Wellcome Trust Genome Campus
Hinxton Cambridge CB10 1SA
T 01223 834244
F 01223 494919
www.sanger.ac.uk

Gene: Epas1

Colony prefix: DAQM

Allele: *Epas1*$^{em1(IMPC)Wtsi}$

Allele type: Crispr/Cas9 mediated Point mutation H194R

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/alleles/MGI:109169/em1%2528IMPC%2529Wtsi?

Mouse QC information

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

Guide RNAs and mutant oligos used in initial experiment

Sequence	Chr	Chr Start	Chr End
CCCCAGGTCCTGCACTGCACCGG	17	86809578	86809600
CTCCCTCTTCTCGGCCGTCTCGGCCTTGTCTTACTTCTGTGCTTTGACCCCAGGTCCTGCGCTGCACCGGGCAAGTGAGAGTCTACAACAACTGCCCCCCTCACAGTAGCCTCTGTGGCT	17	86809531	86809650

Mutant allele sequence:

TGGGAAGAAGAGCAAAGACGTGTCCACCGAGCGTGACTTCTTCATGAGGATGAAGTGCACGGTCACCAACAGAGGCCGGACTG
TCAACCTCAAGTCGGCCACCTGGAAGGTAGGATTCGTGGAGTCTCAAGAAAGAGCCAGGAGCAGGAGGTGCCTGAGGCCTCTC
CCTCTTCTCGGCCGTCTCGGCCTTGTCTTACTTCTGTGCTTTGACCCCAGGTCCTGC**[A/G]**CTGCACCGGGCAAGTGAGAGTCTAC
AACAACTGCCCCCCTCACAGTAGCCTCTGTGGCTCCAAGGAGCCCCTGCTGTCCTGCCTTATCATCATGTGTGAGCCAATCCAGCA
CCCATCCCACATGGACATCCCCCTGGACAGCAAGACTTTCCTGAGCCGCCACAGCATGGACATGAAGT

Report Generated on: 26-February-2019

Wellcome Trust Genome Campus
Hinxton Cambridge CB10 1SA
T 01223 834244
F 01223 494919
www.sanger.ac.uk

Genotyping by end-point PCR

PCRs primer pairs and expected size bands

Assay Type	Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Standard PCR	Screening*	Epas1_PM_WT_F	Epas1_PM_WT_R	403

*The screening PCR flanks the SNP region and can be used for sequence verification of the allele. The PCR will not distinguish wild type from mutant mice, however, as a product will be amplified in all cases.

We recommend that mice are sequence-verified with the screening primers to confirm the genotyping qPCR results when establishing the colony, in case of any cross-talk between the assays.

Primer sequences

Primer Name	Primer Sequence (5' > 3')
Epas1_PM_WT_F	TGGGAAGAAGAGCAAAGACG
Epas1_PM_WT_R	ACTTCATGTCCATGCTGTGG

Reaction setup

Reagent	µl
DNA (~50-100 ng)	1
10x Buffer	2
MgCl2 (50 mM)	0.6
Platinum Taq (Invitrogen)	0.2
dNTPs (100 mM)	0.2
Primer 1 (10 µM)	0.4
Primer 2 (10 µM)	0.4
ddH20	15.2
Total	20

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

Report Generated on: 26-February-2019

Wellcome Trust Genome Campus
Hinxton Cambridge CB10 1SA
T 01223 834244
F 01223 494919
www.sanger.ac.uk

Genotyping by SNP qPCR

We have observed that the clustering for this SNP qPCR assay is not optimal, and would recommend genotyping by sequencing

Primers for LoA qPCR assay

Gene	Source	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
Epas1	Life Technologies	GCCTTGTCTTACTTCTGTGCTTTG	GGCAGTTGTTGTAGACTCTCACTTG	[VIC]CCAGGTCCTGCACT [FAM]CCCAGGTCCTGCGCT

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-SNP™ kit (Applied Biosystems) from mouse ear biopsies. GTXpress™ buffer is also used (Applied Biosystems).

Reagent	µl
2x GTXpressTM buffer	5
40x target assay	0.25
ddH2O	3.75
DNA	1

Amplification conditions

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

Links to information and frequently asked questions

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

How the "critical" exon is decided:
http://www.i-dcc.org/kb/entry/102/

Report Generated on: 26-February-2019

Wellcome Trust Genome Campus
Hinxton Cambridge CB10 1SA
T 01223 834244
F 01223 494919
www.sanger.ac.uk

Relevant 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

Report Generated on: 26-February-2019