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-*Tlr9*^{m4Btlr}/Mmcd (*CpG5*), MMRRC #030344-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
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): *CpG5* 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. The same primers are used for PCR amplification and 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	60	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:CpG5_typeF	AACTCTTCCTGGTTCCAAGGTCTG
2:CpG5_typeR	GGGTATGAATGTCATTGTGTGCC
3:	
4:	

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (bp)	genotype
(i.e. 1&2)	**850**	
(i.e. 3&4)		
(i.e. 1&2&3)		

The flanking sequences of mutation sites:

Wt CCAAACTCCACAC
CpG5 **CCAAACCCCACAC**