invitrogen

Gateway cloning technology

The easy-to-use choice for cloning in multiple expression systems

ThermoFisher SCIENTIFIC

The trusted leader in cloning technology

Invitrogen™ Gateway™ cloning technology has been cited by life science researchers more than 2,000 times. It's no wonder Gateway cloning has been the go-to choice for years, by researchers with varying experience—from beginners to advanced—for protein expression, functional analysis, and much more.

Circumvent the roadblocks of traditional restriction enzyme cloning—no need for ligase, subcloning steps, or the hours spent to screen countless colonies. Experience Gateway cloning technology.

- **Fast reactions**—1-hour room temperature cloning reactions
- **Accurate results**—cloning reactions achieve >95% efficiency to deliver the clone you need
- **Versatile technology**—easily shuttle DNA material/insert from vector to vector
- **Streamlined protocol**—no need for resequencing; use the same clone from target identification to validation

Contents

Basic cloning methodology: three steps to better efficiency

Entry clones, Clonase enzymes, and Destination vectors

Determine the Entry clone

The Entry clone is how and where you start your experiment, as it contains your gene of interest or DNA fragment flanked by *att*L sequences, which are then used to recombine with *att*R sequences to create your desired expression clone. Choose one of our Invitrogen™ TOPO™ cloning vectors to create your Entry clone, or purchase a premade clone from our validated Invitrogen™ Ultimate™ ORF Clone Collection*.

Mediate the reaction with Clonase enzymes

Once the Entry clone is ready, the gene of interest is easily shuttled to a secondary plasmid, the Destination vector. This reaction is mediated by Invitrogen™ LR Clonase™ enzyme mix, which contains the protein machinery necessary to excise the gene of interest from the Entry clone and integrate it into the Destination vector, which then becomes your expression clone. Reversing this reaction is simple: it requires a BP reaction (recombination between *att*B and *att*P sites) using Invitrogen™ BP Clonase™ enzyme mix.

Both LR Clonase and BP Clonase enzyme mixes are supplied in easy-to-use master mix formats, ensuring consistency and reliability from reaction to reaction.

Select the Destination vector

Once you have cloned your gene of interest or DNA fragment into a Gateway™ vector, you can shuttle it to as many expression and functional analysis systems as you need.

The diverse selection of expression vectors available with Gateway cloning technology is vast and broad. From expression proteins in *E. coli*, yeast, insect, or mammalian cells to RNAi studies, from crystallography to protein–protein interaction functional studies, there is a Destination vector for your application. And for those applications that require a specialized or customized vector, the Invitrogen™ Gateway™ Vector Conversion System can convert any vector into one compatible for Gateway cloning.

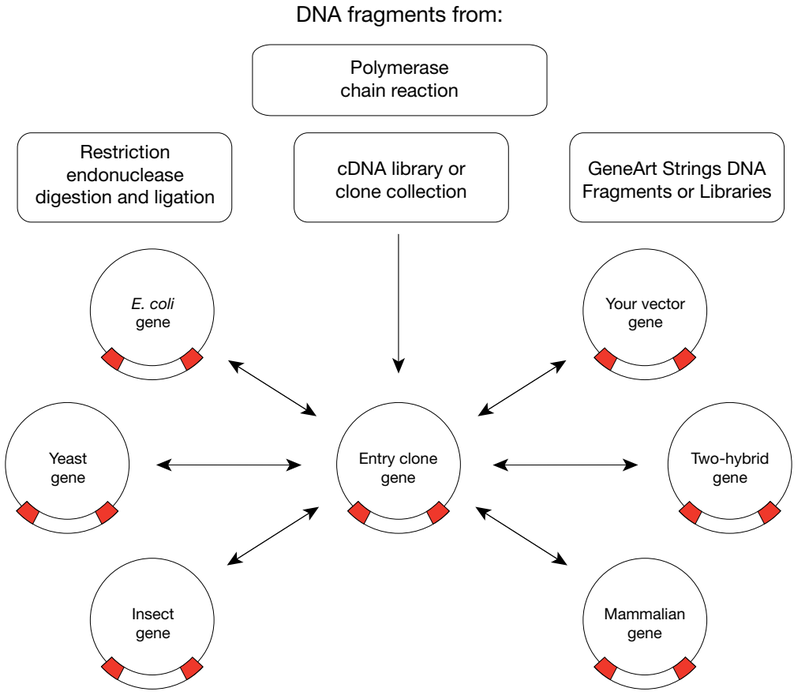

Figure 1. Gateway technology facilitates cloning of genes into and back out of multiple vectors via site-specific recombination. Once a gene is cloned into an Entry clone, you can then move the DNA fragment into one or more Destination vectors simultaneously.

*Ultimate ORF Clones are currently not available from Fisher Scientific. For more information, go to **thermofisher.com/orf**

Product selection guide

Learn which products to implement at each stage

Creating an Entry clone

Using TOPO vectors or PCR amplification/restriction-enzyme vectors is the most common way to construct your own Entry clone.

TOPO vectors—both options offer 5-minute cloning and >95% efficiency

pCR8/GW/TOPO Cloning Kits	pENTR/D-TOPO Vectors
• Convenient sequencing • Robust selection in *E. coli* with spectinomycin resistance • Easy excision of insert DNA with flanking EcoRI sites	• Fast Directional TOPO cloning • Delivers insert in correct orientation • Contains necessary *att*L sequences for recombination into any Destination vector • Select versions carry a TEV protease cleavage site for producing native proteins after expression

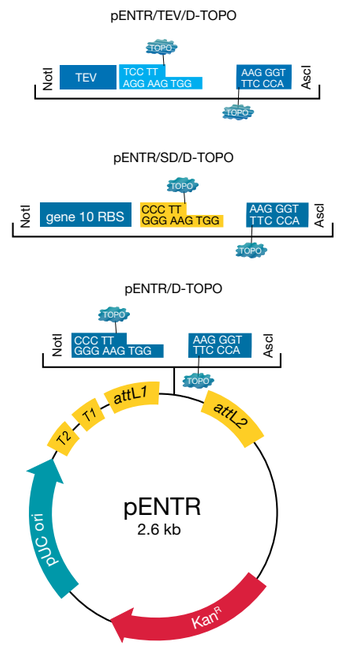

Figure 2. Several Invitrogen™ pENTR™ vectors are available for directional TOPO cloning and direct access to the multitude of Gateway™ expression vectors.

Purchasing a premade clone

You can also utilize Gateway technology with a ready-to-use clone from our extensive clone collection. The Ultimate ORF Clone Collection consists of high-quality, full-insert–sequenced human and mouse open reading frames already cloned into the Invitrogen™ pENTR™ 221 vector for limitless downstream analysis capabilities. Clones contain DNA- and amino acid sequence–verified, expression-ready cDNAs for kinases, G-protein–coupled receptors (GPCRs), phosphatases, ion channels, chemokines, nuclear receptors, and cytokines.

View the Ultimate ORF Clone Collection at **thermofisher.com/orf**

PCR amplification or restriction-enzyme cloning vectors

pDONR and pENTR Vectors

These vectors allow you to clone a PCR product amplified with primers containing *att*B sequences (Invitrogen™ pDONR™ vector) or specific restriction sites (Invitrogen™ pENTR™ vector). Using PCR to generate the Entry clone, two short artificial *att*B sequences (*att*B1 and *att*B2) must flank your gene of interest and be added to specific primers that are used to amplify the gene of choice. The DNA fragment is combined with a donor vector that contains *att*P1 and *att*P2 sequences and with BP Clonase II enzyme.

- >90% of the colonies contain the Entry clone with the gene of interest in the correct orientation
- Final Entry clones are ready for recombination with any Invitrogen™ Gateway™ Destination vector

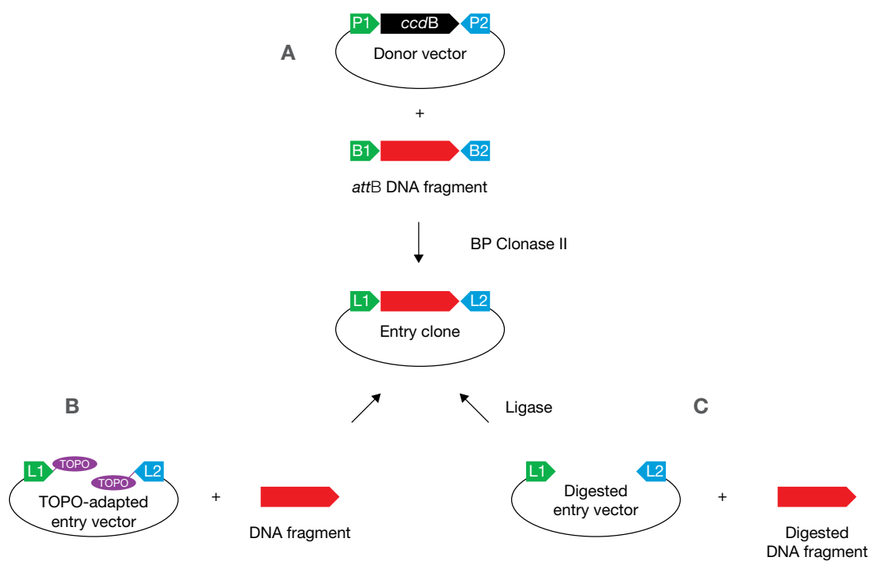

Figure 3. Strategies to build the Entry clone. The three possible methods that lead to the Entry clone are depicted: **(A)** BP cloning, **(B)** TOPO cloning, and **(C)** restriction enzyme and ligase cloning. Red arrows represent the fragment of interest. Adapted from Katzen F (2007) *Expert Opin Drug Discov* 2(4):571–589.

Clonase enzyme mix selection guide

	BP Clonase II Enzyme Mix	LR Clonase II Plus Enzyme Mix
Application	**Creating Entry clones**	**Creating expression clones**
Proteins involved in site-specific recombination	• Int (integrase) • IHF (integration host factor)	• Int (integrase) • IHF (integration host factor) • Xis (excisionase)
Activity	• DNA recombinase • DNA-binding protein • High efficiency for Entry clone construction • Single-mix format eliminates pipetting steps and hands-on errors	• DNA recombinase • DNA-binding protein • Highest cloning efficiency for single- and multiple-fragment cloning • Optimized for difficult cloning reactions • Works with MultiSite Gateway Pro technology
Advantages	• Easy-to-use, single-mix format ensures enzyme stability • Convenient 10 µL reaction setup	• Easy-to-use, single-mix format ensures enzyme stability • Convenient 10 µL reaction setup

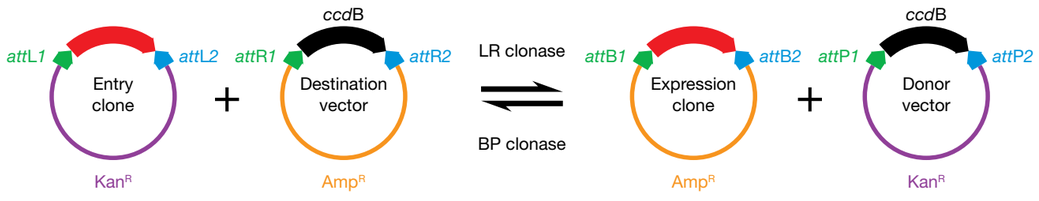

Figure 4. The Gateway reactions. The scheme shows the four types of plasmids and enzyme mixes involved in Gateway cloning reactions. Red arrows represent the fragment of interest. Adapted from Katzen F (2007) *Expert Opin Drug Discov* 2(4):571–589.

Destination vector selection guide

Gateway cloning technology is especially noted for its utility in protein expression. The flexibility and diverse selection of Destination vectors and host systems is particularly attractive for multidisciplinary protein expression studies.

Destination vectors for protein expression

Host system for protein expression	Gateway Destination vector family
E. coli	• pDEST 14, 15, 17, and 24 • pET160 and pET161 DEST vectors
Yeast	pYES2-DEST52
Insect cells	BaculoDirect C-Term Expression Kit
Mammalian cells (constitutive expression)	pcDNA Mammalian Expression vector family
Mammalian cells (regulated expression)	• pT-REx-DEST30 • pT-REx-DEST31 vectors
Mammalian cells (viral delivery)	ViraPower Lentiviral Expression Systems

Destination vectors for additional application areas

Application	Gateway Destination vector family
Antibody or antigen production	Champion pET Expression systems
Localization	Vivid Colors pcDNA GFP Destination vector family
Protein array	Expressway Plus Expression System
Protein–protein interaction studies	ProQuest Two-Hybrid System using Gateway technology
Reporter assay	GeneBLAzer pcDNA vector family
RNAi	GeneBLAzer pcDNA vector family

MultiSite Gateway Pro technology

Mix and match fragments while maintaining orientation

MultiSite Gateway Pro Kits

What if you could easily and accurately assemble multiple DNA fragments in the order and orientation of your desire? This approach, called Invitrogen™ MultiSite Gateway™ Pro technology, allows the mixing and matching of functional fragments in a concerted fashion to generate multi-segment constructs. MultiSite Gateway Pro technology enables you to perform pathway reconstitution, multiple gene expression and regulation, protein interaction studies, and more.

This approach has several applications covering the engineering of proteins, pathways, and cells, and provides a highly flexible platform for functional analysis.

The full power of Gateway cloning is realized with MultiSite Gateway Pro technology, which allows for the simultaneous assembly of multiple fragments into a single vector in a predefined order, orientation, and reading frame (Figures 5 and 6).

1. PCR-amplify your DNA elements of interest with specific *att*B-containing PCR primers.

2. Generate Entry clones through Gateway BP recombination with *att*B-flanked PCR fragments and the appropriate pDONR vectors.

3. Recombine entry clones with a Gateway Destination vector (with *att*R1 and *att*R2 sites) to generate an expression plasmid.

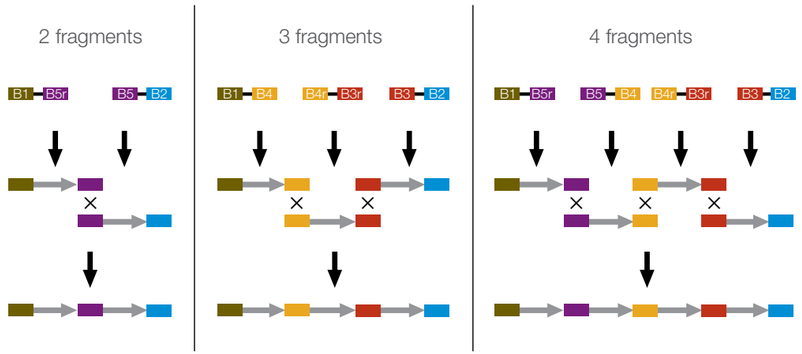

Figure 5. How MultiSite Gateway Pro technology works.

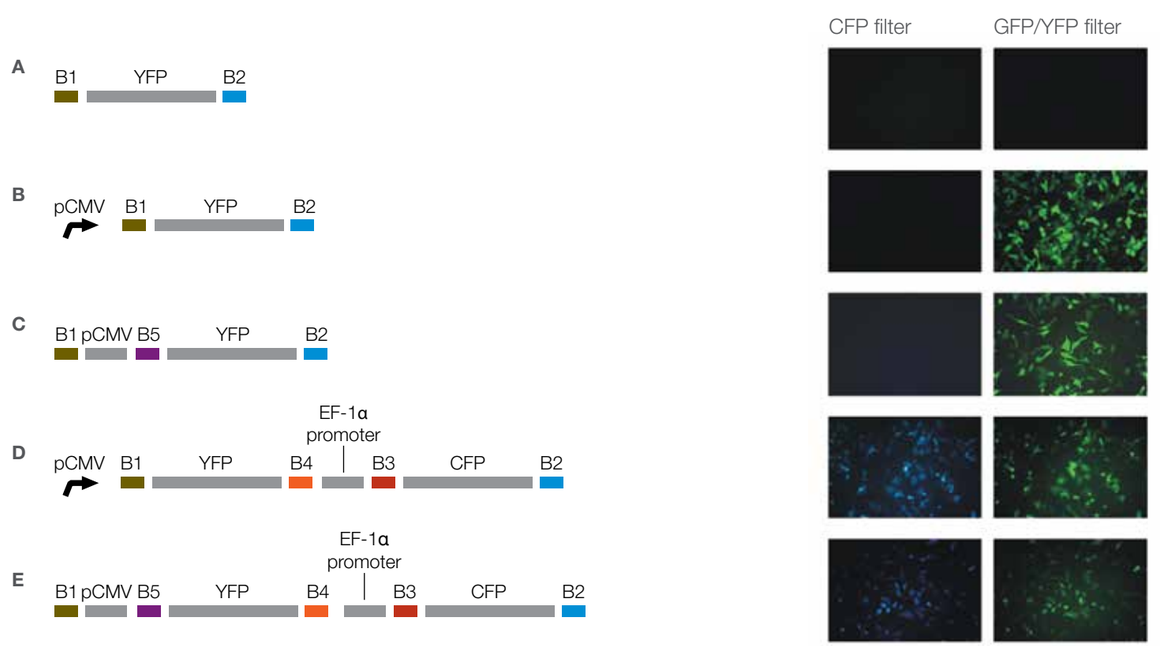

Figure 6. An example of using MultiSite Gateway Pro technology to study expression of multiple genes in human cells. Entry clones containing genes for YFP and CFP and the CMV and EF-1*α* promoters were recombined into the Invitrogen™ pcDNA™ 6.2/V5-PL-DEST Vector (**A**, **C**, and **E**) or into the Invitrogen™ pcDNA™ 6.2/V5-DEST Vector (**B** and **D**). The resulting expression clones were used to transfect HeLa cells. Expression was verified under a fluorescence microscope. The Invitrogen™ pcDNA™ 6.2/V5-PL-DEST Vector is a promoterless derivative of the Invitrogen™ pcDNA 6.2/V5-DEST Vector, which carries the CMV promoter.

Ordering information

TOPO TA cloning

Product	Description	Quantity	Cat. No.
pCR8/GW/TOPO TA Cloning Kit	Efficient TOPO TA cloning kit simplifies Entry clone construction	20 reactions	K250020
pCR8/GW/TOPO TA Cloning Kit	Efficient TOPO TA cloning with fast-growing competent *E. coli* that shortens the time for Entry clone construction	20 reactions	K252020
pCR8/GW/TOPO TA Cloning Kit	Efficient TOPO TA cloning with fast-growing competent *E. coli* and plasmid purification drastically shortens and simplifies Entry clone construction, saving time and hassle	20 reactions	K252002

Directional TOPO cloning

Product	Description	Quantity	Cat. No.
pENTR/D-TOPO Cloning Kit	Directional TOPO cloning kit that produces expression-ready Entry clones	20 reactions	K240020
pENTR/SD/D-TOPO Cloning Kit	Directional TOPO cloning kit, including the Shine-Dalgarno sequence that creates an *E. coli* expression–ready Entry clone	20 reactions	K242020
pENTR/TEV/D-TOPO Cloning Kit	Directional TOPO cloning kit that creates expression-ready Entry clones with 5´ TEV sequence for N-terminal tag removal (creating native proteins)	20 reactions	K252520
pENTR/TEV/D-TOPO Cloning Kit	Directional TOPO cloning kit with fast-growing competent *E. coli* that shortens the time for Entry clone construction while creating expression-ready Entry clones with a 5´ TEV sequence for N-terminal tag removal (creating native proteins)	20 reactions	K253520

PCR cloning using BP recombination

Product	Description	Quantity	Cat. No.
PCR Cloning System with Gateway technology	Complete kit for directional cloning into a Gateway vector with pDONR 221 vector with kanamycin selection	20 reactions	12535029
PCR Cloning System with Gateway technology	Complete kit for directional cloning into a Gateway vector with pDONR/Zeo vector with Zeocin antibiotic selection	20 reactions	12535037
pDONR 221 Vector	Contains a pUC origin for high plasmid yields and universal M13 sequencing sites for ease of use	6 µg	12536017
pDONR Zeo Vector	Contains a pUC origin for high plasmid yields and universal M13 sequencing sites for ease of use. Also supplied with 1.25 mL Zeocin Selection Reagent	6 µg	12535035

Ordering information

Restriction enzyme cloning

Product	Description	Quantity	Cat. No.
pENTR 1A Vector	Restriction enzyme cloning vector that produces in-frame (rf = 0), expression-ready Entry clones, including both Shine-Dalgarno and Kozak sequences	10 µg	11813011
pENTR 2B Vector	Restriction enzyme cloning vector that produces in-frame (rf = +1), expression-ready Entry clones	10 µg	11816014
pENTR 3C Vector	Restriction enzyme cloning vector that produces in-frame (rf = +2), expression-ready Entry clones	10 µg	11817012
pENTR 4 Vector	Same as pENTR 1A Vector except with NcoI instead of DraI in MCS that produces in-frame (rf = 0), expression-ready Entry clones	10 µg	11818010
pENTR 11 Vector	Same as pENTR 1A Vector except with NspV instead of DraI in MCS that produces in-frame (rf = 0), expression-ready Entry clones	10 µg	11819018

Multifragment assembly with Gateway technology

Product	Description	Quantity	Cat. No.
MultiSite Gateway Pro Plus Kit	Allows for flexible cloning of up to four fragments into a Gateway Destination vector	20 reactions	12537100
pcDNA 6.2/V5 PL-DEST Vector	A promoterless version of our most popular pcDNA vector for use with any of the MultiSite Gateway Pro Kits. Vector has C-terminal V5 and blasticidin selection	6 µg	12537162

Ordering information

BP Clonase enzymes

Product	Description	Quantity	Cat. No.
Gateway BP Clonase II Enzyme Mix	A proprietary blend of both Int (integrase) and IHF (integration host factor) proteins that catalyze the *in vitro* recombination of PCR products or DNA segments from clones and a donor vector	20 reactions	11789020
		100 reactions	11789100
Gateway BP Clonase Enzyme Mix		20 reactions	11789013
		100 reactions	11789021

LR Clonase enzymes

Product	Description	Quantity	Cat. No.
Gateway LR Clonase II Plus Enzyme Mix	A proprietary blend of Int (integrase), IHF (integration host factor), and Xis (excisionase) enzymes that catalyze *in vitro* recombination between an Entry clone and a Destination vector	20 reactions	12538120
		100 reactions	12538200
Gateway LR Clonase II Enzyme Mix		20 reactions	11791020
		100 reactions	11791100
Gateway LR Clonase Enzyme Mix		20 reactions	11791019
		100 reactions	11791043

Ordering information

Competent cells

Product	Description	Quantity	Cat. No.
One Shot *ccd*B Survival 2 T1^{R} Competent Cells	Designed for propagation of plasmids containing the *ccd*B gene	10 transformations	A10460

Converting your proprietary cloning vectors with Gateway technology

Product	Description	Quantity	Cat. No.
Gateway Vector Conversion System	Convert any cloning vector into a Gateway Destination vector using restriction endonucleases and ligase	20 reactions	11828029

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