09.30.04
08.04.10 MS
07.21.11 MS
3-24-12 ECB
03.30.12 MS

Genotyping Protocol: **MMRRC 21**

Assay Type: PCR- can distinguish heterozygous/hemizygous 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 fresh or frozen tails were performed with a few minor modifications as follows: use 50 µl of Extraction Solution and 12.5 µl of Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Strain Characteristics: Targeted knockout of X-linked inhibitor of apoptosis gene (*Xiap*). Details can be found in Harlin *et al* (2001) Characterization of XIAP-deficient mice. Mol Cell Biol. 21(10):3604-3608.

Primer Information:
1) Name: ML 21: WT1 Sequence: 5'-TGG AGA GTT TGT TGA ATT TGG G-3'
2) Name: ML 21: WT2 Sequence: 5'-TGG GAA ATA GAA ATC CTT TTG C-3'
3) Name: ML 21: KO1 Sequence: 5'-TTT GAA GTT CCT AAT GCA ATG TTC TC-3'
4) Name: ML 21: KO2 Sequence: 5'-ATC GAG CGA GCA CGT ACT TCG GAT G-3'

Primer location: WT allele: ML21:WT1 and ML21:WT2
KO allele: ML21:KO1 and ML21:KO2

Assay Name: XIAP PCR

PCR Master Mix Components:
Run separate reaction for KO gene and WT gene:

Master Mix for WT gene:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
ML21 WT1	IDT	25µM	0.3
ML21 WT2	IDT	25µM	0.3
FastStart *Taq*	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			13

Master Mix for KO gene:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	4
ML21 KO1	IDT	25µM	0.3
ML21 KO2	IDT	25µM	0.3
FastStart *Taq*	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			13

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PCR Setup:
WT Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)
KO 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:

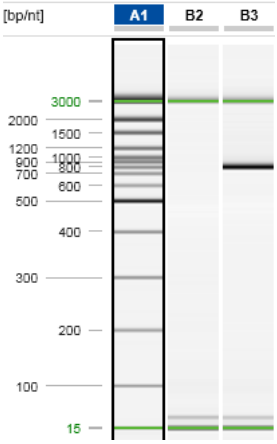
1)	94°C	5 minutes
2)	94°C	1 minute
3)	63°C KO / 63°C WT	1 minute
4)	72°C	1 minute
5)	Repeat steps 2-4 34 times for a total of 35 cycles	
6)	72°C	7minutes
7)	4°C	hold until refrigerate product

Product Analysis:
All products can be analyzed on a 3% agarose gel with ethidium bromide staining
Wild type gene product: 400 bp
Knockout gene product: 600 bp

Note: Because this gene is on the X chromosome, males will be hemizygous (1 copy) for either the wild type or knockout allele. Females can be either wild type, heterozygous, or homozygous for the knockout allele.

Example Gels:

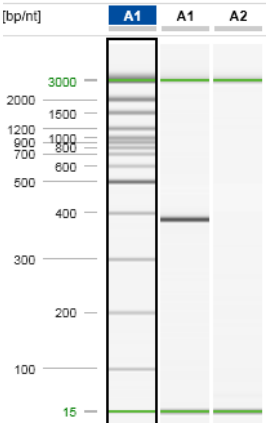
KO PCR:



Lane A1 displays a 15bp-3kb size marker
Lane B2 displays a sample negative for the KO allele (no product)
Lane B3 displays a sample positive for the KO allele (600bp product)

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

WT PCR:



Lane A1(highlighted in blue) displays a 15bp-3kb size marker
Lane A1 (unhighlighted) displays a sample positive for the WT allele (400 bp product)
Lane A2 displays a sample negative for the WT allele (no product)

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