

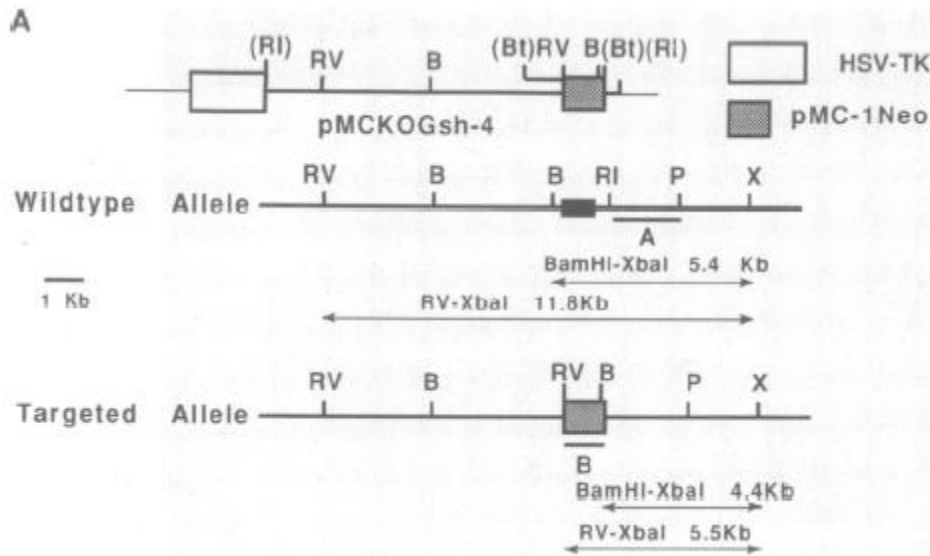
01.27.10 MS
 07.30.10 HB updated
 02.27.14 MLS

Genotyping Protocol: **MMRRC 30090**

Assay Type: PCR- can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Strain Description: This strain carries a Neomycin selection cassette in the LIM homeobox protein 4 gene (*Lhx4*), which replaces 0.7kb of sequence encoding the homeobox domain. Details can be found in Li et al (1994) The EMBO Journal 13:2876-85.



Primer Information:

- | | |
|-------------------|---|
| 1) Name: Lhx4.F | Sequence: 5'-CTC GGA AGC TCA GCT CAC TGT CAC TAA CCC-3' |
| 2) Name: Lhx4.R | Sequence: 5'-CAG AAA ATC CGA CTG TGA CCA CAG CTT GAA-3' |
| 3) Name: Lhx4.Neo | Sequence: 5'-ATC AGG ATG ATC TGG ACG AAG AGC ATC AGG-3' |

Primer location: Lhx4.F is located in exon 5 of the *Lhx4* gene. Lhx4.R is located in intron 3 of the *Lhx4* gene. Lhx4.Neo is located in the inserted cassette.

Assay name: Lhx4 KO PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
Lhx4.F	Sigma	25µM	0.3
Lhx4.R	Sigma	25µM	0.3
Lhx4.Neo	Sigma	25µM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			12.7

PCR Setup:

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

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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) 95°C 3 minutes
- 2) 94°C 1 minute
- 3) 65°C 1 minute
- 4) 72°C 1 minute 15 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

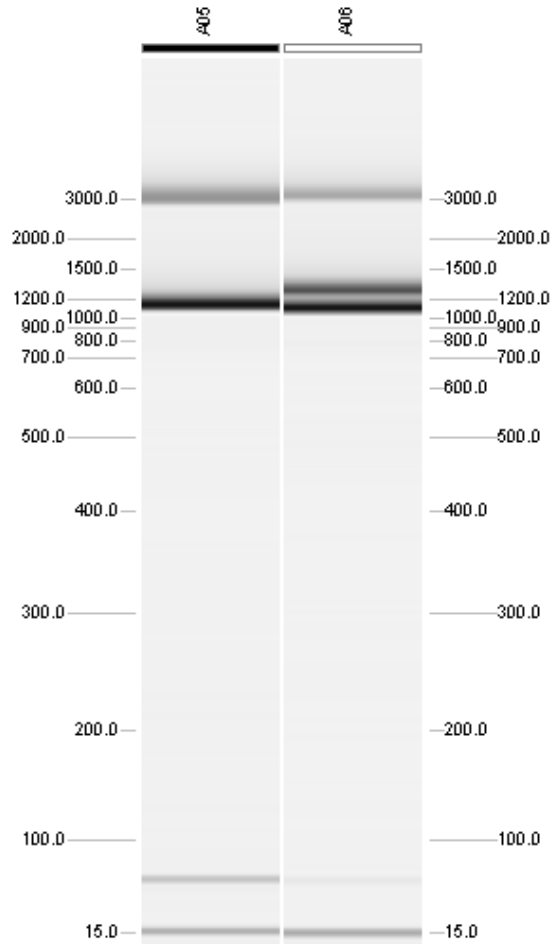
Separation: 320s at 6KV

Expected products:

WT product: 1.1kb

Mutant product: 1.3kb

Example gel:



Lane A05 displays a WT sample (1.1kb band).
Lane A06 displays a Heterozygous sample (1.1kb and 1.3kb bands).

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