

Gene: Syt1

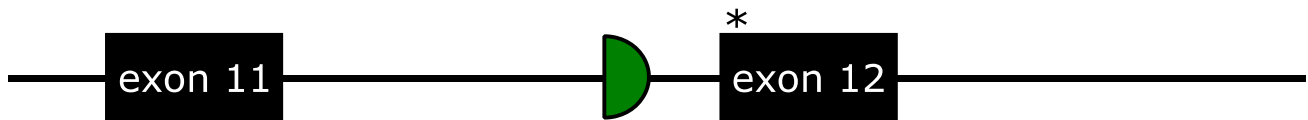
Colony prefix: TCBK

Allele: Syt1^{I367T_2.2}

Allele type: Point mutation + cassette insertion (removed via flp-mediated recombination)

Allele information:

Post flp-excision allele



I367T plus silent mutation

Mouse QC information

Loss of WT Allele (LOA qPCR)	Pass	Mutation Sequence confirmed	Pass
Mutant Specific SR-PCR	Pass	Neo count correct	N/A

Mutant allele sequence:

AGTGCAAGTGGTGGTAACTGTTTTGGACTATGACAA[GATT/AACT]GGCAAGAACGACGCCATCGGCAAAGTCTTT
GTGGG[C/G]TACAACAGCACCGG

I367T mutation = ATT -> ACT

Other SNPs are silent mutations introduced

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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	Wild type	Syt1_I367T_F	Syt1_I367T_R	698
Standard PCR	WT*	Syt1_I367T_F	Syt1_I367T_R	928
Standard PCR	Mutant	CasF1_Term	Syt1_I367T_R	626

* Due to the presence of remaining linker sequences post-flp excision, the *Syt1_I367T_F* + *Syt1_I367T_R* assay will produce a larger PCR band in mutant mice than in wild type mice. This size difference can be used to genotype the mice: 698bp band only = WT, 698p + 928bp bands = het, 928bp band only = hom. Please note that this assay has not been verified in our laboratory as we routinely genotype samples by LoA qPCR.

The *CasF1_Term* + *Syt1_I367T_R* mutant-specific assay can be used to confirm the results from the assay above, and to verify the sequence of the mutation.

Primer sequences

Primer Name	Primer Sequence (5' > 3')
Syt1_I367T_F	TTCATGAGCTTTCAGCATCTTT
Syt1_I367T_R	GGCATCAACCTCCTCCTCT
CasF1_Term	GCTTTCTTGTACAAAGTGGTTGA

Reaction setup

Reagent	µl
DNA (~50-100 ng)	1
10x Buffer	2
MgCl ₂ (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
ddH ₂ O	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

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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 wild type 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	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
SYT1_I367T_WT	CTGTACAATGGAAATATTAATAA AATCTATTGAAAAATAACTGA	AGATTACAGGTAGGCGTCATTA TGC	ATGTGTAGGTATGAACATGA CCC

Reactions are performed in a 10µl volume using an Applied Biosystems 7900HT Fast Real-Time PCR System or Applied Biosystems Vii7 with DNA prepared using the Sample-to-SNPTM kit (Applied Biosystems) from mouse ear biopsies. GTXpress™ buffer is also used (Applied Biosystems).

Reagent	µl
2x GTXpress™ buffer	5
20x target assay	0.5
ddH2O	3
Tfrc endogenous 20x	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	-

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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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