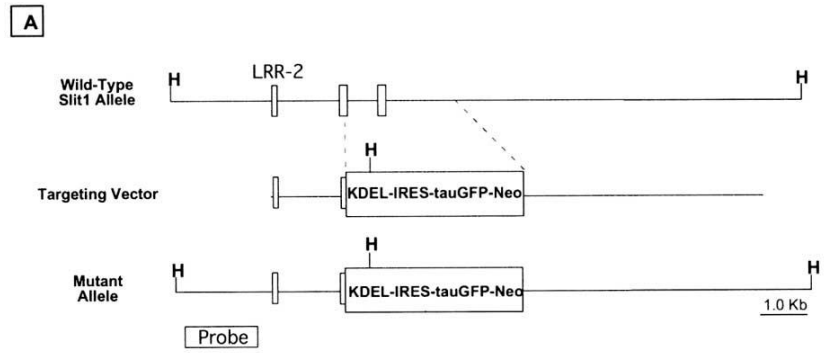
3-9-09 MS
11.01.11 MS updated
02.19.14 MLS

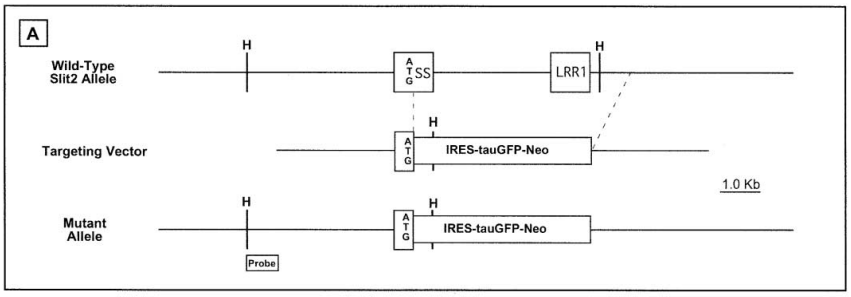
Genotyping Protocol: MMRRC 30404

Strain Characteristics: This strain carries mutations in both the *Slit1* and *Slit2* genes. In both cases, the genes were disrupted by insertion of targeting cassettes. Details can be found in Pump et al (2002) Neuron 33:219-232.

Slit1



Slit2



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.

Assay names: Slit-1 PCR, Slit-2 PCR

A. Slit-1 PCR:

Primer Information:

1) Name: M30404 OAP17	Sequence: 5'-TCT CCT TTG ATC TGA GAC CG-3'
2) Name: M30404 OAP18	Sequence: 5'-AGG TTT CTC GAG CGT CAT AG-3'
3) Name: M30404 OAP 19	Sequence: 5'-ACC CTT AGC TTC TAC CAA CC-3'
4) Name: M30404 OAP 16	Sequence: 5'-AAG ATG CCT CCT CTG ACT TC-3'

Primer Location: Both M30404 OAP16 and 19 are located in the *Slit1* gene on Chromosome 19. M30404 OAP17 and 18 are located in the inserted targeting cassette.

PCR Master Mix Components:
Mutant PCR (Slit1 Mut):

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component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30404 OAP17	Sigma	25µM	0.3
M30404 OAP18	Sigma	25µM	0.3
FastStart *Taq*	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			13

WT PCR (Slit1 WT):

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTPs	Promega	1.25mM	3.2
M30404 OAP16	Sigma	25µM	0.3
M30404 OAP19	Sigma	25µM	0.3
FastStart *Taq*	Roche	5 U/µl	0.2
sterile water			13

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:
Mut PCR:

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:

1)	95°C	5 minutes
2)	94°C	1 minute
3)	66°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 a 3% agarose gel with ethidium bromide staining.

Heterozygous: 250bp, 400bp
Homozygous mutant: 400bp
Wild Type: 250bp

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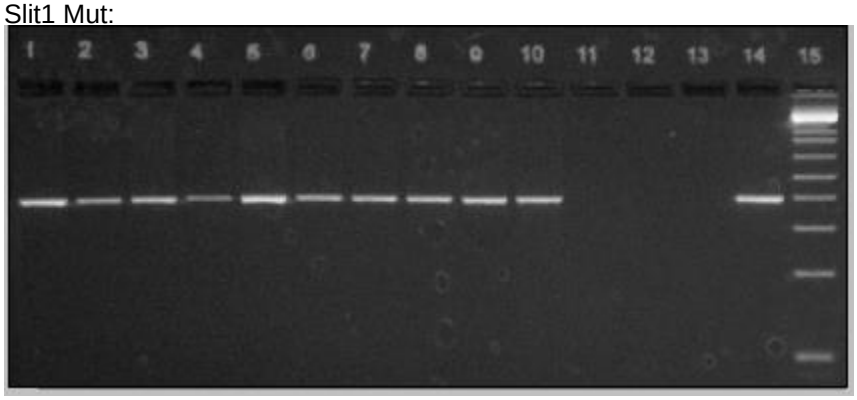
Example gel:

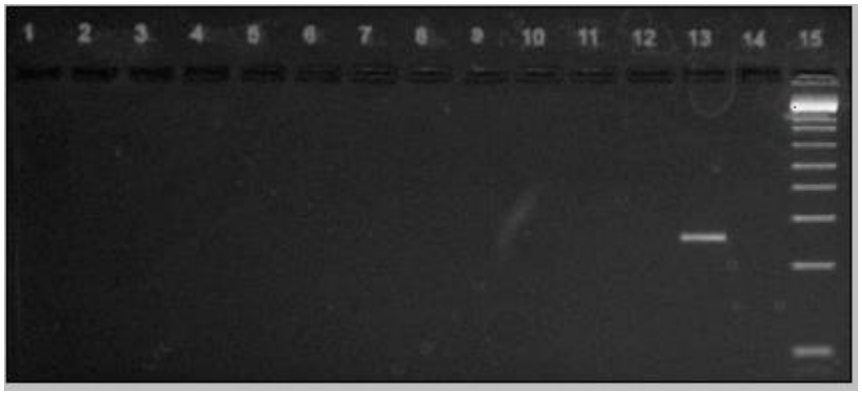
Slit1 Mut:



<400bp

Wells 1-10 are positive for the mutant allele. Wells 11 and 12 are blanks. Well 13 is a WT control and Well 14 is a homozygous *Slit1* mutant control. Well 15 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).

Slit1 WT:



< 250bp

Wells 1-10 are negative for the WT allele. Wells 11 and 12 are blanks. Well 13 is a WT control and Well 14 is a homozygous *Slit1* mutant control. Well 15 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).

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B. Slit-2 PCR:

Primer Information:

1) Name: M30404 OAP20 Sequence: 5'-AAG ACC TGT GCT TCT GTC AG-3'
2) Name: M30404 OAP21 Sequence: 5'-AAG TCT AGT AGA GTC GAG CG-3'
3) Name: M30404 OAP22 Sequence: 5'-AAA CAG GTT TCT ACC GCA CG-3'

Primer location: M30404 OAP20 and 22 are located within the *Slit2* gene on Chromosome 5. M30404 OAP 21 is located in the inserted targeting cassette.

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30404 OAP20	Sigma	25µM	0.3
M30404 OAP 21	Sigma	25µM	0.3
M30404 OAP22	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 Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

1) 95°C 5 minutes
2) 94°C 1 minute
3) 67°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 a 3% agarose gel with ethidium bromide staining.

Heterozygous: 300bp, 500bp
Homozygous mutant: 300bp
Wild Type: 500bp

1Kb+ Ladder (Invitrogen Cat# 10787-018)