EMMA ID: *EM:13830*
Gene: *Mmachc*
Common name: *Mmachc flox/flox*
Allele: *Mmachc*$^{tm1.1Pg}$

Allele Information

To generate the Mmachc-flox/flox mice, a cassette of foreign DNA containing loxP-FRT-neomycin-FRT sequences was inserted into intron 2, 143 bp upstream of exon 3.
The second loxP site was inserted into the 3'UTR of exon 4, 220 bp downstream of the STOP codon. After Flp-mediated recombination to delete the neomycin gene, a single FRT site remained in intron 2.

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. In addition to the expected product, the mutant assay may also amplify the endogenous wild type sequence, which will appear as a larger band on an agarose gel. The presence of this extra band will depend on the size of the original deletion.

PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Wild type	Mmachc_F	Mmachc_R	375
Mutant	Mmachc_F	Mmachc_R	522

Primer sequences

Primer Name	Sequence 5' --> 3'
Mmachc_F	GGTGAATTCCAGCAGTAG
Mmachc_R	CTGCATCCACATCTTGTC

PCR setup

Component	Volume (µl) 1x
DNA (~ 50-100 ng)	2
5x Green GoTaq Flexi Buffer	5
dNTPs (2.5mM each)	1
$MgCl_2$ (25 mM)	1,5
Primer 1 (10 pmol/µl)	1
Primer 2 (10 pmol/µl)	1
DMSO	1
GoTaq G2 Flexi DNA Polymerase	0,15
H_2O*	12,35
Final volume	25

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	94°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 56°C 72°C	30 sec 45 sec 45 sec	35
3 Polymerisation	72°C	10 min	1
4 Cooling	4°C	hold	1

Gel Image

Mmachc flox/flox Genotyping controls: