

Gene: Cd96

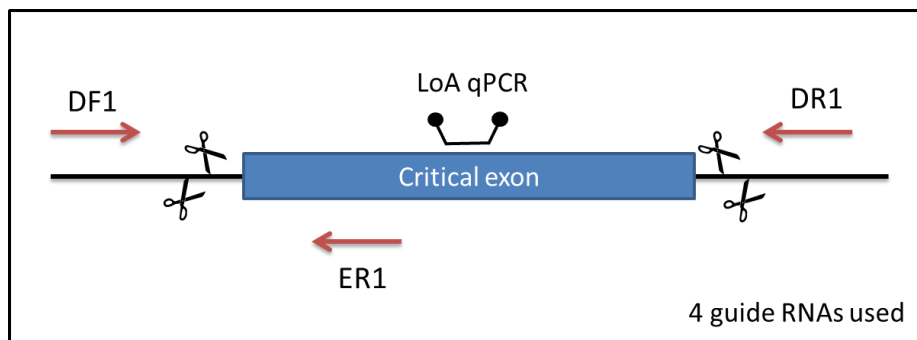
Colony prefix: DAEH

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:

GCTTATTCTATTAGCTGAATAATTATTCTTTAGCCTTCCACCCTTGTCCTACCCTCAATGAGAATGATA
GGAATTAAGGCAAACAGAATTGCAGGAGTTGCTGGTTTGAGATACCCAATGTACCATTTCTCTGGAA
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 |
| GAATTCAGTGGAACTAGAGCTGG | 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 |
| 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 |

Links to information and frequently asked questions

MGP mouse phenotype data:

<http://www.mousephenotype.org>

Useful publications

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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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