

PCR FOR TNFΔAREneo

PCR REACTION:

10x PCR buffer	2λ
MgCl ₂ 25mM	depends on Taq polymerase
dNTP's 2.5mM	1.5λ
Primer A (116)	5 pmol /reaction
Primer B (119)	5 pmol / reaction
Primer C (255)	5 pmol /reaction
Primer D (256)	5 pmol / reaction
Taq polymerase	Variable
DNA	1λ
milliQ H2O	Up to volume 20λ

Primer A : 5'-GAG-CCA-GCC-CCC-CTC-GGA-AGG-CCG-GGG-TG-3'

Primer B : 5'-AAT-TAG-GGT-TAG-GCT-CCT-GTT-TCC-3'
Position in relating gene: 7143-7166

Primer C: 5'-CTG-AAT-GAA-CTG-CAG-GAC-GA-3'
Position in relating gene:neor marker amplification

Primer D: 5'-ATA-CTT-TCT-CGG-CAG-GAG-CA-3'
Position in relating gene:neor marker amplification

PCR CONDITIONS:

- 1) 94, 3 min
 - 2) 93, 1:30 min
 - 3) 57, 1 min
 - 4) 72, 1 min
 - 5) GOTO to 2, for 29 cycles
 - 6) 72, 8 min
 - 7) 20, 1 min
- END

PCR PRODUCT:

Mutated band: ~ 960bp
Wild-type band: ~420bp