

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 μ l 10X PFU buffer
 - X μ l (5-50ng) dsDNA template
 - X μ l (125ng) primer #1
 - X μ l (125ng) primer #2
 - X μ l 10X dNTPs
 - ddH₂O to a final volume of 50 μ l
 - 1 μ l 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 1 μ l DpnI. Mix and incubate at 37°C for 1 hour to digest the parental supercoiled dsDNA.
3. Transform 1 μ l 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 T_m=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