

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	31041
MMRRC Strain Name	C57BL/6J-Tg(Cd4-Il7r)1Wjl/Mmnc
Gene Name(s)	CD4 antigen (Cd4) Interleukin 7 receptor (Il7r)
Breeding Protocol(s)	Sib-Mated (-/- mice are not viable)
Protocol Date	11/4/13

MMRRC# 31041 PCR - Phusion Protocol

NOTE: Dilute DNA 1:5 in nuclease free H₂O before adding to reaction! Set up 1, two primer reaction.

Initial: 94°C for 5 min
 Denaturing: 94°C for 45 sec
 Annealing Temp.: 58°C for 45 sec
 Extension: 72°C for 60 sec
 Cycles: 38

	<u>1X</u>
DNA	1.0
5X Phusion Buffer HF	4.0
10mM dNTPs	1.0
20 uM Primer 1 & 2	1.0
Phusion Taq	0.2
ddH ₂ O	12.7
DMSO	0.6

Taq: **NEB Phusion Poly. Kit**

Bands: MUTANT: 1.4kb

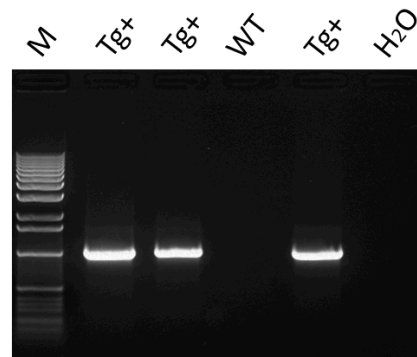
Primer sequences 5' to 3': Primers are 10uM with respect to each primer (20uM total).

Ex2+: 5'-CTC AGA TTC CCA ACC AAC AAG-3'

IL7R2-: 5' GGA AGA TCA TTG GGC AGA AA 3'

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Run on 1% agarose gel in TAE.



M: 1Kb DNA ladder (Invitrogen)