Wellcome Trust Sanger Institute
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Gene: Cd96

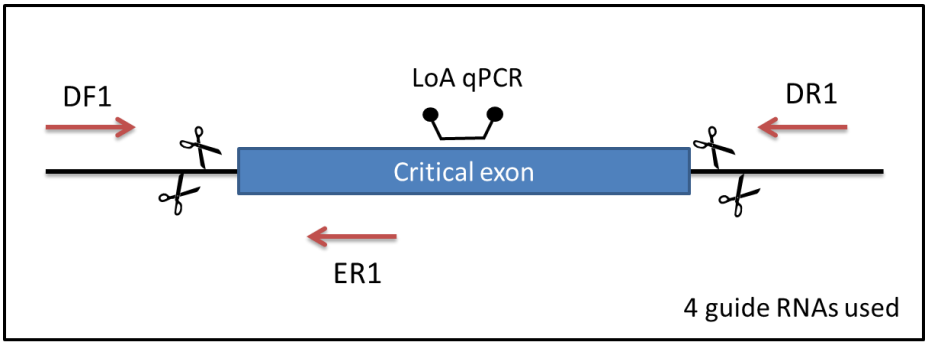
Colony prefix: DAEH

Allele: *Cd96*$^{em1(IMPC)Wtsi}$

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:1934368



Mouse QC information

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

Mutant Allele sequence:

GCTTATTCTATTAGCTGAATAATTATTCTTTAGCCTTCCACCCTTGTCCCTACCCTCAATGAGAATGATA
GGAATTAAGGCAAACAGAATTGCAGGAGTTGCTGGTTTGAGATACCCAATGTACCATTTCCTCTGGAA
GAGCAACAGGGACTTTAAAATGGGTTAAGCATGCGTTAAAATGGGGAGAGACTGAGTATGCT

Deletion size (bp): 512

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Guide RNAs used in initial experiment

Sequence	Chr	Chr Start	Chr End
CCCTACCCTCAATGAGAATGATA	16	46098740	46098762
ATTGCAGGAGTTACCCTGTGTGG	16	46098781	46098803
CGCATGAGGGCAGATTCTTAGGG	16	46099246	46099268
GAATTCACTGGAACTAGAGCTGG	16	46099287	46099309

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

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Assay Type	Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Standard PCR	Wild type	Cd96_DF1	Cd96_ER1	299
Standard PCR	Mutant	Cd96_DF1	Cd96_DR2	313

Primer sequences

Primer Name	Primer Sequence (5' > 3')
Cd96_DF1	TAAAGAGATTTCTGGTACCCTTGGG
Cd96_ER1	CTATGATCTCTTTGCCACCTTTGTG
Cd96_DR2	GCACAGCAATACTTTCTGTAGTAGC

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

Links to information and frequently asked questions

MGP mouse phenotype data:
http://www.mousephenotype.org

Useful publications

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