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 #30336-UCD, C57BL/6J-*Sox10*^{Dal}

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 } steps 2-3-4 will cycle in sequence	94	0.5	35
3. Annealing	56	0.5	35
4. Elongation	72	1	35
5. Amplification (i.e., 72^{o}C, 10 min)	72	10	**1**
6. Finish (i.e., 4^{o}C, indefinite)	4	n/a	n/a

Primers:

Primer Name	Nucleotide Sequence (5^{-} - 3^{-})
1: Dal(F)	TTTGCGATGGGAGAGTCTGACACC
2: Dal(R)	ACACTGTGAATGTGCGATCTAGTGG
3:	
4:	

Electrophoresis Protocol:

% Agarose: _____ **V :** _____

Estimated Running Time (min): _____

Primer combination	Band (kB)	genotype
(i.e. 1&2)	1.3	
(i.e. 3&4)		
(i.e. 1&2&3)		

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

The *Dalmation* mutation introduces an *EcoN* I restriction enzyme sequence. *Dalmation* genotyping is performed by amplifying the followed by *EcoN* I restriction enzyme digestion.

The following sequence of 1313 nucleotides (from Genbank genomic region **NC_000081** for linear genomic sequence of *Sox10*) is amplified:

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1501        tttg cgatgggaga gtctgacacctgcaggcag gcctgcgtcc ccccattctg

1561 ctcccctagg ctgtcagagc agacgagggg aaagagaggt gagcgaaaag gtgggaaatt

1621 ccaggggccc ggattagagc cgaataaagg gtccgttttt cttcctttcc atcaacaaac

1681 ctccacccaa aaggaggttt aggggaaaaa aacgaaaaaa aaaaaaaaaa aaaaacaccc

1741 acacctagag acggttggga tcaggggtg ggaagacgtg gaggcgggac gcacccacct

1801 agggtctggc atgtgcacgc gcccaggccc gagtgggttt agcgcaggca gggagagtcg

1861 ccacttcggg gctgcacccg cgtgccggga gtggcgccca gcgcctctcc atcgcgccct

1921 ccttcccgtc caggtgggcg ttgggctctt cacgaggacc ccggcggcgg gcccggggga

1981 ggcggccgaa gcggcggcgg ccgggagcga catggccgag gaacaagacc tatcagaggt

2041 ggagctgagc cctgtgggct cggaggaacc ccgctgcctg tccccaggca gcgcgccgtc

2101 gctgggaccc gacggcggcg gcggtggctc gggcttgcga gccagcccgg ggcccggtga

2161 actgggcaag gtcaagaagg aacagcagga cggcgaggcg gacgatgaca agttccccgt

2221 gtgcatccgc gaggcggtca gccaggtgct cagcggctac gactggacgc tggtgcccat

2281 gcccgtgcgc gtcaacggtg ccagcaagag caagccgcac gtcaagaggc ccatgaacgc

2341 cttcatggtg tgggcacagg cggcacgcag aaagctagcc gaccagtacc ctcacctcca

2401 caatgctgag ctcagcaaga cactaggcaa gctctggagg tgagcgctgc cccggcccaa

2461 caccccctc gtgcgcgcgc actagggcta gcctgggacc ccagaacagg atcgcccagg

2521 cctctctctc aggggagacc aacctatgga agtttggtct cttggaagac cagatttgga

2581 gatctcgccc acccatctac ccaaagtgcc tctgtggggg gctcctctgg ctgcccactc

2641 tggaactcag aaccccagcg gcctaggctg ggcctcccca ccctgggacg gcgggcggga

2701 aggcgccccc tcgtggttgg aattctgtgg gtggtgggca gggaaggcaa catgggggtg

2761 tgggggaaag cttcacacca gacctccact agatcgcaca ttcacagtgt
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The primer binding sites are underlined; *EcoN* I sites are highlighted in gray; novel *EcoN* I site caused by the *Dalmation* mutation is highlighted in dark gray; the mutated T is shown in red text.

Restriction Digest

Digest PCR reactions with *EcoN* I. Run on 2% agarose gel with heterozygous and C57BL/6J controls.

Products: *Dalmatian* allele- 366 bp, 400 bp, 500 bp. Wild type allele- 500 bp, 766 bp.