EMMA ID: *02339*
Gene: *Kcnh1*
Common name: *LEXKO-1588, CHA302N1*
Allele: *Kcnh1* ^{tm1Lex}

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.

PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Wildtype	LEXKO-1588-21	LEXKO-1588-22	586
Mutant	Neo3a	LEXKO-1588-23	292

Primer sequences

Primer Name	Sequence 5' --> 3'
Neo3a	GCAGCGCATCGCCTTCTATC
LEXKO-1588-23	AGAGATGGGGCACACTAGACC
LEXKO-1588-21	TGCCCTTAGACACTTGGTTGGTTA
LEXKO-1588-22	CCATGGTCATCCTCCCGACT

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
DNTP mix (10 mM)	0,5	0,2
$MgCl_2$ (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
H_2O*	13,7	
Final volume	25	

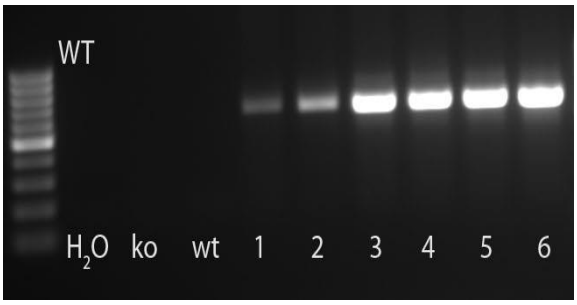
*** The amount of H_2O is adjusted with the number of primer.**

Amplification conditions

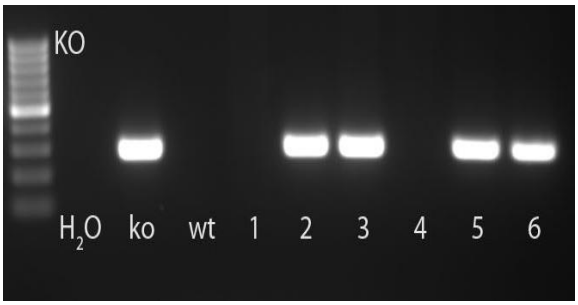
PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 65-55 (↓1°C/Cycle) 72°C	30 sec 45 sec 45 sec	39
3 Polymerisation	72°C	10 min	1
4 Cooling	4°C	hold	1

use Touch-Down cycling protocol: first 10 cycles anneal at 65°C, decreasing 1°C per cycle, next 30 cycles aneal at 55°C
These PCR conditions have been optimized for our methods and preparation kits. Adaptions may be required.

Gel Image



WT-PCR



Mutant-PCR

Separated by gel electrophoresis on a 2% agarose gel.