GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

Investigator/PI: Dr. Bruce Beutler Address: 1055 North Torrey Pines Rd, Mailstop: SP-293 Contact: Dr. Bruce Beutler
Telephone: 858-784-8610 FAX: _____ email: bruce@scripps.edu

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

Protocol: **NAME OF PCR:** MMRRC Strain #30338-UCD, C57BL/6J-*Crygd*^{L23}

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 } steps 2-3-4 will cycle in sequence	94	0.5	35
3. Annealing	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: L23(F)	TCCCATCCGACCTGCCAA
2: L23(R)	GCCAGGAACACACAGAAAAT
3:	
4:	

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	1.59	
(i.e. 3&4)		
(i.e. 1&2&3)		

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

L23 genotyping is performed by amplifying the region containing the mu
of the amplified region to detect the single nucleotide change. The sam
and for sequencing. The following sequence of 1586 nucleotides (from Genbank genomic region **NC_000067** for linear genomic sequence of *Crygd* plus 19 additional nucleotides from the 5'UTR of *Crygd* taken from **NCBI m37 mouse assembly** Chromosome 1: 65108417:65110002) is amplified:

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 -19                                                tcccatccg acctgccaac
   1 accagccatg gggaaggtga gcccagggca tcctcatttt gggaagggac ctgccctggg
  61 ggccaggcca catcaggcct ctgagccctg ccttgccttg ccttacagat caccttctat
 121 gaggaccgcg gcttccaggg ccgccactat gagtgcagca ccgaccactc caacctgcag
 181 ccctacttca gccgctgcaa ctctgtgcgc gtggacagtg gctgctggat gctctatgag
 241 cagcccaact tcacgggctg ccagtacttc ctgcgtcgcg gggactaccc tgactaccag
 301 cagtggatgg gtttcagtga ctctgtccgc tcctgccgcc tcatccccca cgtgagtcca
 361 gattctcaag actgaggcac tgaagaccct gactgcagtt gccagtataa ggttaaatgt
 421 tgaaagcaga gctgagcctg cttgtaaaga aaaacccata gctagaatta attaggtcaa
 481 tagttcccac aacatccaaa aagcaaggtg ttacccagtt acaactattc tattggcccc
 541 tacgtatttg tggcataaga acgcattggc agcaagcggg ctgtatgaaa tctgagtcct
 601 gtcatgcaat catttaggat ggaaaaatag aatggtggct actgaacaca agagtaatta
 661 tgcttaaagt ccccctcctc ctttttgtcc tctacgtgca tatctggggg ttcctccatc
 721 taattcggtc gaagtccttt cgacagagca acacagatgt tttacagctg aaaaatggct
 781 cttgtgctta ctgattttat ctctccattt ttccctcatt atatttataa attattaact
 841 gagcagtcat tgttgtctga gacctaaaat attgaatatt ttaatacatt ttaatataaa
 901 aacattttcc ccaatcagca aaccaagtag taagaagctg ttgacagtca tgatttctct
 961 ctggtgtttg ctttacagta tgctgcacag gaggtggttt gggccctgca gtttctgaac
1021 acactcatag ttccttctcc aattcatttc ctcatacttc ctgatcttag aaaagcctgg
1081 aatctgccct catagtgacg agtttataag aagtctgagt taattaatgc atttttatc
1141 tagttcaaat ggcttgatgc agctgaggcc atcctaacgc attagaatcg cttcaccgtt
1201 gctttttact gtttctgggc ctgccaggcc ggctctcaca ggatcagact gtacgagagg
1261 gaagagtaca gaggccagat gatagagttc acggaggact gcccctctct ccaggaccga
1321 ttccacttca atgagatcta ctccctcaat gtgctagagg gctgctgggt cctctacgac
1381 atgaccaact accggggaag gcagtacttg ctcagacccg gggagtacag gcgctaccac
1441 gactggggcg ccatgaatgc cagggtgggc tctctgagga gagtcatgga tttctactga
1501 attggttttt ttactctacc ctttctccat ttggacgcta ataaaatatt ttctgtgtgt
1561 tcctggc
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Primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated T is shown in red text.