

PCR for TgK21

PCR REACTION:

| | |
|-------------------------|---------------------------|
| 10x PCR buffer | 2 λ |
| MgCl ₂ 25mM | depends on Taq polymerase |
| dNTP's 2.5mM | 1 λ |
| primer A (50) | 5 pmol / reaction |
| primer B (51) | 5 pmol / reaction |
| Taq polymerase | variable |
| DNA | 1 λ |
| milliQ H ₂ O | up to 20 λ |

Primer A(sense): 5`-TAC-CCC-CTC-CTT-CAG-ACA-CC-3`
position in relating gene: (HS-TNFA), 6124-6143

Primer B(antisense): 5`-GCC-CTT-CAT-AAT-ATC-CCC-CA-3`
position in relating gene: (HS- β -globin), 3' untranslated region

PCR CONDITIONS:

- 1) 94.0, 3 min
 - 2) 93.0, 1 min
 - 3) 58.0, 45 sec
 - 4) 72.0, 1 min
 - 5) GOTO 2, for 29 cycles
 - 6) 72.0, 5 min
 - 7) 20.0, 1min
- END

PCR PRODUCT: Band at ~350 bp.

PCR FOR p55 k-o

PCR REACTION:

| | |
|----------------|-------------------------------|
| 10X PCR buffer | 2λ |
| MgCL2 25mM | Depends on the Taq polymerase |
| dNTP'S 2,5mM | 1λ |
| Primer A | 5 μmol per reaction |
| Primer B | 5 μmol per reaction |
| Primer C | 5 μmol per reaction |
| Taq | Variable |
| DNA | 1λ |
| H2O | Up to volume 20λ |

Primer A: 5'-ctc-tct-tgt-gat-cag-cac-tg-3'

Primer B: 5'-ctg-gaa-gtg-tgt-ctc-ac-3'

Primer C (sense): 5'-ctg-aat-gaa-ctg-cag-gac-ga-3'

Position in relating gene: neo^r marker amplification

PCR CONDITIONS:

1. 94C for 4min.
2. 56C for 45sec.
3. 72C for 1:30min.
4. 93C for 45sec.
5. 56C for 45sec.
6. 72C for 1:30sec.
7. Steps 4-6 for 33 more cycles.
8. 72C for 10min.
9. 16C for 1min.
10. End.

PCR PRODUCT: Mutated band: 947 bp
Wild type band:1375 bp