Wellcome Trust Sanger Institute
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Gene: Igsf8

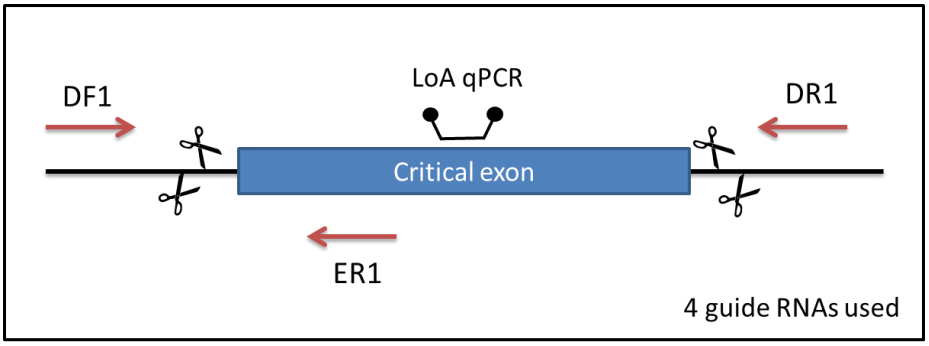
Colony prefix: DAEK

Allele: Igsf8^{em2Wtsi}

Allele type: Crispr/Cas9 mediated deletion

Allele information:

Further information about the allele can be found on the 'International Mouse Phenotyping Consortium' (IMPC) web site at http://www.mousephenotype.org/data/genes/MGI:2154090



Mouse QC information

Loss of WT Allele (LOA) qPCR	Pass	**Mutation Sequence confirmed**	Pass
Mutant Specific SR-PCR	Pass	**Off-target analysis complete**	na

Flanking sequence:

100bp 5' and 3' of the deletion

[CTGTCTATACCCCAGACTCCGGATGGACTCCTCTGTTCATGTAGGTGCTCTGCCTCTAGTTAGCTAGAGTATTCC
TCTGTTCAGGTGGATCTGAACCTAC][AGCACTCTACTAAGCTATATTCCCTGTGTTGTCTTTTGAGGGCTCACTCT
TGTAGCCCAGGCTAGCTTACAACTAGCCTAGGTTGGCCTGAAACTGGTGA]

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Deletion sequence:

[GTGATGTGCTGGGATGCTGGTGTCTCGTGTCCCATCTTGTGTTCCCATCTGTGTCTTCGCCCAGCTATGTTCTAA
CCATCCCTAGGTTTCCCTTTCCTTCACAGGCAGCAGTCAAGAATGGTGCTGATGATACTGGCCATCAAGTGGGGA
CTCGTTCTCTCTAAAAAGATTCCAGTTCTTTCTGCTTTTACCCCACAGGAACCAGGTGCTACGCCCGGCAGGTGC
ATGTCCCCAGGGGACCTCTTTACCGGGTGGCTGGCACCGCTGTCTCTATCTCCTGCAACGTGAGTGACTATGAG
GGCCCTGCCCAGCAAGACTTCGAGTGGTTCATGTACAGACCAGAGGCCCCAGCTACGTCCCTGGGCATTGTCAG
CACCAAGGATAGCCAGTTCTCCTATGCTGTCTTTGGGCCTCGTGTGGCATCTGGTGACCTGCAGGTGCAGCGCC
TGAAGGGAGATTCGGTGGTGCTCAAGATTGCTCGCCTGCAGGCCCAGGACTCTGGCTTTTATGAGTGCTACACC
CCCTCCACAGATACGCAGTACCTGGGCAACTACAGTGCCAAGGTGGAGCTGAGAGGTACTGAGCTCTGAGCTAG
GTGGTGAGAGAGCCTCACAAGCACTGGGGTTATAGGTATAAGCCAGCACGCTCAGATCTATTTTTGCTTCGTCTT
CCTCCTTCTCTGTCATCTTCTTCCTTCTCCTCTTCTCCCCCCACCCCCATCTTCTCAGTACTGGAAATTGAACCTAC
AGCGTGGAACATACCAAGGG]

Guide RNAs used in initial experiment

Sequence	Chr	Chr Start	Chr End
CCTACGTGATGTGCTGGGATGCT	1	172315948	172315970
CCATCTGTGTCTTCGCCCAGCTA	1	172315997	172316019
TGGAAATTGAACCTACAGCGTGG	1	172316684	172316706
CCAAGGGAGCACTCTACTAAGCT	1	172316713	172316735

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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	Igsf8_DF1	Igsf8_ER1	247
Standard PCR	Mutant	Igsf8_DF1	Igsf8_DR1	235

Primer sequences

Primer Name	Primer Sequence (5' > 3')
Igsf8_DF1	TTTTACTTACCGGGCTGTCTATACC
Igsf8_ER1	GTATCATCAGCACCATTCTTGACTG
Igsf8_DR1	CAGTGAATTGTGGGAGTTTTGTCAC

Reaction setup

Reagent	µl
DNA (~50-100 ng)	1
10x Buffer	1.5
MgCl2 (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
ddH20	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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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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