PROTEIN FUNCTION & ANALYSIS

PROBLEM >

Introducing mutations at multiple sites in proteins or in DNA elements typically requires repeating many time-consuming steps. Even creating only a few mutations in a single clone can take weeks using traditional methods.

Mutagenesis Made Easy and Economical

SOLUTION >

Our QuikChange® Multi Site-Directed Mutagenesis Kit offers a fast and reliable method for site-directed mutagenesis of multiple sites simultaneously, saving several days to weeks over traditional methods. The QuikChange Multi kit is now available in convenient 10- and 30-reaction pack sizes to accommodate your research needs.

Accelerating Discovery

Our QuikChange® Multi Site-Directed Mutagenesis Kit[a] can be used to study structure-function relationships or to evolve protein function. You can create specific multi-site mutants by incorporating up to five mutant oligonucleotides into a gene of interest. Introducing mutations at multiple sites simultaneously represents a significant savings in both time and effort compared to traditional methods (Table 1). Furthermore, the QuikChange Multi kit can be used to create diverse semi-random mutant libraries that can be screened for changes in protein function.

Engineered Mutant Clone™ Collections

Efforts to elucidate protein structure-function relationships typically begin by identifying key residues using methods including 1) predictions from structural data, 2) identifying changes in activity accompanying single-site mutagenesis[1], or 3) identifying sequence changes in mutants with altered activity isolated from random mutant libraries[2]. Once key residues are identified, you often need to construct additional mutants that contain various combinations of key mutations or alternative side-chain substitutions.

Our QuikChange Multi kit provides you a one-day method for creating Engineered Mutant Clone™ Collections that encompass all possible substitutions at one position (saturation mutagenesis) or all possible combinations of specific point mutations (multiple mutant oligonucleotides), or both. As outlined in Figure 1, you can perform saturation mutagenesis by incorporating degenerate-codon primers with the QuikChange Multi kit[3]. These Engineered Mutant Clone Collections can be screened directly to identify the best amino acid type for a desired function. Alternatively, a portion of the library can be sequenced to identify a unique panel of mutants for further study. Using our QuikChange Multi kit to generate all possible variations at a particular amino acid position is easier and more economical than performing 19 independent QuikChange reactions. As shown in Figure 2, we used the QuikChange Multi kit to perform saturation mutagenesis on a DNA polymerase to identify four unique mutations which significantly enhanced nucleotide analog incorporation. Thus, saturation mutagenesis is a very powerful tool for evolving protein function.

Save Time and Money

Now available in both 10- and 30-reaction pack sizes, the QuikChange Multi kit allows you to create both multi-site mutants and semi-random clone collections in a single day. In addition to saving valuable research time and money, the QuikChange Multi kit provides you a powerful tool for the detailed exploration of protein structure-function relationships and protein evolution.

+ Create diverse, semi-random mutant libraries
+ Simple one-day protocol
+ Convenient and economical pack sizes

REFERENCE

1. Miyazaki and Arnold (1999) *J. Mol. Evol.* 49: 716-720.
2. *Strategies* (2001) 14 (3): 74-75.
3. Hogrefe, H., et al. (2002) *BioTechniques* 33: 1158-1165.

a. Patents pending. See license reference 10 on page 1.

# sites	Time (days) Includes mutagenesis, miniprep and sequencing reactions	
	Conventional Methods	QuikChange Multi
2	6	3
3	9	3
4	12	3
5	15	3

Table 1
The QuikChange Multi Kit Saves as Much as 2 Weeks on Multi-Site Mutagenesis

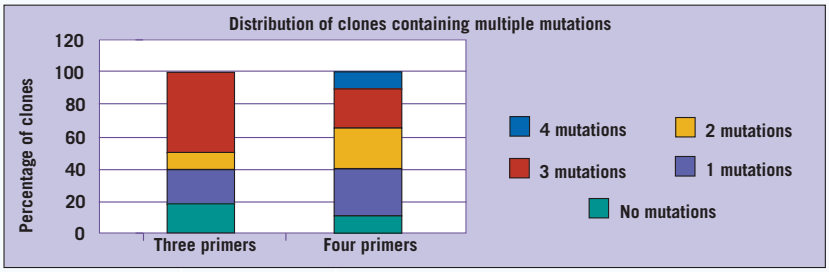

Figure 1
Produce Engineered Mutant Clone™ Collections Using 3 or 4 Mutant Primers in a Single Reaction
The QuikChange® Multi Kit is optimized to incorporate multiple primers; however, contributing factors such as the number of primers employed, their design, and the template sequence can affect efficiency and distribution of multi-site mutagenesis. Overall, 85 to 90% of selected clones should contain one or more desired mutation or subset of mutations. Also, 35 to 50% of the selected clones should contain all of the intended mutations in 3 or 4 primer experiments.

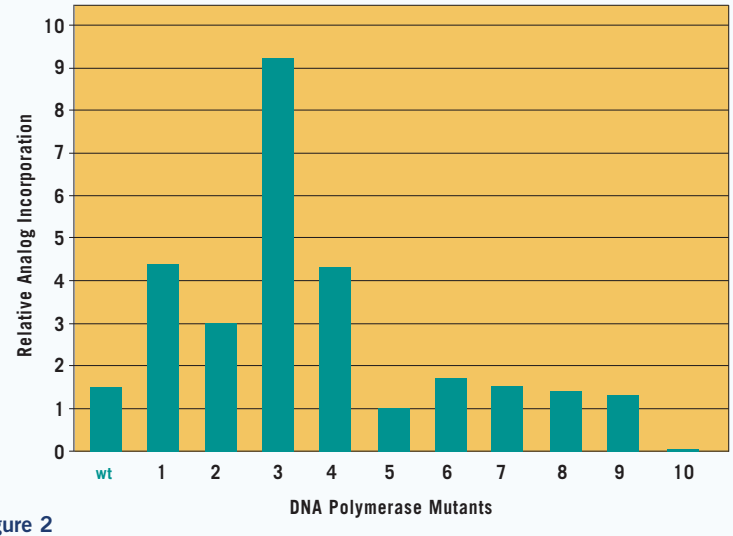

Figure 2
Enhanced Enzyme Activity
We used the QuikChange® Multi Site-Directed Mutagenesis Kit for site specific saturation mutagenesis of a DNA polymerase to enhance nucleotide analog incorporation. Using a single degenerate codon oligo, one QuikChange Multi reaction produced a diverse Engineered Mutant Clone™ collection. Thirty-two randomly selected clones were sequenced to identify 10 different mutants. These mutants were assayed for DNA polymerase activity and nucleotide analog incorporation. One mutation resulted in complete loss of enzyme activity (#10), five different mutations had no effect on enzyme activity (#5-9), and four unique mutations resulted in improved incorporation of a nucleotide analog (#1-4). Three of the four beneficial mutations required 2 to 3 bp changes per codon, and thus, would likely not have been produced using an error-prone PCR approach.[2]

QuikChange® Multi Site-Directed Mutagenesis Kit

		Contents	Catalog	Price
Academic Version		10 rxn	200515	$299
		30 rxn	200514	$799
Commercial Version	new	10 rxn	200531	$599
		30 rxn	200513	$1595