

## PCR Screening for IL-4<sup>-/-</sup> Mice.

### Principle:

The IL-4 gene contains 4 exons and 3 introns and has been inactivated by insertion of the *neo* sequence into exon-2.

### Oligos:

1. D38028 = Sense exon 1 IL-4                    5'-GCACAGAGCTATTGATGGGTC
2. D38027 = Antisense exon 2 IL-4            5'-GCTGTGAGGACGTTTGGC  
The complimentary sequence to this oligo is removed in the gene targeting process and replaced with the neo insert.
3. D38026 = *neo*-specific antisense        5'-TCAGGACATAGCGTTGGC

### PCR:

- |                |  |
|----------------|--|
| Incubation     | <ol style="list-style-type: none"><li>1. Genomic DNA (1 : 1)</li><li>2. 10 mM dNTPs (1 : 1)</li><li>3. 10X Perkin Elmer buffer (2 : 1)</li><li>4. 25 mM Perkin Elmer MgCl<sub>2</sub> (1.6 : 1 for final concentration 2mM)</li><li>5. Oligos, 1 : 1 each add all 3 together.<br/>Total reaction volume - 20 : 1 brought up with PCR water</li></ol> |
| Program<br>IL4 | <ol style="list-style-type: none"><li>1. Add Taq DNA polymerase (0.3 : 1) (Promega).<br/><b>NO HOT START NEEDED.</b></li><li>2. 35 cycles - 94°C for 1 min, 58°C for 1 min, 72°C for 1 min</li><li>3. 1 cycle - 72°C 5 min</li><li>4. Hold at 4°C.</li></ol>   |

### Expected bands on TBE agarose gel electrophoresis:

1. D38028 and D38027 - 444 bp for WT allele, no product for KO allele.
2. D38028 and D38026 - 576 bp for neo insert in exon-2.

### Screening double KO mice

This strategy may be used to detect the IL-4 alleles in double KO, as the amplification of the disrupted allele depends on the presence of the *neo* cassette in exon-2 of IL-4, and not simply the random presence of the *neo* cassette, as in the strategy for LDL receptors.

### Reference

Kuhn et al Science (1991) 254:707-710

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