

## Put the Power of TOPO® Cloning into Your Vector



With a custom TOPO®-Adapted vector you can:

- Accelerate your high-throughput cloning
- Reduce your effort and save money
- Increase your cloning efficiencies

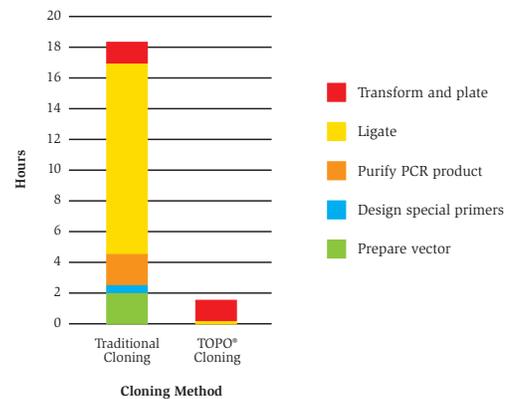
# Accelerate Your Research with TOPO® Cloning in the Vector of Your Choice

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. Invitrogen's Custom TOPO® Adaptation Service can help by putting the power of TOPO® Cloning into your vector. With your own TOPO®-adapted vector you can take advantage of 5-minute bench-top TOPO® Cloning technology, accelerate your research, and get the competitive advantage you need.

## Save hours of time

Identifying, cloning, expressing, and validating gene sequences requires a huge investment of time and resources. TOPO® Cloning technology reduces this time by enabling 5-minute cloning. Custom TOPO® Adaptation Services enable you to use Invitrogen's patented TOPO® Cloning technology in your high-throughput cloning processes. Invitrogen's Custom Service Scientists will TOPO® adapt the vector of your choice so you can save hours in high-throughput cloning by taking advantage of the rapid TOPO® Cloning method (Figure 1).

Figure 1 - Comparison of time required to clone a PCR amplified gene



## Use your own vector

The Custom TOPO® Adaptation Service allows you to put the power of TOPO® Cloning technology into your vector. With a TOPO® adapted vector you can:

- TOPO® Clone with 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—TOPO® adapting your own vector doesn't change your downstream studies, but it will get you there faster

# TOPO® Cloning **works better** than traditional cloning

Traditional procedures for cloning PCR products require the synthesis of special primers, vector and PCR product manipulation, lengthy incubations with DNA ligase—and typically result in only 50-70% recombinants. Traditional cloning is not only inefficient; it costs you time and money. The TOPO® Cloning method takes advantage of the unique activity of *Vaccinia* DNA topoisomerase I to eliminate the need for these

procedures and make PCR product cloning rapid and efficient. The speed and efficiency of TOPO® Cloning will save you time and money (Table 1). Not only is the procedure faster, but it's also efficient. With your custom TOPO® vector you won't waste time repeating failed or low efficiency ligations so you can move on with your research.

**Table 1 - Traditional cloning vs. TOPO® Cloning**

Steps	Traditional Cloning	TOPO® Cloning
Order or prepare PCR primers	Add 10 extra bases to each PCR primer to create restriction sites (6 for the restriction site, 4 for spacing) Cost: \$0.60 to \$1.00/base x 20 bases = \$12.00 to \$20.00/rxn	Special primers containing extra bases are not required
Prepare the vector and PCR product for ligation	Digest vector and PCR product with restriction enzyme(s) Time: 0.5 to 2 hours Cost: \$0.80 to \$1.60/rxn	Your linearized Custom TOPO® vector comes to you ready for direct ligation of unmodified, unpurified PCR products
	Gel purify the digested PCR product using low-melt agarose Time: 1 to 3 hours Cost: \$1.43/rxn	
Obtain ligation reagents	Purchase ligase, ATP, and ligation buffer Cost: \$0.28 to \$0.55/rxn	Your TOPO® vector comes with the required cloning reagents
Incubate the ligation	Time: 4 to 12 hours	Time: 5 minutes
Transform competent <i>E. coli</i>	Time: 1 hour Cost: \$7.00-\$10.00/rxn	Your Custom TOPO® vector includes One Shot® Competent <i>E. coli</i> Time: 1 hour
Percent recombinants	~ 60%	≥95%
Total time required	6.5 to 18 hours	1 hour, 5 minutes
Total cost per reaction	\$21.51 to \$33.58	\$20.00*

\* Price for 5,000 or fewer reactions. Prices per reaction decrease with orders for 5,000 reactions or more.

## High-efficiency cloning

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Invitrogen's TOPO® Cloning technology is more efficient than typical ligase-mediated protocols. DNA ligase may contain impurities, such as nucleases, that nick the ends of linearized DNA, resulting in reduced cloning efficiencies. Because topoisomerase is covalently bound to the linearized vector, it protects the vector from exonuclease digestion and prevents self ligation. TOPO® Cloning efficiencies are exceptionally high, regardless of PCR product size, and yield minimal background (Table 2). Using TOPO® technology in your vector enables you to get your clone the first time.

**Table 2 - TOPO® Cloning results in high efficiencies**

PCR Product Size	Percent Recombinants
700 bp	99%
800 bp	98%
2,000 bp	97%
3,100 bp	89%

Various PCR products were TOPO® Cloned into Invitrogen's pCR®2.1-TOPO® vector, transformed into One Shot® TOP10 Chemically Competent *E. coli*, and plated onto X-gal/Amp plates. The percent recombinants equals the number of white colonies/the number of total colonies.

## You supply the vector and we'll do the work

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To start your Custom TOPO® Adaptation pilot study, simply supply us with an *E. coli* glycerol stock containing your vector. We will:

- Prepare your vector for TOPO® adaptation, covalently attach topoisomerase I and prepare the ends for TOPO® Cloning
- Purify your TOPO®-adapted vector and test its cloning efficiency in a TOPO® Cloning reaction

- Provide a bulk 500-reaction supply for you to test in your high-throughput cloning applications

You will also receive sufficient competent *E. coli*, either in bulk or in 96-well format, for your transformations. Typical turn-around time for the pilot study is 4-6 weeks.

## Easily re-order more

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Once you have experienced the speed and simplicity of using TOPO® Cloning in your high-throughput cloning applications, you'll want to come back for more. Our Custom Service

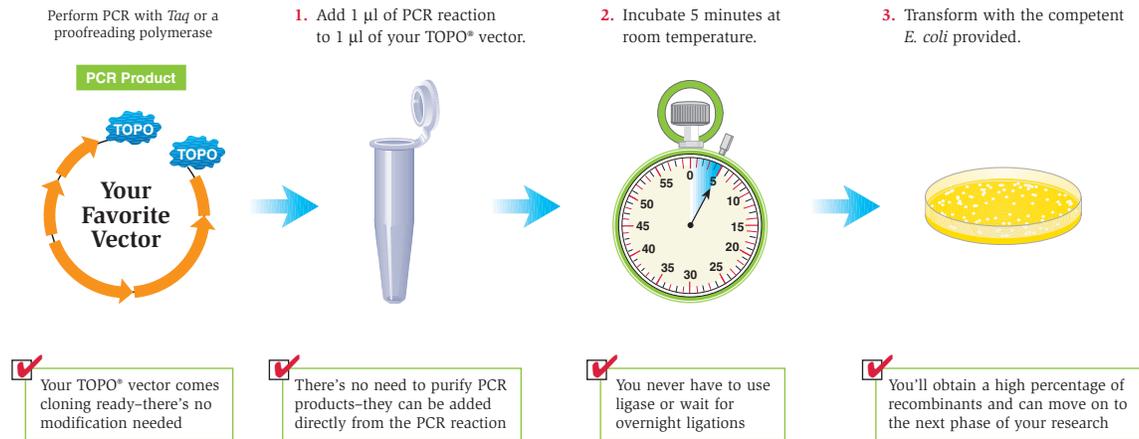
Scientists can supply you with your TOPO®-adapted vector and competent *E. coli* for thousands of reactions in as little as two weeks.

## The advantage of TOPO®

Invitrogen's TOPO® Cloning technology dramatically simplifies high-throughput cloning by eliminating the time-consuming steps associated with conventional cloning methods (Figure 2). Whether you're

cloning hundreds or thousands of PCR-amplified genes, TOPO®-adapting your own vector will save you time and money.

**Figure 2 - The TOPO® Cloning technology**

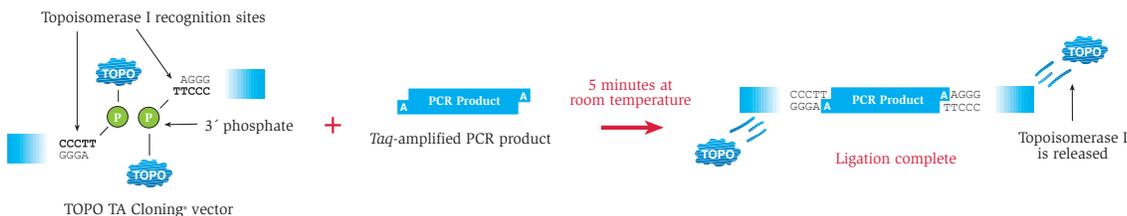


## How TOPO® Cloning works

With the TOPO® Cloning technology, DNA topoisomerase I replaces ligase in standard ligations. The role of topoisomerase I *in vivo* is to cleave and rejoin DNA during replication. To harness its rejoining activity for cloning, topoisomerase is covalently bound to the 3' phosphate of a linearized vector. This topoisomerase I-activated vector will ligate to DNA fragments lacking 5' phosphates such as PCR

products (Figure 3). The TOPO® Cloning reaction is complete in just 5 minutes saving up to 16 hours compared to traditional ligation methods. In addition, the TOPO® Cloning reaction can be carried out at room temperature making it ideal for robotic workstations. The rapid ligation time of TOPO® Cloning in your vector will save you hours of time in your high-throughput cloning processes.

**Figure 3 - TOPO® TA Cloning of *Taq*-amplified DNA**



## Put the power of TOPO® Cloning to work for you

Put the power of the TOPO® Cloning technology into your vector with Invitrogen's Custom TOPO® Adaptation Service. We will TOPO® adapt the vector of your choice for TOPO TA Cloning®, Blunt TOPO® Cloning, or Directional TOPO®

Cloning. Choose the TOPO® Adaptation Service that's right for you (Table 3) so you can reap the benefits of rapid, effective ligation in your high-throughput cloning applications.

**Table 3 - Types of TOPO® Adaptation Service**

TOPO® Adaptation Service	Best When Used For...
Custom TOPO® TA Adaptation	PCR products amplified with Platinum® <i>Taq</i> DNA Polymerase
Custom TOPO® Blunt Adaptation	PCR products amplified with proofreading polymerases (i.e. Platinum® <i>Pfx</i> DNA Polymerase)
Custom Directional TOPO® Adaptation	PCR products amplified with proofreading polymerases that are to be cloned in the correct orientation for expression (5' to 3'). Requires the addition of 4 extra bases to the 5' PCR primer.

## Saving time is priceless

Custom TOPO® Adaptation, available exclusively from Invitrogen, allows you to use TOPO® Cloning instead of traditional ligase-mediate methods in your own vector for your high-throughput cloning experiments. You'll save hours of time and get the

competitive advantage you need to accelerate your research efforts. You can't put a price on that. Call Invitrogen's Custom Services Specialist today at 800 955 6288 ext. 67265 to get started.

Service	Quantity*	Cat. no.
Custom TOPO® Adaptation Service (TA Cloning® or Blunt)	Pilot Study (500 rxns)	M00001
Custom Directional TOPO® Adaptation Service	Pilot Study (500 rxns)	M10001

\* TOPO®-adapted vector is supplied in bulk. Competent *E. coli* are provided in bulk or 96-well format. Please inquire for pricing information.

