Invitrogen™
life technologies

Direct your cloning future.
TOPO® Cloning Technology

With TOPO® Cloning Technology You Can:

- Clone *Taq*-amplified, blunt-end, and long PCR products
- Sequence or clone directly into an expression vector
- Ligate in 5 minutes at room temperature and obtain up to 99% recombinants

Powerful PCR Cloning Tools

For optimal cloning results, you need a technology that you can rely on. TOPO® Cloning is the most effective technology available for cloning PCR products and other DNA molecules. It yields up to 99% recombinants via a simple 5-minute, bench-top ligation.

Topoisomerase greatly improves cloning

TOPO® Cloning makes ligation faster and more successful. It enables 5-minute, bench-top ligation and yields up to 99% recombinants. This speed and efficiency will save you hours of time over other methods of cloning.

The technology behind TOPO® Cloning

The key to TOPO® Cloning is the enzyme, DNA topoisomerase I, which functions both as a restriction enzyme and as a ligase. Its biological role is to cleave and rejoin DNA during replication. *Vaccinia* virus topoisomerase I specifically recognizes the pentameric sequence 5´-(C/T)CCTT-3´ and forms a covalent bond with the phosphate group of the 3´ thymidine. It cleaves one DNA strand, enabling the DNA to unwind. The enzyme then religates the ends of the cleaved strand and releases itself from the DNA.

To harness the religating activity of topoisomerase, TOPO® vectors are provided linearized with topoisomerase I covalently bound to each 3´ phosphate. This enables the vectors to readily ligate DNA sequences with compatible ends (Figures 1, 2, and 3) (1). In only 5 minutes at room temperature, the ligation is complete and ready for transformation into *E. coli*.

Figure 1 - TOPO TA Cloning® of *Taq*-amplified DNA

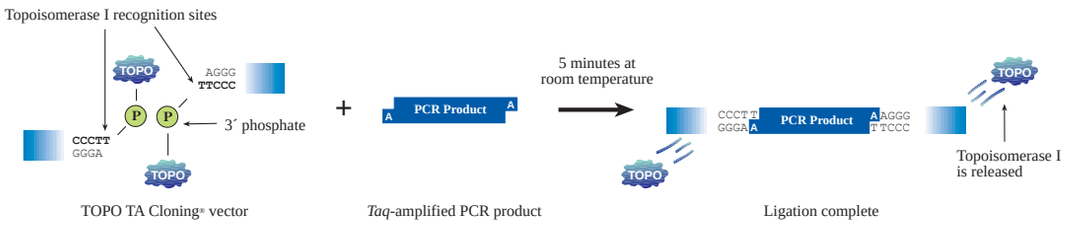


Figure 2 - Zero Blunt® TOPO® Cloning of blunt-end DNA

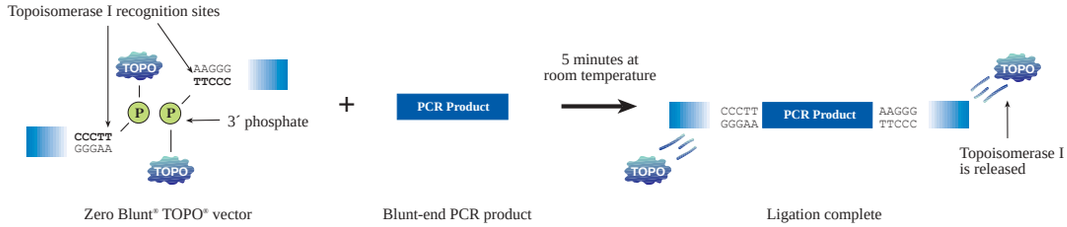
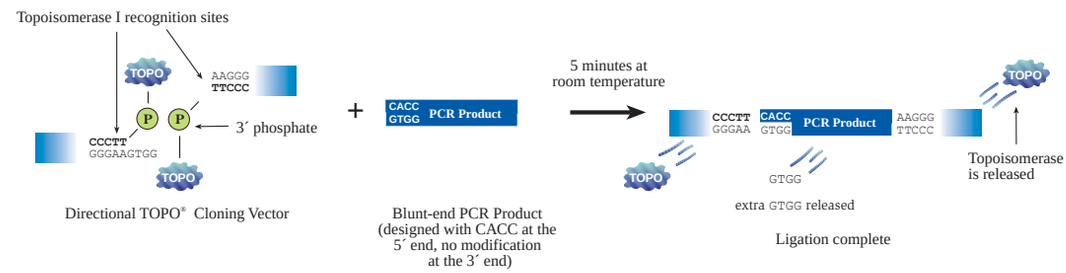


Figure 3 – Directional TOPO® Cloning of blunt-end DNA



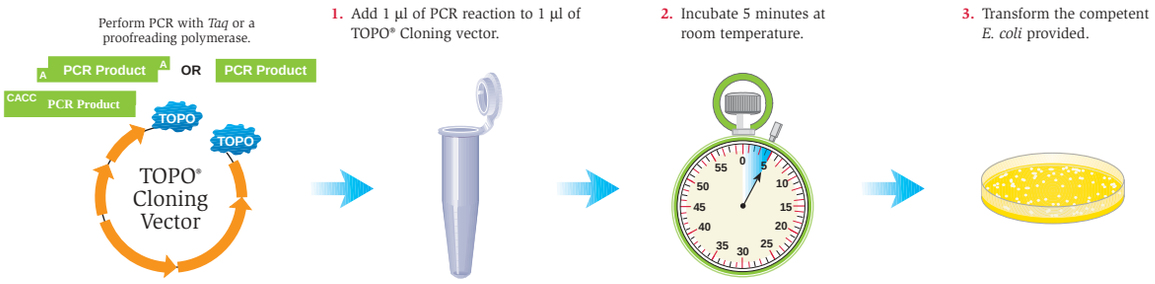
Three simple steps

TOPO® Cloning requires just three easy steps. Simply combine your PCR product and a TOPO® Cloning vector in the provided solution, wait five minutes, then transform *E. coli* (Figure 4). With TOPO® Cloning, the additional time, steps, and reagents required for ligase-mediated cloning are eliminated. **Table 1** provides conservative estimates of the time saved using TOPO® Cloning versus other methods.

TOPO® Cloning means:

- Utilizing standard PCR primers
- Cloning efficiently without ligase or overnight incubations
- Using PCR templates without post-PCR modification or gel purification*

Figure 4 - The TOPO® Cloning protocol



*The TOPO® XL PCR Cloning Kit requires a 15-minute gel purification step.

Table 1 – TOPO® TA Cloning vs. other methods of cloning

Steps	TOPO TA Cloning®	TA/UA Cloning	Restriction Enzyme Cutback and Ligation
Order or prepare PCR Primers	Special primers containing extra bases are not required	Special primers containing extra bases are not required	Add 10 extra bases to each 5´ and 3´ PCR primer to create restriction sites (6 for the restriction site, 4 for the spacing)
Prepare the vector and PCR product for ligation	Linearized TOPO® Cloning Vectors are ready for direct ligation of unmodified, unpurified PCR products	TA/UA Cloning vectors are ready for direct ligation of unmodified, unpurified PCR products	1) Digest vector and PCR product with restriction enzyme(s) 2) Gel purify the digested PCR product using low-melt agarose
Obtain ligation reagents	All required cloning reagents are included	All required cloning reagents are included	Purchase ligase, ATP, and ligation buffer
Prepare or purchase competent cells	TOPO® Cloning Kits include One Shot® Competent *E. coli*	1) With competent cells = 0 hrs 2) Without competent cells = Up to 6 hours	1) Purchase: 0 hours 2) Prepare: 6 hours
Incubate the ligation	5 minutes	1 hour	2 to 23 hours
Recombination efficiency	up to 99%	60% to 80%	~ 60%
Time required for cloning	5 minutes	1 to 12 hours	2 to 23 hours

Complete product offering

Whether you're PCR cloning with *Taq* DNA polymerase or a proofreading enzyme, there is a TOPO® Cloning Kit available to take you quickly and efficiently to the next step. Use Tables 2 and 3 to find the right products for your specific application. For a complete list of products, please visit our web site at **www.invitrogen.com/topo.**

Table 2 – TOPO® Cloning product guide

Amplification Enzyme	Application of Cloned PCR product	Product	Page no.
Taq DNA polymerase: Platinum® *Taq* DNA Polymerase†	General subcloning	TOPO TA Cloning® Kit	4
	in vitro transcription	TOPO TA Cloning® Kit Dual Promoter	
	Sequencing	TOPO TA Cloning® for Sequencing	7
	High-throughput studies	HTP TOPO TA Cloning® Kit HTP TOPO TA Cloning® Kit Dual Promoter HTP TOPO TA Cloning® for Sequencing	13 13 13
	Non-directional expression in *E. coli*, yeast, insect, or mammalian cells	Various non-directional TOPO® Expression Vectors	8
Proofreading polymerase: Platinum® *Pfx* DNA Polymerase†	General subcloning	Zero Blunt® TOPO® Cloning Kit	5
	in vitro transcription		
	Sequencing	Zero Blunt® TOPO® Cloning Kit for Sequencing	7
	High-throughput studies	HTP Zero Blunt® TOPO® PCR Cloning Kit for Sequencing HTP Directional TOPO® pENTR™ vectors	13 13
Proofreading polymerase: Platinum® *Pfx* DNA Polymerase†*	Directional expression in *E. coli*	Champion™ pET Directional TOPO® Expression Kit	10
	Directional expression in mammalian cells	pcDNA™3.1 Directional TOPO® Expression Kit pcDNA™4/HisMax TOPO® TA Expression Kit ViraPower™ Lentiviral Directional TOPO® Expression System	11
	Expression of cloned PCR products in multiple hosts (via Gateway® System)	Directional TOPO® pENTR™ vectors pcDNA/GW/D-TOPO®	12
Polymerase mixtures for long PCR (3-10 kb): Platinum® *Taq* DNA Polymerase High Fidelity†	General subcloning *in vitro* transcription Sequencing	TOPO® XL PCR Cloning Kit	6

* The use of Platinum® *Pfx* DNA polymerase requires a PCR purification step prior to cloning.

† These products may be covered by one or more Limited Use Label Licenses (See the Invitrogen catalog or our web site, www.invitrogen.com). By the use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses.

Table 3 – Sequencing tools from Invitrogen

Kit Name	Application	Template	Page no.
TOPO® Shotgun Subcloning Kit	Construction of shotgun libraries for sequencing	BACs, YACs, cosmids, and genomic DNA	14
TOPO® Walker Kit	Determination of unknown gap sequence	Partially sequenced BACs, YACs, and PACs	15

Please visit our web site at **www.invitrogen.com/topo**

Fast cloning of *Taq*-amplified PCR products

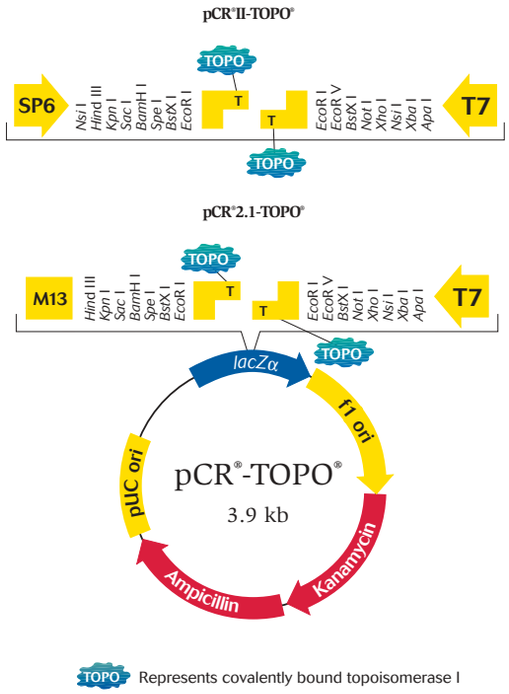
TOPO TA Cloning® Kits

TOPO TA Cloning® Kits combine the TOPO® and TA Cloning® (see box below) technologies to enable fast, efficient cloning of *Taq*-amplified PCR products. The pCR®-TOPO® vectors (Figure 5) are provided linearized with 3´-T overhangs and topoisomerase I-activated to readily accept PCR products with 3´-A overhangs. This enables fast, 5-minute TOPO® Cloning and yields up to 99% recombinants. The pCR®-TOPO® vectors are ideal for applications such as probe generation, *in vitro* transcription, or general subcloning. Some of their convenient features include:

- *Eco*R I sites flanking the PCR product insertion site for easy removal of inserts
- Kanamycin and ampicillin resistance genes for your choice of selection in *E. coli*
- Easy blue/white screening of recombinant colonies
- T7 (pCR®2.1-TOPO®) or T7 and SP6 (pCR®II-TOPO®) promoter/priming sites for *in vitro* transcription
- M13 forward (-20) and reverse priming sites for sequencing or PCR screening

The consistency and speed of the TOPO TA Cloning® Kits make them the best way to clone all of your *Taq*-amplified PCR products.

Figure 5 - pCR®-TOPO® vectors

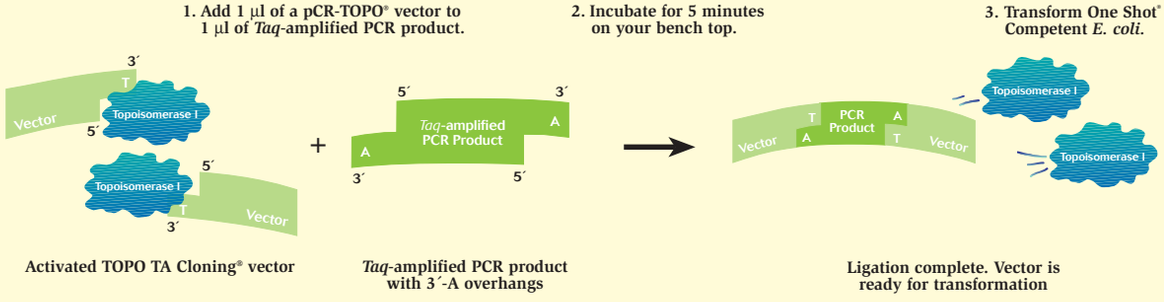


TOPO Represents covalently bound topoisomerase I

Direct ligation with TA Cloning® Technology

The TA Cloning® technology makes it possible to easily clone PCR products produced by *Taq* polymerase. *Taq* has a terminal transferase activity that adds a single 3´-A overhang to each end of the PCR product. TOPO TA Cloning® vectors contain 3´-T overhangs that enable the direct ligation of *Taq*-amplified PCR products (Figure 6)(2,3).

Figure 6 - How TOPO TA Cloning® works



Simplified blunt-end cloning

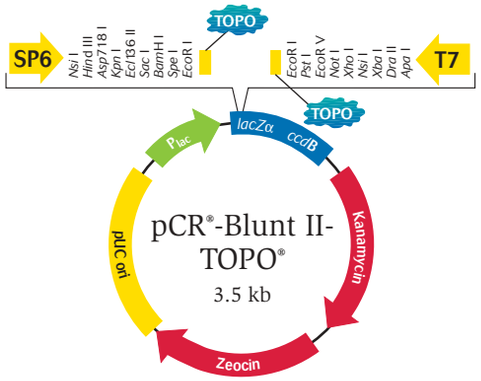
Zero Blunt® TOPO® PCR Cloning Kit

The Zero Blunt® TOPO® PCR Cloning Kit combines the unique Zero Background™ technology (see box below) with TOPO® Cloning to allow easy, high-efficiency cloning of blunt-end PCR products. The pCR®-Blunt II-TOPO® vector (Figure 7) is provided linearized and topoisomerase I-activated so it readily accepts blunt-end PCR products. It produces ≥95% recombinants via rapid 5-minute, benchtop ligation. The pCR®-Blunt II-TOPO® vector features:

- The *ccd*B gene to eliminate background
- *Eco*R I sites flanking the PCR product insertion site for easy removal of inserts
- Kanamycin and Zeocin™ resistance genes for your choice of selection in *E. coli*
- T7 and SP6 promoter/priming sites for *in vitro* RNA transcription and sequencing
- M13 forward (-20) and reverse priming sites for sequencing or PCR screening

The Zero Blunt® TOPO® PCR Cloning Kit offers the easiest, most effective method available for cloning blunt-end PCR products.

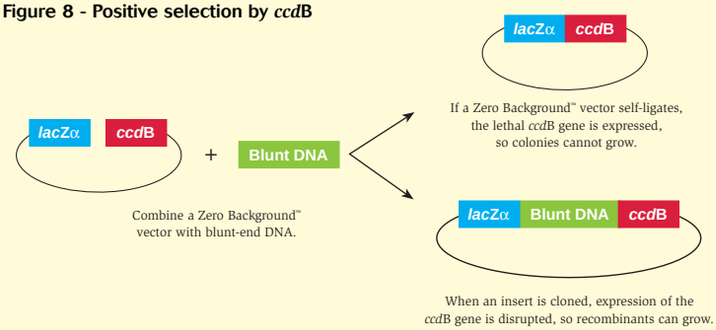
Figure 7 - pCR®-Blunt II-TOPO® vector



Eliminate high backgrounds

Due to high background, cloning blunt-end and long PCR products can be difficult and often yields a low percentage of recombinants. Invitrogen's unique Zero Background™ technology uses the lethal *ccd*B (control of cell death) gene to enable high-efficiency cloning. The *ccd*B gene is incorporated into the cloning site of Zero Background™ vectors. The *ccd*B protein poisons bacterial DNA gyrase, causing degradation of the host chromosome and cell death (4,5). When an insert is ligated into the vector, the *ccd*B gene is disrupted, enabling only recombinant colonies to grow (Figure 8). By eliminating high vector background, the Zero Background™ technology yields nearly 100% recombinants, freeing you from having to screen hundreds of background colonies.

Figure 8 - Positive selection by *ccd*B



Efficient cloning of long PCR products

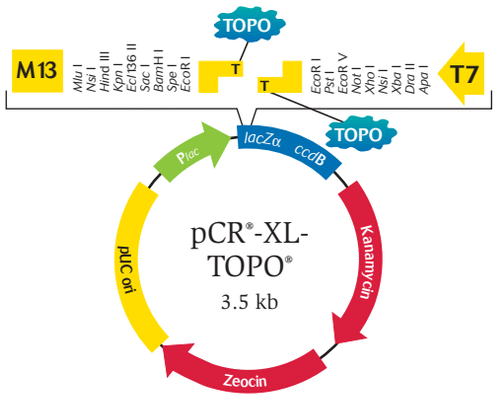
TOPO® XL PCR Cloning Kit

The TOPO® XL PCR Cloning Kit combines TOPO® Cloning, Zero Background™ technology (see box, page 5), and a unique gel purification step (see box below) to optimize the conditions for cloning long PCR products (3-10 kb). The pCR®-XL-TOPO® vector (Figure 9) is provided linearized and topoisomerase I-activated to enable 5-minute, bench-top ligation and ≥80% recombinants‡. It contains 3´-T overhangs for cloning PCR products produced by most thermostable polymerase mixtures†. Some of the convenient features of pCR®-XL-TOPO® include:

- The *ccd*B gene to eliminate background
- Kanamycin and Zeocin™ resistance genes for your choice of selection in *E. coli*
- T7 promoter/priming site for *in vitro* RNA transcription and sequencing
- M13 forward (-20) and reverse priming sites for sequencing or PCR screening

The TOPO® XL PCR Cloning Kit combines gel purification, TOPO® Cloning and Zero Background™ technology to enable high-efficiency cloning of long PCR products.

Figure 9 - pCR®-XL-TOPO® vector



Unique gel purification step improves results

Long PCR often yields multiple products, making gel purification necessary prior to cloning. However, gel purification requires exposure to ethidium bromide and UV light, which can nick and damage DNA (6). To protect against nicking, the TOPO® XL PCR Cloning Kit uses crystal violet to enable visualization of DNA bands in an agarose gel under ambient light. This eliminates ethidium bromide and exposure to UV light, ensuring safe gel purification. Crystal violet staining results in many more colonies and a greater percentage of recombinants than using ethidium bromide and UV light (Table 4).

Table 4 - Crystal violet protects long PCR products for safe gel purification and efficient TOPO® Cloning

	Crystal Violet	Ethidium Bromide
Total Colonies (Kan^R)	275	15
Colonies w/insert (Kan^R-Amp^R)	258	9
Percent Recombinants	94%	60%

A 7 kb ampicillin resistance gene sequence was PCR amplified with Expand™ polymerase. PCR products were loaded onto one gel with crystal violet and another gel stained with ethidium bromide. The 7 kb products were gel purified and cloned into the pCR®-XL-TOPO® vector. The number of recombinants was determined by plating 125 µl of each transformation on LB plates containing either kanamycin or kanamycin and ampicillin.

‡ When using chemically competent *E. coli*, you can expect ≥70% recombinants. For the best results, electrocompetent cells are recommended because electroporation yields higher transformation efficiencies than chemical methods and does not bias against larger constructs.

† The TOPO® XL PCR Cloning Kit has been tested with Expand™ and eLONGase®.

TOPO® Cloning vectors for optimized sequencing

TOPO® Cloning Kits for Sequencing

The TOPO® Cloning Kits for Sequencing allow fast cloning and streamlined sequencing of PCR products. The kits contain TOPO® Cloning vectors with a minimized multiple cloning site that positions the T7 and T3 priming sites only 33 bp away from the PCR product insertion site (Figure 10). This means you'll sequence more of your insert and less of the vector.

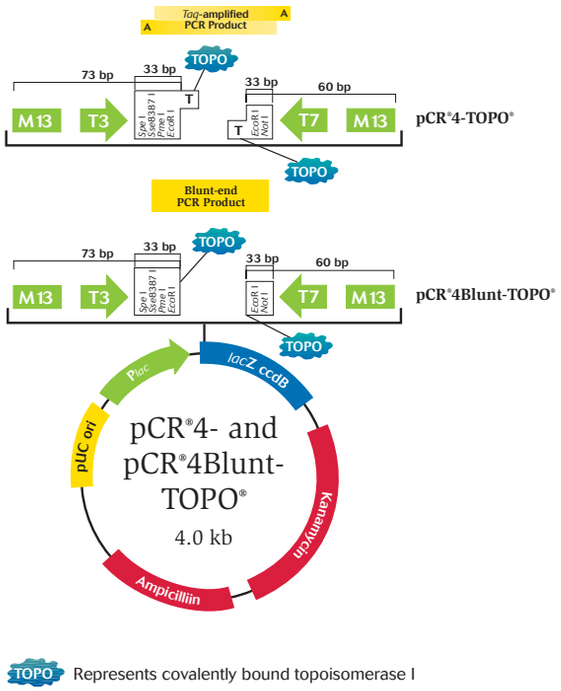
Choice of vectors

The pCR®4-TOPO vector supplied in the TOPO TA Cloning® Kit for Sequencing has 3´-T overhangs for cloning *Taq*-amplified PCR products. The pCR®4Blunt-TOPO® vector supplied in the Zero Blunt® TOPO® PCR Cloning Kit for Sequencing has blunt ends for cloning PCR products amplified with proofreading polymerases. Each vector is supplied linearized and topoisomerase I-activated, enabling 5-minute, bench-top ligation and yielding ≥95% recombinants. Some of their convenient features include:

- T7 and T3 promoter/priming sites for sequencing and *in vitro* transcription/translation
- M13 forward (-20) and reverse priming sites for sequencing or PCR screening
- The *ccd*B gene to eliminate background and improve results (see box, page 5)
- *Eco*R I sites flanking the PCR product insertion site for easy removal of inserts
- Unique *Sse*8387 I site to produce nested deletions for sequencing internal regions of your insert
- Kanamycin and ampicillin resistance genes for your choice of selection in *E. coli*

With their minimized multiple cloning sites, the TOPO® Cloning Vectors for Sequencing enable effective cloning and streamlined sequencing for all of your PCR products.

Figure 10 - TOPO® Cloning Vectors for Sequencing



For shotgun sequencing and cloning unknown sequences, refer to TOPO® Cloning Kits for Genomic Analysis, pages 14 and 15

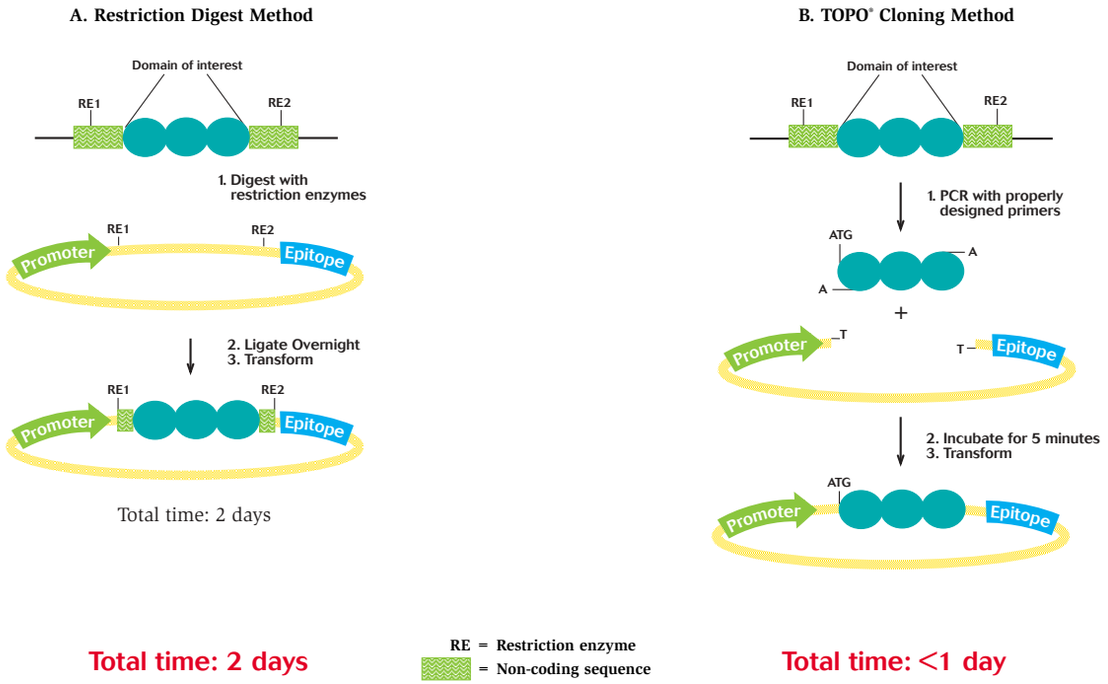
Effective way to express

Non-Directional TOPO® Cloning Expression Kits

Using restriction enzymes to clone your gene of interest into an expression vector often forces you to compromise the final sequence of your insert, especially when there are no useful restriction sites close to your gene's coding sequence.

TOPO® Cloning Expression vectors eliminate potential expression problems by allowing you to insert the exact DNA sequence you require by performing PCR with appropriately designed primers. The resulting recombinant expression vector contains your exact DNA sequence without any non-coding regions (Figure 11). Non-directional TOPO® Cloning Expression vectors are available in the prokaryotic, yeast, insect, and mammalian expression systems.

Figure 11 - Comparison of cloning strategies



For more information on TOPO® Cloning Expression vectors, see the Invitrogen catalog or visit our web site at **www.invitrogen.com/topo**

Time-tested expression results

Directional TOPO® Expression Technology

Directional TOPO® Cloning enables you to clone your blunt-end PCR products in a 5′→3′ orientation into a proven expression vector using a 5-minute ligation reaction. Directional TOPO® Cloning vectors contain a single-strand GTGG overhang on the 5′ end and a blunt end on the 3′ end. The four-nucleotide overhang invades the double-strand DNA of the PCR product and anneals to the CACC sequence that you place in your 5′ primer. Topoisomerase I then ligates the PCR product in the correct orientation.

With Directional TOPO® Cloning Expression Kits, you will:

- Save time – TOPO® Cloning of your PCR product takes just five minutes (Figure 12)
- Obtain efficient cloning results - >90% of your recombinant clones will be in the correct orientation for expression (Figure 13)
- Achieve high-level expression – vectors carry a powerful promoter for expression

Figure 12- Time comparison of cloning strategies

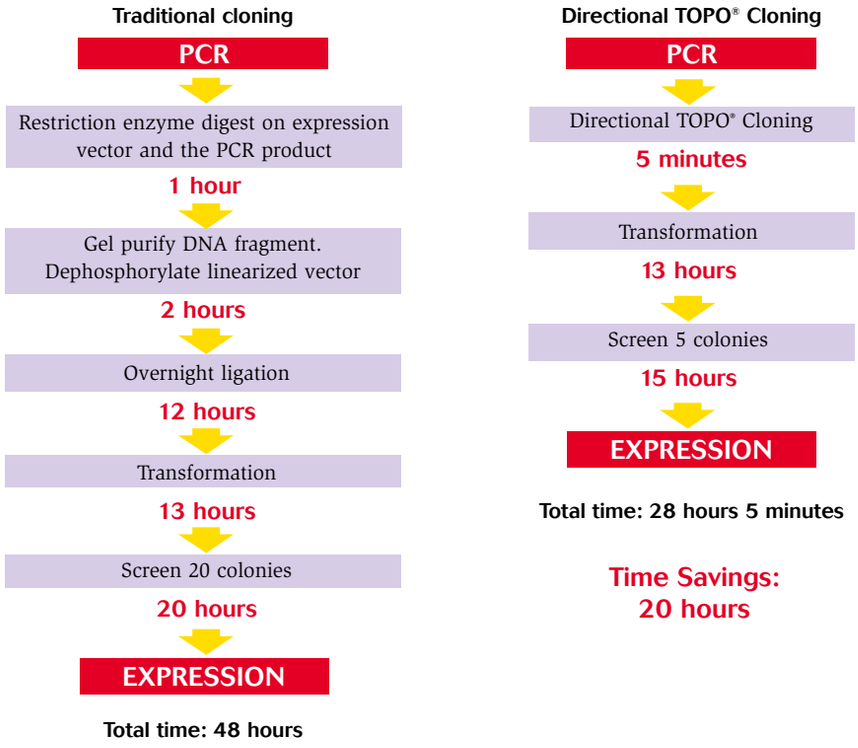


Figure 13 – Directional cloning of human open reading frames into pcDNA3.1D/V5-His-TOPO® vector

Clone	No. in correct orientation	No. in reverse orientation	% Correct
D32129 (1171 bp)	18	2	90%
AF016582 (1504 bp)	20	0	100%
AF020833 (1036 bp)	19	1	95%

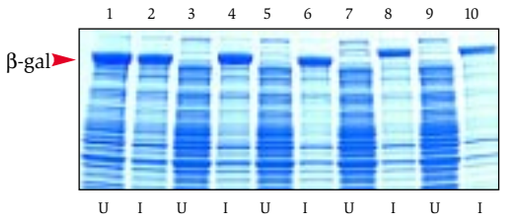
High-level protein production in *E. coli*

Champion™ pET Directional TOPO® Expression Kits

Champion™ pET vectors are powerful *E. coli* expression vectors that use the highly-efficient T7 RNA polymerase to achieve strong transcription levels and high protein yields. T7 RNA polymerase is expressed by host *E. coli* under the control of the IPTG-inducible *lacUV5* promoter. This allows you to regulate transcription with IPTG. The additional *lac*O element found in the T7*lac* promoter used in the pET vectors further reduces the basal expression levels while maintaining strong transcriptional activity upon induction with IPTG. Reported yields of recombinant proteins from the pET vectors are typically in the range of tens to hundreds of milligrams per liter of culture (Figure 14). The pET Directional TOPO® Expression Kits offer:

- Advanced cloning technology
- High-level and regulated expression
- Multiple purification, detection, and cleavage options

Figure 14 – Strong expression in pET Directional TOPO® vectors



U = Uninduced I = Induced

The *lacZ* gene was cloned directionally into pET100/D-TOPO®, pET101/D-TOPO®, and pET102/D-TOPO® vectors and cloned using the restriction digest method into pET15b and pET32a vectors. Constructs were transformed into BL21 Star™(DE3) *E. coli*. A single colony from each transformation was used to inoculate 1 ml LB medium supplemented with 100 µg/ml ampicillin. Induction with 1 mM IPTG was performed at OD_{600} = 0.5. Two and one-half hours post induction, cultures were harvested by centrifugation. Pellets were resuspended in 300 µl sample buffer. Ten microliters of each sample was analyzed on a 4-20% Novex® Tris-Glycine gel.

Note: pET15b contains an N-terminal 6xHis tag while pET32a contains an N-terminal thioredoxin fusion and a C-terminal 6xHis tag.

Lanes 1 and 2: pET15b/*lacZ*
Lanes 3 and 4: pET101/D-TOPO®/*lacZ*
Lanes 5 and 6: pET100/D-TOPO®/*lacZ*
Lanes 7 and 8: pET102/D-TOPO®/*lacZ*
Lanes 9 and 10: pET32a/*lacZ*

BL21 Star™: for highest expression

BL21 Star™(DE3) One Shot® Competent *E. coli* is designed to give you significantly improved expression levels of recombinant protein. BL21 Star™ is the only strain that contains a mutation in the endonuclease RNase. This mutation causes less RNA degradation and as a result more RNA is available for translation. With BL21 Star™ you can get up to a ten-fold increase in protein production. Use BL21 Star™ with any T7 expression system. For added convenience BL21 Star™ are available in the convenient, cost-effective One Shot® format.

Product	Efficiency	Quantity	Cat. no.
BL21 Star™ One Shot® Chemically Competent *E. coli*	1 x 10^8	20 x 50 µl	C6010-03

BL21-AI™: for tightly regulated, highly inducible expression

BL21-AI™ One Shot® Competent *E. coli* is an arabinose-inducible strain designed to give you the maximum expression with the tightest regulation available from a T7 expression system. It's the only strain that gives you both tight regulation and high yields, making it great for high-level expression of toxic proteins. Because it has the tightly regulated arabinose-inducible *araBAD* promoter upstream of the T7 RNA polymerase gene, you can use it with any T7 promoter-based vector. All that in the convenient, cost-effective One Shot® format.

Product	Efficiency	Quantity	Cat. no.
BL21-AI™ One Shot® Chemically Competent *E. coli*	> 1 x 10^8	20 x 50 µl	C6070-03

High-level, constitutive mammalian expression

pcDNA™3.1 Directional TOPO® Expression Kit

The pcDNA3.1™ Directional TOPO® Expression Kit offers efficient and directional cloning of blunt-end PCR products. The pcDNA3.1D/V5-His-TOPO® vector contains the following features (Figure 15):

- The CMV promoter for high-level constitutive expression in a wide range of mammalian cell hosts
- C-terminal V5-His epitope for easy detection of recombinant protein and rapid purification using ProBond™ resin
- Neomycin resistance gene for selection of stable mammalian cell lines with Geneticin® selection agent

To demonstrate the high-level expression achieved with pcDNA3.1D/V5-His-TOPO® vectors, expression of *lac*Z from the vector was compared to pcDNA™3.1/V5-His/*lac*Z (Figure 16). The results show that the equivalent expression levels are achieved.

Figure 15 – pcDNA3.1D/V5-His-TOPO® Vector

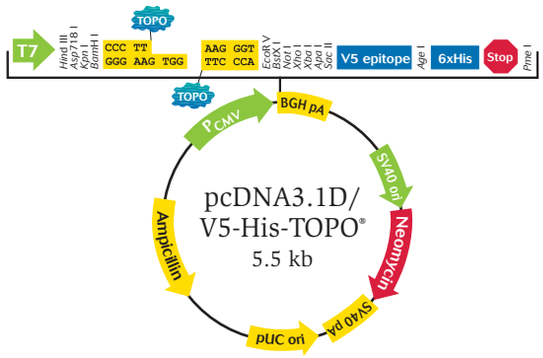
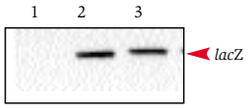


Figure 16 – Expression of β-galactosidase from pcDNA3.1D/V5-His-TOPO®



The *lac*Z gene was PCR amplified and cloned into pcDNA3.1/V5-His-TOPO® and pcDNA3.1D/V5-His-TOPO® directional vectors. These constructs were each transfected into 7.5 x 10^4 COS-7 cells using the calcium phosphate method. Forty-eight hours post transfection, cells were harvested. Ten micrograms of total protein was loaded on each lane of an SDS-PAGE gel. Expression was analyzed by western blot using an anti-β-gal antibody. Lane 1: Mock; Lane 2: pcDNA™3.1/V5-His/*lac*Z; Lane 3: pcDNA™3.1D/V5-His/*lac*Z.

High-level, stable expression in mammalian cells

ViraPower™ Lentiviral Expression System

The ViraPower™ Lentiviral Expression System provides stable gene expression and reproducible delivery to both dividing and non-dividing cells. You can use the pLenti6/V5-D-TOPO® vector to take advantage of a simple, 5-minute TOPO® Cloning reaction to prepare your Lentiviral Expression Vector (Figure 17). The vector contains:

- CMV promoter for high-level expression
- C-terminal V5 tag for convenient detection
- Blasticidin resistance gene for fast, efficient stable selection
- Components for efficient packaging of your gene of interest

The ViraPower™ Lentiviral Directional TOPO® Expression System provides you with the high levels of stable gene expression necessary for valid results in virtually any cell line (Figure 18).

Figure 17 – pLenti6/V5-D-TOPO® Vector

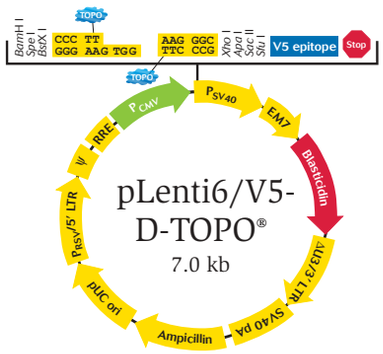
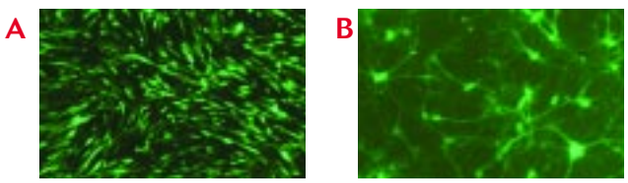


Figure 18 - Lentivirus readily transduces non-dividing cell types



Primary fibroblasts

Adult rat hippocampal neurons

Contact-inhibited, non-dividing, quiescent primary human foreskin fibroblasts (A) or adult hippocampal neurons (B) were transduced with pLenti6/CMV/V5-GFP at an MOI of 5 or 1, respectively. GFP was detected 48 hours post-transduction by fluorescence microscopy.

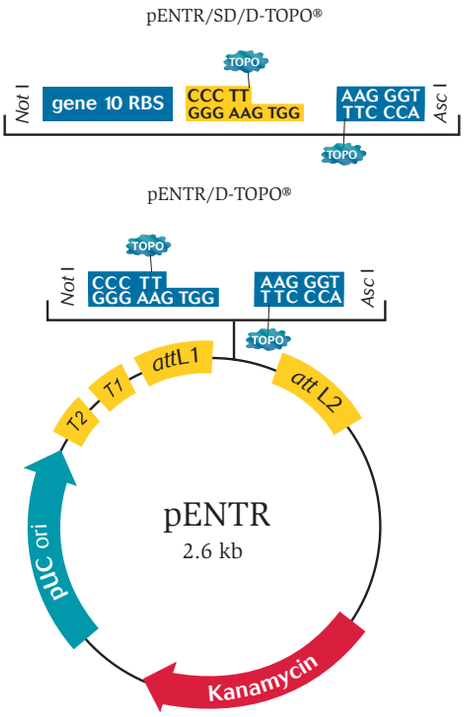
Quick and easy way to enter the Gateway® System

Gateway® entry clones with Directional TOPO® Cloning

Two Directional TOPO® entry vectors are available for creating a Gateway® entry clone. Once you have cloned your PCR product into an entry vector, it can be rapidly shuttled to a wide variety of Gateway® destination vectors for your downstream expression and functional analysis needs. pENTR/D-TOPO® and pENTR/SD/D-TOPO® vectors offer the following features (Figure 19):

- *att*L recombination sites for efficient transfer into any Gateway® destination vector
- Universal M13 sites to facilitate sequencing
- pUC-based ori for high plasmid yields
- Kanamycin resistance gene for selection in *E. coli*

Figure 19 - Gateway™ Directional TOPO® entry vectors

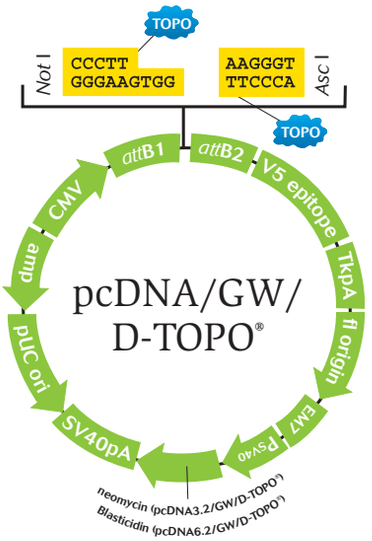


pcDNA/GW/D-TOPO® Vectors

pcDNA/GW/D-TOPO® vectors give you high-level expression in mammalian cells, and allow you to save significant cloning and screening time. Once your gene of interest is cloned into the vector, it will immediately express from the built-in CMV promoter. This powerful promoter drives high-level constitutive expression in a wide variety of mammalian cells. pcDNA/GW/D-TOPO® vectors offer the following (Figure 20):

- *att*B recombination sites for rapid conversion into a Gateway® entry clone
- Universal M13 sites to facilitate sequencing
- pUC-based ori for high plasmid yields
- Choice of neomycin or Blasticidin resistance genes for selection in mammalian cells

Figure 20 - pcDNA/GW/D-TOPO® expression vectors



Easily clone thousands of PCR products

High-Throughput TOPO® Cloning

HTP TOPO® Cloning kits couple TOPO® technology to high-throughput format, enabling you to easily and simultaneously clone thousands of PCR products. With 500 reactions of TOPO® vector supplied in a single tube, you can quickly set up your TOPO® reactions in multi-well plates, incubate for only 5 minutes, then transform the supplied chemically competent *E. coli* with a multi-channel pipette (Table 5). With the speed and high efficiency of TOPO® Cloning, you'll not only get your clones fast, you'll get them the first time, eliminating time wasted repeating unsuccessful reactions.

Table 5 – HTP TOPO® Cloning Kits

Description	Vector*	TOP10 *E. coli* Format†	Reactions	Cat. no.
HTP TOPO TA Cloning® Kit	pCR®2.1-TOPO®	Bulk	500	K4500-500
		MultiShot™	480	K4500-480
		MultiShot™ StripWell	480	K4500-05
HTP TOPO TA Cloning® Kit Dual Promoter	pCR®II-TOPO®	Bulk	500	K4600-500
		MultiShot™	480	K4600-480
		MultiShot™ StripWell	480	K4600-05
HTP TOPO TA Cloning® Kit for Sequencing	pCR®4-TOPO®	Bulk	500	K4575-500
		MultiShot™	480	K4575-480
		MultiShot™ StripWell	480	K4575-05
HTP Zero Blunt® TOPO® PCR Cloning Kit for Sequencing	pCR®4Blunt-TOPO®	Bulk	500	K2875-500
		MultiShot™	480	K2875-480
		MultiShot™ StripWell	480	K2875-05
Directional TOPO® pENTR™ Vectors	pENTR/D-TOPO®	Bulk	500	K2400-500
		MultiShot™ StripWell	480	K2400-480
	pENTR/SD/D-TOPO®	Bulk	500	K2420-500
		MultiShot™ StripWell	480	K2420-480

* For more information on these vectors, see the catalog or visit our web site at www.invitrogen.com/topo

† Bulk (five 5-ml aliquots)
• MultiShot™ (five 96-well plates)
• MultiShot™ StripWell (five stripwell plates)

Streamlined shotgun subcloning

TOPO® Shotgun Sequencing Kit

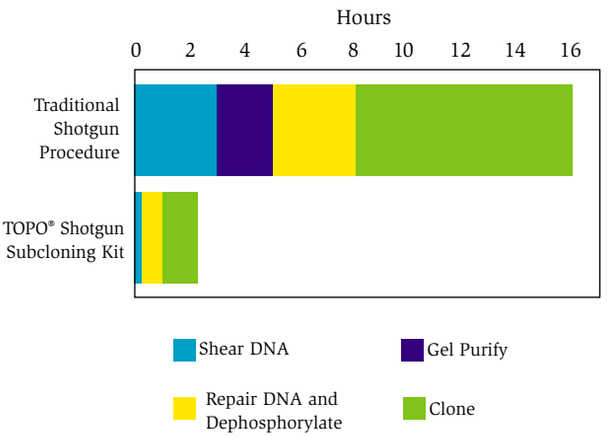
The TOPO® Shotgun Subcloning Kit is specifically designed to expedite traditional shotgun subcloning procedures by saving both time and effort in each step.

TOPO® Shotgun technology was built upon examining each step of a traditional shotgun subcloning protocol and eliminating tedious steps and lengthy incubations (Figure 21).

This kit includes a specialized vector with numerous features to make shotgun subcloning easier than ever before. The TOPO® Shotgun Subcloning Kit includes pCR®4Blunt-TOPO® vector (Figure 10, page 7) that allows you to:

- Easily construct shotgun libraries—readily accepts blunt-ended DNA fragments. Cloning takes just 25 minutes.
- Eliminate multiple inserts—only vectors containing single inserts will circularize and propagate.
- Keep background low—expression of a lethal *ccd*B gene ensures only recombinant clones will grow (page 5).
- Streamline sequencing—increase efficiency by reading more insert and less vector.

Figure 21 – TOPO® Shotgun Subcloning Kit saves over 13 hours of time versus traditional shotgun method



The TOPO® Shotgun Subcloning Kit utilizes a nebulizer—a small plastic device used to atomize liquids—and compressed air to shear large DNA into 2 kb fragments suitable for cloning into the pCR®4Blunt-TOPO® vector.

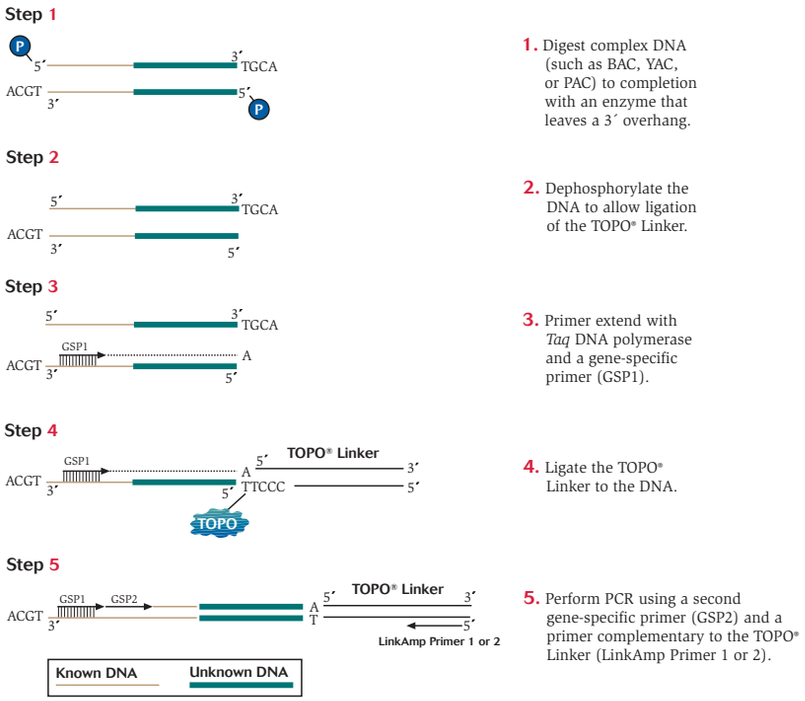
The fast way to fill in sequence gaps

TOPO® Walker Kit

The TOPO® Walker Kit eliminates the need for hybridization-based library screens when isolating unknown DNA sequences. Instead, the kit uses a simple 5-step protocol to save time in your chromosome walking experiments (Figure 22). Once the unknown DNA fragment is amplified, the PCR product can be sequenced directly. There's no need to carry out cloning experiments that can add additional days to your experiment. The TOPO® Walker Kit saves you time by:

- Ligating a topoisomerase-activated linker to the unknown end of a DNA fragment in just 5 minutes.
- Using PCR to rapidly amplify the unknown sequence
- Sequencing the PCR product directly—no subcloning steps are required

Figure 22 – How TOPO® Walker works

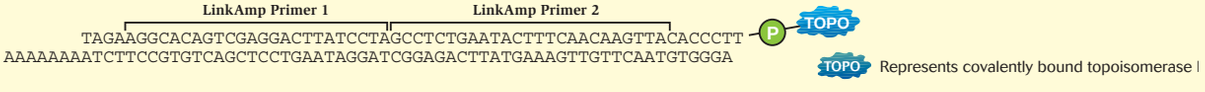


1. Digest complex DNA (such as BAC, YAC, or PAC) to completion with an enzyme that leaves a 3´ overhang.
2. Dephosphorylate the DNA to allow ligation of the TOPO® Linker.
3. Primer extend with *Taq* DNA polymerase and a gene-specific primer (GSP1).
4. Ligate the TOPO® Linker to the DNA.
5. Perform PCR using a second gene-specific primer (GSP2) and a primer complementary to the TOPO® Linker (LinkAmp Primer 1 or 2).

PCR makes it easy — TOPO® Technology makes it fast

The key to the speed of the TOPO® Walker Kit is the TOPO® Linker (Figure 23). The TOPO® Linker is a 58 bp, double-stranded DNA sequence containing two PCR primer sites, and a 3´- T overhang in which topoisomerase I is covalently bound. In just 5 minutes you can ligate the TOPO® Linker to the 3´-A overhang of your DNA sequence. Subsequent PCR using a primer specific to the linker and a gene-specific primer from your known sequence amplifies the sequence gap.

Figure 23 – The topoisomerase-activated TOPO® Linker



Custom TOPO® Cloning Adaptation Service

The development of gene-based therapeutics and diagnostics products requires the rapid analysis of a vast number of gene sequences. When screening gene targets that are of commercial importance, being the first to identify, clone, express, and validate these genes is crucial.

Invitrogen's Custom TOPO® Cloning Adaptation Service puts the power of TOPO® Cloning into your vector.

With your own vector adapted to TOPO® Technology, you can:

- TOPO® Clone your favorite vector – you won't have to compromise on vector features to meet your needs
- Save time – TOPO® Cloning takes only 5 minutes and is so effective, you won't have to repeat experiments
- Maintain your current experimental strategy – adapting your own vector for TOPO® Cloning doesn't change your downstream studies, but it will get you there faster

Your results are guaranteed

Every TOPO® Cloning Kit is functionally tested to ensure that you get successful results. To test each kit, a control DNA fragment is PCR amplified with the appropriate DNA polymerase. The size of the PCR product is verified and TOPO® Cloned. The resulting percent recombinants must meet our stringent standards in order to pass quality control.

Clone it today

With unrivaled consistency and speed, TOPO® Cloning Kits are the most effective way to clone PCR products. The rapid 5-minute, bench-top ligation enables you to perform your PCR, TOPO® Cloning, and transformation reactions all in the same day. And up to 99% recombinants ensures that you'll get your clones the first time, every time. For PCR cloning results you can count on, order a TOPO® Cloning Kit today.

References:
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4. Bernard, P. and Couturier, M. (1992) *J. Mol. Biol.* **226:** 735-745.
5. Bernard, P. *et al.* (1993) *J. Mol. Biol.* **234:** 534-541.
6. Rand, K.N. (1996) *Elsevier Trends Technical Tips Online.*

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With TOPO® Cloning Kits You Can:

- Clone *Taq*-amplified, blunt-end, and long PCR products
- Ligate in 5 minutes at room temperature
- Obtain up to 99% recombinants

Invitrogen™ life technologies

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