

Reaction conditions for TNFR1I PCR

| <u>Component</u> | <u>vol/rxn.</u> |
|----------------------------|---|
| distilled H ₂ O | 30 μ L |
| 5X buffer, pH 9.5 | 10 |
| 2 mM dNTP's | 2 |
| KM oligo #888 | 2 |
| KM oligo #889 | 2 |
| KM oligo #890 | 2 |
| genomic DNA | 2 (from 100 μ l ear buffer preparation) |
| Taq pol | 0.5 |

Add one 2.5 mM Mg 'Hotwax' bead

Cycles: 94°C 1min / 55° C 1min / 72° C 1 min 35X then 72° C 7', 4° C hold

Gel: 2% Nusieve agarose in TBE

expected rxn products:

| <u>+/+ wild-type mouse</u> | <u>KO/+ heterozygous mouse</u> | <u>KO/KO mutant mouse</u> |
|----------------------------|--------------------------------|---------------------------|
| single band: 275 bp | two bands: 160 bp 275 bp | single band: 160 bp |

Oligos

KM #888: 5'-CCGGTGGATGTGGAATGTGTG-3'

K-M #889: 5'-AGAGCTCCAGGCACAAGGGC-3'

K-M #890 (FASET-2): 5'-AACGGGCCAGACCTCGGGT-3'