Site-Directed Mutagenesis Using Dpn1

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[Abstract] Site-directed mutagenesis is an important and widely used tool in molecular biology to generate specific changes in the DNA sequence of a given gene/genome. The protocol described here is for making nucleotide changes at specific loci in a large vector (>= 10 kb), and is based on the QuikChange II XL Site-Directed Mutagenesis Kit.

Materials and Reagents

1. Dpn1 restriction enzyme
2. XL10-Gold Ultracompetent Cells
3. QuikChange II XL Site-Directed Mutagenesis Kit (Guidechem)
4. LB agar (Sigma-Aldrich)
5. Antibiotics (Sigma-Aldrich)
6. NZ amine (casein hydrolysate)
7. Yeast extract
8. NaCl
9. NaOH
10. MgCl2
11. MgSO4
12. Glucose
13. NZY+ broth (see Recipes)

Equipment

1. Thermal cyclers (Bio-Rad Laboratories)
2. Bench-top centrifuge

Procedure

A. Mutant strand synthesis reaction

1. Prepare the sample reaction as follows:

5 µl of 10x reaction buffer
X µl (10 ng) of dsDNA template
1.25 µl (125 ng) of oligonucleotide primer 1
1.25 µl (125 ng) of oligonucleotide primer 2
1 µl of dNTP mix
3 µl of QuikSolution
ddH_2O to a final volume of 50 µl
Then add 1 µl of PfuUltra HF DNA polymerase (2.5 U/µl)

2. Cycle each reaction using the following parameters (be sure to adhere to the 18 cycle limit):

Segment	Cycles	Temperature	Time
1	1	95 °C	1 min
2	18	95 °C	50 sec
		60 °C	50 sec
		68 °C	1 min/kb of plasmid length*
3	1	68 °C	7 min

*For example, a 5 kb plasmid requires 5 min at 68 °C per cycle.

3. Following cycling, place reaction tubes on ice for 2 min to cool the reactions to 37 °C.
4. Check product by electrophoresis of 10 µl of product on 1% agarose gel. While gel is running, start Dpn1 digestion.

B. Dpn1 Digestion of the Amplification Products

1. Add 1 µl of the Dpn1 restriction enzyme (10 U/µl) directly to each amplification reaction using a small, pointed pipet tip.
2. Gently mix each reaction mixture by pipetting up and down several times. Spin for 1 minute and incubate the reaction at 37 °C for 1 h to digest the parental supercoiled dsDNA.
3. Transformation of XL10-Gold Ultracompetent Cells.
4. Gently thaw XL10-Gold ultracompetent cells on ice. For each control and sample reaction to be transformed, aliquot 45 µl of the cells to a pre-chilled polypropylene tube.
5. Add 2 µl of the B-ME mix provided with the kit to 45 µl cells.
6. Swirl the contents of the tube gently. Incubate on ice for 10 min, swirling gently every 2 min.
7. Transfer 2 µl of the Dpn1-treated DNA from each control and sample reaction to separate aliquots of the ultracompetent cells.
   For a positive control, add 1 µl of 0.01 ng/µl pUC18 control plasmid.

8. Preheat NZY+ broth in a 42 °C water bath.
9. Heat-pulse tubes in a 42 °C water bath for 30 sec.
10. Incubate the tubes on ice for 2 min.
11. Add 0.5 ml of preheated (42 °C) NZY+ broth to each tube, then incubate the tubes at 37 °C for 1 h with shaking 225-250 rpm.
12. Plate the appropriate volume of each transformation reaction as indicated in the table below on the appropriate selection plates.

Reaction type	Volume to plate
pWhitescript mutagenesis control	250 µl
pUC18 transformation control	5 µl (in 200 µl NZY+ broth)
Sample mutagensis	250 µl on each of two plates (entire transformation reaction)

13. Incubate plates at 37 °C for >16 h.

Recipes

1. NZY+ Broth (per Liter)
   10 g of NZ amine (casein hydrolysate)
   5 g of yeast extract
   5 g of NaCl
   Add deionized H_2O to a final volume of 1 L
   Adjust to pH 7.5 using NaOH and then autoclave
   Add the following filer-sterilized supplements prior to use:
   12.5 ml of 1 M $MgCl_2$
   12.5 ml of 1 M $MgSO_4$
   20 ml of 20% (w/v) glucose (or 10 ml of 2 M glucose)

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