GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: NaOH _____ Proteinase K __X___ Other_____

Protocol: **NAME OF PCR: C57BL/6J-*Rag1*^{m1Btlr}/Mmcd (*maladaptive*), MMRRC #031719-UCD**

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
Taq Polymerase	2.5
water	39.1
TOTAL VOLUME OF REACTION:	**50 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): *Maladaptive* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide transversion. Use primers 1 & 2 for PCR amplification and primers 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 } steps 2-3-4 will cycle in sequence	94	30 seconds	
3. Annealing	56	30 seconds	30
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: Rag1_maladaptive_F	TCTTCAGGGGCACTGGATACGATG
2: Rag1_maladaptive_R	TCAATGCCCAAAGGGTCCCCTAAG
3: Rag1_maladaptive_seqF	AAGCTTCTGGCTCAGTCTAC
4: Rag1_maladaptive_seqR	TACAGCCAGTGATGTTTCAGGAC

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (bp)	genotype
(i.e. 1&2)	**985**	
(i.e. 3&4)		
(i.e. 1&2&3)		

Mutation site (red) and flanking sequence:

WT ggacctttacctgaagatg

maladaptive ggacctttaactgaagatg