DES® TOPO®
TA Expression Kit

Version F
100301
25-0211

DES® TOPO® TA Expression Kit

For 5 minute cloning and inducible expression of PCR products in *Drosophila* S2 cells

Catalog no. K4125-01, K4125-40

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Table of Contents

Important Information

Shipping and Storage

The DES® TOPO® TA Expression Kit is shipped on dry ice. Each kit contains a box with DES® TOPO® TA Cloning reagents (Box 1) and a box with One Shot® TOP10 chemically competent cells (Box 2).

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

DES® TOPO® TA Expression Kits

Ordering information for the DES® TOPO® TA Expression Kits is provided below.

Kit	Reactions	Catalog no.
DES® TOPO® TA Expression Kit	20	K4125-01
	40	K4125-40

DES® TOPO® TA Cloning Reagents

DES® TOPO® TA Cloning reagents (Box 1) are listed below. Please note that *Taq* polymerase must be supplied by the user. **Store Box 1 at -20°C.**

Item	Concentration	Amount
pMT/V5-His-TOPO® vector	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

continued on next page

DES® TOPO® TA Cloning Reagents, continued

Item	Concentration	Amount
MT Forward Sequencing Primer	0.1 µg/µl in TE Buffer, pH 8	20 µl
BGH Reverse 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
Expression Control Plasmid pMT/*lac*Z	0.5 µg/µl in TE Buffer, pH 8	10 µl

One Shot® TOP10 Reagents

The table below describes the items included in the One Shot® TOP10 chemically competent cell kit. **Store at -80°C.**

Item	Composition	Amount
SOC 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

Sequencing Primers

The table below lists the sequence and amount of the primers included in the DES® TOPO® TA Expression Kit. If you wish to order additional primers, please see **Additional Reagents**, next page.

Primer	Sequence	Amount
MT Forward	5′-CATCTCAGTGCAACTAAA-3′	368 pmoles
BGH Reverse	5′-TAGAAGGCACAGTCGAGG-3′	358 pmoles

Genotype of TOP10 Cells

TOP10: Use this strain for general cloning. 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 *deo*R *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L (Str^R) *end*A1 *nup*G

Accessory Products

Introduction

The products listed in this section are intended for use with the DES® TOPO® TA Expression Kit. For more information, please refer to our World Wide Web site (www.invitrogen.com) or call Technical Service (see page 25).

Additional Reagents

Many of the reagents supplied in the DES® TOPO® TA Expression Kit are available separately from Invitrogen. Ordering information for these reagents is provided below.

Item	Amount	Catalog no.
One Shot® Kit (TOP10 **Chemically Competent** Cells)	10 reactions	C4040-10
	20 reactions	C4040-03
MT Forward Primer	2 µg (368 pmoles)	N560-02
BGH Reverse Primer	2 µg (358 pmoles)	N575-02

Additional Products

The table below lists additional products available from Invitrogen which you may use in conjunction with the DES® TOPO® TA Expression Kit. Please note that the DES® kits include your choice of selection vector (pCoHygro or pCoBlast) needed to generate stable cell lines.

Item	Amount	Catalog no.
DES® Constitutive Kit *with pCoHygro*	1 kit	K4110-01
DES® Constitutive Kit *with pCoBlast*	1 kit	K5110-01
DES® Inducible Kit *with pCoHygro*	1 kit	K4120-01
DES® Inducible Kit *with pCoBlast*	1 kit	K5120-01
DES® Inducible/Secreted Kit *with pCoHygro*	1 kit	K4130-01
DES® Inducible/Secreted Kit *with pCoBlast*	1 kit	K5130-01
DES® Blasticidin Support Kit	1 kit	K5150-01
Schneider (S2) Cells	1 ml vial, 1 x 10^7 cells	R690-07
Schneider's *Drosophila* Medium	500 ml	11720-034
Calcium Phosphate Transfection Kit	75 reactions	K2780-01
Hygromycin-B	1 gram	R220-05
Blasticidin S HCl	50 mg	R210-01
One Shot® Kit (TOP10 **Electrocompetent** Cells)	10 reactions	C4040-50
S.N.A.P.™ MidiPrep Kit	20 reactions	K1910-01
β-Gal Assay Kit	1 kit	K1455-01
β-Gal Staining Kit	1 kit	K1465-01

continued on next page

Accessory Products, continued

Detection of Recombinant Proteins

Expression of your recombinant fusion protein can be detected using an antibody to the appropriate epitope. The table below describes the antibodies available for detection of C-terminal fusion proteins expressed using pMT/V5-His-TOPO®. Horseradish peroxidase (HRP) or alkaline phosphatase (AP)-conjugated antibodies allow one-step detection using colorimetric or chemiluminescent detection methods.

The amount of antibody supplied is sufficient for 25 westerns.

Product	Epitope	Catalog no.
Anti-V5 Antibody	Detects 14 amino acid epitope derived from the P and V proteins of the paramyxovirus, SV5 (Southern *et al.*, 1991) GKPIPNPLLGLDST	R960-25
Anti-V5-HRP Antibody		R961-25
Anti-V5-AP Antibody		R962-25
Anti-His (C-term) Antibody	Detects the C-terminal polyhistidine (6xHis) tag (requires the free carboxyl group for detection (Lindner *et al.*, 1997) HHHHHH-COOH	R930-25
Anti-His(C-term)-HRP Antibody		R931-25
Anti-His(C-term)-AP Antibody		R932-25

Purification of Recombinant Protein

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

Product	Quantity	Catalog no.
ProBond™ Purification System (includes six 2 ml precharged, prepacked ProBond™ resin columns and buffers for native and denaturing purification)	6 purifications	K850-01
ProBond™ Purification System with Anti-V5-HRP Antibody	1 kit	K854-01
ProBond™ Purification System with Anti-His(C-term)-HRP Antibody	1 kit	K853-01
ProBond™ Metal-Binding Resin	50 ml	R801-01
(precharged resin provided as a 50% slurry in 20% ethanol)	150 ml	R801-15
Purification Columns (10 ml polypropylene columns)	50	R640-50

Introduction

Overview

Introduction

The DES® TOPO® TA Expression Kit uses TOPO® Cloning technology to facilitate rapid cloning of *Taq* polymerase-amplified PCR products into the pMT/V5-His-TOPO® vector for transient or stable inducible expression of the gene of interest in *Drosophila* S2 cells. TOPO® Cloning requires no ligase, post-PCR procedures, or PCR primers containing special, additional sequences. For more information about TOPO® Cloning, please see the next page.

pMT/V5-His-TOPO® Vector

pMT/V5-His-TOPO® is a 3.6 kb expression vector designed to facilitate rapid cloning and expression of PCR products and is intended for use with the *Drosophila* Expression System (DES®, Catalog nos. K4110-01, K4120-01, K4130-01, K5110-01, K5120-01, and K5130-01) available from Invitrogen. Upon transfection, the vector allows transient inducible expression of your protein of interest in *Drosophila* cells. When cotransfected with the selection vectors, pCoHygro or pCoBlast, pMT/V5-His-TOPO® allows selection of stable cell lines exhibiting inducible expression of the protein of interest. The pMT/V5-His-TOPO® vector contains the following elements:

- The *Drosophila* metallothionein (MT) promoter for high-level, metal-inducible expression of the gene of interest in S2 cells (Angelichio *et al.*, 1991; Bunch *et al.*, 1988; Maroni *et al.*, 1986; Olsen, 1992)
- TOPO® Cloning site for rapid and efficient cloning of *Taq*-amplified PCR products (see the next page for more information)
- C-terminal peptide containing the V5 epitope and a polyhistidine (6xHis) tag for detection and purification of recombinant protein
- Ampicillin resistance gene for selection of transformants in *E. coli*

The control plasmid, pMT/*lacZ*, is included for use as a positive control for transfection and expression.

For more information about the DES® kits, pCoHygro, and pCoBlast, please refer to the *Drosophila* Expression System manual. The *Drosophila* Expression System manual is supplied with each DES® kit, but is also available for downloading from our World Wide Web site (www.invitrogen.com) or by contacting Technical Service (see page 25).

Description of MT Promoter

The *Drosophila* MT promoter allows high-level, inducible expression of the gene of interest in *Drosophila* S2 cells. When used to express heterologous proteins, the promoter is extremely efficient and tightly regulated, even at high copy number (Johansen *et al.*, 1989). The MT promoter is well characterized (Angelichio *et al.*, 1991; Bunch *et al.*, 1988; Maroni *et al.*, 1986; Olsen, 1992), with regulatory elements and the start of transcription well defined.

The MT promoter is inducible by addition of copper sulfate or cadmium chloride to the culture medium (Bunch *et al.*, 1988). Copper sulfate is generally the preferred inducer due to its reduced toxicity as compared to cadmium. While cadmium is an effective inducer, it also induces a heat-shock response in S2 cells.

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Overview, continued

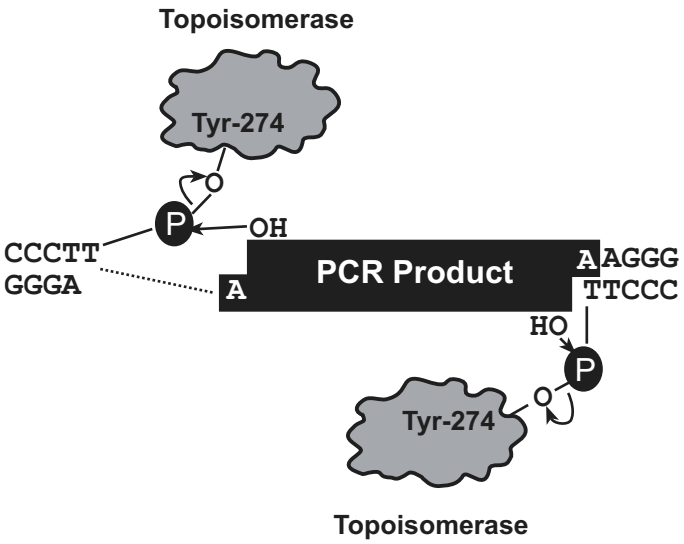
How TOPO® Cloning Works

The plasmid vector, pMT/V5-His-TOPO®, is supplied linearized with:

- Single 3´ thymidine (T) overhangs for TA Cloning®
- Topoisomerase 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´ deoxythimidine (T) residues. This allows PCR inserts to ligate efficiently with the vector.

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 (see below).



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Overview, continued

Experimental Outline

The table below describes the general steps needed to clone and express your gene of interest. For more details, please refer to the manual and pages indicated.

Step	Action	Source
1	Design PCR primers to clone your gene of interest in frame with the C-terminal peptide containing the V5 epitope and the polyhistidine (6xHis) tag (if desired). Consult the diagram on page 5 to help you design your PCR primers.	Pages 4-5, this manual
2	Produce your PCR product.	Page 6, this manual
3	TOPO® Clone your PCR product into pMT/V5-His-TOPO® and transform into One Shot® TOP10 *E. coli*. Select transformants on LB plates containing 50-100 μg/ml ampicillin.	Pages 7-10, this manual
4	Analyze your transformants for the presence and orientation of insert by restriction digestion.	Page 10, this manual
5	Select a transformant with the correct restriction pattern and sequence it to confirm that your gene is cloned in frame with the C-terminal peptide.	Page 10, this manual
6	Transfect your pMT/V5-His-TOPO® construct into S2 cells and induce expression of the gene of interest with copper sulfate.	Page 13, this manual and DES® manual
7	Assay for transient expression of your recombinant protein.	Page 13, this manual and DES® manual
8	To generate stable cell lines, cotransfect your pMT/V5-His-TOPO® construct and pCoHygro or pCoBlast into S2 cells and select for hygromycin resistant clones.	DES® manual
9	Scale up expression for purification.	DES® manual
10	Purify your recombinant protein by chromatography on metal-chelating resin (i.e. ProBond™).	DES® manual

Methods

PCR Primer Design

Introduction

It is important to properly design your PCR primers to ensure that you obtain the recombinant protein you need for your studies. Please use the information below and the diagram on page 5 to design your PCR primers. **Remember that your PCR product will have single 3′ adenine overhangs.**

Note

Do not add 5′ phosphates to your primers for PCR. The PCR product synthesized will not ligate into pMT/V5-His-TOPO®.

General Molecular Biology Techniques

For help with *E. coli* transformations, restriction enzyme analysis, DNA sequencing, and DNA biochemistry, please refer to *Molecular Cloning: A Laboratory Manual* (Sambrook *et al.*, 1989) or *Current Protocols in Molecular Biology* (Ausubel *et al.*, 1994).

Translation Initiation

Your PCR product should contain a Kozak translation initiation sequence and an ATG start codon for proper initiation of translation (Kozak, 1987; Kozak, 1991; Kozak, 1990). You may need to design a Kozak sequence into your forward PCR primer. An example of a Kozak consensus sequence is provided below. Please note that other sequences are possible, but the G or A at position –3 and the G at position +4 are the most critical for function (shown in bold). The ATG start codon is shown underlined.

(**G/A**)NN<u>ATG</u>**G**

Fusion to the C-terminal Peptide

If you wish to include the C-terminal peptide for detection with either the V5 or His(C-term) antibodies or purification using the polyhistidine (6xHis) tag, you must design your reverse PCR primer to remove the native stop codon and maintain the frame through the DNA encoding the C-terminal peptide.

If you do not wish to include the C-terminal peptide, include the native stop codon in the reverse PCR primer or design the primer to anneal downstream of the native stop codon.

Note: Cloning efficiencies may vary depending on the 5′ nucleotide sequence of your primer (see page 21).

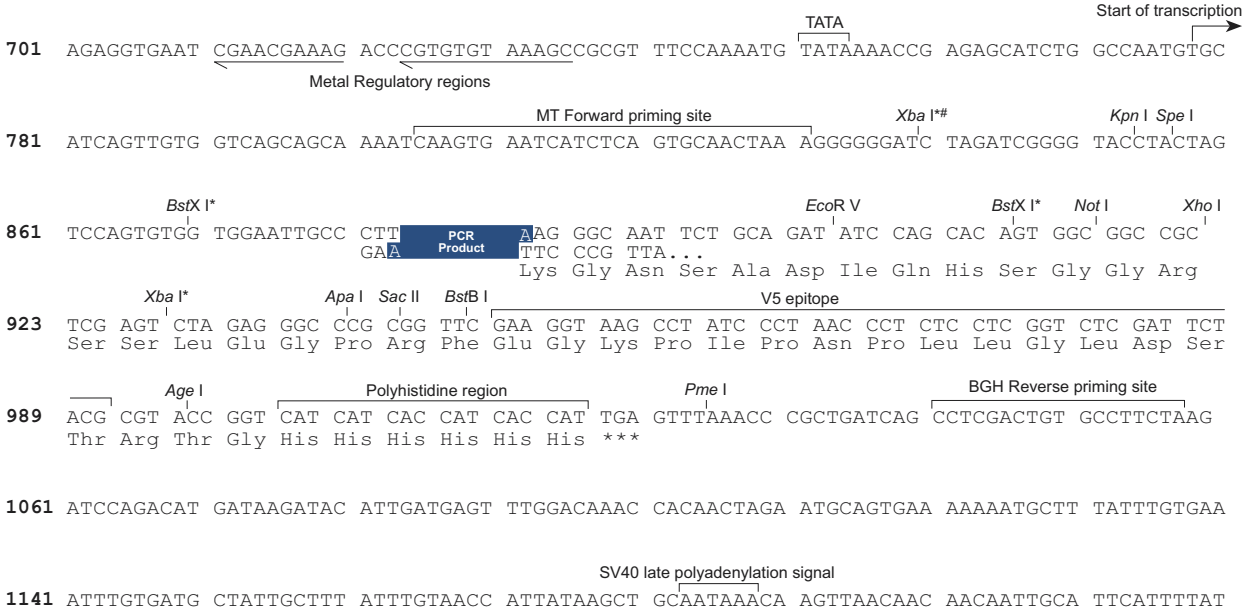
Use the diagram on the next page to design your PCR primers. Once you have designed your PCR primers, proceed to page 6.

continued on next page

PCR Primer Design, continued

TOPO Cloning® Site of pMT/V5-His-TOPO®

The diagram below is supplied to help you design appropriate PCR primers to correctly clone and express your PCR product using pMT/V5-His-TOPO®. Restriction sites are labeled to indicate the actual cleavage site. The vector is supplied linearized between base pair 883 and 884. This is the TOPO® Cloning site. **The complete sequence of pMT/V5-His-TOPO® may be downloaded from our World Wide Web site (www.invitrogen.com) or requested from Technical Service (see page 25).** For a map and a description of the features of pMT/V5-His-TOPO®, please refer to the Appendix, pages 22-23.



*Please note that there are two *Bst*X I sites and two *Xba* I sites in the polylinker.

#This *Xba* I site is *dam* methylated in some *E. coli* strains.

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.

- *Taq* polymerase
- Thermocycler
- DNA template and primers for PCR product

Polymerase Mixtures

If you wish to use a mixture containing *Taq* polymerase and a proofreading polymerase, *Taq* must be used in excess of a 10:1 ratio to ensure the presence of 3′ A-overhangs on the PCR product (i.e. Expand™ or eLONGase™).

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 and be sure to end with a 7 to 30 minute extension at 72°C 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)	1 µM 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, please 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 using the DES® TOPO® TA Expression Kit (see pages 16-17). Take special care to avoid sources of nuclease contamination and long exposure to UV light. Alternatively, you may elect to optimize your PCR to eliminate multiple bands and smearing (Innis *et al.*, 1990). The PCR Optimizer™ Kit (Catalog no. K1220-01) available from Invitrogen can help you optimize your PCR. Call Technical Service (see page 25) for more information.

TOPO® Cloning Reaction and Transformation

Introduction

TOPO® Cloning technology allows you to produce your PCR products, ligate them into pMT/V5-His-TOPO® (or any 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 you obtain the best possible results. If this is the first time you have TOPO® Cloned, you may wish to perform the control reactions on pages 19-20 in parallel with your samples. If you have previously TOPO® Cloned, read the **Note** below.

Note

Recent experiments at Invitrogen demonstrate that inclusion of salt (200 mM NaCl, 10 mM $MgCl_2$) in the TOPO® Cloning reaction results in the following:

- A 2- to 3-fold increase in the number of transformants.
- Allows for longer incubation times (up to 30 minutes). Longer incubation times can result in an increase in the number of transformants obtained.

Including salt in the TOPO® Cloning reaction 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.

If you do **not** include salt in the TOPO® Cloning reaction, the number of transformants obtained generally decreases as the incubation time increases beyond 5 minutes.

Important

Because of the above results, we recommend adding salt to the TOPO® Cloning reaction. A stock salt solution is provided in the kit for this purpose. **Please 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 below).** For this reason two different TOPO® Cloning reactions are provided to help you obtain the best possible results. Please read the following information carefully.

Chemically Competent *E. coli*

For TOPO® Cloning and transformation into chemically competent *E. coli*, adding sodium chloride and magnesium chloride to a final concentration of 200 mM NaCl, 10 mM $MgCl_2$ in the TOPO® Cloning reaction increases the number of colonies over time. A Salt Solution (1.2 M NaCl, 0.06 M $MgCl_2$) is provided to adjust the TOPO® Cloning reaction to the recommended concentration of NaCl and $MgCl_2$.

Electrocompetent *E. coli*

For TOPO® Cloning and transformation of electrocompetent *E. coli*, salt must also be included in the TOPO® Cloning reaction, but the amount of salt **must be reduced** to 50 mM NaCl, 2.5 mM $MgCl_2$ to prevent arcing when electroporating. The Salt Solution provided in the kit must be diluted 4-fold to prepare a 300 mM NaCl, 15 mM $MgCl_2$ solution for convenient addition to the TOPO® Cloning reaction (see next page).

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TOPO® Cloning Reaction and Transformation, continued

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 50-100 μg/ml ampicillin (two for each transformation)
- Reagents and equipment for agarose gel electrophoresis
- 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 sequenced to confirm the presence and orientation of the insert. The MT Forward and BGH Reverse Sequencing primers included with the kit can be used to sequence across the insert in the multiple cloning site to confirm orientation.

Preparation 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*.
- For electroporation, dilute a small portion of the Salt Solution 4-fold to prepare Dilute Salt Solution (e.g. add 5 μl of the Salt Solution to 15 μl sterile water).
- Warm the vial of SOC medium from Box 2 to room temperature.
- Warm selective plates at 37°C for 30 minutes.
- Thaw **on ice** 1 vial of One Shot® cells for each transformation.

Setting Up the TOPO® Cloning Reaction

The table below describes how to set up your TOPO® Cloning reaction (6 μl) for eventual transformation into either chemically competent One Shot® TOP10 *E. coli* (provided) or electrocompetent *E. coli*. Additional information on optimizing the TOPO® Cloning reaction for your needs can be found on page 12.

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

Reagent*	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 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

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

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TOPO® Cloning Reaction and Transformation, continued

Performing the TOPO® Cloning Reaction

1. Mix reaction gently and incubate for 5 minutes at room temperature (22-23°C).

   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 **One Shot® Chemical Transformation** (below) or **Transformation by Electroporation** (below). **Note**: You may store the TOPO® Cloning reaction at -20°C overnight.

One Shot® Chemical Transformation

1. Add 2 µl of the TOPO® Cloning reaction from Step 2 above 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 (see above).

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 (200 rpm) at 37°C for 1 hour.
7. Spread 25-200 µl from each transformation on a prewarmed selective plate and incubate overnight at 37°C. We recommend that you plate 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 **Analysis of Positive Clones**, next page).

Transformation by Electroporation

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

   Note: If you have problems with arcing, see next page.

3. Immediately add 250 µl of room temperature SOC 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 gene.
5. Spread 10-50 µl from each transformation on a prewarmed selective plate and incubate overnight at 37°C. To ensure even spreading of small volumes, add 20 µl of SOC. We recommend that you plate 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 **Analysis of Positive Clones**, next page).

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TOPO® Cloning Reaction and Transformation, continued

Note

Addition of the Dilute Salt Solution in the TOPO® Cloning Reaction brings the final concentration of NaCl and $MgCl_2$ in the TOPO® Cloning reaction to 50 mM and 2.5 mM, respectively. 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 suggestions:

- 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

Analysis of Positive Clones

1. Culture 10 transformants overnight in 2-5 ml LB or SOB medium containing 50-100 µg/ml ampicillin.
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 (Catalog no. K1910-01).
3. Analyze the plasmids for the presence and orientation of insert by restriction analysis. We recommend sequencing your constructs to confirm that your gene of interest is cloned in frame with the C-terminal peptide. Sequencing primers are included to help you sequence your insert (see page vi). Please refer to the diagram on page 5 for the sequence surrounding the TOPO® Cloning site.

   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. You may use either the forward **or** reverse sequencing primers included in the kit **and** a primer that hybridizes within your insert. You will have to determine the amplification conditions.

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. Both 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.

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. 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 using the appropriate conditions (see text above).
5. For the final extension, incubate at 72°C for 10 minutes. Hold at +4°C.
6. Visualize by agarose gel electrophoresis.

continued on next page

TOPO® Cloning Reaction and Transformation, continued

Important

If you have problems obtaining transformants or the correct insert, perform the control reactions described on pages 19-20. These reactions will help you troubleshoot your experiment.

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. We recommend that you also store the purified plasmid DNA at –20°C.

1. Streak the original colony on LB plates containing 50-100 µg/ml ampicillin. Incubate the plate overnight at 37°C.
2. Isolate a single colony and inoculate into 1-2 ml of LB containing 50-100 µg/ml ampicillin.
3. Grow the culture to mid-log phase (OD_{600} = 0.5-0.7).
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

The information below will help you optimize the TOPO® Cloning reaction for your particular needs.

Faster Subcloning

The high efficiency of TOPO® Cloning technology 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

Transfection and Analysis

Introduction

Once you have cloned your gene of interest into pMT/V5-His-TOPO® and have prepared purified plasmid DNA, you are ready to transfect your construct into *Drosophila* S2 cells. If you are assaying for transient, inducible expression of your gene of interest, you may transfect your pMT/V5-His-TOPO® construct alone into S2 cells. If you wish to generate stable cell lines, you **must** cotransfect your pMT/V5-His-TOPO® construct with pCoHygro or pCoBlast into S2 cells. Please note that the pMT/V5-His-TOPO® vector does not contain a resistance marker for selection in *Drosophila* cells. We recommend that you include the pMT/*lacZ* positive control vector (see below) and a mock transfection (negative control) in your experiments to evaluate your results. Specific guidelines and protocols for culturing S2 cells, performing transient transfection, and generation of stable cell lines can be found in the DES® manual.

Note: The DES® kits include your choice of pCoHygro or pCoBlast. For detailed information about pCoHygro and pCoBlast, please refer to the DES® manual.

Plasmid Preparation

Plasmid DNA for transfection into S2 cells must be very clean and free from phenol and sodium chloride. Contaminants will kill the cells, decreasing transfection efficiency. We recommend isolating plasmid DNA using the S.N.A.P.™ MiniPrep Kit (10-15 µg DNA, Catalog no. K1900-01), the S.N.A.P.™ MidiPrep Kit (10-200 µg DNA, Catalog no. K1910-01), or CsCl gradient centrifugation.

Positive Control

pMT/*lac*Z is provided as a positive control vector for transfection and expression in S2 cells (see page 24 for a map) and may be used to optimize transfection conditions for S2 cells. Transfection of pMT/*lacZ* into S2 cells results in induction of β-galactosidase expression upon addition of copper sulfate. A successful transfection will result in β-galactosidase expression that can be easily assayed (see below).

Assay for β-galactosidase Activity

You may assay for β-galactosidase expression by activity assay using cell-free lysates (Miller, 1972) or by staining the cells for activity. Invitrogen offers the β-Gal Assay Kit (Catalog no. K1455-01) and the β-Gal Staining Kit (Catalog no. K1465-01) for fast and easy detection of β-galactosidase expression.

Induction of Recombinant Protein Expression

Once you have transfected your pMT/V5-His-TOPO® construct into S2 cells, you will use copper sulfate to induce expression of your recombinant protein. In general, we recommend that you add copper sulfate directly to the culture medium to a final concentration of 500 µM and incubate the cells for 24 hours to obtain maximal induction of your recombinant protein of interest. Please note that expression conditions may vary depending on the nature of the recombinant protein of interest, therefore, you may want to perform a time course to optimize expression of the recombinant protein. For more details, please refer to the DES® manual. Copper sulfate is provided in the DES® kit.

continued on next page

Detection and Purification of Recombinant Fusion Protein

You may use a functional assay to detect the protein encoded by your PCR product or Western blot analysis if you have an antibody to the protein. If you have elected to express your PCR product as a fusion to the V5 epitope and the polyhistidine tag, you may use the Anti-V5 antibodies or Anti-His(C-term) antibodies available from Invitrogen (see page viii for ordering information) to detect the fusion protein. If you wish, the fusion protein may be purified using metal-chelating resins including ProBond™. For specific guidelines on expression and purification of fusion proteins, please refer to the DES® manual and/or the ProBond™ Purification System manual.

Note

The C-terminal peptide containing the V5 epitope and the polyhistidine region will add approximately ~5 kDa to the size of your protein.

Appendix

Recipes

LB (Luria-Bertani) Medium and Plates

Composition:

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 antibiotic (50-100 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 antibiotic (50 µg/ml of ampicillin), and pour into 10 cm plates.
4. Let harden, then invert and store at +4°C, in the dark.

X-Gal Stock Solution

1. To prepare a 40 mg/ml stock solution, dissolve 400 mg X-Gal in 10 ml dimethyl-formamide.
2. Protect from light by storing in a brown bottle at -20°C.
3. To add 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.

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 (Ausubel *et al.*, 1994) for the most common protocols. Three simple protocols are provided below for your convenience.

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 6).

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. Borate will interfere with the NaI step (Step 2.)
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 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 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 (see page 8). 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 prefer to use low-melt agarose, use the procedure below. Please note that the gel purification will result in a 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 8.
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 chemically competent One Shot® TOP10 cells using the method on page 9.

Addition of 3´ A-Overhangs Post-Amplification

Introduction

Direct cloning of DNA amplified by *Vent*® or *Pfu* polymerases into TOPO® Cloning vectors is often difficult because of very low cloning efficiencies. These low efficiencies are caused by the 3′ to 5′ exonuclease activity, which removes the 3′ A-overhangs necessary for TOPO® Cloning. Invitrogen has developed a simple method to clone these blunt-ended fragments.

Before Starting

You will need the following items:

- *Taq* polymerase
- 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 *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. The DNA amplification product is now ready for ligation into pMT/V5-His-TOPO®.

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.

DES® TOPO® Cloning 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 using the reagents included in the kit involves producing a control PCR product containing the *lac* promoter and the LacZα protein. Successful TOPO® Cloning of the control PCR product in either orientation will yield blue colonies on LB agar plates containing ampicillin and X-gal.

Before Starting

Be sure to prepare LB plates containing 50-100 µg/ml ampicillin **and X-gal** (see page 15 for recipe) before performing the control reaction.

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 PCR Template (50 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	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 the **Control TOPO® Cloning Reactions**, next page.

continued on next page

DES® TOPO® Cloning Control Reactions, continued

Control TOPO® Cloning Reactions

Using the control PCR product produced on the previous page and the pMT/V5-His-TOPO® vector, 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 9).
4. Spread 10-50 µl of each transformation mix onto LB plates containing 50-100 µg/ml ampicillin and X-gal (see page 15). **Note**: No IPTG is required. We recommend that you plate two different volumes to ensure that the colonies are well-spaced. For plating smaller 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' control should be produced (foreground). Greater than 85% of these will be blue and contain the 500 bp insert.

The 'Vector Only' control (background) should produce very few colonies (<15% of the vector + PCR insert plate) and these should all be white.

Transformation Control

pUC19 plasmid is included to check the transformation efficiency of the One Shot® TOP10 competent cells. Transform one vial of One Shot® TOP10 cells with 10 pg of pUC19 DNA using the protocol on page 9. Plate 10 µl of the transformation mixture on LB plates containing 50 µg/ml ampicillin. The transformation efficiency should be ~1 x 10^9 cfu/µg DNA.

continued on next page

DES® TOPO® Cloning Control Reactions, continued

Factors Affecting Cloning Efficiency

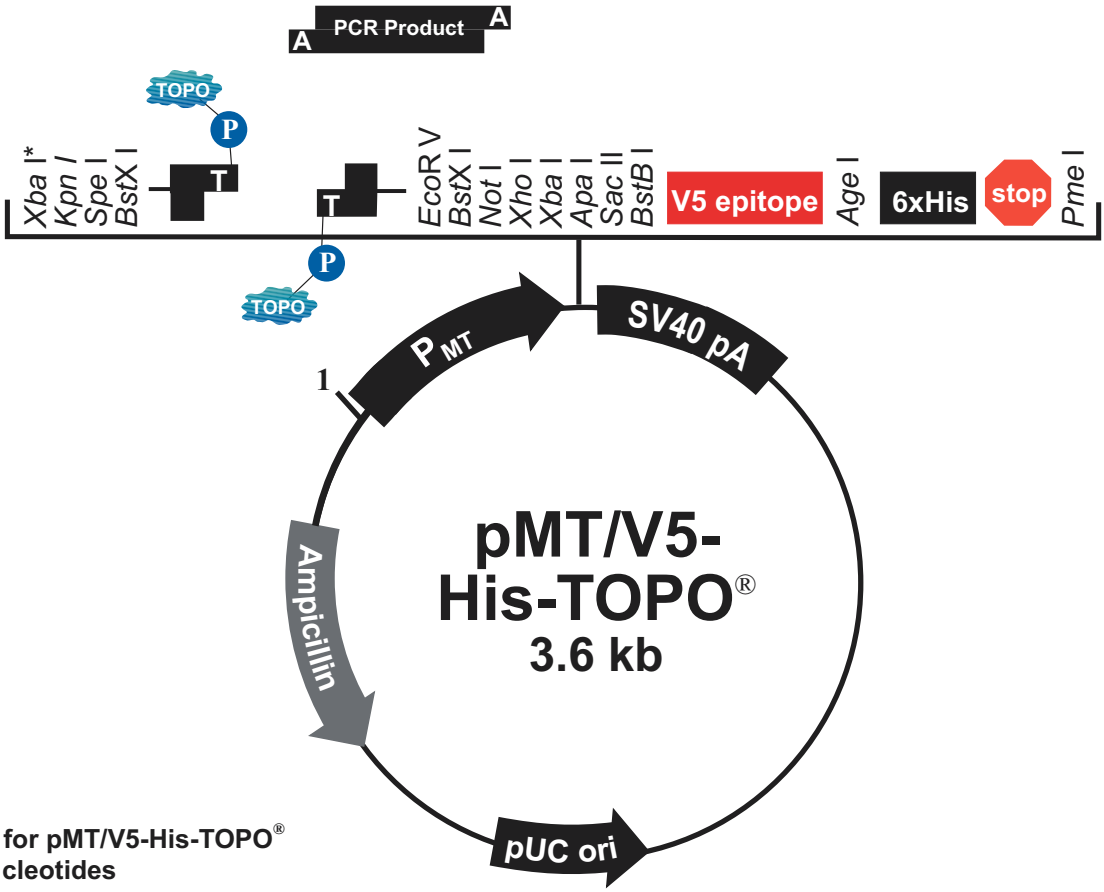
Please note that lower cloning efficiencies will result from the following variables. Most of these are easily corrected, but if you are cloning large inserts, you may not obtain the expected 85% (or more) cloning efficiency.

Variable	Solution
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 at 72°C during PCR. Longer PCR products will need a longer extension time.
Cloning large inserts (>3 kb)	Increase amount of insert. Or gel purify as described on pages 16-17.
Excess (or overly dilute) PCR product	Reduce (or concentrate) the amount of PCR product. Please note that you may add up to 4 µl of your PCR reaction to the TOPO® Cloning reaction (page 8).
Cloning blunt-ended fragments	Add 3´ A-overhangs by incubating with *Taq* polymerase (page 18).
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 (pages 16-17) or optimize your PCR. If your template DNA carries an ampicillin marker, carryover into the TOPO® Cloning reaction from the PCR may lead to false positives. Linearize the template DNA prior to PCR to eliminate carryover.
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).

pMT/V5-His-TOPO® Vector

Map

The figure below summarizes the features of the pMT/V5-His-TOPO® vector. The vector is supplied linearized between base pairs 883 and 884. This is the TOPO® Cloning site. For a more detailed explanation of the features of the vector, please see the next page. **The complete nucleotide sequence is available for downloading from our World Wide Web site (www.invitrogen.com) or from Technical Service (page 25).**



Comments for pMT/V5-His-TOPO®
3558 nucleotides

Metallothionein promoter: bases 406-833
Start of transcription: base 778
MT Forward priming site: bases 814-831
TOPO® Cloning Site: bases 883-884
V5 epitope tag: bases 950-991
Polyhistidine region: bases 1001-1018
BGH Reverse priming site: bases 1041-1058
SV40 late polyadenylation signal: bases 1183-1188
pUC origin: bases 1621-2354 (complementary strand)
bla promoter: bases 3360-3458 (complementary strand)
Ampicillin (*bla*) resistance gene ORF: bases 2499-3359 (complementary strand)

*This *Xba* I site is subject to *dam* methylation in some strains of *E. coli*.

continued on next page

pMT/V5-His-TOPO® Vector, continued

Features of pMT/V5-His-TOPO®

pMT/V5-His-TOPO® contains the following elements. All features have been functionally tested.

Feature	Benefit
Drosophila metallothionein (MT) promoter	Permits strong, inducible expression of your recombinant protein (Bunch *et al.*, 1988; Maroni *et al.*, 1986)
MT Forward priming site	Allows sequencing through the insert from the promoter sequence
TOPO® Cloning site	Allows insertion of your PCR product in frame with the V5 epitope and polyhistidine C-terminal tag
V5 epitope (Gly-Lys-Pro-Ile-Pro-Asn-Pro-Leu-Leu-Gly-Leu-Asp-Ser-Thr)	Allows detection of your recombinant protein with the Anti-V5 Antibody (Catalog no. R960-25) or Anti-V5-HRP Antibody (Catalog no. R961-25) (Southern *et al.*, 1991)
C-terminal polyhistidine tag	Permits purification of your recombinant protein on metal-chelating resin such as ProBond™ In addition, the C-terminal polyhistidine tag is the epitope for the Anti-His (C-term) Antibody (Catalog no. R930-25) and the Anti-His(C-term)-HRP Antibody (Catalog no. R931-25) (Lindner *et al.*, 1997)
BGH reverse priming site*	Permits sequencing through the insert
SV40 late polyadenylation signal	Allows efficient transcription termination and polyadenylation of mRNA (Angelichio *et al.*, 1991)
pUC origin	Allows high-copy number replication and growth in *E. coli*
bla promoter	Allows expression of the ampicillin (*bla*) resistance gene
Ampicillin resistance gene (β-lactamase)	Permits selection of vector in *E. coli*

***Note**: The multiple cloning site, V5 epitope, C-terminal polyhistidine tag, and the BGH Reverse priming site are derived from the vector pcDNA3.1/V5-His©.

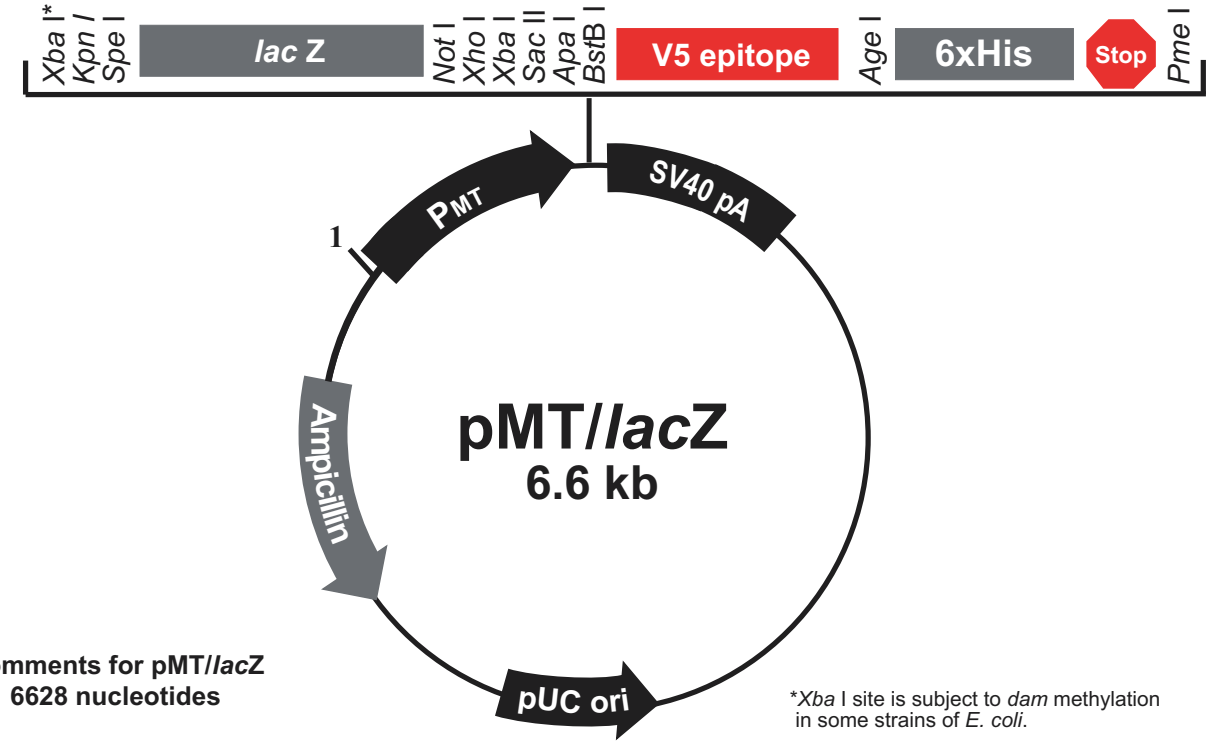
Map of pMT/*lacZ*

Description

pMT/*lacZ* is a 6628 bp control vector containing the gene for β-galactosidase. The 3.1 kb *lacZ* gene was amplified and TOPO® Cloned into pMT/V5-His-TOPO® such that it is in frame with the C-terminal peptide.

Map of Control Vector

The figure below summarizes the features of the pMT/*lacZ* vector. **The complete sequence for pMT/*lacZ* is available for downloading from our World Wide Web site (www.invitrogen.com) or by contacting Technical Service (see page 25).**



Xba I site is subject to *dam* methylation in some strains of *E. coli*.

Comments for pMT/*lacZ*
6628 nucleotides

Metallothionein promoter: bases 406-833
Start of transcription: base 778
MT Forward priming site: bases 814-831
LacZ ORF: bases 894-3950
V5 epitope: bases 4020-4061
Polyhistidine region: bases 4071-4088
SV40 late polyadenylation signal: bases 4253-4258
pUC origin: bases 4751-5424 (complementary strand)
bla promoter: bases 6430-6528 (complementary strand)
Ampicillin (*bla*) resistance gene ORF: bases 5569-6429 (complementary strand)

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 (Netscape 3.0 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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1. On the home page, go to the left-hand column under 'Technical Resources' and select 'MSDS Requests'.
2. Follow instructions on the page and fill out all the required fields.
3. To request additional MSDSs, click the 'Add Another' button.
4. All requests will be faxed unless another method is selected.
5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.

continued on next page

Technical Service, continued

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

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

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continued on next page

Purchaser Notification, continued

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Product User Registration Card

Please complete and return the enclosed Product User Registration Card for each DES® TOPO® TA Expression Kit that you purchase. This will serve as a record of your purchase and registration and will allow Invitrogen to provide you with critical product updates. The agreement outlined above becomes effective upon our receipt of your Product User Registration Card or 10 days following the sale of the DES® TOPO® TA Expression Kit to you. Use of the kit at any time results in immediate obligation to the terms and conditions stated in this Agreement.

Product Qualification

Introduction

This section describes the criteria used to qualify the components in the DES® TOPO® TA Expression Kit.

pMT/V5-His-TOPO® Vector

The pMT/V5-His B plasmid (parental vector of pMT/V5-His-TOPO®) 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. The table below lists the restriction enzymes and the expected fragments. Note that restriction sites used to qualify the parental vector may no longer be present in the topoisomerase I-adapted vector.

Restriction Enzyme	Expected Fragments (bp)
*Hin*d III	654, 2888
Sac II	3542
Xba I	3542

TOPO® Cloning Efficiency

Once the supercoiled vector has been adapted with topoisomerase I, it is lot-qualified using the control reagents included in the kit. Under conditions described on pages 19-20, a 500 bp control PCR product is TOPO® Cloned into pMT/V5-His-TOPO® and transformed into the One Shot® TOP10 competent *E. coli* included with the kit.

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

Sequencing Primers

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

One Shot® 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.

References

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