H/M Genotyping Protocol: **MMRRC Line 132**

Strain Characteristics: Random integration of the human interferon-mediated double-stranded RNA-activated protein kinase (PKR) cDNA.

Assay Type: PCR - can not distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy kit. 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: CMV-JM1 Sequence: CGC TAT TAC CAT GGT GAT GCG G
2) Name: PKR-CMV Sequence: TAC TTC ACG CTC CGC CTT CTC G

Primer location: CMV-JM1 = cytomegalovirus (CMV) promoter
PKR-CMV = Homo sapiens PKR cDNA spanning exons 11 & 12

PCR Master Mix Components:

Master Mix:

component	manufacturer	concentration	µl/rxn
buffer	Roche	10X	2
dNTP	Roche	1.25mM	3.2
CMV-JM1	IDT	25µM	0.3
PKR-CMV	IDT	25µM	0.3
FastStart taq	Roche	5 U/µl	0.2
sterile water			13

PCR Setup:
Final Reaction: 19µl master mix & 1µl DNA template

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) 94°C 5 minutes
2) 94°C 30 seconds
3) 69°C 30 seconds
4) 72°C 1 minute 30 seconds
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 7minutes
7) 4°C hold until refrigerate product

Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining
Expected product: 1.4 Kb