Site directed mutagenesis by PCR

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1 Works for me

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Mikaelian& Sergeant (1992) A general and fast method to generate multiple site directed mutations. *Nucleic Acids Res.* **20:** 376.

1 Design four primers in the following way:

Primer 1 anneals upstream of the region to be mutated
Primer 2 anneals downstream of the region to be mutated, in reverse direction, and has a 6 nt mismatch to the target at its 3'-end.
Primer 3 anneals downstream of primer 2 (not overlapping with the primer 2 site).
Primer M anneals of the site to be mutated, containing the mutation itself and 15 nt on either side of the mutation.

If Primers 1, 2 and 3 are designed to anneal to vector sequences 5' and 3' of a multiple cloning site, they can be reused for any mutagenesis performed in this vector.

2 Perform two PCR reactions with your template DNA: the first with primers 1 and 2, the second with primers M and 3. You should use a proof-reading polymerase that does not append 'A' overhangs to the pCR product - if you use Taq polymerase, the nucleotide before the primer annealing site for primer M must be a T (this will allow base-peiring with the A residues appended to PCR products by Taq).

Clean up the PCR products by resolbing them on an agarose gel, excising the bands, and using a gel extraction kit to retrieve the DNA.

3 Perform the second round PCR, using primers 1 and 3, and ~1 µl each of the two PCR producst from round 1 as template.

In the initial rounds of the PCR, the two product will anneal and the product incorporating the mutation can be extended to full length - this contains annealing sites for both primers 1 and 3, and will be amplified in subsequent rounds. The other strand cannot be extended to full length due to the 3'-end mismatch.

4 Use the final PCR product for cloning as for any other PCR product.