

## MMRRC UNC – Genotyping Protocol

<b>MMRRC Strain ID</b>	36918
<b>MMRRC Strain Name</b>	MRL.129P2(Cg)- <i>Igkj1</i> <sup>tm1(IgkHyHEL10)Mnz</sup> /Mmnc
<b>Gene Name(s)</b>	immunoglobulin kappa joining 1 (Igkj1)
<b>Breeding Protocol(s)</b>	Sib-mating
<b>Protocol Date</b>	9/23/13

### MMRRC #36918 PCR Reaction

**Thermal Cycler:**

Step 1: 95°C for 5min  
 Step 2: 95°C for 60 sec  
 Step 3: 60°C for 60 sec  
 Step 4: 72°C for 90 sec  
 Step 5: Step 2 to 4; Cycles: 35  
 Step 6: 72°C for 7 min

	<b>1X</b>
ddH <sub>2</sub> O	13
5X Buffer	5
25mM MgCl <sub>2</sub>	2
10mM dNTPs	0.5
10 μM Forward Primer	1
10 μM Reverse Primer	1
Taq	0.5
DNA	2

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

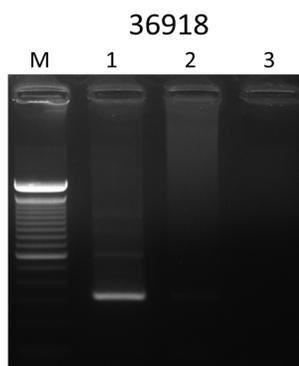
**Primer sequences 5' to 3':** Primers are 10 μM with respect to each primer.

Hel 3 (36918): ACG TTT TAT TTC CAG CTT GGT CCC  
 Hel 5 (36918): TTG TGC TAA CTC AGT CTC CAG CCA C  
 MJK1 (36918): GCA CCA AGC TGG AAA TCA ATC  
 MJK2 (36918): CTG CTT CTT TGA GCA TTT CTG

**Bands expected:**

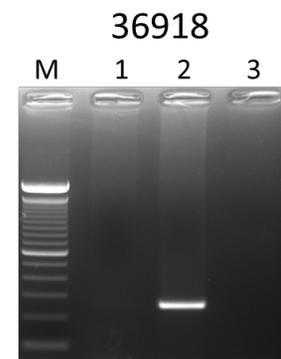
Primers: Hel 3 + Hel 5, MUTANT: 300 bp  
 Primers: MJK1 + MJK2, WT: 250 bp

Run on 2.0% agarose gel in TAE.



Primers: Hel 3 + Hel

Lane 1: Homo; Lane 2: WT; Lane 3: H<sub>2</sub>O;  
 M: 100 bp DNA ladder (Invitrogen)



36918  
Primers: MJK1 + MJK2

Lane 1: Homo; Lane 2: WT; Lane 3: H<sub>2</sub>O;  
 M: 100 bp DNA ladder (Invitrogen)