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:___CRE_____**

Reagent/ Constituent	Volume (uL)
DNA Sample	**5.0**
10x Buffer	**5.0**
dNTPs (stock concentration is 10mM)	**1.0**
Primer 1 (stock concentration is 5 uM) working stock is 0.5ug/uL	**0.5**
Primer 2 (stock concentration is 5 uM) working stock is 0.5ug/uL	**0.5**
50mM MgCl2	**1.5**
H2O	**36.25**
Taq Polymerase	**0.25**
Additives if applicable:	
TOTAL VOLUME OF REACTION:	**50 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): **We usually make the PCR mix then add the TAQ before we add the mix to the PCR tube. We usually add 5uL of our DNA to each PCR tube and 45uL of the PCR mix for a total reaction volume of 50ul per sample.**

Strategy:

Steps	Temp (^{o}C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	**94**	**5:00 min**	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	**94**	**0:30 sec**	
3. Annealing	**58.7**	**0:30 sec**	**35x**
4. Elongation	**72**	**0:30 sec**	
5. Amplification (i.e., 72^{o}C, 10 min)	**72**	**2:00 min**	**1**
6. Finish (i.e., 4^{o}C, indefinite)	**4**	**n/a**	**n/a**

Primers:

Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: CRE 1	**GATGGATTTCCGTCTCTGGTGTAG**
2: CRE 2	**AGCTTGCATGATCTCCGGTATTG**
3:	
4:	

Electrophoresis Protocol:

% Agarose: 1% **V : 150**

Estimated Running Time (min): ~45-60 min

Primer combination	Band (kB)	genotype
(i.e. 1&2)	**268**	**TG/+**

In the gel picture, # 9 is negative, #10-14 are positive, and #15 is undertermined.