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: C57BL/6J-*Ifnar1*^{m1Btlr}/Mmcd (*macro-1*), MMRRC Strain #030878-UCD**

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*:** *Macro-1* 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. Use primers 1 & 2 for amplification and 3 & 4 for sequencing. The 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	0.50	30
3. Annealing	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:

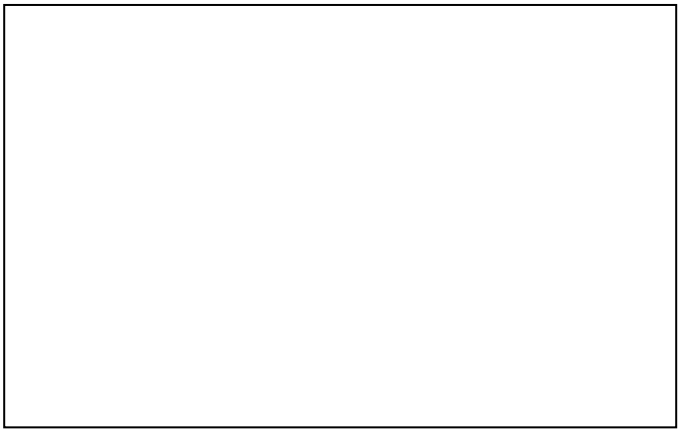
Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: Macro-1 (F)	AGAACAGCTTGCCACTTCACTGG
2: Macro-1 (R)	GCAGAGAAGCCTTAGCCTTAGAAGAAC
3: Macro-1 _seq(F)	CCCAGGGTAGCTTCAAACTTATG
4: Macro-1 _seq(R)	TCACAAAGTTCCTGGGTAGC

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	1.72	
(i.e. 3&4)		
(i.e. 1&2&3)		



The following sequence of 1717 nucleotides (from Genbank genomic region **NC_000082** for linear DNA sequence of *Ifnar1*) is amplified:

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13927       agaa cagcttgcca cttcactggg aattccccac acactctaga gcacccgtgg
13981 ccttcctttg ttggggatct gtgtctcctc cattgtacca ggagttccct aagtggggaa
14041 actgccctca ggtccacagt gcttactaca gtgtcttagg tattatagag cctcagtaca
14101 tagtgaatta attcatgatt ggattggatt ttgtttttgc tttctgtttt caaagtggct
14161 attcaaaaag cagttctgga agccgttcag ataaatggaa accaatacca acctgtgcaa
14221 atgtccagac tacgcactgt gtcttttctc aagatactgt ctacacagga acgttctttc
14281 tccatgtaca agcctcagag ggaaatcaca catccttttg gtctgaagag aagtttattg
14341 attctcaaaa acacagtaag ccgagttttc tttgagacag tctgacactg tagcccaggc
14401 tggcctggaa ctcacggtgt agcccagggt agcttcaaac ttatggcagt cctcctgcct
14461 gagctcctga gagctgaggt tgtgggtgtg gtccatgcct gctgtatagc aagcgctttc
14521 tggagtgtaa ttcctcatgt agggcgagtc ccggaaggtt gtttgaaggt gtcttagtgt
14581 gcaatttctg tttgcattct tccccagttc tccctcctcc tccggtcatt actgtcaccg
14641 ccatgagtga caccttgctt gtttatgtca actgtcagga cagcacatgt gatggactca
14701 attacgaaat catcttttgg gaaaacactt ccaatactaa ggtaaaaagc tacccaggaa
14761 ctttgtgact tagcctcata ccggtgatga tgggaaagaa agttagtggg ggagggaggg
14821 caagagcaag cagtcacagc tctaaggttt gggaggcctt ttaatcttga tggtggcctg
14881 tctgcagtag agcgaatgtc agcctttcc atggtctgca tgacagtaca ggtcgcctgc
14941 atctgggcct gacttttctg tgtgtgaata tacaggagtg tgtgcaagca tgtgtgtgtg
15001 tgtgtgtgtg tgtgtgtgtg tgtgtgtgtg tgtgtgtgtg actgtgcatg agtatgtgaa
15061 tgtatgtatg tgtgaatata tgtgtgtgta tgtgtgaatg tacgtgtgtg tgtatgagtg
15121 tatgtgtgtg tgtgagtgta tgtgtgaatg tgtgggagga tgtgtgtgtg tgtgtgtgtg
15181 tgtgtgtgtg tgagtgactc tccgtgagta tgtgagtata tgtatgtgaa tctgtgtgaa
15241 tgtgtgtgag gatgtgagtg taagtgtgtg tgtgtgtgtg tgaatgactt tgcatgagta
15301 tgtgtgtgta tatatgtgtg aatgtacgtg tttgtgtgtg tgtgtgtgtg tgtgtgtgtg
15361 tgtgaggatg tgaatgtgcg tgtgcgcgtg tgtgttgaca attgcttatg tactatatgt
15421 tcatcacagg acactcatgg cggccagacg ataagctgtg gaagtgagct ctctctcctg
15481 ccttataact cccggggatt gaattaagtc atcaggtttt gcagcaagca tccttatgta
15541 ctaggccatc ttgattttca actatatctt gagcctccta ctgctgaatt agaaccatta
15601 ttacactttc ccaagtgttc ttctaaggct aaggcttctc tgc
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PCR primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated A is shown in red text.