EMMA ID: *13642*
Gene: *Uqox (Uqcrh + Aox)*
Common name: *Uqox (EPD0378_3_C07 + Rosa26 Aox)*

Allele Information

Uqox mice were produced by mating in a first step C57BL/6NCrl AOXRosa26-TG heterozygous mice (AOX het) to C57BL/6NCrl Uqcrh-tm1b heterozygous animals (Uqcrh het) to produce double heterozygous animals. **To determine the genotype of animals two separate pcrs are necessary.**

Genotyping Information

Genotyping by end-point PCR based on gel is composed of a genespecific short range PCR using primers on wild type allele and a mutant allele-specific short range PCR. The combined results show the genotype of the mice. For example: mutant positive, wild type positive = Heterozygous.

Rosa26 Aox PCR

PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Wild type	Rosa26 s	Rosa26 as	523
Mutant	Aox s	Aox as	317

Primer sequences

Primer Name	Sequence 5' --> 3'
Rosa26 s	GACCTCCATCGCGCACTCCG
Rosa26 as	CTCCGAGGCGGATCACAAGC
Aox s	GCGATGCAAGATGGAGGGTA
Aox as	TGAATCCAACCGTGGTCTCG

PCR setup (Qiagen, Hot Start Plus)

Component	Volume (µl) 1x	Final conc.
DNA (~ 50-100 ng)	2	
Q-Solution (5x)	2,5	0,5
PCR-Buffer (10x)	2,5	1
DMSO	0,5	0,02
DNTP mix (10 mM)	0,5	0,2
MgCl2 (25 mM)	1,5	1,5
Primer 1 (10 pmol/µl)	1	0,4
Primer 2 (10 pmol/µl)	1	0,4
Taq Polymerase (5 U/µl)	0,3	0,06
H2O*	13,2	
Final volume	25	

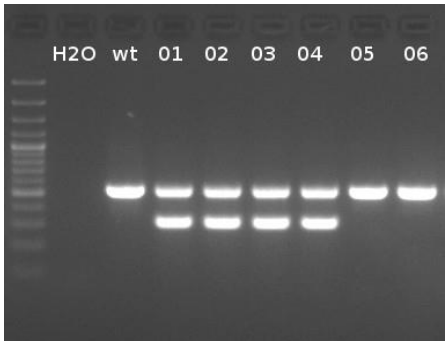
* The amount of H2O is adjusted with the number of primer.

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C	30 sec	39
	68-58 (↓1°C/Cycle)	45 sec	
	72°C	45 sec	
3 Polymerisation	72°C	10 min	1
4 Cooling	4°C	hold	1

use Touch-Down cycling protocol: first 10 cycles anneal at 68°C, decreasing 1°C per cycle, next 30 cycles aneal at 58°C
These PCR conditions have been optimized for our methods and preparation kits. Adaptions may be required.

Gel Image



Separated by gel electrophoresis on a 2% agarose gel.

EPD0378_3_C07 PCR

PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Wild type	Uqcrh 5'arm	Uqcrh 3'arm	652
Mutant	Uqcrh 5'arm	LAR3	518

Primer sequences

Primer Name	Sequence 5' --> 3'
Uqcrh 5'arm	aacttggactcctggtcctct
Uqcrh 3'arm	cttgccttcccagaactagct
LAR3	CAACGGGTTCTTCTGTTAGTCC

PCR setup (Qiagen, Hot Start Plus)

Component	Volume (µl) 1x	Final conc.
DNA (~ 50-100 ng)	2	
Q-Solution (5x)	2,5	0,5
PCR-Buffer (10x)	2,5	1
DMSO	0,5	0,02
DNTP mix (10 mM)	0,5	0,2
MgCl2 (25 mM)	1,5	1,5
Primer 1 (10 pmol/µl)	1	0,4
Primer 2 (10 pmol/µl)	1	0,4
Taq Polymerase (5 U/µl)	0,3	0,06
H2O*	13,2	
Final volume	25	

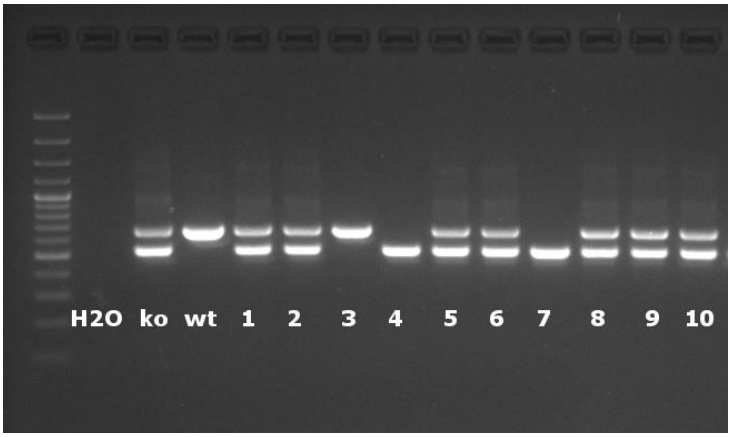
* The amount of H2O is adjusted with the number of primer.

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 65°C 72°C	30 sec 45 sec 45 sec	39
3 Polymerisation	72°C	10 min	1
4 Cooling	12°C	hold	1

These PCR conditions have been optimized for our methods and preparation kits. Adaptions may be required.

Gel Image



Separated by gel electrophoresis on a 2% agarose gel.