

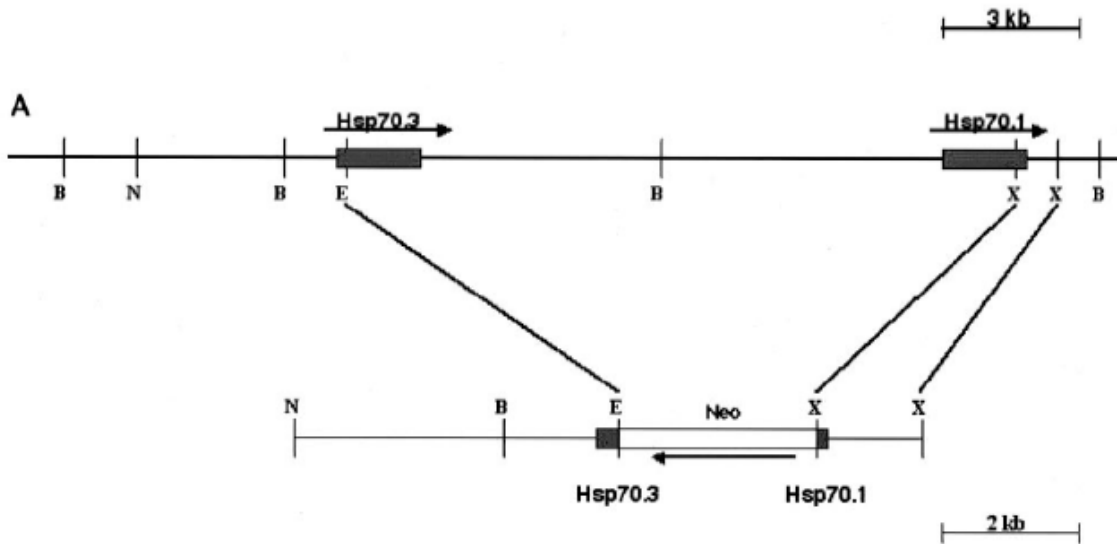
02.24.10 MS  
 08.02.10 HB updated  
 08.21.13 MLS

## Genotyping Protocol: MMRRC 30411

**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 carries a 3.1kb neo gene driven by an RNA polymerase II promoter, which was inserted in place of 11kb of genomic DNA separating the 5' end of heat shock protein 1A (*Hsp70.3* or *Hspa1a*) and the 3' end of heat shock protein 1B (*Hsp70.1* or *Hspa1b*). Details can be found in Hunt et al (2004) Mol. Cell. Biol. 24:899-911.



### Primer Information:

- |                        |  |
|------------------------|--|
| 1) Name: M30411 F12489 | Sequence: 5'-GAA CGG AGG ATA AAG TTA GG-3' |
| 2) Name: M30411 R13269 | Sequence: 5'-AGT ACA CAG TGC CAA GAC G-3'  |
| 3) Name: M30411 F191   | Sequence: 5'-GTA CAC TTT AAA CTC CCT CC-3' |
| 4) Name: M30411 R644   | Sequence: 5'-CTG CTT CTC TTG TCT TCG-3'    |

**Primer location:** M30411 F12489 is located in the inserted fragment. M30411 R13269 is located at the end of the *Hspa1b* gene, after exon 1. M30411 F191 and R644 are located on Chromosome 17 between *Hspa1a* and *Hspa1b*.

**Assay name:** MMRRC Line 30411 PCR

### Mutant PCR:

#### PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl <sub>2</sub> (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
M30411 F12489	Sigma	25µM	0.3
M30411 R13269	Sigma	25µM	0.3
FastStart <i>Taq</i>	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 45 seconds
- 3) 61°C 45 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

**Product Analysis:**

All products were analyzed on a 3% agarose gel with ethidium bromide staining.

**Expected product:** 780bp mutant band

**WT PCR:**

**PCR Master Mix Components:**

component	manufacturer	concentration	µl/rxn
Buffer with MgCl <sub>2</sub> (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
M30411 F191	Sigma	25µM	0.3
M30411 R644	Sigma	25µM	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 Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

**Cycle Parameters:**

- 1) 95°C 3 minutes
- 2) 94°C 20 seconds
- 3) 58°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

**Product Analysis:**

All products were analyzed on a 3% agarose gel with ethidium bromide staining.

**Expected product:** 454bp wild type band

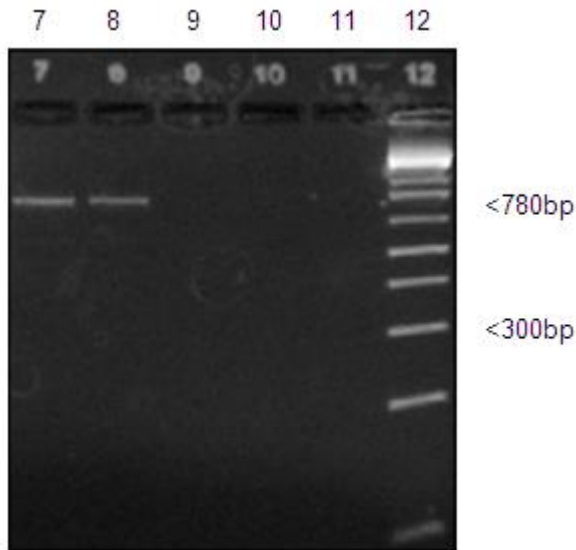
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**Product Analysis:**

<u>Genotype</u>	<u>WT PCR assay</u>	<u>Mutant PCR assay</u>
Wild Type	454 bp	no product
Heterozygous	454 bp	780 bp
Homozygous	no product	780 bp

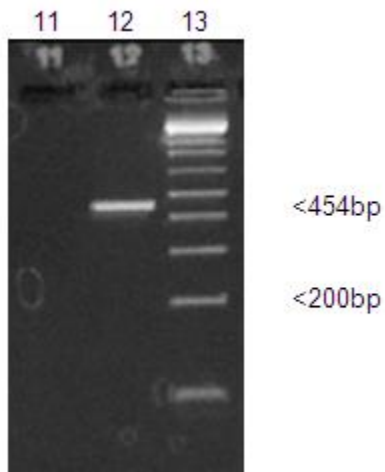
**Example Gels:**

**Mut PCR:**



Lanes 7 and 8 display samples positive for the mutant allele (780bp band).  
 Lanes 9 and 10 displays extraction and PCR controls, respectively.  
 Lane 11 displays a sample negative for the mutant allele (no product).  
 Lane 12 displays 1 Kb+ Ladder (Invitrogen Cat# 10787-018).

**WT PCR:**



Lane 11 displays a sample negative for the WT allele (no product).  
 Lane 12 displays a sample positive for the WT allele (454bp band).  
 Lane 13 displays 1 Kb+ Ladder (Invitrogen Cat# 10787-018).