

Reaction conditions for TNFRI PCR

<u>Component</u>	<u>vol/rxn.</u>
distilled H ₂ O	28 μ L
5X buffer, pH 9.5	10
2 mM dNTP's	2
KM oligo #885	2
KM oligo #886	2
KM oligo #887	2
KM oligo #888	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: 120 bp	two bands: 120 bp 155 bp	single band: 155 bp

Oligos

KM #885: 5'-GGATTGTCACGGTGCCGTTGA-3'

K-M #886: 5'-TGACAAGGACACGGTGTGTGGC -3'

K-M #887: 5'-TGCTGATGGGGATACATCCATC -3'

K-M #888: 5'-CCGGTGGATGTGGAATGTGTG-3'