

Site-specific recombinase-based vector system

Mammalian Expression Vectors with Interchangeable Selectable Markers

Lisa Marsh • Carsten Carstens
Stratagene

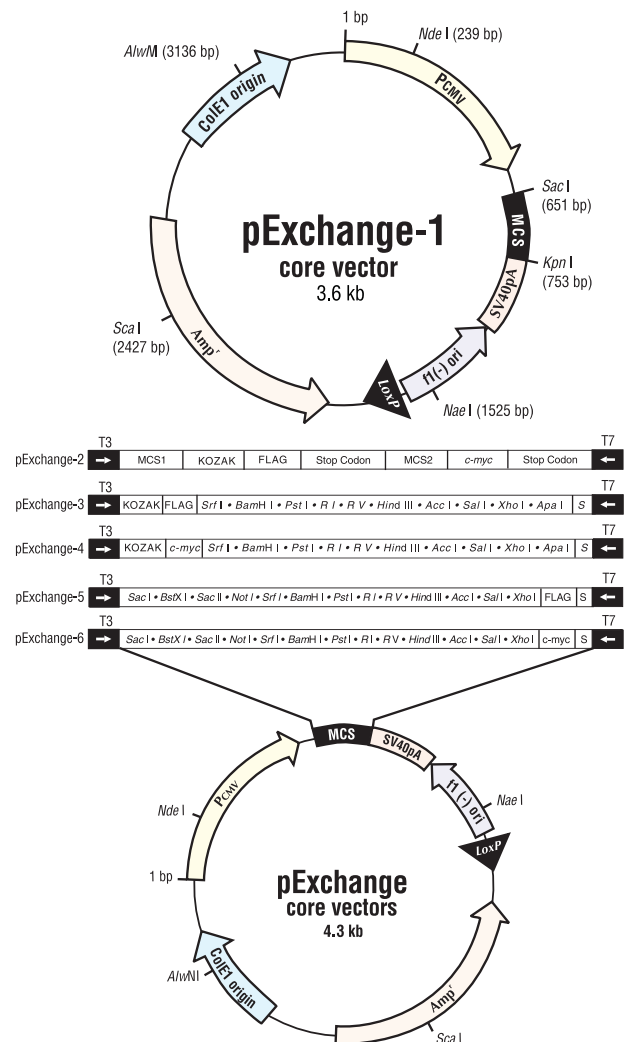
Stratagene's Exchanger™ system of eukaryotic expression vectors offers unparalleled convenience. The drug-resistance genes of these vectors can be exchanged without subcloning. Through site-specific recombination, hygromycin, puromycin, and neomycin functional cassettes are quickly and efficiently inserted into the pExchange core vectors. Insertion is carried out *in vitro* by a Cre-mediated recombination of LoxP sites, and recombination products are selected via drug resistance. The components of the Exchanger system include Cre recombinase; one of the six different pExchanger core vectors; linearized insertion modules, which are flanked by LoxP sites and provide hygromycin, puromycin, or neomycin resistance for selection in mammalian cells; the corresponding luciferase core-vector control; and supercompetent *Epicurian Coli*® XL1-Blue cells. The entire site-specific recombination procedure consists of a 30-minute recombination reaction, a 15-minute heat inactivation, and transformation of suitable hosts with the recombination reaction. Desired reaction products are obtained with greater than 90% efficiency.

Stratagene's pCMV-Script® and pCMV-Tag mammalian expression vectors^{1,2,***} offer easy cloning and high-level expression; accordingly, they are used in a variety of standard molecular biology applications. These vectors, in addition to containing an expression cassette for inserting the gene of interest into a multiple cloning site, usually include a cassette that contains a selectable marker. However, when it is necessary for the expression vector to carry a different selectable marker, the most common method is to subclone the gene of interest into a different vector, which is very inconvenient. Therefore, it would be ideal to be able to quickly and efficiently exchange drug-resistance elements without subcloning into such vectors.

Cre-Mediated Recombination

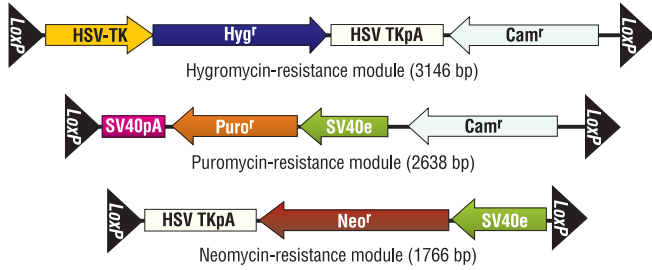
To eliminate conventional restriction digestion and ligation subcloning procedures, site-specific recombination is used to manipulate DNA fragments. Site-specific recombinases, such as the P1-derived Cre recombinase, catalyze the exchange of DNA located between specific recognition sequences.^{3,4,5} For Cre recombinase, the recognition site (*LoxP*) consists of two

Figure 1
Core Expression Vectors



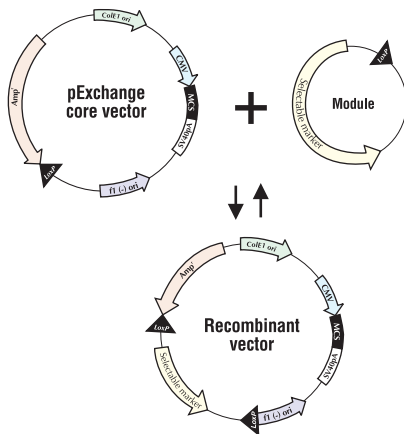
The pExchange core vectors are derived from the pCMV-Script® and pCMV-Tag vectors. Each vector includes the ampicillin-resistance gene, the cytomegalovirus (CMV) immediate-early promoter, the SV40 polyadenylation sequence, a multiple cloning site, and a *LoxP* site. In addition, the pExchange 2 vector contains the synthetic FLAG and the c-myc epitopes for tagging of the expressed protein. The pExchange vectors 3 through 6 allow FLAG^{11 11} or c-myc tagging at either the N-terminus or C-terminus.

Figure 2
Antibiotic-Resistance Cassettes



Each cassette contains the specified eukaryotic resistance marker and a chloramphenicol marker flanked by two *LoxP* sites in parallel orientation for bacterial selection of the recombinants. Since the neomycin expression cassette already confers kanamycin resistance to the bacterial host, the chloramphenicol marker was omitted in the neomycin cassette.

Figure 3
Insertion of Selectable-Marker Cassettes by Site-Specific Recombination



inverted 13-bp repeats and an intervening, 8-bp nonpalindromic region.³ Intermolecular and intramolecular recombination between the two sites can occur.

Exchange of Selectable Markers

Stratagene’s Exchanger cloning system is based on Cre-mediated, site-specific recombination, which allows fast and efficient insertion of prefabricated, selectable DNA fragments into core expression vectors. The system consists of a pExchange core expression vector, drug-resistance modules and Cre recombinase protein. The pExchange core vectors are based on the pCMV-Script and pCMV-Tag vectors (Figure 1) that contain a *LoxP* site that serves as the acceptor site for the eukaryotic selectable-marker cassettes and offer a variety of expression options. The hygromycin-, puromycin-, or neomycin-resistance modules (Figure 2) are designed to be inserted into the *LoxP* site of either core vector. Each cassette is linearized and flanked by *LoxP* sites. A luciferase-containing core vector control for verifying expression in any cell line is provided along with Epicurian Coli XL-1 Blue supercompetent cells for simplifying transformation. All components are available separately.

The essential process for Cre-mediated insertion of antibiotic-resistance cassettes into the core vectors of the Exchanger system is described in Figure 3. Recombination requires a 30-minute incubation of the core vector, antibiotic-resistance cassette, and Cre recombinase. This reaction is followed by heat inactivation of Cre recombinase at 65°C for 15 to 30 minutes. The inactivated recombination reaction is then used to transform host cells, and the transformation mixture is selected on the appropriate antibiotic-containing nutrient plates. After incubation, colonies are picked and screened using standard miniprep procedures, and plasmid DNA can be analyzed by restriction digestion. Successful insertion rates of greater than 90% are achieved routinely.

Functional Testing of the Selectable Cassettes

To test the performance of the eukaryotic selectable markers provided by each cassette, we transfected Chinese hamster ovary (CHO) and NIH3T3 cells with the core vector containing the selectable marker that was introduced by site-specific recombination. Each construct conferred resistance to the respective drug at a frequency equal to or better than comparable vectors with the same drug resistance marker (data not shown).

To confirm the functionality of the CMV expression cassettes of the core vectors, we compared luciferase expression from the pExchange core vectors to luciferase expression from the parental pCMV-Script and pCMV-Tag vectors. In these transient transfections of CHO cells, the presence of the integrated drug-resistance cassettes did not affect the performance of the CMV expression cassette in luciferase expression assays. The observed expression level of the Exchanger system vectors did not differ from their respective parental pCMV-Script and pCMV-Tag vector constructs (data not shown).

Conclusions

Stratagene’s Exchanger vector system allows a single expression construct to be used for multiple applications without subcloning the gene of interest. This system features the pExchange core vectors into which, through Cre-mediated site-specific recombination, hygromycin-, puromycin-, or neomycin-resistance selection cassettes can be introduced. Several test experiments have verified that the desired recombinants are generated with greater than 90% efficiency. While eliminating tedious subcloning procedures, the Exchanger vector system provides an easy mechanism for inserting selectable markers.

REFERENCES

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Exchanger™ System	
With pExchange-1 core vector (1 µg/µl) supercoiled	#211184
With pExchange-2 core vector (1 µg/µl) supercoiled	#211185
With pExchange-3 core vector (1 µg/µl) supercoiled	Coming soon!
With pExchange-4 core vector (1 µg/µl) supercoiled	
With pExchange-5 core vector (1 µg/µl) supercoiled	
With pExchange-6 core vector (1 µg/µl) supercoiled	

