EMMA ID: *13451*
Gene: *Zmym6*
Common name: *Zmym6-em1_3_1*
Allele: *Zmym6* $^{em1(IMPC)Hmgu}$

Allele Information

For more information on production,guides and mutation, search for gene/project, go to project summary, go to production plan, go to production outcome and "more details"
https://www.gentar.org

IMPC mouse phenotype data, search by the gene name
http://www.mousephenotype.org/

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.

Zmym6-em1_3_1 contains a +1bp insertion and therefore needs to be sequenced.

PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Wild type	Zmym6 for	Zmym6 rew	420
Mutant	same as wt	same as wt	421

Primer sequences

Primer Name	Sequence 5' --> 3'
Zmym6 for	gagagtagttattactttcttactgtt
Zmym6 rew	agagagaccttaactaagaaatcagaa

PCR setup (LongAmp® Taq DNA Polymerase)

Component	Volume (µl) 1x
DNA (~ 50-100 ng)	2-4
100% DMSO	0,4
PCR-Buffer (5x)	4
DNTP mix (10 mM)	0,5
Primer 1 (10 pmol/µl)	1
Primer 2 (10 pmol/µl)	1
Primer 3 (10 pmol/µl)	1
Taq Polymerase (2,5U/µl	0,5
H2O*	7,6
Final volume	20

*** The amount of H2O is adjusted with the number of primer.**

Amplification conditions

PCR Settings	Temperature (°C)	Time	# of cyles
1 Denaturation (Melting)	94°C	3 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 68-58 (↓1°C/Cycle) 65°C	30 sec 20 sec 1 min	39
3 Polymerisation	65°C	10 min	1
4 Cooling	4°C	hold	1

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.

Sequence / mutation

Wt:

caacctacctaccggaaggccggatttgcccagctttactgctccatgggatgcatcatcagattctcctccgcagtctgcgtgccacctcttcccaagagaacctgtgcacattgctc
caagtacagttcctaagcttgcactttcccttccttctgatttcttagttaaggtctctctctgtagccatggctgtcctagaactctgtgtagaccaggctagccctgaactcacagaga
tcctcctgtctttgcctgaagtgctgggattaagg

Mut:

caacctacctaccggaaggccggatttgcccagctttactgctccatgggatgcatcatcagattctcctccgccagtctgcgtgccacctcttcccaagagaacctgtgcacattgct
ccaagtacagttcctaagcttgcactttcccttccttctgatttcttagttaaggtctctctctgtagccatggctgtcctagaactctgtgtagaccaggctagccctgaactcacagag
atcctcctgtctttgcctgaagtgctgggattaaggtgtgcaccaccatg