

PCR for TNF k/o

PCR REACTION:

10x PCR buffer	2 λ
MgCl ₂ 25mM	depends on the Taq polymerase
dNTP's 2.5mM	1.5 λ
Primer A (148)	5 pmol /reaction
Primer B(149)	5 pmol /reaction
Taq polymerase	Variable
DNA	1 λ
milliQ H ₂ O	Up to volume 20 λ

Primer A (sense): 5'-aac-cag-gca-ggt-tct-gtc-cc-3'

Position in relating gene:(MM-TNFAB):4459-4478

Primer B (antisense): 5'-ctc-ttg-ctt-atc-ccc-tct-tcc -3'

Position in relating gene:(MM-TNFABKNO): 5687-5707

PCR CONDITIONS:

- 1) 94.0, 4 min
 - 2) 94.0, 1 min
 - 3) 58.0, 1 min
 - 4) 72.0, 1:30 min
 - 5) GOTO 2, for 4 cycles
 - 6) 94.0, 30 sec
 - 7) 58.0, 40 sec
 - 8) 72.0, 1:30 min
 - 9) GOTO 6, for 25 cycles
 - 10) 72.0, 10 min
 - 11) 20.0, 1 min
- END

PCR PRODUCT: Mutated band: 1228bp
Wild-type band: 481bp