GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: **B6.129X1-*Gpd2*^{tm1Lbr}/Mmcd** **MMRRC #** **009980-UCD**

Protocol:

Reagent/ Constituent	Volume (µL)
Water	5.25
10x Buffer (contains 15mM $MgCl_2$)	1.0
dNTPs (stock concentration is 2mM)	1.0
Primer 1 (stock concentration is 20µM) InF	0.5
Primer 2 (stock concentration is 20µM) NeoR	0.25
Primer 3 (stock concentration is 20µM) E5R	0.5
Taq Polymerase (1Units/µL)	0.5
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**10µL**

Comments on protocol:

If the wild-type band does not PCR well, decrease the relative amount of the NeoR primer to below 50% that of the E5R. Alternatively, PCR may be performed separately for the two alleles. Times below are for a 10ul reaction, and may need lengthened for larger volumes.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	95	5:00	**1**
2. Denaturation	} steps 2-3-4 will cycle in sequence	94	0:20	} **30x**
3. Annealing		58	0:30	
4. Elongation		72	0:40	
5. Amplification		72	5:00	**1**
6. Finish		4	∞	n/a

Primers:

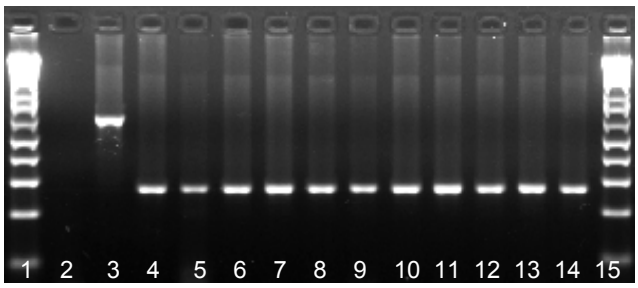
Primer Name	Nucleotide Sequence (5' - 3')
1: InF	CGT TTC TCT TCA GCA TCC GTG A
2: NeoR	TTC CTG ACT AGG GGA GGA GTA GAA G
3: E5R	GAA GGG CTT CTT TCA CCA TCC T

Electrophoresis Protocol:

% Agarose: 2% in TAE **V:** 100V/60-80mA

Estimated Running Time (min): 30-60

Expected Bands	Genotype
672 bp	Wild-type +/+
269 bp	KO -/-



Lanes:
1. 1Kb+ ladder
2. H_2O
3. B6 wild-type +/+ control
4. Homozygous Mutant control -/-
5-14. samples (Hom -/-)
15. Ladder