

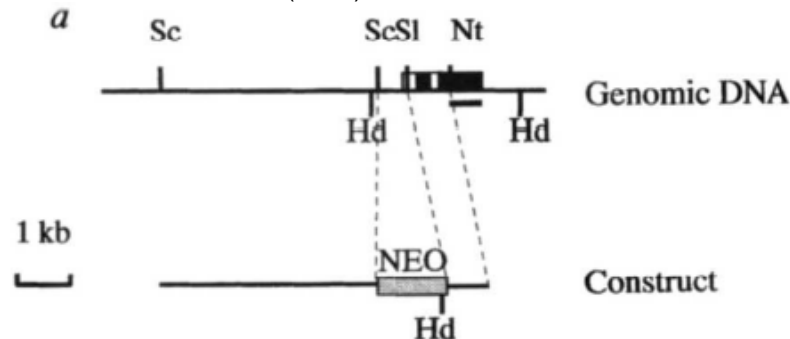
05.11.10 MS  
08.02.10 HB updated  
03.03.14 MLS

## Genotyping Protocol: **MMRRC 30510**

**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 has a targeted deletion of the 5' end of the coding region of the SRY-box containing gene 4 gene (*Sox4*) on Chromosome 13. The 5' end of the *Sox4* coding sequence is replaced by a Neo gene. Details can be found in Schilham et al (1996) Nature 380:711-714.



### Primer Information:

- |                            |   |
|----------------------------|---|
| 1) Name: M30510 Sox4 II-R3 | Sequence: 5'-CCA CAC CAT AAA GGC GTT CAT GG-3'  |
| 2) Name: M30510 Sox4 III   | Sequence: 5'-GGT CTG TTG CAT GCA AGC TTC-3'     |
| 3) Name: Neo F             | Sequence: 5'-CAT TCG ACC ACC AAG CGA AAC ATC-3' |

**Primer location:** M30510 Sox4 II-R3 and M30510 Sox4 III are located in exon 1 of the *Sox4* gene. Neo F is located in the inserted neo gene.

**Assay name:** Sox4 KO PCR

### WT PCR:

#### PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl <sub>2</sub> (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30510 Sox4 II-R3	Sigma	25µM	0.3
M30510 Sox4 III	Sigma	25µM	0.3
DMSO	Sigma	5%	1
FastStart Taq	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			12

### PCR Setup:

Final Reaction: 19µl master mix & 1µl 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.

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**Cycle Parameters:**

- 1) 95°C 3 minutes
- 2) 94°C 30 seconds
- 3) 66°C 30 seconds
- 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

**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 100bp-3kb (Cat# 929553)

Method: AH320 Injection: 20s at 2kV  
Separation: 320s at 6kV

**Expected product:** 716bp

**Mut PCR:**

**PCR Master Mix Components:**

component	manufacturer	concentration	µl/rxn
Buffer with MgCl <sub>2</sub> (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30510 Sox4 II-R3	Sigma	25µM	0.3
Neo F	Sigma	25µM	0.3
DMSO	Sigma	5%	1
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			12

**PCR Setup:**

Final Reaction: 19µl master mix & 1µl 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) 95°C 3 minutes
- 2) 94°C 30 seconds
- 3) 66°C 30 seconds
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 7 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 100bp-3kb (Cat# 929553)

Method: AH320 Injection: 20s at 2kV  
Separation: 320s at 6kV

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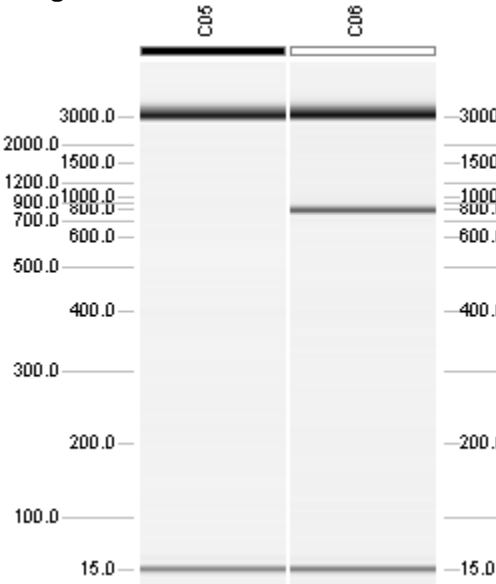
Expected product: 775bp

Product Analysis:

	WT PCR	Mut PCR
Homozygous	no product	775bp
Heterozygous	716bp	775bp
WT	716bp	no product

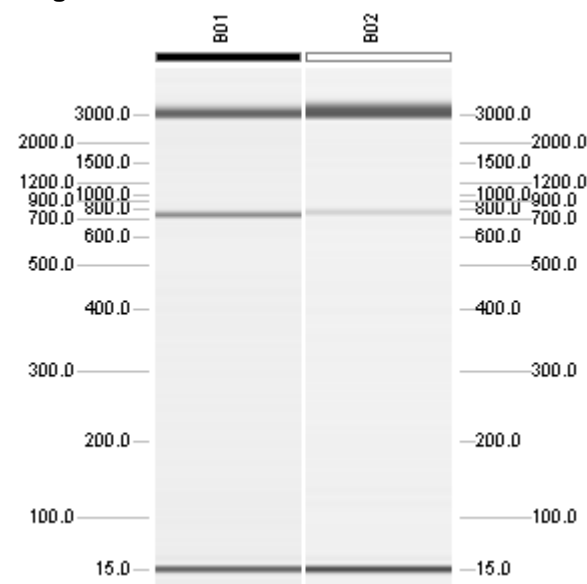
Example Gels: (\*Please note: the 15bp and 3kb bands are reference markers specific to the QIAxcel method and do not represent expected products.\*)

KO gel:



Lane C05 displays a sample negative for the KO allele (no product)  
Lane C06 displays a sample positive for the KO allele (775bp product)

WT gel:



Lanes B01 and B02 display samples positive for the WT allele (716bp product)