GENOTYPING PROTOCOL

MUTANT MOUSE RESOURCE & RESEARCH CENTER: UC DAVIS

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Protocol Name: **CR11562 Miox EXDEL**

Protocol: ***GoTaq® G2 Colorless Master Mix(Promega)***

Reagent/Constituent	Volume (µL)
Water	4.5
GoTaq® G2 Colorless Master Mix,2X	7.5
Primer 1. (stock concentration is 20µM) comF	0.5
Primer 2. (stock concentration is 20µM) wtR	0.5
Primer 3. (stock concentration is 20µM) mutR	0.5
DNA (example) extracted w/ "Qiagen DNeasy columns or other similar silica based kits"	1.5
TOTAL VOLUME OF REACTION:	**15.00 µL**

Comments on protocol:

- Protocol may work with other DNA extraction methods.

Strategy:

Steps		Temp (ºC)	Time (m:ss)	# of Cycles
1. Initiation/Melting	HOT START? ☐	94	2:00	**1x**
2. Denaturation		94	0:10	
3. Annealing	steps 2-3-4 cycle in sequence	65 (↓1ºC/cycle)	0:30	**10x**
4. Elongation		68	2:00	
5. Denaturation		94	0:15	
6. Annealing	steps 5-6-7 cycle in sequence	55	0:30	**25x**
7. Elongation		68	2:00 (↑20sec/cycle)	
8. Finish		4	∞	n/a

Primers:

Name	Nucleotide Sequence (5' - 3')
1. CR_Miox_comF	CAGAGTGATCCCAGTTTATCTGAAGG
2. CR_Miox_wtR*	CACTGCCTCTTCTGTTGAGCC
3. CR_Miox_mutR	GAGGTCCTAAATTCAATTCCCAACAATGC

Electrophoresis Protocol:

Agarose: **1.5%** **V:** **90**

Estimated Running Time: **90** min.

Primer Combination	**Band** (bp)	**Genotype**
1 & 2,1 & 3	349,3119	wildtype
1 & 3	480	mutant

Allele Description: Exon 1-10 (ENSMUSE00000868307, ENSMUSE00001253592, ENSMUSE00001239661, ENSMUSE00001252294, ENSMUSE00001236571, ENSMUSE00001245068, ENSMUSE00001277067,ENSMUSE00001249530, ENSMUSE00000128827, ENSMUSE00000858990)were constitutively deleted from the 5'UTR through the 3' UTR from the Miox Gene ENSMUSG00000022613 using CRISPR Cas9 gene editing technology in mouse zygotes. The subsequent 2639bp deletion from Chr 15: 89218608 - 89221246 GRCm39 was screened by PCR analysis. The selected founder animal was backcrossed to C57BL6/N to produce sequence confirmed heterozygous animals for establishing and archiving the line.

*wtR primer untested (ePCR verified**)**