GENOTYPING BY PCR PROTOCOL FORM

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

Investigator/PI: MMRRC Address: 2795 2nd Street, Suite 400, Davis, CA 95618 Contact: Reneé Araiza
Telephone: 530-754-MMRRC FAX: 530-757-3284 email: mmrrc@ucdavis.edu

DNA Extraction Method: NaOH _____ Proteinase K _____ Other__Any___

Protocol: **NAME OF PCR: C57BL/6J-*Hr*^{m1Btlr}/Mmcd (*prune*), MMRRC #030821-UCD**

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*:** *Prune* 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. Use primers 1 & 2 for amplification and 3 & 4 for sequencing. The 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	0.50	30
3. Annealing } steps 2-3-4 will cycle in sequence	56	0.50	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: Prune(F)	TCCTGGGTTTTGGAGAAAGGGACAC
2: Prune (R)	AGCCGCCTTTACTGCTTGGAACAC
3: Prune _seq(F)	CCAGGGTCACAGAAGCTATG
4: Prune _seq(R)	GAATGCTGTATGATCAGGACTCC

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	1.25	
(i.e. 3&4)		
(i.e. 1&2&3)		

The following sequence of 1248 nucleotides (from Genbank genomic region **NC_000080** for linear DNA sequence of *Hr*) is amplified:

```
16975                                                          tcctgg
16981 gttttggaga aagggacacg gagagaacaa ggggtcaggc cagggtcaca gaagctatga
17041 aaagcacatc ccagggaagg cagggattta gatccctcct cccaccccca gcttgagtgt
17101 cccttactct aagctccttc caatccaacc tggacctacc cccatcctgt cctatccctt
17161 accaccaacc ctcctctctc ctacctcttc cttcttttgc ctgcccttca agacagcttc
17221 agcaccctat tgaacctgat cttccaaaga gtccactccc cttcctctct gaatgcttgc
17281 cttcatcccc actgacattc atcttccttt ctcttcttga aggtgtgccc agctggagca
17341 ggaaccttgg agcctggtgc cccaggcagc tgctacttgg atgcagggtt gcgccgacgg
17401 ctaagagaag agtggggtgt gagctgctgg accctgctgc aggctcctgg ggaagcggtg
17461 ctggtcccgg ctggggcgcc ccatcaggtg cttacctggt gggtgggggt taacaaaaga
17521 tcagagtatc agaagtagca aggagtcctg atcatacagc attctctacc tgatagaaag
17581 ctaggcaggg caggaagcca tggagataga aagccaaggg tcttgaagtc ttcaaagcta
17641 tgtgggactc tctccagcat ccctcaggtc ttgttctggc catggccttt tgaatgtctg
17701 gttctgtaac tagggttggt tactgttttc tgaaagttgg ataaggaagt agacttagtg
17761 atcagaggca tggaggggga atgtggtttg agcctcaact caccctctct tgcccttgtt
17821 ccgcccccct ccctgcccac cccacggcac aggtgcaggg cctggtgagc acaatcagtg
17881 tcactcagca ctttctgtct cctgagacct ctgccctctc tgctcagctc taccaccagg
17941 gagccagcct acccccctgac caccgtatgc tttatgccca ggtgagtgtg gtactggctt
18001 gaaggcagag tgtgctgctg ccctagtctc cttggggcac agccgcagta ggcaggacag
18061 atctgtctga gtggaatggg ctgctagcct gtcagtgcta gataaaggtg ttcttgagaa
18121 gggtagggag tgagggttgg tgctggaacg cgattgaaaa ttctgtgtca tttttctttt
18181 tcccagatgg accgggctgt gttccaagca gtaaaggcgg ct
```

PCR primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated T is shown in red text.