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:**_____

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	30
3. Annealing } steps 2-3-4 will cycle in sequence	56	0.5	30
4. Elongation	72	1	30
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: Slug(F) (for PCR)	CAAAACCGTGAGCTTTCCTGCTG
2: Slug(R) (for PCR)	AGGCACCATGTTACAATCTGTCCG
3: Slug_seq(F)	ACACTAGGTGGGTTCCTGAC
4: Slug_seq(R)	TAGGAATGCTCAACTGGTCC

Electrophoresis Protocol:

% Agarose: __1%____ 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

Sluggish/030499 genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide change.. The following sequence of 1147 nucleotides (from Genbank genomic region **NC_000084** for linear DNA sequence of *Map3k8*) is amplified:

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13116                                        caaaa ccgtgagctt tcctgctggc
13141 tgtagctcct agctgcccac actaggtggg ttcctgacag gcctcaggtg ttttcacctg
13201 actgctcccc tacttctctc ttctggggtg aaccacctga gtcattatca gaagcgtttc
13261 cttctgctct gcttggaact tgctgttcta gatgattttc ctgttgggtt aggatgcatt
13321 aactcacatt aaaaacccct ctcgttgcag ctagcaacat tgtattcatg tctacaaaag
13381 ctgttttggt agattttggc ctgagtgtta agatgactga agatgtctat cttcccaagg
13441 acctccgggg aacagaggta attgattgat tgtattggtg atggtctgga aatgctgtga
13501 caatcagcaa tgaaggggac aaaatggcaa cattggatga tcgttgtcac cagaacaaat
13561 tcattttaca ttttaccttt tttactattt ctatgataag actttaaata tgcatggtta
13621 tatttataga tatttttttat tactgtttat atatacaaca caaagcactt acagacaatt
13681 cagtgccaaa acagaggacc agttgagcat tcctaatcca aacgtctaaa aagctagaca
13741 agacctagag agaggttgcc aagtaaagtg cttgctacac aagcccaggg acctgagttc
13801 aaatcctgag aacccaaata aagccatgta tggtagacct agtgtgacta tgggtagatc
13861 taagtagaca ggagtcttcc ccaacgctca caggagagta gcctggaata caggactatg
13921 aataagagat cccgcctcaa ggtgggaagt gtcatatgtg cagtgtcgta tatattacac
13981 agacagacag acaaggtgaa aagaaaagcc cataaaaata tcaaatattc caaacatgag
14041 cttctaaatc tgatttttga ttctaagttt ctcaagtaag ggatactcag aattcatctt
14101 taaaactgtt agagaacaat agtcaactta tagttcactt agtaccttaa atgaattatt
14161 tcttccttaa aattattgtg tgtaaatact ggtataacag gcagatgact gctattatcc
14221 tattttataa gaagacaacg gacagattgt aacatggtgc ct
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PCR primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated T is shown in red text.