09.22.10 MS
07.09.14 MLS

Genotyping Protocol: **MMRRC 12039**

Strain Type: Lysozyme-GFP Knock-in gene

Assay Type: PCR- can distinguish heterozygous animals from homozygous animals.

DNA Extraction: DNA from tail snips was extracted using Sigma's Extract-N-Amp Tissue PCR Kit (Cat#XNAT2R). Kit directions for fresh or frozen tails were performed with a few minor modifications as follows: use 50 µl of Extraction Solution and 12.5 µl of Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Strain Description: This strain has an EGFP gene and a Tk-Neo cassette inserted into the first exon of the mouse lysozyme 2 (*Lyz2*) gene. This deletes 350bp of *Lyz2*, including the coding region of exon 1 and the start codon. Details can be found in Faust et al (2000) Blood 96:2.

Primer Information:
1) Name: mlysup Sequence: 5'- AAG CTG TTG GGA AAG GAG GG -3'
2) Name: EGFPDWN Sequence: 5'- GTC GCC GAT GGG GGT GTT CT -3'
3) Name: MLP1 Sequence: 5'- TCG GCC AGG CTG ACT CCA TA -3'

Primer Location :
Mlysup and MLP1 are located in the mouse *Lyz2* gene, mlysup just before exon 1 and MLP1 at the end of exon 1 (on either side of the GFP insertion site). EGFPDWN is located in the inserted GFP.

Assay Name: MMRRC Line 12039 Lysozyme EGFP PCR

Master Mix for M12039 Assay:

Component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
mlysup	Sigma	25µM	0.3
EGFPDWN	Sigma	25µM	0.3
MLP1	Sigma	25µM	0.3
sterile water			5.1

PCR Setup:
Final reaction: 16µl master mix & 4µl 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) 94°C 3 minutes
2) 94°C 30 seconds
3) 61°C 30 seconds
4) 72°C 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 10minutes
7) 4°C hold until refrigerate product

Product Analysis:
Products were analyzed on a 3% agarose gel with ethidium bromide staining and run for 1 hour at 100 volts.
WT product: 220 bp
Knock-in product: 680 bp

Homozygous KI mice= 680 bp product
WT mice= 225 bp product

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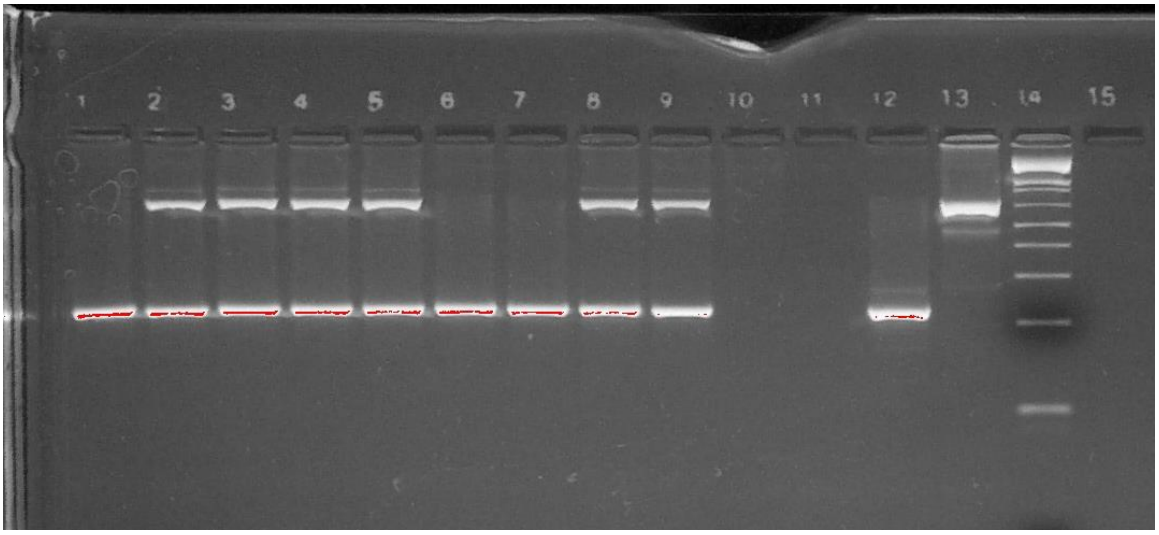
Heterozygous mice= 225 & 680 bp product

Example of Gel:



Lanes 1, 6, 7 display WT samples (220bp band).
Lanes 2-5, 8, 9 display heterozygous samples (220bp and 680bp bands).
Lanes 10 and 11 are extraction and PCR blanks, respectively.
Lane 12 is a WT control (220bp band) and Lane 13 is a homozygous control (680bp band).
Lane 14 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).