Faster Cloning Means Faster Results

The Flexi® Vector Systems: The Easy Way to Clone

By Michael Slater, Ph.D., Promega Corporation

Abstract

The Flexi® Vector Systems facilitate the study of multiple facets of protein function by reducing the cloning burden of shuttling protein-coding regions between vectors with different functional capabilities. Once your protein-coding region is cloned into a Flexi® Vector, you can easily shuttle it into many other Flexi® Vectors, without the need for re-sequencing, to compare various expression systems and obtain your best yields and results.

The Flexi® Vector Systems allow you to easily and efficiently transfer a protein-coding region between a wide variety of expression vectors without the need to re-sequence.

Introduction

A better understanding of the function of protein-encoding genes can be gained by combining different approaches to study the encoded protein. These include characterizing interactions with other proteins, nucleic acids or small molecules, determining the protein's chemical activities, determining its subcellular localization and determining its three-dimensional structure. Promega offers a variety of Flexi® Vectors specialized for these approaches (Figure 1). Flexi® Vector Systems[a,b,c] provide an efficient and high-fidelity method for transferring protein-encoding DNA into vectors capable of expressing native (untagged) protein or protein with an amino- (N) or carboxy- (C) terminal tag in bacterial, mammalian or cell-free expression systems (1).

The Flexi® Vector Systems use two rare-cutting restriction enzymes, *Sgf* I[d] and *Pme* I[c], in a simple, yet powerful, directional cloning method for protein-coding sequences. *Sgf* I and *Pme* I have the first and second fewest restriction sites in the protein-coding regions of human cDNA sequences, respectively, making them ideally suited for cloning. This enzyme pair also cuts infrequently in the open reading frames of many other organisms (1). In the unlikely event your protein-coding region contains one of these sites, you should consider cloning a portion of the protein-coding region, using RecA protein to protect the *Sgf* I or *Pme* I sites from digestion (2), or using PCR[e]-based, site-directed mutagenesis methods (3,4) to mutate restriction enzyme sites without changing the amino acid sequence of the protein-coding region.

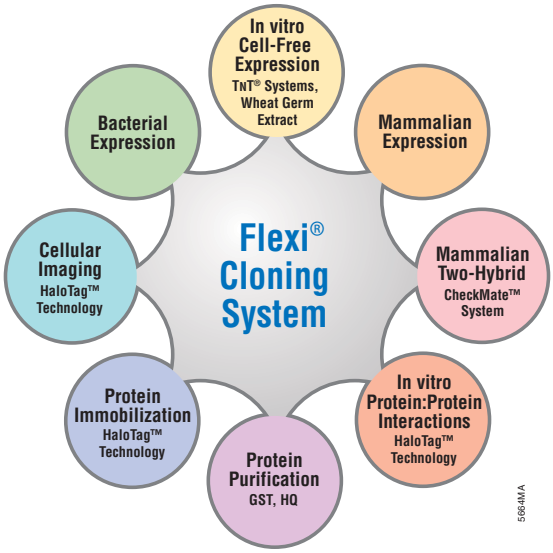

Figure 1. Functional protein analysis capabilities provided by the Flexi® Vectors. This system provides multiple points of entry, eliminating the need for an archival "entry" vector, while retaining easy shuttling of the protein-coding region to other Flexi® Vectors, enabling a wide range of functional analysis options.

Cloning into the Flexi® Vectors

Any Flexi® Vector can accept a protein-coding region flanked by *Sgf* I and *Pme* I sites. PCR can be used to append *Sgf* I and *Pme* I sites to the protein-coding region (1; Figure 2). The primers are designed so that an *Sgf* I site is incorporated into the amino-terminal PCR primer and a *Pme* I site into the carboxy-terminal PCR primer. A tool for designing primers is available at: **www.promega.com/techserv/tools/flexivector/**

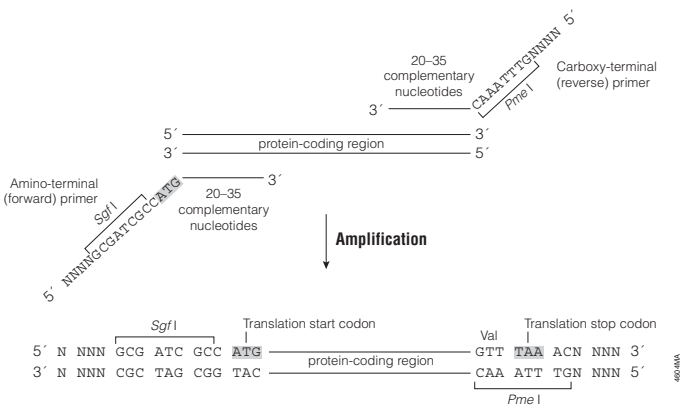

Figure 2. PCR primer design.

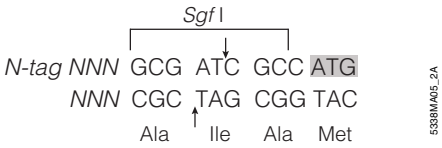

Figure 3. The *Sgf* I site in N-terminal Flexi® Vectors. Protein-coding regions transferred into N-terminal Flexi® Vectors allow translational readthrough of the *Sgf* I site, which encodes the peptide sequence Ala-Ile-Ala.

The *Sgf* I site is placed one base upstream of the start codon of the protein-coding region. This allows expression of native protein in Flexi® Vectors designed to express native proteins. In N-terminal Flexi® Vectors, readthrough of the *Sgf* I site allows expression of an N-terminal-tagged protein (Figure 3). The *Pme* I site contains the stop codon for the protein-coding region and appends a single valine residue to the C-terminus of the protein (Figure 2). The valine codon, GTT, is immediately followed by an ochre stop codon, TAA. When a protein-coding region flanked by *Sgf* I and *Pme* I sites is cloned into a vector cut with *Sgf* I and *Pme* I, the translation stop codon is recreated. As this is a flexible system, you can also design your PCR primers to place the *Pme* I site downstream of the native stop codon, so translation terminates at the native stop codon, and a valine residue is not appended to the protein. However, in doing so, you lose the ability to express the protein with a carboxy-terminal tag.

Expressing Native or N-Terminal Fusion Proteins

To express native or N-terminal fusion proteins, the protein-coding region can be ligated directly into an appropriate Flexi® Vector or shuttled from one Flexi® Vector (donor) to another Flexi® Vector (acceptor) (Figure 4). To transfer a protein-coding region, donor and acceptor vectors are mixed and cut with *Sgf* I and *Pme* I. After ligating, the DNA mixture is transformed into *E. coli*, and colonies are selected for the appropriate drug resistance for the acceptor vector. All Flexi® Vectors contain a lethal gene, barnase, which must be replaced with an insert for the desired clone to survive, allowing high-efficiency transfer of a protein-coding region between vector backbones.

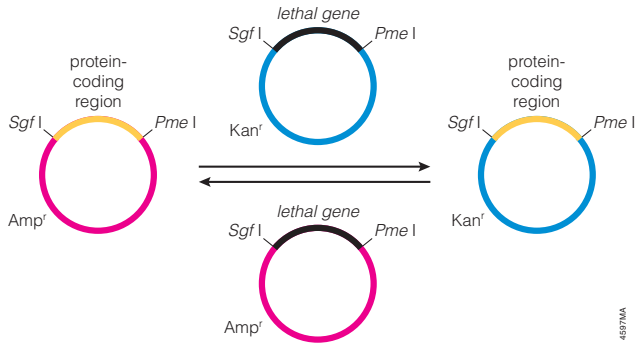

Figure 4. Reversible transfer between native and N-terminal Flexi® Vectors.

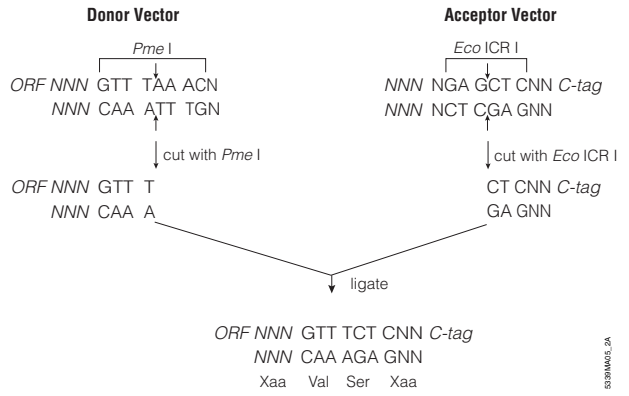

Figure 5. Cloning into a C-terminal Flexi® Vector. In transferring the DNA fragment from a donor Flexi® Vector to a C-terminal Flexi® Vector, the blunt ends of *Pme* I and *Eco*ICR I are joined to generate the sequence: GTT TCT CNN, which translates to Val-Ser-Xaa, allowing readthrough into the C-terminal tag of the C-terminal Flexi® Vectors

Expressing C-Terminal Fusion Proteins

A carboxy-terminal fusion protein can be created by fusing the blunt *Pme* I end of the protein-coding region with the blunt end generated by a different restriction enzyme (e.g., *Eco*ICR I). C-terminal Flexi® Vectors lack a *Pme* I site and contain an *Eco*ICR I site, so they can accept protein-coding regions flanked by *Sgf* I and *Pme* I sites. To transfer a protein-coding region, the donor plasmid is cut with *Sgf* I and *Pme* I, while the acceptor plasmid is cut separately with *Sgf* I and *Eco*ICR I. When the blunt *Pme* I and *Eco*ICR I ends are joined, the stop codon is not recreated, allowing readthrough into the C-terminal peptide sequence (Figures 5 and 6). Both blunt ends are destroyed, so this joined sequence cannot be cut by *Pme* I or *Eco*ICR I, and the protein-coding region cannot be transferred out of these vectors. For this reason, do not clone a protein-coding PCR fragment directly into a C-terminal Flexi® Vector if you plan to transfer the protein-coding region to a different Flexi® Vector in the future. By cloning the PCR fragment first into a native or N-terminal Flexi® Vector, the ability to transfer it to any other Flexi® Vector is preserved.

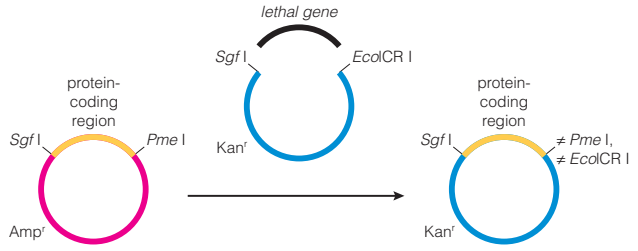

Figure 6. Transfer of protein-coding regions into a C-terminal Flexi® Vector. After ligation, both the *Pme* I or *Eco*ICR I sites are destroyed, so transfer is not reversible.

Advantages of Flexi® Vectors

The simple design of the Flexi® Vector directional cloning method is easily adapted to high-throughput formats through the use of automated liquid-handling systems or multichannel pipettors. Blommel *et al*. compared the Flexi® Vector System with a popular high-throughput recombination-based transfer system (5). Cloning protocols for both systems were tested in parallel on 96 target genes; cDNA sequences were amplified from plasmids. The frequency of capture of target genes was similar for both systems (90–92%), but the number of protocol steps and the occurrence of missense and silent mutations in the PCR clones were twofold lower for the Flexi® Vector System. This was primarily due to the shorter PCR primers needed for the Flexi® Vector System. This resulted in quicker production of sequence-validated clones: 6–8 days for Flexi® Vector clones versus 12 days for the other system. In addition, it was simpler to capture the remaining Flexi® Vector clones. For the Flexi® Vectors, another clone was selected and sequenced, whereas the other system required reworking two steps of the cloning process. Also, the rate of transfer of the protein-coding region to another acceptor vector was equally high for both systems (95–98%). Blommel *et al*. concluded that the Flexi® Vector System offered time and cost savings for high-throughput production of expression clones.

Another advantage of the Flexi® Vectors is that fewer amino acids are appended to the N- or C-terminus of the protein of interest compared to other site-specific recombination vector systems. Addition of unstructured amino acids to the termini of proteins can sometimes interfere with protein folding and function. This is more problematic for recombination-based systems due to the large size of the recombination sites that must be incorporated. The restriction sites used in Flexi® Vectors do not impede DNA sequencing reactions when validating target gene clones, unlike some site-specific recombination vector systems (6). The sites also do not impair the transcription or translation of the protein-coding region in mammalian, bacterial or cell-free expression systems (1,5,7,8). Flexi® Vector Systems do not require an archival entry vector or a specialized donor vector for most applications and allow direct entry into the type of vector best suited to the experimental design. Protein-coding regions can be shuttled from any native or N-terminal Flexi® Vector to another vector to provide alternative expression and functional analysis options. For these reasons, Flexi® Vector Systems offer advantages in elucidating protein function.

References

1. Slater, M. *et al.* (2005) *Promega Notes* **89**, 11–5.
2. Schoenfeld, T., Harper, T. and Slater, M. (1995) *Promega Notes* **50**, 9–13.
3. Higuchi, R., Krummel, B. and Saiki, R.K. (1988) *Nucl. Acids Res.* **16**, 7351–67.
4. Ho, S.N. *et al.* (1989) *Gene* **77**, 51–9.
5. Blommel, P.G. *et al.* (2006) *Protein Expr. Purif.*, in press.
6. Esposito, D., Gillette, W.K. and Hartley, J.L. (2003) *BioTechniques* **35**, 914–20.
7. Godat, B. (2005) *Promega Notes* **91**, 17–20.
8. Slater, M. *et al.* (2005) *Promega Notes* **91**, 21–5.

Protocols

◆ *Flexi® Vector Systems Technical Manual* TM254, Promega Corporation. www.promega.com/tbs/tm254/tm254.html

Ordering Information

Product	Size	Cat.#
Flexi® System, Entry/Transfer	5 entry and 20 transfer reactions	C8640
Flexi® System, Transfer	100 transfer reactions	C8820
Carboxy Flexi® System, Transfer	50 transfer reactions	C9320
10X Flexi® Enzyme Blend (*Sgf* I and *Pme* I)	25µl	R1851
	100µl	R1852
pF1A T7 Flexi® Vector	20µg	C8441
pF1K T7 Flexi® Vector	20µg	C8451
pFN2A (GST) Flexi® Vector	20µg	C8461
pFN2K (GST) Flexi® Vector	20µg	C8471
pF3A WG (BYDV) Flexi® Vector	20µg	L5671
pF3K WG (BYDV) Flexi® Vector	20µg	L5681
pF4A CMV Flexi® Vector	20µg	C8481
pF4K CMV Flexi® Vector	20µg	C8491
pF5A CMV-neo Flexi® Vector	20µg	C9401
pF5K CMV-neo Flexi® Vector	20µg	C9411
pFN6A (HQ) Flexi® Vector	20µg	C8511
pFN6K (HQ) Flexi® Vector	20µg	C8521
pFC7A (HQ) Flexi® Vector	20µg	C8531
pFC7K (HQ) Flexi® Vector	20µg	C8541
pFC8A (HaloTag™) CMV Flexi® Vector	20µg	C3631
pFC8K (HaloTag™) CMV Flexi® Vector	20µg	C3641
CheckMate™/Flexi® Mammalian Two-Hybrid System*		C9360

*includes the pFN10A (ACT) Flexi® Vector and pFN11A (BIND) Flexi® Vector.

The "pF" indicates that the vector is a Flexi® Vector. The letter after "pF" indicates the position of any expression tags (e.g., "N" for an N-terminal expression tag; "C" for a C-terminal expression tag). The number associated with the vector specifies the type of expression and application. The letters "A" and "K" designate the bacterial drug selection for the vector (A = ampicillin and K = kanamycin).

[a] For research use only. Persons wishing to use this product or its derivatives in other fields of use, including without limitation, commercial sale, diagnostics or therapeutics, should contact Promega Corporation for licensing information.

[b] Patent Pending.

[c] Licensed under U.S. Pat. No. 5,945,288.

[d] U.S. Pat. No. 5,391,487.

[e] Patents for the foundational PCR process, European Pat. Nos. 201,184 and 200,362, expired on March 28, 2006. In the U.S., the patents covering the foundational PCR process expired on March 29, 2005.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.