EMMA ID: *08134*
Gene: *Mcam*
Common name: *B6-Mcamtm1Pyu*
Allele: *Mcam* ^{tm1Pyu}

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	U3D5	UCYAS	561
Mutant	MCAMNeoIn 297	MCAM KO12	896

Primer sequences

Primer Name	Sequence 5' --> 3'
U3D5	CTGGAGACAGGTGCAGAGT
UCYAS	ATCTCCTGTTTTCCCGAGCGT
MCAMNeoIn 297	CGCCTTCTATCGCCTTCTTGA
MCAM KO12	GGTTAGGGGTTACACAGGGT

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	

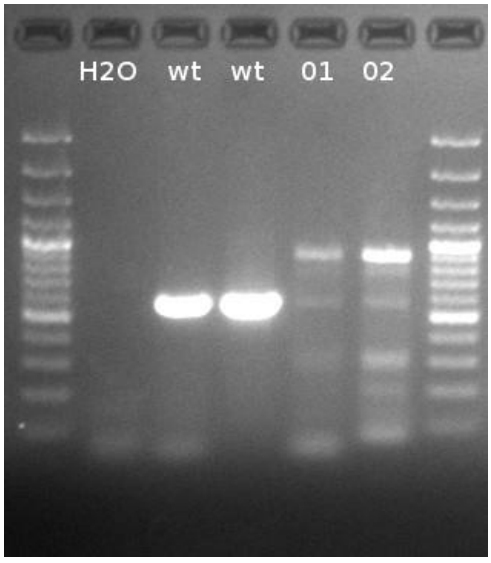
*** The amount of H_2O is adjusted with the number of primer.**

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 58°C 72°C	30 sec 45 sec **1:30 min**	39
3 Polymerisation	72°C	15 min	1
4 Cooling	4°C	hold	1

These PCR conditions have been optimized for our methods and preparation kits. Adaptions may be required.
Use longer elongationtime to amplify the mutant-band.

Gel Image



Separated by gel electrophoresis on a 2% agarose gel.