

Rxn conditions for RAG-2 KO PCR

<u>Component</u>	<u>vol/rxn.</u>
Distilled H ₂ O	18 μ L
5X buffer, pH 9.5	10
2 mM dNTP's	5
oligo #879	5
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genomic DNA	2
Taq pol	0.5

Add one 3.5 mM 'Hotwax' bead

Cycles (PCR 9700): 94°C 45''/ 60° C 45''/ 72° C 45''//35X then 72° C 7', 4° C hold

Gel: 2% Nusieve agarose in TBE

expected rxn products:

+/+ wild-type mouse
single band: 1.11 kbp

KO/+ heterozygous mouse
two bands: 1.11kbp
0.97kbp

KO/KO mutant mouse
single band: 0.97 kbp

Oligos

Neo-3' CCA ACG CTA TGT CCT GAT AGC GGT-3'

RAG2-1 TTA ATT CAA CCA GGC TTC TCA CTT-3'

RAG2-3 GCC TGC TTA TTG TCT CCT GGT ATG-3'

