SITE DIRECTED MUTAGENESIS

This technique can be used to generate single point mutations or to delete/insert single or multiple amino acids.

Procedure:

1. PCR reaction
   5 µl 10X PFU buffer
   X µl (5-50ng) dsDNA template
   Xµl (125ng) primer #1
   Xµl (125ng) primer #2
   Xµl 10X dNTPs
   ddH2O to a final volume of 50µl
   1µl PFU polymerase

Cycling parameters:

Segment	Cycles	Temperature	Time
1	1	95 C	30 seconds
2	12-18	95 C	30 seconds
		55 C	1 minute
		68 C	2minutes/kb of plasmid length

Type of mutation desired	Number of cycles
Point mutations	12
Single amino acid changes	16
Multiple amino acid changes	18

2. Chill PCR reaction on ice and add 1µl DpnI. Mix and incubate at 37 C for 1 hour to digest the parental supercoiled dsDNA.
3. Transform 1µl of the DpnI treated DNA and plate on appropriate medium. Screen colonies for desired mutants.

Comments:

1. It is very important that the primers be designed to have a Tm=78.
2. It is useful to set up a series of PCR reactions with 5,10,20, and 50ng of template DNA to determine optimal concentration.

Reference:

QuikChange Site Directed Mutagenesis Kit Manual at www.stratagene.com