GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS
2795 2nd Street, Suite 400, Davis, CA 95618
mmrrc@ucdavis.edu
530-754-MMRRC

NAME OF PCR: **B6.129S6-*Btla*^{tm1Kay}/Mmcd** **MMRRC #** **030472-UCD**

DNA Extraction Method: ☐ NaOH ☐ Proteinase K ☐ Other: _____

Protocol:

Reagent/ Constituent	Volume (µL)
Water	38.8
10x Buffer	5.0
50 mM MgCl2	2.0
dNTPs (stock concentration is 10 mM)	1.0
Primer 1 (stock concentration is 5 mM)	2.0
Primer 2 (stock concentration is 5 mM)	2.0
Primer 3 (stock concentration is 5 mM)	2.0
Taq Polymerase 5 Units/mL	0.2
DNA Sample	1.0
TOTAL VOLUME OF REACTION:	**50 µL**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc):

_____

Strategy:

Steps		Temp (°C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE ☐		94	5:00	**1**
2. Denaturation		94	0:30	
3. Annealing	steps 2-3-4 will cycle in sequence	58	0:40	**33x**
4. Elongation		72	0:30	
5. Amplification (i.e., 72°C, 10 min)		72	5:00	**1**
6. Finish (i.e., 4°C, indefinite)			n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1:	5'-TCCAAACAGTCTGCCAGGACAGGA
2:	5'-TGGCGCCTACCGGTGGATGTGGAATG
3:	5'-TGGAGAACAAAAACCGGAACTGATTGA

Electrophoresis Protocol:

% Agarose: _____ **V:** _____

Estimated Running Time (min): _____

Primer Combination	Band	Genotype
	150 bp	wild-type
	250 bp	knockout