Wellcome Sanger Institute
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Gene: Triqk

Colony prefix: DARH

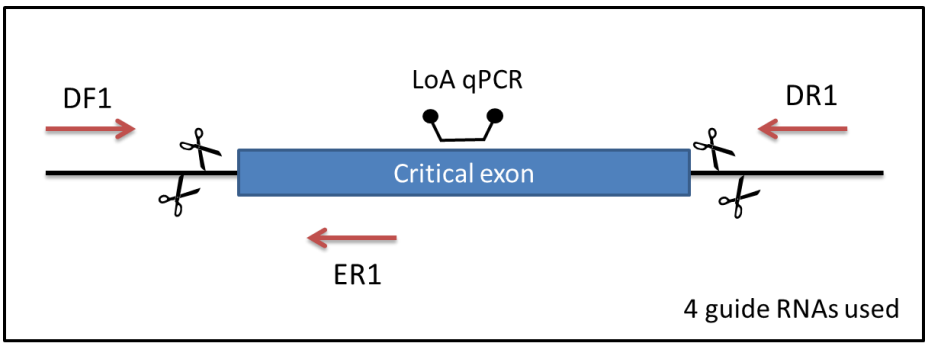
Allele: *Triqk*$^{em1(IMPC)Wtsi}$

Allele type: Crispr/Cas9 mediated deletion

Anticipated mutation: Exon deleted and out of frame

Allele information:

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



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:

ATAAATTTATCAGGAATTATTTTGAAATTTCTAAAACAAAGTTAACTTTACAAAATTATATGATAATATAATTATGTAT
GCATTATTTATCAGTGCCTTTAAAAAAGGTATAGTTAAAGTATATAACATAAACAGTACATAGAAAATAAGTTATTCC
TAAAGTTATCATTCTAGCTGTTATAATTATTGTTCAAACATTTCAG

Deletion size (bp): 589

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Guide RNAs used in initial experiment

Sequence	Chr	Chr Start	Chr End
CCTTTATTTCCAAAAGGTGCACT	4	12974436	12974458
CCGTGAATAAGCATGTAGTTCTC	4	12974602	12974624
AATTCTGGACATCTTAAGCTAGG	4	12974916	12974938
AAATTTTGAGGGCTCTCAAAAGG	4	12975015	12975037

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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	Triqk_DF1	Triqk_ER1	497
Standard PCR	Wild type	Triqk_EF1	Triqk_DR1	308
Standard PCR	Mutant	Triqk_DF1	Triqk_DR1	278

Primer sequences

Primer Name	Primer Sequence (5' > 3')
Triqk_DF1	AACAGCAAATATTTCTTAAACTAATCC
Triqk_ER1	GCTTTTAATTTGGTTGCTCGT
Triqk_EF1	TGTGTGTATGCCCGTTCATT
Triqk_DR1	TCTGACGAATCCCACAGACA

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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Genotyping by loss of WT allele qPCR Assay (gene-specific assay)

The wild type loss of allele (LoA) qPCR assay uses a hydrolysis probe assay (for example Applied Biosystems TaqMan® technology) to determine the copy number of the wild type allele in a sample. Homozygotes will show no amplification, heterozygotes one copy and wild type mice will show two copies when compared to a wild type control.

The number of copies of the wildtype allele can be detected using a FAM-labelled custom qPCR TaqMan® assay. These are multiplexed with a VIC® labelled endogenous control assay (for example TaqMan® Copy Number Reference Assay, Mouse, Tfrc; Applied Biosystems part #4458366). Reference DNA controls of known genotypes should also be included to facilitate correct analysis.

Primers for LoA qPCR assay

Gene	Source	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
Triqk	Life Technologies	GCAGAAGCAAAGAAAACAGCAA	CGGGCATACACACATCAAATTATAA	AGGCATAAAGGTATACTTC

Reactions are performed in a 10µl volume using an Applied Biosystems 7900HT Fast Real-Time PCR System or Applied Biosystems Viia7 with DNA prepared using the Sample-to-SNPTM kit (Applied Biosystems) from mouse ear biopsies. GTXpressTM buffer is also used (Applied Biosystems).

Reagent	µl
2x GTXpressTM buffer	5
20x target assay	0.5
ddH2O	3
Tfrc endogenous 20x assay	0.5
DNA	1

Amplification conditions

Step	Conditions	Time
1	95°C	20 sec
2	95°C	10 sec
3	60°C	30 sec
4	Go to '2' + 34 cycles	-

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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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