

PCR FOR mp55TNFR^{FLX} mice

PCR REACTION:

10x PCR buffer	2 λ
MgCl ₂ 25 mM	1.2 λ
dNTP's 2.5 mM	1.5 λ
Primer A	10 pmol / reaction
Primer B	10 pmol / reaction
Taq polymerase	0.2 λ
DNA	1 λ
milliQ H ₂ O	Up to volume 20 λ

Primer A (sense): 5'-CAA-GTG-CTT-GGG-GTT-CAG-GG -3'

Primer B (antisense): 5'- CGT-CCT-GGA-GAA-AGG-GAA-AG-3'

PCR CONDITIONS:

1. 94^oC for 3 min.
2. 94^oC for 0:30 sec.
3. 60^oC for 1 min.
4. 72^oC for 2 min.
5. Steps 2-4 for 29 more cycles.
6. 72^oC for 5 min.
7. 16^oC for 5 min.
8. End

PCR PRODUCT:

Mutated band: ~195 bp

Wild-type band: ~134 bp

PCR FOR mp55TNFR^{dFLX} mice (detection of deletion)

PCR REACTION:

10x PCR buffer	2 λ
MgCl ₂ 25 mM	1.2 λ
dNTP's 2.5 mM	1.5 λ
Primer A	10 pmol / reaction
Primer B	10 pmol / reaction
Taq polymerase	0.2 λ
DNA	1 λ
milliQ H ₂ O	Up to volume 20 λ

Primer A (sense): 5'- CCT-GCA-GAC-ACA-CGG-GGA-AA -3'

Primer B (antisense): 5'- TGA-ACT-CAG-GTT-GCC-AGA-CG -3'

PCR CONDITIONS:

1. 94^oC for 3 min.
2. 94^oC for 0:30 sec.
3. 60^oC for 1 min.
4. 72^oC for 2 min.
5. Steps 2-4 for 29 more cycles.
6. 72^oC for 5 min.
7. 16^oC for 5 min.
8. End

PCR PRODUCT:

~300bp