

Agilent QuikChange Lightning Multi Site-Directed Mutagenesis Kits

Data Sheet

Introduction

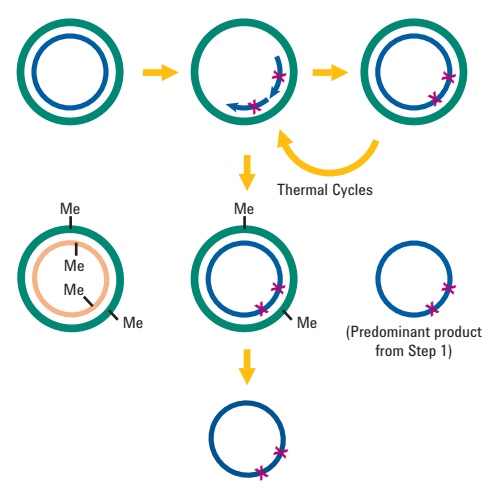
The Agilent QuikChange Lightning Multi Site-Directed Mutagenesis Kits enable you to rapidly and efficiently create point mutations in plasmid DNA at up to five sites simultaneously. QuikChange Lightning Multi Kits are optimized for plasmids up to 8 Kb. The QuikChange technology employs a simple, 3-step method (Figure 1) that does not require subcloning, single-stranded DNA rescue, or *in vitro* methylation.

Generate mutants in under four hours (plus overnight transformation)

Achieve greater than 50% mutagenesis efficiency for three sites (or fewer)

Increase accuracy and decrease errors with ultra-high fidelity QuikChange Lightning Multi Enzyme

Free online access access to the QuikChange Primer Design Program



- 1. Mutant Strand Synthesis**
Perform thermal cycling to:
 - Denature DNA template
 - Anneal mutagenic primers
 - Extend primers and ligate nicks with QuikChange Lightning Multi Enzyme**Total reaction time: 2 h***
 - 2. Dpn I Digestion of Template**
 - Digest methylated and hemimethylated DNA with enhanced *Dpn I* enzyme**Total reaction time: 5 min**
 - 3. Transformation**
 - Transform mutated ssDNA into XL10-Gold Ultracompetent Cells**Total reaction time: 1.5 h**
- * Based on a 5 Kb plasmid; excludes ramp times

Figure 1. QuikChange Lightning Multi Site-Directed Mutagenesis Kit method.

The QuikChange Method

QuikChange kits provide a fast, easy and efficient non-PCR alternative to other site-directed mutagenesis techniques, and commercially-available kits. Unlike PCR-based techniques, where errors are propagated with each round of thermal cycling, the QuikChange method utilizes a linear amplification strategy whereby only the parental strand serves as the DNA template and, additionally, mutagenic primers are incorporated into the new mutant strand. Combining this with our highest fidelity enzymes leads to a significant reduction in unwanted second-site errors. The existence of such errors is likely to complicate and delay downstream analysis.

The Lightning Advantage

The QuikChange Lightning Multi Kits contain enzymes specially designed to shorten the time necessary to complete our signature 3-step protocol. Extension times for the thermal cycling process have been reduced by 75%, and digestion of the non-mutated parental template has been decreased to only five minutes. From the adjacent graph, when introducing four point mutations into three different template sizes, there is a significant reduction in thermal cycling and digestion times when comparing QuikChange Lightning Multi to QuikChange Multi (Figure 2). The QuikChange Lightning Multi Kits provide the same performance and ease-of-use as previous versions but with the added benefit of saving valuable research time.

Featured Application: Engineering Mutant Clone Collections

The QuikChange Lightning Multi Kits are well suited for constructing a diverse collection of engineered mutant clones. Such a collection can be created by performing site-specific saturation mutagenesis using a single QuikChange Lightning Multi reaction, and then screening for mutants with improved activity using the appropriate functional assay. For example, the system may be used to create mutant collections containing all possible amino acids at one site as well as combinations of different amino acids at multiple sites. The QuikChange Lightning Multi overcome issues associated with mutagenesis methods that employ mutagenic primers corresponding to both strands of DNA, where representation of mutants in the collection may be limited by the preferential binding of complementary strands of mutagenic primers to each other.

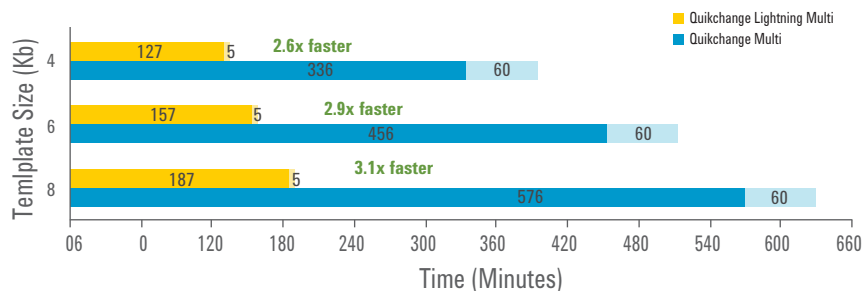


Figure 2. Reaction times (excluding ramp times) of QuikChange Lightning Multi and QuikChange Multi for different template sizes. Compared to QuikChange Multi, QuikChange Lightning Multi reduces both thermal cycling and digestion times for a variety of construct sizes.

Ordering information

Our QuikChange kits provide all of the necessary components, including enzymes and competent cells, to successfully and rapidly perform site-directed mutagenesis.*

Product Description	Reactions	Part Number
QuikChange Lightning Multi Site-Directed Mutagenesis Kit, Academic	10 30	210515 210513
QuikChange Lightning Multi Site-Directed Mutagenesis Kit, Commercial	10 30	210516 210514

*The Commercial QuikChange Lightning Multi Site-Directed Mutagenesis Kits include a limited use license, which is required for purchases by commercial entities.

Related Products

Product Description	Reactions	Part Number
QuikChange Lightning Site Directed Mutagenesis Kit	10 30	210518 210519

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