

Genotyping Protocol: **MMRRC 41472**

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 200ul of AE buffer once.

Strain Description: This strain carries two mutations:

- Exons 1, 2, and 3 of mouse *H2-D1* (histocompatibility 2, D region locus 1) were replaced with a neomycin cassette.
- Exons 2 and 3 of mouse *H2-K1* (histocompatibility 2, K region locus 2) were replaced by a HPRT minigene.

Primer Information:

1) Name: M41472 F1	Sequence: 5'-CGG AAG TCG CCT TAC CTG ATT G-3'
2) Name: M41472 F2	Sequence: 5'-CAG CAG AAA CAT ACA AGC TGT C-3'
3) Name: M41472 R	Sequence: 5'-GTC TCC TCT GGC ACC TAT GGG-3'
4) Name: M41472 Kb mut F	Sequence: 5'-TAA AGC GCA TGC TCC AGA CT-3'
5) Name: M41472 Kb mut R	Sequence: 5'-GTG ACT ATT GCA GCT CCA AGG-3'
6) Name: M41472 Kb wt F	Sequence: 5'-CCC TGC TCG GCT ACT ACA AC-3'

Primer location: M41472 F1 and R are located in Intron 3 of the mouse *H2-D1* gene. M41472 F2 is located in the neomycin cassette.

M41472 Kb mut F is located in PGK of the insertion. M41472 mut R is located in Exon 5 of mouse *H2-K1*. M41472 wt F is located in Exon 2 of mouse *H2-K1*.

Assay names: Db KO PCR; Kb KO PCR

Db KO PCR:**PCR Master Mix Components:**

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25 mM	3.2
M41472 F1	Sigma or IDT	25 µM	0.3
M41472 F2	Sigma or IDT	25 µM	0.3
M41472 R	Sigma or IDT	25 µM	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 (10-20ng/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:

- 94°C 4 minutes
- 94°C 30 seconds
- 68°C 30 seconds
- 72°C 30 seconds
- Repeat steps 2-4 34 times for a total of 35 cycles
- 72°C 7 minutes
- 4°C hold until refrigerate product

Product Analysis:

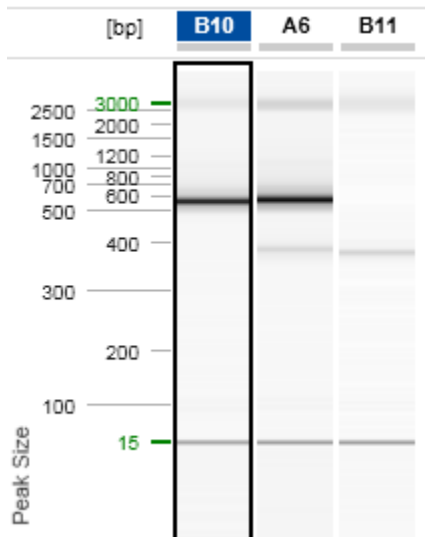
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

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:

Wild-type: 520bp
 Homozygous mutant: 400bp
 Heterozygous: 400bp, 520bp

Example gel:



Lane B10 displays a Wild-type sample (520bp product)
 Lane A6 displays a heterozygous sample (400bp and 520bp products)
 Lane B11 displays a homozygous mutant sample (400bp product)

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

Kb KO MUTANT PCR:

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25 mM	3.2
M41472 Kb mut F	Sigma or IDT	25 µM	0.3
M41472 Kb mut R	Sigma or IDT	25 µM	0.3
FastStart Taq	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 Eppendorf Master Cycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 94°C 4 minutes
- 2) 94°C 1 minute
- 3) 70°C 1 minute
- 4) 72°C 1 minute 45 seconds
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 7 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/5Kb (Cat# 929524)

Size Marker: QX DNA Size Marker 250-4Kb (Cat# 929562)

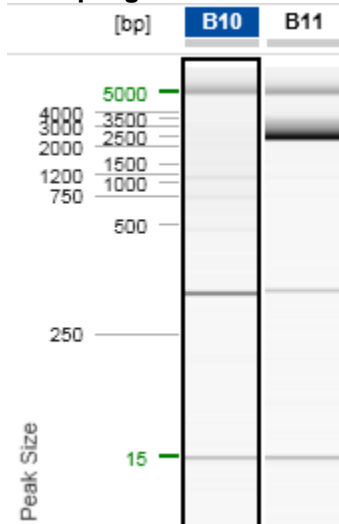
Method: AM320 Injection: 10s at 5KV

Separation: 320s at 6KV

Expected product:

Mutant allele product: 1800bp

Example gel:



Lane B10 displays a sample negative for the mutant allele (no product)
 Lane B11 displays a sample positive for the mutant allele (1800bp product)

This assay produces a ~340bp non-specific band. This band is not used for genotyping

Please note: the 15bp and 5kb bands are reference markers specific to the QIAxcel method and do not represent expected products

Kb KO WILD-TYPE PCR:

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25 mM	3.2
M41472 Kb wt F	Sigma or IDT	25 µM	0.3
M41472 Kb mut R	Sigma or IDT	25 µM	0.3
FastStart Taq	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 Eppendorf Master Cycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 94°C 4 minutes
- 2) 94°C 30 seconds
- 3) 66°C 30 seconds
- 4) 72°C 2 minutes 30 seconds
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 7 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/5Kb (Cat# 929524)

Size Marker: QX DNA Size Marker 250-4Kb (Cat# 929562)

Method: AM320

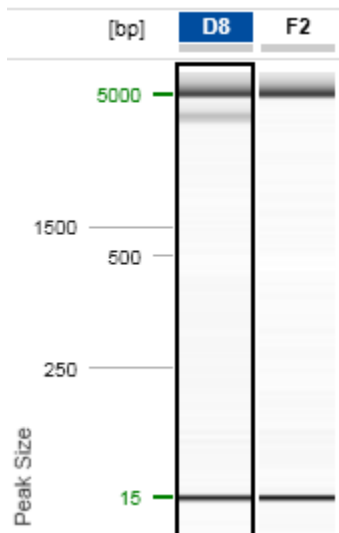
Injection: 10s at 5KV

Separation: 320s at 6KV

Expected product:

Wild-type allele product: 2600bp

Example gel:



Lane D8 displays a sample positive for the wild-type allele (2600bp product)
 Lane F2 displays a sample negative for the wild-type allele (no product)

Please note: the 15bp and 5kb bands are reference markers specific to the QIAxcel method and do not represent expected products

Genotype analysis:

	<u>Kb KO Mutant PCR</u>	<u>Kb KO Wild-type PCR</u>
Wild-type	1800bp	no product
Heterozygous	1800bp	2600bp
Homozygous mutant	no product	2600bp