GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: B6;129S5-*Gpr56*^{tm1Lex}/Mmcd **MMRRC #** 032341-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
Water	10.775
10x Buffer (15mM $MgCl_2$)	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M)	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO	0.325
Primer 1 (stock concentration is 20µM) DNA085-5	0.5
Primer 2 (stock concentration is 20µM) DNA085-14	0.5
Primer 3 (stock concentration is 20µM) Neo3a	0.5
Taq Polymerase 5Units/µL	0.2
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? CHECK HERE ☐	94	5:00	**1**
2. Denaturation		94	0:15	
3. Annealing	steps 2-3-4 will cycle in sequence	65 to 55 (↓1°C/cycle)	0:30	**40x**
4. Elongation		72	0:40	
5. Amplification		72	5:00	**1**
6. Finish		4	∞	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: DNA085-5	CGA GAA GAC TTC CGC TTC TG
2: DNA085-14	AAA GTA GCT AAG ATG CTC TCC
3: Neo3a	GCA GCG CAT CGCCTT CTA TC

Electrophoresis Protocol:

% Agarose: 1.5 **V:** 90

Estimated Running Time (min): 90

Primers	Expected Bands	Genotype
1 and 2	639 bp	Wild-type
2 and 3	369 bp	Mutant