Wellcome Trust Sanger Institute
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Gene: Ceacam3

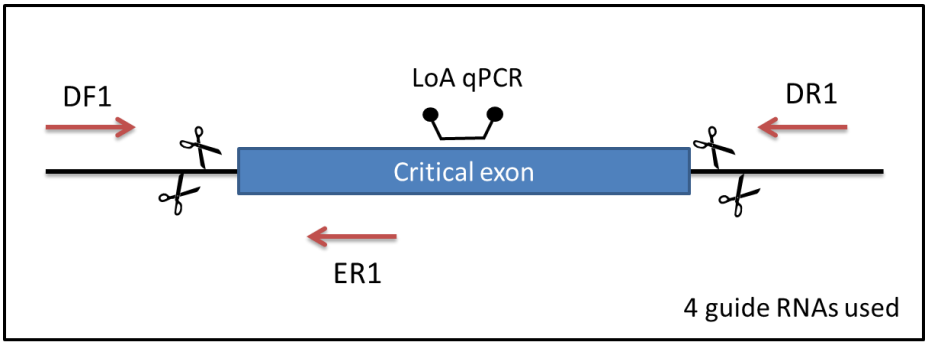
Colony prefix: DAEG

Allele: *Ceacam3*^{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:3646296



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

[AAGTAGGGCAGGGAGACACCCACCCCACACACCTCGGCTGAGCAATGGAGACACCTGCTCACAACACAGGGCG
CCATCTTAGTCAAATACAAGCATCCCC][AAGCAACTCAGCCTAAGGTGTAAGACACTGTCCAGTCTTGTGGGGAT
CCCAATGTATGTTCCCCTAAGAAAGACCCTGTGGGCATTAGGCAGGGGCTGAT]

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Deletion sequence:

[ATATTGATGGATCTCTCTCTTCTCTTCTGCTCAGGACACAGGGCAGCATCTTAGTCAAATACAAGCGTCCCCATAT
TAATGGAACTCTCTCTTCCCTTGTAGCCTGTCTTTCAATCTACGAGAACCCTCCTACCTCTGCCCAGCTCACTGTT
GAATCAGCACCGACCAGTGTTGCTGAAGGGGAAAGTGTTCTTCTACTTGTTCACAATCTCCCAGAGAATCTCCGA
GCCGTTTTCTGGTACAAAGGGGCGATTGTGTTCAAGGACCTTGAGGTTGCACGGTATGTAATAGCCAAGAATTCA
AGTGTTCCGGGCCCTGCCCACAGCCGTAGAGAGACAGTGTACAGCAATGGATCCCTCCTGATTCAGAATGTCAC
TCGGAATGATGCCGGATTCTACACCTTACGAACTCTGAGTACAGATCTGAAAGCTGAAGTAGCGCATGTGCAACT
CCAGGTGGACAGTAAGTGGTTCTCTGTGATTAGTCAGTGCTGTGTGGGGCTTAAACACACAGAACTGTCCTTTCT
GGCCTGTGCATAGTGTTCCCATGTTAAGTTTTGAGCACTTCATGCAAGACACACATGGTGGAGACAAATGACCAT
AGATCAGACTCCATTTTCTGATTCCCTTCTGCATCCAGAAAGACCTGGTTGGA]

Guide RNAs used in initial experiment

Sequence	Chr	Chr Start	Chr End
CCTGCTCACAACACAGGGCGCCA	7	17157954	17157976
TACAAGCATCCCCATATTGATGG	7	17157988	17158010
CCTTTCTGGCCTGTGCATAGTGT	7	17158520	17158542
TGCATCCAGAAAGACCTGGTTGG	7	17158631	17158653

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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	Ceacam3_DF1	Ceacam3_ER1	434
Standard PCR	Mutant	Ceacam3_DF1	Ceacam3_DR1	595

Primer sequences

Primer Name	Primer Sequence (5' > 3')
Ceacam3_DF1	CAGAACAACAATAAGTCAGAGAGCC
Ceacam3_ER1	TCGTAGATTGAAAGACAGGCTACAA
Ceacam3_DR1	AATAACTACCCCAGTTTGACATGGA

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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