Genotyping Protocol: **MMRRC 11155**

**Assay Type**: PCR- cannot distinguish heterozygous animals from homozygous animals. Detects presence of Rat Insulin Promoter with Cre Recombinase transgene.

**DNA Extraction**: DNA from tail snips was extracted using Sigma’s Extract-N-Amp Tissue PCR Kit (Cat#XNAT2R). Kit directions for fresh or frozen tails were performed with a few minor modifications as follows: use 50 µl of Extraction Solution and 12.5 µl of Tissue Preparation Solution and 50 µl of Neutralization Solution B.

**Primer Information**:
1) Name: Ripyasu  
   Sequence: 5’ - TAA GGC TAA GTA GAG GTG T -3’
2) Name: Bglobrev  
   Sequence: 5’- TCC ATG GTG ATA CAA GGG AC -3’

**Assay Name**: RIP-Cre PCR

**PCR Master Mix Components**:

<table>
<thead>
<tr>
<th>component</th>
<th>manufacturer</th>
<th>concentration</th>
<th>µl/rxn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract-N-Amp PCR Reaction Mix</td>
<td>Sigma (Cat#XNAT2R)</td>
<td>2X</td>
<td>10</td>
</tr>
<tr>
<td>Ripyasu</td>
<td>IDT or Sigma</td>
<td>25µM</td>
<td>0.3</td>
</tr>
<tr>
<td>Bglobrev</td>
<td>IDT or Sigma</td>
<td>25µM</td>
<td>0.3</td>
</tr>
<tr>
<td>sterile water</td>
<td></td>
<td></td>
<td>5.4</td>
</tr>
</tbody>
</table>

**PCR Setup**:
Final Reaction: 16µl master mix & 4µl DNA template (10-20 ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Applied Biosystems 2700 thermocycler.

**Cycle Parameters**:
1) 94°C 3 minutes  
2) 94°C 30 seconds  
3) 55°C 30 seconds  
4) 72°C 1 minute  
5) Repeat steps 2-4 39 times for a total of 40 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**: 350bp

**Example Gel**:

Lane 6 displays a wild-type sample (no product)  
Lane 7 displays a transgene positive sample (350bp product)  
Lane 8 displays 1kb+ ladder (Invitrogen Cat #10787-018).