Mutagenesis

1) Design the mutagenesis primers (fwd and rev) with 15-18 bp each side of the mutated codon

Ex : Carm1 P409A

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Primer fwd TGGCTATCCACAGCCGCAACAGAGCCCCTGAC
Primer rev GTCAGGGGCTCTGTTGCGGCTGTGGATAGCCA
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2) PCR :

- 1 µl plasmid DNA (200ng/µl)
- 5 µl 10x pfu buffer
- 5 µl dNTP 2mM
- 5 µl DMSO
- 0.5 µl primer fwd 100µM
- 0.5 µl primer rev 100µM
- 1 µl pfu polymerase
- qsp H2O 50 µl

Cycles : 20x(94°C 30'', 55°C 30'', 68°C 20')

3) Digest *Dpn*I : add 6 µl tampon 4 Biolab's + 4 µl *Dpn*I 2h 37°C.

4) Transform DH5a with 10 µl. Spread on LB+AB plate.