

Genotyping Protocol: **MMRRC 30988**

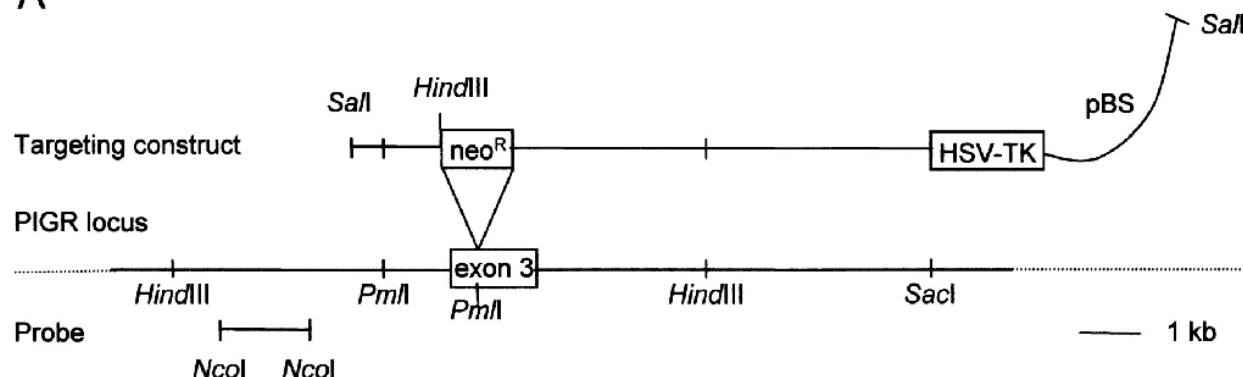
Assay Type: PCR- can distinguish heterozygous animals from homozygous animals

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 has a knock-out of the polymeric immunoglobulin receptor gene (*Pigr*) on Chromosome 1. Details can be found in Johansen et al (1999) J Exp Med 190:915–922.

Current background strain: C57BL/6

A



Primer Information:

- 1) Name: *Pigr* KO A Sequence: 5'-GAA CTC TTG TCT TTT GTC TCC-3'
- 2) Name: *Pigr* KO B Sequence: 5'-TCC AGA CTG CCT TGG GAA A-3'
- 3) Name: *Pigr* WT A Sequence: 5'-GAA CTC TTG TCT TTT GTC TCC-3'
- 4) Name: *Pigr* WT B Sequence: 5'-CTC GCC TGA ATA CTC CTT G-3'

Primer location: *Pigr* WT A is located immediately upstream of exon 3, and *Pigr* WT B is located within exon 3 of the *Pigr* gene. *Pigr* KO A is located immediately upstream of exon 3 and *Pigr* KO B is located in the targeting vector.

Assay name: *Pigr* PCR

Mutant 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
<i>Pigr</i> KO A	Sigma	25µM	0.3
<i>Pigr</i> KO B	Sigma	25µM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup:

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

09.04.09 MS
02.09.12 MS updated

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 20 seconds
- 3) 61°C 25 seconds
- 4) 72°C 30 seconds
- 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

Expected product: 150bp mutant band

WT 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
Pigr WT A	Sigma	25µM	0.3
Pigr WT B	Sigma	25µM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup:

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

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Cycle Parameters:

- 1) 95°C 3 minutes
- 2) 94°C 20 seconds
- 3) 58°C 25 seconds
- 4) 72°C 30 seconds
- 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

Expected product: 226bp wild type band

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/1000bp (Cat# 929521)

Size Marker: QX DNA Size Marker 50-800bp (Cat# 929556)

Method: AH320 Injection: 20s at 2kV

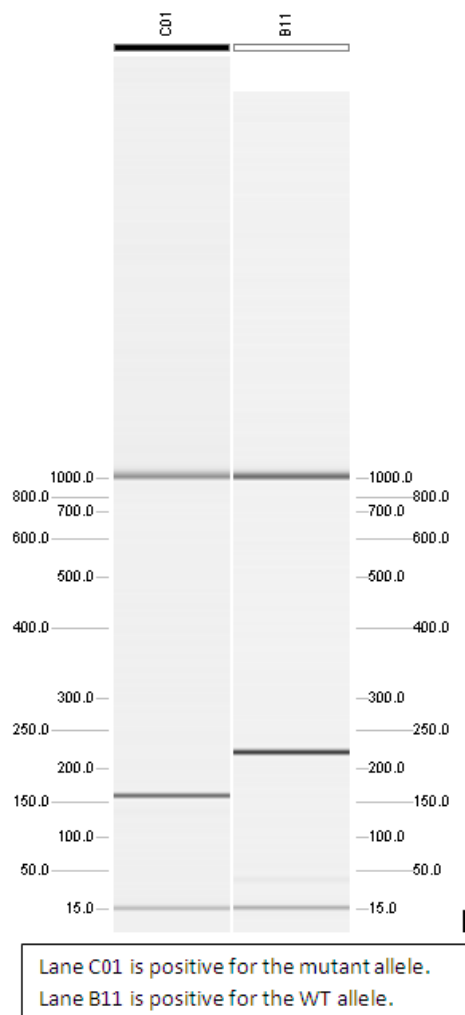
Separation: 320s at 6kV

09.04.09 MS
02.09.12 MS updated

Product Analysis:

Genotype	WT PCR assay	Mutant PCR assay
Wild Type (WT)	226 bp	no product
Heterozygous	226 bp	150 bp
Homozygous mutant	no product	150 bp

Example Gel:



Please note: the 15bp and 1kb bands are markers specific to the Qiaxcel method and do not represent expected products.