

Gene: Lfng

Colony prefix: DAMM

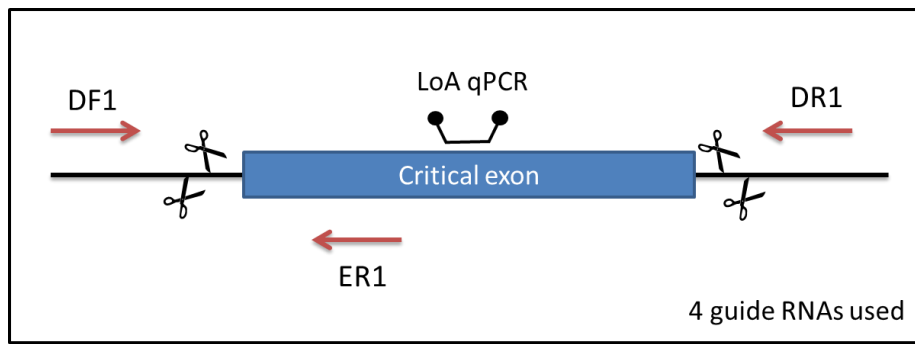
Allele: Lfng^{em1(IMPC)Wtsi}

Allele type: Crispr/Cas9 mediated deletion

Anticipated mutation: Exon deleted and out of frame

Allele information:

Further information about the allele can be found on the 'International Mouse Phenotyping Consortium' (IMPC) web site at <http://www.mousephenotype.org>



Mutant Allele sequence:

TGCAGAGGAGACCCTTTTCAGTCCTCTGGGGACCCTTTGAGTCCCGCTTAGCAGCAACAGGTGGCGGGTACTGG
GGGAAAAAAACTGGCTGAATGAATGGGGCCACTACCCCTGACAGCAGACTGTTCCCTGGTGCTCGCTCTTGGTC
CTGGGTTCCCTGCAGCAGCAGCGTCTGGCTTGGGCTATTCTCTCCCTGCCTTAGG

Deletion size (bp): 698

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Guide RNAs used in initial experiment

Sequence	Chr	Chr Start	Chr End
GCTGAATGAATGGGCAGCGGTGG	5	140612155	140612177
CCTGCCGCTTGTGCGGACCAGGC	5	140612241	140612263
CCTAGTTGCCACTACCCCTGACA	5	140612860	140612882
CCTGGTGCTCGCTCTTGGTCCTG	5	140612893	140612915

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Genotyping by end-point PCR

These mice may be genotyped through a combination of separate PCR reactions that detect the gene-specific wild type allele and a mutant allele-specific short range PCR. Interpretation of the consolidated results produces the genotype of the mice. 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.

PCRs primer pairs and expected size bands

Assay Type	Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Standard PCR	Wild type	Lfng_DF3	Lfng_ER1	370
Standard PCR	Wild type	Lfng_EF1	Lfng_DR2	245
Standard PCR	Mutant	Lfng_DF3	Lfng_DR2	333

Primer sequences

Primer Name	Primer Sequence (5' > 3')
Lfng_DF3	TGGTCCCAAGCAACTTCTTC
Lfng_ER1	GTTGGTGAGCACCACATTGC
Lfng_EF1	GTCTCCTGACAGCCCTTGTT
Lfng_DR2	GCTACCTAAGGCAGGGAGAGA

Reaction setup

Reagent	µl
DNA (~50-100 ng)	1
10x Buffer	1.5
MgCl ₂ (50 mM)	0.45
Platinum Taq (Invitrogen)	0.15
dNTPs (100 mM)	0.15
Primer 1 (10 µM)	0.3
Primer 2 (10 µM)	0.3
ddH ₂ O	11.15
Total	15

Amplification conditions

Step	Conditions	Time
1	94°C	5 min
2	94°C	30 sec
3	58°C	30 sec
4	72°C	1:30 sec
5	Go to '2' + 34 cycles	-
6	72°C	5 min
7	12°C	forever

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Genotyping by loss of WT allele qPCR Assay (gene-specific assay)

The wild type loss of allele (LoA) qPCR assay uses a hydrolysis probe assay (for example Applied Biosystems TaqMan® technology) to determine the copy number of the wild type allele in a sample. Homozygotes will show no amplification, heterozygotes one copy and wild type mice will show two copies when compared to a wild type control.

The number of copies of the wildtype allele can be detected using a FAM-labelled custom qPCR TaqMan® assay. These are multiplexed with a VIC® labelled endogenous control assay (for example TaqMan® Copy Number Reference Assay, Mouse, Tfrc; Applied Biosystems part #4458366). Reference DNA controls of known genotypes should also be included to facilitate correct analysis.

Primers for LoA qPCR assay

Gene	Source	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
Lfng	Life Technologies	CGCCTTGTGTTTCAGGTGG TT	CCCGGAGGTTGACGTAGTTG	TGCCACGTGGATGAT

Reactions are performed in a 10µl volume using an Applied Biosystems 7900HT Fast Real-Time PCR System or Applied Biosystems Viiia7 with DNA prepared using the Sample-to-SNPTM kit (Applied Biosystems) from mouse ear biopsies. GTXpress™ buffer is also used (Applied Biosystems).

Reagent	µl
2x GTXpress™ buffer	5
20x target assay	0.5
ddH ₂ O	3
Tfrc endogenous 20x assay	0.5
DNA	1

Amplification conditions

Step	Conditions	Time
1	95°C	20 sec
2	95°C	10 sec
3	60°C	30 sec
4	Go to '2' + 34 cycles	-

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Links to information and frequently asked questions

MGP mouse phenotype data:

<http://www.mousephenotype.org>

Useful publications

White, J.K., Gerdin, A.-K., Karp, N.A., Ryder, E., Buljan, M., Bussell, J.N., Salisbury, J., Clare, S., Ingham, N.J., Podrini, C., et al. (2013). Genome-wide Generation and Systematic Phenotyping of Knockout Mice Reveals New Roles for Many Genes. *Cell* 154, 452–464.

Mali P, Yang L, Esvelt KM, et al (2013) RNA-guided human genome engineering via Cas9. *Science* 339:823–6. doi: 10.1126/science.1232033

Jinek M, Chylinski K, Fonfara I, et al (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 337:816–21. doi: 10.1126/science.1225829

Cong L, Ran FA, Cox D, et al (2013) Multiplex genome engineering using CRISPR/Cas systems. *Science* 339:819–23. doi: 10.1126/science.1231143

Singh P, Schimenti JC, Bolcun-Filas E (2014) A Mouse Geneticist's Practical Guide to CRISPR Applications. *Genetics* genetics.114.169771–. doi: 10.1534/genetics.114.169771

Brandl C, Ortiz O, Röttig B, et al (2015) Creation of targeted genomic deletions using TALEN or CRISPR/Cas nuclease pairs in one-cell mouse embryos. *FEBS Open Bio* 5:26–35. doi: 10.1016/j.fob.2014.11.009

Zhou J, Wang J, Shen B, et al (2014) Dual sgRNAs facilitate CRISPR/Cas9 mediated mouse genome targeting. *FEBS J*. doi: 10.1111/febs.12735

Kraft K, Geuer S, Will AJ, et al (2015) Deletions, Inversions, Duplications: Engineering of Structural Variants using CRISPR/Cas in Mice. *Cell Rep*. doi: 10.1016/j.celrep.2015.01.016

Shen B, Zhang J, Wu H, et al (2013) Generation of gene-modified mice via Cas9/RNA-mediated gene targeting. *Cell Res* 23:720–3. doi: 10.1038/cr.2013.46

Wang H, Yang H, Shivalila CS, et al (2013) One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. *Cell* 153:910–8. doi: 10.1016/j.cell.2013.04.025

Yang H, Wang H, Shivalila CS, et al (2013) One-Step Generation of Mice Carrying Reporter and Conditional Alleles by CRISPR/Cas-Mediated Genome Engineering. *Cell* 154:1370–1379. doi: 10.1016/j.cell.2013.08.022

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