

08.24.10 MS  
 01.07.14 MLS  
 08.02.18 MLS

## 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.

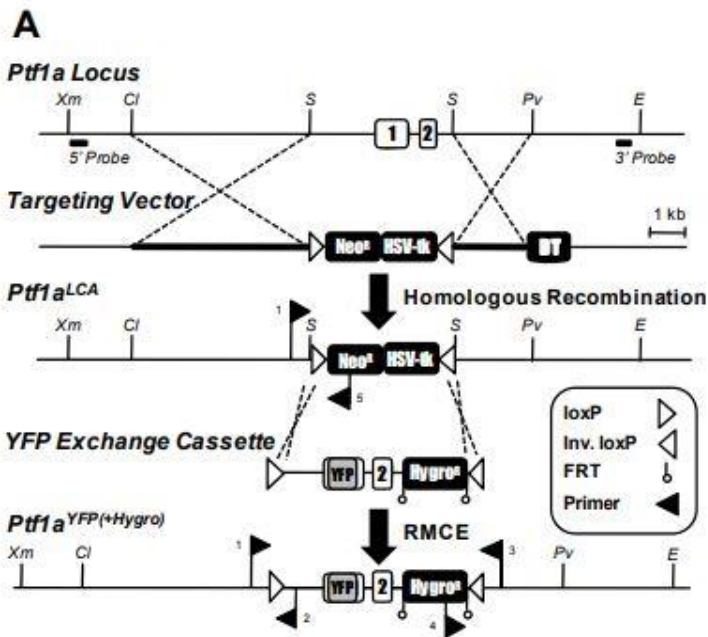


Image from Burlison et al (2008) Dev Biol 316(1): 74-86.

### 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:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
F-p48 M29175	IDT or Sigma	25µM	0.3
R-5' p48 M29175	IDT or Sigma	25µM	0.3
sterile water			5.4

#### PCR Setup:

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

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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

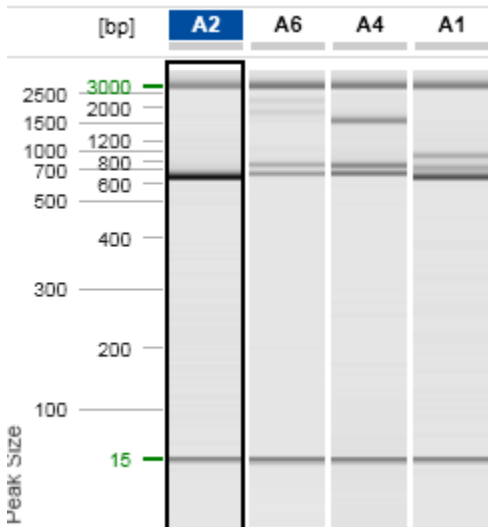
Separation: 320s at 6KV

**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.\*