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:

<table>
<thead>
<tr>
<th>Segment</th>
<th>Cycles</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>95°C</td>
<td>30 seconds</td>
</tr>
<tr>
<td>2</td>
<td>12-18</td>
<td>95°C</td>
<td>30 seconds</td>
</tr>
<tr>
<td></td>
<td></td>
<td>55°C</td>
<td>1 minute</td>
</tr>
<tr>
<td></td>
<td></td>
<td>68°C</td>
<td>2 minutes/kb of plasmid length</td>
</tr>
</tbody>
</table>

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: