



Ordering information (continued)

LR Clonase® enzymes

Product	Description	Quantity	Cat. no.
Gateway® LR Clonase® II Plus Enzyme Mix		20 rxns	12538120
		100 rxns	12538200
Gateway® LR Clonase® II Enzyme Mix	A proprietary blend of Int (Integrase), IHF (Integration Host Factor) and Xis (Excisionase) enzymes that catalyze the <i>in vitro</i> recombination between an Entry clone and a Destination vector	20 rxns	11791020
		100 rxns	11791100
Gateway® LR Clonase® Enzyme Mix		20 rxns	11791019
		100 rxns	11791043

Competent cells

Product	Description	Quantity	Cat. no.
One Shot® <i>ccdB</i> Survival™ Competent Cells	Designed for propagation of plasmids containing the <i>ccdB</i> gene	10 transformations	C751003

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 rxns	11828029

Please visit www.invitrogen.com/gateway for the latest in Gateway® technology.

Amazingly versatile
Gateway® recombination cloning technology



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Gateway[®] recombination cloning

- Fast, one hour, room temperature cloning reactions with >99% efficiency deliver the clone you need
- Maintaining orientation and reading frame without using restriction enzymes or ligation makes expression-ready clones
- Eliminating resequencing ensures consistent results throughout your experiment using the same clone from target identification to validation
- Shuttling insert DNA from one expression vector to another affords flexibility while simplifying your cloning workflow

The typical cloning workflow involves many steps, particularly, traditional restriction enzyme cloning. This traditional method limits your cloning success. For example, certain restriction enzymes cannot be used because they might cut within your gene of interest, truncating the insert and making the gene useless for downstream expression. Additional clean-up steps are needed with this method, you experience low-efficiency recovery of recombinants from cloning large fragments, and you waste time screening colonies to find the clone you need—all of these steps take considerable time and effort and success is not guaranteed.

In contrast, Gateway[®] recombination cloning technology circumvents these cloning limitations, enabling you to access

virtually any expression system. Gateway[®] recombination cloning uses a one hour, 99%-efficient, reversible recombination reaction, without using restriction enzymes, ligase, subcloning steps, or screening of countless colonies, thereby saving you time, money, and effort. Widely adopted in the research community with more than 1,500 references since its launch, Gateway[®] technology makes collaboration across research disciplines easy and convenient and enables access to a multitude of vectors from these research groups for truly multidisciplinary scientific studies. Finally, new advancements such as MultiSite Gateway[®] technology makes Invitrogen's Gateway[®] cloning the ideal cloning method for protein expression and functional analysis.

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Gateway[®] terminology

- att site**—a defined length of DNA that constitutes a recombination site; there are 4 classes of att sites: attB, attP, attL, and attR
- ccdB gene**—a counter-selectable gene that allows for negative selection of unwanted by-product plasmids after recombination
- Donor (pDONR) vector**—a vector with attP sites flanking a counter-selectable gene that recombines with a gene of interest flanked by attB sites
- BP reaction**—a recombination event between attB and attP sites catalyzed by BP Clonase[®] II enzyme mix
- Entry (pENTR) clone**—a vector that contains a gene of interest flanked by attL or attR sites
- LR reaction**—a recombination event between attL and attR sites catalyzed by LR Clonase[®] II enzyme mix
- Destination (pDEST) vector**—an application-g geared vector with attR sites flanking a counter-selectable gene that will recombine with one or more Entry clones
- MultiSite Gateway[®] technology**—a system that allows simultaneous assembly of multiple DNA fragments into a single Destination vector

How it works

Invitrogen's Gateway® technology was developed using the well-characterized bacteriophage lambda site-specific recombination pathway (Figure 1). In *E. coli*, there is a short sequence of DNA called *attB* (B for bacteria), and in the phage, there is a stretch of DNA called *attP* (P for phage). After infection, the lambda DNA recombines with the corresponding bacterial DNA via the *att* sites using phage-encoded protein integrase (Int) and the host-encoded accessory protein integration host factor (IHF). The outcome of the recombination is integration of the phage DNA into the bacterial genome. After integration, the phage chromosome is flanked by *attL* and *attR*, which consist of DNA sequences from *attB* and *attP*. The reverse reaction between *attL* (L for left) and *attR* (R for right) results in excision of the phage DNA, and regenerates the original *attB* and *attP* sequences. The Gateway® BP reaction is a simple *in vitro* recombination reaction in which two *attB* sites on one DNA molecule react with two *attP* sites on a second DNA molecule to create *attL* and *attR* sites, as depicted in Figure 1. Note that *attB1* will only react with *attP1* and not *attP2*, ensuring the directionality of the reaction. The specificity is determined by a unique, 7-bp

core sequence that allows minimal cross-reactivity among *att* sequences. After the reaction, the hybrid recombination sites are again called *attL* and *attR* and are located on separate DNA molecules. The reaction is reversible by doing a separate

in vitro "LR" reaction. These recombination reactions ("LR" and "BP") are the basis of Gateway® cloning.

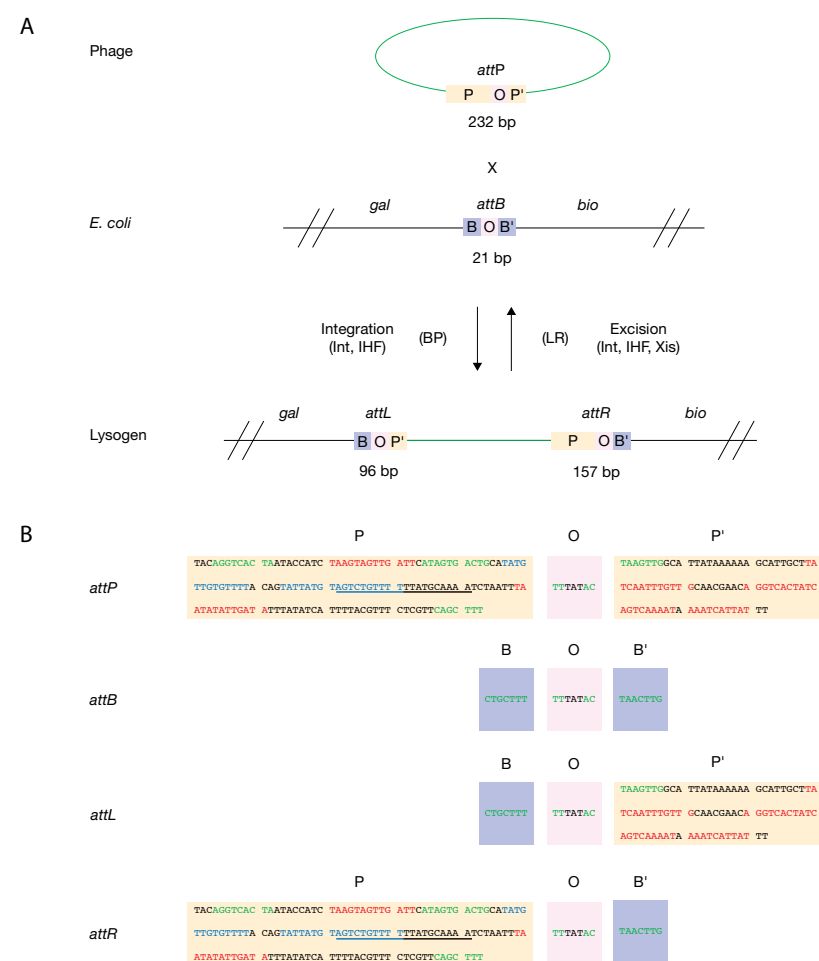


Figure 1—Site-specific recombination in phage λ. A. The integrative and excisive λ recombination pathways are shown. The labels *gal* and *bio* represent adjacent gene markers. BP stands for 'attL x attP recombination' whereas LR stands for 'attL x attR recombination'. B. The DNA sequence and protein binding sites for the four different *att* sequences depicted in A are shown; the arms and core (O) IHF, red; XLS; blue; Fis, underlined. Adapted from Katzen, F. (2007) *Expert Opin Drug Discov* 2(4): 571–589.

There are essentially two parts to Gateway® cloning: 1) constructing an Entry clone and 2) constructing the expression clone.

Part 1: constructing an Entry clone

There are various methods to enter the Gateway® platform, including use of TOPO® cloning vectors containing Gateway® *att* sites, or purchasing an Ultimate™ ORF Clone already inserted into a Gateway® vector. These will be discussed more on pages 6–7.

Part 2: constructing the expression clone

Site-specific recombination between *att* sites in an Entry clone and a Destination vector creates an expression clone with the gene of interest (see page 8 for reaction details). Following transformation and selection in *E. coli*, the Expression clone is ready for downstream expression and analysis.

Successful cloning made simple

Both BP and LR recombination reactions deliver >99% cloning efficiency, practically guaranteeing recovery of your recombinant clone with minimal screening. A simple 1 hour, 3-step protocol to set up either a BP or LR reaction saves you time by eliminating tedious subcloning steps, overnight ligations, and PCR clean-up procedures.

Eliminate resequencing after subcloning

One of the main benefits of Gateway® cloning is that you only have to clone and sequence your DNA fragment one time to create an Entry clone, ensuring consistent results. Using the same clone throughout your studies eliminates subcloning or worrying about sequence integrity and erroneous results. Moreover, because each recombination reaction is directional, both orienta-

tion and reading frame are maintained throughout your study, delivering expression-ready clones.

Ultimate flexibility

Unlike traditional cloning methods, Gateway® cloning is based on a reversible recombination reaction which enables unparalleled flexibility to access every expression system. Once you clone 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 (Figure 2). And with our new MultiSite Gateway® Pro technology, cloning multiple fragments has never been easier (see pages 10–11 for more details).

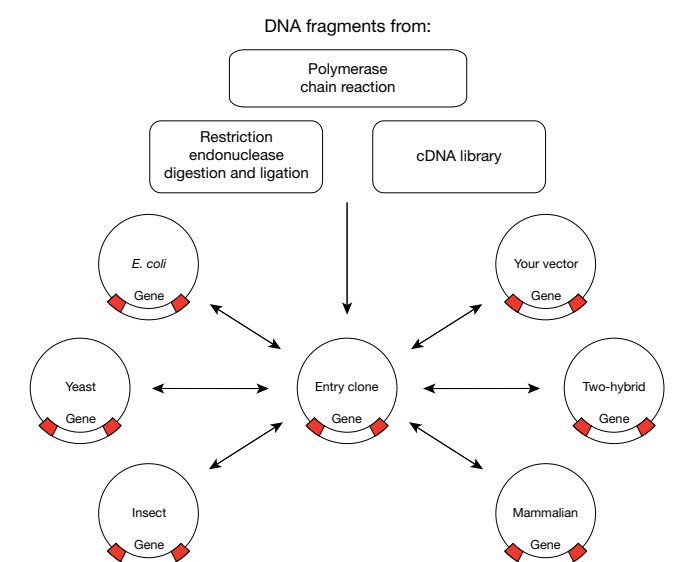


Figure 2—Gateway® technology facilitates cloning of genes, into and back out of, multiple vectors via site-specific recombination. Once a gene is cloned into an Entry clone you can then move the DNA fragment into one or more destination vectors simultaneously.

Entry clones—where you start

An Entry clone contains your gene of interest flanked by *attL* sequences, which are then used to recombine with *attR* sequences to create your desired expression clone. There are several methods you can use to produce an Entry clone: 1) use a TOPO® cloning Gateway® Entry vector, 2) perform restriction enzyme cloning into a pDONR vector, through PCR and a BP recombination reaction, or 3) purchase your clone from the Ultimate™ ORF Clone collection, which is in a Gateway®-compatible vector. Each method is briefly detailed below.

TOPO® cloning

We offer two types of TOPO® vectors that offer easy TOPO® cloning to create a Gateway® Entry vector. pCR®8/GW/TOPO® vector kits and pENTR™/D-TOPO® vector families both offer 5-minute, efficient TOPO® cloning, with >95% efficiency.

The pCR®8/GW/TOPO® vector enables efficient TOPO®-TA cloning. These vectors easily facilitate multipurpose use: rapid recombination into a variety of Gateway® Destination vectors, convenient sequencing, robust selection in *E. coli* with spectinomycin resistance, and easy excision of insert DNA with flanking *EcoR* I sites (Figure 3).

pENTR™/D-TOPO® vectors take advantage of fast, efficient Directional TOPO® cloning that delivers your insert in the correct orientation for expression. These vectors contain the necessary *attL* sequences for recombination into any Destination vector and certain versions carry a TEV protease cleavage site for producing native proteins after expression (Figure 4).

PCR amplification or a restriction-enzyme cloning vector

pDONR™ and pENTR™ vectors allow you to clone a PCR product amplified with primers containing *attB* sequences or specific restriction sites, respectively. Using PCR to generate the Entry clone, two artificial short *attB* sequences (*attB1* and *attB2*) 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 *attP1* and *attP2*

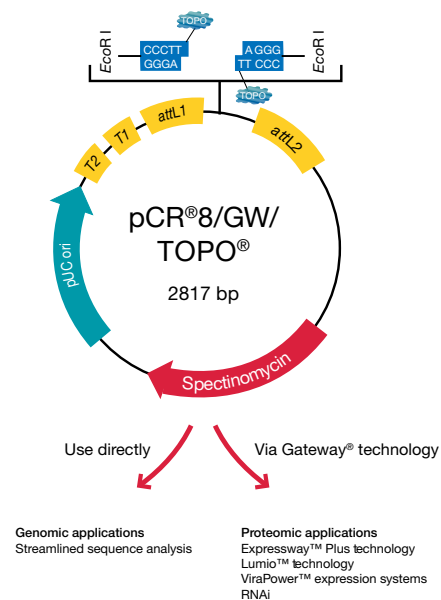


Figure 3—The pCR®8/GW/TOPO® Entry vector allows TOPO® TA Cloning® for multiple downstream applications.

sequences and BP Clonase® II enzyme.

Greater than 90% of the colonies contain the Entry clone with the gene of interest in the correct orientation. The resulting Entry clones are ready for recombination with any Gateway® Destination vector (Figure 5).

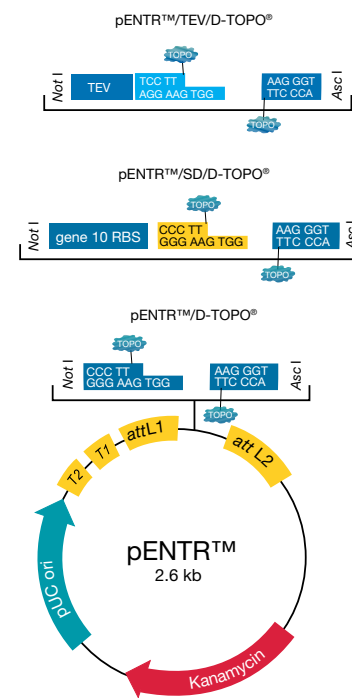


Figure 4—Several pENTR™ vectors are available for Directional TOPO® cloning and direct access to the multitude of Gateway® expression vectors.

A purchased clone

You can also enter Gateway® technology with a ready-to-use clone from an 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 pENTR™221 Gateway®

Entry vector for limitless downstream analysis capabilities.

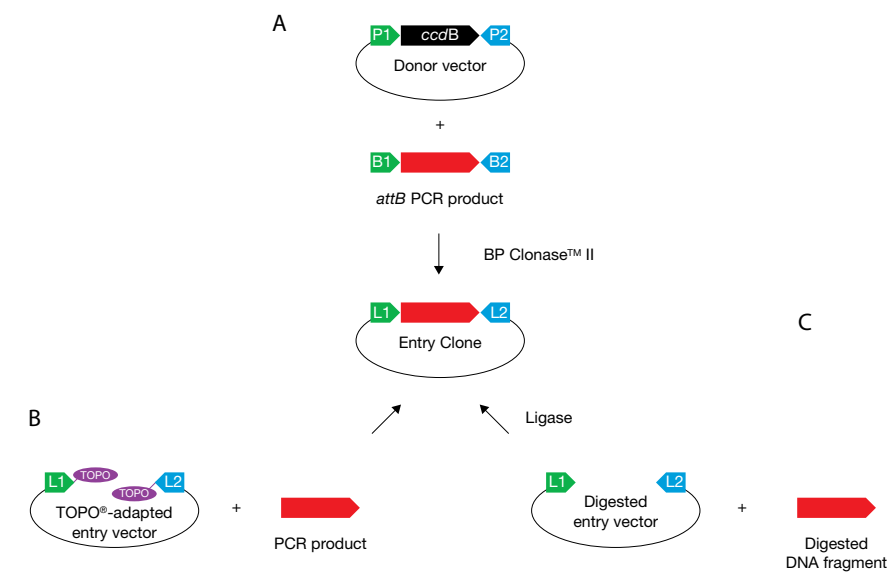


Figure 5—Strategies to build the Entry clone. The three possible methods that lead to the Entry clone are depicted: A. BP cloning, B. TOPO® cloning and C. restriction enzyme and ligase cloning. Red arrows represent the fragment of interest. Adapted from Katzen, F. (2007) *Expert Opin Drug Discov* 2(4): 571–589.

Clonase® enzymes— mediating the recombination reaction

As explained, the core of Gateway® cloning is the Entry vector. 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 a robust enzyme mixture called LR Clonase®, which contains the necessary protein activity to excise the gene

of interest from the Entry clone and integrate it into the Destination vector, which then becomes your expression clone (Figure 6). Reversing this reaction simply entails performing a BP reaction with BP Clonase® enzyme mix. Both LR and BP Clonase® enzyme mixtures are easy-to-use master mix formats ensuring consistency and reliability from reaction to reaction (Table 1).

Table 1—Applications of BP and LR Clonase® enzymes.

Application	Recommended Clonase® enzyme mix	Proteins involved in site-specific recombination	Activity	Advantage
Creating Entry clones	BP Clonase® II	Int (Integrase) IHF (Integration Host Factor)	DNA recombinase DNA binding protein	<ul style="list-style-type: none"> High efficiency cloning for Entry clone construction Single-mix format eliminates pipetting steps and error Easy-to-use, single-mix format ensures enzyme stability Convenient 10 µl reaction set up
Creating expression clones	LR Clonase® II Plus	Int (Integrase) IHF (Integration Host Factor) Xis (Excisionase)	DNA recombinase DNA binding protein DNA recombination (site specific, directional)	<ul style="list-style-type: none"> Highest cloning efficiency for single and multiple fragment cloning Optimized for difficult cloning reactions Works with MultiSite Gateway® Pro technology Easy-to-use, single-mix format ensures enzyme stability Convenient 10 µl reaction set up

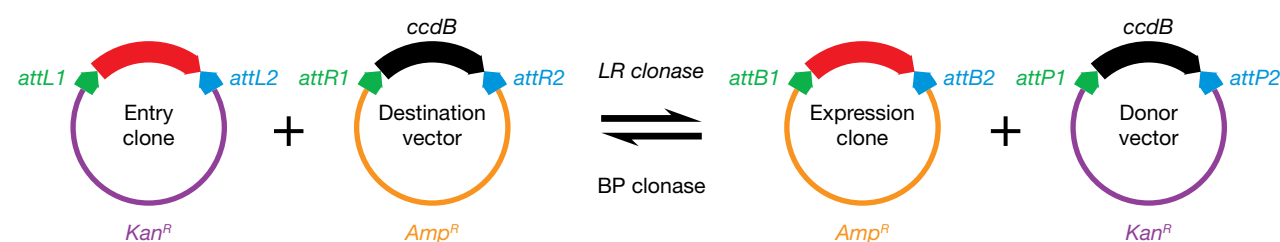


Figure 6—The Gateway® reactions. The scheme shows the four types of plasmids and enzyme mixes involved in Gateway® cloning reactions. Red arrows represent the fragment of interest. Adapted from Katzen, F. (2007) *Expert Opin Drug Discov* 2(4): 571–589.

Destination vectors—where you want to go

A unique advantage to Gateway® cloning is the unparalleled selection of expression vector options (Table 2). From expressing 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. For applications for which you require a special vector not available commercially, our Gateway® Vector Conversion System converts any vector into a Gateway®-compatible vector.

Table 2—Gateway® expression vector options.

Application	Gateway® Destination vector family
Protein array	Expressway™ Plus Expression system
Antibody or antigen production	Champion™ pET Expression systems
Protein expression in <i>E. coli</i>	pDEST™14, 15, 17, and 24 pET160 and pET161 DEST™ vectors
Protein expression in yeast	pYES2-DEST™52
Protein expression in insect cells	BaculoDirect™ C-term Expression Kit
Protein expression in mammalian cells (constitutive expression)	pcDNA® mammalian expression vector family
Protein expression in mammalian cells (regulated expression)	pT-REx-DEST30 and pT-REx-DEST31 vectors
Protein expression in mammalian cells (viral delivery)	ViraPower™ Lentiviral Expression Systems
Protein-protein interaction studies	ProQuest™ Two-Hybrid System using Gateway® technology
Localization	VividColors™ pcDNA GFP Destination vector family
RNAi	BLOCK-iT™ vector family
Reporter assay	GeneBLAzer™ pcDNA vector family

For a complete listing of all our Gateway® Destination vectors, visit www.invitrogen.com/gateway.

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 MultiSite Gateway® Pro technology, allows the mixing and matching of functional fragments in a concerted fashion to generate multisegment 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 technology is realized with MultiSite Gateway® Pro kits, which allow for the simultaneous assembly of multiple fragments into a single vector in a pre-defined order, orientation, and reading frame (Figures 7 and 8).

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

2. Generate entry clones through Gateway® BP recombination with *attB*-flanked PCR fragments and the appropriate pDONR™ vectors provided in MultiSite Gateway® Pro kits.

3. Recombine entry clones with a Gateway® Destination vector (with *attR1* and *attR2* sites) to generate an expression plasmid.

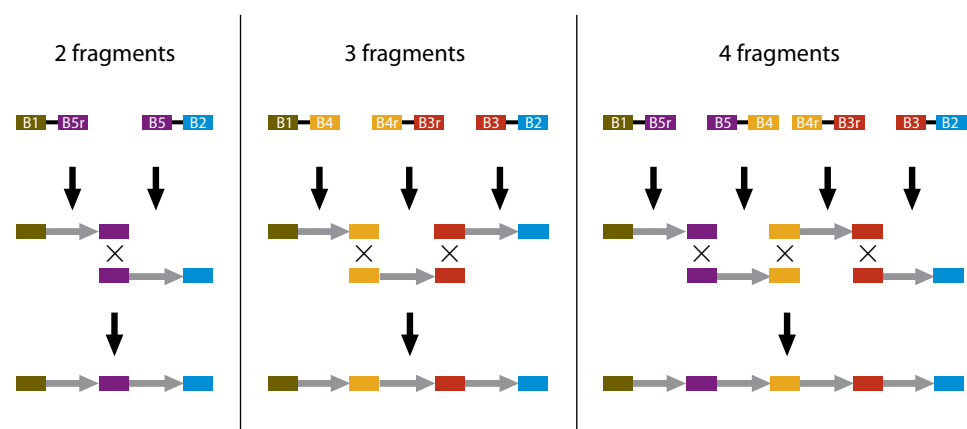


Figure 7—How MultiSite Gateway® Pro technology works.

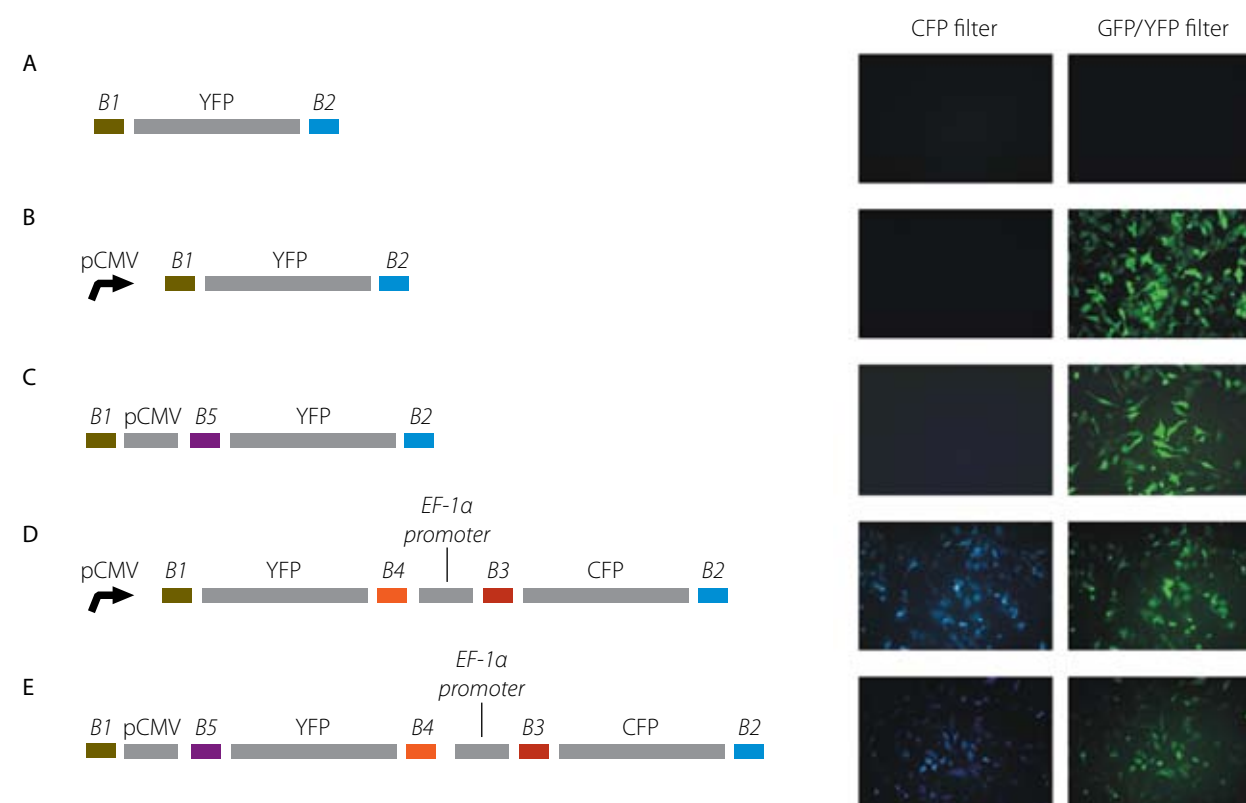


Figure 8—An example of using MultiSite Gateway® Pro technology to study expression of multiple genes in human cells. Entry clones encoding genes for YFP and CFP and the CMV and EF-1α promoters were recombined into pcDNA™ 6.2/V5-PL-DEST (A, C, and E) or into pcDNA™ 6.2/V5-DEST (B and D). The resulting expression clones were used to transfect HeLa cells. Expression was verified under a fluorescence microscope. The plasmid pcDNA™ 6.2/V5-PL-DEST is a promoterless derivative of pcDNA™ 6.2/V5-DEST, which carries the CMV promoter.

Vector NTI® software

Invitrogen's Vector NTI Advance® sequence analysis software is the most intelligent, powerful application for designing, executing, and tracking Gateway® cloning experiments (Figure 9).

- Easy-to-use wizard functionality that guides you through complete Gateway® cloning workflows (“in silico” cloning)
- Built-in biological knowledge of Gateway® technology to alert you to possible inconsistencies in recombination sites and selection markers
- Graphically rich sequence maps, step-by-step cloning protocols, and comprehensive data management and tracking capabilities
- Enables batch Gateway® clone construction for high-throughput projects

Download your copy from www.invitrogen.com/vectornti.

Gateway® open architecture policy

Gateway® open architecture is a new licensing policy that makes the use and distribution of nucleic acid fragments cloned using Gateway® technology even easier. By facilitating access and distribution of Gateway® clones, Gateway® architecture demonstrates Invitrogen's commitment to supporting cutting-edge life science research.

Please visit www.invitrogen.com/gateway for details.

Let us do the work

You can also access the advantages of Gateway® technology through our Custom Services department. Invitrogen can help you save time by being more productive by performing cloning, protein expression, and vector conversion services for you.

For complete details, go to www.invitrogen.com/gateway or call Custom Services at 1 800 955 6288 (in the USA).



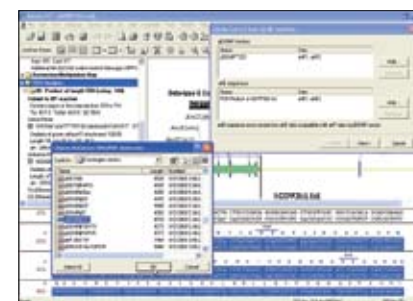
Step 1. Select DNA sequence.

Easily select the DNA sequence you want to clone—by highlighting an annotated feature graphically, for example—and choose the “Amplify selection to use in BP reaction” option under the Gateway® Cloning menu.



Step 2. Design PCR primers.

Let the application design PCR primers for you. Vector NTI Advance® will automatically add the correct *attB* recombination sites, and you can easily include additional sequences—such as an acTEV protease cleavage site—to your sense and antisense primers.



Step 3. Choose Entry vector.

Choose the Entry vector you wish to use from the pre-loaded list present in the Vector NTI Advance® database. The Gateway® cloning wizard alerts you as to which kind of *attP* sites are required on the Entry vector.



Step 4. Create your recombinant molecule.

With compatible recombination sites on the DNA fragment and Entry vector, complete your workflow by simply clicking the Finish button. The Gateway® cloning wizard recombines the input sequences in the correct orientation, and automatically creates a graphical map of your recombinant molecule.



Results

Vector NTI Advance® automatically creates a graphical map of the recombinant molecule you have designed. The map includes a step-by-step cloning protocol that you can use at the bench. There's also an innovative hyperlink—VectorLink™—that takes you to a custom-built web page with information on Invitrogen products relevant to the features of your Entry clone.

Figure 9—Vector NTI Advance® sequence analysis software workflow.

Ordering information

TOPO® TA Cloning

Product	Description	Quantity	Cat. no.
pCR®8/GW/TOPO® TA Cloning Kit	Efficient TOPO® TA cloning kit simplifies Entry clone construction	20 rxns	K250020
pCR®8/GW/TOPO® TA Cloning Kit	Efficient TOPO® TA cloning with fast-growing competent <i>E. coli</i> that shortens the time for Entry clone construction	20 rxns	K252020
pCR®8/GW/TOPO® TA Cloning kit	Efficient TOPO® TA cloning with fast growing competent <i>E. coli</i> and plasmid purification drastically shortens and simplifies Entry clone construction, saving time and hassle.	20 rxns	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 rxns	K240020
pENTR™/SD/D-TOPO® Cloning Kit	Directional TOPO® Cloning, including the Shine-Dalgarno sequence that creates an <i>E. coli</i> expression-ready Entry clone	20 rxns	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 rxns	K252520
pENTR™/TEV/D-TOPO® Cloning Kit	Directional TOPO® Cloning kit with fast-growing competent <i>E. coli</i> 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 rxns	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 rxns	12535029
PCR Cloning System with Gateway® technology	Complete kit for directional cloning into a Gateway® vector with pDONR™/Zeo vector with Zeocin™ selection	20 rxns	12535037
pDONR™ 221 Vector		6 µg	12536017
pDONR™ Zeo Vector		6 µg	12535035

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 produce in-frame (rf = +1), expression-ready Entry clones	10 µg	11816014
pENTR™ 3C Vector	Restriction enzyme cloning vector that produce in-frame (rf = +2), expression-ready Entry clones	10 µg	11817012
pENTR™ 4 Vector	Same as pENTR™ 1A Vector except with <i>Nco</i> I instead of <i>Dra</i> I 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 <i>Nsp</i> V instead of <i>Dra</i> I 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 2.0 Kit	Cloning two fragments into a Gateway® Destination vector	20 rxns	12537102
MultiSite Gateway® Pro 3.0 Kit	Cloning three fragments into a Gateway® Destination vector	20 rxns	12537103
MultiSite Gateway® Pro 4.0 Kit	Cloning four fragments into a Gateway® Destination vector	20 rxns	12537104
MultiSite Gateway® Pro Plus Kit	Allows for flexible cloning of up to four fragments into a Gateway® Destination vector	20 rxns	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

Ultimate™ ORF Clones

Product	Description	Cat. no.
Ultimate™ Human ORF Clone	The Ultimate™ ORF Clone Collection contains DNA- and amino acid sequence-verified and expression-ready cDNAs, including kinases, G-protein-related, phosphatases, ion channels, GPCRs, chemokines, nuclear receptors, and cytokines; visit www.invitrogen.com/ORF to select your clone today	HORF01
Ultimate™ Mouse ORF Clone		MORF01

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 <i>in vitro</i> recombination of PCR products or DNA segments from clones and a donor vector	20 rxns	11789020
		100 rxns	11789100
Gateway® BP Clonase® Enzyme Mix		20 rxns	11789013
		100 rxns	11789021