



Gene: Htra3

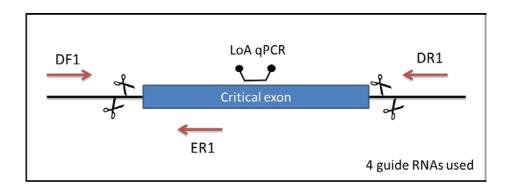
Colony prefix: DAFM

Allele: Htra3em1(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 <a href="http://www.mousephenotype.org/data/alleles/MGI:1925808/em1%2528IMPC%2529Wtsi">http://www.mousephenotype.org/data/alleles/MGI:1925808/em1%2528IMPC%2529Wtsi</a>



#### **Mouse QC information**

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

### **Mutant Allele sequence:**

TTGGTGGCCAGAGTAAGTGTGGGCACAGAGGTGAAAGGTGGGGACTGACCTCCCTGGTCTGTCAGCCCACAGGGACAAAGGTGGGATCTGACTAGAACATTTGCTGCAGTGAGACAAAGTACCACTGGTATCAGCATTAACAGCCCACAGTGGCGCCAACCTAGATGAGCTGCCCTCAGGCAGACTCTGTAAGCCCTC

Deletion size (bp): 559

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## **Guide RNAs used in initial experiment**

| Sequence                | Chr | Chr Start | Chr End  |
|-------------------------|-----|-----------|----------|
| ATCTGACTAGAACAGTAAAGAGG | 5   | 35668053  | 35668075 |
| CCCTCCTGGGCCCCGCTTCAGTC | 5   | 35668102  | 35668124 |
| CTGAGGCTGCCACTTGCGTGTGG | 5   | 35668529  | 35668551 |
| CCATAAAGATGTGACCGTCATCT | 5   | 35668592  | 35668614 |

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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 | Htra3_DF1      | Htra3_ER1      | 315                     |
| Standard PCR | Mutant    | Htra3_DF1      | Htra3_DR2      | 250                     |

# **Primer sequences**

| Primer Name | Primer Sequence (5' > 3') |
|-------------|---------------------------|
| Htra3_DF1   | AACAGCACCTGGATTTCTAGATTCT |
| Htra3_ER1   | GACATTGCCACGATTGTAATCCAC  |
| Htra3_DR2   | CTGAGGGCAGCTCATCTAGG      |

## **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: <a href="http://www.mousephenotype.org">http://www.mousephenotype.org</a>

### **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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