

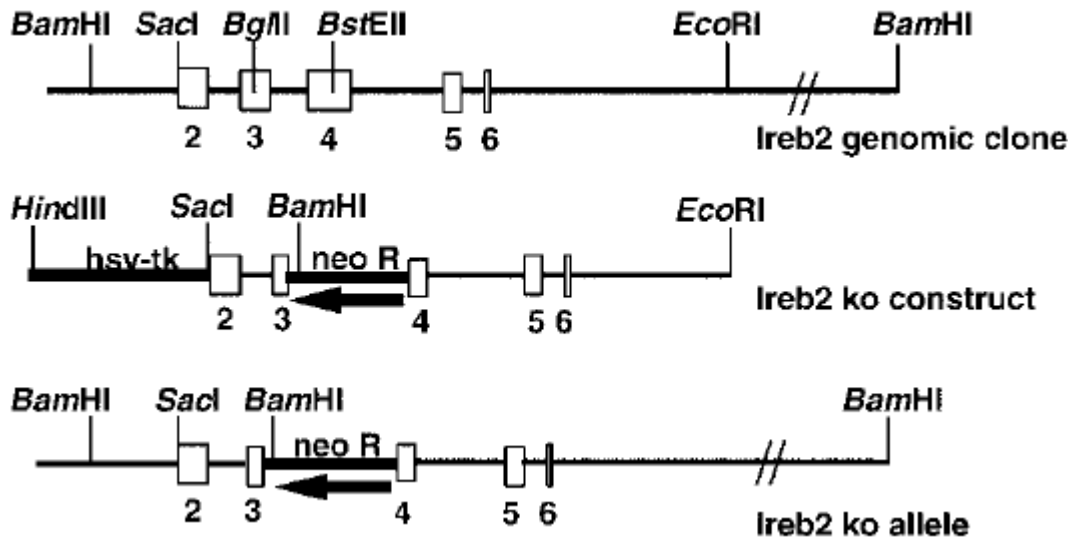
02.24.10 MS  
 08.02.10 HB updated  
 02.08.12 MS updated

## Genotyping Protocol: **MMRRC 30490**

**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 neo cassette inserted between exons 3 and 4 of the iron responsive element binding protein 2 gene (*Ireb2*) on Chromosome 9, deleting parts of exons 3 and 4. Details can be found in LaVaute et al (2001) Nature Genetics 27:209-214.



**Primer Information:**

- |                     |                                      |
|---------------------|--------------------------------------|
| 1) Name: M30490 SC1 | Sequence: AAA GAG TGG TCC ACT GTG AG |
| 2) Name: M30490 SC2 | Sequence: TGG AAT ACC GGC AAT GGT GG |
| 3) Name: M30490 SC3 | Sequence: AGC TCA TTC CTC CAC TCA TG |

**Primer location:** M30490 SC1 is located in exon 4, and M30490 SC2 is located just upstream of exon 4 of the *Ireb2* gene on Chromosome 9. M30490 SC3 is located in the inserted cassette.

**Assay name: Ireb2 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
M30490 SC1	Sigma	25µM	0.3
M30490 SC2	Sigma	25µM	0.3
M30490 SC3	Sigma	25µM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			12.7

**PCR Setup:**

Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

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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) 61°C 30 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:**

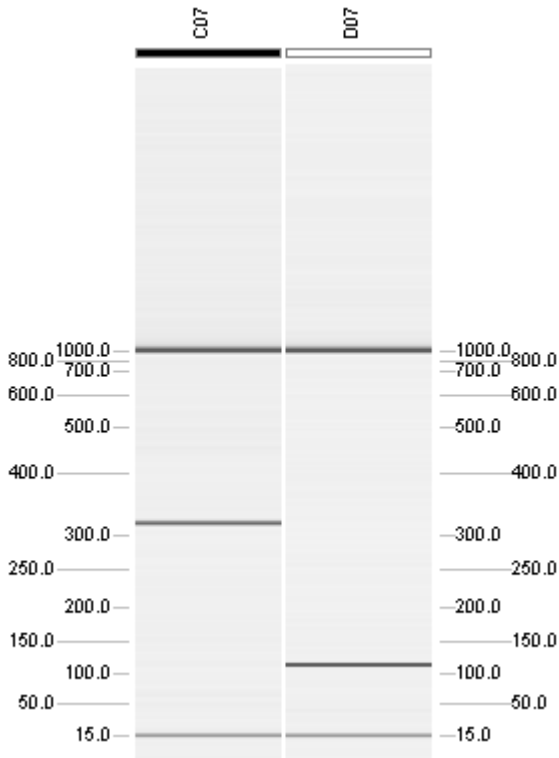
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

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:**

WT: 107bp  
Mut: 300bp

**Example gel:**



Lane C07 displays a homozygous sample (300bp).  
Lane D07 displays a WT sample (107bp).  
  
\*Please note: the 1000bp and 15bp bands are reference markers specific to the Qiaxcel method and do not represent expected products.\*