GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

2795 2nd Street, Suite 400, Davis, CA 95618
mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: C57BL/6J-*Tlr9*^{CpG2}/Mmcd, (*CpG2*) **MMRRC #** 030020-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
Water	37.0
10x Buffer Sigma Red Taq Buffer	5.0
dNTPs (stock concentration is 25mM) (D-7295- Sigma)	2.5
Primer 1 (stock concentration is 20µM) Tlr9-CpG2 PCR F	1.0
Primer 2 (stock concentration is 20µM) Tlr9-CpG2 PCR R	1.0
JumpStart REDAccuTaq LA DNA Polymerase (D-1313- Sigma)	2.5
gDNA template (50-100ng/µl) extracted with ☐ NaOH ☐ Proteinase K ☒ Other: Any	1.0
TOTAL VOLUME OF REACTION:	**50µL**

Comments on protocol:

- *CpG2* 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.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐		94	2:00	**1**
2. Denaturation		94	0:30	
3. Annealing	steps 2-3-4 will cycle in sequence	55	0:30	**40x**
4. Elongation		72	1:00	
5. Amplification		72	7:00	**1**
6. Finish		4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: Tlr9-CpG2 PCR F	TAA AGG CCC TGA CCA ATG GCA C
2: Tlr9-CpG2 PCR R	GGC AGA GAA TGA ACT CCA GTC CTG
3: Tlr9-CpG2_Seq F	GGA GCC GCA AGA CTC TAT TTG
4: Tlr9-CpG2_Seq R	TCA CTC TCC TGA AAG ATG CAT GG

Electrophoresis Protocol:

Agarose: 2% **mV:** 80 **Estimated Running Time:** 90 **min**

Primer Combination	Band	Genotype
1 and 2	1268 bp	*CpG2*
SNP found at position ~ 167 of sequencing		

Mutation site (red) and flanking sequence:

WT gactgcgcc**A**gcgtctctg
CpG2 gactgcgcc**T**gcgtctctg