GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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DNA Extraction Method: NaOH _____ Proteinase K __0__ Other_____

Protocol: **NAME OF PCR:**_____

Reagent/ Constituent	Volume (uL)
DNA Sample	1ul (10ng)
10x Buffer (contains 15mM MgCl2)	1
dNTPs (stock concentration is 25mM)	1
Primer **1** (stock concentration is 20 uM)	1 (10pm)
Primer **2** (stock concentration is 20 uM)	1 (10pm)
Primer **3** (stock concentration is 20 uM)	
Primer **4** (stock concentration is 20 uM)	
Taq Polymerase	1
Additives if applicable:	14
TOTAL VOLUME OF REACTION:	**20 ul**

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

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Strategy:

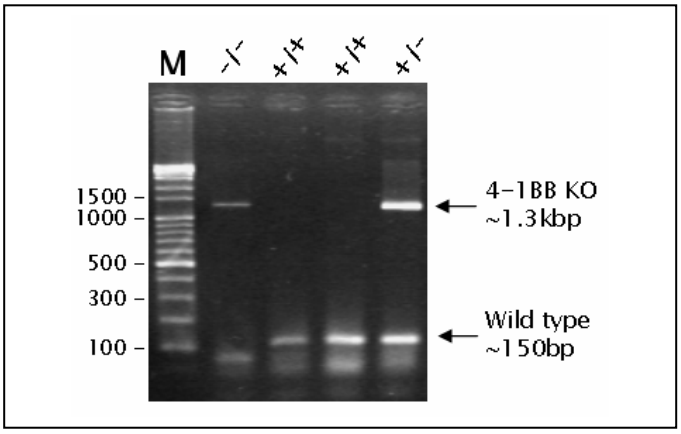
Steps	Temp (^{o}C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	95	5	**1**
2. Denaturation	94	1	
3. Annealing } steps 2-3-4 will cycle in sequence	58	1	35 cycle
4. Elongation	72	2	
5. Amplification (i.e., 72^{o}C, 10 min)	72	7	**1**
6. Finish (i.e., 4^{o}C, indefinite)		n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: forward	**AGT GTC CTG TGC ATG TGA C**
2: reverse	**AGT TAT CAC AGG AGT TCT GC**
3:	
4:	

Electrophoresis Protocol:

% Agarose: 1.5 **V : 100**

Estimated Running Time (min): _35_

Primer combination	Band (kB)	genotype
1&2	~150bp	Wild-type
1&2	~1.3kbp	4-1BB KO