Agilent Genomics

Enabling Fast, Efficient, and High Fidelity Site-Directed Mutagenesis through Continuous Innovation

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Mutagenesis experiments allow researchers to modulate protein activity and characterize structure/function relationships, which enriches our understanding of basic cellular processes and complex diseases and fuels discoveries in translational research. Site-directed mutagenesis is the method of choice for altering a gene or vector sequence at a selected location. Point mutations, insertions or deletions are introduced by incorporating primers containing the desired modification(s) with a DNA polymerase.

The QuikChange site-directed mutagenesis method, first introduced in 1995 (see: Site-Directed Mutagenesis in One Day with >80% Efficiency" (1996) Papworth, C., Bauer, J. Braman, J, and Wright, D. Strategies 9(1): 3-4.) utilizes a signature three-step, one-day method to introduce point mutations, amino acid substitutions, deletions and small insertions in virtually any double stranded plasmid template with high rates of efficiency. The success and prevalent adoption of the QuikChange method, can be attributed to the assay's high rate of efficiency (>80% colonies with desired mutation) and relative ease of use. Continued improvements in the following decade led to enhancements in fidelity, robustness, and the number of sites that can be mutagenized simultaneously.

The QuikChange method has a number of advantages over PCR-based approaches. For example, the frequency of unintended errors is higher in PCR strategies (copies are copied), which increases the amount of sequencing and error-correction required downstream. In contrast, QuikChange employs a linear amplification method (only parental strands are copied) in addition to high-fidelity Pfu polymerases, to minimize the number of clones with undesired mutations. This is reflected downstream, by less need for the sequencing of clones and correction of clones with incorrect mutations. Additionally, PCR-based strategies have been hampered by laborious cloning steps and low efficiencies (e.g., long range PCR of vector backbones); a contrast to QuikChange's >80% efficiency and three-step one-day protocol. Finally, robustness, or the ability to introduce mutations in larger templates, is achieved through the introduction of ultra-high-competency XL-10 Gold competent cells in the QuikChange II XL and Lightning kits.

Continued Innovation Leads to Lightning Fast Enhancements

While QuikChange represented a marked improvement over traditional PCR-based methods, in 2008 and 2009, QuikChange Lightning and QuikChange Lightning Multi Site-Directed Mutagenesis methods were introduced to address the demand for faster workflows and increased productivity. What distinguished QuikChange Lightning from its predecessors was the introduction of a new enzyme, Pfu fusion DNA polymerase. Pfu fusion's secret: tighter binding and increased processivity through the presence of a C-terminal DNA binding domain, in order to enable faster PCR cycling. In addition, optimized protocols and reagents reduced the length of the PCR and DpnI selection steps, providing up to a three-fold reduction in overall mutagenesis turn-around time.

QuikChange Lightning Multi, builds on Lightning's speediness with the ability to create mutations at multiple sites simultaneously. While conventional PCR-based methods are typically limited to two simultaneous mutational sites, by employing a patented multi-enzyme polymerase blend, QuikChange Lightning Multi is capable of introducing mutations at up to five sites simultaneously in about 3 hours. Conventional techniques to perform site-directed mutagenesis at five sites (along with accompanying miniprep and sequencing reactions) by contrast, could take up to 15 days to complete.

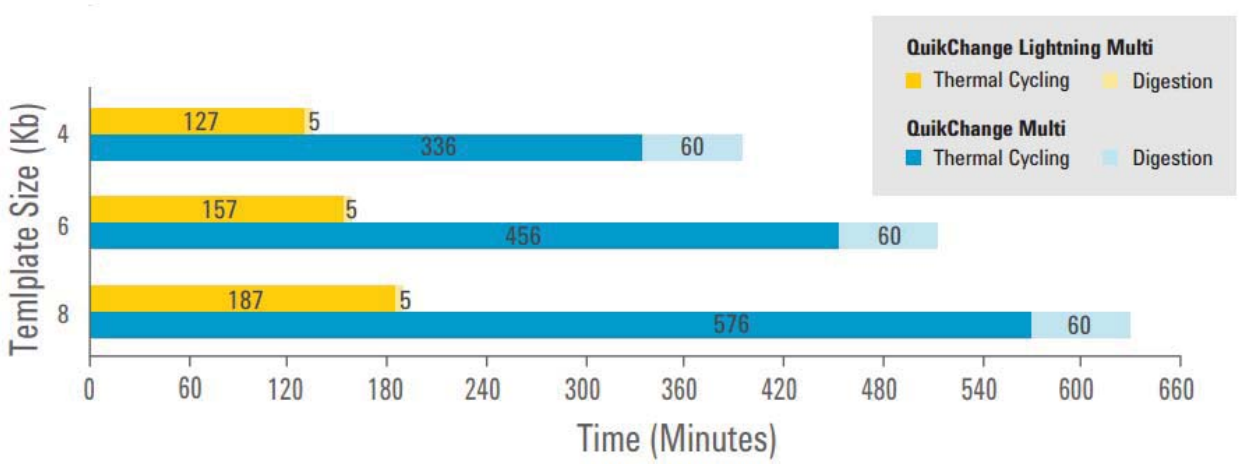

Figure 1: Reaction times (excluding ramp times) of QuikChange Lightning Multi and QuikChange Multi for different template sizes. Compared to QuikChange Multi, QuikChange Lightning Multi reduces both thermal cycling and digestion times for a variety of construct sizes

Synthetic Biology as the Next Step in Site-Directed Mutagenesis

The efficiency and ease-of-use of the QuikChange method has facilitated engineering of promoter and coding regions of numerous genes. Continuing improvements to Pfu polymerase have led to enhancements to the QuikChange method, permitting protocols with higher fidelity, longer length-capability, multi-site targeting, and significantly faster time-to-completion. (e.g., Agilent's QuikChange II, QuikChange II XL, QuikChange Multi and QuikChange Lightning). Looking forward, leading the drive for continued innovation in the area of mutagenesis means embracing novel technologies which may yield

new enhancements in speed, efficiency, fidelity, or throughput. One such area, synthetic biology, may enable broad scanning mutagenesis approaches and permit the sequential adding of mutations through a domain in order to understand the role of each amino acid in a protein, find improved variants, and outline structural linkages. Synthetic biology approaches allow the scaling up of mutagenesis experiments with accompanying significant reductions in time and cost for large-scale assays.

Request more information at
agilent.com/genomics/QuikChangeHT
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