GENOTYPING BY PCR PROTOCOL FORM
MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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NAME OF PCR: B6.129X1-*Prkar1a*^{tm1Rpk}/Mmcd MMRRC# 032879-UCD

DNA Extraction Method: NaOH _____ Proteinase K __X___ Other_____

Protocol:

Reagent/ Constituent	Volume (uL)
DNA Sample	1
10x Buffer (contains 15mM MgCl2)	2.5
dNTPs (stock concentration is 25mM)	0.2
Primer **1** (stock concentration is 20 uM)	0.25
Primer **2** (stock concentration is 20 uM)	0.25
Taq Polymerase	0.5
Additives if applicable: 100X BSA NEB	0.25
ddH2O	20
TOTAL VOLUME OF REACTION:	**25 ul**

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

Strategy:

Steps	Temp (^{o}C)	Time (m:ss)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	94	1:00	**1**
2. Denaturation }	94	0:30	2
3. Annealing } steps 2-3-4 will cycle in sequence	62	0:30	**29x**
4. Elongation }	72	1:00	2
5. Amplification (i.e., 72^{o}C, 10 min)	72	10:00	**1**
6. Finish (i.e., 4^{o}C, indefinite)		n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1:BSW-1 FOR	5'-CGCTTGAGGATGTCTGAGCAC-3'
2:BSW-2 REV	5'-GTGATAGTCGTTACTGTCTC-3'

After PCR reaction, 10% of the reaction is digested with NEB restriction enzyme HphI:

Reagent	Volume (uL)
NEB HphI enzyme	0.5
NEB Buffer #4	2
100X BSA	1
10% of PCR reaction	2.5
ddH20	14
Total	**20**

Electrophoresis Protocol:

14% Acrylamide: **V: 180**

Estimated Running Time (min): 1 hour

Band (kB)	Genotype
153bp	WT
126bp & 27bp	RIaB