EMMA ID: *12394*
Gene: *Tg(Adipoq-cre)1Evdr, Mgll*
Common name: *MGL flox/AdiQ-Cre*
Allele: *Mgll $^{tm1.2Rzim}$ Tg(Adipoq-cre)1Evdr*

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)
MGL wildtype	MGL flox FW	MGL flox RV	400
MGL flox	same as wt	same as wt	490
AdiQ-Cre	AdiQ-Cre FW	Cre RV	950

Primer sequences

Primer Name	Sequence 5' --> 3'
MGL flox FW	TTGCAGCTGGAGTCTGTGTC
MGL flox RV	GTCAGTCGAGGCTGGAAGAG
AdiQ-Cre FW	AGACCTCCTGGGAGAGTGAGG
Cre RV	TAGCTGGCTGGTGGCAGATG

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 68-58 TD 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 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