GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: NaOH _____ Proteinase K _____ Other VIAGEN Direct PCR (tail)

Protocol: **NAME OF PCR:** MMRRC Strain #031076-UCD, B6;129-OoepGt(139A2-3)Cmhd

Reagent/ Constituent	Volume (uL)
GE Healthcare Ready-To-Go-Beads (Cat. #27-9557-01)	
Primer **1** (stock concentration is 20 pmol/ul)	0.5
Primer **2** (stock concentration is 20 pmol/ul)	0.5
Primer **3** (stock concentration is 20 pmol/ul)	0.5
Genomic DNA	0.5—1.0 ul
dH_2O	Make up difference
TOTAL VOLUME OF REACTION:	**25 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): _____

Strategy:

Steps	Temp (^oC)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	95	3	**1**
2. Denaturation	94	0.5	
3. Annealing } steps 2-3-4 will cycle in sequence	55	0.5	} 30
4. Elongation	68	1.0	
5. Amplification (i.e., 72^{o}C, 10 min)			**1**
6. Finish (i.e., 4^{o}C, indefinite)		n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5^- - 3^-)
1: p1-5'	**CCC TGC TGA CAG TGG ACT C**
2: p2-5'	**CCA GCC AGT TTT AGC CCT TT**
3: p3-5'	**TGC GCA ACT GTT GGG AAG**
4:	

Electrophoresis Protocol:

% Agarose: 0.8 **V : 95**

Estimated Running Time (min): 20 min

Primer combination	Band (kB)	genotype
1 & 2 & 3	512 (bp)	Normal
1 & 2 & 3	890 (bp)	Null

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