

Genotyping Protocol: **MMRRC 32099**

**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.

**\*MMRRC 32099, MMRRC 32826 and MMRRC 32828 all carry the same mutation. The difference between the three strains is the background strain \***

**M32099 Current Background Strain:** 129S6/SvEvTac

**Strain Description:** This strain has a Neomycin cassette inserted into Exon 5 of the peroxisomal membrane protein 3 gene (*Pex2* or *Pxmp3*) on Chromosome 3. This replaces the entire coding region of the gene. Details can be found in Faust P.L. and Hatten M.E. (1997) Journal of Cell Biology vol. 139 no. 5: 1293-1305.

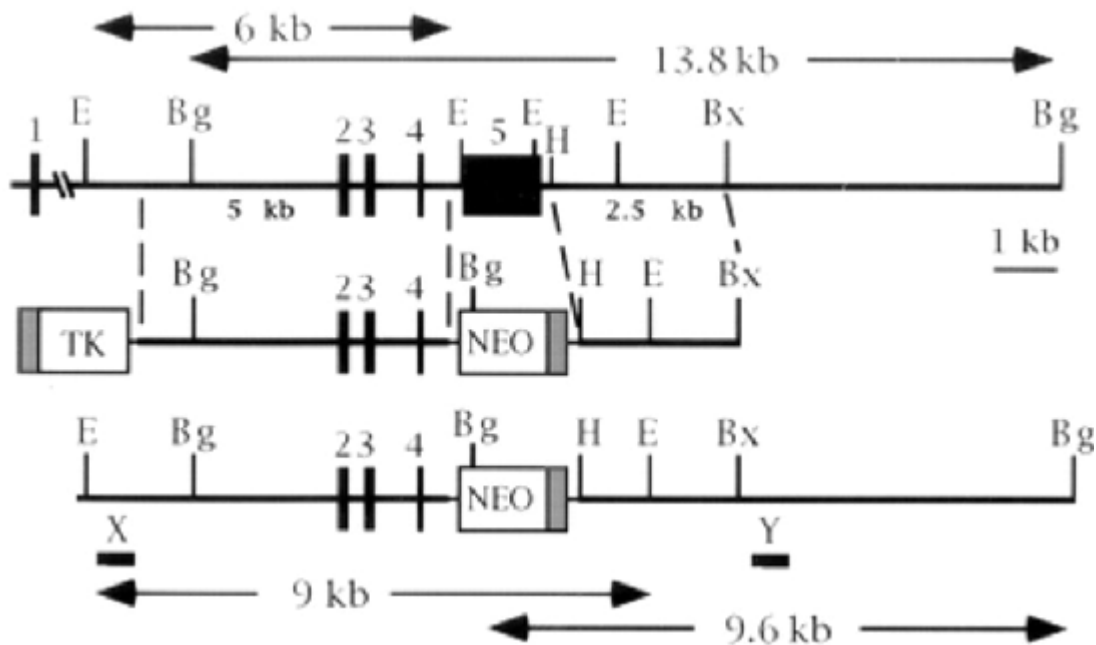


Image from Faust P.L. and Hatten M.E. (1997) Journal of Cell Biology vol. 139 no. 5: 1293-1305.

**Primer Information:**

- |                     |   |
|---------------------|---|
| 1) Name: Pxmp3.F    | Sequence: 5'-GGG ATA GGG TCA AGA TAT AAA GG-3'  |
| 2) Name: Pxmp3.wtR  | Sequence: 5'-TAG ATG GTG AAC CTC CAC AGG AAA-3' |
| 3) Name: Pxmp3.mutR | Sequence: 5'-ATG CCT GCT TGC CGA ATA TCA TG-3'  |

**Primer location:** Pxmp3.F is located in intron 4. Pxmp3.wtR is located in exon 5 after the start codon. Pxmp3.mutR is located in the inserted Neomycin cassette.

**Assay name:** Pex2 PCR

**PCR Master Mix Components:**

component	manufacturer	concentration	µl/rxn
Buffer with MgCl <sub>2</sub> (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
Pxmp3.F	Sigma or IDT	25µM	0.3
Pxmp3.wtR	Sigma or IDT	25µM	0.3
Pxmp3.mutR	Sigma or IDT	25µM	0.3
FastStart <i>Taq</i>	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) 58°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/5kb (Cat# 929524)

Size Marker: QX DNA Size Marker 250bp-4kb (Cat# 929562)

Method: AM320

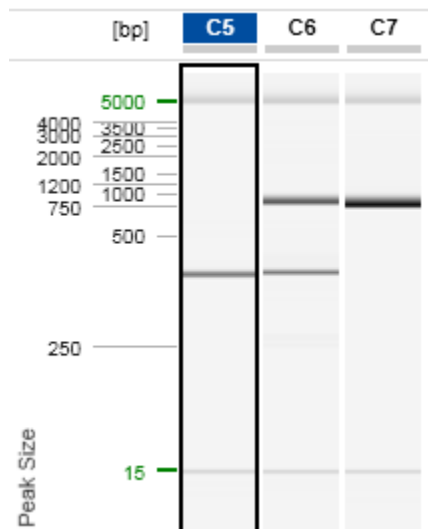
Injection: 10s at 5KV

Separation: 320s at 6KV

**Expected products:**

Wild Type allele: 400 bp

Mutant allele: 800 bp

**Example gel:**

Lane C5 displays a wild type sample (400bp product only)  
 Lane C6 displays a heterozygous sample (400bp and 800bp products)  
 Lane C7 displays a homozygous mutant sample (800bp product only)

\*Please note: the 15bp and 5kb bands are reference markers specific to the QIAxcel method and do not represent expected products.\*