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www.sanger.ac.uk

Gene: Slc29a3

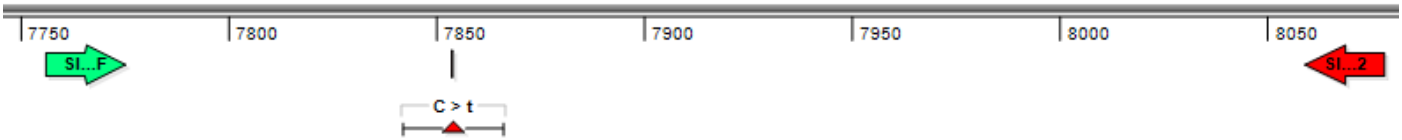
Colony prefix: DARW

Allele: *Slc29a3* *em1(IMPC)Wtsi*

Allele type: Crispr/Cas9 mediated Point mutation

Allele information: E447K

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	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
GTGGCCTCAGCCAGCTCCCGGGG	10	60715918	60715940
GGCTACCTCAGCACGCTGGTGCTCATCTATGGGCCCAAGATTG TGCCCCGGGAGCTGGCTAAGGCCACCAGTGTTGTGATGTTGTT CTATATGTCTGTGGGCTTGATGCTGGGCTCAGCCTGC	10	60715863	60715985

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Mutant allele sequence:

CGCCCCCTCCTAGATAAAGTGTTCAAGCAGGGCCGCGCAGGCTGAGCCCAGCATCAAGCCCACAGACATATAGAACAACATCAC
AACACTGGTGGCCT[C/t]AGCCAGCTCCCGGGGCACAATCTTGGGCCCATAGATGAGCACCAGCGTGCTGAGGTAGCCGTTGCT
GAGCCCCAGGAGGCAGGTGAAAAGCACTGGGTAGATGTCCGACTGGAAAAGCACCTTGGTCAAGTGTGAGCGCGGCTGGTAG
TTACAGAGCAAGAAGAGAGGCACAAGGCAGAACCGAGAGACCACCAGTCCGGGGAGCAGCTTGCTCCTAGGACCTG

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*	Slc29a3_PM_WT_F	Slc29a3_PM_WT_R2	323

*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')
Slc29a3_PM_WT_F	CGCCCCCTCCTAGATAAAGT
Slc29a3_PM_WT_R2	CAGGTCCTAGGAGCAAGCTG

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

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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

Genotyping by SNP qPCR

Primers for LoA qPCR assay

Gene	Source	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
Slc29a3	Life Technologies	GCATCAAGCCCACAGACA TATAGAA	GCTCATCTATGGGCCCAAGA	[VIC]TGGCTGAGGCCACC [FAM]CTGGCTAAGGCCACC

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-read	60°C	30 sec

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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/

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

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