GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: Tissue lysis buffer with Proteinase K

Protocol: **NAME OF PCR:** MMRRC #030511-UCD, B6.129-*Chrna4*^{tm2Jbou}

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)	1.25
Primer **2** (stock concentration is 20 uM)	1.25
Primer **3** (stock concentration is 20 uM)	-
Primer **4** (stock concentration is 20 uM)	-
Taq Polymerase	0.25
Additives if applicable: "Q" solution from Qiagen, Inc. (for Hot Star Taq)	10
TOTAL VOLUME OF REACTION:	**50 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): Final Mg2+ concentration is 2.5mM

Strategy:

Steps	Temp (°C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START	95	15	**1**
2. Denaturation	95	0.5	
3. Annealing steps 2-3-4 will cycle in sequence	55	1.0	33 cycles total
4. Elongation	72	2.0	
5. Amplification (i.e., 72°C, 10 min)	72	5.0	**1**
6. Finish (i.e., 4°C, indefinite)	4	Indefinite	

Primers:

Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: A4loxP.1	CAG CCT GAA CAC TTC AAA GGA AGA (24-mer, T_m = 51° C)
2: A4loxP.2	AGG GTA CAT AGT GTA GTC TCT GCC (24-mer, T_m = 52° C)
3:	
4:	

Electrophoresis Protocol:

% Agarose: 1.5% **V :** 90 - 100

Estimated Running Time (min): 120 - 150

Primer combination	Band (kB)	genotype
(i.e. 1&2)	0.200	wild type allele
(i.e. 3&4)	0.240	targeted allele
(i.e. 1&2&3)