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: C57BL/6J-*Hr*^{m2Btlr}/Mmcd (*mister clean*), MMRRC #031039-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*:** *Mister clean* 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 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:

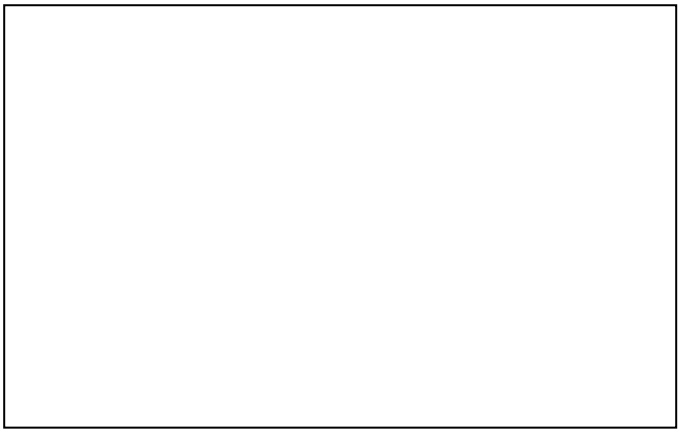
Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: MrClean (F)	CCATCCGTCCACGTTGGAATCATC
2: MrClean (R)	TGCACTGGCTGTTTCCTCCATAAG
3: MrClean_seq (F1)	CTTCCTAGAGACTTGGGCTGAAC
4: MrClean_seq (F2)	AGACTTGTGGATACATCCTGC
5: MrClean_seq (R)	GACTGGACCAAGTCTTTAGACTC

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	1.03	
(i.e. 3&4)		
(i.e. 1&2&3)		



The following sequence of 1033 nucleotides (from Genbank genomic region **NC_000080** for linear DNA sequence of *hr*) is amplified:

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5210                                                    c catccgtcca
5221 cgttggaatc atctcacagt gtcacatagc cacttagcct ttagctgacc tacccaaagt
5281 ctatactggc tggattctgc acgtgtacaa ggaccattgc tcaggcttcc tagagacttg
5341 ggctgaacat gctaaacaac ccctgagcct ccattcctac cagggacatc tccagctact
5401 gctcagcttt gtgtgtgaaa tatactggac tgatcctctt tcgggttctg aattggtcct
5461 cacaagcctg gggtccaggg gctgaattgg ctgcatgtgg acaagggtgg gtgttaattc
5521 agggctttga ctagcataag ccccagaaac cagactccct aagcaactgt attgtctccc
5581 cagggccccg agatggcagg attaggctcc aggagtccag acttgtggat acatcctgcc
5641 agcatcactt agcaggtgtc acccagtgcc aaagctgtgt ccaggcagct ggagaggtag
5701 gggtactgac cggccactcc cagaaatcac gtaggtgagt gttgtgtctg acagtcagag
5761 ccagcagcac tccatcccca cccaggggct ccctcctcaa gcttcatcca tttccaggtc
5821 acccctggag gagaagcagt tggaggagga ggattcctct gccacttccg aagaaggagg
5881 aggagggcct ggcccagaag cttcactcaa caagggcctg gccaagcacc tgctgagtgg
5941 tttgggggac cgactctgcc gcctgctgcg gaaggagcgg gaggcccttg cctgggcaca
6001 gcgagaaggt gagccaattt cccttgtggg cctgctcctg catgtccctc ccaacccacg
6061 tttaccagtg ctttggggtt cccaggccta ctcaggagag ctccgtcctt ctttgttcct
6121 agctatccct agcacggact cttggtcaat cctgaggttt ctggcctcct ggggagtcta
6181 aagacttggt ccagtctagt tctctaagaa caactgggct tatggaggaa acagccagtg
6241 ca
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PCR primer binding sites are underlined; sequencing primer binding sites are highlighted in gray; the mutated G is shown in red text.