GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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

Protocol: **NAME OF PCR:** MMRRC Strain #30337-UCD, C57BL/6J-*Crygb*^{Clapper}

Reagent/ Constituent	Volume (uL)
DNA Sample	0.5 (50-100ng/ul)
10x Buffer (contains 15mM MgCl2)	2.5
dNTPs (stock concentration is 25mM)	0.5
Primer **1** (stock concentration is 20 uM)	0.5
Primer **2** (stock concentration is 20 uM)	0.5
Primer **3** (stock concentration is 20 uM)	
Primer **4** (stock concentration is 20 uM)	
Taq Polymerase	0.5
Additives if applicable:	
TOTAL VOLUME OF REACTION:	**25 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): lab uses JumpStart®REDTaq®ReadyMix® (P1107-Sigma) 12.5 ul in a 25 ul reaction (includes Taq, buffer, dNTPs)

Strategy:

Steps	Temp (^{o}C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [x]	94	2	**1**
2. Denaturation	94	0.5	35
3. Annealing } steps 2-3-4 will cycle in sequence	55	0.5	35
4. Elongation	72	1	35
5. Amplification (i.e., 72^{o}C, 10 min)	72	5	**1**
6. Finish (i.e., 4^{o}C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5' - 3')
1: L10(F)	CAGCAGTCATGACAGCTATA
2: L10(R)	CACCCACTCTTTGCTCTAGA
3:	
4:	

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	0.52	
(i.e. 3&4)		
(i.e. 1&2&3)		

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

Clapper genotyping is performed by amplifying the region containi
the amplified region to detect the single nucleotide change. The sa
sequencing. The following sequence of 520 nucleotides (from Genbank genomic region **NC_000067** for linear genomic sequence of *Crygb* plus 69 additional nucleotides from the 5'UTR of *Crygb* taken from **NCBI m37 mouse assembly** Chromosome 1: 65128391:65128910) is amplified:

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 -69                                                                cagcagtc
-61 atgacagcta tatataccag gggagctccc ctagagtctc cagctcccag ggcatctctt
  1 actctcagcg agatgggaaa ggtaagtcct ggaaccctga cctttgcccg caagcagcat
 61 ccttgctggc agaaatcact tatttgtctg gtccctttct gcgcttacag atcaccttct
121 tcgaggaccg cagcttccag ggccgctgct atgagtgcag cagcgactgc cccaacctgc
181 agacctactt cagccgctgc aattctgtcc gcgtggacag tggctgctgg atgctctatg
241 agcgccccaa ctaccagggc caccagtact tcctgagacg tggagagtac cctgactacc
301 agcagtggat gggtttcagc gactccattc gttcctgctg cctcatcccc caagtgagtt
361 tggctgtctt tattattgat ctctgggaac acaaattatc ctaaaagatc atcttaaagc
421 aagtcgtttc aactagagca aagagtgggt g
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Primer binding sites are underlined; the mutated A is shown in red text.