

EMMA ID: 13557

Gene: *Tspan8*

Common name: *Tspan8-em1_3*

Allele: *Tspan8*^{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.

Tspan-em1_3 contains a -2bp deletion and therefore needs to be sequenced.

PCR primer pairs and expected size bands

Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Wild type	Tspan8 seq for 2	Tspan 8 seq rev	309
Mutant	same as wt	same as wt	307

Primer sequences

Primer Name	Sequence 5' --> 3'
Tspan8 seq for 2	CAGTGGGGACTCAAGGGGTATTTG
Tspan 8 seq rev	GGTTTCATTAAGCTTGGCAAGGAC

PCR setup (LongAmp® TaqDNA 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/µ)	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 cycles
1 Denaturation (Melting)	94°C	3 min	1
2 Amplification (Melting, Annealing, Polym.)	94°C 68-58 (↓1°C/Cycle)	30 sec 20 sec	39
3 Polymerisation	65°C	1 min 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 anneal at 58°C. These PCR conditions have been optimized for our methods and preparation kits. Adaptions may be required.

Sequence / mutation

Wt:

GCCTGACACCCAGTGGGGACTCAAGGGGTATTTGGAAAATGAGTAAAATAAACATCAAGGGAAGGAATCTATACATCAGTTATTTTC
 TTTTCTTTTTCTAGATCATCACGTCAGGAGACTCTAGCACGAACCCCTTCATTGCTGTGAATATCTTGATTGCAGTTGGGTCTATCATC
 ATGTTCTAGTTTCTGGGATGCTGTGGAGCTGTGAAAGAAAGCCGGTGCATGCTTCTTGGTAGGCAACCCACCCCTCAGACCC
 CCAAGGGTTATCTGTGATGTCAAGACTTGTCTTGCCAAGCTTAATGAAACCCTGACTAAC

Mut:

GCCTGACACCCAGTGGGGACTCAAGGGGTATTTGGAAAATGAGTAAAATAAACATCAAGGGAAGGAATCTATACATCAGTTATTTTC
 TTTTCTTTTTCTAGATCATCACGTCAGGAGACTCTAGCACGAACCCCTTCATTGCTGTGAATATCTTGATTGCAGTTGGGTCTATTCAT
 GTTTCTAGTTTCTGGGATGCTGTGGAGCTGTGAAAGAAAGCCGGTGCATGCTTCTTGGTAGGCAACCCACCCCTCAGACCC
 AAGGGTTATCTGTGATGTCAAGACTTGTCTTGCCAAGCTTAATGAAACCCTGACTAAC