

GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

Investigator/PI: Dr. Bruce Beutler Address: 1055 North Torrey Pines Rd, Mailstop: SP-293 Contact: Dr. Bruce Beutler

Telephone: 858-784-8610 email: bruce@scripps.edu

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 MgCl ₂)	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 (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [x]	94	2	1
2. Denaturation	94	0.25	35
3. Annealing	56	0.50	35
4. Elongation	72	1	35
5. Amplification (i.e., 72°C, 10 min)	72	10	1
6. Finish (i.e., 4°C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: Rsq2(F)	AGCACTCTTCGCAGCAACTAATATGTAA
2: Rsq2 (R)	CCAACCAACAAGGTTGGGAAGAAAATG
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.

21781 caagacaaat aaataaatgt gaaaaaattc ttgaagtttg ttagaaagct aagatggtac
21841 aagcaaaaca taaaaccatt atcaaagtct tcagtgggta ataagtacag tcacagggac
21901 ggaggtgctg tttacactat tacaacaag acctgtgttg tttagtttta ataatgtacc
21961 aaaagagagg aaataaatgg aacttctcaa tcattccttg atatatttta taacaataat
22021 ttttttctct cttttattta caggtgtttt cgatgtggac acggaagaga caaatTTTga
22081 tctttttaaa tatgctctta gtttctagag tctttggggt tcgatgggtt cctaaaactc
22141 taccttgtga agttaaagta aatatcccag aggcccatgt gatcgtggac tgcacagaca
22201 agcatttgac agaaatccct gagggcattc ccactaacac caccaatctt acccttacca
22261 tcaaccacat accaagcatc tctccagatt ccttccgtag gctgaacat 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 caaaactggtt attatcgaaa tccttgcaat gtttcttatt
22621 ctattgaaaa agatgctttc ctagttatga gaaatttgaa ggttctctca ctaaaagata
22681 acaatgtcac agctgtcccc accactttgc cacctaattt actagagctc tatctttata
22741 acaatatcat taagaaaatc caagaaaatg attttaataa cctcaatgag ttgcaagttc
22801 ttgacctaa gggaaattgc cctcgatggt ataatgtccc atatccgtgt acaccgtgtg
22861 aaaataattc cccttacag atccatgaca atgctttcaa ttcattgaca gaattaaaag
22921 ttttacgttt acacagtaat tctcttcagc atgtgcccc aacatggttt aaaaacatga
22981 gaaacctcca ggaactagac ctctcccaa actacttggc cagagaaatt gaggaggcca
23041 aatTTTtgc ttttcttccc aaccttggtg agttgg

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