GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

Investigator/PI: Bruce Beutler, M.D. Address: N. Torrey Pines Rd., SP293 Contact: Xin Du
Telephone: 858-784-8610 FAX: 858-784-8444 email: bruce@scripps.edu

DNA Extraction Method: NaOH _____ Proteinase K __X___ Other_____

Protocol: **NAME OF PCR:** MMRRC Strain 30229-UCD, C57BL/6J-*Tlr7*^{m1Btlr}, (Rsq1)

Reagent/ Constituent	Volume (uL)
DNA Sample	1
10x Buffer (contains 15mM MgCl2)	5
dNTPs (stock concentration is 25mM)	0.4
Primer **1** (stock concentration is 20 uM)	1
Primer **2** (stock concentration is 20 uM)	1
Primer **3** (stock concentration is 20 uM)	
Primer **4** (stock concentration is 20 uM)	
Taq Polymerase	2.5
water	39.1
TOTAL VOLUME OF REACTION:	**50 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): **using primers 1 & 2 for amplification and 3 & 4 for sequencing.**

Strategy:

Steps	Temp (^{o}C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	94	2	**1**
2. Denaturation	94	15 seconds	30
3. Annealing } steps 2-3-4 will cycle in sequence	56	30 seconds	
4. Elongation	72	1 min	
5. Amplification (i.e., 72^{o}C, 10 min)	72	7	**1**
6. Finish (i.e., 4^{o}C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: Rsq1_typeF	AGCACTCTTCGCAGCAACTAATATGTAA-
2: Rsq1_typeR	CCAACTCAACAAGGTTGGGAAGAAAATG
3: Rsq1_seqF	TACTTGTGGACAAGACTGATGACC
4: Rsq1_seqR	TTTCCATCCAGGTAAAGGGC

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (bp)	genotype
(i.e. 1&2)	**1911**	
(i.e. 3&4)		
(i.e. 1&2&3)		

The flanking sequences of mutation sites:

Wt ACACCACCAATCT
Rsq1 ACACCATCAATCT