

Genotyping Protocol: MMRRC 37491

Assay Type: PCR; can distinguish heterozygous and homozygous animals.

DNA Extraction: DNA from tail snips was extracted using the following protocol:

1. Place a ~3mm piece of tail in 180ul of 50mM NaOH in a 0.2mL tube and vortex
2. Incubate at 95°C for 10 minutes, then at 40°C for 5 minutes.
3. Add 20ul of 1M Tris-HCl (pH 8.0) and vortex well. Use this lysate as your DNA template in KOD Xtreme PCR reactions.

Strain Description: The first two coding exons of mouse *Folr4* have been flanked by *loxP* sites. A neomycin resistance gene and a third *loxP* site follow the second *loxP* site. Details can be found in Salbaum *et al.* (2013) *Biochimica et Biophysica Acta* 1832:1653-1661.

Primer Information:

- | | |
|-------------------|---|
| 1) Name: M37491 F | Sequence: 5'-CTT TTC CCC TGT TGG TCA GT TTG-3' |
| 2) Name: M37491 R | Sequence: 5'-TGA CCT GCC ACA GAG AGA AGC CCA T-3' |

Primer Location: M37491 F and R are located before the first coding exon of the mouse *Folr4* gene. They are on either side of a *loxP* site in the mutant allele.

Assay Name: Folr4 floxed PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
KOD Xtreme Buffer	Millipore	2X	10
KOD Xtreme dNTPs	Millipore	2mM	4
M37491 F	Sigma or IDT	25µM	0.3
M37491 R	Sigma or IDT	25µM	0.3
KOD Xtreme <i>Taq</i>	Millipore (Cat# 71975-3)	1 U/µl	0.4
sterile water			3

PCR Setup:

Final Reaction: 18 µl master mix & 2 µl extracted DNA (10-20ng/µl).

All reactions were performed in 200 µl 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 seconds |
| 3) | 66°C | 30 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:

For analysis 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 100bp-2.5kb (Cat# 929559)

Method: AH320 Injection: 20s at 2kV

Separation: 320s at 6kV

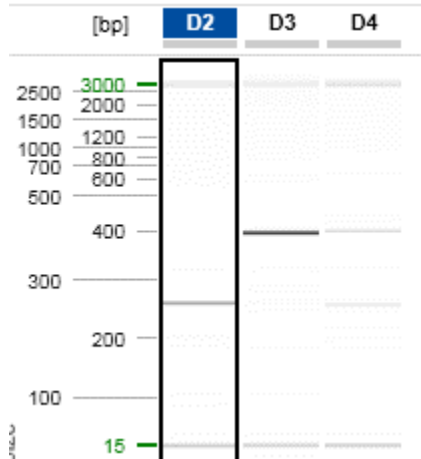
Expected products:

Wild type: 268bp product

Heterozygous: 268bp and 415bp products

Homozygous mutant: 415bp products

Example gel:



Lane D2 displays a wild-type sample (268bp product)
Lane D3 displays a homozygous mutant sample (415bp product)
Lane D4 displays a heterozygous sample (268bp and 415bp products)

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