EMMA ID: *07800*
Gene: *Map3k14*
Common name: *C57BL/6J-Map3k14<aly> N12*
Allele: *Map3k14* ^{aly}

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.

The PCR produces a 509 bp product, which has to be cut into different fragments by MspA1l digestion.

Assay	Forward Primer	Reverse Primer	PCR product cut into (bp)
Wildtype	Primer 310	Primer 311	152
Mutant	Primer 310	Primer 311	179

Primer sequences

Primer Name	Sequence 5' --> 3'
Primer 310	GCCTACTGACATCCCGAGCTACTTCAGC
Primer 311	CTGTTACAGACGCCCTGGTAAGATGC

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 56°C 72°C	30 sec 45 sec 45 sec	39
3 Polymerisation	72°C	10 min	1
4 Cooling	12°C	hold	1

These PCR conditions have been optimized for our methods and preparation kits. Adaptions may be required.

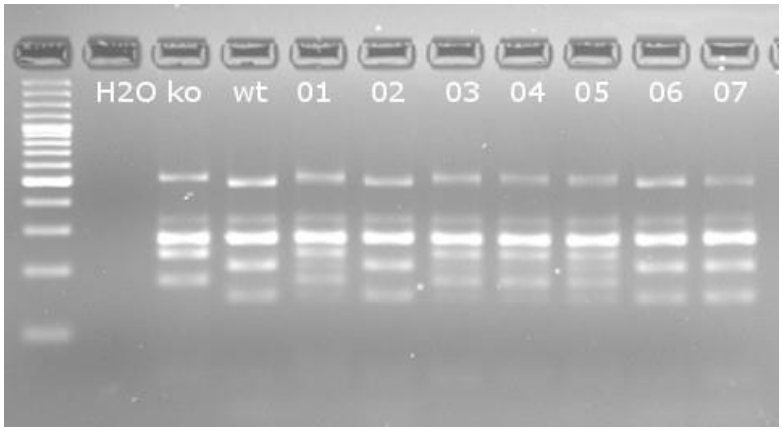
Restriction digest

Digest the PCR product by adding digestion-premix:	7.7 ml Water
	0.2 ml BSA (10 mg/ml)
	0.9 ml NEB buffer 4
	0.2 ml MspA1I
digest for one to four hours, 37°C	add 9.0 ml per 11 ml PCR

MspA1I digest of 509 bp PCR product:

for wt allele:	PCR product cut into:
	274 bp, 152 bp, 57 bp, 27 bp
for aly allele:	PCR product cut into:
	274 bp, 179 bp, 57 bp.

Gel Image



Separated by gel electrophoresis on a 3-4% agarose gel.