

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	36917
MMRRC Strain Name	MRL.B6-Igk-J ^{tm1Mwg} /Mmnc
Gene Name(s)	immunoglobulin kappa chain, joining region (Igk-J)
Breeding Protocol(s)	Sib-mating
Protocol Date	9/23/2013

MMRRC #36917 PCR Reaction

Thermal Cycler:

Step 1: 95°C for 5min
 Step 2: 95°C for 60 sec
 Step 3: 55°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

	1X
ddH ₂ O	13
5X Buffer	5
25mM MgCl ₂	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.

VK4-MW119 (36917): TCT CTC TAC GTT GCT TTT TTT CCA G

VK4-MW161 (36917): CCC CGA GCC GAA CGT GAA CGG

Jk3-5 (36917): CTG TAA ATC ACA TTC AGT GAT GG

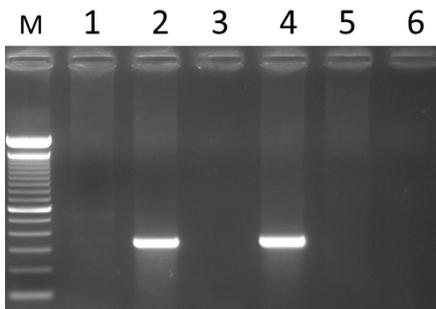
JK4-Rev (36917): CCG AGC CGA ACG TGA ATC ACA

Bands expected: Primers: (VK4-MW119 + VK4-MW161) MUTANT: 350 bp

Primers: (Jk3-5 + JK4-Rev) WT: 347 bp

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Run on 2.0% agarose gel in TAE.



Lane 1 to 3: Primers: JK3-5 + JK4; Lane 4 to 6: Primers: VK4119 + VK4161

Lane 1, 4: Homo; Lane 2, 5: WT; Lane 3, 6: H₂O; M: 100 bp DNA ladder (Invitrogen)

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