

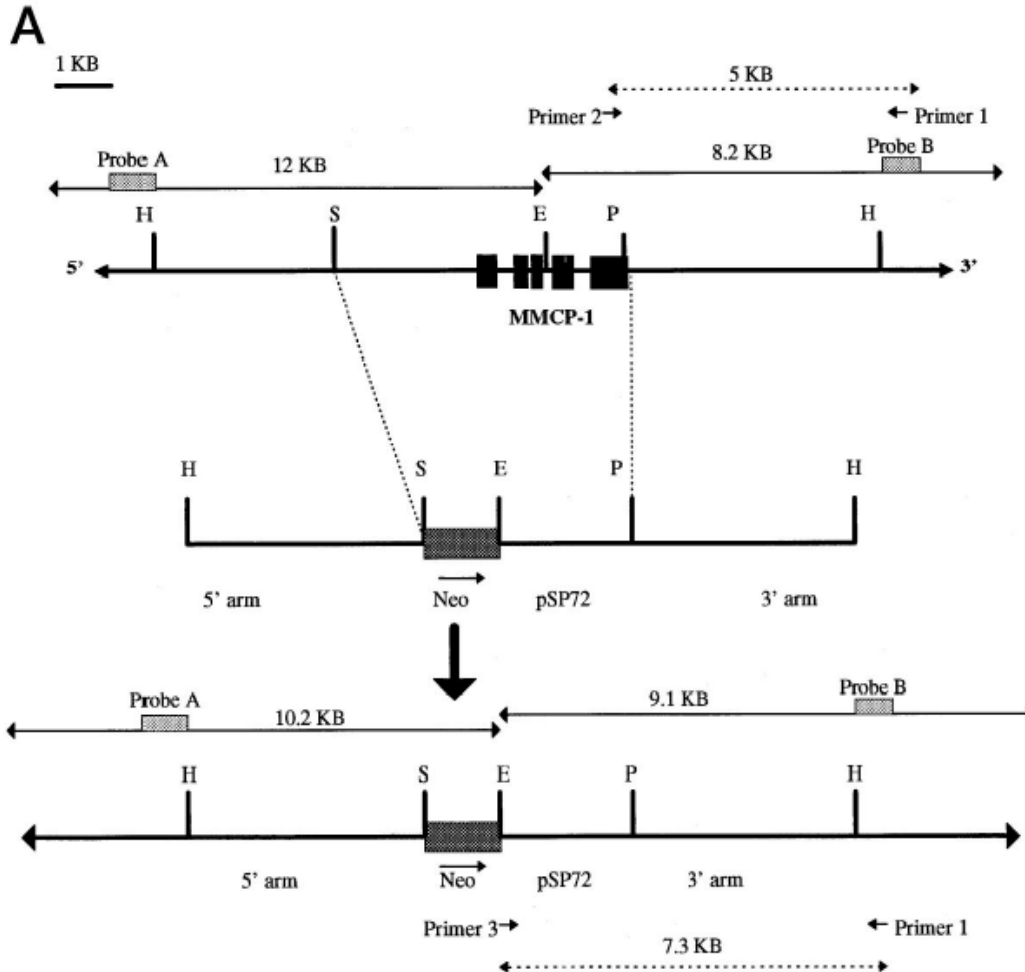
09.30.09 MS
 08.02.10 HB updated
 03.03.14 MLS

Genotyping Protocol: **MMRRC 30488**

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 has all 5 exons of the Mast cell protease 1 gene (*Mcpt1*) replaced by the neo gene. Details can be found in Wastling *et al.* (1998) Am J Pathol 153:491-504.



Primer Information:

- | | |
|----------------------|---|
| 1) Name: M30488Mut-F | Sequence: 5'-CTT GGG TGG AGA GGC TAT TC-3' |
| 2) Name: M30488Mut-R | Sequence: 5'-AGG TGA GAT GAC AGG AGA TC-3' |
| 3) Name: M30488WT-F | Sequence: 5'-GGA AAA CTG GAG AGA AAG AAC CTA C-3' |
| 4) Name: M30488WT-R | Sequence: 5'-GAC AGC TGG GGA CAG AAT GGG G-3' |

Primer location: M30488WT-F and M30488WT-R are located in the *Mcpt1* gene on Chromosome 14. M30488Mut-F and M30488Mut-R are located in the targeting vector.

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Assay name: Mcpt1 KO PCR

Mutant PCR:

PCR Master Mix Components:

component	manufacturer	stock concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30488Mut-F	Sigma or IDT	25µM	0.3
M30488Mut-R	Sigma or IDT	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 30 seconds
- 3) 58°C 30 seconds
- 4) 72°C 30 seconds
- 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: AH320 Injection: 20s at 2kV
Separation: 320s at 6kV

Expected product: 280bp mutant band

WT PCR:

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30488WT-F	Sigma or IDT	25µM	0.3
M30488WT-R	Sigma or IDT	25µM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

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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 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: AH320 Injection: 20s at 2kV

Separation: 320s at 6kV

Expected products: 853bp wild type band

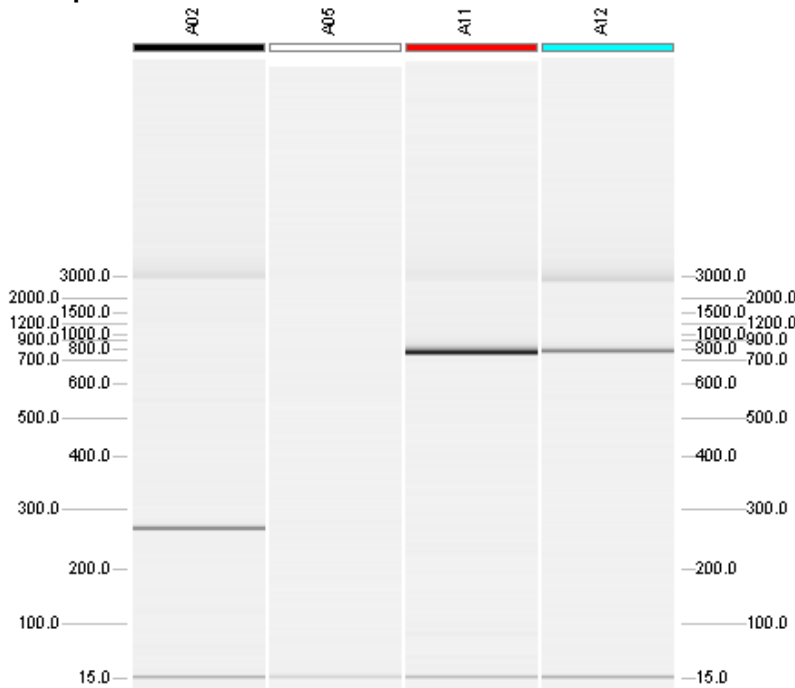
Product Analysis:

Wild Type: 853bp on WT gel, no product on Mut gel

Heterozygous: 853bp on WT gel, 280bp on Mut gel

Homozygous: no product on WT gel, 280bp on Mut gel

Example Gel:



Lanes A02 and A05 are from a Mut PCR.

Lanes A11 and A12 are from a WT PCR.

Lanes A02 and A12 display results for a heterozygous sample. The sample has the 280bp Mut product (A02) and an 853bp WT product (A12).

Lanes A05 and A11 display results for a wild type sample. No Mut product is produced (A05), only a 853bp WT product (A11).

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