

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	30308
MMRRC Strain Name	C.129S6(FVB)- <i>Il7^{tm1Hms}</i> /Mmnc
Gene Name(s)	Interleukin 7 (Il7)
Breeding Protocol(s)	Sib-mating Backcross to Balb/c
Protocol Date	10/7/13

MMRRC #30308 PCR Reaction

NOTE: Run two, 2 primer reactions for each DNA sample: primer sets are 799&23 (WT); Neo3&23(KO). **Combine PCR product and run ~15ul per sample.**

Denaturing: 94°C for 30 sec
 Annealing Temp.: 55°C for 45 sec
 Extension: 72°C for 1min
 Cycles: 30

	1X
DNA	1.0
10X Buffer	2.5
10mM dNTPs	1.0
20 uM Primer F&R	2.0
Taq	1.0
ddH ₂ O	<u>16.0</u>
25mM MgCl ₂	2.0

Taq: **Apex and Chromataq Buffer**

Bands: WT: 200bp
 KO: 700bp

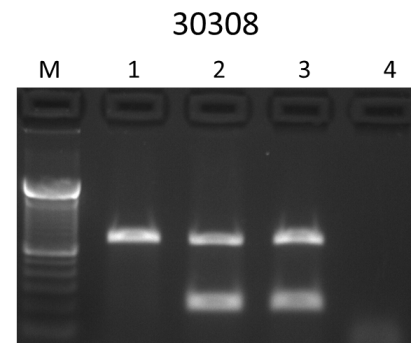
Primer sequences 5' to 3': Primers are 10uM with respect to each primer (20uM total).

Primer 799: AAT CCC ACT GAA ACA ACC

Primer 23: CAA CAC GTA CCA ATT CAT CG

Neo3': GGT ATC GCC GCT CCC GAT TCG CAG

Run on 2% agarose gel in TAE.



Lane 1: WT; Lane 2, 3: Het; Lane 4: H₂O
 M: 100 bp DNA ladder (Invitrogen)