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: C57BL/6J-*Epha4*^{m1Btlr}/Mmcd (*frog*), MMRRC #032237-UCD**

Reagent/ Constituent	Volume (uL)
DNA Sample	1
10x Buffer (contains 15mM MgCl2)	5
dNTPs (stock concentration is 25mM)	0.4
Primer **1** (stock concentration is 20 uM)	1
Primer **2** (stock concentration is 20 uM)	1
Taq Polymerase	2.5
Additives if applicable: H2O	39.1
TOTAL VOLUME OF REACTION:	**50 ul**

Comments on protocol *(e.g.,* different concentration of MgCl2, etc): *Frog* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide transition.

Strategy:

Steps	Temp (^{o}C)	Time (min)	# of Cycles
1. Initiation/Melting HOT START?..CHECK HERE [ ]	95	2	**1**
2. Denaturation } steps 2-3-4 will cycle in sequence	95	30 sec	29
3. Annealing	56	30 sec	29
4. Elongation	72	1	29
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: frog_PCR(F)	5'- ACACATTTCCATGCCCAATTTTGCAAT -3'
2: frog_PCR(R)	5'- ACCCACACCTTTATTTTGAACAGCAGT -3'
3: frog_seq(F)	5'- GTACTGTTGATCCAGACTCAGGC -3'
4: frog_seq(R)	5'- GTGTGTTTCAAGATAACGAGCC -3'

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	1250 bp	
(i.e. 3&4)		
(i.e. 1&2&3)		

Mutation site (red) and flanking sequence:

WT `tatgaaaaggtacttctttct`
frog `tatgaaaaggcacttctttct`

The following sequence of 1250 nucleotides (from Genbank genomic region NC_000067 for linear genomic sequence of *Epha4*, sense strand) is amplified.

```
112988       aca catttccatg cccaattttg caatggccat caggtataga atatccactg
113041 tcctagtctt agttagtgtg tccatttcag tgaagagaca ccatgacaaa ggcaattctt
113101 ataaaggcaa acttttaatt ggggctgggt tacagtttca gaggtcagtc ccttatcacc
113161 ctggcaggaa gcttggcaac ttgcattcag acatggtggt ggtggaagag ctgagagatc
113221 tacctcttga tttgaaggga gcagaagggt tggctggctg tcttttgcac tgggcaaagc
113281 ctgcctccag tgacacacct cctcctataa ggccatacct cctccaataa ggccacacct
113341 cctaatactg ttttaaatac atgaatccat gggaaccaaa cctagtcaga ccaccacagt
113401 cttcctccac ccttccccat tcacccctct acttccagcc ttacaaacca ctgccaacca
113461 cacccagctt ttctgtgggt actgttgatc cagactcagg ccctcatgat tgcagagcaa
113521 gccctaccca cagcgttgtg tcttcagtct cctcacaatt gatcttttaa agacaaaccc
113581 taaacaaagg aaggtctgga gataatggta ataataaatg ctcttgtctg gaaaagggca
113641 acgtgaccaa caggaaggca tgggactctg aagtcaagtg aaattgttga gttgtggttt
113701 ctaaaatatg tttctttcta aagtaagatt attatttctg tctttctgtc tttctccccg
113761 atagcaccat catccattgc tttggtccag gctaaagaag ttacaagata tagcgtagct
113821 ctggcttggc tggaaccaga tcgaccaaat ggagtcatct tagaatatga agtcaaatat
113881 tatgaaaagg tacttctttc tttcttcttt gctttccttc ttcttctttc tccttccctt
113941 tctttcttac tgttccttcc tctgtgttgt tcaagtatta gacagttcta aatcatgaaa
114001 cagcagcaaa aggaaaatca aatgcatctt gattccccac aaatctgatt atgagatgta
114061 atatttggct cgttatcttg aaacacacac acaatgttct aatataatta tattttcttt
114121 cgaataaaag aaatcaagac ctaatctata taccttctat cacagagcat gcctcataac
114181 tgctaaaata atgatatgtt taagcagatc actgctgttc aaaataaagg tgtgggt
```

Primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated T is indicated in red.