invitrogen

TOPO Cloning Technology
Fast, efficient, and simple cloning
When Invitrogen™ TOPO™ cloning technology was launched, it sparked a revolution. Over the past 18 years, TOPO technology has become the most widely used cloning method in the world.

**TOPO cloning technology is:**
- **Efficient**—up to 95% of clones contain desired insert
- **Fast**—5-minute, room temperature reaction
- **Easy**—simple 3-step procedure
- **Proven**—over 20,000 citations
- **Flexible**—available with or without competent cells, in multiple reaction sizes

Whether you’re performing general subcloning, sequencing, TA or blunt-end cloning, long-fragment cloning, expression vector cloning, directional cloning, or using the Gateway™ system, there’s a TOPO cloning solution that’s right for you. Fast, reliable, and direct, TOPO cloning helps you get the right clone sooner, freeing up your time to answer more important questions.
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The technology behind TOPO cloning

TOPO cloning is as easy as 1, 2, 3

1. Add 1 µL of PCR reaction to 1 µL of TOPO cloning vector.
2. Incubate 5 min at room temperature.
3. Transform the competent E. coli provided.

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 overhanging 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**

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
- EcoRI 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/white colony screening for selection of recombinants

**Sequencing**

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 EcoRI sites for simplified excision of cloned PCR products and a unique Sse8387I site for generation of nested deletions prior to sequencing

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

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

**Figure 4. The pCR4-TOPO TA Vector.**
Blunt-end TOPO cloning

Invitrogen™ Zero Blunt™ TOPO™ PCR cloning kits are designed for cloning PCR products amplified with thermostable proofreading polymerases. These polymerases have extensive 3’ to 5’ exonuclease activity, and do not leave 3’-A overhangs. High-fidelity polymerases such as Invitrogen™ Platinum™ Pfx DNA Polymerase have this exonuclease activity, leaving blunt-ended PCR products. Therefore, the vectors supplied in our Zero Blunt TOPO PCR cloning kits have blunt ends as well for effective ligation (Figure 5).

Applications

Subcloning

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 Zeocin™ antibiotic resistance genes for your choice of selection in E. coli
- EcoRI 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

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
- EcoRI sites flanking the PCR product insertion site for easy excision of inserts and a unique Sse8387I site for generation of nested deletions prior to sequencing
- T7 and T3 promoters for in vitro transcription
- M13 forward, M13 reverse, T7, and T3 priming sites for sequencing

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

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

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

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.
**TOPO long-fragment cloning**

Invitrogen™ TOPO™ XL PCR Cloning Kit combines TOPO cloning, the ccdB gene for positive selection, and a unique gel purification step to enhance cloning of PCR products from 3 to 10 kb. 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. To help 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 the need for ethidium bromide and UV light exposure, helping to ensure safe gel purification. This results in significantly more colonies and a greater percentage of recombinants than using ethidium bromide and UV light (Figure 8).

Features of the pCR™-XL-TOPO™ Vector (Figure 9) include:
- *ccdB* gene to help eliminate background of nonrecombinant clones
- Kanamycin and Zeocin antibiotic resistance genes for your choice of selection in *E. coli*
- T7 promoter for *in vitro* transcription and sequencing
- M13 forward (−20) and reverse priming sites for sequencing or PCR screening
- Selection of competent cells for routine cloning, high-speed cloning, or electroporation

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**Figure 8. Crystal violet enhances TOPO cloning of large fragments.** A 7 kb ampicillin resistance gene sequence was PCR-amplified, and PCR products were loaded onto one gel containing crystal violet and another gel containing ethidium bromide. PCR 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. The crystal violet–stained gel allowed for 94% positive recombinants, while the ethidium bromide–stained gel allowed only 60% positive recombinants.

**Figure 9. The pCR-XL-TOPO Vector.**
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 10). 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 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 10. 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 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 Invitrogen™ 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 Geneticin™ antibiotic
- pUC origin for high copy number and maintenance of the plasmid in E. coli

Additionally, the pcDNA 3.4-TOPO TA Vector includes the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) downstream of the cloning site to enhance transcript expression and yields.

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.

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 11). 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 towards accessing multiple expression vectors by simply cloning your gene into a 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.

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](http://thermofisher.com/strings)

Figure 11. 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.

We offer several TOPO cloning kits that allow direct access to the Gateway system. Go to [thermofisher.com/gateway](http://thermofisher.com/gateway) to learn more about Gateway technology.
Custom TOPO Adaptation Service

The development of gene-based therapeutic and diagnostic 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. Our Invitrogen™ Custom TOPO™ Adaptation Service puts the power of TOPO cloning into your vector. With your own vector adapted with TOPO technology, you can:

- **Save time**—the TOPO cloning reaction only takes 5 minutes and is 95% efficient
- **Maintain your current experimental strategy**—no need to change downstream assays or experimental parameters

Flexible solutions

For your convenience and to help maximize your success, many of our TOPO cloning vectors:

- Are now available with or without competent cells
- Now include 25% more reactions for the same price

Cloning just got simpler—to learn more about TOPO cloning and select the best kit for your research, visit [thermofisher.com/topo](http://thermofisher.com/topo)