07.07.17 MLS

Genotyping Protocol: **MMRRC 41409**

Assay Type: PCR; can distinguish homozygous animals from heterozygous samples.

DNA Extraction: DNA from tail snips was extracted using the following protocol:

1. Place a ~3mm piece of mouse or rat tail in 180ul of 50mM NaOH in a 0.2mL tube and vortex
2. Incubate at 95°C for 10 minutes, then at 40°C for 5 minutes to prevent popped tube caps.
3. Add 20ul of 1M Tris-HCl (pH 8.0) and vortex well. Use this lysate as your DNA template in KOD Xtreme PCR reactions.

Strain Description: This strain was created using a gene trap protocol. The vector pGT0lxf was inserted into intron 13 of mouse *Svil*. This vector contains an intron from the murine engrailed 2 gene upstream of a Beta-galactosidase/neomycin resistance (Beta-geo) fusion protein. Details can be found in Edelstein et al (2012) Circulation 125:2762-2771.

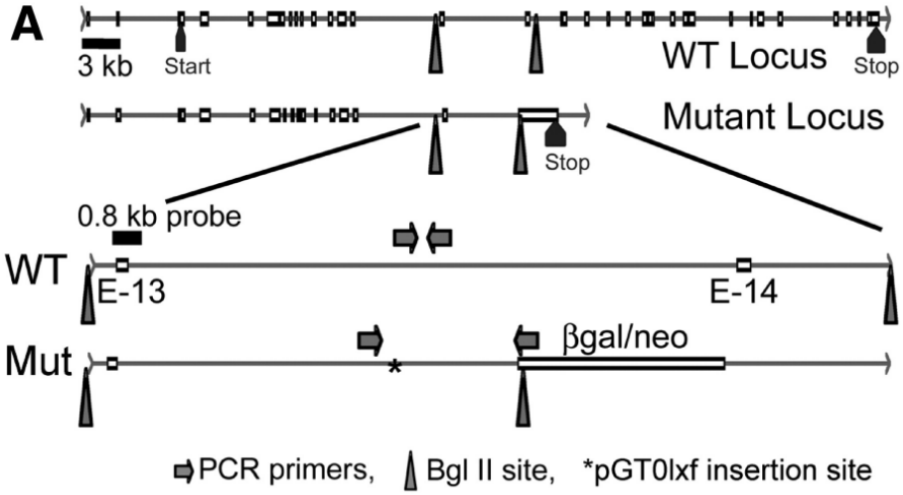


Image from Edelstein et al (2012) Circulation 125:2762-2771.

Primer Information:

1) Name: M41409 WT F Sequence: 5'-CAT GCT GAT GGA TTA GTG TTC TCA GGG ATA AGT CCT TTA C-3'
2) Name: M41409 WT R Sequence: 5'-GCA GAA GCA GAA TAC TGG CAG AAG GTC TAC TTT ACA TCT T-3'
3) Name: M41409 Mut F Sequence: 5'-GCC TTT GGT ATT CTA CCC AAA AGA TGG TTA TAC CCA G-3'
4) Name: M41409 Mut R Sequence: 5'-CAT CAA CAT TAA ATG TGA GCG AGT AAC AAC CCG TC-3'

Primer location: M41409 WT F and R and M41409 Mut F are located in Intron 14 of mouse *Svil*. M41409 Mut R is located in the inserted Beta galactosidase.

Assay Name: Svil KO PCR

Wild Type PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
KOD Xtreme Buffer	Millipore	2X	10
KOD Xtreme dNTPs	Millipore	2mM	4
M41409 WT F	Sigma or IDT	25µM	0.3
M41409 WT R	Sigma or IDT	25µM	0.3
KOD Xtreme *Taq*	Millipore (Cat# 71975-3)	1 U/µl	0.4
sterile water			3

Mutant PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
KOD Xtreme Buffer	Millipore	2X	10
KOD Xtreme dNTPs	Millipore	2mM	4
M41409 Mut F	Sigma or IDT	25µM	0.3
M41409 Mut R	Sigma or IDT	25µM	0.3
KOD Xtreme *Taq*	Millipore (Cat# 71975-3)	1 U/µl	0.4
sterile water			3

PCR Setup:
Final Reaction: 18 µl master mix & 2 µl extracted DNA (10-20ng DNA).

All reactions were performed in 200 µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Wild Type PCR Cycle Parameters:
1) 95°C 4 minutes
2) 94°C 1 minute
3) 70°C 1 minute
4) 72°C 1 minute, 30 seconds
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 7 minutes
7) 4°C hold until refrigerate product

Mutant PCR Cycle Parameters:
1) 95°C 4 minutes
2) 94°C 1 minute
3) 65°C 1 minute
4) 72°C 2 minutes, 30 seconds
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 7 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).

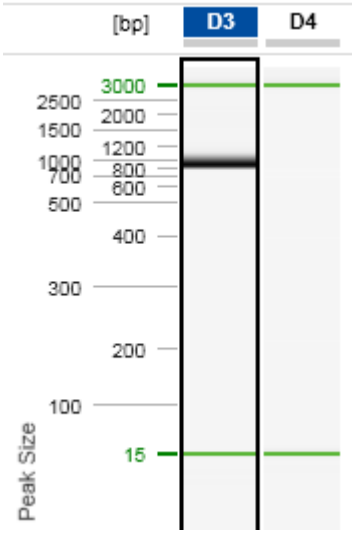
Alignment and Size Markers:
Wild Type PCR: QX Alignment Marker 15bp/3Kb (Cat# 929522) Size Marker: QX DNA Size Marker 100-3Kb (Cat# 929553)
Mutant PCR: QX Alignment Marker 15bp/5Kb (Cat# 929524) Size Marker: QX DNA Size Marker 250-4Kb (Cat# 929562)

Both PCRs use: Method: AM320 Injection: 10s at 5KV, Separation: 320s at 6KV

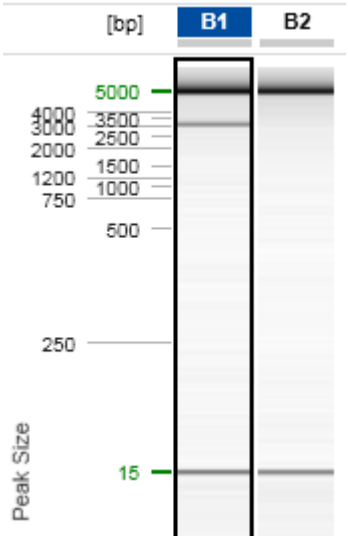
Expected products:
Wild Type PCR: 800bp product
Mutant PCR: 2.5kb product

Example Gels:

Wild Type PCR Gel:



Mutant PCR Gel:



Lane D3 displays a sample positive for the Wild Type allele (800bp product)
Lane D4 displays a sample negative for the Wild Type allele (no product)

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

Lane B1 displays a sample positive for the Mutant allele (2.5kb product)
Lane B2 displays a sample negative for the Mutant allele (no product)

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

Genotyping Analysis:

	Wild Type PCR	**Mutant PCR**
Wild-type	800bp product	no product
Heterozygous	800bp product	2.5kb product
Homozygous mutant	no product	2.5bp product