



Accelerate your research with TOPO® Cloning and Gateway® Technology



With this forward-moving combination you'll:

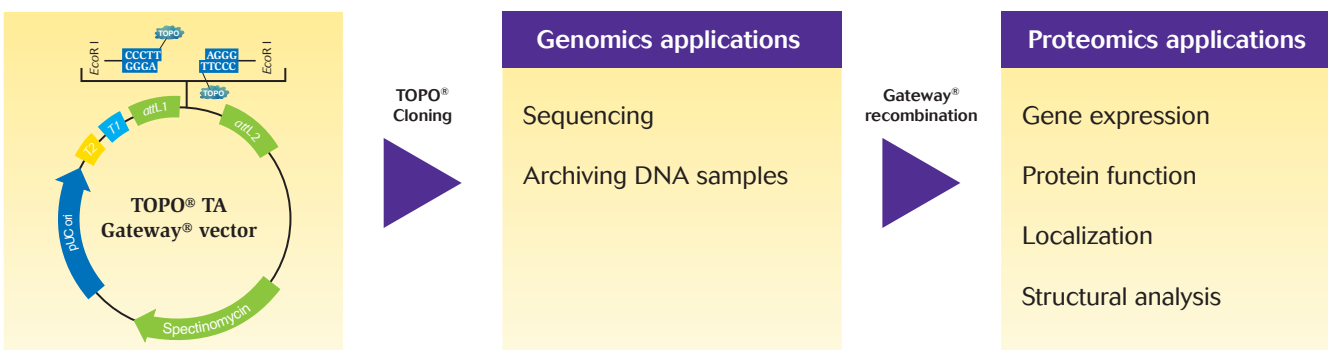
- Achieve 5-minute TOPO TA Cloning® reactions yielding 95% recombinants
- Eliminate tedious, time-consuming cloning/sub-cloning steps
- Be ready for all your downstream research needs

Fast entry to a world of discovery



TOPO® Cloning is the fastest, most effective cloning technology available. Gateway® Technology provides a straightforward, rapid method for flexible protein expression and functional analysis studies. Combining these two technologies quickly satisfies your research needs today and sets you up for future downstream analysis—even if you're not sure where your research will take you tomorrow.

Use TOPO® Cloning for fast, efficient PCR cloning and Gateway® Technology for rapid access to any of your downstream applications



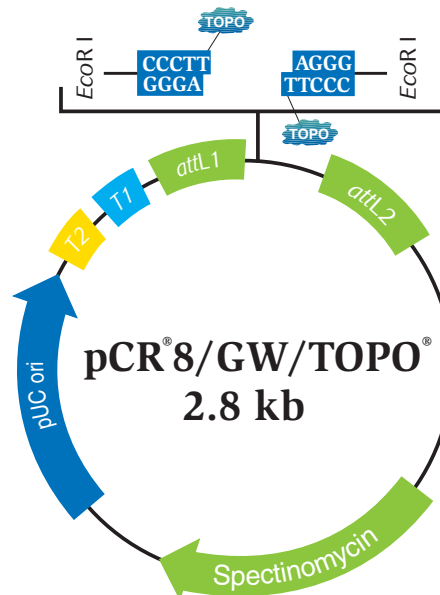
Unique vector design keeps you moving

Fast TOPO® Cloning and flexible Gateway® Technology are combined in the pCR®8/GW/TOPO® vector (Figure 1). It is supplied linearized and activated with topoisomerase I for easy, 5-minute ligations. In addition, pCR®8/GW/TOPO® offers:

- Novel primer sites located less than 55 base pairs from the PCR product insertion site for convenient sequencing
- Spectinomycin resistance gene for robust selection in *E. coli*
- EcoR I sites flanking the PCR product insertion site for easy excision of inserts
- attL sites for rapid recombination into any Gateway® destination vector for your downstream applications

You'll get what you need now for your genomics studies and be ready for the next steps your research requires.

Figure 1 – The pCR®8/GW/TOPO® vector

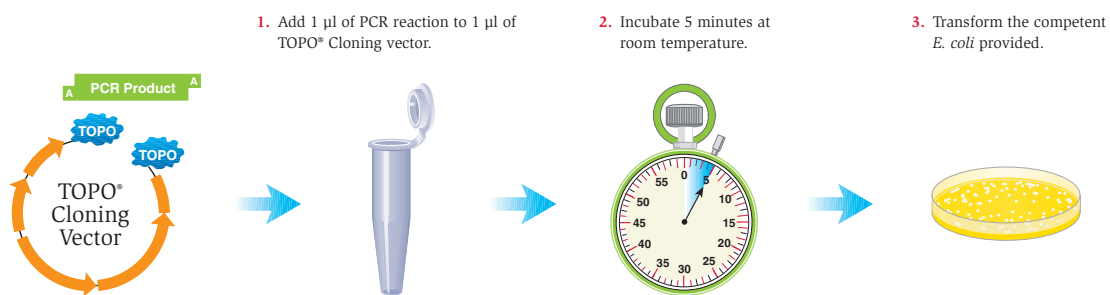


Three simple TOPO® Cloning steps get you in

The TOPO® Cloning method replaces ligase with topoisomerase I to clone PCR products and other DNA molecules in a 5-minute, bench-top ligation yielding 95% recombinants. TOPO® Cloning takes just three easy steps. Simply combine your *Taq*-amplified PCR product and the pCR®8/GW/TOPO® vector, wait five minutes,

then transform *E. coli* (Figure 2). With TOPO® Cloning you'll eliminate the additional time, steps, and reagents required for ligase-mediated cloning and save an entire day. Once cloned, you can rapidly move your PCR product into a number of Gateway® destination vectors for downstream gene expression and protein analysis.

Figure 2 – The TOPO® Cloning protocol



Product*

pCR®8/GW/TOPO® TA Cloning® Kit
with One Shot® Mach1™-T1^R Cells

with One Shot® TOP10 Cells

Quantity

20 rxns

20 rxns

Cat. no.

K2520-20

K2500-20

*The pCR®8/GW/TOPO® vector is provided as part of a complete kit that includes PCR reagents (no polymerase), sequencing primers, and chemically competent cells. Choose One Shot® Mach1™-T1^R cells for high-efficiency transformations and fastest growth or One Shot® TOP10 cells for high-efficiency transformations.

Looking for a gene?

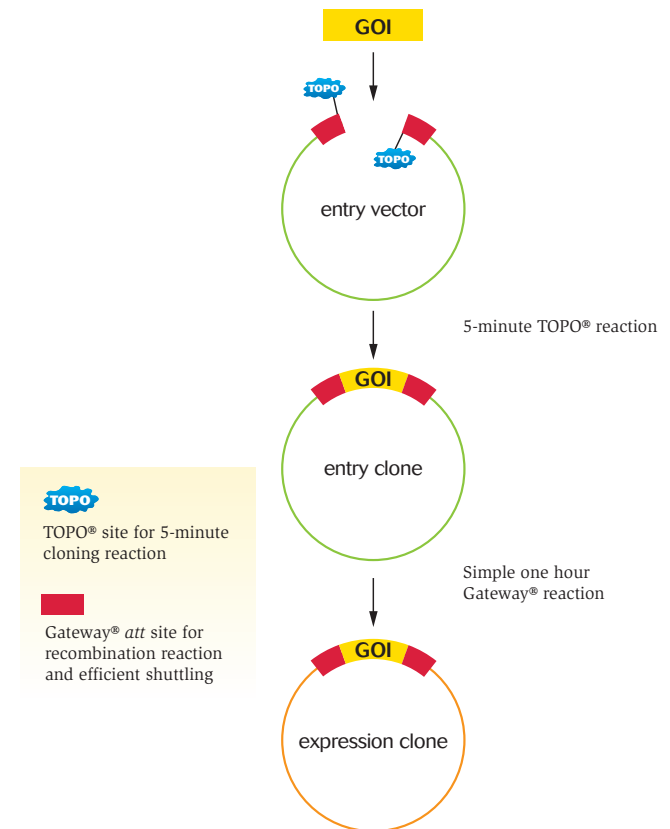
If you don't have your gene of interest, find it in the Ultimate™ ORF Clone Collection. These ready-to-use clones are ready for recombination into any Gateway® destination vector. Visit www.invitrogen.com/clones to learn more.

Gateway® Technology moves you around with ultimate flexibility

Gateway® Technology is an innovative and highly efficient method for protein expression and functional analysis. Based on lambda site-specific recombination, Gateway® Technology offers you the most flexible system for comprehensive gene analysis, protein expression, and functional analysis. Take advantage of a highly efficient, one-hour recombination reaction to transfer fragments from one vector to the next.

Gateway® reactions are robust with greater than 95% recombinants in the correct orientation and reading frame, eliminating time-consuming cloning and subcloning steps. In addition, the sequence of your gene of interest or DNA fragment is entirely conserved, ensuring consistent results. Once you clone your gene of interest or DNA fragment into a Gateway® vector, you can shuttle it into as many expression and functional analysis systems as you need (Figure 3). By comparing a variety of expression systems, you can optimize for the best results and highest yields.

Figure 3 – Rapidly move from one application to the next



Where will your research take you next?

For more information on the systems and analysis vectors currently available in the Gateway® Technology, visit www.invitrogen.com/gateway.

Consistent results

Unlike traditional cloning methods, the TOPO® vector compatible with Gateway® Technology enables you to access an unlimited number of systems from one initial construct. From target identification to validation via biochemical or functional assays, you can use the same clone throughout your studies, ensuring

consistent results. No more subcloning or worrying about sequence integrity and erroneous results. See Table 1 for a wide variety of products that incorporate Gateway® Technology. From initial gene expression in mammalian cells to localization and interaction studies, there is a system made for your convenience.

Table 1 – Gateway® Vectors and Systems are ideal for all stages of research

Stage of research	Application	Gateway® products
Gene Acquisition	Drug target identification	Ultimate™ ORF Clone collection
Cloning	Sequencing	Entry and donor vectors
	Cloning & subcloning	
	Building clone & library collections	CloneMiner™ cDNA Library Construction Kit
Delivery	Gene delivery into challenging mammalian cell lines	ViraPower™ Expression Systems
	<i>In vivo</i> studies in animal model systems	
Protein Production	Protein arrays	Expressway™ Plus Expression System
	Antibody or antigen production	Champion™ pET Expression System
		BaculoDirect™ Expression System
Protein Analysis	Function	pcDNA™ mammalian destination vectors
	Interactions	Two-hybrid systems
	Reporter assays	GeneBLAzer™ Technology
	Localization	GFP and Lumio™-tagged destination vectors
	RNAi	BLOCK-iT™ Technology
	Purification	His-tagged destination vectors

Convenient recombination

Clonase™ II enzymes catalyze highly specific, *in vitro* recombination reactions between vectors and DNA segments containing the appropriate *att* recombination sites.

LR Clonase™ II enzyme mix catalyzes recombination between any entry clone (containing *attL* sites) and destination vector (containing *attR* sites) to create an expression clone. BP Clonase™ II enzyme mix catalyzes the reverse reaction, shuttling the gene of interest from the expression clone (containing *attB* sites) to a donor vector (containing *attP* sites) to create an entry clone. Both reactions are

highly efficient and take just one hour, yielding >95% recombinants in the correct orientation and reading frame.

The Clonase™ II enzyme mixes are:

- Convenient—provided in a pre-mixed, ready-to-use solution of enzyme and buffer
- Easy-to-use—minimizes pipetting steps
- Cost-effective—no need to purchase multiple reagents, new 10-µl half-reaction size minimizes material waste

Product	Quantity	Cat. no.
LR Clonase™ II enzyme mix	20 rxns	11791-020
	100 rxns	11791-100
BP Clonase™ II enzyme mix	20 rxns	11789-020
	100 rxns	11789-100

Open Architecture – No Royalties. No License fees.

Invitrogen has recently relaxed its licensing policy for Gateway® Technology. This Open Architecture policy allows more open access to, and distribution of, Gateway® clones. Under this policy:

- Government and academic researchers can freely share or distribute Gateway® entry clones and expression clones with their colleagues
- All other organizations may freely distribute Gateway® entry clones, developed by government or academic researchers, with a no-fee license from Invitrogen. Expression clones may be distributed for a nominal fee (\$10/clone)
- Therapeutic, clinical diagnostic, vaccine, or prophylactic products developed in research by the use of Gateway® Technology are not subject to infringement provided that none of the products or method claims were used in the manufacture of such product(s)

Innovative online tools

The VectorDesigner™ tool is an innovative set of free online tools from Invitrogen for designing Gateway® and TOPO® experiments. With these tools, you can:

- Create and store your own recombinant molecules on the web
- View created molecules in the most graphically rich DNA sequence viewer available
- Seamlessly connect to Invitrogen's online ordering system to expedite purchases of relevant products and services

The VectorDesigner™ tool includes fully documented online help and is backed by Invitrogen's award-winning Technical Support. You'll find the VectorDesigner™ tool at www.invitrogen.com.

It's easier than ever to try Gateway® Technology:

- Easy TOPO® TA Cloning® vector meets your current and future needs
- Convenient reaction set-up with Clonase™ II enzyme mix simplifies shuttling your gene to other systems with minimal sub-cloning/sequencing steps
- Unrestricted access to a large repository of relevant clones and vectors built by the research community



Top 10 reasons to use Gateway® Technology

1. Proven technology for a wide variety of research needs—**over 150** scientific publications
2. Gateway® Open Architecture[§] empowers your resources to promote rapid scientific advancement
3. Fast and simple—enter into Gateway® Technology in **5 minutes** with TOPO TA Cloning®
4. Easy and more cost-effective set-up with new Clonase™ II enzyme mixes
5. Efficient and robust recombination—shuttle to a variety of systems with **>95%** efficiency every time
6. **>30 Invitrogen systems** available for a number of applications, including expression, solubility, detection, purification, localization, and knockdown experiments
7. Take advantage of the growing research community using Gateway® Technology—collaborate and share collections and knowledge with many of your peers
8. New web-facilitated tools aide in primer design, *in silico* cloning, and ordering
9. Choose from **>9,000** full-insert sequenced Ultimate™ ORF Clones ready for entry into Gateway® Technology
10. Invitrogen is dedicated to your on-going success in Gateway® Technology—we commit to new product and technology innovation and high-level service

For more information on the systems and analysis vectors currently available in the Gateway® Technology, visit www.invitrogen.com/gateway.

§ No license fees, no royalties for research use.



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