

MMRRC UNC – Genotyping Protocol

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| MMRRC Strain ID | 36919 |
| MMRRC Strain Name | MRL.129(Cg)- <i>Igkc</i> ^{tm1(IgkC)Mnz} /Mmnc |
| Gene Name(s) | immunoglobulin kappa constant (Igkc) |
| Breeding Protocol(s) | Sib-mating |
| Protocol Date | 9/23/13 |

MMRRC #36919 PCR Reaction

Thermal Cycler:

Step 1: 95°C for 5 min
 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

| | <u>1X</u> |
|------------------------|------------------|
| 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.

Hck F (36919): ATA AAC TGG TCT GAA TCT CTG TCT G

Hck R (36919): GAG TTA CCC GAT TGG AGG GCG TTA

MK-Toe-F (36919): CAG TGA ACG ACA AAA TGG CG

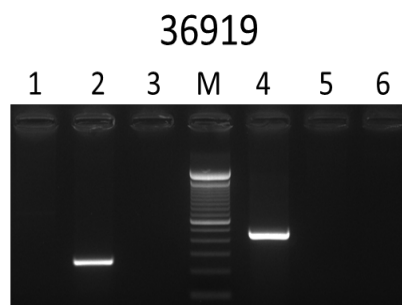
MK-Toe-R (36919): CCT CCA AGA CCT TAG AAG G

Bands expected:

(HckF + HckR) MUTANT: 450 bp

(MK-Toe-F + MK-Toe-FR) WT: 261 bp

Run on 2.0% agarose gel in TAE.



Lane 1 to 3: Primers: MK-F + MK-R; Lane 4 to 6: Primers: HckF + HckR

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