09.01.10 MS
08.01.16 MLS

Genotyping Protocol: **MMRRC 442**

Assay Type: PCR (can distinguish heterozygous animals from homozygous animals) detects presence of mutations in both the *Chrna5* (α5) and *Chrnb4* (β4) genes**.**

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.

Strain Information: A neo cassette replaces most of Exon 5 in the *Chrna5* gene. Most of Exon 5 is deleted in the *Chrnb4* gene. Details can be found in Kedmi et al (2004) Physiol. Genomics 17:221-229.

A5 Primer Information:

1) Name: A5 Wt f Sequence: 5'- GTG AAA GAG AAC GAC GTC CGC -3'
2) Name: A5 Wt R Sequence: 5'- GCC TCA GCC CCT GAA TGG TAG -3'
3) Name: Neo-1 Sequence: 5'- CTT TTT GTC AAG ACC GAC CTG TCC -3'
4) Name: Neo-2 Sequence: 5'- CTC GAT GCG ATG TTT CGC TTG GTG -3'

B4 Primer Information:

1) Name: B4 Rev.wt Sequence: 5'-AGTGAATGACAGATGGACCTC -3'
2) Name: B4 Rev.mut Sequence: 5'- AGTCAAAACCTTGCACCAG -3'
3) Name: B4 Forward Sequence: 5'- AGTCAAAACCTTGCACCAG -3'

Assay names: A5 PCR, B4 PCR

PCR Master Mix Components:

Master Mix for A5 assay:

Component	Manufacturer	Concentration	µl/rxn
Extract-N-Amp Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
A5 Wt F	Sigma-Genosys	25 µl	0.3
A5 Wt R	Sigma-Genosys	25 µl	0.3
Neo-1	Sigma-Genosys	25 µl	0.3
Neo-2	Sigma-Genosys	25 µl	0.3
water			4.8

Master Mix for B4 assay:

Component	Manufacturer	Concentration	µl/rxn
Extract-N-Amp Reaction mix	Sigma	2X	10
B4 Rev.wt	Sigma-Genosys	25 µl	0.3
B4 Rev.mut	Sigma-Genosys	25 µl	0.3
B4 Forward	Sigma-Genosys	25 µl	0.3
water			5.1

PCR Setup:

Both assays final reaction: 16µl master mix & 4µl DNA template (10-20 ng/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) 58°C 1 minute

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

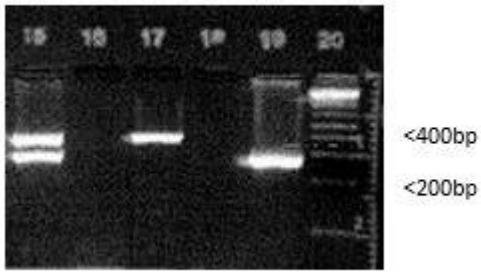
Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining

Expected products:
A5 assay Hom mutant = 290 bp
WT = 380 bp
Het = 290 and 380 bp

B4 assay Hom mutant = 150 bp
WT = 300 bp
Het = 150 and 300 bp

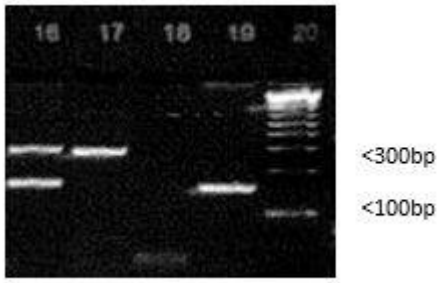
Example gels:

A5 Assay:



Lane 15 displays a heterozygous sample (290bp and 380bp products)
Lane 17 displays a WT sample (380bp product)
Lane 19 displays a homozygous mutant sample (290bp product)
Lanes 16 and 18 are PCR blanks
Lane 20 is 1kb+ Ladder (Invitrogen Cat#10787-018)

B4 Assay:



Lane 16 displays a heterozygous sample (150bp and 300bp products)
Lane 17 displays a WT sample (300bp product)
Lane 19 displays a homozygous mutant sample (150bp product)
Lane 18 is a PCR blank
Lane 20 is 1kb+ Ladder (Invitrogen Cat#10787-018)