GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: 129S6/SvEvTac5-*Mal*^{tm1Lex}/Mmcd **MMRRC #** 032460-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
Water	10.275
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) DNA312-1	0.5
Primer 2 (stock concentration is 20µM) DNA312-2	0.5
Primer 3 (stock concentration is 20µM) UNQ5407-R	0.5
Primer 4 (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: DNA312-1	TCT CGG TCT TCA CCA CCT TCC
2: DNA312-2	AGG ACT CTC AGT GGT ACC TTG
3: UNQ5407-R	GCT GGG TTT GTT TAC TCA GGA GGA CT
4: 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	315 bp	Wild-type
3 and 4	444 bp	Mutant