

PCR FOR TNFΔARE

PCR REACTION:

10x PCR buffer	2 µl
MgCl ₂ 25 mM	1,2 µl
dNTP's 2.5 mM	1 µl
Primer A (116)	5 pmol / reaction
Primer B (119)	5 pmol / reaction
Taq polymerase	0,4 µl
DNA	1 µl
milliQ H ₂ O	Up to volume 20 µl

Primer A(sense): 5'- GAG-CCA-GCC-CCC-CTC-GGA-AGG-CCG-GGG-TG -3'
Position in relating gene: (MM TNFAB),6622-6636/Δ(6637-6706)/6707-6720

Primer B(antisense): 5'- AAT-TAG-GGT-TAG-GCT-CCT-GTT-TCC -3'
Position in relating gene: (MM TNFAB), 7143-7166

PCR CONDITIONS:

1. 94⁰C for 2 min.
2. 93⁰C for 2 min.
3. 57⁰C for 1 min.
4. 72⁰C for 1 min.
5. Steps 2-4 for 29 more cycles.
6. 72⁰C for 10 min.
7. 16⁰C for 5 min.
8. End

PCR PRODUCT:

Mutated band: ~530 bp

Wild-type band: ~420 bp