

Genotyping Protocol: MMRRC 16999

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: Majority of intron 4 and exon 5 of *Mmp-20* gene was replaced by a phosphoglycerate kinase promoter-controlled hypoxanthine-guanine phosphoribosyl transferase (Hprt) minigene to create a knockout allele.

Primer Information:

- | | |
|----------------|---|
| 1) Name: UP41 | Sequence: 5'-AAG TAG ACT GAA GTC AGG AGA GCC-3' |
| 2) Name: DN460 | Sequence: 5'-CTG TAG TGG TGA CCC TAG TCA TCT T-3' |
| 3) Name: UP231 | Sequence: 5'-CTG CGT CCC CAG ACT TTT GAT TT-3' |
| 4) Name: DN861 | Sequence: 5'-GCT TTT CAT GGC CAG AAT GCT CT-3' |

Primer location: UP41 and DN460 bind to the *Mmp-20* gene to amplify the wild-type allele. UP231 binds to the Hprt insert and DN861 binds to the *Mmp-20* gene to amplify the knockout (KO) allele.

Assay Name: Mmp-20 PCR

PCR Master Mix Components: Assays are performed as two separate reactions.

WT assay

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
UP41	Sigma-Genosys	25µM	0.3
DN460	Sigma-Genosys	25µM	0.3
sterile water			5.1

KO assay

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma	2X	10
UP231	Sigma-Genosys	25µM	0.3
DN861	Sigma-Genosys	25µM	0.3
sterile water			5.1

PCR Setup:

Final Reaction: 16µl master mix & 4µl 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:

WT and KO assay

- 1) 94°C 3 minutes
- 2) 94°C 1 minute
- 3) 61°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

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

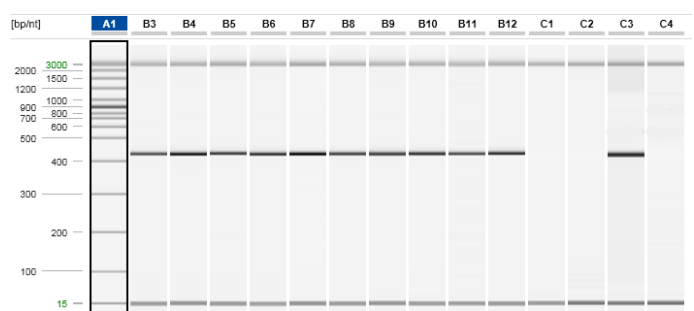
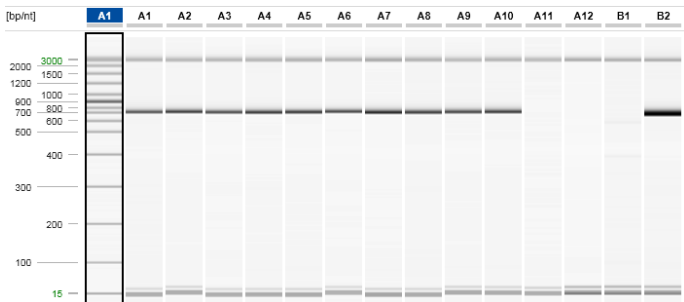
Wild-type allele= 450 bp band
 Knock out allele= 700 bp band

Wild type: 450 bp band with WT assay, no band with KO assay
 Heterozygous: 700 bp band with KO assay and 450 bp band with WT assay
 Homozygous mutant: 700 bp band with KO assay, no band with WT assay

Example Gels:

M16999 KO (Mmp-20 PCR)	
tube	sample
A1	15bp - 3kb Marker
A1	Sample 1
A2	Sample 2
A3	Sample 3
A4	Sample 4
A5	Sample 5
A6	Sample 6
A7	Sample 7
A8	Sample 8
A9	Sample 9
A10	Sample 10
A11	blank
A12	no dna
B1	C57BL/6 WT control
B2	Homozygous control

M16999 WT (Mmp-20 PCR)	
tube	sample
A1	15bp - 3kb Marker
B3	Sample 1
B4	Sample 2
B5	Sample 3
B6	Sample 4
B7	Sample 5
B8	Sample 6
B9	Sample 7
B10	Sample 8
B11	Sample 9
B12	Sample 10
C1	blank
C2	no dna
C3	C57BL/6 WT control
C4	Homozygous control



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

Interpretation of Results
 Samples 1-10: Heterozygous