GENOTYPING BY PCR PROTOCOL

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: **B6.129P2-*Boc*^{tm1Aok}/Mmucd** **MMRRC #** **034378-UCD**

Protocol:

(PCR protocol provided by Donating Investigator)

Reagent/ Constituent	Volume (µL)
Water	13.4
10x Buffer (contains 15mM $MgCl_2$)	2.0
dNTPs (stock concentration is 10mM)	0.8
Primer 1 (stock concentration is 10µM)	0.6
Primer 2 (stock concentration is 10µM)	0.6
Primer 3 (stock concentration is 10µM)	0.6
Taq Polymerase	0.2
Additives / Other (if applicable): 25mM $MgCl_2$	0.8
DNA sample extracted in ☒ NaOH ☐ Proteinase K ☐ Other:	1.0
TOTAL VOLUME OF REACTION:	**20.00µL**

Comments on protocol:

- Amplification for Wild-type and KO reactions are done separately, but run in parallel to obtain genotypes (it has not been possible to combine the reactions). In addition the band sizes are such that WT and KO would be difficult to discern if generated in the same reaction.
- Reaction works best with 10x Qiagen PCR buffer (for standard Taq). Can be sensitive to degradation of primers, probably because the region is highly repetitious. Oligonucleotides should be frozen in aliquots. If reactions start to fail, repeat with freshly made oligonucleotides.

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☒	95	10:00	**1**
2. Denaturation		94	0:30	
3. Annealing	steps 2-3-4 will cycle in sequence	58	0:30	**35**
4. Elongation		72	3:00	
5. Amplification		72	5:00	**1**
6. Finish		4	indefinite	1

Primers:

Name	Nucleotide Sequence (5' - 3')
1: AO 9	5'- TTCGTGTCCTACAACACACACTCC -3'
2: AO12	5'- TAGTATATCCCAGCCAGTAACAAC -3'
3: AO14	5'- CCTGGGACAGGAGAGGACCCTG -3'

Electrophoresis Protocol:

Agarose: 1.5% **V:** 100

Estimated Running Time: 60 **min.**

Primer Combination	Expected Bands	Genotype
1 and 2	~500 bp	KO band
2 and 3	~500 bp	WT band