EMMA ID: *04425*
Gene: *Abca3*
Common name: *Abca3tm1Holz*

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	Abca3_wtF	Abca3_R	708
Mutant	Neo_F	Abca3_R	350

Primer sequences

Primer Name	Sequence 5' --> 3'
Abca3_wtF	CACAGCCTACTACCAGCAAACAGGA
Abca3_R	GAAAGCCCATCCTAAAGTATCAGCC
Neo_F	CTGAAGAGCTTGGCGGCGAATGGGCTG

PCR setup (LongAMP Taq)

Component	Volume (µl) 1x
DNA (~ 50-100 ng)	4
100% DMSO	0,4
PCR-Buffer (5x)	4
DNTP mix (10 mM)	0,5
Primer 1 (10 pmol/µl)	1
Primer 2 (10 pmol/µl)	1
Taq Polymerase (5 U/µl)	0,3
H_2O*	13,7
Final volume	20

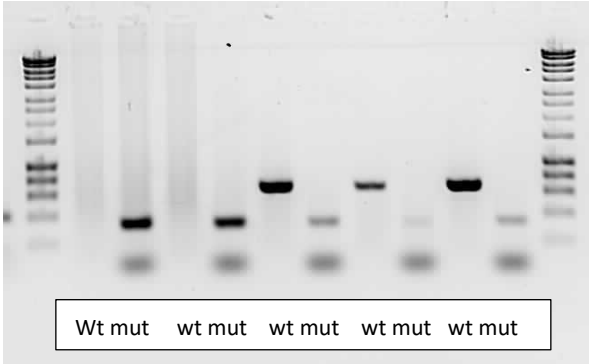
*** The amount of H_2O is adjusted with the number of primer.**

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	94°C	3 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 68-58 (↓1°C/Cycle) 65°C	30 sec 20 sec 60 sec	39
3 Polymerisation	65°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.