DpnI mediated site-directed Mutagenesis

A highly effective simple method for making site directed lesions in plasmids without subcloning based on the work of Fisher and Pei (1997).

1. Amplification of mutant DNA

DNA template plasmid 5-20 ng
10x pfu DNA polymerase buffer 5.0 µl
25uM oligo 1 0.5 µl
25uM oligo 2 0.5 µl
10mM dNTP 1.0 µl
Pfu DNA polymerase (2.5 units) 1.0 µl
fill w/ddH2O to 50 µl

PCR conditions
95° 30 seconds,
18 cycles of : 95° 30 seconds, 55° 1 minute, 68° 2 minutes/kb of plasmid length

2. Degradation of methylated (parental) DNA with Dpn I

Cool down PCR reaction.
Add 1µl Dpn I (10 unit) to PCR reaction 37°C and incubate for 1 hr.

3. Transformation into *E. coli*

Place 200 µl competent cells (1 x 10^8/ug efficiency or greater) DH5α on ice.
Add 1-2 µl of digested PCR reaction.
Incubate on ice 15-20 minutes.
Heat shock 45 seconds, and return to ice for 2 minutes.
Add 1 ml LB broth. Incubate at 37°C for 1 hr.
Plate 100 µl aliquot on appropriate antibiotic plate.

4. Miniprep 6 colonies and digest plasmids looking for mutant.

5. Sequence the lesion to confirm the change.

Notes:

Design of oligos

Oligos should be perfectly complementary (actually haven't checked out if this in required). The oligos should contain 12-15 bp on each site of the lesion. It is useful to engineer a restriction site addition or loss into the oligos for ease of screening. We have used the protocol for deletions using 17 bp on each side of the deletion.

Plasmid size

We have used the protocol for plasmids over 18 kb in length. Plasmids below 10 kb seem to work routinely. In most cases, a vast majority 80-100% of colonies are correct. For longer plasmids it may be necessary to work with PCR to optimize synthesis.

Introduction of lesions

Note that this is a linear amplification, so that the template only gets copied one time. Hence the odds of introducing lesions when using a high fidelity polymerase are very low.

Analysis of PCR product by gel electrophoresis

Run an agarose gel (5 -10 µl). If the product is visible, the mutagenesis will almost certainly work. Even if the product is not visible, one often still gets the mutant.

Using other polymerases

We have started using TaKaRa Ex-Taq instead of Pfu polymersase with great success using large plasmids (15-20 kb size). While getting the amplification to work efficiently with Pfu take a bit of fiddling, amplification with Ex-Taq appears very robust for large plasmids.

References

Fisher, C. L., and Pei, G. K. (1997). Modification of a PCR-based site-directed mutagenesis method. Biotechniques 23, 570-574.