GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: NaOH _____ Proteinase K _X__ Other_____

Protocol: **NAME OF PCR: MMRRC Strain #30113, B6.129X1-*Hipk2*^{tm1Ejhr}**

Reagent/ Constituent	Volume (uL)
DNA Sample	1
10x Buffer (contains 15mM MgCl2)	2.5
dNTPs (stock concentration is 25mM)	0.25
Primer **1** (stock concentration is 20 uM)	2.5
Primer **2** (stock concentration is 20 uM)	2.5
Primer **3** (stock concentration is 20 uM)	2.5
Primer **4** (stock concentration is 20 uM)	
Taq Polymerase	0.2
Additives if applicable: ddH2O	13.55
TOTAL VOLUME OF REACTION:	**25 ul**

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

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Strategy:

Steps	Temp (^{o}C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	94	5	**1**
2. Denaturation	94	45 (sec)	
3. Annealing } steps 2-3-4 will cycle in sequence	56	45 (sec)	} 25
4. Elongation	72	1	
5. Amplification (i.e., 72^{o}C, 10 min)	72	7	**1**
6. Finish (i.e., 4^{o}C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: 5armF	**5'-CCT GTC TCT GTC ACC TAT CTT GC-3'**
2: Exon3R	**5'-CCC AGT TGG AAC TTG GCT CTA C-3'**
3: LacZR1	**5'-CCA CAG ATG AAA CGC CGA GT-3'**
4:	

Electrophoresis Protocol:

% Agarose: __2____ V : __120_____

Estimated Running Time (min): ___15-20____

Primer combination	Band (kB)	genotype
1+2 (i.e. 1&2)	350	Wild type
1+3 (i.e. 3&4)	730	Mutant
1+2+3 (i.e. 1&2&3)	350 & 730	Heterozygote

PASTE GEL PICTURE HERE, CLEARLY INDICATING LADDER, WATER CONTROL, DNA CONTROL, & DIAGNOSTIC SAMPLES