

08.24.10 MS
12.21.15 MLS

Genotyping Protocol: MMRRC 316

Assay Type: PCR (cannot 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.

Primer Information:

1) Name: M316 (Hes5) F Sequence: 5'- TAT ATA GGG CTG GCG TGC TGG -3'
Genomic location: Chromosome 4, -104 to -84 of Hes5 start codon

2) Name: Gensat GFP Rev Sequence: 5'- TAG CGG CTG AAG CAC TGC A -3'
Binds to bases 206-224 of the GFP transgene

TATATACGGCTGGCGTGCTGGGGTCCAGGTCGCGCCAGTCCGGGACGCCTGGCTCTGTCCGCTCCGCTCCGCTCC
GCTCGCTAATCGCCTCCAGAGCTCCAGGCAT**GGCCCAAGTACCGTGGCGGTGGAGATGCTCAGTCCCAAG**AAATC
CCGGGATCCACCGGTCGCCACCATGGT**GAGCAAGGGCGAGGAGCTGTTCCACGGGGTGGTGCCCATCCTGGT**CGA
GCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCT
GACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCACCCTCGTGACCACCCTGACCTACGGC
GTGCAGTGCTTCAGCCGCTA

Chromosomal DNA (upstream and exon 1 of Hes 5 gene) in red (blue= start codon)

Vector sequence in black

EGFP transgene sequence in green

Assay Name: MMRRC Line 316 PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25 mM	3.2
M316 (Hes5) F	IDT	20 µM	0.3
Gensat GFP Rev	IDT	20 µM	0.3
FastStart <i>Taq</i>	Roche (Cat#12032953001)	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 Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 95°C 5 minutes
- 2) 94°C 1 minute
- 3) 68°C 1 minute
- 4) 72°C 1 minute
- 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

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Product Analysis:

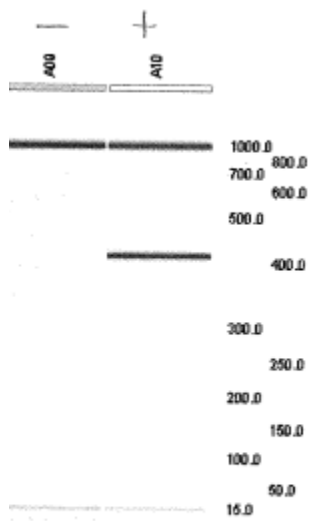
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/1kb (Cat# 929521)
Size Marker: QX DNA Size Marker 50-800bp (Cat# 929556)
Method: AM320 Injection: 10s at 5KV
 Separation: 320s at 6KV

Expected products:

Transgene positive: 396bp
Transgene negative: no product

Example Gel:



Lane A09 displays a transgene negative sample (no product).
Lane A10 displays a transgene positive sample (396bp product)

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