EMMA ID: *EM:16995*
Gene: *Rsph6a*
Common name: *Rsph6a-em1_2*
Allele: *Rsph6a* $^{em1(IMPC)Hmgu}$

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.

PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Wild type	Rsp6a_F	Rsp6a_wtR	311
Mutant	Rsp6a_F	Rsph6a_R	464

Primer sequences

Primer Name	Sequence 5' --> 3'
Rsp6a_F	ATACGTTTTCTCAATCTCTGCCT
Rsph6a_R	AGTTCAAAACAGGGCCTCAA
Rsp6a_wtR	TGGGGGACACCACTCAGTC

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
Primer 3 (10 pmol/µl)	1	0,4
Taq Polymerase (5 U/µl)	0,5	2,5 U/rxn
H_2O*	10	
Final volume	25	

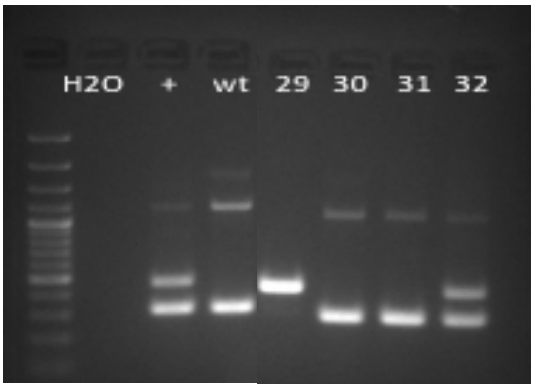
*** The amount of H_2O is adjusted with the number of primer.**

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

Gel Image



+	**het control**
29	**hom**
30,31	**wt**
32	**het**

Separated by gel electrophoresis on a 2% agarose gel.