Genotyping Protocol: **MMRRC 32781**

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.

Strain Description: This strain carries a transgene which contains cre driven by the mouse otoferlin gene (*Otof*) promoter.

Primer Information:

1) Name: Otof.wtF Sequence: 5'-CTG AAG GAG CAG CTT GCT TT-3'
2) Name: Otof.wtR Sequence: 5'-ACT TTG GCA ATC CGG TCA C-3'
3) Name: Cre.R Sequence: 5'-GCA AAC GGA CAG AAG CAT TT-3'

Primer location: Otof.wtF is located before exon 1 and Otof.wtR is located in exon 1 of the *Otof* gene. Cre.R is located in the Cre gene.

Assay name: Otof-Cre PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
Otof.wtF	Sigma	25µM	0.3
Otof.wtR	Sigma	25µM	0.3
Cre.R	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 Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

1) 95°C 3 minutes
2) 94°C 30 seconds
3) 63°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

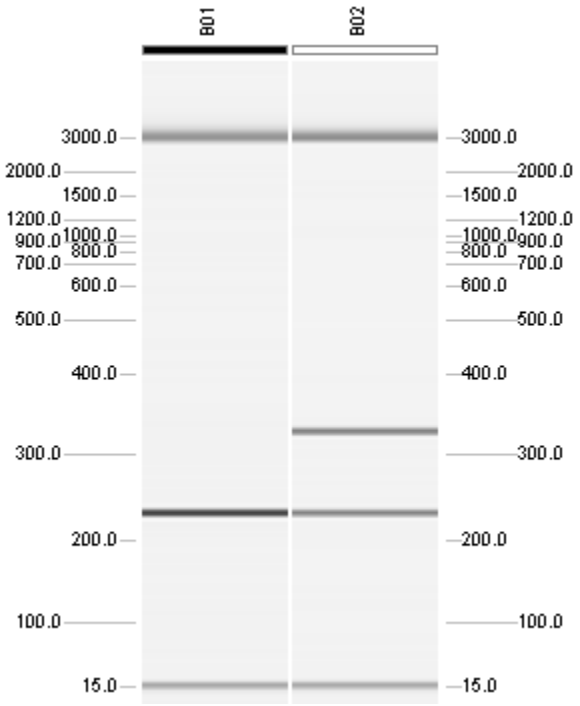
Product Analysis:
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

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 product:
Wild type: 219bp
Mutant: 323bp

Example gel:



Lane B01 displays a WT sample (219bp band).
Lane B02 displays a heterozygous sample (219bp and 323bp bands).

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