

## Genotyping Protocol: MMRRC 30089

**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 200ul of AE buffer once.

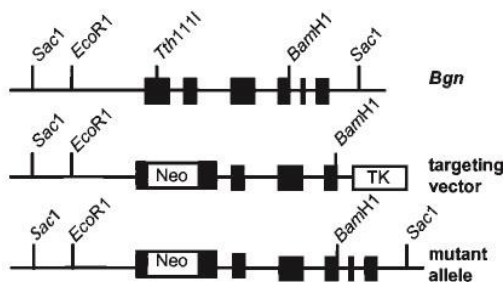
**Strain Description:** This strain has a PGK-neo cassette inserted into exon 2 of the biglycan gene (*Bgn*) at the *Tth1111* site. *Bgn* is located on Chromosome X. Details can be found in Xu et al (1998) Nature Genetics 20, 78-82.

**Primer Information:**

- |                                 |   |
|---------------------------------|---|
| 1) Forward Primer Name: Exon2-5 | Sequence: 5'-CAG GAA CAT TGA CCA TG-3'        |
| 2) Reverse Primer Name: Exon2-3 | Sequence: 5'-GAA AGG ACA CAT GGC ACT GAA G-3' |
| 3) Forward Primer Name: PGK1    | Sequence: 5'-TGG ATG TGG AAT GTG TGC GAG G-3' |

Primer location: Exon 2-5 & Exon 2-3 = *Bgn* Exon 2.

PGK1 = Neo cassette insertion into exon 2 just 5' to the start of translation. Use with Exon2-5 primer to detect mutant allele



**Assay Name: BGN<sup>tm1</sup> 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
Exon2-5	Sigma	25µM	0.3
Exon2-3	Sigma	25µM	0.3
PGK10	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 an Applied Biosystems 2700 thermocycler.

**Cycle Parameters:**

- 1) 95°C 5 minutes
- 2) 94°C 1 minute
- 3) 61°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 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 100bp-3kb (Cat# 929553)

Method: AH320

Injection: 20s at 2kV

Separation: 320s at 6kV

**Expected products:** Wild type allele: 212 bp  
Mutant allele: 310 bp

**Genotyping:**

*Females:*

WT: 212bp band only

Heterozygous: 212bp and 310bp bands

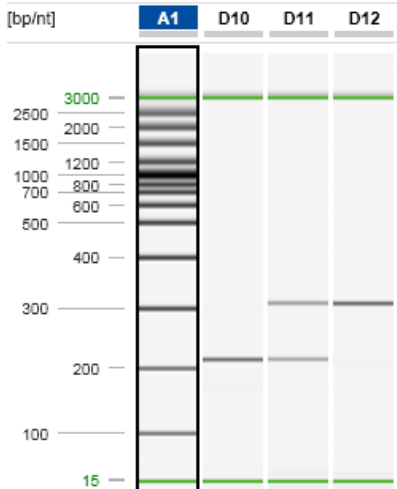
Homozygous mutant: 310bp band only

*Males:*

WT: 212bp band only

Hemizygous: 310bp band only

**Example gel:**



Lane A1 displays a 15bp-3kb size marker  
Lane D10 displays a WT sample (212bp product)  
Lane D11 displays a heterozygous sample (212bp and 310bp products)  
Lane D12 displays a homozygous mutant sample (310bp product only)

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