Genotyping Protocol: **MMRRC 29175**

**Assay Type**: PCR: can distinguish heterozygous animals from homozygous animals.

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

**Strain Description**: This line has a knock-in mutation in the *Ptf1a* gene. Coding sequence for yellow fluorescent protein (YFP) has been inserted into the first exon of *Ptf1a*. This mutation is homozygous lethal. Details can be found in Burlison et al (2008) Dev Biol 316(1): 74-86.

![Diagram of Ptf1a Locus and Targeting Vector](https://example.com/diagram.png)


**Primer Information**:  
1) Name: F-p48 M29175  
   Sequence: 5'- CCT TCT GAC TTC TCC AAG AAG GCA -3'  
2) Name: R-5' p48 M29175  
   Sequence: 5'- CCC TTT ATG CCT GGC ATT TCA CTG -3'

Primer Location: Primers are located on either side of the 5' loxp site. They appear as Primers 1 and 2 in the above image.

**Assay Name**: Ptf1a 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>F-p48 M29175</td>
<td>IDT or Sigma</td>
<td>25µM</td>
<td>0.3</td>
</tr>
<tr>
<td>R-5' p48 M29175</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 (10-20ng/µl)
All reactions were performed in 200µl thin walled PCR tubes and were run in Applied Biosystems 2700 thermocycler.

**Cycle Parameters:**
1) 95°C 3 minutes  
2) 94°C 1 minute  
3) 66°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

**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/3Kb (Cat# 929522)  
- **Size Marker:** QX DNA Size Marker 100-3Kb (Cat# 929553)  
- **Method:** AM320  
  - **Injection:** 10s at 5KV  
  - **Separation:** 320s at 6KV

**Expected products:**
Wild type allele = 636 bp  
Mutant allele = 670 bp

**Example of Gel:**

Lane A2 displays a wild type sample (636bp product)  
Lane A6 displays heterozygous sample (636bp and 670bp products)

*There are two additional band patterns which may result from this PCR (illustrated by lanes A4 and A1) but these have not been confirmed to be the correct genetic alteration.

*Please note: the 15bp and 3 kb bands are reference markers specific to the QIAxcel method and do not represent expected products.*