GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: NaOH_X_ Proteinase K _____ Other_____

Protocol: **NAME OF PCR:** MMRRC Strain #30513-UCD, B6.129X1-*Neurl*^{tm1Ynj}

Reagent/ Constituent	Volume (uL)
DNA Sample	0.5
10x Buffer (contains 15mM MgCl2)	2.5
dNTPs (stock concentration is 25mM)	0.5
Primer **1** (stock concentration is 20 uM)	1
Primer **2** (stock concentration is 20 uM)	1
Primer **3** (stock concentration is 20 uM)	1
Primer **4** (stock concentration is 20 uM)	
Taq Polymerase	0.2
Additives if applicable:	
TOTAL VOLUME OF REACTION:	**25 ul**

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

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Strategy:

Steps	Temp (ºC)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	94	3	**1**
2. Denaturation	94	1	30
3. Annealing } steps 2-3-4 will cycle in sequence	60	1	
4. Elongation	72	2	
5. Amplification (i.e., 72ºC, 10 min)	72	10	**1**
6. Finish (i.e., 4ºC, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5- - 3-)
1:Neun#1	**GAT TGG GCT GAA AGA GTT TCA GGC A**
2:NeuN#2	**GCT ACA TGT CTG CTA ACT CTC TCC A**
3:Neun#3	**CAT AGC GTT GGC TAC CCG TGA TAT T**
4:	

Electrophoresis Protocol:

% Agarose: _1.5%_____ V : _10ul_____

Estimated Running Time (min): _1hr_____

Primer combination	Band (kB)	genotype
#1 & #2	0.19	wildtype
#2 & #3	0.27	knockout
(i.e. 1&2&3)		

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