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:** MMRRC Strain #31706; C57BL/6J-*Muc2*^{m1Btlr} (Schlendrian)

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	0.50	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: Schlen (F)	TCCTGAACTGCTGCCAGTCAAC
2: : Schlen (R)	TGGACCAGGCTCCCTTTCACAAAC
3: Schlen _seq (F)	CTCTGTGGAAACTTCAATGGC
4: Schlen _seq (R)	TGTCCTTGACAAGCCAGTG

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	1.076	
(i.e. 3&4)		
(i.e. 1&2&3)		

PASTE GEL PICTURE HERE, CLEARLY INDICATING LADDER, WATER CONTROL, DNA CONTROL, & DIAGNOSTIC SAMPLES

Schlendrian genotyping is performed by amplifying the region c
by sequencing of the amplified region to detect the single nucleot

Primers for PCR amplification

Schlen(F): 5'- TCCTGAACTGCTGCCAGTCAAC -3'

Schlen(R): 5'- TGGACCAGGCTCCCTTTCACAAAC -3'

PCR program

1) 94°C 2:00

2) 94°C 0:30

3) 56°C 0:30

4) 72°C 1:00

5) repeat steps (2-4) 29X

6) 72°C 7:00

7) 4°C ∞

Primers for sequencing

Schlen_seq(F): 5'- CTCTGTGGAAACTTCAATGGC -3'

Schlen_seq(R): 5'- TGTCCTTGACAAGCCAGTG -3'

The following sequence of 1107 nucleotides (from Genbank genomic region **NC_000073** for linear DNA sequence of *Muc2*) is amplified:

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4800                                                                    t
4801 cctgaactgc tgccagtcaa cagagccagc tgagattcgg acagcctaag agtctgggca
4861 aggagaggcc ttgtgtcctc aggcagactc tattcttggg tccctgctag tggaaggggg
4921 tggtgggcat gtcaccccat agtatggtgg aaccaaggag ccctgcagcc tttcaccatg
4981 tccttccttg tcttatagcc agcttctcca tcttccaacc ctcctcctac cacattgttg
5041 tgaacacgaa gttcgggctg cggctgcaga tccagttgct tccagtcatg cagcttttg
5101 tgactctgga ccaggctgcc cagggacagg tgcagggtga gtggctctct cctgtcttgt
5161 ctctgaaaaa tccccatgag ggtcctattt ttttccccca cccccaggtt cttcctaatc
5221 ctgtcctggc cctttaggtc tctgtggaaa cttcaatggc ctagagagtg atgacttcat
5281 gacgtctggt ggaatggtgg aggccaccgg tgctggcttc gccaatacct ggaaggccca
5341 atcaagctgc cacgacaagc tggactggct agatgacccc tgctccctca acattgagag
5401 tggtaaggct caggagaagc tggttgctgt ccactggctt gtcaaggaca cacaatcctg
5461 acagtccagc ctcagagcag atggggctcc tgatgaagac ataggatgtg ggtgaggagt
5521 gggtgtaggt cacatggcct atggtgctgg gatcagcaca tttacctgct gtttcagttg
5581 acagtggccc catggtggca gcattccagg tgacaaagta aatggactgg gcaggctaac
5641 tgctgggtgg tggctctcat ctcggcctgg cttaatgcta ggatgacatg cccttctatc
5701 catttctcca tcatatctgt ggatttctag ccccttgggg tcgaaaaagg gagaggtggg
5761 tgttatgcag aaaggtctca tcttgttcat agctgcctgg agttatgaag aaaggggtct
5821 ggctttggtc acccaggaga ccatgtcacc tccatcctgg gtgatcagac tttaggctca
5881 gggtttgtga aagggagcct ggtcca
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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.