Accelerate Your Research with QuikChange Lightning Site-Directed Mutagenesis Kits

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An R&D Manager in the Genomics Division. Agilent Technologies
Site-Directed & Random Mutagenesis

**Single & Multiple Site-Directed Mutagenesis:**

**What:** create one or more nucleotide or codon (amino acid) replacements, insertions or deletions  
**When:** desired modification is known  
**Where:** codons identified from analyzing protein structure, amino acid sequence homology, prior mutagenesis data, or host codon preference  
**Why:** determine structure-function relationships in proteins or promoters, map antibody-antigen or receptor-ligand epitopes, create designer enzymes, optimize expression, add/remove restriction sites…  
**How:** QuikChange Site-Directed Mutagenesis Kits

**Random Mutagenesis:**

**What:** create random single-base replacements (typically)  
**When:** desired modification is **UN**known  
**Where:** gene or gene fragment (domain)  
**Why:** identify nucleotide or codon changes that alter function, e.g., promoter activity, antibody binding, enzyme activity  
**How:** GeneMorph Random Mutagenesis Kit
Agilent Mutagenesis Products

Site-Directed Mutagenesis Kits:

**Single Site Kits**
- QuikChange & XL
- QuikChange II, II XL, II E
- QuikChange Lightning

**Multiple Site Kits**
- QuikChange Multi
- QuikChange Lightning Multi

**Mutagenic primer design**
- Free QC Primer Design Program available online: [www.agilent.com/genomics](http://www.agilent.com/genomics)

Random Mutagenesis Kits:
- GeneMorph Random Mutagenesis
- GeneMorph EZ Clone
Mutagenesis Tools are Developed in Parallel with Protein Engineering at Agilent

Novel mutations in Moloney Murine Leukemia Virus reverse transcriptase increase thermostability through tighter binding to template-primer

Bahram Arezi* and Holly Hogrefe
Agilent Technologies, Stratagene Products Division, La Jolla, CA 92037, USA

AffinityScript Multi-Temp RT (MMLV RT pentamutant)

Thermal profile

- MMLV RT
- AffinityScript RT

Identified best combination of thermal resistant mutations

Random mutagenesis & screening
GeneMorph Random Mutagenesis Kit

Located sites that confer thermal resistance
QuikChange Site-Directed Mutagenesis kit (with degenerate codon primers)

Identified side chain replacements that provide greatest thermal resistance
QuikChange Multi Site-Directed Mutagenesis kit (4 mutagenic primers)

Screen combinatorial mutant library
Codon saturation

Located sites that confer thermal resistance

AffinityScript Multi-Temp RT (MMLV RT pentamutant)
The QuikChange Method

- Features a simple 3-step protocol
- Creates single base changes, codon replacements, insertions and deletions with >80% mutation efficiency
- Employs high-fidelity *Pfu* polymerase and linear amplification to avoid unintended mutations
- Referenced in thousands of publications

**MIX**
Denature plasmid and annealing primers containing desired mutation X

**CYCLE**
Temperature cycle to extend and incorporate mutation primers resulting in nicked circular strands

**DIGEST**
Digest parental DNA template

**TRANSFORM**
Transform the resulting annealed double-stranded nicked DNA molecules

After transformation, XL-1Blue *E. coli* cell repairs nicks in plasmid
The QuikChange Multi Method

- Introduces point mutations at 1-5 sites simultaneously

- Provides high mutation efficiencies of >80% for 1 site and >50% for up to 3 sites

- Saves time when constructing multi-site mutants

- Rapid mutant construction facilitates testing of multiple combinations of point mutations

STEP 1
Mutant Strand Synthesis (Thermal Cycling)
Perform thermal cycling to:
1. Denature DNA template
2. Anneal mutagenic primers
3. Extend primers and seal nicks with the QuikChange Multi enzyme

STEP 2
Dpn I Digestion of Template DNA
Digest methylated and hemimethylated DNA with Dpn I

STEP 3
Transformation
Transform mutated ssDNA into XL10-Gold ultracompetent cells
Evolution of QuikChange Kits


- **QuikChange** Site-Directed Mutagenesis Kit
- **QuikChange Multi** Site-Directed Mutagenesis Kit
- **QuikChange II & II XL** Site-Directed Mutagenesis Kit
- **QuikChange LIGHTNING** Site-Directed Mutagenesis Kit
- **QuikChange MULTI** Site-Directed Mutagenesis Kit

- First Pfu Patent Issued
- First of Six QuikChange Patents Issued
- PfuUltra
- PfuUltra II

- Pyrococcus furiosus Isolated
- Native Pfu
- Cloned Pfu
- PfuTurbo
- Fusion
Advantages of QC Lightning & Lightning Multi Kits

• **Improved** DNA polymerase and mutagenesis buffer*

• **Enhanced** *Dpn* I enzyme*

• **Faster** protocol
  • Shorter cycle times
  • Shorter selection step

• **One** kit for both long and short templates**

• **Same** accuracy, efficiency, and ease-of-use as previous kits

* Not sold separately from kit
** Applies to single site kits
QuikChange Lightning Polymerase

**Pfu fusion DNA polymerase**

- C-terminal fusion with DNA-binding protein promotes **tighter binding** and **increased processivity**
- Same polymerase used in Agilent’s *PfuUltra II* & *Herculase II* PCR enzymes to enable **fast cycling** with **high yield** and **fidelity**; however, enzyme and buffer formulas are unique to each application and therefore should not be interchanged.

*PfuUltra II* amplifies a wide range of genomic DNA targets.

*Herculase II* generates robust yields with fast cycling times.
QuikChange Lightning Features

- **Shortened protocols**
  - Reduced cycle times
    - 30 sec vs. 1-2 min per kb extension times
  - Faster selection step
    - 5 min vs. 1 hr

- **One kit for templates sizes up to 14 kb**

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*Previous kits require separate kits for shorter (4-8 kb) & longer templates (8-14 kb).*
We thoroughly optimized enzyme and buffer formulations, \textit{DpnI} digestion conditions, and cycling parameters on multiple thermal cyclers to achieve equivalent performance to our QuikChange II and II XL kits…. \textit{in less than half the time.}

<table>
<thead>
<tr>
<th>Test System</th>
<th>Size (kb)</th>
<th>Mutation type</th>
<th>Selection marker</th>
<th>Mutation Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.5</td>
<td>Point mutation</td>
<td>\textit{lacZ}</td>
<td>&gt;90</td>
</tr>
<tr>
<td>2</td>
<td>5.7</td>
<td>Point mutation</td>
<td>\textit{lacZ}</td>
<td>&gt;90%</td>
</tr>
<tr>
<td>3</td>
<td>6.1</td>
<td>\text{In (+10)/Del (-10)}</td>
<td>Fluorescence</td>
<td>&gt;90%</td>
</tr>
<tr>
<td>4</td>
<td>7.3</td>
<td>\text{In (+2)/Del (-8)}</td>
<td>Restriction sites</td>
<td>&gt;90%</td>
</tr>
<tr>
<td>5</td>
<td>8.1</td>
<td>Point mutation</td>
<td>Restriction sites</td>
<td>&gt;90%</td>
</tr>
<tr>
<td>6</td>
<td>13.8</td>
<td>Point mutation</td>
<td>\textit{lacZ}</td>
<td>&gt;90%</td>
</tr>
</tbody>
</table>
## QuikChange Lightning Kit Provides High Value

<table>
<thead>
<tr>
<th>Component</th>
<th>Purchased Separately</th>
<th>QuikChange Lightning Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-efficiency comp cells (&gt;10⁹)</td>
<td>$18.00 to $21.00</td>
<td>Included</td>
</tr>
<tr>
<td>High-fidelity PCR enzyme</td>
<td>$1.29 to $2.73</td>
<td>Included</td>
</tr>
<tr>
<td><em>Dpn I</em> restriction enzyme</td>
<td>$0.58 to $4.55</td>
<td>Included</td>
</tr>
<tr>
<td>Kit control</td>
<td>N/A</td>
<td>Included</td>
</tr>
<tr>
<td><strong>Cost / Rx ($USD)</strong></td>
<td><strong>$19.87 to $28.28</strong></td>
<td><strong>$22.43</strong></td>
</tr>
</tbody>
</table>

* Based on a 30 rx kit

Our QuikChange kits are priced comparably to purchasing individual reagents. Plus, Agilent provides expert Technical Service support and guarantees >80% efficiency with every kit.
• Updated with faster polymerase and selection enzyme

• Creates multiple mutants in about 3 hours (plus overnight transformation)

• Saves time without compromising performance

• Recommended for incorporating as many as 5 mutant oligonucleotides into plasmids up to 8 kb
Construct Complex Multi-Site Mutants in 3 Hours

Save up to 7 hours by upgrading to QuikChange Lightning Multi!*  

* Based on 8 kb plasmid.
Shorter Protocol without Compromising Performance

The QuikChange Lightning Multi Kit is quality-controlled to ensure same level of performance as the original kit, with efficiencies exceeding 80% and 50% for 1 and 3 sites, respectively.
## Performance at Recommended Limits of Primer Number & Template Size

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Size (kb)</th>
<th># sites</th>
<th>Selection markers</th>
<th>Mutation Efficiency (%)</th>
<th>QCLM</th>
<th>QCM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pWS-3</td>
<td>4.0</td>
<td>3</td>
<td>lacZ+(3)</td>
<td>68.8</td>
<td>51.9</td>
<td></td>
</tr>
<tr>
<td>pWS-3</td>
<td>4.0</td>
<td>4</td>
<td>lacZ+(3)/+Kpn I (1)</td>
<td>48.5</td>
<td></td>
<td>39.0</td>
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<tr>
<td>pWS-3</td>
<td>4.0</td>
<td>5</td>
<td>lacZ+(3)/+Kpn I (2)</td>
<td>52.1</td>
<td></td>
<td>32.4</td>
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<tr>
<td>pWS-3</td>
<td>5.7</td>
<td>2</td>
<td>lacZ+(3)/+Kpn I (1)</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>pWS-3</td>
<td>5.7</td>
<td>5</td>
<td>lacZ+(3)/+Kpn I (2)</td>
<td>30.8</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>pCMVLacI</td>
<td>8.1</td>
<td>2</td>
<td>+Kpn I (2)</td>
<td>51.6</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

*From: Hogrefe et al (02) Biotechniques 33:1158-1165*
QuikChange Lightning Publications
(HighWire Stanford University)

* Through October
QuikChange Lightning Application: Epitope mapping for vaccine development

Human broadly neutralizing antibodies to the envelope glycoprotein complex of hepatitis C virus

Erick Giang, Marcus Dorner, Jannick C. Prentoe, Marlène Dreux, Matthew J. Evans, Jens Bukh, Charles M. Rice, Alexander Ploss, Dennis R. Burton, and Mansun Law

- Used alanine scanning to map the epitope of a monoclonal antibody with exceptionally broad neutralizing activity towards diverse HCV genotypes
- Introduced alanine substitutions at 155 positions in the envelope glycoproteins E1 and E2 to identify amino acids critical to antibody binding
- Results of epitope mapping will aide in the design and development of vaccine candidates that elicit broadly neutralizing antibodies to HCV which currently infects ~2% of the world’s population

Alanine scanning. Mutagenesis strategy for defining structure-function relationships. Set of mutants is constructed with a single alanine replacement at each position within a protein or protein domain
QuikChange Lightning Application: Identifying genetic changes that contribute to cancer

Comprehensive sequencing of tumor suppressor genes in breast cancer patients identified 25 rare missense mutations in CHEK2.

To investigate functional consequences, each CHEK2 variant was constructed and tested in a DNA damage response assay to distinguish causal (red) from benign (green) mutations.

As genomic sequencing becomes more widespread, the number of rare mutations identified in cancer-related genes will continue to grow. Determining the functional consequences of novel variants will be essential to establishing genetic guidelines for assessing risk.
Examined the effect of rare (37W) and common (607F) variants of DISC 1 (Disrupted-in-Schizophrenia 1) on subcellular targeting

Investigated the consequences of mutations in SHANK2 scaffold protein which are linked to autism
QuikChange Lightning Application: 
Defining structure-function relationships

Sought to identify residues that contribute to lesion bypass and to UV-induced mutagenesis in UmuC, a Family Y DNA polymerase

In the absence of a crystal structure, used modeling and sequence alignment to related homologs to identify 2 surface loops that are likely to interact with DNA template and incoming nucleotide

By amino acid replacement, identified 3 amino acids (N32, N33, D34) that play a role in UmuC function, both in UV resistance and modulating homologous recombination
**QuikChange Lightning Application: Engineering tools for biotechnology**

Engineered zinc finger nickases induce homology-directed repair with reduced mutagenic effects

Cherie L. Ramirez1,2, Michael T. Certo3,4, Claudio Mussolino5, Mathew J. Goodwin1, Thomas J. Cradick6, Anton P. McCaffrey6, Toni Cathomen5, Andrew M. Scharenberg4, and J. Keith Joung1,2,3,*

1Molecular Pathology Unit, Center for Cancer Research, and Center for Computational and Integrative Biolog 
Massachusetts General Hospital, Charlestown, MA, 02129, 2Biological and Biomedical Sciences Program, 
Harvard Medical School, Boston, MA, 02115, 3Program in Molecular and Cellular Biology, University of 
Washington, Seattle, WA, 98195, 4Center of Immunity and Immunotherapies, Seattle Children’s Research 
Institute, Seattle, WA, 98105, USA, 5Institute of Experimental Hematology, Hannover Medical School, Hannover, 
30625, Germany, 6School of Internal Medicine, University of Iowa School of Medicine, Iowa City, IA, 
52245, 7Department of Pediatrics, University of Washington, Seattle, WA, 98106 and 8Department of Pathology, 
Harvard Medical School, Boston, MA, 02115, USA

- Converted engineered zinc finger nuclease (ZFN) into zinc finger nickase by introducing a known inactivating mutation in one monomer of a ZFN heterodimer
- In human cell reporter assays, ZFNickases induce homology-directed repair at the nick site with fewer incidences of NHEJ-mediated errors
- May provide a promising strategy for genome engineering

ZFNs are a class of engineered DNA-binding proteins that facilitate targeted editing of the genome by creating double-strand breaks in DNA at user-specified locations. From Genetic Engineering & Biotechnology News, July 1, 2010, Vol 30 No. 13.
Highly thermostable fungal cellobiohydrolase I (Cel7A) engineered using predictive methods

- Constructed 43 point mutants identified by predictive methods to increase thermostability of fungal cellulase CHB I
- Identified 8 stabilizing mutations that when combined increase thermal resistance by 4.7°C and improve sugar production at elevated temperature
Define functional consequences of post-translational modifications

**Glycosylation**
- N residues at 7 sites
  - Autocatalytic Cleavage of Human γ-Glutamyl Transpeptidase Is Highly Dependent on N-Glycosylation at Asparagine 95 (2011)

**Phosphorylation**
- S residues at 2 sites
  - Plk3 Functions as an Essential Component of the Hypoxia Regulatory Pathway by Direct Phosphorylation of HIF-1α (2010)

**Ubiquitination**
- K residues at 7 sites
  - The ocular albinism type 1 (OA1) GPCR is ubiquitinated and its traffic requires endosomal sorting complex responsible for transport (ESCRT) function (2009)
Promoter modifications at 1, 2, or 3 transcription factor binding sites

Rac1-mediated Mitochondrial H₂O₂ Generation Regulates MMP-9 Gene Expression in Macrophages via Inhibition of SP-1 and AP-1

MMP-9 promoter activity

WT
Null
Null
Null
Null
Null
Null
Null
Null
Null
How Can QuikChange Lightning Help You?

Time savings with quicker protocol

- Faster processing DNA polymerase
- Faster digestion

More complete digestion of parental template

- Enhanced \textit{Dpn I} enzyme

ONE kit for short & long templates

- Separate XL kit purchase no longer necessary

Cost savings

- Competitively priced compared to original QC kits, QC II kits, and purchasing individual components
Agilent QuikChange Lightning Site-Directed and Multi Site-Directed Mutagenesis Kits

www.agilent.com/genomics/QCLKits

We bring the Lightning, You bring the Thunder!