EMMA ID: *EM:16178*
Gene: *Mast2*
Common name: *Mast2-em1*
Allele: *Mast2* $^{em1(IMPC)Hmgu}$

Allele Information

Knock-in frameshift mutation in the MAST2 gene (L1196Wfs*92) introduced via CRISPR/Cas9. 4 additional synonymous substitutions were introduced as part of the CRISPR strategy to facilitate genotyping via restriction digest.

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	M2L96 F	M2L96 R	500

sequencing of the PCR product or restriction digestl of 5µl PCR product in 25µl with MluI-HF (NEB) at 37°C for 3 h.

Primer sequences

Primer Name	Sequence 5' --> 3'
M2L96 F	CAAACCCCAGACCGTTCTAA
M2L96 R	TTCCGGAATAGGGAGCTTCT

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	

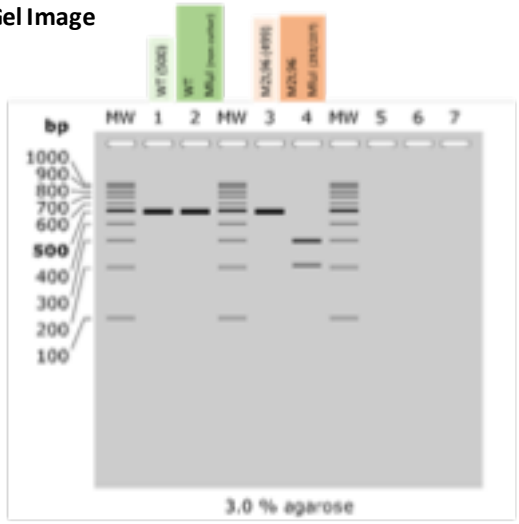
*** The amount of H_2O is adjusted with the number of primer.**

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



Comments/Additional information:

Genotyping is done by PCR followed by restriction digest.
MluI restriction enzyme will only cut the mutant amplification product.
Wildtype => non-cutter (500 bp fragment)
Homozygous => 2 fragments at 300 bp and 200 bp
Heterozygous => mixed, 3 fragments at 500 bp, 300 bp and 200 bp