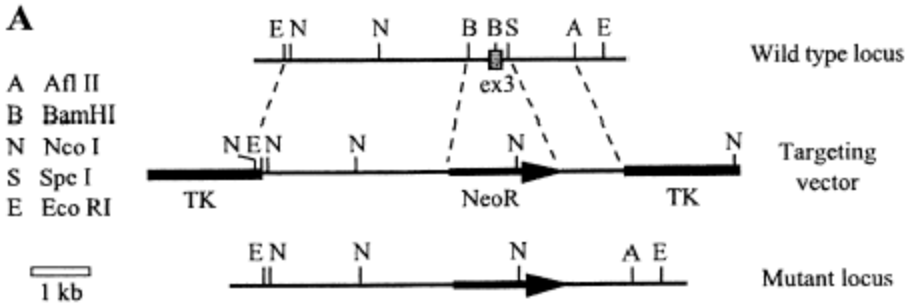
02.10.10 MS
07.30.10 HB updated
02.07.13 MLS

Genotyping Protocol: **MMRRC 25**

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 carries a Neomycin cassette which replaces exon 1 of the protein kinase inhibitor beta, cAMP dependent, testis specific gene (*Pkib*) on Chromosome 10. Details can be found in Belyamani et al (2001) Mol Cel. Biol. 21:3159-63.



Primer Information:

1) Name: Pkib.F	Sequence: 5'-TGA TGA GGA CAG ATT CAT CAG AG-3'
2) Name: Pkib.R	Sequence: 5'-CCA ACG CTT CCA GTT TCA GT-3'
3) Name: Neo F	Sequence: 5'-CAT TCG ACC ACC AAG CGA AAC ATC-3'
4) Name: Neo R	Sequence: 5'-ATA TCA CGG GTA GCC AAC GCT ATG-3'

Primer location: Pkib.F and Pkib.R are both located in exon 1 of the *Pkib* gene on Chromosome 10. Neo F and R are located in the Neomycin cassette.

Assay name: Pkib KO PCR

WT PCR:

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
Pkib.F	Sigma	25µM	0.3
Pkib.R	Sigma	25µM	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 Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

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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
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/1kb (Cat# 929521)
Size Marker: QX DNA Size Marker 50bp-800bp (Cat# 929556)
Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected product: WT: 159 bp

Mut PCR:

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
Neo F	Sigma	25µM	0.3
Neo R	Sigma	25µM	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 Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

1) 95°C 3 minutes
2) 94°C 30 seconds
3) 68°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/1kb (Cat# 929521)
Size Marker: QX DNA Size Marker 50bp-800bp (Cat# 929556)
Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected product: Mut: 289bp

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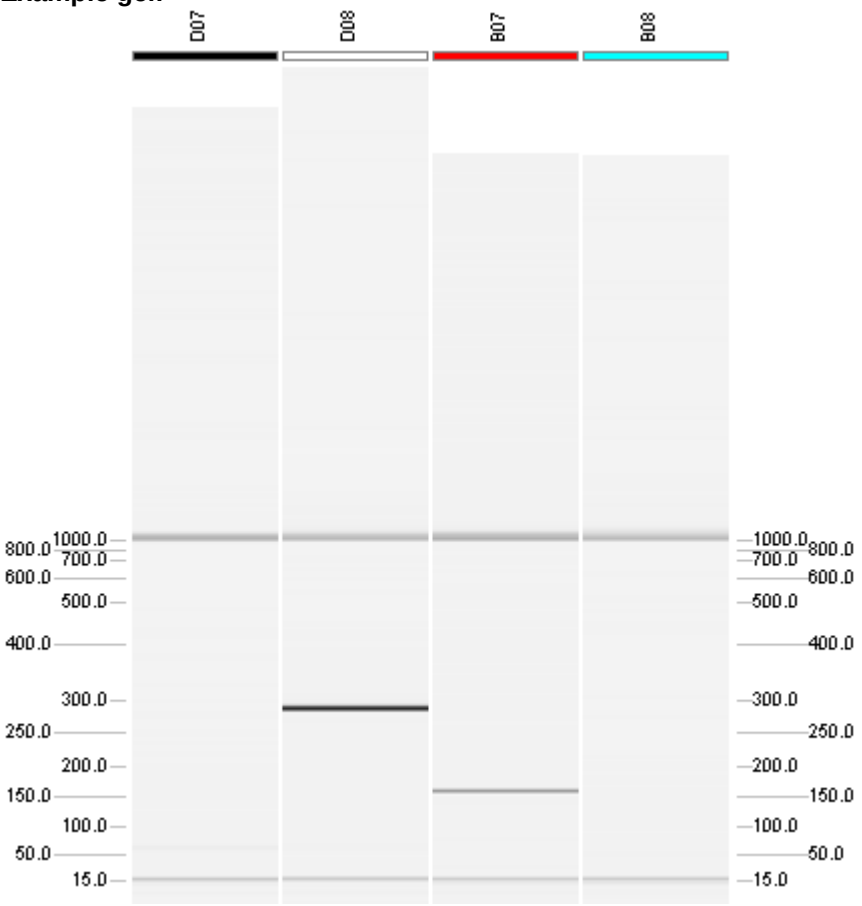
Example gel:



Lane D07 displays a sample negative for the mutant allele (no product)
Lane D08 displays a sample positive for the mutant allele (289bp band)

Lane B07 displays a sample positive for the WT allele (159bp band)
Lane B08 displays a sample negative for the WT allele (no product).

Bands in all lanes at 1000 bp and 15 bp are alignment markers.