>PRIMERS (all primers are listed 5'---->3')
>
>Exon 11: anti-sense
>ttg gtt ctc ttg aac tct gc
>
>Neo 2: anti-sense
>act gca tct gcg tgt tcg aa
>
>Intron: sense; common primer
>taa gag taa tct tcc aga gc
>
>REACTION_____
>
>10.0 uL : DNA (20 ng) of genomic per reaction (I use ~0.3 uL of DNA)
> 5.0 uL : 10xBuffer
> 0.4 uL : 25 mM dNTP
> 2.5 uL : [20 uM] Exon 11
> 2.5 uL : [20 uM] Neo 2
> 5.0 uL : [20 uM] Intron 5
> 0.5 uL : Taq (Fisher)
>26.5 uL : H20
>--------
>50.0 uL Total
>
>CYCLING_____
>
>step 1: 94, 3 min
>step 2: 1.5 degrees/sec to 55
>step 3: 55, 45 sec
>step 4: 72, 1 min
>step 5: 94, 45 sec
>step 6: 1.5 degrees/sec to 55
>step 7: 55, 45 sec
>step 8: 72, 1 min
>step 9: goto step 5, 35 cycles
>step 10: 72, 5 min
>step 11: 4, for ever
>GEL_____
>
>run 25 uL on a 1.0% agarose gel for ~1:30 hours at 90V.
>
>bands: (exon 11, neo 2, intron 5)
>wild-type: 426 bp
>heterozyg: 426 bp + 600 bp
>knock-out: 600 bp