

Genotyping Protocol: MMRRC 30220

Strain Characteristics: This strain has a novel in-frame deletion of 18bp within exon 36 of the *Itpr1* gene. Details can be found in van de Leemput et al (2007) PLoS Genet. 3(6):e108.

Assay Type: PCR- can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy 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 200ul of AE buffer once.

Primer Information:

- 1) Name: M30220_F1 Sequence: 5'- GCC AGC CTG TCT TTG TGC -3'
 2) Name: M30220_F2 Sequence: 5'- CCG CCA GCC TGT CTT TGT -3'
 3) Name: M30220_R Sequence: 5'- TGC CTT ACC TAC GTC AGA GAG -3'

Primer location: All three primers are located within the *Itpr1* gene on Chromosome 6.

Assay Name: Itpr1 PCR

PCR Master Mix Components:

component	manufacturer	concentration	μl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
M30220_F1	Sigma or IDT	25 μM	0.3
M30220_F2	Sigma or IDT	25μM	0.3
M30220_R	Sigma or IDT	25μM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/μl	0.2
sterile water			12.7

PCR Setup:

Final Reaction: 19μl master mix & 1μl DNA template (10-20 ng/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 5 minutes
 2) 94°C 1 minute
 3) 64°C 1 minute
 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 a 3% agarose gel with ethidium bromide staining.

Homozygous mutant (HOM): ~110bp

Heterozygous (HET): ~110bp and ~130bp

Wild type (WT): ~130bp

