

## Genotyping Protocol: MMRRC 31038

**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 an exon trapping element in the first intron of the phosphatidic acid phosphatase type 2A gene (*Ppap2a*) on Chromosome 13. Details can be found in Tomsig et al (2009) *Biochem J* 419(3):611–618.

**Primer Information:**

- |                      |   |
|----------------------|---|
| 1) Name: Ppap2a.F    | Sequence: 5'-GAG AGT GAG CGA GTG TCT GAG TTT CTG ATG-3' |
| 2) Name: Ppap2a.wtR  | Sequence: 5'-AGT ACT GGG CAT CTC ACA CCA CAT-3'         |
| 3) Name: Ppap2a.mutR | Sequence: 5'-CCT TCA AAG GGA AGG GGT AAA GTG GTA GGG-3' |

**Primer location:** Ppap2a.F and Ppap2a.wtR are located in intron 1 of the *Ppap2a* gene on Chromosome 13. Ppap2a.mutR is located in the exon trapping element.

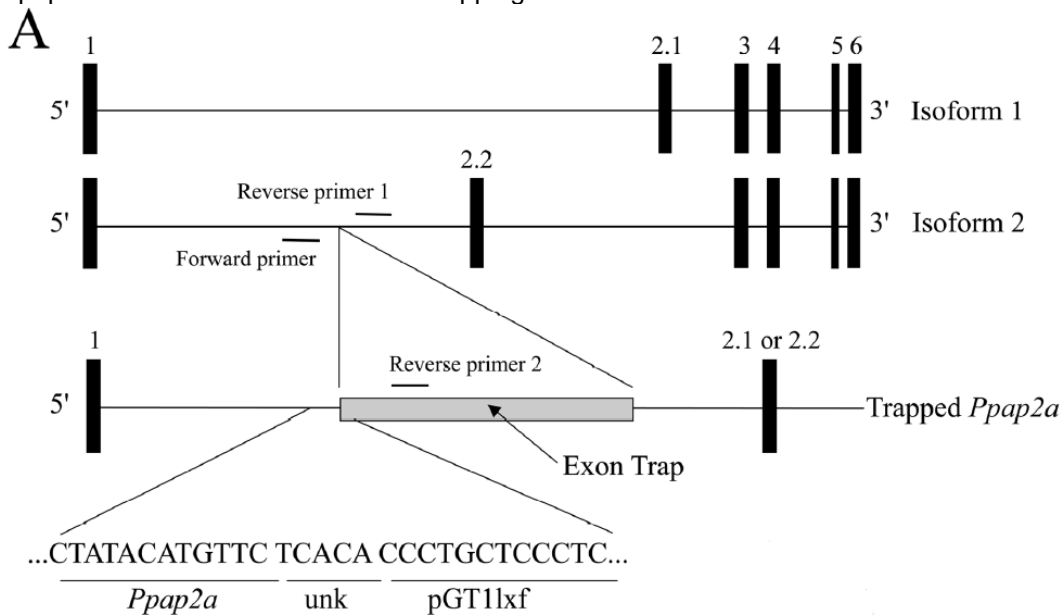


Figure from Tomsig et al (2009) *Biochem J* 419(3):611–618

**Assay name: Ppap2a 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
Ppap2a.F	Sigma	25µM	0.3
Ppap2a.wtR	Sigma	25µM	0.3
Ppap2a.mutR	Sigma	25µM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			12.7

03.16.11 MS  
03.04.14 MLS

**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   | 30 seconds                     |
| 3) | 58°C   | 30 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 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 100bp-3kb (Cat# 929553)

Method: AM320

Injection: 10s at 5KV

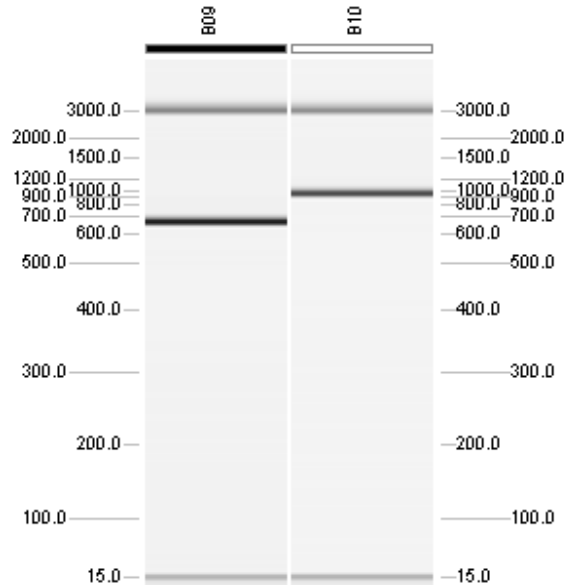
Separation: 320s at 6KV

**Expected products:**

WT: 675bp

Mut: 931bp

**Example gel:**



Lane B09 displays a WT sample (675bp product).  
Lane B10 displays a homozygous sample (931bp product).

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