Stratagene Mutagenesis Solutions for Your Protein Engineering Needs

STRATAGENE

An **Agilent Technologies** Company

Protein engineering via mutagenesis allows researchers to modulate protein activity and characterize structure-function relationships, which enriches our understanding of basic cellular processes and disease mechanisms, fueling discoveries in new therapies for complex diseases such as cancer. Stratagene has been a pioneer in developing innovative mutagenesis tools for the past two decades. Cited in thousands of publications, our QuikChange® site-directed mutagenesis and GeneMorph® random mutagenesis kits are the tools behind many important experiments and scientific breakthroughs.

Stratagene: Your Partner in Targeted Protein Engineering

To help you maximize the power of mutagenesis, Stratagene offers the most comprehensive portfolio of reagents and kits today. Integrating advanced technology, enzyme engineering expertise and proven protocols, our products make it easy for you to achieve accurate, reliable results in the shortest time possible. Our ongoing product innovations continue to set new standards. The new QuikChange Lightning site-directed mutagenesis kit[a], for example, dramatically shortens experiment time while maintaining the same high efficiency and accuracy that you've come to expect from Stratagene. Now, as an Agilent Technologies company, Stratagene brings even more resources to our partnership with our customers.

Mutagenesis Selection Guide

APPLICATION	PRODUCT	DESCRIPTION	UNIQUE FEATURES	PAGE
Site-Directed Mutagenesis	QuikChange Lightning kit **NEW**	Features faster enzymes and shorter reaction protocols, which provide significant time savings with the same efficiency and accuracy as our original QuikChange kits; suitable for site-directed mutagenesis of all plasmid sizes, and quality-controlled with DNA templates up to 14 kb	• Protocol completed in <3 hours plus an overnight transformation • Contains the highly processive, ultra-high-fidelity QuikChange Lightning DNA polymerase fusion • Employs QuikSolution for enhanced amplification • Contains an *enhanced* *Dpn* I enzyme for faster mutant selection • Uses our highest-efficiency XL-10 Gold® ultracompetent cells	6
	QuikChange II kit	Uses an ultra-high-fidelity *Pfu* enzyme to ensure highly accurate and efficient site-directed mutagenesis	• Contains our ultra-high-fidelity *PfuUltra*® DNA polymerase and *Dpn* I selection enzyme • Includes XL1-Blue competent cells	7
	QuikChange II XL kit	Uses an ultra-high-fidelity *Pfu* enzyme to ensure highly accurate and efficient site-directed mutagenesis; additional kit components facilitate mutagenesis of larger (>8 kb) or difficult plasmids	• Contains our ultra-high-fidelity *PfuUltra* DNA polymerase and *Dpn* I selection enzyme • Employs QuikSolution for enhanced amplification • Includes our highest-efficiency XL-10 Gold ultracompetent cells	7
	QuikChange II-E kit	Provides ultra-high-fidelity site-directed mutagenesis, followed by transformation into electroporation-competent cells	• Contains our ultra-high-fidelity *PfuUltra* DNA polymerase and *Dpn* I selection enzyme • Includes XL1-Blue electroporation-competent cells	7
Multi-Site-Directed Mutagenesis	QuikChange Multi kit	Employs simple, three-step protocol for introducing mutations at up to five sites simultaneously; easily adapted to perform saturation mutagenesis or create mutant clone collections for screening	• Uses a multi-enzyme blend containing our *PfuTurbo*® DNA polymerase[b] • Includes *Dpn* I selection enzyme and our highest-efficiency XL-10 Gold ultracompetent cells	8
Random Mutagenesis	GeneMorph II kit	Provides superior method over conventional *Taq*-based methods for error-prone PCR; uses a novel polymerase that produces an unbiased mutational spectrum and robust amplification of targets up to 10 kb	• Contains Mutazyme® II error-prone DNA polymerase blend • Uses balanced nucleotides and manganese-free reaction buffer for robust error-prone PCR • Provides an easy method for achieving desired low, medium or high mutation rate	10
	GeneMorph II EZClone kit	Employs a two-part procedure for random mutagenesis and restriction-free cloning of target sequences up to 3.5 kb; combines the benefits of GeneMorph II random mutagenesis with an easy and accurate cloning method to create large, representative mutant libraries for screening	• Contains all components of the GeneMorph II kit • Additionally contains the ultra-high-fidelity EZClone polymerase for incorporating randomized sequences into nicked circular DNA, and *Dpn* I for mutant enrichment • Includes control primers and template, and our highest-efficiency XL-10 Gold ultracompetent cells	10

Easily Achieve Accurate, Reliable Site-Directed Mutagenesis

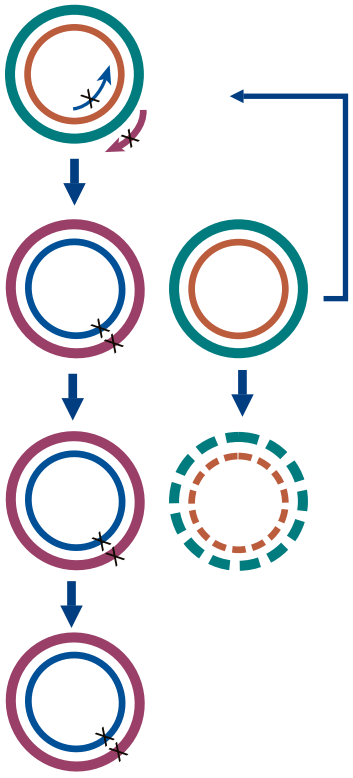
Site-directed mutagenesis is the method of choice for altering a gene or vector sequence at a selected location. Point mutations, insertions or deletions are introduced by incorporating primers containing the desired modification(s) with a DNA polymerase in an amplification reaction. Stratagene offers the most accurate, efficient and easy-to-use mutagenesis kits available today.

Save time with simple, same-day protocol

Our QuikChange mutagenesis kits utilize our signature three-step, one-day method to introduce point mutations, amino acid substitutions, deletions and small insertions in virtually any double-stranded plasmid template at mutagenesis efficiencies greater than 80%. The simple three-step process begins with mutant strand synthesis using a thermal cycler, followed by degradation of parental DNA using *Dpn* I, and is completed by a final transformation step (Figure 1). Time-consuming subcloning, single-stranded DNA rescue or *in vitro* methylation steps are eliminated.

Reduce unwanted errors

Unlike PCR, where errors are propagated with each round of thermal cycling, the QuikChange method uses a linear amplification strategy, where only parental DNA serves as template. Low cycle number and a relatively high input template amount ensure robust linear amplification with minimal errors. To guarantee that only parental strands are replicated, complementary primers are designed to completely overlap. Treatment of this product with *Dpn* I provides the digestion of only dam methylated and hemimethylated parental strands. By combining this non-PCR-based method with the highest-fidelity polymerases available, unwanted second-site errors are virtually eliminated.



1. Mutant Strand Synthesis
Perform thermal cycling to:
- Denature DNA template
- Anneal mutagenic primers containing desired mutation
- Extend and incorporate primers with *PfuUltra*® DNA polymerase

2. *Dpn* I Digestion of Template
Digest parental methylated and hemimethylated DNA with *Dpn* I

3. Transformation
Transform mutated molecule into competent cells for nick repair

Figure 1. QuikChange Method

The one-day QuikChange method gets you the results you need in three simple steps.

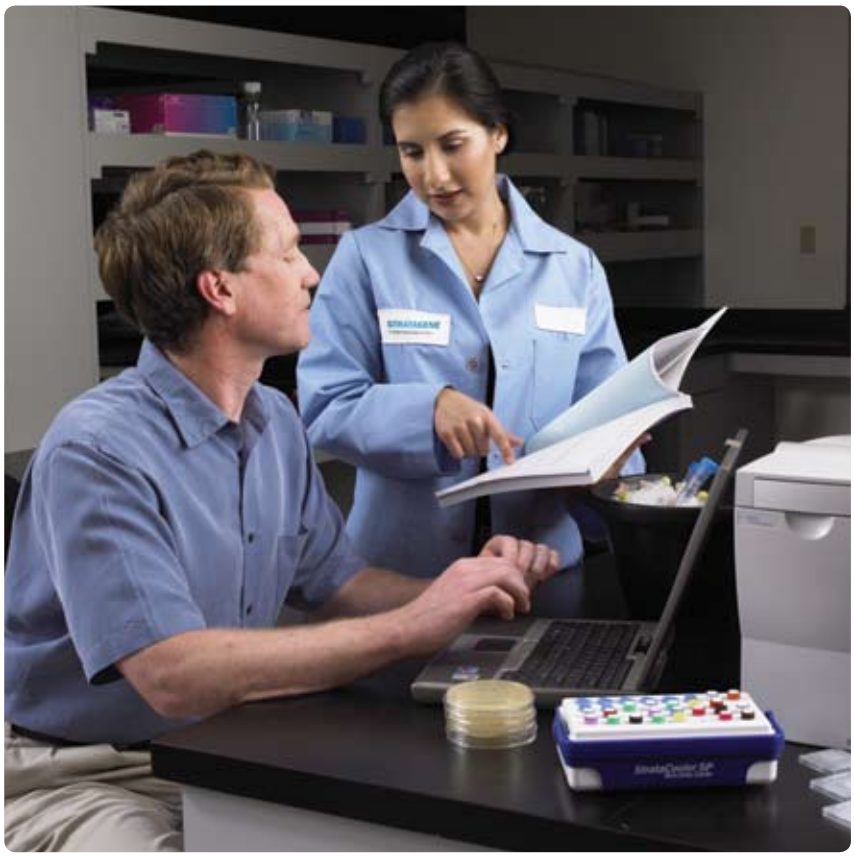
Optimized for your success

QuikChange kits include all required reagents, including controls, so you have everything you need. Enzymes, buffers and dNTPs are optimized, saving you time and ensuring consistent results. In addition, all of our kits include *Dpn* I enzyme and high-efficiency competent cells.

Primer design made easy

The QuikChange Primer Design Program assists you in designing mutagenic primers for your QuikChange site-directed mutagenesis experiments.

- Generates appropriate primer sequences for point mutations, deletions and insertions, with optimal melting temperature and free energy
- Provides suggestions for multi-site and saturation mutagenesis
- Translates imported DNA sequence into amino acid sequence
- Supports all of our QuikChange site-directed mutagenesis kits



Experience the QuikChange Primer Design Program at www.stratagene.com/mutagenesis.

Innovations in Site-Directed Mutagenesis

Stratagene recognizes the important role of site-directed mutagenesis in your research. That is why we continue to develop new, more powerful tools to help you perform your experiments more easily and in less time, in order to address your most challenging applications.

QuikChange Lightning site-directed mutagenesis kit: a powerful new tool

Introduce point mutations, insertions or deletions in less than three hours followed by an overnight transformation, while maintaining the high accuracy and efficiency that you've come to expect with QuikChange kits (Figure 2). Using our signature three-step QuikChange protocol, we have shortened the length of the amplification and selection steps by introducing faster enzymes. These novel enzyme formulations are exclusively available in our QuikChange Lightning site-directed mutagenesis kits.

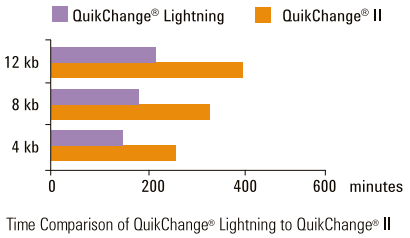


Figure 2. Our QuikChange Lightning products save time
Time savings with the QuikChange Lightning site-directed mutagenesis kit help you accelerate your research.

Enhanced Processivity Reduces Selection Time

Our QuikChange Lightning DNA polymerase fusion is a proprietary formulation that incorporates mutagenic primers with ultra-high-fidelity and faster extension times. The enhanced processivity of our QuikChange Lightning DNA polymerase fusion provides you with extreme accuracy, high efficiency and long target length capability, while dramatically reducing your overall extension times.

- QuikChange Lightning DNA polymerase fusion provides a faster extension time (30 sec/kb).
- The *Dpn* I restriction enzyme is enhanced to effectively digest parental plasmid DNA in just five minutes, offering you up to a 55-minute time savings.

Suitable for large plasmids

Using high-fidelity enzymes for amplifying long targets is critical since mutation frequency increases linearly with amplicon size. The kit is suitable for G/C-rich, difficult and/or large plasmids up to 14 kb. Our new QuikChange Lightning kit includes our QuikSolution reagent that helps to facilitate replication of large plasmids.

- Our XL-10 Gold[c] ultracompetent cells increase the efficiency of DNA uptake during transformation.

Easy domain-swapping

Domain-swapping experiments allow you to exchange regions between related gene sequences to map functional differences between homologous proteins. Traditionally, these experiments were often difficult and time-consuming because they involved generating uracil-containing ssDNA[1] or relied on convenient restriction sites[2]. In contrast, the new modified QuikChange kit method allows you to quickly and easily exchange DNA sequences[3] (Figure 3).

Ideal for insertions and deletions

Insertion and deletion studies offer the ability to map discrete functional areas of genes, modify vectors, introduce affinity tags and correct frame shifts. We have used the QuikChange method for small insertions and deletions (up to 12 bp) and observed greater than 80% efficiency[4]. Many outside researchers have used the QuikChange method or have modified it to introduce deletions of 31 bp[5], 87 bp[6] and 3 kb[7] or insertions of 31 bp[5] and up to ~1 kb[8]. As with domain-swapping experiments, these QuikChange kit modifications are simple and effective and allow maximum versatility with any plasmid DNA template.

QuikChange II site-directed mutagenesis kit: proven technology

Achieve efficient and accurate site-directed mutagenesis with our QuikChange II kits, featuring *PfuUltra* DNA polymerase[d], a genetically engineered mutant of *Pfu* DNA polymerase with enhanced proofreading capability.

QuikChange II XL: For your most challenging applications

Our QuikChange II XL site-directed mutagenesis kit[a] was created for efficient mutagenesis of large or otherwise difficult to mutagenize plasmid templates. The QuikChange II XL kit features components specifically designed for more efficient DNA replication and bacterial transformation.

- QuikSolution reagent facilitates replication of large plasmids
- XL-10 Gold ultracompetent cells offer five-fold higher transformation efficiency compared to XL1-Blue[e] cells employed in the original QuikChange kit

QuikChange II-E: For highest efficiency in transformation by electroporation

Our QuikChange II-E site-directed mutagenesis kit[f] is designed for researchers interested in isolating mutant clones via transformation into electroporation-competent cells. It includes our XL1-Blue electroporation-competent cells, which are resistant to tetracycline and exhibit high electroporation efficiency.

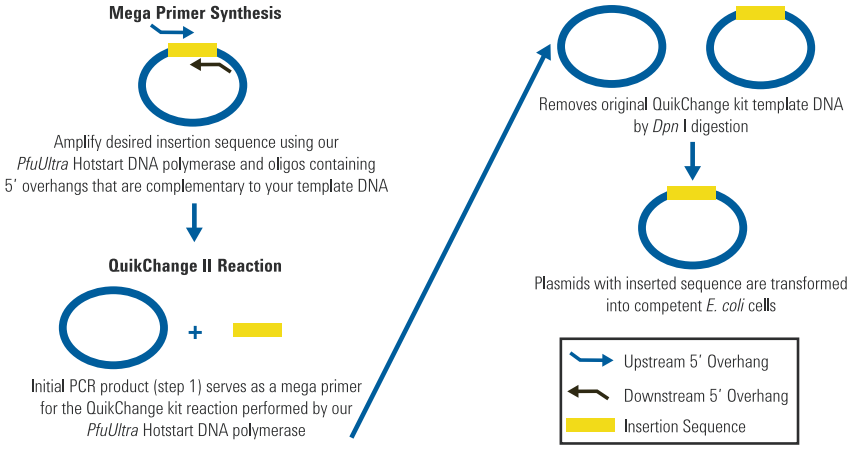


Figure 3. Domain-swapping mechanism
QuikChange kits make it easy to exchange regions between homologous proteins.

The Versatility You Need in Multi Site-Directed Mutagenesis

Efforts to elucidate protein structure-function relationships typically begin by identifying key residues through predictions from structural data, identifying changes in activity accompanying single-site mutagenesis[9] or identifying sequence changes in mutants with altered activity isolated from random mutant libraries.[10] Once you identify key residues, you may need to create numerous multi-site mutants to determine the effects of combining key mutations. Our QuikChange Multi site-directed mutagenesis kit provides a simple method for introducing mutations at up to five sites simultaneously. Moreover, this powerful and versatile kit can be easily adapted to perform site-saturation mutagenesis and to create mutant collections consisting of random combinations of site-specific mutations.

A powerful system for engineering mutants

By introducing mutations concurrently rather than sequentially, you can save days to weeks of time creating multi-site mutants. Our QuikChange Multi site-directed mutagenesis kit[g] offers a rapid and reliable method for site-directed mutagenesis of plasmid DNA at up to five different sites simultaneously. The mutagenic efficiency is approximately 50% with three mutagenic primers and up to 95% efficient using a single mutagenic oligo (Table 1). The kit features a one-day, three-step procedure, and a single mutagenic oligonucleotide is required to mutagenize each site, using a double-stranded DNA template (Figure 4).

Create mutant clone collections in one day

Our QuikChange Multi kit provides a simple, efficient method for creating mutant clone collections that encompass all possible substitutions at one position (site-saturation mutagenesis) or all possible combinations of specific point mutations, or both.

Table 1. QuikChange Multi site-directed mutagenesis[10]
Mutation efficiency and distribution of mutations depend on factors such as the number of primers employed, their design and the template sequence.

Average Efficiencies of Simultaneous Mutations

Number of sites mutated	Mean mutation frequency (%)
1	91
2	91
3	58
4	60
5	30

When performing multi-site mutagenesis with two or more primers, additional mutants are generated through random incorporation of subsets of mutant primers. For example, when introducing mutations at three sites in a protein, the QuikChange Multi reaction will contain all possible single and double mutants, in addition to the desired triple mutant. As shown in Figure 5A, receptor phosphorylation was studied at three tyrosines simultaneously. Although only three products are shown, the resulting mutant clone collection contains seven unique mutants (one triple, three single and three double mutants). These mutant clone collections can be screened directly to identify the best combination of mutations for a desired function.

Mutant clone collections can also be created by adapting the QuikChange Multi kit to perform saturation mutagenesis. Saturation mutagenesis provides a much more comprehensive analysis of structure-function relationships compared to single amino acid replacements or error-prone PCR. Using the QuikChange Multi kit with degenerate-codon primers, you can create a diverse collection of clones, each containing a different amino acid substitution at one or more sites. The QuikChange Multi kit can generate all possible variations at a particular amino acid position more easily and economically than performing numerous independent QuikChange reactions.

As shown in Figure 5B, a key phosphorylation site (tyrosine) was subject to saturation mutagenesis by incorporating a degenerate-(NNN)-codon primer with the QuikChange Multi kit. This example shows three (W, Q, R) of the 19 mutants that are produced in a single reaction. As above, the resulting mutant clone collection can be screened as a library to identify changes in activity, or as individual clones to precisely characterize structure-function relationships. Additional studies have shown that the number of clones per QuikChange Multi reaction is sufficient for creating fully representative libraries with up to three randomized codons.[11]

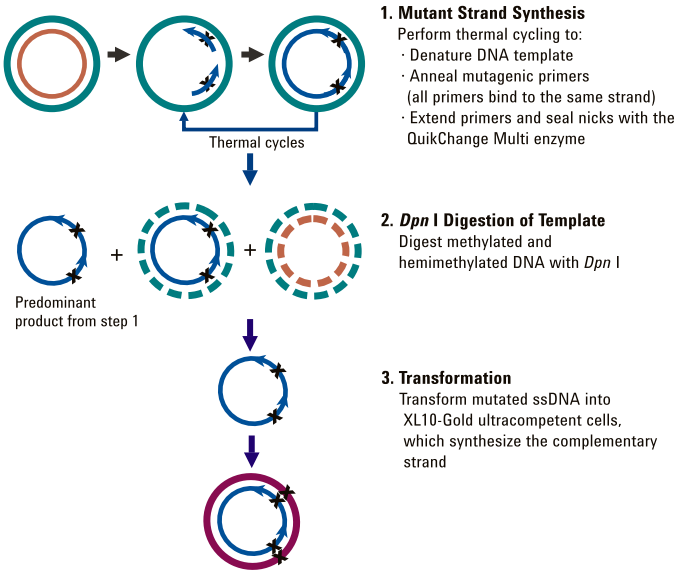


Figure 4. The QuikChange Multi site-directed mutagenesis method
Incorporate up to five mutant primers into plasmid DNA with the QuikChange Multi site-directed mutagenesis kit.

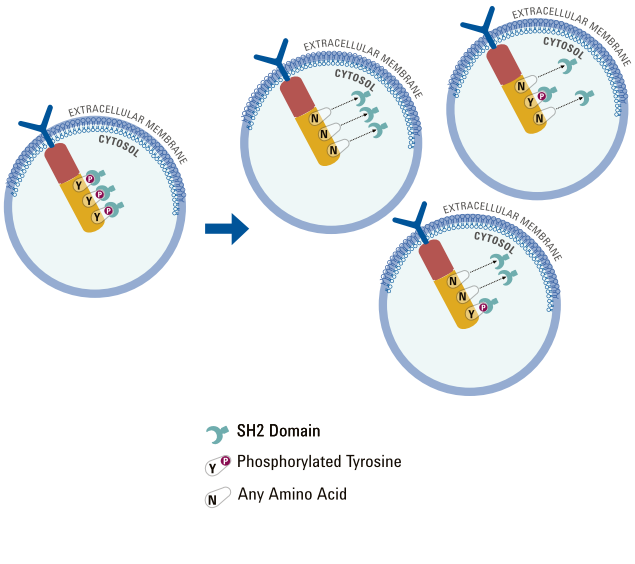


Figure 5A. Random combinations of multiple point mutations

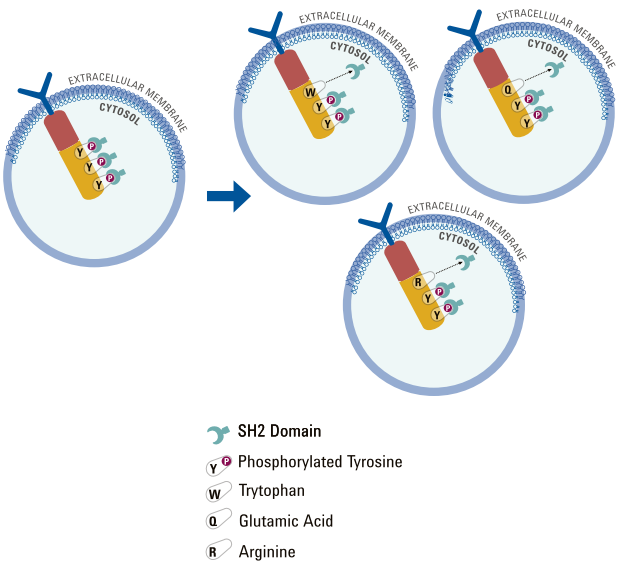


Figure 5B. Saturation mutagenesis

A Superior Choice over Conventional Random Mutagenesis Methods

Random mutagenesis is often the first step on the path to creating a preliminary map of your protein's functional domains. Typically, a gene or gene fragment is randomly mutated by error-prone PCR, mutant sequences are cloned into a suitable expression vector, and libraries are screened to identify clones that exhibit changes in protein function. By correlating changes in activity to changes in amino acid sequence, researchers can begin to identify functional domains and map structure-function relationships. Our GeneMorph products facilitate the process of random mutagenesis by providing superior methods for error-prone PCR and library construction.

A more balanced mutational spectrum with GeneMorph II random mutagenesis kit

The GeneMorph II random mutagenesis kit[h] offers fast and easy protocols for controlling your mutation frequency and ensuring robust yields for easy cloning and library construction. The kit features Mutazyme® II DNA polymerase, a novel blend of Mutazyme DNA polymerase with a *Taq* mutant that has a higher error rate compared to wild-type *Taq* polymerase. Mutazyme II DNA polymerase overcomes the mutational bias of a single PCR enzyme, creating a more uniform spectrum of mutations, where mutations at As and Ts occur at nearly identical frequencies as Gs and Cs (Figure 6). Not only is library construction easier, GeneMorph II-generated libraries should be more representative and contain unique mutants compared to libraries produced using *Taq* under error-prone, low-efficiency conditions (unbalanced dNTPs, manganese).

Generate mutant libraries quickly and efficiently with GeneMorph II EZClone domain mutagenesis kit

The GeneMorph II EZClone domain mutagenesis kit[h] offers an easy and fast method for random mutagenesis and restriction-free cloning of gene sequences up to 3.5 kb. The entire process can be completed in one day followed by an overnight transformation.

With the GeneMorph II EZClone domain mutagenesis kit, you can randomize a plasmid DNA sequence encoding an entire protein or a specific domain, and then swap the wild-type sequence for the randomized sequence using a restriction/ligation-free method. The ease and simplicity of the EZClone method allow you to construct multiple libraries quickly (e.g., different mutation rates or different protein domains), providing you the opportunity to accomplish your research goals faster than ever before.

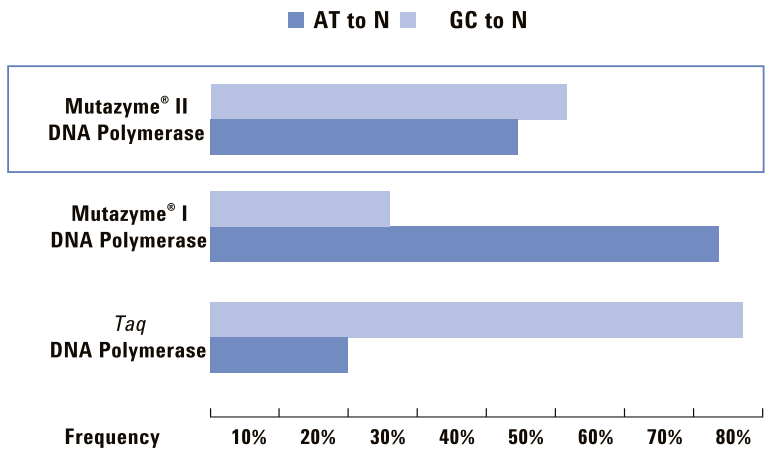


Figure 6. More balanced mutational spectrum
The GeneMorph II kits include the Mutazyme II polymerase, which mutates Gs and Cs at nearly identical rates compared to As and Ts.

As shown in Figure 7, domains are first amplified to introduce random mutations using error-prone PCR with Mutazyme II DNA polymerase. The purified PCR products then serve as megaprimers for the EZClone reaction. They are denatured and annealed to the original donor plasmid and then extended with EZClone high-fidelity DNA polymerase. This reaction is thermal cycled several times and treated with *Dpn* I enzyme to remove background DNA before transformation into competent *E. coli*. After transformation, the resulting mutant library can be screened in your functional assay.

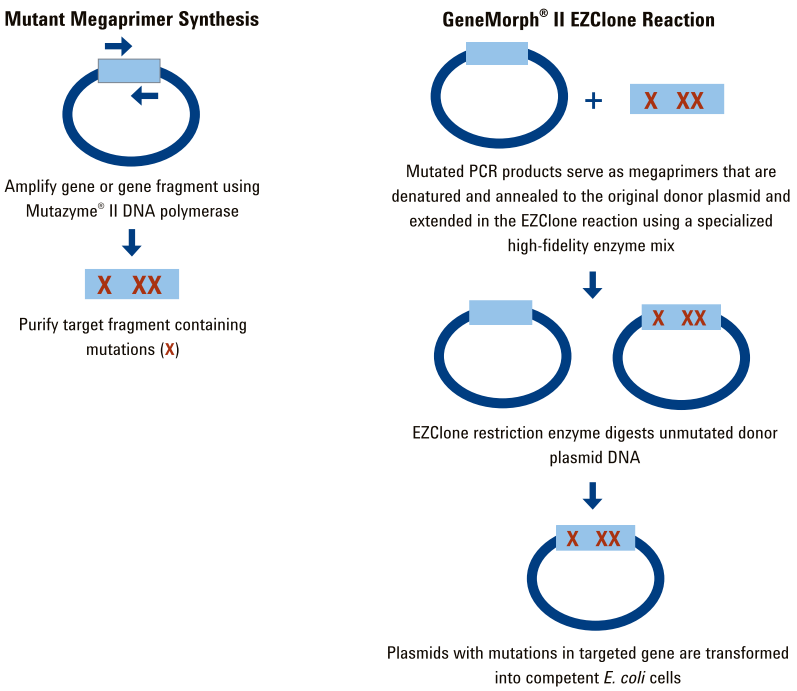


Figure 7. GeneMorph II EZClone method
This procedure allows you to randomly mutate a specific region within your gene of interest without the need for subcloning. Simply create your mutant megaprimer using Mutazyme II, purify and add that product into your next reaction with the EZClone enzyme. Digest any background product with *Dpn* I, and you are ready to transform into our XL-10 Gold ultracompetent cells. Most templates will allow you to finish the protocol in 24 hours.

Notes:

a. U.S. Patent Nos. 7,176,004; 7,132,265; 7,045,328; 6,734,293; 6,713,285; 6,706,525; 6,489,150; 6,444,428; 6,391,548; 6,183,997; 5,948,663; 5,932,419; 5,866,395; 5,789,166; 5,707,841; 5,545,552; 5,512,468; and patents pending.

b. U.S. Patent Nos. 7,045,328; 6,734,293; 6,489,150; 6,444,428; 6,183,997; 5,948,663; 5,866,395; 5,545,552; and patents pending.

c. U.S. Patent Nos. 6,706,525; 5,512,468; 5,707,841; and patents pending and equivalent foreign patents.

d. U.S. Patent Nos. 7,045,328; 6,734,293; 6,489,150; 6,444,428; 6,183,997; 5,948,663; 5,866,395; 5,545,552 and patents pending.

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e. U.S. Patent Nos. 6,586,249; 6,338,965; 6,040,184; and patents pending.

f. U.S. Patent Nos. 7,176,004; 7,132,265; 7,045,328; 6,734,293; 6,489,150; 6,444,428; 6,391,548; 6,183,997; 5,948,663; 5,932,419; 5,866,395; 5,789,166; 5,545,552, and patents pending.

g. U.S. Patent Nos. 6,734,293; 6,489,150; 6,444,428; 6,183,997; 5,948,663; 5,866,395; 5,545,552; and patents pending. Use of the QuikChange® Multi site-directed mutagenesis kit, catalog #200514 and #200515, by commercial entities requires a commercial license from Stratagene. The QuikChange® Multi site-directed mutagenesis kit, catalog #200513 and #200531, is offered for sale to commercial entities with a limited-use license.

h. U.S. Patent Nos. 7,045,328; 6,803,216; 6,734,293; 6,489,150; 6,444,428; 6,183,997; 5,489,523; and patents pending.

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Ordering Information

PRODUCT		QUANTITY	CATALOG NO.
QuikChange Lightning site-directed mutagenesis kit	*NEW*	10 reactions 30 reactions	210518 210519
QuikChange II site-directed mutagenesis kit		10 reactions 30 reactions	200523 200524
QuikChange II-E site-directed mutagenesis kit		10 reactions	200555
QuikChange II XL site-directed mutagenesis kit		10 reactions 30 reactions	200521 200522
QuikChange Multi site-directed mutagenesis kit			
Academic Version		10 reactions	200515
Academic Version		30 reactions	200514
Commercial Version		10 reactions	200531
Commercial Version		30 reactions	200513
GeneMorph II random mutagenesis kit		30 reactions	200550
GeneMorph II EZClone domain mutagenesis kit		10 reactions	200552

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