08.05.10 MS
08.15.14.MLS

Genotyping Protocol: **MMRRC 228**

Strain Characteristics: Phospholamban knockout, maintained homozygous

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.

Primer Information:
1) Name: HLT7 R4 Sequence: 5`-ACA ACC ACT TCC TCT CTG GGA GAT CA-3`
2) Name: L27 NEO 3' (F) Sequence: 5`-TCC TCG TGC TTT ACG GTA TC-3`
3) Name: 27 WT F Sequence: 5`-CAC GTC AGA ATC TCC AGA ACC-3`
4) Name: 27 WT R Sequence: 5`-TCC CCC TTT AAC TCT CATAAG C-3`

Primer location: WT allele: 27 WT F & 27 WT R bind to exon (deleted region in KO)
KO allele: NEO 3' binds to the neomycin cassette and HLT7 binds to Chromosome 10

Run separate PCR assays for KO allele and WT allele:

Assay Name: Phospholamban KO PCR

PCR Master Mix Components:

Master Mix for WT Allele Assay:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
27 WT F	IDT	25µM	0.3
27 WT R	IDT	25µM	0.3
sterile water			5.4

Master Mix for KO Allele Assay:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma	2X	10
L27 NEO 3'	IDT	25µm	0.3
HLT7 R4	IDT	25µm	0.3
sterile water			5.4

PCR Setup:
WT Final Reaction: 16µl master mix & 4µl DNA template (10-20ng/µl)
KO 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:

1)	94°C	3 minutes
2)	94°C	30 sec
3)	59°C KO / 60°C WT	30 sec
4)	72°C	1 minute
5)	Repeat steps 2-4 34 times for a total of 35 cycles	
6)	72°C	10minutes
7)	4°C	hold until refrigerate product

Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining
Wild type Phospolamban allele: 700 bp
Knockout allele: 212 bp

Example Gels:

WT PCR Gel

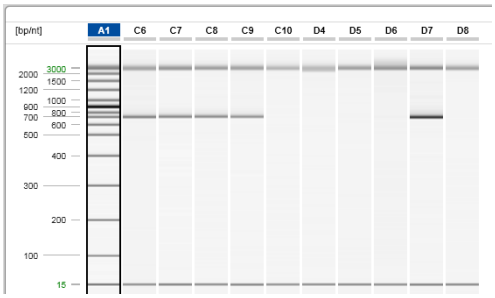
Lane	Sample
A1	15 bp-3 kb Marker
C6	Sample 1
C7	Sample 2
C8	Sample 3
C9	Sample 4
C10	Sample 5
D4	Blank
D5	No DNA
D6	C57BL/6 (WT)
D7	12977-10-1 (het)
D8	11220-09-3 (hom)

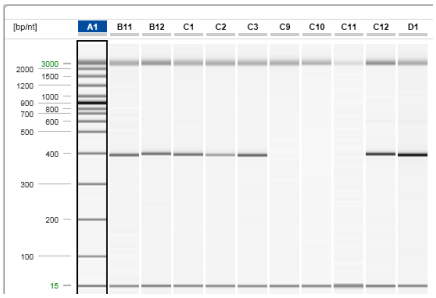
KO PCR Gel

Lane	Sample
A1	15 bp-3 kb Marker
B11	Sample 1
B12	Sample 2
C1	Sample 3
C2	Sample 4
C3	Sample 5
C9	Blank
C10	No DNA
C11	C57BL/6 (WT)
C12	12977-10-1 (het)
D1	11220-09-3 (hom)

Interpretation of Results

Samples 1-4: heterozygous
Sample 5: homozygous for KO allele





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

***** As a backup assay the primer set MMRRC line 27 HLT7 and L27 NEO 3' can be used to test for the KO allele.
Primer Information:
1) Name: MMRRC line 27 HLT7 Sequence: 5'-TGT GGG TTG CAA AGT TAG GC-3'
2) Name: L27 NEO 3' (F) Sequence: 5'-TCC TCG TGC TTT ACG GTA TC-3'

PCR parameters remain the same. Primer set produces an expected KO product of 450 bp.