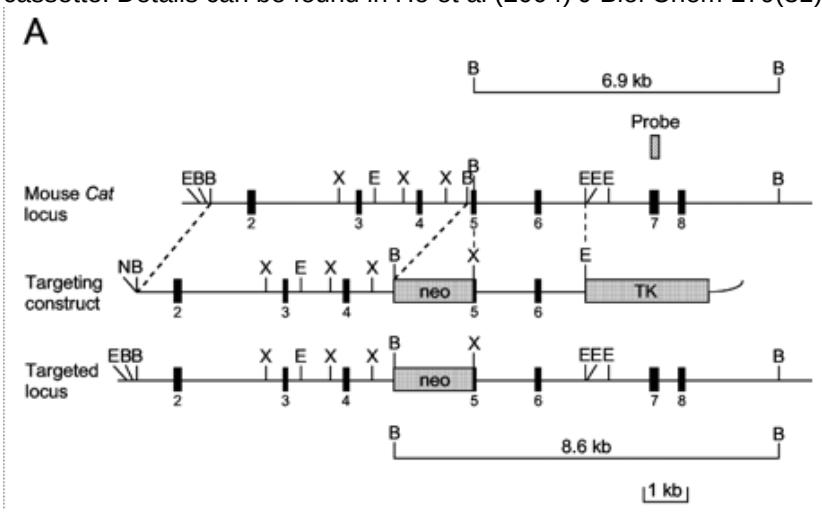
08.15.12 MLS
08.24.15 MLS

Genotyping Protocol: **MMRRC 36739**

Assay Type: PCR; Can distinguish heterozygous animals from homozygous animals.

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Mutation Information: Exon 5 and part of intron 4 of the mouse catalase gene (*Cat*) was replaced by a neomycin cassette. Details can be found in Ho et al (2004) J Biol Chem 279(31):32804-12.



Primer Information:
1) Name: M36739 F Sequence: 5'-GGG ACG AAG ACT CCA GAA GTC CC-3'
2) Name: M36739 R Sequence: 5'-GCC TGG AGA ACA GGC TGT GCC-3'
2) Name: M36739 Neo Sequence: 5'-TTG GCG GCG AAT GGG CTG AC-3'

Primer Location: M36739 F is located in Exon 5 of *Cat,* and M36739 R is located in Intron 4 of the mouse *Cat* gene. M36739 Neo is located in the inserted neomycin cassette.

Assay Name: Cat KO PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M36739 F	Sigma	25µM	0.3
M36739 R	Sigma	25µM	0.3
M36739 Neo	Sigma	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			12.7

PCR Setup:
Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in an Applied Biosystems 2700 thermocycler.

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Cycle Parameters:

1)	95°C	3 minutes
2)	94°C	30 seconds
3)	66°C	30 seconds
4)	72°C	1 minute
5)	Repeat steps 2-4 34 times for a total of 35 cycles	
6)	72°C	10 minutes
7)	4°C	hold until refrigerate product

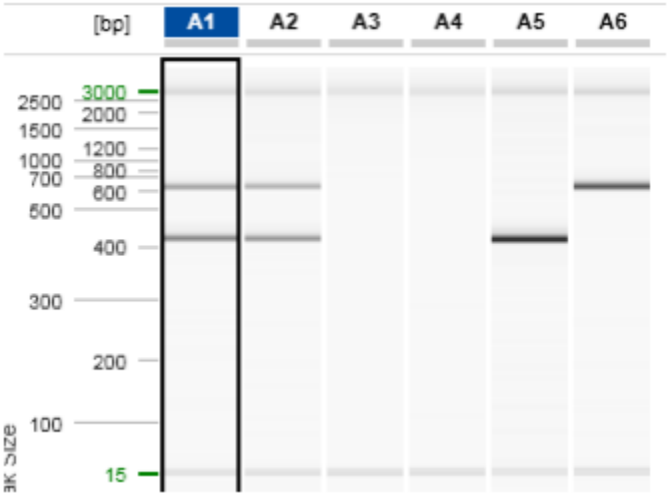
Product Analysis:

All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/3Kb (Cat# 929522)
Size Marker: QX DNA Size Marker 100-3Kb (Cat# 929553)
Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected products: Wild type: 450bp product only
Heterozygous: 450bp and 650bp products
Homozygous: 650bp product only

Example of Gel:



Lanes A1 and A2 display heterozygous samples (450bp and 650bp products).
Lanes A3 and A4 displays extraction and PCR blanks, respectively.
Lane A5 displays a wild type sample (450bp product only).
Lane A6 displays a homozygous mutant sample (650bp product only).

Please note: the 15bp and 3kb bands are reference markers specific to the Qiaxcel method and do not represent expected products.