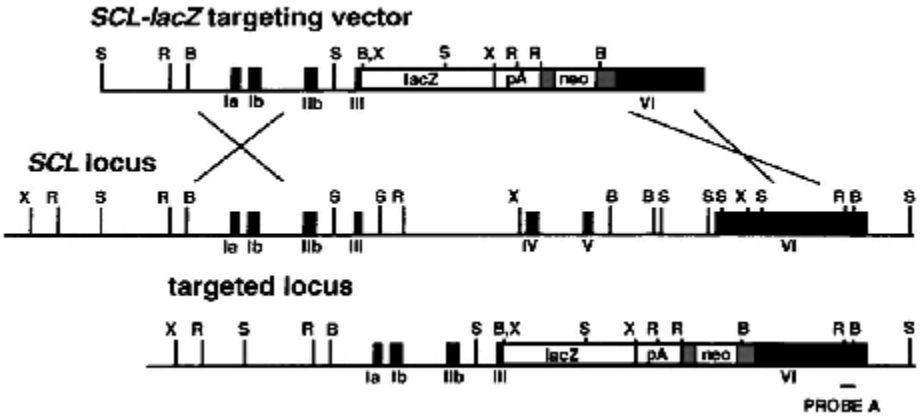
03.15.11 MS
02.24.14 MLS

Genotyping Protocol: **MMRRC 30665**

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 Characteristics: This strain has the LacZ gene knocked in to the T-cell acute lymphocytic leukemia 1 gene (*Tal1*), which results in the deletion of exons 4 and 5. This strain is homozygous lethal at ~day 8.5 due to failure of vascular system development in the embryo. Details can be found in Elefanty et al (1998) Proc Natl Acad Sci USA 95(20):11897-902.



Primer Information:

1) Name: Tal1.mutF	Sequence: 5'-ATA TTG CTG AAG AGC TTG GCG GC-3'
2) Name: Tal1.wtF	Sequence: 5'-GTT TTG GTC TAG AGT TTG TGA GCC-3'
3) Name: Tal1.R	Sequence: 5'-GCA TGC TCA AGG CTG CTG ACT TGG-3'

Primer location: Tal1.mutF is located in the inserted LacZ cassette. Tal1.wtF and Tal1.R are located in exon 5.

Assay name: Tal1 KI PCR

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
Tal1.mutF	Sigma	25µM	0.3
Tal1.wtF	Sigma	25µM	0.3
Tal1.R	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:

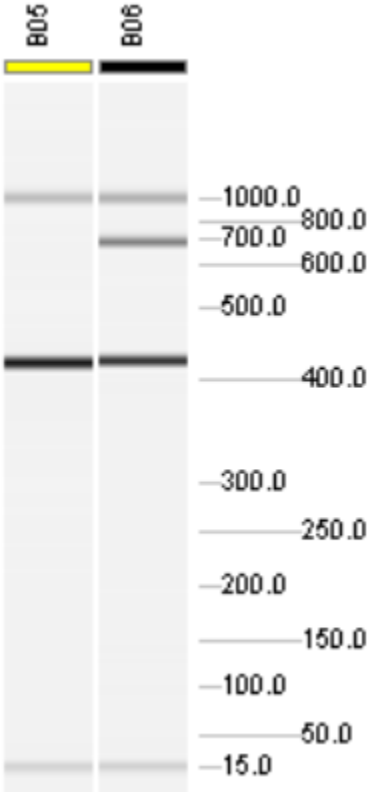
1)	95°C	3 minutes
2)	94°C	30 seconds5
3)	66°C	30 seconds
4)	72°C	30 seconds
5)	Repeat steps 2-4 34 times for a total of 35 cycles	
6)	72°C	10 minutes
7)	4°C	hold until refrigerate product

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: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected products:
WT: 424bp
Mut: ~650bp

Example gel:



Lane B05 displays a WT sample (424bp band).
Lane B06 displays a heterozygous sample (424bp and 650bp bands).

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