MUTAGENESIS

GeneMorph® II EZClone Domain Mutagenesis Kit

The new GeneMorph® II EZClone Domain Mutagenesis Kit* offers an easy and fast cloning method (Figure 1) to perform targeted random mutagenesis on protein domains and promoter elements, while delivering uniform mutational spectrum. The kit contains Mutazyme® II DNA polymerase, which introduces a more uniform mutational spectrum compared to other error-prone PCR enzymes so that mutations at As and Ts occur at the same frequency as Gs and Cs (Figure 2). This allows you to generate all types of random mutations without a bias to more rapidly discover key sites responsible in structure-function relationships. This enhanced version makes cloning and library construction easier than ever.

sy Cloning Simplifies Library Construction

Cloning amplicons generated by error-prone PCR can be difficult and labor-intensive due to low product yields, mutations at the ends which interfere with restriction-based cloning, and/or inefficient synthesis of 3' dA overhangs or blunt ends which reduces the efficiency of TA- or blunt-end cloning strategies. In addition, targeting specific functional domains for random mutagenesis can also be difficult and inefficient using traditional methods. To address the need for efficient and flexible cloning methods, the new GeneMorph II EZClone domain mutagenesis kit offers an easy and fast cloning method for random mutagenesis on targeted protein domains. As illustrated in Figure 1, domains are first amplified to introduce random mutations using error-prone PCR with Mutazyme II DNA polymerase. Purified PCR products serve as mega primers for the EZClone reaction. They are denatured and annealed to the original donor plasmid and then extended with a specialized high-fidelity DNA polymerase. This reaction is thermal cycled several times before being treated with *Dpn* I enzyme to remove background DNA prior to transformation into competent *E. coli*. After transformation, the resulting mutant library can be screened in your functional assay. With the entire process completed in one day plus an overnight transformation, you can generate mutant libraries quickly and efficiently, and target specific domains without the need for unique restriction sites.

Mutazyme® II Polymerase Delivers a Uniform Spectrum

The original Mutazyme DNA polymerase introduces more mutations at Gs and Cs while *Taq* DNA polymerase is biased toward As and Ts. We developed Mutazyme II DNA polymerase to overcome these biases and produce a more uniform mutation spectrum. Mutazyme II polymerase mutates As and Ts as frequently as Gs and Cs giving you both spectra of Mutazyme and *Taq* DNA polymerases (Figure 2). Because of this feature, libraries produced using the GeneMorph II EZClone kit will be more representative than using libraries produced using a single PCR enzyme.

Applications

+ Analysis of structure-function relationship of proteins
+ Directed evolution of proteins
+ Promoter element function

+ Fast three-step cloning method eliminates tedious sub-cloning and the need for specific restriction sites
+ Includes Mutazyme® II DNA polymerase to introduce a more uniform mutational spectrum in which mutations at As and Ts occur at the same frequency as Gs and Cs
+ Simple protocol to control mutation frequency from 1 to 16 mutations per kb
+ Robust yields of targets up to 3.5 kb for library construction

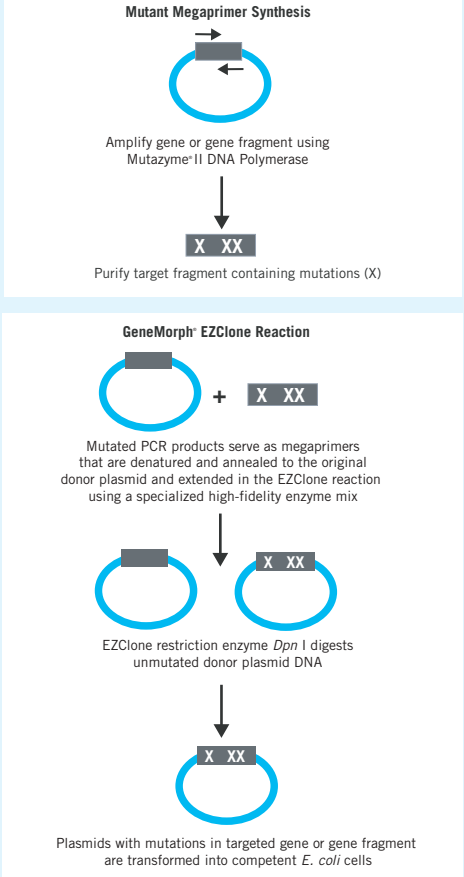

Figure 1
GeneMorph® II EZClone Method

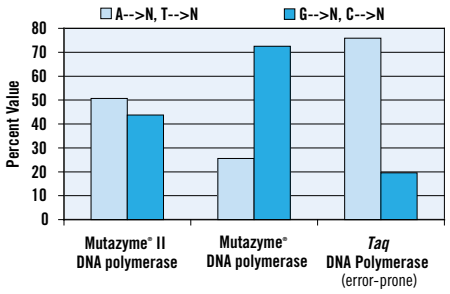

Figure 2
Mutazyme® II DNA Polymerase Introduces Mutations at As and Ts at the Same Frequency as Gs and Cs

The GeneMorph® EZClone approach produces a more comprehensive collection of mutants to discover key functional sites. On the other hand, *Taq* polymerase under error-prone conditions and the original Mutazyme DNA polymerase deliver bias mutational spectra and many key sites often would go undiscovered.

Easily Control Mutation Frequency

In the GeneMorph II EZClone kit, low, medium, and high mutation frequencies are achieved using a single set of optimal reaction conditions ($MgCl_2$ balanced dNTPs). The only parameter varied is DNA template concentration, so the same spectrum of mutations is produced over a broad range of mutation frequencies from 0.1 to 1.6% per PCR (Figure 3). Since mutation levels are varied without altering reaction conditions, the broad spectrum of mutations produced by the GeneMorph kit is maintained over a wide range of mutation frequencies, even at high mutation levels. Additionally, using the error-prone PCR conditions outlined in the manual, we observed a mean mutation frequency of 5.8 mutations/clone with nearly 75% of clones sequenced containing 3-9 mutations (Figure 4). We also generated a library under error-prone conditions for a low mutation frequency and observed a mean mutation frequency of 2 mutations/clone with 66% of clones containing 1-4 mutations (data not shown).

Maximize Library Size and Diversity

With reduced bias compared to error-prone PCR methods employing *Taq* DNA polymerase, the GeneMorph II EZClone kit is the ideal choice for creating diverse mutant collections (Table 1). We include XL10 Gold® Ultracompetent Cells, which exhibit the Hte phenotype that increases the transformation efficiency of large and ligated DNA molecules to produce large, diverse libraries. Screening more representative libraries allows you to more rapidly uncover key sites responsible for structure-function relationships and to accomplish your research goals faster than ever before.

Complete Kit

The GeneMorph II EZClone domain mutagenesis kit includes all the necessary components for random PCR mutagenesis including Mutazyme II DNA polymerase, 10X reaction buffer, dNTPs, and gel standard as well as cloning reagents for the GeneMorph II EZClone method.

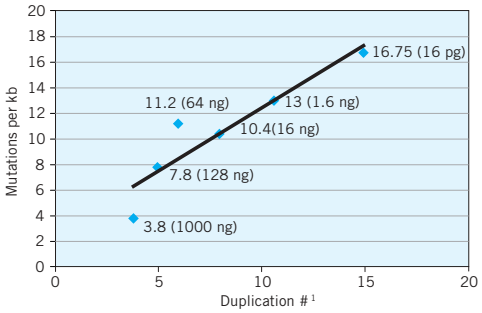

Figure 3
Simply Change the Input Amount for the Desired Frequency

We generated a range of mutation frequencies (mutations per kb) by adjusting the initial template amount from 16 pg to 1000 ng. By increasing the amount of template (in parenthesis) added to the PCR, the mutation frequency decreased from 16 mutations per kb using 16 pg to 3.8 mutations per kb using 1000 ng.
[1] Duplication # equals the number of doublings during PCR.

Gene Fragment (kb)	Library Size (x 10^6 CFU/50 µl)
0.146	3.99
0.209	5.34
0.706	2.58
3.17	4.61
3.41	4.27

Table 1
GeneMorph® II EZClone Method Produces Large Libraries

We used the GeneMorph II® EZClone domain mutagenesis kit to introduce random mutations in gene fragments up to 3.5 kb and subsequently used the GeneMorph EZClone method to create mutant libraries.

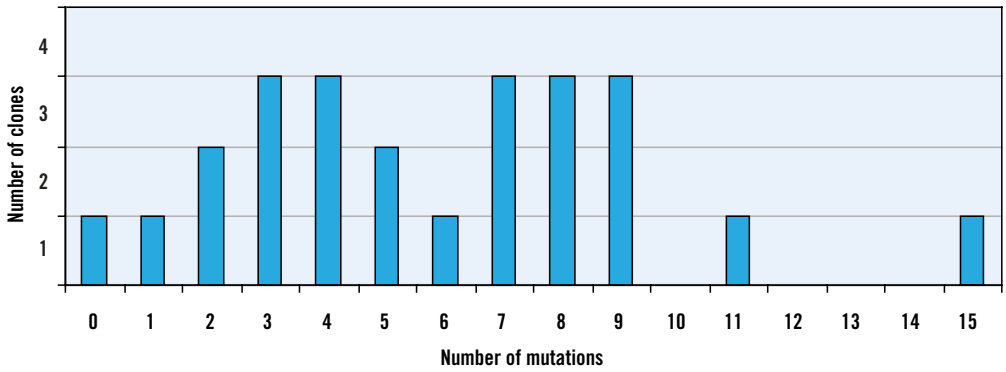

Figure 4
Distribution of Mutations in Random Sampling of Clones Created Using GeneMorph® II EZClone Method

We generated a library of ~2.5 x 10^5 clones using the GeneMorph® II EZClone domain mutagenesis kit. Mutazyme® II DNA polymerase was used to amplify a 0.67 kb fragment under error-prone conditions for a "high" mutation frequency using 1 ng DNA template. We sequenced 24 random clones from this library to assess mutation frequency and distribution. We observed in this sample that there were a total of 140 mutations detected in 24 clones, which yields a mean mutant frequency of 5.8 mutations/clone or 8.7 mutations/kb. This result is consistent with the error rate of Mutazyme II DNA polymerase listed in the kit manual. As shown, these clones contained 0 to 15 mutations, with 75% of the clones exhibiting between 3 and 9 mutations, and only one clone each contained 0, 1, or 15 mutations.

GeneMorph® II EZClone Domain Mutagenesis Kit		
GeneMorph® II EZClone Domain Mutagenesis Kit	contents	catalog
	10 rxn	200552

* Purchase of these products is accompanied by a license to use them in the Polymerase Chain Reaction (PCR) process in conjunction with an Authorized Thermal Cycler.

Printed in USA, April 2008
5989-8297EN

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