

# QuikChange Site-Directed Mutagenesis

Revised on September 22, 2015

## Introduction

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WatCut offers several analysis tools related to the use of restriction enzymes in cloning experiments. It can be used for silent mutation analysis, restriction analysis, and SNP-RELP analysis. Silent mutation analysis is intended for use with oligonucleotide sequences. This tool is useful to introduce restriction sites in primers for QuikChange. It starts from a DNA (*not* protein) sequence, for which you also need to indicate the reading frame of protein translation. WatCut will find sites created by any number of mutations, with both non-degenerate and degenerate recognition sequences – that is, it will find all possible sites.

## Primer Design for QuikChange Site-Directed Mutagenesis

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The programs below support mutagenic primer design for QuikChange mutagenesis experiments.

GeneArt® Primer and Construct Design Tool: <http://www.lifetechnologies.com/order/oligoDesigner/mutagenesis>

PrimerX: <http://www.bioinformatics.org/primerx/>

Quikchange Primer Design Helper: <http://perry.freeshell.org/qc.html>

WatCut: <http://watcut.uwaterloo.ca/watcut/watcut/template.php>

## Materials

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- PfuUltra II Hotstart PCR Master Mix (Agilent Technologies, 600850)
- DpnI (NEW ENGLAND BioLabs, R0176S)

## Procedure

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1. Set up a 50- $\mu$ l reaction in a 0.2-ml microfuge tube at either ambient temperature, or on ice:

<u>Components</u>	<u>Final Concentration</u>	<u>Volume</u>
H <sub>2</sub> O	Not applicable	24.5 $\mu$ l
2X PfuUltra II Hotstart PCR Master Mix	1X	25 $\mu$ l
Forward primer (40 pmol/ $\mu$ l, 40 $\mu$ M)	0.2 $\mu$ M	0.25 $\mu$ l
Reverse primer (40 pmol/ $\mu$ l, 40 $\mu$ M)	0.2 $\mu$ M	0.25 $\mu$ l
Total reaction volume	N/A	50 $\mu$ l

If desired, a master mix can be prepared for multiple reactions, to minimize reagent loss and to enable accurate pipetting.

**Note:** Prepare a reaction without primers as a negative control.

2. Mix contents of the tubes and add 50  $\mu$ l of PCR mixture to each tube.
3. Add template DNA (100 - 300 ng) to tubes.
4. Carry out PCR using the following reaction conditions: initial denaturation at 95°C for 2 min, followed by 12-30 cycles of 95°C for 20 sec,  $T_m$ -5°C for 20 sec, 72°C for 15 sec/kb, and a final incubation of 72°C for 3 min. Reactions are stopped by chilling to 4°C.
5. Run 5  $\mu$ l of PCR products on a 0.9% agarose gel to make sure the production of PCR products.

**Note:** QuickChange product is electrophoresed as a bigger DNA than template.

#### ***Digestion of parental DNA template with Dpn I***

6. Add 1  $\mu$ l of the *Dpn* I restriction enzyme (20 U/ $\mu$ l) directly to each amplification reaction.

**Note:** The *Dpn* I endonuclease (target sequence: 5'-Gm<sup>6</sup>ATC-3') is specific for methylated and hemimethylated DNA and is used to digest the parental DNA template and to select for mutation-containing synthesized DNA. DNA isolated from almost all *E. coli* strains is dam methylated and therefore susceptible to *Dpn* I digestion.

7. Gently and thoroughly mix each reaction mixture by pipetting the solution up and down several times. Spin down the reaction mixtures in a microcentrifuge for 1 minute and immediately incubate each reaction at 37°C for 1-16 hour to digest the parental (i.e., the nonmutated) supercoiled dsDNA.
8. **Optional:** Heat-inactivate *Dpn* I for 20 minutes at 80°C.

**Memo:** Alanine is the most neutral amino acid and also is chemically inert. By using the alanine substitution, we can determine whether the charge has any thing to do with the function of protein.