

09.14.10 MS
12.23.13 MLS

Genotyping Protocol: **MMRRC 11589**

Assay Type: PCR- can distinguish heterozygous animals from homozygous animals.

DNA Extraction: DNA from tail snips was extracted using Sigma's Extract-N-Amp Tissue PCR Kit (Cat#XNAT2R). Kit directions for fresh or frozen tails were performed with a few minor modifications as follows: use 50 µl of Extraction Solution and 12.5 µl of Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Primer Information:

M11589 WT Assay

1) Name: VCLAD F Sequence: 5'- GCC GTG AAA GAG AAG ATC ACA GCT -3'
2) Name: VCLAD R Sequence: 5'- AGC ACA TTC TCT GAT GGC ACC TTC AC -3'

Primer Location: VCLAD F binds to Chromosome 11 at location 69738352:69738975 and the reverse is located at 69737916:69738541

M11589 KO Assay

1) Name: Neo F Sequence: 5'- CAT TCG ACC ACC AAG CGA AAC ATC -3'
2) Name: Neo R Sequence: 5'- ATA TCA CGG GTA GCC AAC GCT ATG -3'

Primer Location: Both F and R bind to the Neo insert

Assay Name: Acadvl KO PCR

PCR Master Mix Components (Run separate reaction for each assay):

Master Mix for M11589 WT Assay:

Component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
VCLAD F	IDT	25µM	0.3
VCLAD R	IDT	25µM	0.3
sterile water			5.4

Master Mix for M11589 KO Assay:

Component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma	2X	10
Neo F	IDT	25µM	0.3
Neo R	IDT	25µM	0.3
sterile water			5.4

PCR Setup:

Both assays final reaction: 16µl master mix & 4µl DNA template (10-20ng/µl DNA)

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

Cycle Parameters (both assays):

- 1) 94°C 3 minutes
- 2) 94°C 1 minute
- 3) 67°C 1 minute
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 10minutes
- 7) 4°C hold until refrigerate product

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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:

M11589 WT Assay product: 450 bp

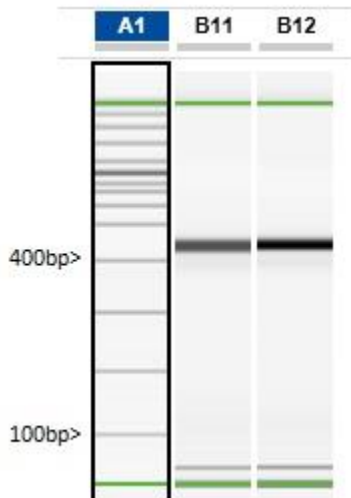
M11589 KO Assay product: 289 bp

Genotype Analysis:

	M11589 WT Assay	M11589 KO Assay
Wild-type	450bp product	No product
Heterozygous	450bp product	289bp product
Homozygous Mutant	No product	289bp product

Example Gels:

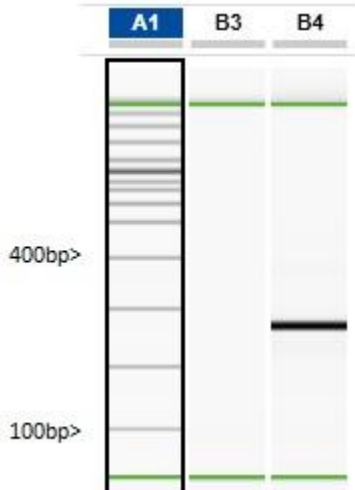
M11589 WT Assay:



Lane A1 displays a 15bp-3kb size marker
Lanes B11 and B12 display samples positive for the wild-type allele (450bp product)

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

M11589 KO Assay:



Lane A1 displays a 15bp-3kb marker
Lane B3 displays a sample negative for the KO allele (no product)
Lane B4 displays a sample positive for the KO allele (289bp product)

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