10.12.09 MS
12-31-09 EB

Genotyping Protocol: **MMRRC 31019**

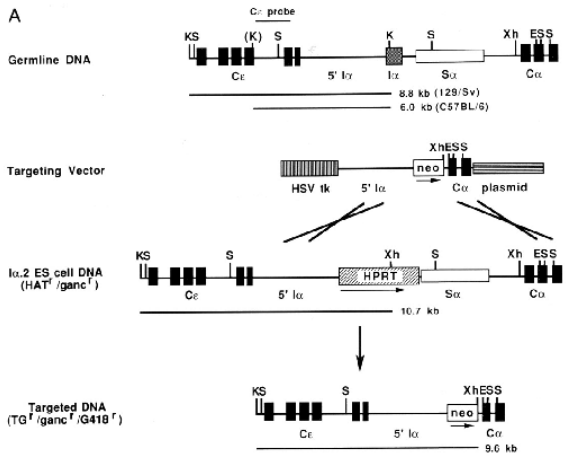
Assay Type: PCR- can distinguish heterozygous animals from homozygous animals

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 targeting vector which knocks-out the Iα exon, the entire switch region (Sα) and the 5' half of the constant region (Cα) of immunoglobulin heavy chain 2 (serum IgA) gene (*Igh-2*) on Chromosome 12. Details can be found in Harriman et al (1999) J Immunol 162:2521-2529.

This strain has an identical genetic alteration to M31020 – the two strains differ in the background strain.

Current background strain: C57BL/6



Primer Information:
1) Name: Iga KO A Sequence: 5'-GGA CAA GAG CTC ATT CAG G-3'
2) Name: Iga KO B Sequence: 5'-CCT TCT ATC GCC TTC TTG ACG-3'
3) Name: Iga WT A Sequence: 5'-CCA TCT GGA CTC CTC TGC TC-3'
4) Name: Iga WT B Sequence: 5'-GTC TCC TGT TGC TGC TTT CC-3'

Primer location: Iga WT A and Iga WT B are located around the Iα exon on Chromosome 12. Iga KO A and Iga KO B are located in the targeting vector.

Assay name: Igh-2 PCR

<u>Mutant (MUT) PCR</u>:

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
Iga KO A	Sigma	25µM	0.3
Iga KO B	Sigma	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

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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 seconds
3) 64°C 30 seconds
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

Expected product: 740bp mutant band

Wild Type (WT) 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
Iga WT A	Sigma	25µM	0.3
Iga WT B	Sigma	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
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3) 64°C 25 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

Expected products: 235bp wild type band

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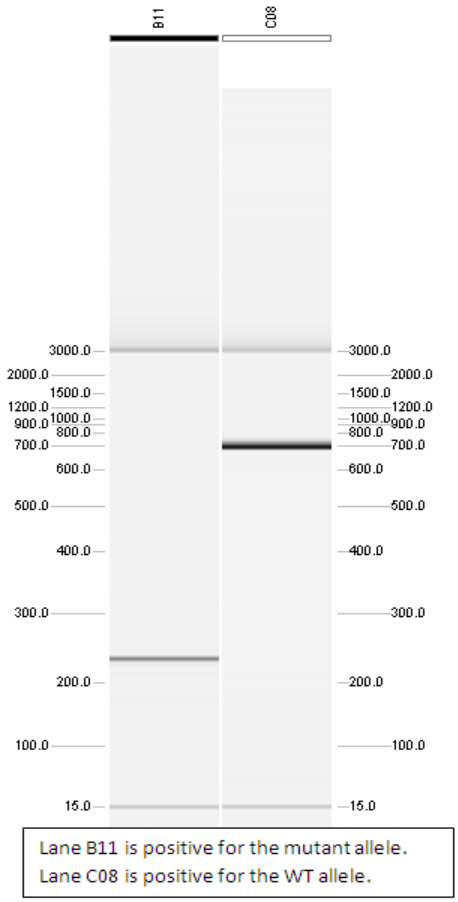
Product Analysis for Both Mutant and Wild Type PCR:

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/3kb (Cat# 929522)
Size Marker: QX DNA Size Marker 100bp-3kb (Cat# 929553)
Method: AH320 Injection: 20s at 2kV
Separation: 320s at 6kV

Wild Type: 235bp with WT PCR, no product with MUT PCR
Heterozygous: 235bp with WT PCR, 740bp with MUT PCR
Homozygous: no product with WT PCR, 740bp with MUT PCR

Example Gel:



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