

Inconvenient or non-existent restriction sites and tedious sub-cloning procedures make it difficult to perform targeted random mutagenesis on protein domains and promoter elements.



Efficient Cloning for Error-Prone PCR

SOLUTION >

The new GeneMorph® II EZClone domain mutagenesis kit offers an easy and fast method for random mutagenesis and subsequent cloning of whole genes, gene fragments encoding protein domains and promoter elements.

Easy Cloning Simplifies Library Construction

We commonly use error-prone PCR to create random mutant libraries, which you can subsequently screen to identify mutants with altered or improved properties. Amplicons generated by error-prone PCR can prove more difficult to clone due to low product yields, mutations at the ends that 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. With current methods, randomizing and sub-cloning a portion of a gene that encodes a functional domain can be tedious and time consuming as there are rarely convenient restriction sites available. To address this need, we developed the GeneMorph® II

EZClone domain mutagenesis kit*, which features an efficient, flexible three-step cloning method that doesn't require sub-cloning or specific restriction enzymes.

As illustrated in Figure 1, we first amplified the domains 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 to minimize unwanted errors during the cloning process. You cycle the reaction several times before treating it with *Dpn* I enzyme to remove background DNA prior to transformation into competent *E. coli*. After transformation, these colonies are ready-to-go for further analysis in your functional assay. With the entire process completed in a day plus an overnight transformation, you save valuable research time that would have been wasted on trying to find, introduce and verify unique restriction sites to excise these domains.

A More Uniform Spectrum

Like the GeneMorph® II random mutagenesis kit, our new GeneMorph® II EZClone kit also features Mutazyme® II DNA polymerase, which produces mutation rates from 1 to 16 mutations/kb, introduces all possible mutation types, and provides equivalent mutation rates at As and Ts compared to Gs and Cs (Figure 2). As a result of reduced mutational bias, libraries created with Mutazyme II DNA polymerase should exhibit greater representation compared to libraries generated with other error-prone PCR enzymes.

Discover More, Faster

With reduced bias compared to error-prone PCR methods employing *Taq* DNA polymerase, the GeneMorph II EZClone kit offers a fast and easy method for cloning. It is the ideal choice for creating large, diverse mutant collections (Table 1). Screening more representative libraries allows you to rapidly uncover key sites responsible for structure-function relationships and to accomplish your research goals faster than ever before.

- + Efficient, time-saving method for cloning randomized PCR amplicons
- + Includes Mutazyme® II DNA polymerase for a more uniform mutational spectra
- + Simple protocol controls mutation frequency

REFERENCE

* See license reference 3 on page 65

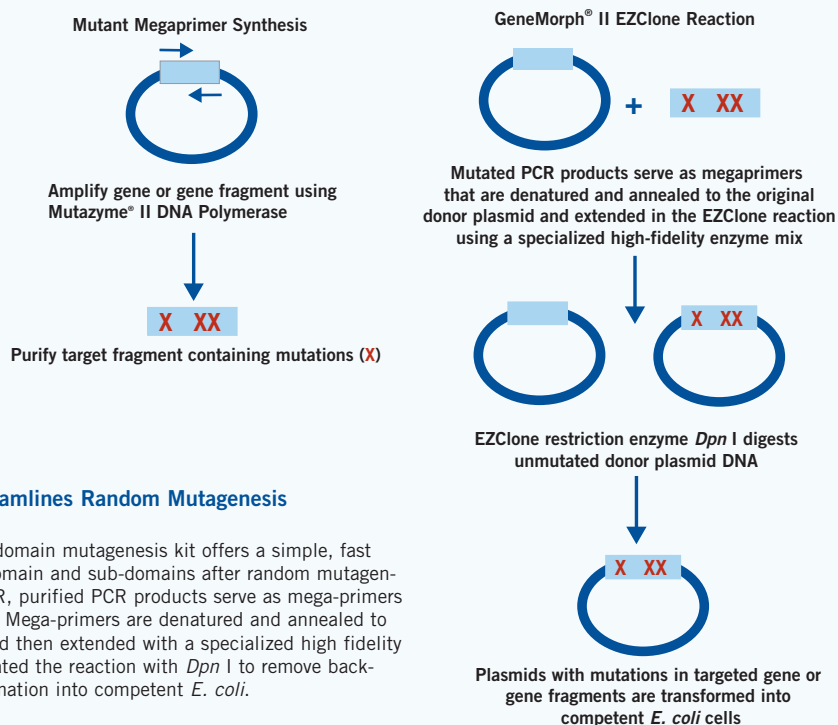


Figure 1:
Easy Cloning Method Streamlines Random Mutagenesis Experiments

The GeneMorph® II EZClone domain mutagenesis kit offers a simple, fast method for cloning protein domain and sub-domains after random mutagenesis: 1.) After error-prone PCR, purified PCR products serve as mega-primers for the EZClone reaction: 2.) Mega-primers are denatured and annealed to the original donor plasmid and then extended with a specialized high fidelity DNA polymerase. 3.) We treated the reaction with *Dpn I* to remove background DNA prior to transformation into competent *E. coli*.

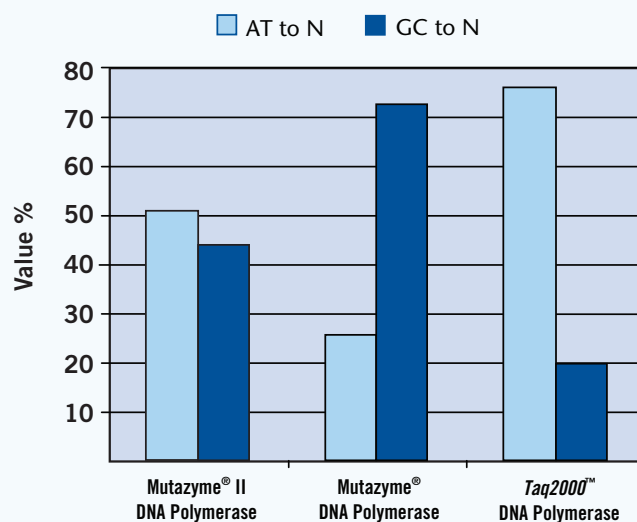


Figure 2
Mutazyme® II Polymerase Delivers Ideal Mutational Spectrum

The Mutazyme® II DNA polymerase contained in the GeneMorph® EZClone Kit introduces a more uniform mutational spectrum in which mutations at As and Ts occur at the same frequency as Gs and Cs. In contrast, *Taq* DNA polymerase under error-prone conditions (Mn^{2+} , unbalanced nucleotides) and the original Mutazyme® DNA polymerase produce biased mutational spectra.

Gene fragment (Kb)	Library size (x 10 ⁶ CFU/50µl)
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 EZClone method to create large mutant libraries.

GeneMorph® II EZClone Random Mutagenesis Kit

	Contents	Catalog
GeneMorph® II EZClone Domain Mutagenesis Kit	10 reactions	200552
GeneMorph® II Random Mutagenesis Kit	30 reactions	200550