HTP Zero Blunt® TOPO® PCR Cloning Kit for Sequencing

Version G
21 July 2004
25-0363

HTP Zero Blunt® TOPO® PCR Cloning Kit for Sequencing

Five-minute cloning of blunt PCR products for high-throughput cloning and sequencing

Catalog nos. K2875-05, K2875-480, K2875-500

Invitrogen™
life technologies

www.invitrogen.com
tech_service@invitrogen.com

Table of Contents

Kit Contents

Shipping and Storage

The HTP Zero Blunt® TOPO® PCR Cloning Kits for Sequencing are shipped on dry ice. Each kit contains a box with TOPO® Cloning reagents and one with TOP10 Chemically Competent *E. coli* in bulk or in a 96-well format (MultiShot™ or MultiShot™ StripWell TOP10 Chemically Competent *E. coli*). **Store TOPO® Cloning reagents at -20°C and store the cells at -80°C.**

Types of Kits

The table below lists the catalog number, type of competent *E. coli*, and number of reactions in the kits available.

Catalog no.	Competent Cells	Reactions
K2875-500	5 x 5 ml; 1 x 50 µl	500
K2875-480	Five 96-well plates, 15 µl/well 1 test plate with 12 wells of cells only	480
K2875-05	Five 96-well plates, 50 µl/well	480

HTP Zero Blunt® TOPO® Reagents

HTP Zero Blunt® TOPO® PCR Cloning reagents are listed below. **Note that the user must supply the proofreading polymerase and buffer.** Store at -20°C.

Item	Concentration	Amount
pCR®4Blunt-TOPO® vector	10 ng/µl plasmid DNA in: 50% glycerol 50 mM Tris-HCl, pH 7.4 (at 25°C) 1 mM EDTA 1 mM DTT 0.1% Triton X-100 100 µg/ml BSA 30 µM bromophenol blue	500 µl
dNTP Mix	12.5 mM each dATP, dCTP, dGTP, and dTTP in water, pH 8	300 µl
Salt Solution	1.2 M NaCl 0.06 M $MgCl_2$	500 µl
Sterile Water	--	3 x 1 ml

continued on next page

HTP Zero Blunt® TOPO® PCR Cloning Reagents, continued

Item	Concentration	Amount
M13 Forward (-20) Primer	0.1 µg/µl in TE Buffer, pH 8	20 µl (407 pmoles)
M13 Reverse Primer	0.1 µg/µl in TE Buffer, pH 8	20 µl (385 pmoles)
T3 primer	0.1 µg/µl in TE Buffer, pH 8	20 µl (329 pmoles)
T7 primer	0.1 µg/µl in TE Buffer, pH 8	20 µl (328 pmoles)

Primer Sequence

The table below lists the sequence of the sequencing primers included in the kit.

Primer	Sequence
M13 Forward (-20)	5´-GTAAAACGACGGCCAG-3´
M13 Reverse	5´-CAGGAAACAGCTATGAC-3´
T3	5´-ATTAACCCTCACTAAAGGGA-3´
T7	5´-TAATACGACTCACTATAGGG-3´

TOP10 Chemically Competent *E. coli*

TOP10 Chemically Competent *E. coli* is supplied in one of three formats (bulk, MultiShot™, or MultiShot™ StripWell) depending on the catalog number (see page v). Reagents supplied with each format are described on the following page.

continued on next page

Kit Contents, Continued

Bulk TOP10

Catalog no. K2875-500 supplies the following reagents.

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	5 x 15 ml
TOP10 cells	--	5 x 5 ml 1 x 50 µl (control reaction)
pUC19 Control DNA	10 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8	50 µl

MultiShot™ TOP10

Catalog no. K2875-480 supplies the following reagents.

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	5 x 15 ml
TOP10 cells	--	5 x 96-well plates (15 µl per well) Test plate with 12 wells of cells (1 row)
pUC19 Control DNA	10 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8	50 µl

continued on next page

MultiShot™ StripWell TOP10

Catalog no. K2875-05 supplies the following reagents.

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	5 x 15 ml
TOP10 cells	--	5 x 96-well plates (50 µl per well)
pUC19 Control DNA	10 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8	50 µl

Genotype of TOP10

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

Introduction

Overview

Introduction

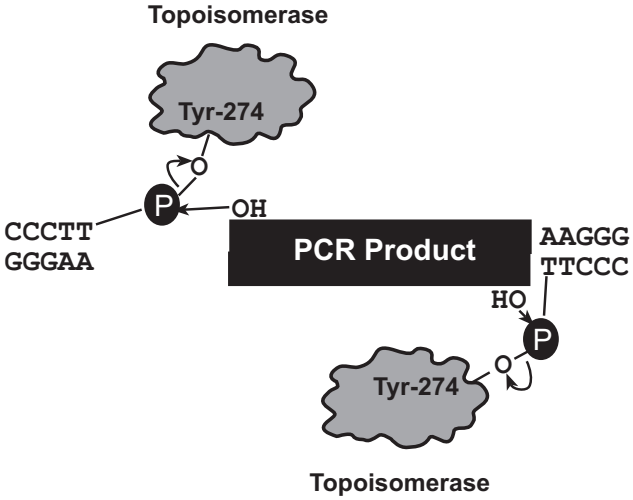
The HTP Zero Blunt® TOPO® PCR Cloning Kits for Sequencing are specifically designed to simplify high-throughput (HTP) applications. Sufficient pCR®4Blunt-TOPO® is provided in bulk to perform 500 TOPO® Cloning reactions. Chemically competent TOP10 *E. coli* are provided in a choice of three formats:

- Cells are provided in bulk aliquots of 5 ml to allow simple transfer of the cells from a sterile trough into a 96-well plate containing the TOPO® Cloning reaction.
- Cells are provided pre-aliquoted in 96-well plates (MultiShot™ format) to allow addition of the TOPO® Cloning reaction to the cells.
- Cells are provided pre-aliquoted in 96-stripwell plates (MultiShot™ StripWell format) to allow addition of the TOPO® Cloning reaction to only those wells needed for your particular application.

How It Works

The plasmid vector (pCR®4Blunt-TOPO®) is supplied linearized with *Vaccinia* virus topoisomerase I covalently bound to the 3´ ends (referred to as "activated" 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).



continued on next page

ccdB Gene

pCR®4Blunt-TOPO® allows direct selection of recombinants via disruption of the lethal *E. coli* gene, *ccd*B (Bernard and Couturier, 1992; Bernard *et al.*, 1994; Bernard *et al.*, 1993). The vector contains the *ccd*B gene fused to the C-terminus of the LacZα fragment. Ligation of a blunt-end PCR product disrupts expression of the *lac*Zα-*ccd*B gene fusion permitting growth of only positive recombinants upon transformation into *E. coli*. Cells that contain non-recombinant vector are killed upon plating. Therefore, blue/white screening is not required.

Experimental Outline

Most users will already have a process for their own high-throughput application. The table below provides a basic outline of the experimental protocol for reference and to evaluate your own protocol.

Step	Action	Page
1	Produce PCR products using your own protocol. Guidelines are provided for your convenience.	3
2	Set up TOPO® Cloning reactions for use with—	
	Bulk cells	5
	MultiShot™ cells	7
	MultiShot™ StripWell cells	9
3	Transform the TOPO® Cloning reaction into—	
	Bulk cells	5
	MultiShot™ cells	7
	MultiShot™ StripWell cells	9
4	Analyze plasmid DNA for the correct clone	12
5	Sequence plasmid DNA using your method of choice.	12

Methods

Producing Blunt PCR Products

Introduction

The HTP Zero Blunt® TOPO® PCR Cloning Kits for Sequencing are specifically designed to clone PCR products for high-throughput sequencing. Blunt-end PCR products are generated by thermostable proofreading polymerases such as Platinum® *Pfx*, *Pfu*, or Vent®. While most users will already have a procedure and reagents for producing PCR products, we include some reminders for your convenience.

Note

We have tested this kit specifically with the *Pfu* proofreading polymerase. Other proofreading polymerases that generate blunt ends may be suitable.

Do not add 5´ phosphates to your primers for PCR. The PCR product synthesized will not TOPO® Clone into pCR®4Blunt-TOPO®.

Producing Blunt PCR Products

Follow the manufacturer's instructions and recommendations for producing blunt-end PCR products. It is very important to optimize PCR conditions to produce a single, discrete PCR product.

Materials Supplied by the User

You will need the following reagents and equipment for PCR.

Note: dNTPs (adjusted to pH 8) are provided in the kit.

- Thermocycler and thermostable proofreading polymerase
- 10X PCR buffer appropriate for your polymerase
- DNA template and primers for your PCR product

Producing PCR Products

Set up a 25 µl or 50 µl PCR reaction using the guidelines below:

- Follow the manufacturer's recommendations
- Use the cycling parameters suitable for your primers and template
- Use a 7 to 30 minute final extension at 72°C to ensure that all PCR products are completely extended.

After cycling, place the tube on ice or store at -20°C for up to 1-2 weeks. Proceed to **Checking the PCR Product**, below.

Checking the PCR Product

Remove 5 to 10 µl from each amplification and analyze by ethidium bromide agarose gel electrophoresis. Be sure you have a single discrete band of the correct size. If you obtain multiple bands, see the next page.

continued on next page

Producing Blunt PCR Products, Continued

Multiple PCR Bands

If you obtain multiple bands from your PCR, there are several options to consider, depending on your application. Note that primer-dimers are preferentially cloned because of their small size. You may--

- Proceed directly to TOPO® Cloning (page 5, 7, or 9).
- Gel-purify your fragment before TOPO® Cloning. A variety of methods and kits are available. We recommend the S.N.A.P.™ Gel Purification Kit (Catalog no. K1999-25).
- Optimize your PCR to eliminate multiple bands and smearing. Follow the manufacturer's recommendations for optimizing your PCR with the polymerase of your choice.

The Next Step

After producing your PCR products, you are ready to TOPO® Clone and transform *E. coli*. Three sample protocols are provided to help you develop a protocol specifically for your needs. These protocols were created in response to specific customer needs. To TOPO® Clone and transform using:

- Bulk chemically competent *E. coli*, see page 5
- MultiShot™ chemically competent *E. coli*, see page 7
- MultiShot™ StripWell chemically competent *E. coli*, see page 9

High-Throughput TOPO® Cloning and Transformation with Bulk Cells

Description

In this protocol, the TOPO® Cloning reaction is set up in a 96-well U bottom, polystyrene plate (Costar, Catalog no. 3366, 330 µl/well) and the competent cells are placed in a trough for dispensing.

Before Starting

- Chill a 96-well metal heating block (VWR, Catalog no. 13259-260) on ice until the block is cold.
- Bring a vial of SOC to room temperature.
- Pre-heat a heat block or thermocycler containing a 96-well metal block to 42°C.

  Note: You can also use a water bath, but be careful not to contaminate the cells.
- Thaw 1 tube (5 ml) of TOP10 chemically competent *E. coli* on ice (30-40 minutes).
- Warm agar plates containing 50-100 µg/ml ampicillin or 50 µg/ml kanamycin to 37°C.
- If you wish to test the transformation efficiency of the cells with pUC19, dilute a portion of the 10 pg/µl stock to 2.5 pg/µl and store on ice.

Controls

A 50 µl aliquot of competent cells is provided to perform a test TOPO® Cloning and transformation reaction. You can also include the pUC19 plasmid as an internal control (see **Procedure** below).

Procedure

1. Set up the 6 µl TOPO® Cloning reaction in each well as follows. If you include pUC19 as a control, leave 2-3 wells empty.

PCR product	1 µl
Salt Solution	1 µl
Sterile Water	3 µl
pCR®4Blunt-TOPO®	1 µl
Final Volume	6 µl

2. Incubate 5-10 minutes at room temperature.
3. Place the 96-well plate on the cooling block for 5 minutes.
4. If you are including pUC19, add 2 µl (5 pg) of the diluted plasmid to 2-3 empty wells.
5. Pour thawed TOP10 *E. coli* into a sterile trough and immediately dispense 45 µl/well. Gently pipet up and down 1-2 times to mix.
6. Cover the plate with Parafilm® and incubate it on the chilled block for 20 minutes. Proceed to the next page.

continued on next page

Procedure, continued

7. Transfer the plate to either the pre-warmed heat block or the thermocycler and heat-shock the cells at 42°C for 30 seconds.
8. Transfer the plate back to the cooling block and press down to ensure the plate is in complete contact with the cooling block. Incubate for 1 minute.
9. Remove the Parafilm® and add 150 µl/well of SOC.
10. Re-cover the plate and incubate the plate at 37°C for 1 hour.

    Note: Gentle shaking (125 RPM) is optional.
11. Plate 50 µl from each well onto selective plates. Use LB plates containing 100 µg/ml ampicillin for the pUC19 controls. Incubate overnight at 37°C.
12. The next day, select 5-10 colonies and process as desired.

Plating Volumes and Expected Results

The table below describes the type of DNA, amount transformed into chemically competent cells, the volume plated, and the number of colonies.

Note: We use pUC19 to qualify the kit. Transformation efficiency should be > 1 x 10^8 cfu/µg and yield 100-300 colonies per plate.

DNA	Type	Amount Transformed	Volume Plated	Number of Colonies
pUC19	Supercoiled	5 pg vector	50 µl	100-300
pCR®4Blunt-TOPO® plus PCR-amplified 750 bp insert	Zero Blunt® TOPO® Cloning	10 ng vector + 20 ng insert	50 µl	150-300

Too Many Colonies

If you obtain too many colonies, reduce the amount plated and/or dilute the transformation with additional SOC.

High-Throughput TOPO® Cloning and Transformation with MultiShot™ Cells

Description

In this protocol, the TOPO® Cloning reaction is set up in a 96-well plate and 2 µl are transferred to each well of a MultiShot™ 96-well plate containing 15 µl of chemically competent cells per well.

Before Starting

- Chill two 96-well metal heating blocks (VWR, Catalog no. 13259-260) on ice until the blocks are cold.
- Bring a vial of SOC to room temperature.
- If you are planning to use pUC19 as a control for transformation, dilute a portion of the 10 pg/µl stock solution to 2.5 pg/µl and store on ice.
- Warm agar plates containing 50-100 µg/ml ampicillin or 50 µg/ml kanamycin to 37°C.
- Pre-heat a heat block or thermocycler containing a 96-well metal block to 42°C.

  Note: You can also use a water bath, but be careful not to contaminate the cells.
- If you are using a thermocycler, program the machine to hold the temperature at 42°C.

Controls

A test plate containing 1 row (12 wells) of cells is included to perform test TOPO® Cloning reactions and transformations. In addition, you can include the pUC19 plasmid as an internal control (see **Procedure** below).

Procedure

1. In a 96-well plate, set up the 6 µl TOPO® Cloning reaction in each well as follows.

PCR product	1 µl
Salt Solution	1 µl
Sterile Water	3 µl
pCR®4Blunt-TOPO®	1 µl
Final Volume	6 µl

2. Incubate 5-10 minutes at room temperature.
3. Place the 96-well plate on one of the cooling blocks for 5 minutes.
4. Remove a MultiShot™ plate from the freezer and place it in the second cooling block. Cells should thaw within 30 seconds.
5. Carefully remove the aluminum foil seal.
6. Use a multi-channel pipet to add 2 µl of each TOPO® Cloning reaction (~3.3 ng) to each well of the MultiShot™ plate. Keep the volume around 2 µl for uniform results. To use pUC19 as an internal control, add 2 µl (5 pg) of the 2.5 pg/µl solution. Proceed to the next page.

continued on next page

High-Throughput TOPO® Cloning and Transformation with MultiShot™ Cells, Continued

Procedure, continued

7. Cover the cells with the supplied plastic lid and incubate the cells and DNA in the chilled block for 20 minutes.
8. Transfer the cell plate to either the pre-warmed heat block or thermocycler and heat-shock for 30 seconds at 42°C.
9. Transfer the cell plate back to a cooling block, press the plate into the block, and allow the plate to cool for 1 minute.
10. Remove the plastic lid and add 90 µl SOC to each well.
11. Cover the plate with the lid and incubate the plate at 37°C for 1 hour.

    Note: Gentle shaking (125 RPM) is optional.
12. Plate 100 µl from each well on selective plates. Plate the pUC19 control reactions on LB plates containing 100 µg/ml ampicillin. Incubate overnight at 37°C. See below for the expected number of colonies.

Plating Volumes and Expected Results

The table below describes the type of DNA, amount transformed into MultiShot™ chemically competent cells, the volume plated, and the number of colonies.

Note: We use pUC19 to qualify the kit. Transformation efficiency should be > 1 x 10^8 cfu/µg and yield 50-200 colonies per plate.

DNA	Type	Amount Transformed	Volume Plated	Number of Colonies
pUC19	Supercoiled	5 pg vector	10 µl	50-200
pCR®4Blunt-TOPO® plus PCR-amplified 750 bp insert	Zero Blunt® TOPO® Cloning	3.3 ng vector + insert	100 µl	50-200

Too Many Colonies

If you obtain too many colonies, you can reduce the amount plated or dilute the TOPO® Cloning reactions with sterile water or TE buffer prior to adding the reaction to the cells.

High-Throughput TOPO® Cloning and Transformation with MultiShot™ StripWell Cells

Description

In this protocol, the TOPO® Cloning reaction is set up in a 96-well plate and 2 µl are transferred to each well of a MultiShot™ StripWell 96-well plate containing 50 µl of chemically competent cells per well.

Before Starting

- Chill a 96-well metal heating block (VWR, Catalog no. 13259-260) on ice until the block is cold.
- Prepare a container of ice large enough to chill the number of wells you will be using.
- Bring the vial of SOC to room temperature.
- If you are planning to use pUC19 as a control for transformation, dilute a portion of the 10 pg/µl stock solution to 2.5 pg/µl and store on ice.
- Warm agar plates containing 50-100 µg/ml ampicillin or 50 µg/ml kanamycin to 37°C.
- Pre-heat a water bath to 42°C.

Procedure

1. In a 96-well plate, set up the 6 µl TOPO® Cloning reaction in the desired number of wells as follows.

PCR product	1 µl
Salt Solution	1 µl
Sterile Water	3 µl
pCR®4Blunt-TOPO®	1 µl
Final Volume	6 µl

2. Incubate 5-10 minutes at room temperature.
3. Place the 96-well plate on the cooling block for 5 minutes.
4. Remove a MultiShot™ StripWell plate from the freezer and remove the number of wells you need. Return any unused wells to the freezer. Place the wells in the container of ice. Cells should thaw within 1 minute.
5. Carefully remove the strip of caps from each set of 8 wells and keep them for further use.
6. Use a multi-channel pipet to add 2 µl of each TOPO® Cloning reaction (~3.3 ng) to the wells. Keep the volume around 2 µl for uniform results. To use pUC19 as an internal control, add 2 µl (5 pg) of the 2.5 pg/µl solution to 2-3 separate wells.

continued on next page

High-Throughput TOPO® Cloning and Transformation with MultiShot™ StripWell Cells, continued

Procedure, continued

7. Cover the cells with the caps and incubate the cells and DNA on ice for 30 minutes.
8. Transfer the wells to the water bath and heat-shock for 30 seconds at 42°C.

   Note: Be careful not to contaminate the cells.
9. Transfer the wells back to the ice and allow the wells to cool for 1 minute.
10. Remove the caps and add 250 µl SOC to each well. **Re-cap the wells tightly.**
11. Incubate the wells at 37°C for 1 hour with shaking (225 RPM). We turn the wells on their side to increase aeration and secure them to the shaker.
12. Plate 25 µl from each well on selective plates. Plate the pUC19 control reactions on LB plates containing 100 µg/ml ampicillin. Incubate overnight at 37°C. See below for the expected number of colonies.

Plating Volumes and Expected Results

The table below describes the type of DNA, amount transformed into MultiShot™ StripWell chemically competent cells, the volume plated, and the number of colonies.

Note: We use pUC19 to qualify the kit. Transformation efficiency should be > 1 x 10^8 cfu/µg and yield 100-300 colonies per plate.

DNA	Type	Amount Transformed	Volume Plated	Number of Colonies
pUC19	Supercoiled	5 pg vector	10 µl	100-300
pCR®4Blunt-TOPO® plus PCR-amplified 750 bp insert	Zero Blunt® TOPO® Cloning	3.3 ng vector + insert	25 µl	100-300

Too Many Colonies

If you obtain too many colonies, you can reduce the amount plated or dilute the TOPO® Cloning reactions with sterile water or TE buffer prior to adding the reaction to the cells.

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 combining the TOPO® Cloning reaction with 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

Analyzing and Sequencing Clones

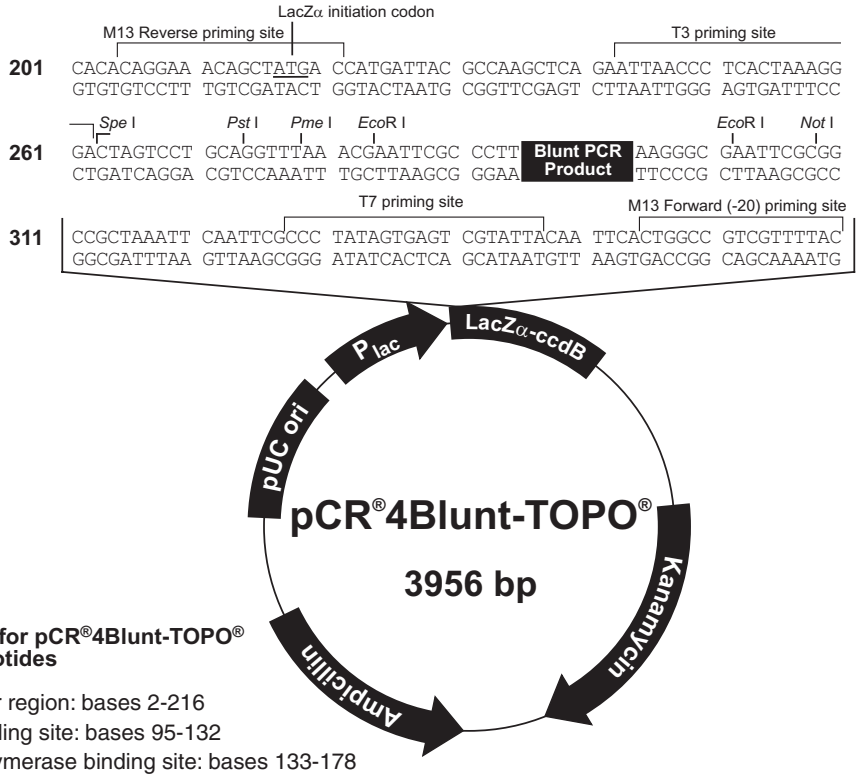
Analysis	Use your own HTP method to analyze clones for the desired insert.
Sequencing	Once you have identified the correct clone, you are ready to sequence your insert. Four primers (M13 Forward (-20), M13 Reverse, T3, and T7) have been included to help you sequence your insert. Refer to the map on page 13 for the sequence surrounding the TOPO® Cloning site. For the full sequence of the vector, visit our Web site or contact Technical Service (page 14).

Appendix

Map of pCR®4Blunt-TOPO®

pCR®4Blunt-TOPO® Map

The map below shows the features of pCR®4Blunt-TOPO® and the sequence surrounding the TOPO® Cloning site. Restriction sites are labeled to indicate the actual cleavage site. **The complete sequence of the vector is available for downloading from our Web site (www.invitrogen.com) or by calling Technical Service (page 14).**



Comments for pCR®4Blunt-TOPO®
3956 nucleotides

lac promoter region: bases 2-216
  CAP binding site: bases 95-132
  RNA polymerase binding site: bases 133-178
  Lac repressor binding site: bases 179-199
Start of transcription: base 179
M13 Reverse priming site: bases 205-221
LacZα-*ccdB* gene fusion: bases 217-810
  LacZα portion of fusion: bases 217-497
  *ccd*B portion of fusion: bases 508-810
T3 priming site: bases 243-262
TOPO® Cloning site: bases 294-295
T7 priming site: bases 328-347
M13 Forward (-20) priming site: bases 355-370
Kanamycin promoter: bases 1021-1070
Kanamycin resistance gene: bases 1159-1953
Ampicillin (*bla*) resistance gene: bases 2203-3063 (c)
Ampicillin (*bla*) promoter: bases 3064-3160 (c)
pUC origin: bases 3161-3834
(c) = 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 (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!

Contact Us

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

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

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

European Headquarters:
Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail: eurotech@invitrogen.com

MSDS 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

Limited Warranty

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

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

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

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

Purchaser Notification

Introduction

Use of the HTP Zero Blunt® TOPO® PCR Cloning Kit for Sequencing is covered under the licenses detailed below.

Limited Use Label License No. 54: *ccdB* Vectors

This product is the subject of one or more of U.S. Patent Numbers 5,910,438 and 6,180,407 and corresponding foreign patents and is sold under license from the Université Libre de Bruxelles for research purposes only. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). For licensing information, please contact: Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, CA 92008. Tel: 760-603-7200; Fax: 760-602-6500.

Limited Use Label License No: 118 TOPO® Cloning Products

The TOPO® Cloning Technology products and their use are the subject of one or more of U.S. Patent Nos. 5,766,891, 6,548,277 and/or other pending U.S. and foreign patent applications licensed to Invitrogen Corporation.

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500.

Product Qualification

Introduction	Invitrogen qualifies the HTP Zero Blunt® TOPO® PCR Cloning Kits for Sequencing as described below.
Restriction Digest	The parental supercoiled pCR®4 vector is qualified by restriction digest prior to adaptation with topoisomerase. Restriction digests must demonstrate the correct banding pattern when electrophoresed on an agarose gel.
TOPO® Cloning Efficiency	Once the pCR®4Blunt-TOPO® vector has been adapted with topoisomerase I, it is lot-qualified using the method described in this kit. A 750 bp control PCR product is TOPO® Cloned into the vector and subsequently transformed into the TOP10 competent *E. coli* included with each kit. Each lot of vector must yield greater than 95% cloning efficiency to qualify.
Primers	The primers included in this kit have been lot-qualified by DNA sequencing experiments using the dideoxy chain termination technique.
TOP10 Competent *E. coli*	All competent cells are qualified as follows: • Cells are tested for transformation efficiency using the control plasmid included in the 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 1×10^8 cfu/µg DNA for TOP10 cells. • To verify the absence of phage contamination, 0.5-1 ml of competent cells are added to LB top agar and poured onto LB plates. After overnight incubation, no plaques should be detected. • Untransformed cells are plated on LB plates 100 µg/ml ampicillin, 25 µg/ml streptomycin, 50 µg/ml kanamycin, or 15 µg/ml chloramphenicol to verify the absence of antibiotic-resistant contamination.

References

Bernard, P., and Couturier, M. (1992). Cell Killing by the F Plasmid CcdB Protein Involves Poisoning of DNA-Topoisomerase II Complexes. J. Mol. Biol. *226*, 735-745.

Bernard, P., Gabant, P., Bahassi, E. M., and Couturier, M. (1994). Positive Selection Vectors Using the F Plasmid *ccd*B Killer Gene. Gene *148*, 71-74.

Bernard, P., Kezdy, K. E., Melderen, L. V., Steyaert, J., Wyns, L., Pato, M. L., Higgins, P. N., and Couturier, M. (1993). The F Plasmid CcdB Protein Induces Efficient ATP-dependent DNA Cleavage by Gyrase. J. Mol. Biol. *234*, 534-541.

Shuman, S. (1994). Novel Approach to Molecular Cloning and Polynucleotide Synthesis Using Vaccinia DNA Topoisomerase. J. Biol. Chem. *269*, 32678-32684.

Shuman, S. (1991). Recombination Mediated by Vaccinia Virus DNA Topoisomerase I in *Escherichia coli* is Sequence Specific. Proc. Natl. Acad. Sci. USA *88*, 10104-10108.