GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: C57BL6/J-*Ticam2*^{tm1Btlr}/Mmcd **MMRRC #** 030018-UCD

Protocol:

Reagent/ Constituent	Volume (µL)
Water	11.275
10x Buffer (contains / without 15mM $MgCl_2$)	2.5
$MgCl_2$ (stock concentration is 25mM)	1.7
Betaine (stock concentration is 5M) *Optional*	6.5
dNTPs (stock concentration is 10mM)	0.5
DMSO *Optional*	0.325
Primer 1 (stock concentration is 20µM) 30018-wtF	0.5
Primer 2 (stock concentration is 20µM) 30018-wtR	0.5
Primer 3 (stock concentration is 20µM) 30018-neoF	0.5
Primer 4 (stock concentration is 20µM) 30018-neoR	0.5
Taq Polymerase 5Units/µL	0.2
DNA extracted with ☐ NaOH ☐ Proteinase K ☒ Other: Qiagen	1.0
TOTAL VOLUME OF REACTION:	**25µL**

Comments on protocol:

- Run as simplex reactions
- Use Touch-Down cycling protocol-first 10 cycles anneal at 65° C decreasing in temperature by 1.0 ° C; next 30 cycles anneal at 55° C.
- Betaine and DMSO have been standardized due to high GC content. Protocol may be tested without. Also, may adjust $MgCl_2$ to increase reaction or decrease non specific amplifications.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START? ☐		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	**10x**
4. Elongation		72	0:40	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 will cycle in sequence	55	0:30	**30x**
7. Elongation		72	0:40	
8. Amplification		72	5:00	**1**
9. Finish		15	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1: 30018-wtF	TCA AGA CGG CCA TGA GTC AGA CTC CAA G
2: 30018-wtR	ATG GTT TGC AGC GCC AAG GGA GTC C
3: 30018-neoF	TGA ACA AGA TGG ATT GCA CGC AGG TTC
4: 30018-neoR	TTC GCC GCC AAG CTC TTC AGC AAT ATC

Electrophoresis Protocol:

Agarose: 2% **V:** 100 **Estimated Running Time:** 60 **min**

Primer Combination	Expected Bands	Genotype
1 and 2	495 bp	WT +/+
	495 / 700 bp	HET +/-
3 and 4	700 bp	KO -/-