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 Strain #31072-UCD,** C57BL/6J-*Ifnar2*^{m1Btlr} (Macro-2)

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	94	0.50	30
3. Annealing } steps 2-3-4 will cycle in sequence	56	0.50	30
4. Elongation	72	1	30
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: Macro-2 (F)	TTGATACCACAGCGGAAGGTGAGC
2: : Macro-2 (R)	AACCATAGGCGGGACACATTAACTG
3: Macro-2 _seq (F)	CCTCTCTGCTTAGAGGACAGATG
4: Macro-2 _seq (R)	CAAGGCCGTTTCCTGAATATG

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

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

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

Macro-2 genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide change.

Primers for PCR amplification

Macro-2(F): 5'- TTGATACCACAGCGGAAGGTGAGC -3'

Macro-2(R): 5'- AACCATAGGCGGGACACATTAACTG -3'

PCR program

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

5) repeat steps (2-4) 29X

6) 72°C	7:00
7) 4°C	8

Primers for sequencing

Macro2_seq(F): 5'- CCTCTCTGCTTAGAGGACAGATG -3'

Macro2_seq(R): 5'- CAAGGCCGTTTCCTGAATATG -3'

The following sequence of 1002 nucleotides (from Genbank genomic region **NC_000082** for linear DNA sequence of *Ifnar2*) is amplified:

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10580                    t tgataccaca gcggaaggtg agctaagcaa gtcccaggga

10621 tcaaggaagg actgaaacca gaatttggct acttttaat ttatttttaa agacaggtag

10681 ctcctctagc tggcttcaaa cttgctatgt agccgaggat gaccttgaat gcctgacctt

10741 tcccccacct ctctgcttag aggacagatg tgacacgcac agtaaactca tgcaagttta

10801 accctaatcc taaccaatcc agggctacca cggggccgca tctgcagcta aatctggctc

10861 gttcttactc gtctctcgtt agcgtgtatg tgtctatcat gtaaattaca atataattgg

10921 gtgcttctga gttttgacca actcaatatt gatctctttc aggtgtgaga gcagaaaaac
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10981 ggacttaaga gctgagcagg **a**tgcgttcac ggtgcaccgt ctctgccgtc ggtctcctca

11041 gcttgtgtct tgtgggtaag ggctacttct cagcacagcc cttagaggag aaagcctctg

11101 tttctgtcat cacagagagc cctggtgtgg agcagcacac tgatgtccat atctggagaa

11161 cccagatcag cacggccagc atcaggcacc ccacgggggt cttcccttc attttagcta

11221 agccagaata atataggcta cagccatatt caggaaacgg ccttgtttat aattcaaagg

11281 gttgcggctc tgcacaccct gaatctcacg cccggtggcg tttagaaggt ggccatccct

11341 ttatctcttc ccatataaac taacttgaaa aatccatccc tacacattga tttatactct

11401 tcctttcttt ttagccctta tcttttctat atctgtattt cttcacgtcc tttctgttcc

11461 ttcctctgaa ctgtttgaat tccacaatgt catctctccc attcattgtc ctcagcggat

11521 ccaggagtat gacaaatgtc tcagtgtgga catccacagt taatgtgtcc cgcctatggt

11581 t

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