

MMRRC UNC – Genotyping Protocol

MMRRC Strain ID	209
MMRRC Strain Name	B6.129S1-Kcnj10 ^{tm1Lst} /Mmnc
Gene Name(s)	Potassium inwardly-rectifying channel, subfamily J, member 10 (Kcnj10)
Breeding Protocol(s)	Intra-strain mating or Backcross to C57BL/6NTac
Protocol Date	8/12/13

MMRRC #209 PCR Reaction

	<u>1X</u>
ddH ₂ O	12
5X Buffer	4
25mM MgCl ₂	2
10mM dNTPs	0.5
10 uM KIR4.1FWD	1
10 uM KIR4.1REV	1
10 uM KIR4.1NEO	1
Taq	0.5
DNA	2

Thermal Cycler:

Step 1: 94^oC for 5 min
 Step 2: 94^oC for 45 sec
 Step 3: 65^oC for 1 min
 Step 4: 72^oC for 1 min
 Step 5: Step 2 to 4; Cycles: 35
 Step 6: 72^oC for 7 min

Taq: Apex and Chromataq 5X Buffer

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

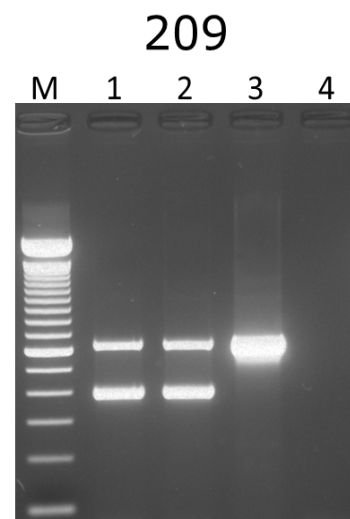
KIR4.1FWD: TGG ACG ACC TTC ATT GAC ATG CAG TGG

KIR4.1REV: CTT TCA AGG GGC TGG TCT CAT CTA CCA CAT

KIR4.1NEO: GAT TCG CAG CGC ATC GCC TTC TAT C

Expected band: WT ~634bp
 Mut ~ 383 bp

Run on 2% agarose gel.



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