

Genotyping Protocol: MMRRC 29877

Assay Type: PCR - can distinguish heterozygous 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.

Strain Description: This strain carries a floxed allele of the *Klf* gene on the C57BL/6 genetic background. Introns 1 and 3 of the *Klf* gene have loxP sites to create the floxed allele. See Katz, J.P et al. (2002) Development 129:2619-2628 for details.

Primer Information:

- 1) Name: Klf4 exon 1 Sequence: 5'-CTG GGC CCC CAC ATT AAT GAG-3'
2) Name: Klf4 exon 2 Sequence: 5'-CGC TGA CAG CCA TGT CAG ACT-3'

Klf4 exon 1 and Klf4 exon 2 bind to exons 1 and 2 of *Klf4* respectively.

Assay Name: Klf4loxP PCR

PCR Master Mix Components:

Component	Manufacturer	Concentration	µl/rxn
Extract-n-amp solution	Sigma (Cat#XNAT2R)	2X	10
Klf4 exon 1	IDT	25µM	0.3
Klf4 exon 2	IDT	25µM	0.3
sterile water			5.4

PCR Setup:

Final Reaction: 16µl master mix & 4 ul DNA template (10-20ng DNA). All reactions were performed in 200µl thin walled PCR tubes and were run in Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 94°C 5 minutes
- 2) 94°C 45 seconds
- 3) 58°C 45 seconds
- 4) 72°C 90 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/3Kb (Cat# 929522)

Size Marker: QX DNA Size Marker 100-3Kb (Cat# 929553)

Method: AM320 Injection: 10s at 5KV

Separation: 320s at 6KV

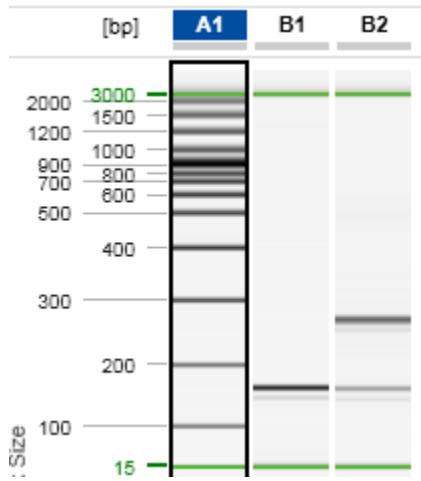
Expected Products:

Wild type = 172 bp

Homozygous mutant = 296 bp

Heterozygous = 296 bp and 172 bp band both present

Example Gel:



Lane A1 displays a 15bp-3kb size marker
Lane B1 displays a wild-type sample (172bp product)
Lane B2 displays a heterozygous sample (172bp and 296bp products)

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