

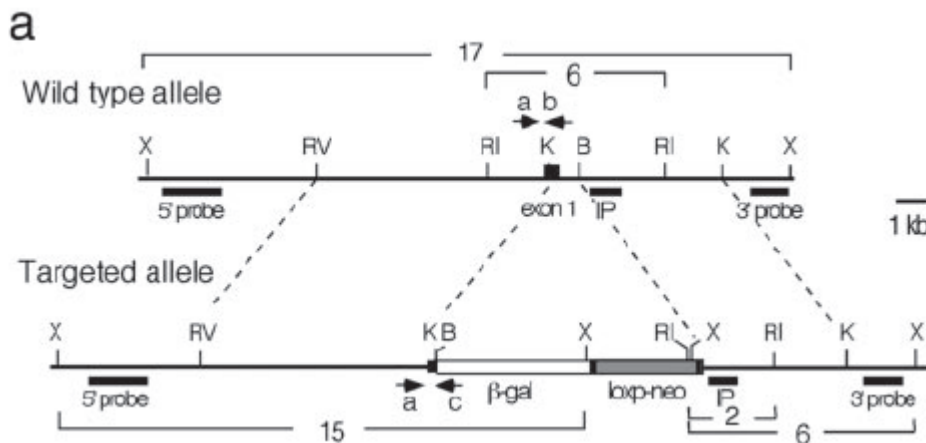
09.25.09 MS  
 08.02.10 HB updated  
 12.17.10 MS updated  
 02.19.14 MLS

## Genotyping Protocol: **MMRRC 30759**

**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 1kb deletion encompassing 194bp of the 3' end of exon 1 and ~800bp of intron 1 of the slit homolog 3 gene (*Slit3*) on Chromosome 11. The deletion is replaced by a beta-galactosidase gene and a loxp-PGK-neo selection cassette. Details can be found in Yuan et al (2003) Proc Natl Acad Sci USA 100:5217-22.



### Primer Information:

- |                            |  |
|----------------------------|--|
| 1) Name: M30400 Z2046 anti | Sequence: 5'-TCG TCT GCT CAT CCA TGA CC-3'     |
| 2) Name: M30400 Z1924      | Sequence: 5'-GTC TCG TTG CTG CAT AAA CC-3'     |
| 3) Name: M30756 slit3 F    | Sequence: 5'-GTA CCT GGG CAC CGT GAG TGA TG-3' |
| 4) Name: M30756 slit3 R    | Sequence: 5'-GCG GAG CAG GTA CAC TTG GTG G-3'  |

**Primer location:** M30400 Z2046 anti and Z1924 are both located within the insertion. M30756 slit3 F and R are both located within the *Slit3* gene on Chromosome 11.

### Assay name: Slit-3 PCR

### PCR Master Mix Components:

Mut PCR:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl <sub>2</sub> (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30400 Z2046 anti	Sigma	25µM	0.3
M30400 Z1924	Sigma	25µM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

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WT PCR:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl <sub>2</sub> (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30756 slit3 F	Sigma	25µM	0.3
M30756 slit3 R	Sigma	25µM	0.3
DMSO		5%	2.5
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			10.5

**PCR Setup (both mixes):**

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.

Mut PCR:

**Cycle Parameters:**

- 1) 95°C 5 minutes
- 2) 94°C 1 minute
- 3) 63.5°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

WT PCR:

**Cycle Parameters:**

- 1) 95°C 5 minutes
- 2) 94°C 1 minute
- 3) 68°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/1kb (Cat# 929521)

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

Method: AH320 Injection: 20s at 2kV

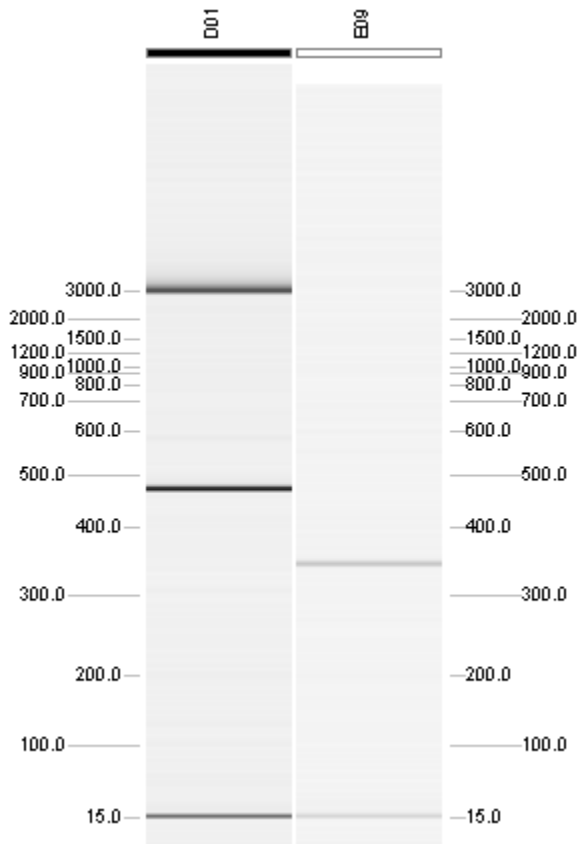
Separation: 320s at 6kV

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**Expected products: Slit3:** WT: 362bp  
Mutant: 500 bp

Genotype:	Mut PCR:	WT PCR:
WT	no product	362bp
Het	500bp	362bp
Hom	500bp	no product

**Example gel:**



Lane D01 displays a mutant band (500bp), and Lane E09 displays a wild type band (362bp).

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