GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: NaOH _____ Proteinase K _____ Other__Any___

Protocol: **NAME OF PCR:** MMRRC #30753, C57BL/6J-*Tlr7*^{rsq2} (Rsq2)

Reagent/ Constituent	Volume (uL)
DNA Sample	0.5 (50-100ng/ul)
10x Buffer (contains 15mM MgCl2)	2.5
dNTPs (stock concentration is 25mM)	0.5
Primer **1** (stock concentration is 20 uM)	0.5
Primer **2** (stock concentration is 20 uM)	0.5
Primer **3** (stock concentration is 20 uM)	
Primer **4** (stock concentration is 20 uM)	
Taq Polymerase	0.5
Additives if applicable:	
TOTAL VOLUME OF REACTION:	**25 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): lab uses JumpStart®REDTaq®ReadyMix® (P1107-Sigma) 12.5 ul in a 25 ul reaction (includes Taq, buffer, dNTPs)

Strategy:

Steps	Temp (^{o}C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [x]	94	2	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	94	0.25	35
3. Annealing	56	0.50	35
4. Elongation	72	1	35
5. Amplification (i.e., 72^{o}C, 10 min)	72	10	**1**
6. Finish (i.e., 4^{o}C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: Rsq2(F)	AGCACTCTTCGCAGCAACTAATATGTAA
2: Rsq2 (R)	CCAACTCAACAAGGTTGGGAAGAAAATG
3: Rsq2_seq(F)	GTGGACTCTCTGACTTAAAAGCC
4: Rsq2_seq(R)	ATGCAAAAATTTGGCCTCCTC

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	1.91	
(i.e. 3&4)		
(i.e. 1&2&3)		

PASTE GEL PICTURE HERE, CLEARLY INDICATING LADDER, WATER CONTROL, DNA CONTROL, & DIAGNOSTIC SAMPLES

Rsq2 genotyping is performed by amplifying the region containing sequencing of the amplified region to detect the single nucleotide as for *rsq1* genotyping.

Primers for PCR amplification

Rsq1(F): 5'- AGCACTCTTCGCAGCAACTAATATGTAA -3'

Rsq1(R): 5'- CCAACTCAACAAGGTTGGGAAGAAAATG -3'

PCR program

1) 94°C	2:00
2) 94°C	0:15
3) 56°C	0:30
4) 72°C	1:00

5) repeat steps (2-4) 35X

6) 72°C	10:00
7) 4°C	∞

Primers for sequencing

Rsq2_seq(F): 5'- GTGGACTCTCTGACTTAAAAGCC -3'

Rsq2_seq(R): 5'- ATGCAAAAATTTGGCCTCCTC -3'

The following sequence of 1911 nucleotides (from Genbank genomic region **NC_000086** for linear DNA sequence of *Tlr7*) is amplified:

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21166                                                agcac tcttcgcagc

21181 aactaatatg taatgctgct tctgtatctt aaaaactgtt tggggcagca agagacatgg

21241 ctcagtgggt aagtatggtg atgtgaatgt gaatgacccc gtaggctcat gtttgaatac

21301 ttggttccca gtcagtggaa cttttggcaa ggattaggag gtgtggcttt gttgaaagag

21361 gtgtgccact agaggtaggc tgtgagattt caaaagtcca tgcccatccc agtctcattc

21421 cttctctgcc tacaatttgc agataacatg tgagctctca gcagctgctc cagtgtcatg

21481 cctacctgtc tgctaccatg tttcctgtca tgatatcata aactaaccct ctaaaactct

21541 aggcaagaac cagagcaaat gctttgtttt ctaaggtgtt gtggtcatag tggttcatca

21601 cagcaataga aacagtaatg agacagtaag tgtacttgtg gacaagactg atgacctgcg

21661 tttgatctgc aggatccaca aggtggaagg agataacaga ctctcacaag ttatcctctg

21721 accgccacaa tcacgtcatg gtatgtgcac actcactgac atatgcaaag catactcaca
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21781 caagacaaat aaataaatgt gaaaaaattc ttgaagtttg ttagaaagct aagatggtac

21841 aagcaaaaca taaaaccatt atcaaagtct tcagtggtta ataagtacag tcacagggac

21901 ggaggtgctg tttacactat tacaaacaag acctgtgttg tttagtttta ataatgtacc

21961 aaaagagagg aaataaatgg aacttctcaa tcattccttg atatatttta taacaataat

22021 ttttttctct cttttattta caggtgtttt cgatgtggac acggaagaga caaattttga

22081 tctttttaaa tatgctctta gtttctagag tctttgggtt tcgatggttt cctaaaactc

22141 taccttgtga agttaaagta aatatcccag aggcccatgt gatcgtggac tgcacagaca

22201 agcatttgac agaaatccct gagggcattc ccactaacac caccaatctt acccttacca

22261 tcaaccacat accaagcatc tctccagatt ccttccgtag gctgaaccat ctggaagaaa

22321 tcgatttaag atgcaattgt gtacctgttc tactggggtc caaagccaat gtgtgtacca

22381 agaggctgca gattagacct ggaagcttta gtggactctc tgacttaaaa gccctttacc

22441 tggatggaaa ccaacttctg gagataccac aggatctgcc atccagctta catcttctga

22501 gccttgaggc taacaacatc ttctccatca cgaaggagaa tctaacagaa ctggtcaaca

22561 ttgaaacact ctacctgggt caa**a**actgtt attatcgaaa tccttgcaat gtttcctatt

22621 ctattgaaaa agatgctttc ctagttatga gaaatttgaa ggttctctca ctaaaagata

22681 acaatgtcac agctgtcccc accactttgc cacctaattt actagagctc tatctttata

22741 acaatatcat taagaaaatc caagaaaatg attttaataa cctcaatgag ttgcaagttc

22801 ttgacctaag tggaaattgc cctcgatgtt ataatgtccc atatccgtgt acaccgtgtg

22861 aaaataattc ccccttacag atccatgaca atgctttcaa ttcattgaca gaattaaaag

22921 ttttacgttt acacagtaat tctcttcagc atgtgccccc aacatggttt aaaaacatga

22981 gaaacctcca ggaactagac ctctcccaaa actacttggc cagagaaatt gaggaggcca

23041 aatttttgca tttttcttccc aaccttgttg agttgg

PCR primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated A is shown in red text.