GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

Investigator/PI: _Lawrence A. Tabak____ Address: __NIH/NIDCR_____ Contact: __Timothy A. Fritz___

Telephone: __301-451-6319_____ FAX: __301-480-4214_____ email:_tim.fritz@nih.gov_____

DNA Extraction Method: NaOH _____ Proteinase K _____ Other_:Qiagen: DNAeasy Tissue kit

Protocol: **NAME OF PCR:_____T5 genotyping (Strain 29583)_____**

Reagent/ Constituent	Volume (uL)
DNA Sample (10-15ng/ul)	15
10x Buffer (contains 15mM MgCl2)	2.5
dNTPs (stock concentration is 25mM)	1
Primer **1** (stock concentration is 20 uM)	0.5 or 0
Primer **2** (stock concentration is 20 uM)	0.5 or 0
Primer **3** (stock concentration is 20 uM)	0 or 0.5
Primer **4** (stock concentration is 20 uM)	0 or 0.5
Taq Polymerase	0.125
H2O	4.875
Additives if applicable: DMSO	0.5
TOTAL VOLUME OF REACTION:	**25 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): ____Use primers 1 & 2, and 3 & 4 separately

Strategy:

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	94	5 min	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	94	30sec	35
3. Annealing	56	30sec	
4. Elongation	72	40sec	
5. Amplification (i.e., 72°C, 10 min)	72	1	**1**
6. Finish (i.e., 4°C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1:mT5-5'WT	**CGAGAAAGCAAGGTGTGGAGATAC**
2:mT5-3'WT	**TTAGGAGATTGACAGGCTGGGAG**
3:mT5-5'KO	**CATCGCATTGTCTGAGTAGGTGTC**
4:mT5-3'KO	**GCTTCTCCGAGTTTCCCTTTGC**

Electrophoresis Protocol:

% Agarose: ___2.0___ **V : ____70_____**

Estimated Running Time (min): ____15___

Primer combination	Band (kB)	genotype
1&2(i.e. 1&2)	0.63	WT
3&4(i.e. 3&4)	0.34	KO
(i.e. 1&2&3)