Instruction Manual

pGeneBLAzer™ TOPO® TA Expression Kits

For five-minute cloning of *Taq* polymerase-amplified PCR products into a vector for promoter function analysis in mammalian cells using the β-lactamase reporter gene

Catalog nos. 12578-019 and 12578-027

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TOPO® Cloning Procedure for Experienced Users

Introduction

This quick reference sheet is provided for experienced users of the TOPO® Cloning procedure. If you are performing the TOPO® Cloning procedure for the first time, we recommend that you follow the detailed protocols provided in the manual.

Step	Action
Produce the PCR product	1. Amplify your promoter sequence of interest using *Taq* polymerase and your own protocol. End the PCR reaction with a final 7 to 10 minute extension step. 2. Use agarose gel electrophoresis to check the integrity and yield of your PCR product.
Perform the TOPO® Cloning Reaction	1. Set up the following TOPO® Cloning reaction. **Note:** If you plan to transform electrocompetent *E. coli*, use Dilute Salt Solution in the TOPO® Cloning reaction. Fresh PCR product — 0.5 to 4 µl Salt Solution — 1 µl Sterile water — add to a final volume of 5 µl TOPO® vector — 1 µl Total volume — 6 µl 2. Mix gently and incubate for 5 minutes at room temperature. 3. Place on ice and proceed to transform One Shot® TOP10 chemically competent *E. coli*, below.
Transform One Shot® TOP10 Chemically Competent *E. coli*	1. Add 2 µl of the TOPO® Cloning reaction into a vial of One Shot® TOP10 chemically competent *E. coli* and mix gently. 2. Incubate on ice for 5 to 30 minutes. 3. Heat-shock the cells for 30 seconds at 42°C without shaking. Immediately transfer the tube to ice. 4. Add 250 µl of room temperature S.O.C. Medium. 5. Incubate at 37°C for 1 hour with shaking. 6. Spread 50-200 µl of bacterial culture on a prewarmed LB agar plate containing 100 µg/ml ampicillin and incubate overnight at 37°C.

Control Reaction

We recommend using the Control PCR Template and the Control PCR Primers included with the kit to perform the control reaction. See pages 20-21 for instructions.

Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Note: Each kit is also supplied with an appropriate GeneBLAzer™ Detection Kit and the GeneBLAzer™ Detection Kit manual.

Kit	Reactions	Catalog no.
pGeneBLAzer™ TOPO® TA Expression Kit for *In Vitro* Detection	20	12578-019
pGeneBLAzer™ TOPO® TA Expression Kit for *In Vivo* Detection	20	12578-027

Kit Components

The pGeneBLAzer™ TOPO® TA Expression Kits include the following components. For a detailed description of the contents of each component, see pages viii-ix.

Component	Catalog no.	
	12578-019	12578-027
pGeneBLAzer™ TOPO® Reagents	√	√
One Shot® TOP10 Chemically Competent *E. coli*	√	√
GeneBLAzer™ *In Vitro* Detection Kit	√	
GeneBLAzer™ *In Vivo* Detection Kit		√

Shipping and Storage

Each pGeneBLAzer™ TOPO® TA Expression Kit is shipped as described in the table below, and contains the following boxes. Note that each kit contains Box 3a **or** 3b, as appropriate. Upon receipt, store the boxes as detailed below.

Box	Item	Shipping	Storage
1	pGeneBLAzer™ TOPO® Reagents	Dry ice	-20°C
2	One Shot® TOP10 Chemically Competent *E. coli*	Dry ice	-80°C
3a	GeneBLAzer™ *In Vitro* Detection Kit	Dry ice	**CCF2-FA:** -20°C, dessicated and protected from light
3b	GeneBLAzer™ *In Vivo* Detection Kit	Room temperature	**CCF2-AM:** -20°C, dessicated and protected from light **Solutions:** Room temperature, protected from light

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pGeneBLAzer™ TOPO® Reagents

The pGeneBLAzer™ TOPO® reagents (Box 1) are listed below. **Note that the user must supply *Taq* polymerase. Store Box 1 at -20°C.**

Item	Concentration	Amount
pGeneBLAzer-TOPO® vector, linearized and TOPO®-adapted	10 ng/µl plasmid DNA in: 50% glycerol 50 mM Tris-HCl, pH 7.4 (at 25°C) 1 mM EDTA 2 mM DTT 0.1% Triton X-100 100 µg/ml BSA 30 µM phenol red	20 µl
10X PCR Buffer	100 mM Tris-HCl, pH 8.3 (at 42°C) 500 mM KCl 25 mM $MgCl_2$ 0.01% gelatin	100 µl
dNTP Mix	12.5 mM dATP 12.5 mM dCTP 12.5 mM dGTP 12.5 mM dTTP neutralized at pH 8.0 in water	10 µl
Salt Solution	1.2 M NaCl 0.06 M $MgCl_2$	50 µl
Sterile Water	--	1 ml
T7 Sequencing Primer	0.1 µg/µl in TE Buffer, pH 8	20 µl
Control PCR Template	0.05 µg/µl in TE Buffer, pH 8	10 µl
Control PCR Primers	0.1 µg/µl **each** in TE Buffer, pH 8	10 µl
pGeneBLAzer™/UbC expression control plasmid	0.5 µg/µl in TE Buffer, pH 8	10 µl

continued on next page

One Shot® TOP10 Reagents

The table below describes the reagents included in the One Shot® TOP10 Chemically Competent *E. coli* kit (Box 2). Transformation efficiency is $\geq 1 \times 10^9$ cfu/μg DNA. **Store Box 2 at -80°C.**

Item	Composition	Amount
S.O.C. Medium (may be stored at +4°C or room temperature)	2% Tryptone 0.5% Yeast Extract 10 mM NaCl 2.5 mM KCl 10 mM $MgCl_2$ 10 mM $MgSO_4$ 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	50 μl

GeneBLAzer™ Detection Kit

The pGeneBLAzer™ TOPO® TA Expression Kits include either the GeneBLAzer™ *In Vitro* Detection Kit or the GeneBLAzer™ *In Vivo* Detection Kit for fluorescence detection of β-lactamase reporter activity. Refer to the GeneBLAzer™ Detection Kit manual for detailed information pertaining to each kit and a description of the reagents provided in each kit.

Genotype of TOP10 Cells

Use this strain for general cloning. 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

Sequencing Primers

The table below provides the sequence and amount supplied of the T7 Sequencing Primer.

Primer	Sequence	Amount
T7 Sequencing	5´-TAATACGACTCACTATAGGG-3´	328 pmoles

Accessory Products

Additional Products

The table below lists additional products that may be used with the pGeneBLAzer™ TOPO® TA Expression Kits. For more information, refer to our Web site (www.invitrogen.com) or call Technical Service (see page 30).

Note: Other reagent sizes may be available.

Item	Amount	Catalog no.
Platinum® *Taq* DNA Polymerase	100 reactions	10966-018
	250 reactions	10966-026
	500 reactions	10966-034
	5000 reactions	10966-083
Taq DNA Polymerase, Recombinant	100 units	10342-053
	500 units	10342-020
Platinum® *Taq* DNA Polymerase High Fidelity	100 units	11304-011
One Shot® Kit (TOP10 Chemically Competent *E. coli*)	10 reactions	C4040-10
	20 reactions	C4040-03
	40 reactions	C4040-06
One Shot® Kit (TOP10 Electrocompetent *E. coli*)	10 reactions	C4040-50
	20 reactions	C4040-52
T7 Promoter Primer	2 μg (328 pmoles)	N560-02
PureLink™ HQ Mini Plasmid Purification Kit	100 reactions	K2100-01
Geneticin®	1 g	11811-023
	5 g	11811-031
	20 ml (50 mg/ml)	10131-035
	100 ml (50 mg/ml)	10131-027

Introduction

Overview

Introduction

The pGeneBLAzer™ TOPO® TA Expression Kits provide a highly efficient, 5 minute, one-step cloning strategy ("TOPO® Cloning") for the direct insertion of promoter sequences amplified by *Taq* polymerase into a reporter vector. You will insert your promoter sequences upstream of the β-lactamase reporter gene. Recombinant vectors can then be transfected into mammalian cells and assayed for promoter function and strength *in vivo* or *in vitro* using the GeneBLAzer™ *In Vivo* or *In Vitro* Detection Kit, respectively. No ligase, post-PCR procedures, or PCR primers containing specific sequences are required.

For more information about TOPO® Cloning and the GeneBLAzer™ Technology, see pages 2-3.

Features of the pGeneBLAzer-TOPO® Vector

The pGeneBLAzer-TOPO® vector contains the following elements:

- TOPO® Cloning site for rapid and efficient cloning of *Taq* polymerase-amplified PCR products
- *bla*(M) reporter gene to facilitate *in vitro* or *in vivo* analysis of promoter function using fluorescence detection
- Herpes Simplex Virus thymidine kinase (TK) polyadenylation sequence for efficient transcription termination and polyadenylation of mRNA (Cole and Stacy, 1985)
- Neomycin resistance gene for selection of stable cell lines (Southern and Berg, 1982)
- pUC origin for high-copy replication and maintenance of the plasmid in *E. coli*
- Ampicillin resistance gene for selection in *E. coli*

The control plasmid, pGeneBLAzer™/UbC, is included for use as a positive control for transfection and expression in mammalian cells.

Advantages of the GeneBLAzer™ Detection System

Using the GeneBLAzer™ Technology and the GeneBLAzer™ Detection System as a reporter of gene expression in mammalian cells provides the following advantages:

- Suitable for use as a sensitive reporter of gene expression in living mammalian cells using fluorescence microscopy.
- Provides a ratiometric readout to minimize differences due to variability in cell number, substrate concentration, fluorescence intensity, and emission sensitivity.
- Compatible with a wide variety of *in vivo* and *in vitro* applications including microplate-based transcriptional assays and flow cytometry.
- Provides a flexible and simple assay development platform for gene expression in mammalian cells.
- Using a non-toxic substrate allows continued cell culturing after quantitative analysis.

How TOPO® Cloning Works

How Topoisomerase I Works

Topoisomerase I from *Vaccinia* virus binds to duplex DNA at specific sites and cleaves the phosphodiester backbone after 5′-CCCTT in one strand (Shuman, 1991). The energy from the broken phosphodiester backbone is conserved by formation of a covalent bond between the 3′ phosphate of the cleaved strand and a tyrosyl residue (Tyr-274) of topoisomerase I. The phospho-tyrosyl bond between the DNA and enzyme can subsequently be attacked by the 5′ hydroxyl of the original cleaved strand, reversing the reaction and releasing topoisomerase (Shuman, 1994). TOPO® Cloning exploits this reaction to efficiently clone PCR products.

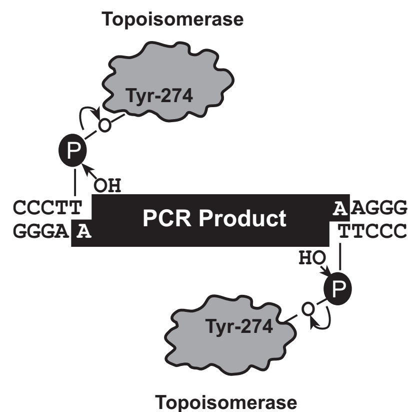
TOPO® Cloning

The pGeneBLAzer-TOPO® vector is 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 products to ligate efficiently into the vector.

TOPO® Cloning exploits the ligation activity of topoisomerase I 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.



The GeneBLAzer™ Technology

Components of the GeneBLAzer™ System

The GeneBLAzer™ System facilitates fluorescent detection of β-lactamase reporter activity in mammalian cells, and consists of two major components:

- The β-lactamase reporter gene, *bla*(M), a truncated form of the *E. coli bla* gene. When fused to promoter sequences in the pGeneBLAzer-TOPO® vector, the *bla*(M) gene functions as a reporter of promoter activity in mammalian cells. For more information about the *bla*(M) gene, see below.
- A fluorescence resonance energy transfer (FRET)-enabled substrate, CCF2 to facilitate fluorescence detection of β-lactamase reporter activity. In the absence or presence of β-lactamase reporter activity, cells loaded with the CCF2 substrate fluoresce green or blue, respectively. Comparing the ratio of blue to green fluorescence in a population of live cells or in a cell extract of your sample to a negative control provides a means to quantitate gene expression. For more information about the CCF2 substrate and how FRET works, refer to the GeneBLAzer™ Detection Kits manual. This manual is supplied with the pGeneBLAzer™ TOPO® TA Expression Kits, but is also available for downloading from our Web site (www.invitrogen.com) or by calling Technical Service (see page 30).

β-Lactamase (*bla*) Gene

β-lactamase is the product encoded by the ampicillin resistance gene (*bla*) and is the bacterial enzyme that hydrolyzes penicillins and cephalosporins. The *bla* gene is present in many cloning vectors and allows ampicillin selection in *E. coli*. β-lactamase is not found in mammalian cells.

bla(M) Gene

The GeneBLAzer™ Technology uses a modified *bla* gene as a reporter in mammalian cells. This *bla* gene is derived from the *E. coli TEM-1* gene present in many cloning vectors (Zlokarnik *et al.*, 1998), and has been modified in the following ways:

- 72 nucleotides encoding the first 24 amino acids of β-lactamase were deleted from the N-terminal region of the gene. These 24 amino acids comprise the bacterial periplasmic signal sequence, and deleting this region allows cytoplasmic expression of β-lactamase in mammalian cells.
- The amino acid at position 24 was mutated from His to Asp to create an optimal Kozak sequence for improved translation initiation.

This modified reporter gene is named *bla*(M).

Note: The *TEM-1* gene also contains 2 mutations (at nucleotide positions 452 and 753) that distinguish it from the *bla* gene in pBR322 (Sutcliffe, 1978).

Experimental Outline

Flow Chart

The flow chart below outlines the experimental steps necessary to clone and test your promoter sequences. For more details, refer to the pages indicated.

Step	Action	Page
1	Design PCR primers to clone your promoter sequence of interest into pGeneBLAzer-TOPO®.	5
2	Produce your PCR product.	7
3	TOPO® Clone your PCR product into pGeneBLAzer-TOPO® and transform the reaction into One Shot® TOP10 *E. coli*. Select for transformants on LB agar plates containing 100 μg/ml ampicillin.	8-11
4	Analyze transformants for the presence and orientation of the insert by restriction digestion, PCR, or sequencing.	13
5	Prepare purified plasmid DNA for transfection.	16
6	Transfect your pGeneBLAzer-TOPO® construct into the mammalian cell line using your method of choice. Select for stable transfectants using Geneticin® (if desired).	16-18
7	Assay for β-lactamase reporter activity using the appropriate GeneBLAzer™ Detection Kit.	19

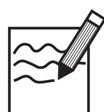
Methods

General Cloning Considerations

Introduction

In general, promoter reporter vectors can be used to analyze:

- Tissue and cell-specific promoter function
- Transcriptional enhancers in a known promoter
- Deletions within a promoter



Note

When analyzing promoters in a reporter vector, it is important to realize that sequences within the native gene can influence regulation of its own promoter. In addition, sequences within the reporter gene can also affect transcription from the promoter under study. We recommend that you verify any observations of transcriptional control of the fusion gene with expression of the native gene. S1 mapping can be used to confirm that the subcloned promoter initiates transcription at the correct site. For more information about S1 mapping, see *Current Protocols in Molecular Biology*, pages 4.6.1 to 4.6.13 (Ausubel *et al.*, 1994).

Important

Since initiation of translation in eukaryotes occurs at the first available AUG codon, it is important that there are no AUG codons between the start of transcription and the AUG of the reporter gene.

PCR Primer Design

Use the diagram on the next page and the sequence of your promoter to design PCR primers. Unique restriction sites may be included in the 5´ and 3´ primers to excise the fragment or facilitate analysis once it is TOPO® Cloned.

Note

Do not add 5´ phosphates to your PCR primers. Phosphates will inhibit topoisomerase I and the synthesized PCR product will not ligate into the pGeneBLAzer-TOPO® vector. Note that cloning efficiencies may vary depending on the 5´ nucleotide in the primers (see page 22).

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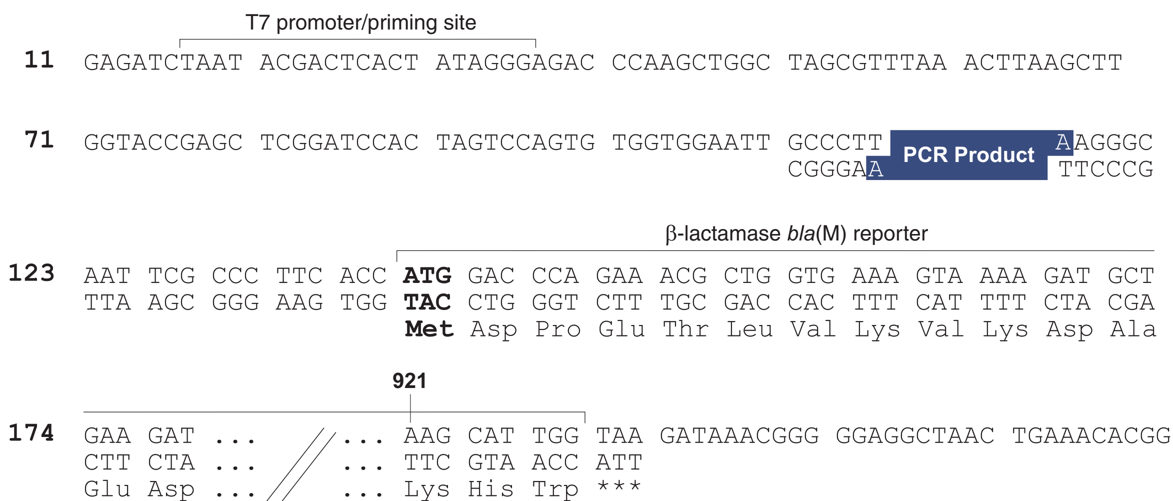
TOPO® Cloning Site of pGeneBLAzer-TOPO®

Use the diagram below to help you design appropriate PCR primers to clone your promoter sequence of interest into pGeneBLAzer-TOPO®. The pGeneBLAzer-TOPO® vector is supplied linearized between base pair 116 and 117. This is the TOPO® Cloning site. **The complete sequence of pGeneBLAzer-TOPO® is available for downloading from our Web site (www.invitrogen.com) or by contacting Technical Service (see page 30).** For a map and a description of the features of pGeneBLAzer-TOPO®, refer to the **Appendix**, page 26.



Producing PCR Products

Introduction

Once you have decided on a PCR strategy and have synthesized the primers you are ready to produce your PCR product.

Materials Supplied by the User

You will need the following reagents and equipment. **Note:** dNTPs (adjusted to pH 8) are provided in the kit.

- *Taq* polymerase or other suitable DNA polymerase

  Note: For improved specificity and higher yields, we recommend using Platinum® *Taq* DNA Polymerase available from Invitrogen (see page x for ordering information) to generate your PCR product.
- Thermocycler
- DNA template and primers for PCR product

Polymerase Mixtures

You may use a polymerase mixture containing *Taq* polymerase and a proofreading polymerase to produce your PCR product; however, the mixture must contain a ratio of *Taq* polymerase:proofreading polymerase in excess of 10:1 to ensure the presence of 3′ A-overhangs on the PCR product.

If you use polymerase mixtures that do not have enough *Taq* polymerase or a proofreading polymerase only, you may add 3′ A-overhangs to your PCR product using the method on page 25.

Producing PCR Products

1. Set up the following 50 µl PCR reaction. Use less DNA if you are using plasmid DNA as a template and more DNA if you are using genomic DNA as a template. Use the cycling parameters suitable for your primers and template. Be sure to include a 7 to 30 minute extension at 72°C after the last cycle to ensure that all PCR products are full length and 3′ adenylated.

DNA Template	10-100 ng
10X PCR Buffer	5 µl
50 mM dNTPs	0.5 µl
Primers	100-200 ng 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 you do not see a single band, refer to the **Note** below.

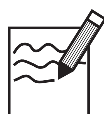
Note

If you do not obtain a single, discrete band from your PCR, you may gel-purify your fragment before proceeding to TOPO® Cloning (see page 23). Take special care to avoid sources of nuclease contamination and long exposure to UV light. Alternatively, you may 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.

Setting Up the TOPO® Cloning Reaction

Introduction

TOPO® Cloning technology allows you to produce your PCR products, ligate them into pGeneBLAzer-TOPO®, and transform the recombinant vector into One Shot® TOP10 *E. coli*, all in one day. It is important to have everything you need set up and ready to use to ensure you obtain the best possible results. We suggest that you read this section and the section entitled **Transforming One Shot® TOP10 Competent Cells** before beginning. If this is the first time you have TOPO® Cloned, perform the control reactions on pages 20-22 in parallel with your samples.



Note

We have found that including salt (200 mM NaCl, 10 mM $MgCl_2$) in the TOPO® Cloning reaction can increase the number of transformants 2- to 3-fold. In addition, incubating the reaction mixture for greater than 5 minutes in the presence of salt can also increase the number of transformants. This is in contrast to earlier experiments **without salt** where the number of transformants decreases as the incubation time increases beyond 5 minutes.

Including salt in the TOPO® Cloning reaction allows for longer incubation times because it prevents topoisomerase I from rebinding and potentially nicking the DNA after ligating the PCR product and dissociating from the DNA. The result is more intact molecules, leading to higher transformation efficiencies.

Using Salt Solution in the TOPO® Cloning Reaction

You will perform TOPO® Cloning in a reaction buffer containing salt (*i.e.* using the stock salt solution provided in the kit). **Note that the amount of salt added to the TOPO® Cloning reaction varies depending on whether you plan to transform chemically competent cells (provided) or electrocompetent cells (see page x for ordering information).**

- If you are transforming chemically competent *E. coli*, use the stock Salt Solution as supplied and set up the TOPO® Cloning reaction as directed on the next page.
- If you are transforming electrocompetent *E. coli*, the amount of salt in the TOPO® Cloning reaction **must be reduced** to 50 mM NaCl, 2.5 mM $MgCl_2$ to prevent arcing during electroporation. Dilute the stock Salt Solution 4-fold with water to prepare a 300 mM NaCl, 15 mM $MgCl_2$ Dilute Salt Solution. Use the Dilute Salt Solution to set up the TOPO® Cloning reaction as directed on the next page.

Materials Needed

You should have the following materials on hand before beginning:

- Your PCR product (freshly prepared)
- pGeneBLAzer-TOPO® vector (supplied with the kit; keep at -20°C until use)
- Salt Solution (supplied with the kit) or Dilute Salt Solution as appropriate
- Sterile water (supplied with the kit)

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Setting Up the TOPO® Cloning Reaction, continued

Performing the TOPO® Cloning Reaction

Use the procedure below to perform the TOPO® Cloning reaction. Set up the TOPO® Cloning reaction using the reagents in the order shown, and depending on whether you plan to transform chemically competent *E. coli* or electrocompetent *E. coli*.

Note: The red or yellow color of the TOPO® vector solution is normal and is used to visualize the solution.

Reagents*	Chemically Competent *E. coli*	Electrocompetent *E. coli*
Fresh PCR product	0.5 to 4 μl	0.5 to 4 μl
Salt Solution	1 μl	--
Dilute Salt Solution (1:4)	--	1 μl
Sterile Water	add to a final volume of 5 μl	add to a final volume of 5 μl
TOPO® vector	1 μl	1 μl
Final Volume	**6 μl**	**6 μl**

*Store all reagents at -20°C when finished. Salt solutions and water can be stored at room temperature or +4°C.

1. Mix reaction gently and incubate for 5 minutes at room temperature.

   Note: For most applications, 5 minutes will yield plenty of colonies for analysis. Depending on your needs, the length of the TOPO® Cloning reaction can be varied from 30 seconds to 30 minutes. For routine subcloning of PCR products, 30 seconds may be sufficient. For large PCR products (> 1 kb) or if you are TOPO® Cloning a pool of PCR products, increasing the reaction time will yield more colonies.

2. Place the reaction on ice and proceed to **Transforming One Shot® TOP10 Competent Cells** (next page).

   Note: You may store the TOPO® Cloning reaction at -20°C overnight.

Transforming One Shot® TOP10 Competent Cells

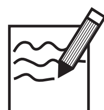
Introduction

Once you have performed the TOPO® Cloning reaction, you will transform your pGeneBLAzer-TOPO® construct into competent *E. coli*. One Shot® TOP10 Chemically Competent *E. coli* (Box 2) are included with the kit to facilitate transformation, however, you may also transform electrocompetent cells (see page x for ordering information). Protocols to transform chemically competent or electrocompetent *E. coli* are provided in this section.

Materials Supplied by the User

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

- 42°C water bath (or electroporator with cuvettes, optional)
- LB plates containing 100 μg/ml ampicillin (two for each transformation)
- 37°C shaking and non-shaking incubator



Note

There is no blue-white screening for the presence of inserts. Individual recombinant plasmids need to be analyzed by restriction analysis or sequencing for the presence and orientation of insert. The T7 Sequencing Primer is included in the kit to facilitate sequencing across the junction of the TOPO® Cloning site and your PCR product to confirm orientation.

Preparing for Transformation

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

- Equilibrate a water bath to 42°C (for chemical transformation) or set up your electroporator if you are using electrocompetent *E. coli*.
- Warm the vial of S.O.C. Medium from Box 2 to room temperature.
- Warm selective plates at 37°C for 30 minutes.
- Thaw on ice 1 vial of One Shot® TOP10 cells from Box 2 for each transformation.

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Transforming One Shot® TOP10 Competent Cells, continued

One Shot® TOP10 Chemical Transformation

1. Add 2 µl of the TOPO® Cloning reaction from Step 2, page 9 into a vial of One Shot® TOP10 Chemically Competent *E. coli* and mix gently. **Do not mix by pipetting up and down.**
2. Incubate on ice for 5 to 30 minutes.

   Note: Longer incubations on ice seem to have a minimal effect on transformation efficiency. The length of the incubation is at the user's discretion.
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 S.O.C. Medium.
6. Cap the tube tightly and shake the tube horizontally (200 rpm) at 37°C for 1 hour.
7. Spread 25-200 µl from each transformation on a pre-warmed selective plate and incubate overnight at 37°C. We recommend plating two different volumes to ensure that at least one plate will have well-spaced colonies.
8. An efficient TOPO® Cloning reaction will produce hundreds of colonies. Pick ~10 colonies for analysis (see **Analyzing Transformants**, next page).

Transformation by Electroporation

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

1. Add 2 µl of the TOPO® Cloning reaction from Step 2, page 9 into 50 µl of electrocompetent E. coli and mix gently. **Do not mix by pipetting up and down. Avoid formation of bubbles.** Transfer the electrocompetent cells into a 0.1 cm cuvette.
2. Electroporate your samples using your own protocol and your electroporator.

   Note: If you have problems with arcing, see the next page.
3. Immediately add 250 µl of room temperature S.O.C. Medium.
4. Transfer the solution to a 15 ml snap-cap tube (*i.e.* Falcon) and shake for at least 1 hour at 37°C to allow expression of the antibiotic resistance marker.
5. Spread 10-50 µl from each transformation on a pre-warmed selective plate and incubate overnight at 37°C. To ensure even spreading of small volumes, add 20 µl of S.O.C. Medium. We recommend plating two different volumes to ensure that at least one plate will have well-spaced colonies.
6. An efficient TOPO® Cloning reaction will produce hundreds of colonies. Pick ~10 colonies for analysis (see **Analyzing Transformants**, page 13).

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Transforming One Shot® TOP10 Competent Cells, continued

To prevent arcing of your samples during electroporation, the volume of cells should be between 50 and 80 µl (0.1 cm cuvettes) or 100 to 200 µl (0.2 cm cuvettes).

If you experience arcing during transformation, try one of the following options:

- Reduce the voltage normally used to charge your electroporator by 10%.
- Reduce the pulse length by reducing the load resistance to 100 ohms.
- Ethanol precipitate the TOPO® Cloning reaction and resuspend in water prior to electroporation.

Analyzing Transformants

Analyzing Positive Clones

1. Pick 10 colonies and culture them overnight in 3-5 ml LB medium containing 100 μg/ml ampicillin.
2. Isolate plasmid DNA using your method of choice. We recommend using the PureLink™ HQ Mini Plasmid Purification Kit (Catalog no. K2100-01) available from Invitrogen.
3. Analyze the plasmids for the presence of the insert and orientation by restriction analysis or by sequencing. Use the T7 Sequencing Primer included in the kit to help you verify the orientation of your insert by sequencing. Refer to the diagram on page 6 for sequence surrounding the TOPO Cloning® site. For the complete sequence of pGeneBLAzer-TOPO®, see our Web site (www.invitrogen.com) or contact Technical Service (page 30).

Analyzing Transformants by PCR

You may analyze positive transformants using PCR. For PCR primers, use the T7 Sequencing Primer and a primer that binds within your insert. You will have to determine the amplification conditions. If you are using this technique for the first time, we recommend performing restriction analysis in parallel. Artifacts may be obtained because of mispriming or contaminating template. The protocol below is provided for your convenience. Other protocols may be suitable.

Materials Needed

PCR SuperMix High Fidelity (Invitrogen, Catalog no. 10790-020)

Appropriate forward and reverse PCR primers (20 μM each)

Procedure

1. For each sample, aliquot 48 μl of PCR SuperMix High Fidelity into a 0.5 ml microcentrifuge tube. Add 1 μl each of the forward and reverse PCR primer.
2. Pick 10 colonies and resuspend them individually in 50 μl of the PCR cocktail from Step 1, above. Don't forget to make a patch plate to preserve the colonies for further analysis.
3. Incubate the reaction for 10 minutes at 94°C to lyse the cells and inactivate nucleases.
4. Amplify for 20 to 30 cycles.
5. For the final extension, incubate at 72°C for 10 minutes. Store at +4°C.
6. Visualize by agarose gel electrophoresis.

Important

If you have problems obtaining transformants or the correct insert, perform the control reactions described on page 20-22. These reactions will help you troubleshoot your experiment.

continued on next page

Long-Term Storage

Once you have identified the correct clone, be sure purify the colony and make a glycerol stock for long-term storage. We recommend that you store a stock of plasmid DNA at -20°C.

1. Streak the original colony on LB plates containing 100 µg/ml ampicillin.
2. Isolate a single colony and inoculate into 1-2 ml of LB containing 100 µg/ml ampicillin.
3. Grow until culture reaches stationary phase.
4. Mix 0.85 ml of culture with 0.15 ml of sterile glycerol and transfer to a cryovial.
5. Store at -80°C.

Optimizing the TOPO® Cloning Reaction

Introduction

Use the information below to help you optimize the TOPO® Cloning reaction for your particular needs.

Faster Subcloning

The high efficiency of TOPO® Cloning allows you to streamline the cloning process. If you routinely clone PCR products and wish to speed up the process, consider the following:

- Incubate the TOPO® Cloning reaction for only 30 seconds instead of 5 minutes.

  You may not obtain the highest number of colonies, but with the high efficiency of TOPO® Cloning, most of the transformants will contain your insert.

- After adding 2 µl of the TOPO® Cloning reaction to chemically competent cells, incubate on ice for only 5 minutes.

  Increasing the incubation time to 30 minutes does not significantly improve transformation efficiency.

More Transformants

If you are TOPO® Cloning large PCR products, toxic genes, or cloning a pool of PCR products, you may need more transformants to obtain the clones you want. To increase the number of colonies:

- Incubate the salt-supplemented TOPO® Cloning reaction for 20 to 30 minutes instead of 5 minutes.

  Increasing the incubation time of the salt-supplemented TOPO® Cloning reaction allows more molecules to ligate, increasing the transformation efficiency. Addition of salt appears to prevent topoisomerase from rebinding and nicking the DNA after it has ligated the PCR product and dissociated from the DNA.

Cloning Dilute PCR Products

To clone dilute PCR products, you may:

- Increase the amount of the PCR product
- Incubate the TOPO® Cloning reaction for 20 to 30 minutes
- Concentrate the PCR product by precipitation

Transfecting Cells

Introduction

Once you have obtained the desired pGeneBLAzer-TOPO® construct, you are ready to transfect the plasmid into the mammalian cell line of choice. Guidelines for transfection are provided below. If you wish to generate stable cell lines, see additional guidelines provided in the next section.

If you plan to detect β-lactamase reporter activity *in vivo* using the GeneBLAzer™ *In Vivo* Detection Kit (supplied with Catalog no. 12578-027 only), note that a number of factors including cell type and cell density can influence the degree of the fluorescence signal detected. We recommend taking these factors into account when designing your transfection experiment. For more information, refer to the section entitled **General Guidelines to Use the GeneBLAzer™ *In Vivo* Detection Kit** in the GeneBLAzer™ Detection Kit manual.

Plasmid Preparation

Plasmid DNA for transfection into eukaryotic cells must be very clean and free from phenol and sodium chloride. Contaminants will kill the cells and salt will interfere with lipid complexing decreasing transfection efficiency. We recommend isolating plasmid DNA using the PureLink™ HQ Mini Plasmid Purification Kit (Catalog no. K2100-01) or CsCl gradient centrifugation.

Controls

We recommend including a positive and a negative control to evaluate expression and detection β-lactamase. A negative control can be either a mock transfection or a pGeneBLAzer-TOPO® construct containing non-promoter DNA sequences (*i.e.* stuffer DNA). For a positive control, use the pGeneBLAzer™/UbC plasmid included in the kit (see below for more information).

Positive Control

pGeneBLAzer™/UbC is provided as a positive control for mammalian cell transfection, expression, and detection (see page 28 for a map) and may be used to optimize detection conditions in your cell line. In this vector, the human ubiquitin C (UbC) promoter (Nenoi *et al.*, 1996) controls expression of the β-lactamase reporter gene.

To propagate and maintain the plasmid:

1. Use the stock solution to transform a *rec*A, *end*A *E. coli* strain such as TOP10, DH5α™, or equivalent.
2. Select transformants on LB agar plates containing 100 μg/ml ampicillin.
3. Prepare a glycerol stock of a transformant containing plasmid for long-term storage.

continued on next page

Methods of Transfection

For established cell lines (*e.g.* HeLa), consult original references or the supplier of your cell line for the optimal method of transfection. We recommend that you follow exactly the protocol for your cell line. Pay particular attention to medium requirements, when to pass the cells, and at what dilution to split the cells. Further information is provided in *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

Methods for transfection include calcium phosphate (Chen and Okayama, 1987; Wigler *et al.*, 1977), lipid-mediated (Felgner *et al.*, 1989; Felgner and Ringold, 1989) and electroporation (Chu *et al.*, 1987; Shigekawa and Dower, 1988). For high efficiency transfection in a broad range of mammalian cell lines, we recommend using Lipofectamine™ 2000 Reagent (Catalog no. 11668-027) available from Invitrogen. For more information about Lipofectamine™ 2000 and the other transfection reagents available from Invitrogen, visit our Web site (www.invitrogen.com) or contact Technical Service (see page 30).

Creating Stable Cell Lines

Introduction

The pGeneBLAzer-TOPO® vector contains the neomycin resistance gene to allow selection of stable cell lines using Geneticin®. If you wish to create stable cell lines, transfect your construct into the mammalian cell line of choice and select for foci using Geneticin®. General information and guidelines are provided below.

Geneticin®

Geneticin® blocks protein synthesis in mammalian cells by interfering with ribosomal function. It is an aminoglycoside, similar in structure to neomycin, gentamycin, and kanamycin. Expression in mammalian cells of the bacterial aminoglycoside phosphotransferase gene (APH), derived from Tn*5*, results in detoxification of Geneticin® (Southern and Berg, 1982).

Geneticin® Selection Guidelines

Geneticin® is available from Invitrogen (see page x for ordering information). Use as follows:

1. The liquid form of Geneticin® is supplied as a 50 mg/ml stock solution and is ready-to-use. To use the powder form of Geneticin®, prepare a 50 mg/ml stock solution in buffered solution (*e.g.* 100 mM HEPES, pH 7.3).
2. Use 100 to 1000 µg/ml of Geneticin® in complete growth medium.
3. Test varying concentrations of Geneticin® on your cell line to determine the concentration that kills your cells (kill curve). Cells differ in their susceptibility to Geneticin®.

Note: Cells will divide once or twice in the presence of lethal doses of Geneticin®, so the effects of the drug take several days to become apparent. Complete selection can take from 2 to 4 weeks of growth in selective medium.

Generating Stable Cell Lines

Once you have determined the appropriate Geneticin® concentration to use for selection, you can generate a stable cell line expressing your pGeneBLAzer-TOPO® construct.

1. Transfect the mammalian cell line of interest with the pGeneBLAzer-TOPO® construct using your transfection method of choice.
2. 24 hours after transfection, wash the cells and add fresh growth medium.
3. 48 hours after transfection, split the cells into fresh growth medium such that they are no more than 25% confluent. If the cells are too dense, the antibiotic will not kill the cells. Antibiotics work best on actively dividing cells.
4. Incubate the cells at 37°C for 2-3 hours until they have attached to the culture dish.
5. Remove the growth medium and replace with fresh growth medium containing the Geneticin® at the pre-determined concentration required for your cell line.
6. Feed the cells with selective media every 3-4 days until Geneticin®-resistant colonies can be identified.
7. Pick at least 5 Geneticin®-resistant colonies and expand them to assay for recombinant protein expression.

Detecting β-Lactamase Reporter Activity

Introduction

Depending on the kit you are using, you will assay for β-lactamase reporter activity using *in vivo* or *in vitro* detection methods. A brief description of each GeneBLAzer™ Detection Kit is provided below. For detailed information about each kit, refer to the GeneBLAzer™ Detection Kits manual.

***In vitro* Detection**

Using the GeneBLAzer™ *In Vitro* Detection Kit allows you to quantitate the amount of intracellular β-lactamase in cells based on the β-lactamase enzyme activity in lysates.

To detect β-lactamase reporter activity in mammalian cell lysates, you will use the CCF2-FA substrate. CCF2-FA is the non-esterified, free acid form of CCF2, and is recommended for *in vitro* use because it is readily soluble in aqueous solution and may be added directly to pre-made cell lysates. Once added to cell lysates, you may quantitate the fluorescence signal using a fluorescence plate reader or a fluorometer.

To prepare cell lysates from mammalian cells containing the *bla*(M) reporter gene, you must use a method that will preserve the activity of the β-lactamase enzyme. Refer to the GeneBLAzer™ Detection Kits manual for detailed guidelines and protocols to prepare the CCF2-FA stock solution, prepare cell lysates and samples, and detect CCF2-FA fluorescence signal.

***In vivo* Detection**

Using the GeneBLAzer™ *In Vivo* Detection Kit allows you to measure β-lactamase reporter activity in live mammalian cells. Once β-lactamase reporter activity has been measured, cells may be cultured further for use in additional assays or other downstream applications.

To detect β-lactamase reporter activity in live mammalian cells, you will use the CCF2-AM substrate. CCF2-AM is the membrane-permeable, esterified form of CCF2, and is recommended for *in vivo* use because it is non-toxic, lipophilic and readily enters the cell. Once cells are "loaded" with CCF2-AM, you may quantitate fluorescence signal using a variety of methods.

Refer to the GeneBLAzer™ Detection Kits manual for detailed guidelines and protocols to prepare the CCF2-AM stock solution, load cells with CCF2-AM substrate, and detect CCF2 fluorescence signal.

Appendix

Performing the Control Reactions

Introduction

We recommend performing the following control TOPO® Cloning reactions the first time you use the kit to help you evaluate your results. Performing the control reactions involves producing a control PCR product (containing the *lac* promoter and the LacZα protein) using the reagents included in the kit and using this product directly in a TOPO® Cloning reaction. Successful TOPO® Cloning of the control PCR product will yield blue colonies on LB agar plates containing ampicillin and X-gal.

Before Starting

Prepare the following reagents before performing the control reaction:

- 40 mg/ml X-gal in dimethylformamide (Catalog no. 15520-034)
- LB plates containing 100 µg/ml ampicillin and X-gal (two per transformation)

  To add X-gal to previously made agar plates, warm the plate to 37°C. Pipette 40 µl of the 40 mg/ml stock solution onto the plate, spread evenly, and let dry 15 minutes. Protect plates from light.

Producing the Control PCR Product

1. To produce the 500 bp control PCR product containing the *lac* promoter and LacZα, set up the following 50 µl PCR:

Control DNA Template (50 ng)	1 µl
10X PCR Buffer	5 µl
50 mM dNTPs	0.5 µl
Control PCR Primers (0.1 µg/µl each)	2 µl
Sterile Water	40.5 µl
Taq Polymerase (1 unit/µl)	1 µl
Total Volume	50 µl

2. Overlay with 70 µl (1 drop) of mineral oil, if required.
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	60°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 500 bp band should be visible. Proceed to **Control TOPO® Cloning Reactions**, next page.

continued on next page

Performing the Control Reactions, continued

Control TOPO® Cloning Reactions

Use the control PCR product produced on the previous page and pGeneBLAzer-TOPO® to set up two 6 µl TOPO® Cloning reactions as described below.

1. Set up control TOPO® Cloning reactions:

Reagent	"Vector Only"	"Vector + PCR Insert"
Sterile Water	4 µl	3 µl
Salt Solution or Dilute Salt Solution	1 µl	1 µl
Control PCR Product	--	1 µl
TOPO® vector	1 µl	1 µl

2. Incubate at room temperature for **5 minutes** and place on ice.
3. Transform 2 µl of each reaction into separate vials of One Shot® TOP10 cells (page 11).
4. Spread 10-50 µl of each transformation mix onto LB plates containing 100 µg/ml ampicillin and X-Gal. Be sure to plate two different volumes to ensure that at least one plate has well-spaced colonies. To plate small volumes, add 20 µl of S.O.C. Medium to allow even spreading.
5. Incubate overnight at 37°C.

What to Expect

Hundreds of colonies from the vector + PCR insert reaction should be produced. Greater than 85% of these will be blue and contain the 500 bp insert. Very few colonies (<10% of the vector + PCR insert) will be present on the vector only plate. These colonies should be white.

Transformation Control

pUC19 plasmid is included to check the transformation efficiency of the One Shot® TOP10 competent *E. coli*. Transform one vial of One Shot® TOP10 cells with 10 pg of pUC19 using the protocol on page 11. Plate 10 µl of the transformation mixture plus 20 µl S.O.C. Medium on LB plates containing 100 µg/ml ampicillin. Transformation efficiency should be ~1 x 10^9 cfu/µg DNA.

continued on next page

Factors Affecting Cloning Efficiency

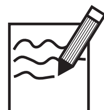
Note that the following variables may influence the cloning efficiency. Most of these are easily corrected, but if you are cloning large inserts, you may not obtain the expected ≥ 85% cloning efficiency.

Variable	Solution
pH>9	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 (>3 kb)	Gel-purify as described on page 23.
Excess (or overly dilute) PCR product	Reduce (or concentrate) the amount of PCR product. Note that you can add up to 4 µl of the PCR reaction to the TOPO® Cloning reaction.
Cloning blunt-ended fragments	Add 3´ A-overhangs by incubating with *Taq* polymerase (see page 25).
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 (see page 23).
PCR product does not contain sufficient 3´ A-overhangs even though you used *Taq* polymerase	Increase the time of the final extension to ensure that the 3´ ends are adenylated. Note that *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).
Size of promoter sequences cloned	For large plasmids, you may have to use electroporation to transform into *E. coli.* **Do not use the chemically competent TOP10 cells included in the kit for electroporation.** Use electrocompetent TOP10 cells (see page x for ordering information).

Gel 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. Refer to *Current Protocols in Molecular Biology*, Unit 2.6 (Ausubel *et al.*, 1994) for the most common protocols. Three simple protocols are provided below.



Note

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 7).

Using the S.N.A.P.™ Gel Purification Kit

The S.N.A.P.™ Gel Purification Kit (Catalog no. K1999-25) allows you to rapidly purify PCR products from regular agarose gels.

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

   Note: Do not use TBE to prepare agarose gels. Borate will interfere 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 sodium iodide solution.
3. Add 1.5 volumes of Binding Buffer.
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.
7. Centrifuge 1 minute at full speed in a microcentrifuge and discard the flow-through.
8. Repeat Step 7.
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 9.

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 9). Be sure to make the gel slice as small as possible for best results.

continued on next page

Low-Melt Agarose Method

If you prefer to use low-melt agarose, use the procedure below. Note that gel purification will result in dilution of your PCR product and a potential loss of cloning efficiency.

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. Add 4 µl of the melted agarose containing your PCR product to the TOPO® Cloning reaction as described on page 9.
6. Incubate the TOPO® Cloning reaction **at 37°C for 5 to 10 minutes**. This is to keep the agarose melted.
7. Transform 2 to 4 µl directly into One Shot® TOP10 chemically competent *E. coli* using the procedure on page 11.

Adding 3´ A-Overhangs Post-Amplification

Introduction

Direct cloning of DNA amplified by a thermostable, proofreading polymerase such as Platinum® *Pfx* DNA Polymerase into TOPO TA Cloning® vectors is often difficult because of very low cloning efficiencies. These low efficiencies are caused by the 3´ to 5´ exonuclease activity associated with proofreading polymerases which removes the 3´ A-overhangs necessary for TA Cloning®. A simple method is provided below 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 (optional)
- 3 M sodium acetate (optional)
- 100% ethanol (optional)
- 80% ethanol (optional)
- TE buffer (optional)

Procedure

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

1. After amplification with a proofreading 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. Proceed to TOPO® Cloning (see page 9).

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.

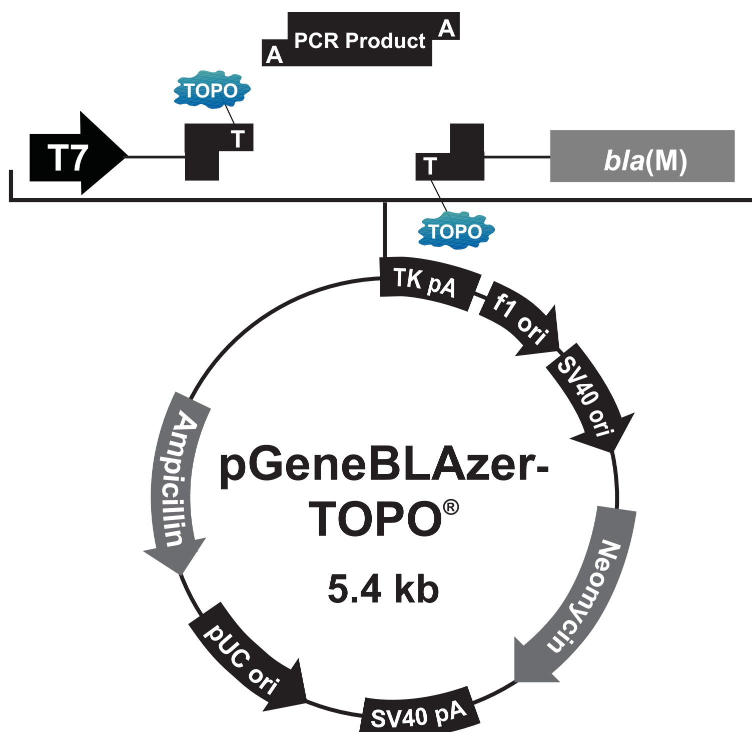
Note

You may also gel-purify your PCR product after amplification with proofreading polymerase (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.

Map and Features of pGeneBLAzer-TOPO®

Map

The figure below summarizes the features of the pGeneBLAzer-TOPO® vector. The vector is supplied linearized between base pairs 116 and 117. This is the TOPO® Cloning site. **The complete nucleotide sequence is available for downloading from our Web site (www.invitrogen.com) or from Technical Service (see page 30).**



Comments for pGeneBLAzer-TOPO®
5380 nucleotides

T7 promoter/priming site: bases 17-36
TOPO® Cloning site: bases 116-117
β-lactamase *bla*(M) reporter gene: bases 138-932
TK polyadenylation signal: bases 940-1210
f1 origin: bases 1247-1675
SV40 early promoter and origin: bases 1702-2010
Neomycin resistance gene: bases 2085-2879
SV40 early polyadenylation signal: bases 3053-3183
pUC origin (c): bases 3566-4239
Ampicillin resistance gene (c): bases 4384-5244
Ampicillin promoter (c): bases 5245-5343

(c) = complementary strand

continued on next page

Features of the Vector

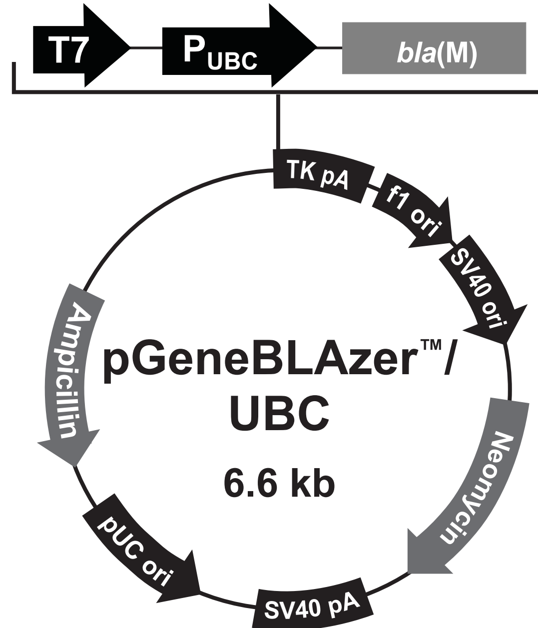
The pGeneBLAzer-TOPO® vector (5380 bp) contains the following elements. All features have been functionally tested.

Feature	Benefit
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation and sequencing through the insert.
TOPO® Cloning site	Allows insertion of your PCR product containing the promoter of interest upstream of the *bla*(M) reporter gene.
bla(M) reporter gene	Allows assay of promoter function either *in vitro* or *in vivo*.
Herpes Simplex Virus thymidine kinase (TK) polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA (Cole and Stacy, 1985).
f1 origin	Allows single-stranded DNA rescue.
SV40 early promoter and origin	Allow efficient, high-level expression of the neomycin resistance gene and episomal replication in cells expressing the SV40 large T antigen.
Neomycin resistance gene	Allows selection of stable transfectants in mammalian cells (Southern and Berg, 1982).
SV40 polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA.
pUC origin	Allows high-copy number replication and maintenance in *E. coli*.
Ampicillin resistance gene	Allows selection of transformants in *E. coli*.

Map of pGeneBLAzer™/UbC

Description

pGeneBLAzer™/UbC (6620 bp) is a control vector containing the human ubiquitin C (UbC) promoter (Nenoi *et al.*, 1996; Wulff *et al.*, 1990) upstream of the *bla*(M) reporter gene. The vector was constructed by amplifying a DNA fragment containing the UbC promoter and TOPO® Cloning the PCR product into pGeneBLAzer-TOPO®. **The complete nucleotide sequence for pGeneBLAzer™/UbC is available for downloading from our Web site (www.invitrogen.com) or from Technical Service (see page 30).**



Comments for pGeneBLAzer™/UBC
6620 nucleotides

T7 promoter/priming site: bases 17-36
UbC promoter: bases 129-1347
β-lactamase *bla*(M) reporter gene: bases 1378-2172
TK polyadenylation signal: bases 2180-2450
f1 origin: bases 2487-2915
SV40 early promoter and origin: bases 2942-3250
Neomycin resistance gene: bases 3325-4119
SV40 early polyadenylation signal: bases 4293-4423
pUC origin (c): bases 4806-5479
Ampicillin resistance gene (c): bases 5624-6484
Ampicillin promoter (c): bases 6485-6583

(c) = complementary strand

Recipes

LB (Luria-Bertani) Medium and Plates

1.0% Tryptone
0.5% Yeast Extract
1.0% NaCl
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. Autoclave on liquid cycle for 20 minutes at 15 psi. Allow solution to cool to 55°C and add 100 μg/ml ampicillin, if needed.
4. Store at room temperature or at +4°C.

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 100 μg/ml of ampicillin, and pour into 10 cm plates.
4. Let harden, then invert and store at +4°C, in the dark.

Technical Service

World Wide Web

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

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

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

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E-mail: eurotech@invitrogen.com

MSDS Requests

To request an MSDS, visit our Web site at www.invitrogen.com. On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

continued on next page

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Introduction

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Product Qualification

Introduction

The pGeneBLAzer™ TOPO® TA Expression Kits are qualified as described below.

Vectors

The pGeneBLAzer™ (parental vector of pGeneBLAzer-TOPO®) and pGeneBLAzer™/UbC plasmids are qualified by restriction analysis. The pGeneBLAzer™ vector is qualified by restriction digest prior to adaptation with topoisomerase I. Restriction digests must demonstrate the correct banding pattern when electrophoresed on an agarose gel.

TOPO® Cloning Efficiency

Once adapted with topoisomerase I, the pGeneBLAzer™ vector is lot-qualified using the control reagents included in the kit. Under conditions described on pages 20-22, a 750 bp control PCR product is TOPO® Cloned into pGeneBLAzer-TOPO® and subsequently transformed into the One Shot® TOP10 chemically competent *E. coli* included with the kit.

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

Primers

Primers are lot-qualified by DNA sequencing experiments using the dideoxy chain termination technique.

One Shot® TOP10 Chemically Competent *E. coli*

All competent cells are tested for transformation efficiency using the control plasmid included in the One Shot® kit. 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 greater than 1 x 10^9 cfu/μg plasmid DNA.

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

GeneBLAzer™ Detection Kits

For information about how the GeneBLAzer™ *In Vitro* Detection Kit and the GeneBLAzer™ *In Vivo* Detection Kit are qualified, refer to the GeneBLAzer™ Detection Kits manual.

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