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-*Muc2*^{m1Btlr}/Mmcd *(Muskatenwein)*, MMRRC #032797-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: *Muskatenwein* 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:

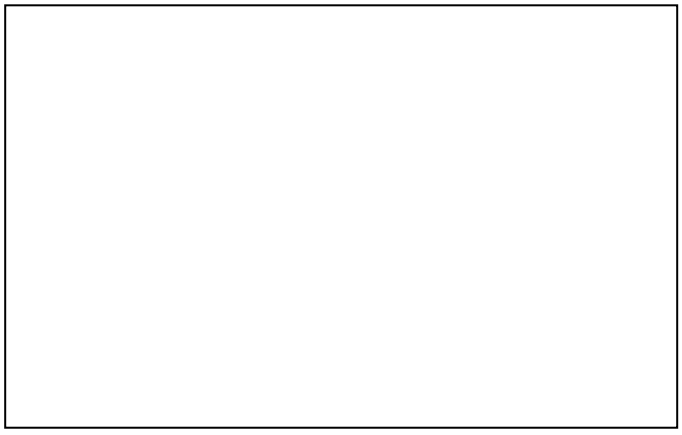
Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: Musk (F)	AGCAGACCCATTGCATCATCGAG
2: Musk (R)	TCCTTCATGACGGAGACAGCAGAG
3: Musk _seq (F)	GATACTCATTCCTGGAGCTAGGAC
4: Musk _seq (R)	TTTGAGGAACTGAGTCCCAAC

Electrophoresis Protocol:

% Agarose: _1.5_____ V : _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	1.390	
(i.e. 3&4)		
(i.e. 1&2&3)		



The following sequence of 1390 nucleotides (NCBI Mouse Genome Build 37.1, Chromosome 7 bases 148938628 to 148940017) is amplified:

agcagaccca ttgcatcatc gaggggccca agcagcagta cattattctg aaggtgtgtg
cgcttctggc tccacccaca agctaggcag ccactgaggg caaggctagg atgacttcgg
gcttgagtca tcctaaatcc cacatagcat atttgggtgg ctaaaagcaa catatatggt
tgctccactt ctggagctct gaagtgtaag atcaaggctg gcagatgctg tgaggagtcc
ttcctgcctc ttctttgggc tccagccaat tttggtttgt gtttgaatca cctctgatgt
agtagtgtcc ttctatctga gcctgtgcct cctcttctgt ctcttataag gatactcatt
cctggagcta ggacctcctt catgtagggt gacctcatat ccagattatt ttcttgagct
tttcaaagat catttcccca ctcagagtca ctttctagtt cacaagtcag acctaggtta
gagatttctg tgttatgtaa ccacagtagc tatctccaag actgtaacgg atgccacttg
cccagtgact ctggacctcc tttctgtggc gctttgcctc ccaggtcttc ctgggctggc
ctctggcgta cacctgctca tcctcattct gttgcgtcca cagcctgggg agattcacaa
aaaccccagc aacaagtgca ccttcttcag ctgcatgaaa atcaacaacc agctcatctc
ctcggtctcc aacatcacct gtcccgactt caacccaagt gattgtgttt cagtgagtgg
gaccctatgg ccctgtccat gtatgcgttg ccaggagcag ggaaccttaa tgcaccagct
acctggtttc caggggcctt gttccacacc ctcctgtagg acagccctta tgcatcccat
gacaagaagg ggcactcact tgctgggggt gctctggact cctttcttac tctcctttct
cttcagggct ccatcacata catgcctaat ggctgctgta agacatgtga gtacggctgg
ggaaatgttg ggtgatgggt gagttttact cagagccttg aggggaagat gcaggacagg
atgcttcagg actgtgtctc ctacactcat gcagatgtat aggaatctga gtctccttcc
agggagaggc tgggtggagc ccagctgcat gttgggactc agttcctcaa aggaaagtgg
gactcagtta agggctagag acatgctcgg agaaggaaga agaaatggca ccagagaagg
agagggaggg gaagtgggga gagagaggga aggggcaagg ggagctagct agctagagtc
atttttcccc cacaggcatc cctcaaaacc agaccagagt cccttgctct gctgtctccg
tcatgaagga

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