Site-Directed Mutagenesis

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Reference: Stratagene manual

Primer design

25 <= Length of primers <= 45
Tm >= 78 °C
Tm = 81.5 + 0.41(%GC) – 675/N - % mismatch
(Can be estimated at
http://depts.washington.edu/bakerpg/primertemp/primermelttemp.html)
N is the primer length in bases
Values for %GC and % mismatch are whole numbers

The desired mutation should be in the middle of the primer with ~10-15 bases of correct sequence on both sides.

The primers optimally should have a minimum GC content of 40% and should terminate in one or more C or G bases.

PCR

Prepare 4 PCR reactions (control (no Pfu), 10 ng, 20 ng and 40 ng)

Forward Primer (5 μM)	2 μl
Reverse Primer (5 μM)	2 μl
Template: 10 ng, 20 ng or 40 ng	
H_2O	
Total 20 μl	

Use a Hot Start method.

95 °C	5 min	
95 °C	30 sec	-----
55 °C	1 min	| 13 cycles
68 °C	1.5 min/kb of plasmid length	-----
72 °C	10 min	

Add 1 µl of the Dpn I (10 U/µl) directly to the PCR reactions, and incubate for 1 hour @ 37 °C.

Load 5 µl of each digestion product on the agarose gel.

Choose the reaction showing the faintest intensity of band.

Transfer 2 µl of the Dpn I-treated DNA from selected reaction to competent cells (50 µl).

Transform and spread.