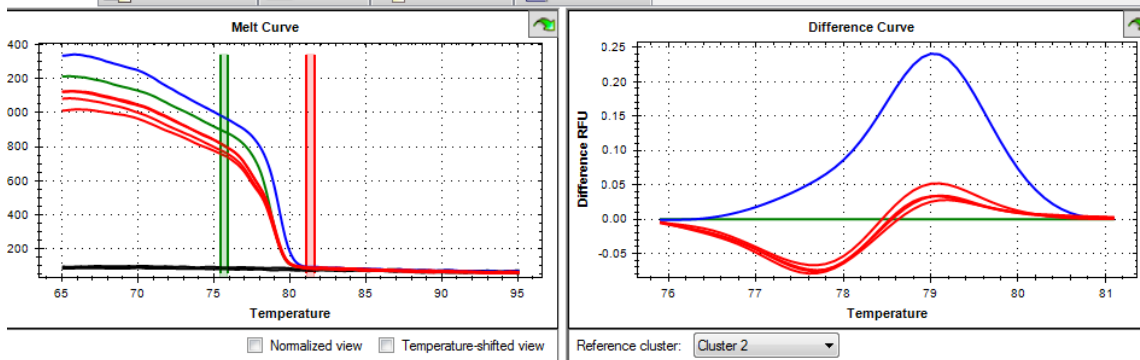




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**BioRad Data Output:**



YBR Step Number: 8

1	2	3	4	5	6	7	8	9	10	11	12	Well	Content	Sample	Cluster	Percent Confidence
Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	A03	Unkn	2272-14-1	Cluster 1	99.8
Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	B03	Unkn	2273-14-1	Cluster 1	100.0
Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	C03	Unkn	2273-14-2	Cluster 1	99.8
Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	D03	Unkn	2274-14-1	Cluster 1	99.6
Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	H03	Unkn	2455-14-1 (hom)	Cluster 2	99.9
Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	G03	Unkn	C57BL/6 (wt)	Cluster 3	99.9
Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	E03	Unkn	blank	<Excluded>	N/A
Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	Unk	F03	Unkn	No DNA	<Excluded>	N/A

In this example, Cluster 1 are heterozygous samples, Cluster 2 are homozygous samples, and Cluster 3 are WT samples.

**For Genotype Confirmation via Sequencing (Optional):**

**Primer Information:**

- 1) Name: M36192 seq F      Sequence: 5'-CCA TTG CTA TTG CCA GTC CT-3'
- 2) Name: M36192 seq R      Sequence: 5'-GGC ACA GGT TAG CCA TCT CT-3'

**Primer location:** Primers are located on either side of the G to A transversion at nucleotide 12212 of mouse *Tgm3*.

**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
M36192 seq F	Sigma	25µM	0.3
M36192 seq R	Sigma	25µM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

**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      20 seconds
- 3) 63°C      25 seconds
- 4) 72°C      30 seconds
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C      10 minutes

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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 product:** 213bp

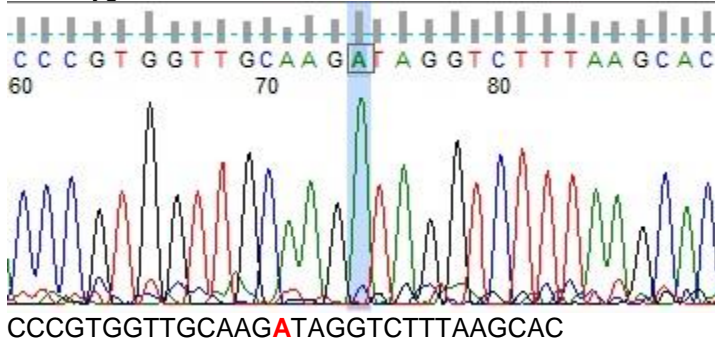
**Sequencing:** To confirm that the correct point mutation is present.

PCR product is purified with the QIAquick PCR Purification Kit (Qiagen Cat# 28104). Kit directions are performed, and DNA is eluted with 50µl nuclease-free water. 15µl of the DNA is then added to 1µl M36192 seq F primer (25µM) in a 1.5ml tube. The tube is submitted to the MU DNA Sequencing Core.

**Analysis:** In mutant allele, G changes to A

**Example Chromatograms:**

Homozygous mutant:



Wild Type:

