EMMA ID: *EM:16101*
Gene: *Mast3*
Common name: *Mast3-em1*
Allele: *Mast3* $^{em1(IMPC)Hmgu}$

Allele Information

CRISPR/Cas9-mediated knock-in point mutation in Mast3 resulting in a glycine-to-serine substitution at amino acid position 516 (p.Gly516Ser). CRISPR/Cas9 genome editing using guide RNA targeting Mast3 and a donor template introducing the G516S substitution (induced knock-in point mutation). Cas9 was delivered by electroporation in the form of a ribonucleoprotein complex. For this, we obtained Cas9 protein commercially from IDT (Integrated DNA Technologies).

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)
Mutant/wild type	mM3G516S_R_Caro	mM3G516S_F_long_	577
Sequencing of the PCR product			

Primer sequences

Primer Name	Sequence 5' --> 3'
mM3G516S_R_Caro	TGAGGCTAGCCTAGCCTG
mM3G516S_F_long_Caro	catttagcgcacagacagaag

Touch-Down cycling protocol: first 10 cycles anneal at 65°C, decreasing 1°C per cycle, next 30 cycles aneal at 55°C.These PCR conditions have been optimized for our methods and preparation kits.

PCR setup (AllTaq)

Component	Volume (µl) 1x	Final conc.
DNA (~ 50-100 ng)	2	
Q-Solution (5x)	2,5	0,5
PCR-Buffer (5x)	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)	0,5	2,5 U/rxn
H_2O*	11	
Final volume	25	

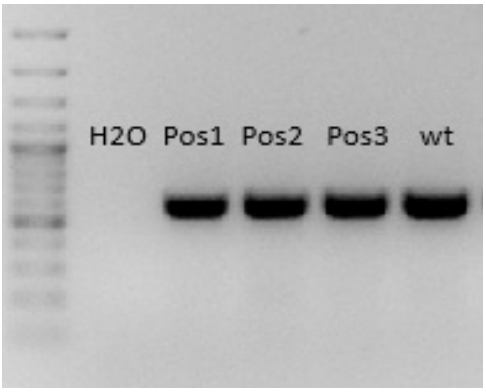
*** The amount of H_2O is adjusted with the number of primer.**

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	95°C	5 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 65-55 TD 72°C	30 sec 45 sec 45 sec	39
3 Polymerisation	72°C	10 min	1
4 Cooling	4°C	hold	1

Touch-Down cycling protocol: first 10 cycles anneal at 65°C, decreasing 1°C per cycle, next 30 cycles aneal at 55°C.These PCR conditions have been optimized for our methods and preparation kits. Adaptions may be required.

Gel Image



Pos1,Pos2,Pos3 mutant

Separated by gel electrophoresis on a 2% agarose gel.