Molecular biology

Gateway cloning technology

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

invitrogen

The trusted leader in cloning technology

Invitrogen™ Gateway™ cloning technology has more than 10,000 citations by life science researchers. It's no wonder Gateway cloning has been the go-to choice for years, by researchers with varying experience—from beginners to advanced scientists—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

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 the Invitrogen™ TOPO™ cloning vectors to create your Entry clone, or custom fragment in the desired vector through **Invitrogen™ GeneArt™ services**.

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, helping ensure consistency and reliability from reaction to reaction.

Select the Destination vector

Once you have cloned your gene of interest or DNA fragment into an Invitrogen™ 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.

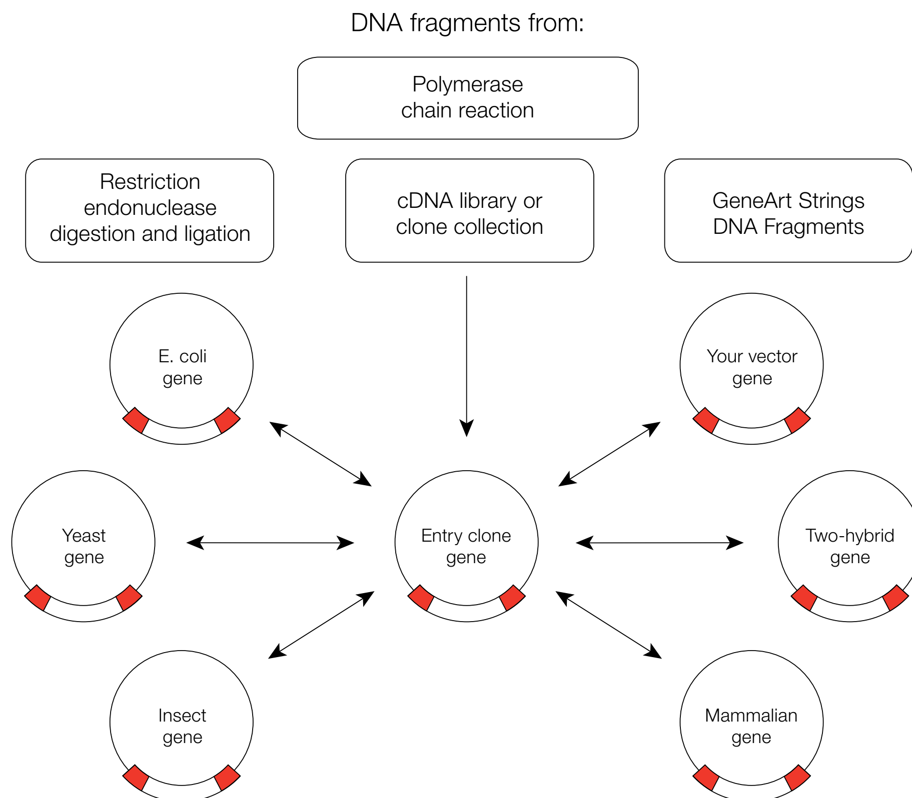


Figure 1. Gateway technology facilitates cloning of genes into and back out of multiple vectors via site-specific recombination. Once a gene is cloned into an Entry clone, you can then move the DNA fragment into one or more Destination vectors simultaneously.

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 TA Cloning Kit	pENTR/D-TOPO Vectors
• Convenient sequencing	• Fast directional TOPO cloning
• Robust selection in *E. coli* with spectinomycin resistance	• Delivers insert in correct orientation
• Easy excision of insert DNA with flanking EcoRI sites	• 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

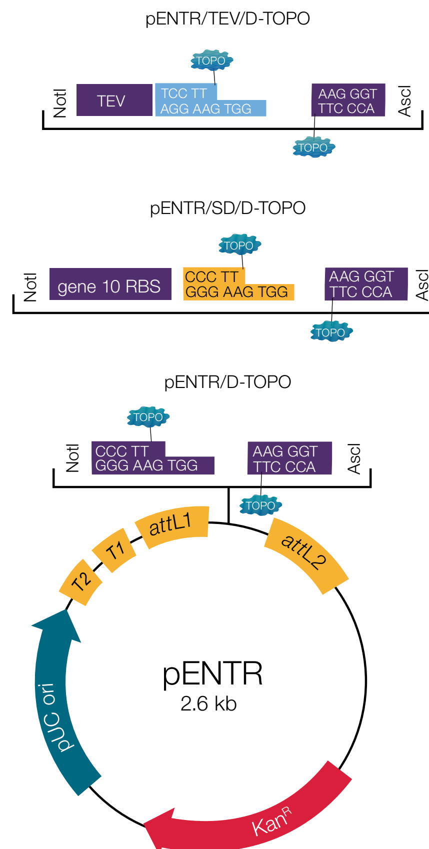


Figure 2. Several Invitrogen™ pENTR™ vectors are available for directional TOPO cloning and direct access to the multitude of Invitrogen™ Gateway™ expression vectors.

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 Invitrogen™ 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

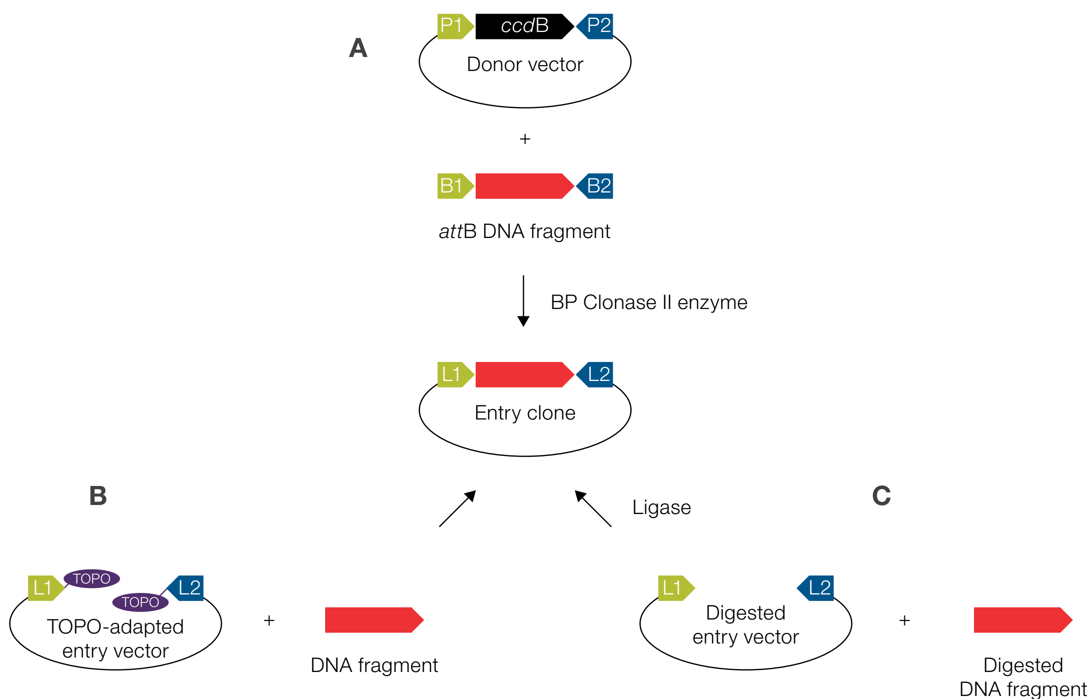


Figure 3. Strategies to build the Entry clone. The three possible methods that lead to the Entry clone are depicted: **(A)** BP cloning, **(B)** TOPO cloning, and **(C)** restriction enzyme and ligase cloning. Red arrows represent the fragment of interest. Adapted from Katzen F (2007) *Expert Opin Drug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

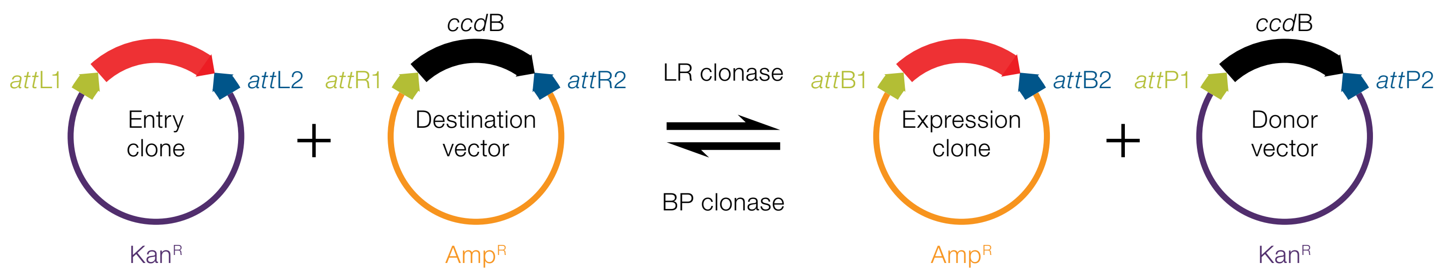
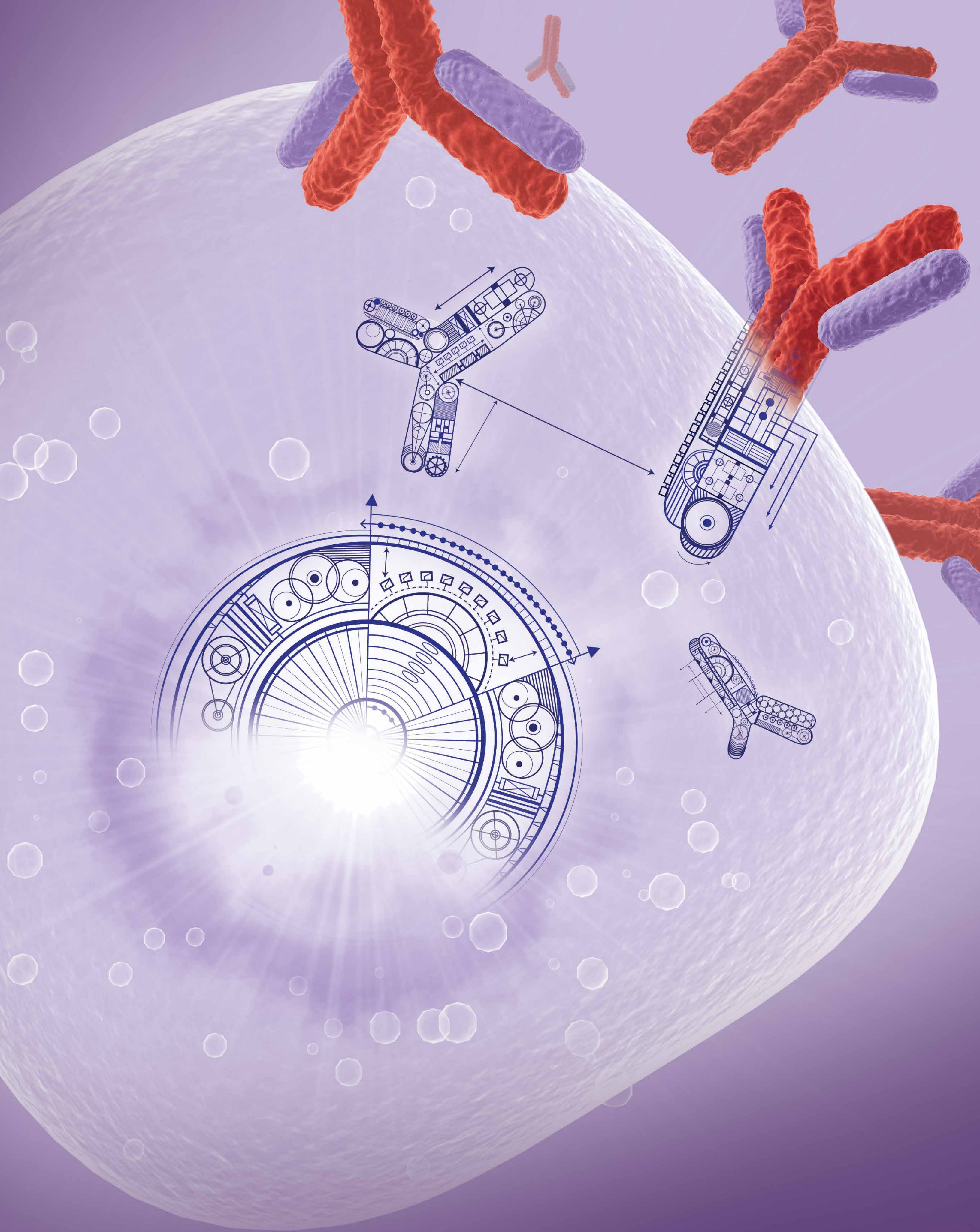


Figure 4. The Gateway reactions. The scheme shows the four types of plasmids and enzyme mixes involved in Gateway cloning reactions. Red arrows represent the fragment of interest. Adapted from Katzen F (2007) *Expert Opin Drug Discov* 2(4):571–589.



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 that you 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).

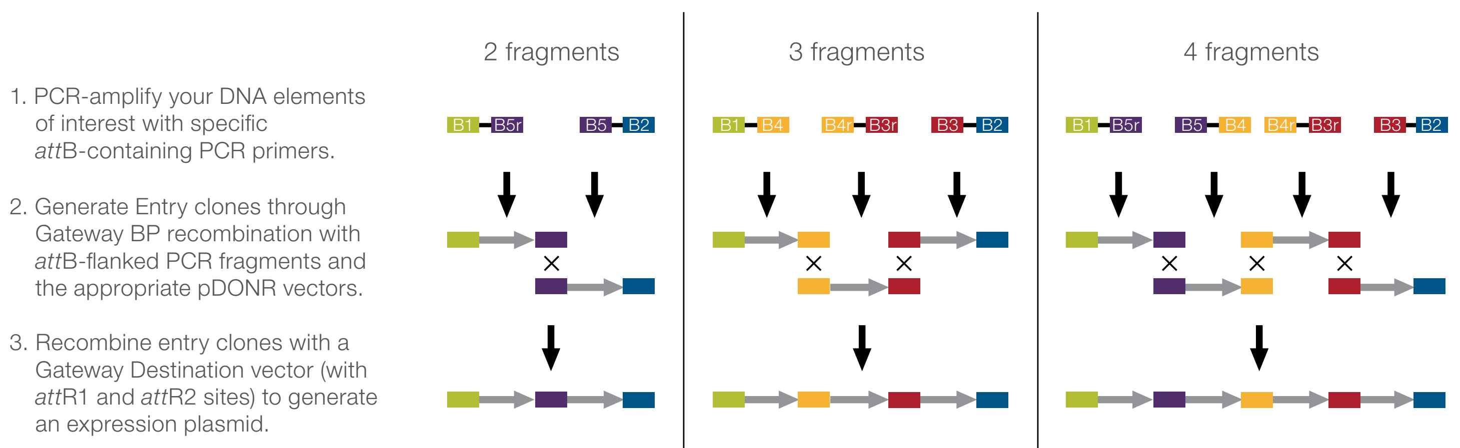


Figure 5. How MultiSite Gateway Pro technology works.

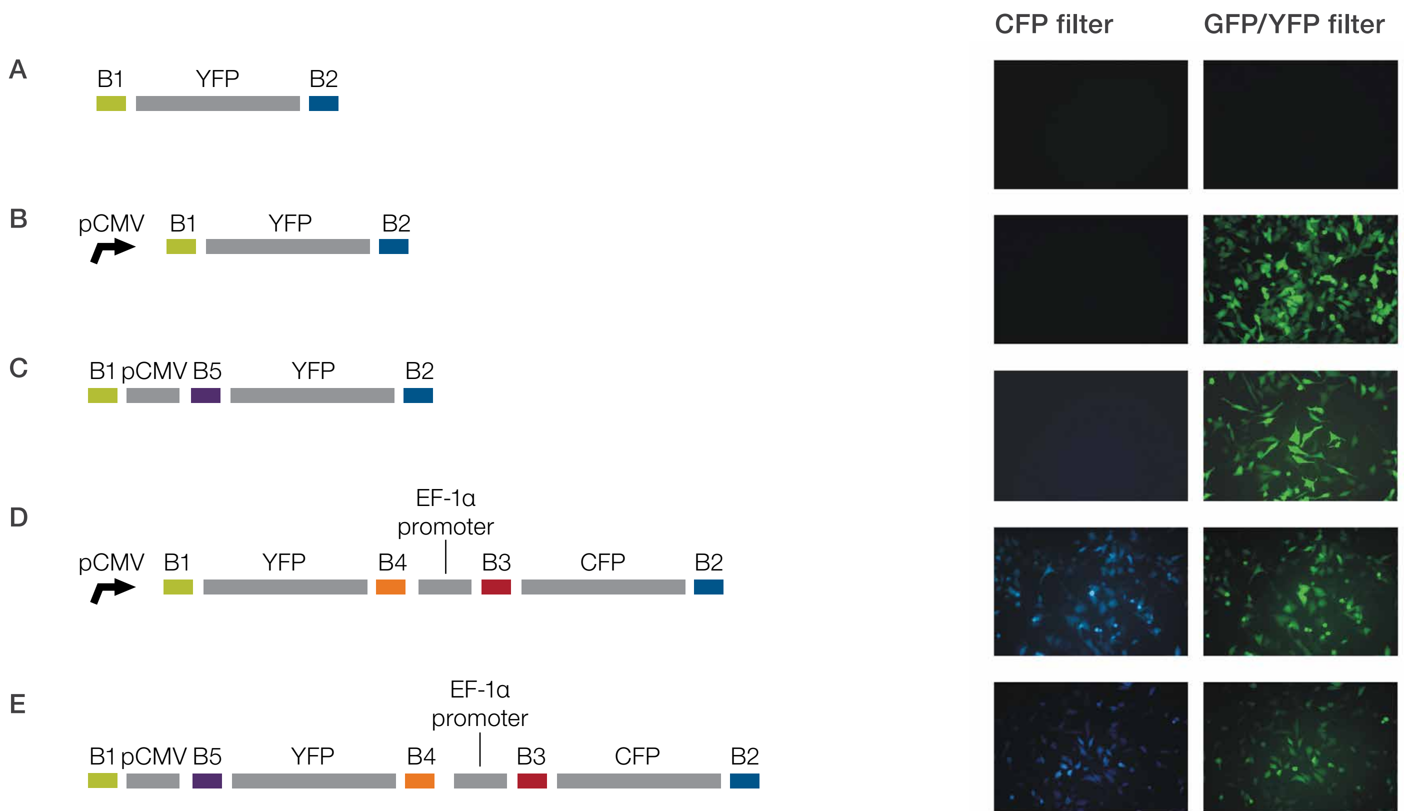
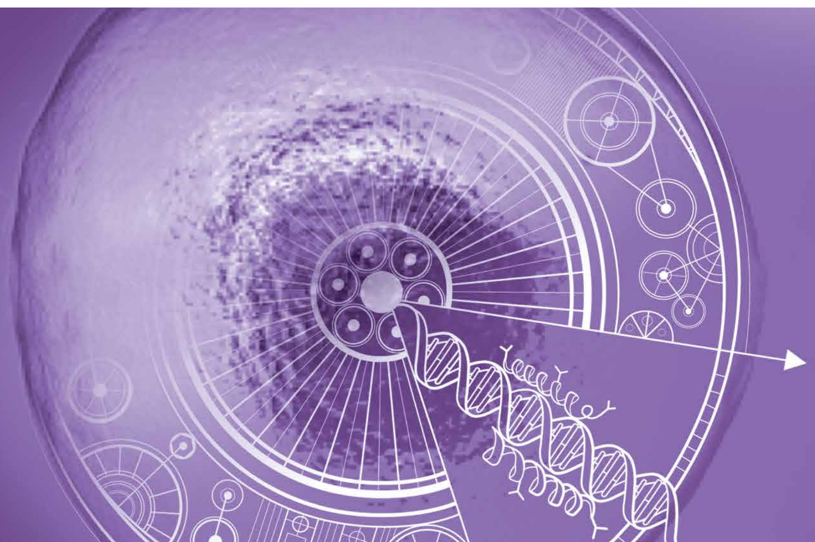


Figure 6. An example of using MultiSite Gateway Pro technology to study expression of multiple genes in human cells. Entry clones containing genes for YFP and CFP, and the CMV and EF-1*α* promoters, were recombined into the Invitrogen™ pcDNA™ 6.2/V5-PL-DEST Vector (**A**, **C**, and **E**) or into the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



Destination vector selection guide

Gateway cloning technology is widely adapted to generate clones for protein expression in a variety of hosts.

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	pYES-DEST52
Insect cells	BaculoDirect C-Term Expression Kit
Mammalian cells (constitutive expression)	pcDNA Mammalian Expression vector family
Mammalian cells (regulated expression)	pT-REx-DEST30 and 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

Ordering information

Product	Description	Quantity	Cat. No.*
TOPO TA cloning			
pCR8/GW/TOPO TA Cloning Kit	Efficient TOPO TA cloning kit for simplified Entry clone construction	20 reactions	K250020 K252020 K252002
Directional TOPO cloning			
pENTR/D-TOPO Cloning Kit	Directional TOPO cloning kit to produce expression-ready Entry clones	20 reactions	K240020
pENTR/SD/D-TOPO Cloning Kit	Directional TOPO cloning kit with a Shine-Dalgarno sequence to create an *E. coli* expression-ready Entry clone	20 reactions	K242020
pENTR/TEV/D-TOPO Cloning Kit	Directional TOPO cloning kit to create expression-ready Entry clones with a 5´ TEV sequence for N-terminal tag removal (creating native proteins)	20 reactions	K252520 K253520
PCR cloning using BP recombination			
PCR Cloning System with Gateway technology	A complete directional cloning kit with a pDONR 221 Vector (kanamycin selection) or a pDONR Zeo Vector (zeocin selection)	20 reactions	12535029 12535037
pDONR 221 Vector	Contains pUC origin, M13 sequence sites, T7 promoter, *ccd*B gene, chloramphenicol resistance gene, kanamycin resistance gene	6 µg	12536017
pDONR Zeo Vector	Contains pUC origin, M13 sequence sites, T7 promoter, *ccd*B gene, zeocin resistance gene, supplied with 1.25 mL Zeocin Selection Reagent	6 µg	12535035
Restriction enzyme cloning vectors			
pENTR 1A Dual Selection Vector	Restriction enzyme cloning vector that produces in-frame (rf = 0), expression-ready Entry clones, including both Shine-Dalgarno and Kozak sequences	10 µg	A10462
pENTR 2B Dual Selection Vector	Restriction enzyme cloning vector that produces in-frame (rf = +1), expression-ready Entry clones	10 µg	A10463
pENTR 3C Dual Selection Vector	Restriction enzyme cloning vector that produces in-frame (rf = +2), expression-ready Entry clones	10 µg	A10464
pENTR 4 Dual Selection 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	A10465
pENTR 11 Dual Selection 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	A10467

* When multiple Cat. Nos. are listed for one product name, each Cat. No. includes a different cell line.

Ordering information (continued)

Product	Description	Quantity	Cat. No.
Multifragment assembly with Gateway technology			
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 pcDNA vector with C-terminal V5 and blasticidin selection to use with MultiSite Gateway Pro Kits	6 µg	12537162
BP Clonase enzymes			
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			
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
Competent cells			
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			
Gateway Vector Conversion System	Convert any cloning vector into a Gateway Destination vector using restriction endonucleases and ligase	20 reactions	11828029

Go to **thermofisher.com/gateway** for the latest in Gateway technology.

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