

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	15980
<b>MMRRC Strain Name</b>	B6;129S-Crlf2 <sup>tm1Wjl</sup> /Mmnc
<b>Gene Name(s)</b>	Cytokine receptor-like factor 2 (Crlf2)
<b>Breeding Protocol(s)</b>	Sib-mating
<b>Protocol Date</b>	8/19/13

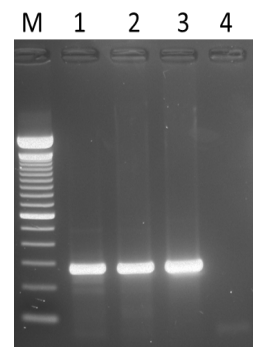
### MMRRC #15980 PCR Reaction

	<u>1X</u>
ddH <sub>2</sub> O	13
5X Buffer	5.0
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10 μM Primer F	1
10 μM Primer R	1
Taq	0.5
DNA	2

**Thermal Cycler:**

Step 1: 94°C for 5 min  
 Step 2: 94°C for 30 sec  
 Step 3: 58°C for 45 sec  
 Step 4: 72°C for 45 sec  
 Step 5: 35x from step 2 to step 4  
 Step 6: 72°C for 7 min

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Primers: mTSLPR1 + P3

Lane 1, 2: Het; Lane 3: WT; Lane 4: H<sub>2</sub>O  
 M: 100 bp DNA ladder Invitrogen

**Taq: Apex and Chromataq 5X Buffer**

**Primer sequences 5' to 3':** Primers are 10 μM with respect to each primer.

mTSLPR1: AGACTTTACCTGATTCCCTGCCTTG

P3: AACCTCTCCCACAAGAAGTCCAGAAG

mTSLPR Neo: ATCGCCTTCTATCGCCTTCTT

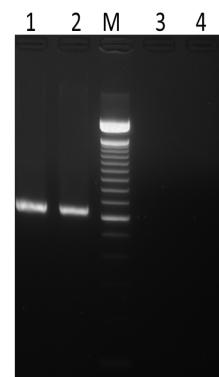
**Bands expected:**

(mTSLPR1 + P3) WT: 279

(P3 + mTSLPR Neo) MUTANT: ~650 bp

Run on 2% agarose gel in TAE.

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Primers: P3 + mTSLPR Neo

Lane 1, 2: Het; Lane 3: WT; Lane 4: H<sub>2</sub>O  
 M: 100 bp DNA ladder Invitrogen