 993-1050
Ampicillin resistance gene (*bla*): bases 1051-1911
pBR322-derived origin: bases 2056-2729
Lac Repressor (*lacI*$^{q}$) ORF: bases 3383-4342

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# Map and Features of pTrcHis2-TOPO®, continued

## Features of pTrcHis2-TOPO®

The important elements of pTrcHis2-TOPO® (4381 bp) are described in the following table. All features have been functionally tested.

| Feature | Benefit |
|---|---|
| *trc* promoter region | Provides high-level, inducible expression of recombinant proteins in *E. coli*. It is a hybrid promoter consisting of the -35 region from *trpB* and the -10 region from the *lacUV5* promoter (Egon *et al.*, 1983). |
| *lac* operator (*lacO*) | Binding site of the *lac* repressor to provide regulated expression of the *trc* promoter (Jacob and Monod, 1961). |
| *rrnB* antitermination signal | Sequence from the *rrnB* gene that reduces the level of premature transcription termination (Li *et al.*, 1984). |
| T7 gene 10 translational enhancer | Sequence from bacteriophage T7 gene 10 that optimizes translation initiation (Olins *et al.*, 1988). |
| pTrcHis Forward priming site | Permits sequencing of your insert from the 5´ end. |
| Minicistron, RBS, and Initiation ATG | A short open reading frame containing nucleotide sequences that is efficiently translated in prokaryotic cells. A ribosome binding site (RBS) is present within the coding sequence 5´ to the translation termination codon. This RBS and termination codon are positioned in frame and three nucleotides upstream from the translation initiation codon used to express the fusion protein of interest. Following translation of the open reading frame of the minicistron, ribosomes efficiently reinitiate translation at the second initiation site (Schoner *et al.*, 1986). |
| TOPO® Cloning site | Allows fast insertion of your PCR product for expression. |
| C-terminal *myc* epitope (optional) | Allows detection of the fusion protein by the Anti-*Myc* Antibody (Catalog no. R930-25) (Evan *et al.*, 1985). |
| C-terminal polyhistidine region (optional) | Forms metal-binding site for affinity purification of recombinant fusion protein on metal-chelating resin (*i.e.* ProBond™).<br>In addition, it allows detection of the recombinant protein with Anti-His (C-term) Antibody (see page 4). |
| pTrcHis2 Reverse priming site | Permits sequencing of your insert from the 3´ end. |
| *rrn*B transcription termination region | Strong transcription termination region (Orosz *et al.*, 1991). |
| Ampicillin resistance gene (β-lactamase) | Allows selection of the plasmid in *E. coli*. |
| pBR322-derived origin | Medium copy replication and growth in *E. coli*. |
| *lacI*$^{q}$ gene | Encodes the *lac* repressor for regulation of the *trc* promoter (Müller-Hill *et al.*, 1968). |

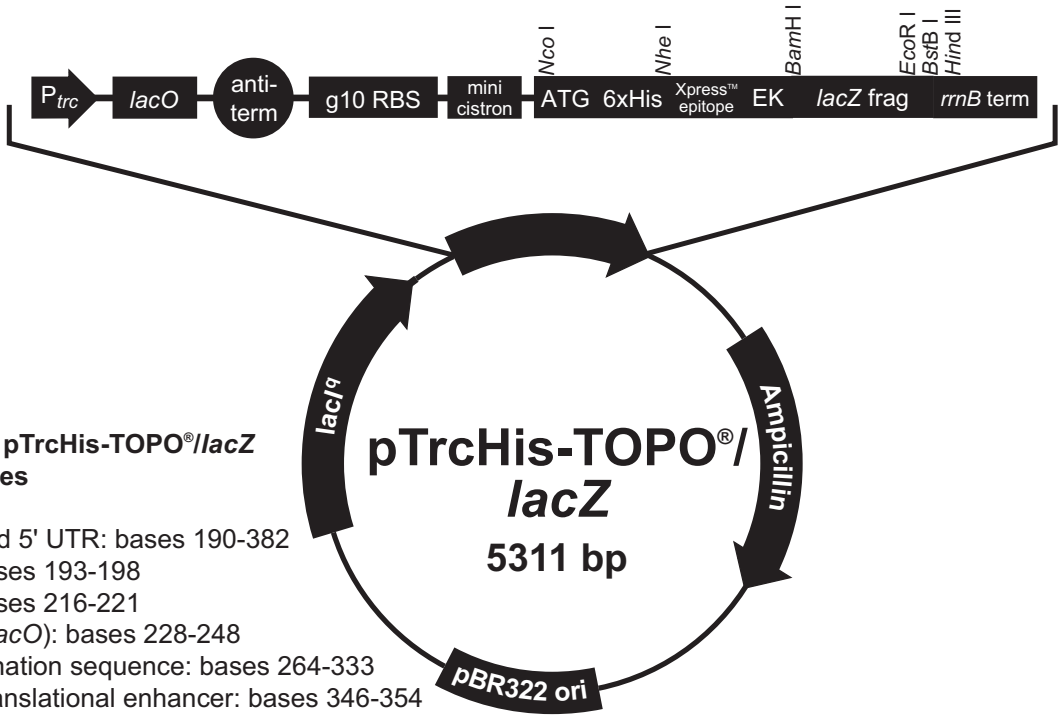
# pTrcHis-TOPO®/*lacZ*

## Description

pTrcHis-TOPO®/*lac*Z is a 5311 bp control vector containing a fragment of the *lacZ* gene fused to the N-terminal peptide. It was constructed by amplifying a 921 bp *lac*Z gene fragment and TOPO® Cloning it into pTrcHis-TOPO®. It yields a 40 kDa expression product.

## Map of Control Vector

The figure below summarizes the features of the pTrcHis-TOPO®/*lac*Z vector. For the full sequence of the vector, you may download it from our Web site (www.invitrogen.com) or call Technical Service (page 33).




**Comments for pTrcHis-TOPO®/*lacZ***
**5311 nucleotides**

*trc* promoter and 5' UTR: bases 190-382
  -35 region: bases 193-198
  -10 region: bases 216-221
  *lac* operator (*lacO*): bases 228-248
  *rrnB* antitermination sequence: bases 264-333
  T7 gene 10 translational enhancer: bases 346-354
  Ribosome binding site: 369-373
  pTrcHis Forward priming site: bases 370-390
Minicistron: bases 383-409
Reinitiation RBS: bases 398-403
*lacZ* fusion protein: bases 413-1480
  Initiation ATG: bases 413-415
  6xHis tag: bases 425-442
  Xpress™ epitope: bases 482-505
  Xpress™ forward priming site: bases 445-463
  Enterokinase cleavage site: bases 491-505
  *lacZ* gene fragment: bases 518-1438
pTrcHis reverse priming site: bases 1495-1512
*rrnB* $T_1$ and $T_2$ transcription termination sequence: bases 1545-1702
*bla* promoter: bases 1923-1980
Ampicillin resistance gene (*bla*): bases 1981-2841
pBR322-derived origin: bases 2986-3659
Lac Repressor (*lacI*$^q$): bases 4188-5272

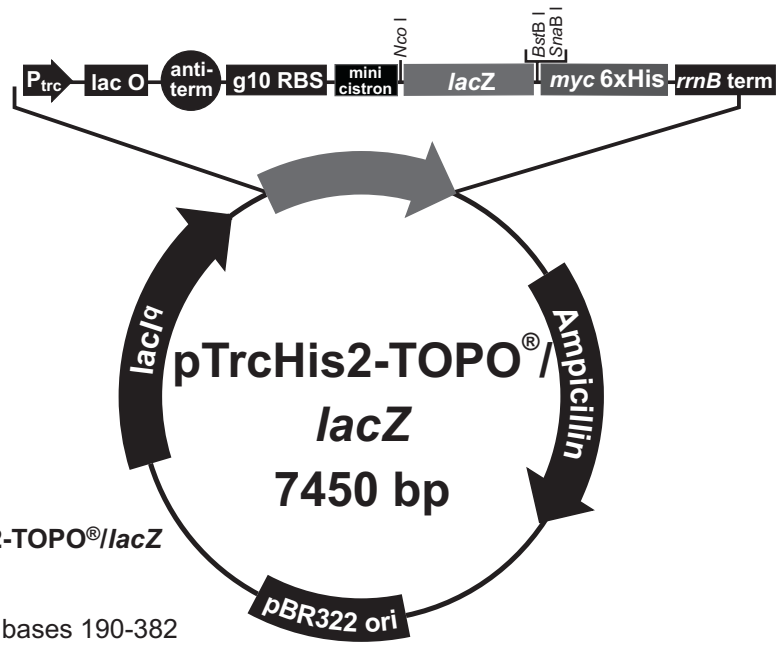
# pTrcHis2-TOPO®/*lacZ*

## Description

pTrcHis2-TOPO®/*lac*Z is a 7450 bp control vector containing the gene for β-galactosidase fused to the C-terminal peptide. It was constructed by amplifying the *lac*Z gene from pTrcHis2/*lacZ* and TOPO® Cloned into pTrcHis2-TOPO®. It yields a 120 kDa expression product.

## Map of Control Vector

The figure below summarizes the features of the pTrcHis2-TOPO®/*lac*Z vector. For the full sequence of the vector, you may download it from our Web site (www.invitrogen.com) or call Technical Service (page 33).




**Comments for pTrcHis2-TOPO®/*lacZ***
**7450 nucleotides**

*trc* promoter and 5' UTR: bases 190-382
-35 region: bases 193-198
-10 region: bases 216-221
*lac* operator (*lacO*): bases 228-248
*rrn*B antitermination signal: bases 264-333
gene 10 region: bases 346-354
Ribosome binding site: bases 369-373
pTrcHis Forward priming site: bases 370-390
Minicistron ORF: bases 383-409
Reinitiation RBS: bases 398-403
Initiation ATG: bases 413-415
LacZ fusion protein: bases 413-3580
LacZ portion of fusion: bases 413-3487
*myc* epitope: bases 3515-3544
Polyhistidine region: bases 3560-3577
pTrcHis2 Reverse priming site: bases 3633-3650
*rrn*B T1 and T2 transcriptional terminators: bases 3683-3840
Ampicillin resistance gene (*bla*): bases 4120-4980
pBR322-derived origin: bases 5125-5798
Lac Repressor (*lacI*$^{q}$) ORF: bases 6327-7411

# Technical Service

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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 service contact information
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## Contact Us

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

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# Qualifying the Product

## Restriction Digest

Supercoiled pTrcHis, pTrcHis2, pTrcHis-TOPO®/*lacZ*, and pTrcHis2-TOPO®/*lacZ* are qualified by restriction digest. The table below lists the restriction enzymes and the expected fragments.

| Restriction Enzyme | pTrcHis | pTrcHis2 | pTrcHis-TOPO®/*lacZ* | pTrcHis2-TOPO®/*lacZ* |
|---|---|---|---|---|
| *Bam*H I | 4400 bp (linearizes) | N/A | N/A | N/A |
| *Hin*d III | 4400 bp (linearizes) | N/A | N/A | 4371, 3079 bp |
| *Xmn* I/*Nco* I | 3900, 700, 160 bp | N/A | N/A | N/A |
| *Apa* I | N/A | 4405 bp (linearizes) | N/A | N/A |
| *Sna*B I | N/A | 4405 bp (linearizes) | N/A | N/A |
| *Cla* I | N/A | N/A | 4558, 707, 46 bp | N/A |
| *Bam*H I/*Eco*R I | N/A | N/A | 4371, 940 bp | N/A |
| *Nco* I | N/A | N/A | N/A | 7450 bp (linearizes) |

## TOPO® Cloning Efficiency

Once pTrcHis and pTrcHis2 have been adapted with topoisomerase I, they are lot-qualified using the control reagents included in the kit. Under conditions described on pages 24-26, a 750 bp control PCR product was TOPO®-Cloned into each vector and subsequently transformed into the One Shot® competent *E. coli* included with the kit.

Each lot of vector should yield greater than 85% cloning efficiency.

## Primers

All primers have been lot-qualified by DNA sequencing experiments using the dideoxy chain termination technique.

## One Shot® TOP10 Competent *E. coli*

All competent cells are tested for transformation efficiency using the control plasmid. Transformed cultures are plated on LB plates containing 100 µg/ml ampicillin and the transformation efficiency is calculated. Test transformations are performed in duplicate. Transformation efficiency should be ~1 x $10^9$ cfu/µg DNA for chemically competent cells.

In addition, untransformed cells are tested for appropriate antibiotic sensitivity and lack of phage contamination..

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