SYNTHETIC BIOLOGY

QUIKCHANGE HT

Protein Engineering System

Save Research Time and Learn More – all in a Single Experiment

- Quickly identify functional sites – Site Saturation Mutagenesis across the entire protein
- Targeted Combinatorial Mutagenesis uncovers enhanced variants with minimal screening
- Target one protein or a family of variants

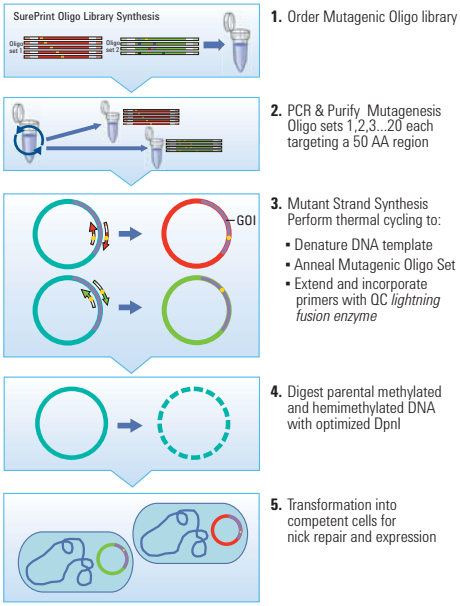


Figure 1. QuikChange HT Method

Overview

Site Directed Mutagenesis (SDM) is the method of choice for rationally mutating proteins delivering the highest fidelity and targeted action.

The QuikChange HT Protein Engineering System improves on the proven power of the QuikChange technology with the addition of a high fidelity custom oligonucleotide library. QuikChange HT allows rapid resolution of structural and functional questions by creating libraries of rationally designed mutants, at a price point that enables broad utilization of comprehensive mutant libraries for applications such as single amino acid scanning, site saturations scanning or targeted combinatorial mutagenesis.

The best of both worlds

Rationally designed mutant libraries facilitate efficient and comprehensive identification of protein functional sites responsible for activity or binding efficiency, and physical properties such as solubility, permeability, conformation and in cell location.

Rationally designed mutant libraries have traditionally required structural information, consequently high throughput mutagenesis experiments have been costly and untargeted. Additionally, error-prone PCR (EP-PCR) is not comprehensive, generates many unwanted mutations, and may identify multi-site mutants, which require follow up SDM experiments in order to confirm key mutations.

Agilent's SurePrint technology for high throughput, high quality oligo library synthesis enables rationalized single amino acid scanning, site saturation scanning and targeted combinatorial mutagenesis, providing structural and functional linkage while at the same time identifying improved variants. SurePrint minimizes the number of clones to screen without the expense of synthesizing the entire gene.

QUIKCHANGE HT APPLICATIONS	
Antibody Engineering	Phosphorylation sites
Enzyme Engineering	Protein/Protein Interactions
Protein Folding and Solubility	Protein Expression Codon Optimization
ID functional sites	SNP validation

QUIKCHANGE HT PROTEIN ENGINEERING SYSTEM

Discover faster, learn more

- QC HT rational design eliminates codon redundancy and bias as well as non-coding, wild type and non-target mutations seen when using degenerate oligos and EP-PCR
  - Uncover structural and functional relations with confidence – only rational mutations included, saving time and money by eliminating screening of large numbers of non-functional mutants
  - Rapidly create relevant mutants with improved function with optimized codons for the host organism
  - Efficient screening reduces sequencing burden. QuikChange HT requires 1.7 – 3.5x less colony screening compared to degenerate mutants (NNX/NNN) in order to find any given variant.
  - Comprehensive coverage with every mutation included for improved intellectual property creation and protection.

QC HT Workflow Including Sub-libraries, QuikScan, and QuikCombine

The QuikChange HT Protein Engineering System provides researchers the ability to precisely target and mutate every codon within a 50 amino acid region in less than one day with a single oligo set. Additional oligo sets can mutate several distinct sites e.g. other 50 amino acid regions of the same or of different proteins, in parallel reactions.

This technology provides researchers the ability to design large mutagenic libraries from as few as 1,000 and up to 120,000 user-defined sequences via free access to a mutagenesis workspace software user-interface. The library may consist of numerous oligo sets (up to 20) targeted at distinct regions of a single protein or of multiple proteins. Each oligo set will correspond to a homologous DNA sequence that hybridizes to the same gene fragment and varies in sequence by one or up to four codons. Each DNA sequence contains fully complementary ends for PCR amplification of the oligo set. Each mutagenesis library is customized for each experiment and once received, researchers can generate libraries of transformed competent cells in less than a day.

	QuikChange HT	QuikChange + Degenerate Oligos	GeneMorph II
Method	Site Directed Mutagenesis	Site Directed Mutagenesis	Random Mutagenesis
Requires Structural Information	No	Yes	No
Provides Structural Information	**YES**	**YES**	**NO**
Coverage (of intended mutations)	95–100%	60–100%	10–50%
Library size/clones to screen (equal representation low abundant codons)	1x	1.7–3.5x	100x
All in one system	Yes	No	No
Cost	$$	$$$	$

Figure 2. QuikChange HT mutagenesis methods comparison

The QuikChange Lightning performance allows site specific mutation in virtually any double-stranded plasmid, thus eliminating the need for subcloning and for ssDNA rescue, achieving the highest fidelity DNA synthesis and efficient transformation of >80%.

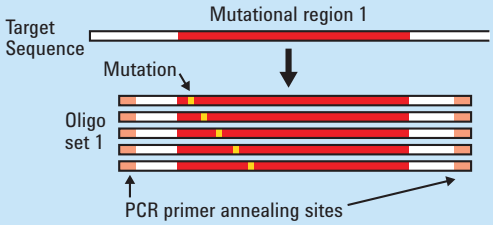
Mutagenesis methods: The free-to-use QC HT mutagenesis workspace in eArray (agilent.com/genomics/earray) helps you design complex oligo libraries with ease. Create 1–20, 15–50 amino acid regions to cover the entire protein or selected domains. Select one of three mutagenesis methods per mutational region to make an oligo set and combine oligo sets for a single protein or family of proteins to make up a library of up to 120,000 mutants.

Validation: Codon Saturation Scanning of GFP

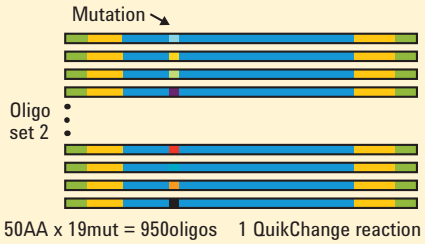
To validate the power of rationally designed mutant libraries, a site saturation experiment was designed to uncover single amino acid variants to the well-known, highly optimized enhanced GFP. The single experiment resulted in the identification of 40 single point mutants, each brighter than the enhanced GFP and 15X brighter than the super bright Vitality hrGFP II.

Interestingly, these 15 super-bright variants were identified as having mutations in the two domains that did not directly make up the chromophore, thus identifying target regions for subsequent combinatorial mutagenesis.

Use QuikScan1 to determine relevant sites for structure, function, and stability: Separately replaces each amino acid in the wild type mutational region with a particular amino acid. Often used for Alanine scanning to quickly identify key functional or structural amino acids.



Use QuikScan19 to identify single codon replacements that improve binding, function, or stability: Codon saturation scanning, systematically replaces each amino acid in the wild type mutational region with all 19 other amino acids, resulting in 19 mutagenic oligos for each amino acid position in the mutational region.



Use QuikCombine to discover a multisite mutant with improved structure, function, and stability: Combine multiple mutants in groups of 1–4 position with defined variation at each site. Make up to $1.2x10^4$ libraries for a single 50AA set or combine a few identified variants and validate functional relevance .

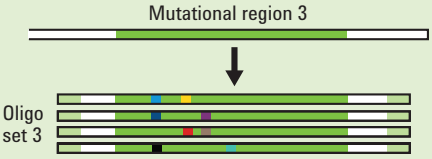
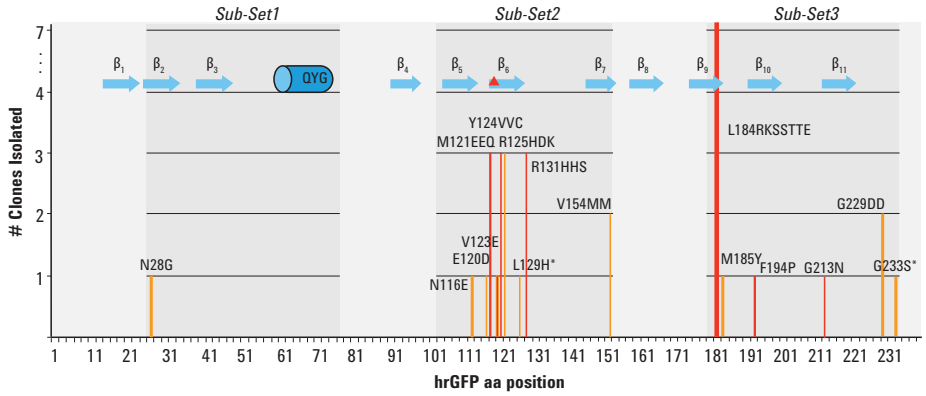


Figure 3. QC HT Methods



	GFP Set1 N-terminal (chromophore)	GFP Set2 Middle	GFP Set3 C-terminal
Sub-library size	7,800	14,000	8,900
# Colonies Screened	13,200	25,000	15,400
% fluorescent clones	0.5%	5.9%	7.7%
# positives brighter than hrGFP/hrGFP II (*E. coli*)	1/0	14/8	10/7

Figure 4. Codon saturation scanning of GFP. The GFP structure appears to be very sensitive to modification, as seen by the overall low number of mutants that retain fluorescence. The conformation of the beta barrel structure is critical to orientation, maturation, and fluorescence emitted by the chromophore. Other enzymes (e.g., beta gal) appear to be much more tolerant of mutations.

QUIKCHANGE HT PROTEIN ENGINEERING SYSTEM

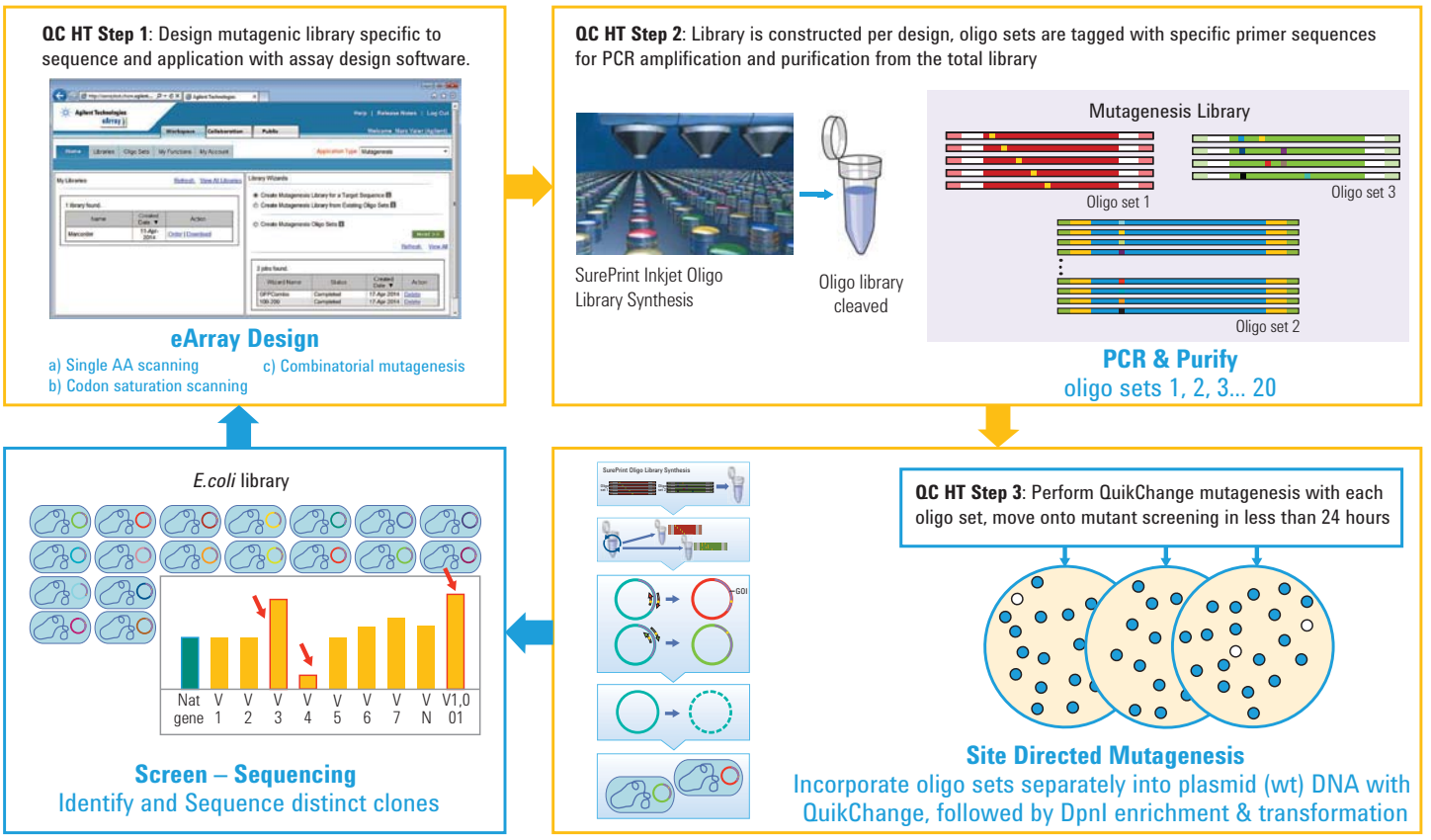


Figure 5. Simple QuikChange HT workflow

QC HT product ordering and structure

QuikChange HT Protein Engineering System contains all you need to create libraries of mutants utilizing up to 120,000 mutagenic oligonucleotides, oligo amplification primers and reagents, QuikChange enzymes and reagents, and SoloPack Gold SuperCompetent cells.

Scope	Description	Part Number
Commercial	QuikChange HT Protein Engineering System 150nt, 10 sites	G5900A
	QuikChange HT Protein Engineering System 150nt, 20 sites	G5900B
	QuikChange HT Protein Engineering System 200nt 10 sites	G5901A
	QuikChange HT Protein Engineering System 200nt 20 sites	G5901B
Academic	G5902A QuikChange HT Protein Engineering System 150nt-Academic, 10 sites	G5902A
	G5902B QuikChange HT Protein Engineering System 150nt-Academic, 20 sites	G5902B
	G5903A QuikChange HT Protein Engineering System 200nt-Academic, 10 sites	G5903A
	G5903B QuikChange HT Protein Engineering System 200nt-Academic, 20 sites	G5903B

Figure 6. Ordering Part numbers

Request more information at **www.agilent.com/genomics** or call your Agilent service representative for a demo.

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