QuikChange Lightning Site-Directed Mutagenesis Kit

Data Sheet

Generate mutants in <3 hours

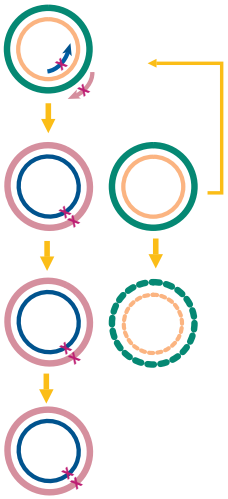
> 85% mutagenesis efficiency, equivalent to QuikChange & QuikChange II

Eliminate unintended mutations with ultra-high fidelity QuikChange Lightning Enzyme

Free online access to the QuikChange Primer Design Program

Introduction

The Agilent QuikChange Lightning Site-Directed Mutagenesis Kit lets you rapidly and efficiently create point mutations, amino acid substitutions, insertions, and deletions in virtually any double-stranded plasmid. QuikChange Lightning Mutagenesis Kits are optimized for performing mutagenesis on difficult-to-replicate sequences and large plasmids up to 14 Kb. The QuikChange Lightning technology employs a simple, 3-step protocol (Figure 1) that makes it easier and faster than other available methods or kits. Other methods are more cumbersome and prone to human error as they often require subcloning, single-stranded DNA rescue, or *in vitro* methylation.



1. **Mutant Strand Synthesis**
   Perform thermal cycling to:
   - Denature DNA template
   - Anneal mutagenic primers containing desired mutation
   - Extend and incorporate primers with our exclusive QuikChange Lightning fusion enzyme

   Total reaction time: 1 hour*
2. **Faster *Dpn* I Digestion of Template**
   - Digest parental methylated and hemimethylated DNA with enhanced *Dpn* I enzyme

   Total reaction time: 5 minutes
3. **Transformation**
   - Transform mutated molecules into competent cells for nick repair

   Total reaction time: 1.5 hours

* Based on a 5 Kb plasmid; excludes ramp time.

Figure 1. Overview of the QuikChange Lightning Site-Directed Mutagenesis method.

The QuikChange Method

QuikChange kits have provided researchers with a fast, easy, and efficient non-PCR method to reliably perform site-directed mutagenesis since 1996. Other commercially-available kits utilize PCR-based techniques, which can propagate errors with each successive round of thermal cycling. The QuikChange method uses a linear amplification strategy with only the parental strand serving as the DNA template. Combining this with our highest fidelity polymerases leads to a significant reduction in unwanted second-site errors. The existence of such errors is likely to complicate and delay downstream screening and analysis.

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The Lightning Advantage

The QuikChange Lightning Kit contains specially engineered enzymes that have been 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 performing site-directed mutagenesis on three different template sizes, there is a significant reduction in thermal cycling and digestion times when comparing QuikChange Lightning to either QuikChange or QuikChange II (Figure 2). The QuikChange Lightning Kit provides the same performance and ease-of-use as previous versions but with the added benefit of saving valuable research time.

Featured Application: Domain Swapping

Domain-swapping experiments allow for the exchange of 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 or relied on convenient restriction sites. In contrast, the QuikChange Lightning Kit method allows for the quick and easy exchange of DNA sequences.

QuikChange Lightning Kits enable domain-swapping in two simple steps. First, PCR-amplify the desired insertion sequence using a high fidelity DNA polymerase, such as our *PfuUltra* II Fusion HS DNA Polymerase, to create a mega primer. This mega primer will serve as the complimentary primers required in the subsequent QuikChange Lightning reaction. Second, add the gel-purified mega primer to the QuikChange Lightning Kit reaction.

References

Kirsch, J. and Joly, E. (1998) Nucleic Acid Res. 26(7): 1848-50.

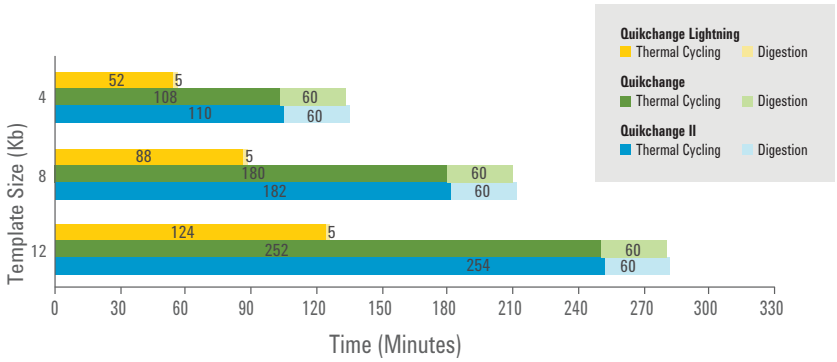

Figure 2. **Reaction times (excluding ramp times) of QuikChange Lightning, QuikChange, and QuikChange II for different template sizes.** Compared to QuikChange and QuikChange II, QuikChange Lightning reduces both thermal cycling and digestion times for a variety of construct sizes.

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 Site-Directed Mutagenesis Kit	10	210518
	30	210519

Related Products

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

For more information

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