

09.22.10 MS
03.10.17 MLS

Genotyping Protocol: **MMRRC 16831**

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 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.

Primer Information:

Name: XP26 Sequence: 5'- GTG TCA GGC ATA AGA TCT ATG ACA A -3'
Name: XP47 Sequence: 5'- AGG CAA GCA CCT GCA GCT GT -3'
Name: PGK2 Sequence: 5'- GGC CAC TTG TGT AGC GCC AA -3'

XP26 and Xp47 are located in xeroderma pigmentosum, complementation group A (*Xpa*). Xp26 is the forward primer. PGK2 is located in the PGK promoter. PGK2 is a reverse primer.

Assay Name: Xpa KO PCR

PCR Master Mix Components:

KO (Mutant) Master Mix

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
XP26	Sigma or IDT	25 µM	0.3
PGK2	Sigma or IDT	25 µM	0.3
sterile water			5.4

Wild-type Master Mix

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
XP26	Sigma or IDT	25 µM	0.3
XP47	Sigma or IDT	25 µM	0.3
sterile water			5.4

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:

- 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
- 7) 4°C hold until refrigerate product

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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

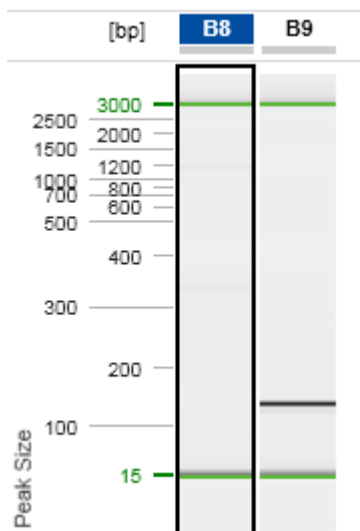
Expected products:

Wild-type PCR: 214bp

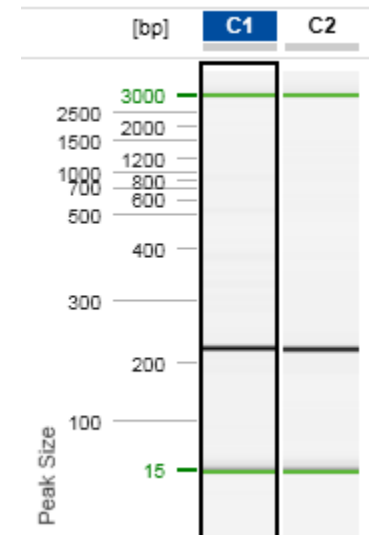
Mutant (KO) PCR: 132bp

Example of Gels:

Mutant (KO) gel:



Wild-type gel:



Lane B8 displays a sample negative for the KO allele (no product)
 Lane B9 displays a sample positive for the KO allele (132bp product)

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

Lanes C1 and C2 display samples positive for the WT allele (214bp product)

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

Genotype Interpretation:

Genotype:	Wild-type PCR	KO (Mutant) PCR
Wild-type	214bp product	No product
Heterozygous	214bp product	132bp product
Homozygous mutant	No product	132bp product