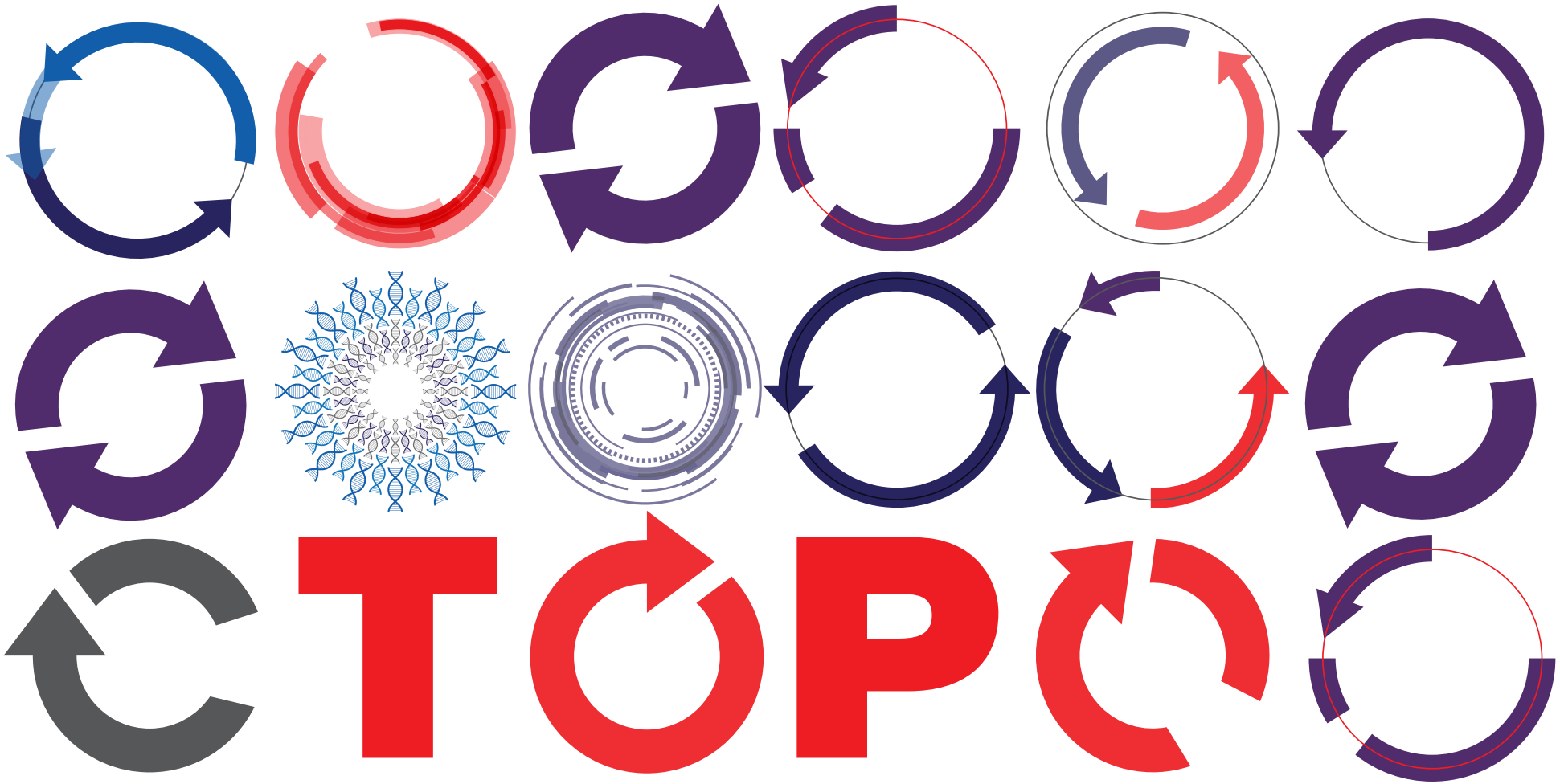
invitrogen



TOPO PCR cloning solutions

Simple. Flexible. Exceptional.

ThermoFisher SCIENTIFIC

Get the cloning results you can count on

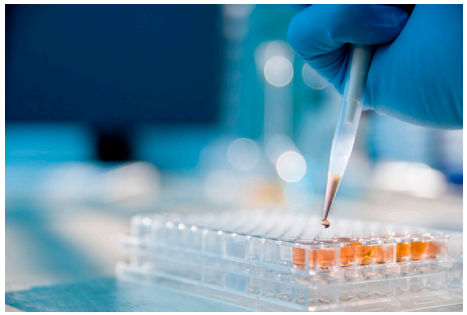
Twenty years ago, Invitrogen™ TOPO™ technology became known for superior quality and game-changing innovation in cloning. Today, Invitrogen™ TOPO™ PCR cloning kits are still among the fastest, simplest, and most flexible cloning solutions available.

TOPO cloning technology is:

- **Efficient**—up to 95% of clones contain desired insert
- **Fast**—5-min, room-temperature reaction
- **Easy**—simple 3-step procedure
- **Proven**—over 20,000 citations
- **Flexible**—available with or without competent cells, and in multiple reaction sizes and formats to accommodate your choice of polymerase

Whether you're performing general subcloning, sequencing, TA or high-fidelity blunt-end cloning, long-fragment cloning, expression-vector cloning, or directional cloning, or you're using the Invitrogen™ Gateway™ system, there's a TOPO cloning solution that's right for you. TOPO PCR cloning kits help you get the right clone sooner, freeing up your time to answer more important questions.

Contents



The technology behind TOPO cloning

TOPO cloning is as easy as 1, 2, 3

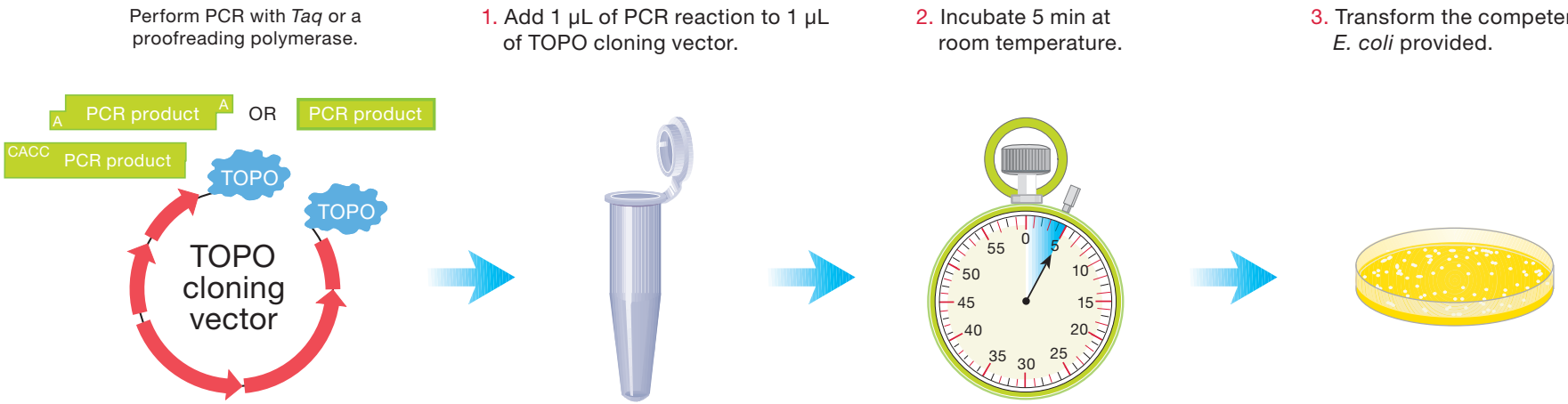


Figure 1. TOPO PCR cloning requires just three easy steps. Simply combine your PCR product and a TOPO cloning vector in the provided reaction buffer, wait 5 minutes, then transform an *E. coli* strain. With TOPO cloning, the additional time, steps, and reagents required for ligase-mediated cloning are eliminated.

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 attached to the 3′ thymidine. It cleaves one DNA strand, enabling the DNA to unwind. The enzyme then religates the ends of the cleaved strand and is released from the DNA.

To harness the religating activity of topoisomerase, TOPO™ vectors are provided linearized with topoisomerase I covalently bound to the 3′ phosphate on each end. This enables the vectors to readily ligate DNA sequences with compatible ends. The ligation is complete in only 5 minutes (Figure 1).

TOPO TA cloning

Invitrogen™ TOPO™ TA Cloning™ Kits are designed for cloning PCR products amplified by *Taq* DNA polymerase, which leaves an adenine at the 3´ end of the product, creating overhangs or sticky ends (Figure 2). The TOPO™ TA vectors include 3´-thymine overhangs for fast, effective, and direct cloning of *Taq*-amplified PCR products.

Applications

Subcloning

Invitrogen™ TOPO™ TA subcloning kits utilize our pCR™2.1-TOPO™ TA Vector (Figure 3), which features:

- T7 promoter and M13 forward- and reverse-primer sites for *in vitro* transcription and sequencing
- *Eco*RI sites flanking the PCR product insertion site for easy excision of inserts
- 15 convenient and validated restriction sites flanking the insert for easy, directional subcloning
- Ampicillin- and kanamycin-resistance genes for your choice of selection in *E. coli*
- Easy blue or white colony screening for selection of recombinants

Sequencing

Invitrogen™ TOPO™ TA sequencing kits utilize our pCR™4-TOPO™ TA Vector, which allows you to directly select recombinants by disrupting the lethal *E. coli* gene, *ccdB*. The vector contains the *ccdB* gene fused to the C-terminus of the *lacZα* gene (Figure 4). Ligation of a PCR product disrupts expression of the *lacZα–ccdB* gene fusion, permitting growth of only positive recombinants upon transformation. Competent cells that contain nonrecombinant vector are killed upon plating, so blue/white screening is not required. The pCR4-TOPO TA Vector features:

- Minimal multiple cloning site to shorten the distance between sequencing primer sites and the insert site to as little as 33 bp
- Ampicillin- and kanamycin-resistance genes and a *lacZα–ccdB* gene fusion for positive selection
- Flanking *Eco*RI sites for simplified excision of cloned PCR products and a unique *Sse*8387I site for generation of nested deletions prior to sequencing

Helpful tips

Add restriction sites and/or universal primer sites to either end of a PCR product using our TOPO™ subcloning and sequencing vectors

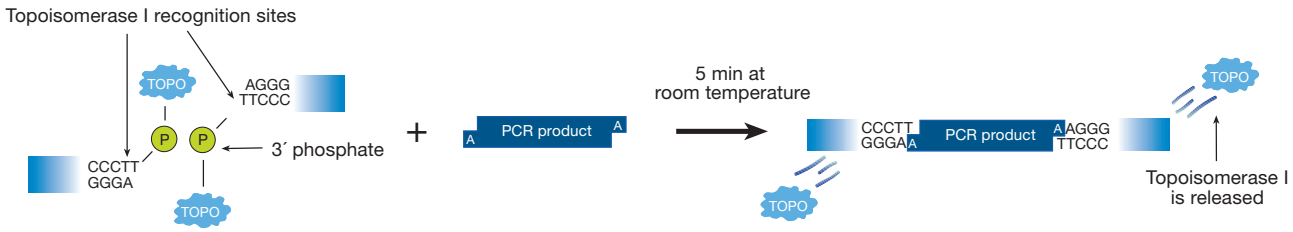


Figure 2. TOPO TA cloning of *Taq*-amplified DNA.

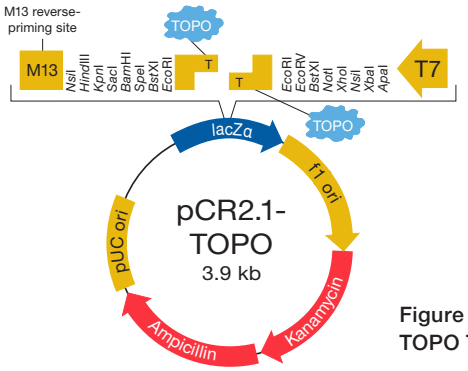


Figure 3. The pCR2.1-TOPO TA Vector.

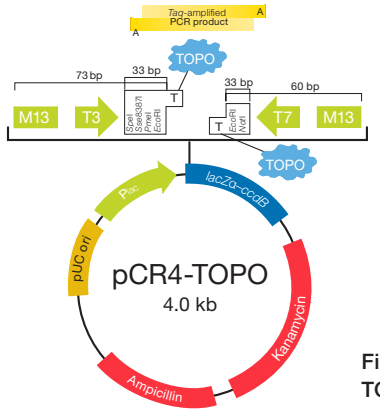


Figure 4. The pCR4-TOPO TA Vector.

High-fidelity blunt-end TOPO cloning

Invitrogen™ Zero Blunt™ TOPO™ PCR cloning kits are designed for cloning PCR products amplified with high-fidelity polymerases such as Invitrogen™ Platinum™ SuperFi™ DNA Polymerase, which features >100x *Taq* fidelity and robust amplification of even the most difficult targets. These polymerases have extensive 3´ to 5´ exonuclease activity, and leave blunt-ended PCR products rather than 3´-A overhangs. Therefore, the vectors supplied in our Zero Blunt TOPO PCR cloning kits have blunt ends as well for effective ligation (Figure 5).

Applications

Subcloning

Invitrogen™ Zero Blunt™ TOPO™ subcloning kits utilize our pCR™-Blunt II-TOPO™ Vector (Figure 6). It features:

- The *ccdB* gene for positive selection, only permitting growth of plasmid vectors with recombinants
- Kanamycin- and Gibco™ Zeocin™ antibiotic–resistance genes for your choice of selection in *E. coli*
- *Eco*RI sites flanking the PCR product insertion site for easy excision of inserts
- SP6 promoter/primer site for *in vitro* transcription and sequencing
- M13 forward- and reverse-primer sites for sequencing or PCR screening

Sequencing

Invitrogen™ Zero Blunt™ TOPO™ sequencing kits utilize our pCR™4Blunt-TOPO™ Vector (Figure 7). It features:

- Minimal multiple cloning site to shorten the distance between sequencing primer sites and the insert site to as little as 33 bp
- The *ccdB* gene for positive selection, only permitting growth of plasmid vectors with recombinants
- Ampicillin- and kanamycin-resistance genes for your choice of selection in *E. coli*
- *Eco*RI sites flanking the PCR product insertion site for easy excision of inserts and a unique *Sse*8387I site for generation of nested deletions prior to sequencing

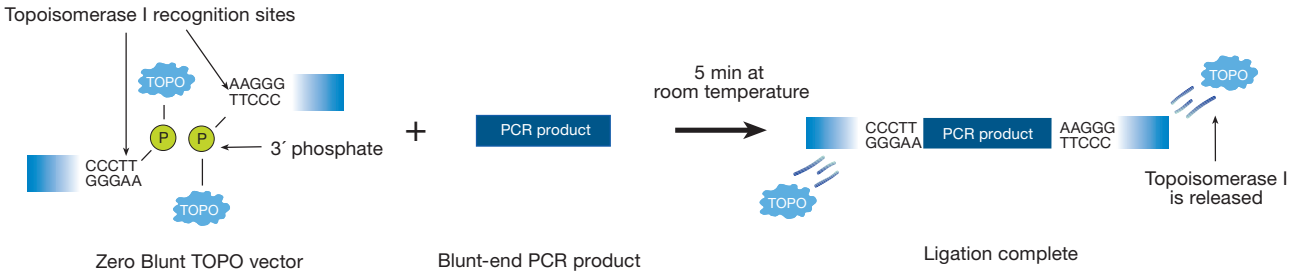


Figure 5. Zero Blunt TOPO cloning of blunt-end DNA.

Did you know

All Zero Blunt TOPO vectors contain the lethal *ccdB* gene. With this positive selection method, only clones with an insert will grow as colonies on your plate, giving you the confidence you need in your blunt-end cloning results.

- T7 and T3 promoters for *in vitro* transcription
- M13 forward, M13 reverse, T7 and T3 priming sites for sequencing

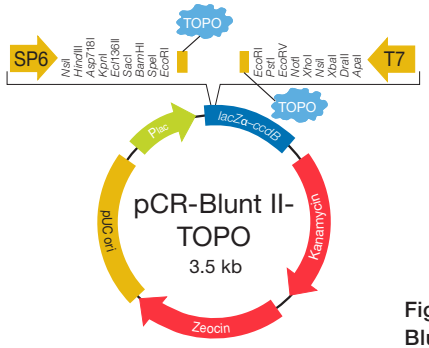


Figure 6. The pCR-Blunt II-TOPO Vector.

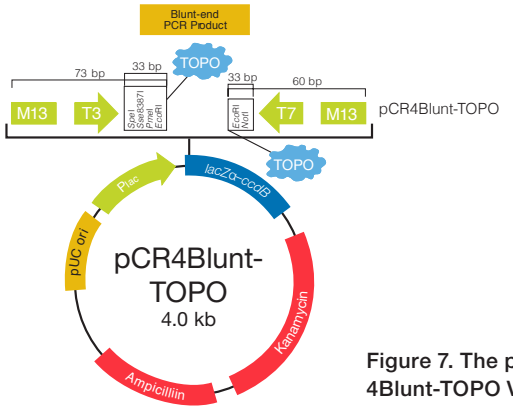


Figure 7. The pCR-4Blunt-TOPO Vector.

TOPO long-fragment cloning

The Invitrogen™ TOPO™ XL-2 Complete PCR Cloning Kit provides all the necessary elements for highly efficient cloning of extra-long PCR products (up to 13 kb). The kit uses the linearized and topoisomerase 1-activated pCR™-XL-2-TOPO™ vector, which is compatible with the cloning of blunt-end PCR fragments. Amplification of long PCR fragments is enabled by Invitrogen™ Platinum™ SuperFi™ Green PCR Master Mix, which is included in the kit (Figure 8). Topoisomerase I activation of the vector allows PCR products to be ligated in just 5 minutes on your benchtop, resulting in high cloning efficiencies (Figure 9).

The TOPO XL-2 Complete PCR Cloning Kit includes:

- **TOPO XL-2 PCR Cloning Kits** containing the pCR-XL-2-TOPO vector
- **Platinum SuperFi Green PCR Master Mix**—featuring a high-fidelity (>100x *Taq* fidelity), proofreading DNA polymerase to help generate more accurate long PCR amplicons
- **Invitrogen™ PureLink™ Quick Gel Extraction and PCR Purification Combo Kit**—designed to purify DNA fragments in less than 30 minutes from agarose gels or direct PCR purification using a silica-based spin cartridge
- **Invitrogen™ One Shot™ OmniMAX™ 2 T1^{R} Chemically Competent *E. coli* Cells**—a highly efficient chemically competent cell line, well-suited for use in all cloning applications

Features of the pCR-XL-2-TOPO vector (Figure 10) include:

- *ccdB* gene for positive selection
- *Eco*RI site flanking the PCR product insertion site for easy excision of inserts
- Ampicillin- and kanamycin-resistance genes for your choice of antibiotic selection
- T7 promoter/priming site for *in vitro* transcription
- T7, T3, and M13 forward- and reverse-primer sites for sequencing

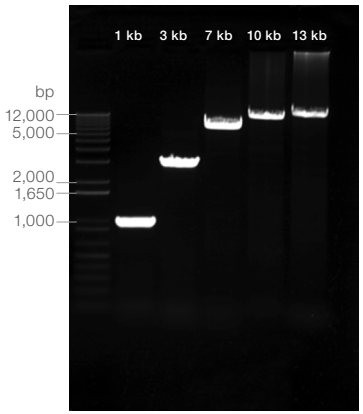


Figure 8. Platinum SuperFi DNA Polymerase exhibits high specificity while amplifying a wide range of target sizes. 1 kb, 3 kb, 7 kb, 10 kb, and 13 kb targets from Thermo Scientific™ Lambda genomic DNA were amplified using Platinum SuperFi DNA Polymerase. Manufacturer's standards for PCR cycling parameters were followed.

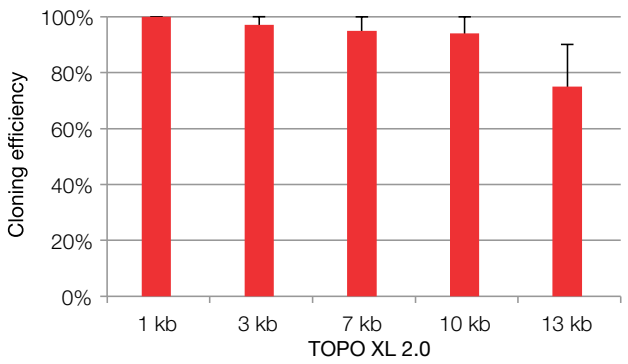


Figure 9. TOPO XL-2 PCR Cloning Kits show high cloning efficiencies for a wide range of targets, up to 13 kb in length. 1 kb, 3 kb, 7 kb, 10 kb, and 13 kb Lambda DNA targets were cloned using the TOPO XL-2 PCR Cloning Kit workflow.

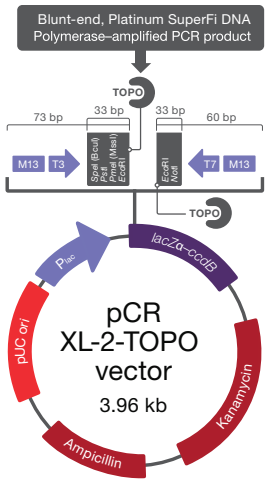


Figure 10. The pCR-XL-2-TOPO vector.

Directional TOPO cloning

Directional TOPO cloning enables cloning of blunt-ended PCR products directly into an expression vector in a single orientation. With a 5-minute ligation reaction, this technology eliminates subcloning steps and saves you time. Directional TOPO cloning vectors have a single-stranded GTGG overhang at one end and a blunt end at the other (Figure 11). The GTGG overhang invades the double-stranded DNA of the PCR product and anneals to the CACC sequence that you place in your primer. Topoisomerase I then ligates the PCR product in the correct orientation for expression. With Invitrogen™ directional TOPO™ cloning expression kits, you can:

- **Save time**—TOPO cloning of your PCR product takes just 5 minutes
- **Obtain efficient cloning results**—more than 90% of recombinant clones will be in the correct orientation for expression
- **Achieve high-level expression**—vectors carry powerful promoters for expression in *E. coli* or mammalian cells

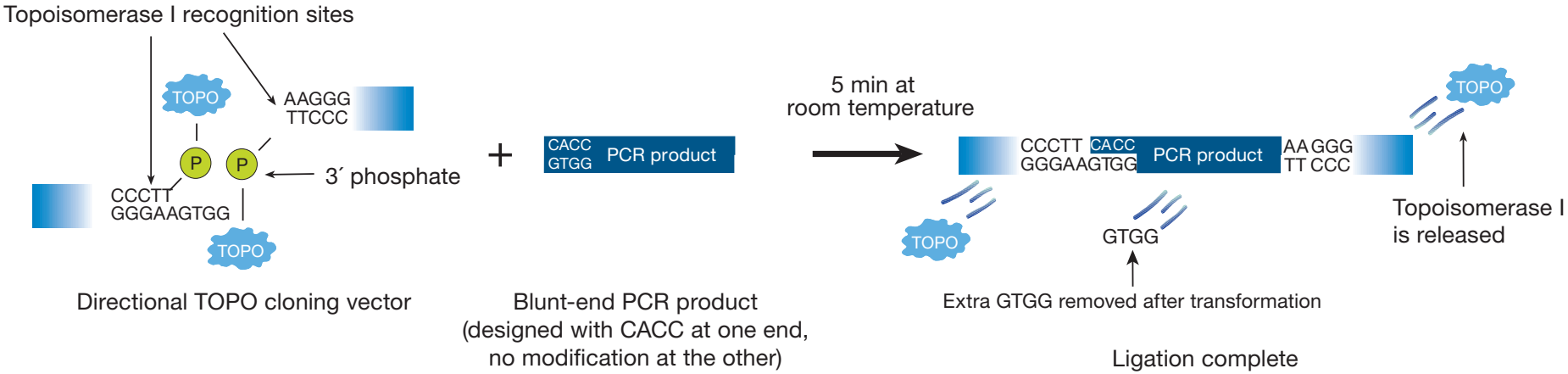
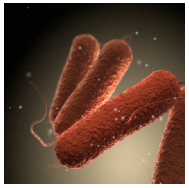


Figure 11. Directional TOPO cloning of blunt-end DNA.

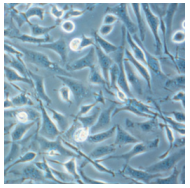
TOPO expression vector cloning

TOPO cloning for *E. coli* expression



Invitrogen™ Champion™ pET Directional TOPO™ vectors are powerful *E. coli* expression vectors that use the highly efficient T7 RNA polymerase to achieve 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 *lacO* element found in the T7 *lac* promoter used in these vectors further reduces basal expression levels while enabling strong transcriptional activity upon induction with IPTG. Reported yields of recombinant proteins from pET vectors are typically in the range of tens to hundreds of milligrams per liter of culture.

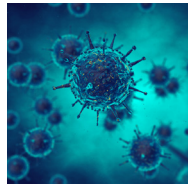
TOPO cloning for mammalian expression



For constitutive mammalian expression, the Invitrogen™ pcDNA™ mammalian expression vector is one of the most popular expression vectors available today. The newest versions are the pcDNA™ 3.3-TOPO™ TA Vector and pcDNA™ 3.4-TOPO™ TA Vector, which enable expression of exceptionally high levels of recombinant protein in mammalian cells and are ideal for use with our Gibco™ Expi293™ and FreeStyle™ Expression Systems. These vectors offer:

- Two- to five-fold higher protein yields compared to other expression vectors
- Generation of native (or tagged) proteins without extraneous amino acids—ideal for antibody production and structural biology
- Full-length human cytomegalovirus (CMV) immediate-early promoter/enhancer for high-level gene expression in a wide range of mammalian cells
- TOPO cloning site for rapid and efficient (>85%) cloning of *Taq*-amplified PCR products
- Neomycin-resistance gene for selection of stable cell lines with Gibco™ Geneticin™ antibiotic
- pUC origin for high copy number and maintenance of the plasmid in *E. coli*

ViraPower HiPerform Lentiviral Expression Systems



Invitrogen™ ViraPower™ HiPerform™ Lentiviral Expression Systems are designed to provide stable gene expression and reproducible delivery to both dividing and nondividing cells. This system offers:

- Greater than four-fold increase in protein expression compared to other lentiviral vectors
- Efficient gene delivery into cells that are virtually impossible to transfect
- Accurate and fast 2-day titer determination of functional lentivirus
- A choice of Gateway or TOPO TA cloning vectors

ViraPower expression systems enable high levels of stable gene expression necessary for valid results in virtually any cell line, especially in primary or difficult-to-transfect cells.

Did you know

We also carry TOPO expression vectors for yeast, algae, and insect cells. Find the right vector for your research at **thermofisher.com/vectors**

Entry into the Gateway system

The Gateway system is a powerful cloning technology that offers a rapid and highly efficient route to multiple expression systems (Figure 12). There are many ways to clone a gene, but only Gateway technology lets you rapidly transfer your gene into different expression vectors with minimal cloning effort. Take the first step toward accessing multiple expression vectors by simply cloning your gene into an Invitrogen™ Gateway™ entry vector. Whether you prefer TOPO or restriction enzyme cloning with PCR products or Invitrogen™ GeneArt™ Strings™ DNA Fragments, we have a Gateway entry vector for you.

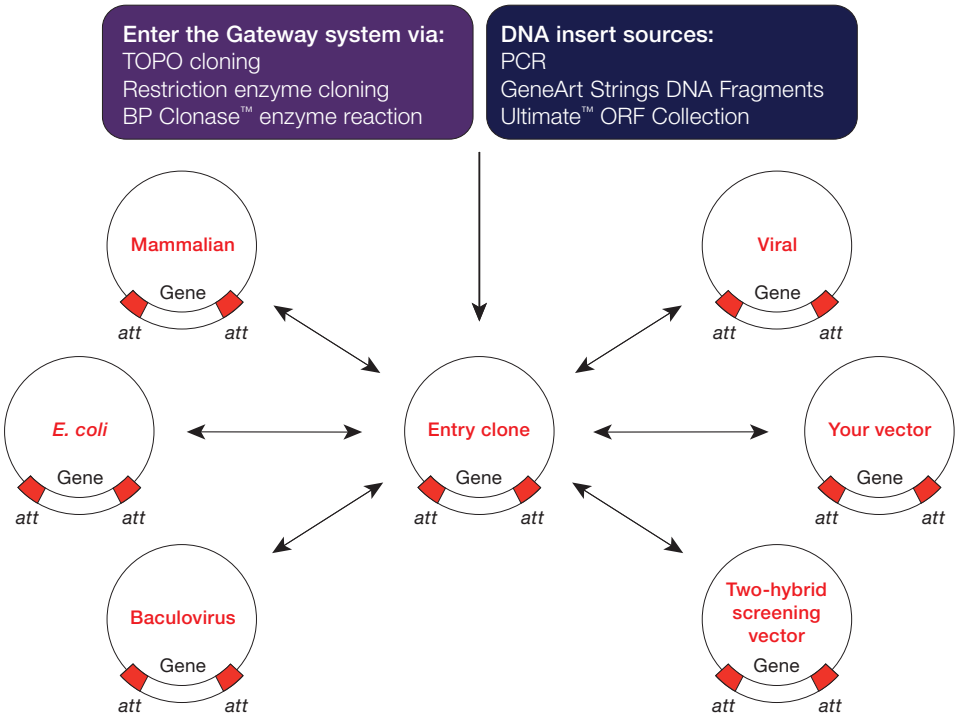


Figure 12. The flexibility of Gateway technology. This powerful technology is designed to simplify cloning and provide a rapid and highly efficient route to multiple expression and functional analysis options.

Helpful tips

Looking for an alternative to PCR for your cloning?

GeneArt Strings DNA Fragments are custom-made, uncloned, double-stranded linear DNA fragments up to 3,000 bp in length, assembled from synthetic oligonucleotides using the same high-quality process developed for Invitrogen™ GeneArt™ Gene Synthesis. Strings DNA Fragments are delivered dried and ready for resuspension, cloning, and screening to enable identification of the correct clone.

- **Affordable**—Strings DNA Fragments are a cost-effective alternative to complete gene synthesis
- **Flexible**—full gene design and cloning flexibility with no template required; clone with any downstream method of choice, including TOPO TA cloning and Zero Blunt TOPO cloning
- **Streamlined**—enter your gene sequence and directly edit, optimize, and order through our online GeneArt™ portal
- **Fast**—at least 200 ng of Strings DNA Fragments are produced within 5 (for up to 1,000 bp) or 8 (for 1,000–3,000 bp) business days

Find out more at **thermofisher.com/strings**

We offer several TOPO cloning kits that allow direct access to the Gateway system. Go to **thermofisher.com/gateway** to learn more about Gateway technology.

Ordering information*

Product	Size	Cat. No.
TOPO TA cloning		
TOPO TA Cloning Kit for Subcloning, without competent cells	25 reactions	450641
	10 reactions	451641
TOPO TA Cloning Kit for Subcloning, with One Shot TOP10 Chemically Competent *E. coli* cells	50 reactions	K450040
	25 reactions	K450001
	10 reactions	K4500J10
TOPO TA Cloning Kit for Sequencing, without competent cells	25 reactions	450030
TOPO TA Cloning Kit for Sequencing, with One Shot TOP10 Chemically Competent *E. coli* cells	50 reactions	K457540
	25 reactions	K457501
	10 reactions	K4575J10
High-fidelity blunt end TOPO cloning		
Zero Blunt TOPO PCR Cloning Kit, without competent cells	25 reactions	450245
	10 reactions	451245
Zero Blunt TOPO PCR Cloning Kit, with One Shot TOP10 Chemically Competent *E. coli* cells	50 reactions	K280040
	25 reactions	K280020
	10 reactions	K2800J10
Zero Blunt TOPO PCR Cloning Kit for Sequencing, without competent cells	25 reactions	450031
	10 reactions	450159
Zero Blunt TOPO PCR Cloning Kit for Sequencing, with One Shot TOP10 Chemically Competent *E. coli* cells	50 reactions	K287540
	25 reactions	K287520
	10 reactions	K2875J10
TOPO long-fragment cloning		
TOPO XL-2 Complete PCR Cloning Kit, with One Shot OmniMAX 2 T1^{R} Chemically Competent *E. coli* Cells	20 reactions	K8050-20
	10 reactions	K8050-10
TOPO directional and expression vector cloning		
pcDNA 3.1/V5-His TOPO TA Expression Kit	40 reactions	K480040
	20 reactions	K480001
pcDNA 3.4 TOPO TA Cloning Kit	1 kit	A14697
pcDNA 3.3 TOPO TA Cloning Kit	20 reactions	K830001
Champion pET101 Directional TOPO Expression Kit with BL21 Star (DE3) One Shot Chemically Competent *E. coli*	20 reactions	K10101
Entry into the Gateway system		
pENTR/D-TOPO Cloning Kit, with One Shot TOP10 Chemically Competent *E. coli*	20 reactions	K240020
pCR8/GW/TOPO TA Cloning Kit with One Shot TOP10 *E. coli*	20 reactions	K250020
pENTR/SD/D-TOPO Cloning Kit, with One Shot TOP10 Chemically Competent *E. coli*	20 reactions	K242020

* Additional product formats and sizes available.

invitrogen

Cloning just got simpler—for a full list of products and to select the best kit for your research, go to **thermofisher.com/topo**

ThermoFisher SCIENTIFIC