

**Gene:** Slc25a4

**Colony prefix:** MDKZ

**ESC clone ID:** EPD0369\_5\_A05

**Allele:** Slc25a4<sup>tm1a(EUCOMM)Wtsi</sup>

**Allele type:** Knockout First, Reporter-tagged insertion with conditional potential

**Allele information:**

Further information about the allele can be found on the 'International Mouse Phenotyping Consortium' (IMPC) web site at [http://www.mousephenotype.org/martsearch\\_ikmc\\_project/martsearch/ikmc\\_project/24960](http://www.mousephenotype.org/martsearch_ikmc_project/martsearch/ikmc_project/24960)  
Details on how to determine the floxed exon can be found at <http://www.i-dcc.org/kb/entry/21/>

**Mouse QC information**



<b>Southern Blot</b>	na	<b>TV Backbone Assay</b>	na	<b>5' LR-PCR</b>	na
<b>Loss of WT Allele (LOA) qPCR</b>	na	<b>Homozygous Loss of WT Allele (LOA) SR-PCR</b>	na	<b>Neo Count (qPCR)</b>	na
<b>LacZ SR-PCR</b>	na	<b>5' Cassette Integrity</b>	na	<b>Neo SR-PCR</b>	na
<b>Mutant Specific SR-PCR</b>	na	<b>LoxP Confirmation</b>	na	<b>3' LR-PCR</b>	na
<b>Genotyping Comment</b>					

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## Southern blot confirmation:

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## Links to information and frequently asked questions about the EUCOMM/KOMP alleles and MGP projects

General targeting strategies:

[http://www.mousephenotype.org/martsearch\\_ikmc\\_project/about/targeting-strategies](http://www.mousephenotype.org/martsearch_ikmc_project/about/targeting-strategies)

MGP mouse phenotype data:

<http://www.sanger.ac.uk/mouseportal/>

IKMC allele types:

<http://www.i-dcc.org/kb/entry/89/>

MGP mouse quality control tests :

<http://www.i-dcc.org/kb/25/>

Allele conversion guide - genotyping tm1b, tm1c and tm1d mice:

<http://www.i-dcc.org/kb/entry/105/>

How the "critical" exon is decided:

<http://www.i-dcc.org/kb/entry/102/>

## Genotyping Information

### Genotyping by end-point PCR

These mice may be genotyped through a combination of separate PCR reactions that detect the cassette, 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.

For example: cassette positive, mutant positive, wild type positive = heterozygous.

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## PCRs primer pairs and expected size bands

Assay Type	Assay	Forward Primer	Reverse Primer	Expected Size Band (bp)
Standard PCR	Cassette	LacZ_2_small_F	LacZ_2_small_R	108
Standard PCR	Mutant	Slc25a4_47324_F	CAS_R1_Term	258
Standard PCR	Wildtype	Slc25a4_47324_F	Slc25a4_47324_R	466

## Primer sequences

Primer Name	Primer Sequence (5' > 3')
CAS_R1_Term	TCGTGGTATCGTTATGCGCC
LacZ_2_small_F	ATCACGACGCGCTGTATC
LacZ_2_small_R	ACATCGGGCAAATAATATCG
Slc25a4_47324_F	ACCTTCTTATCTGCATCCACCC
Slc25a4_47324_R	CTTTACTCGAATGTCCCCCG

## Reaction setup

Reagent	µl
DNA (~50-100 ng)	1
10x Buffer	2
MgCl <sub>2</sub> (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 <sub>2</sub> O	15.2
<b>Total</b>	<b>20</b>

## Amplification conditions

Step	Conditions	Time
1	94°C	5 min
2	94°C	30 sec
3	58°C	30 sec
4	72°C	45 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 Slc25a4 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, Tfr; Applied Biosystems part #4458366). Reference DNA controls of known genotypes should also be included to facilitate correct analysis.

#### Primers for LoA qPCR assay

Primer type	Assay Name	Forward Primer Seq.	Reverse Primer Seq.	Probe Primer Seq.
LoA	SLC25A4_WT	CCAATCTGTTGGAGCATTTTGTG TT	CTCAAAGGCAAACGCTGTTCAA	CCAAACGGCTCTTCCCA

#### Reaction setup

Reaction setup and amplification conditions are the same as those used for the neo cassette qPCR assay.

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## Relevant publications

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- Ryder, E., Gleeson, D., Sethi, D., Vyas, S., Miklejewska, E., Dalvi, P., Habib, B., Cook, R., Hardy, M., Jhaveri, K., et al. (2013). Molecular Characterization of Mutant Mouse Strains Generated from the EUCOMM/KOMP-CSD ES Cell Resource. *Mamm Genome*, 24, 286–294.
- 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.
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- Bradley, A., Anastassiadis, K., Ayadi, A., Battey, J.F., Bell, C., Birling, M.-C., Bottomley, J., Brown, S.D., Bürger, A., Bult, C.J., et al. (2012). The mammalian gene function resource: the international knockout mouse consortium. *Mamm Genome* 23, 580–586.
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**ESC clone ID:** EPD0369\_5\_A05

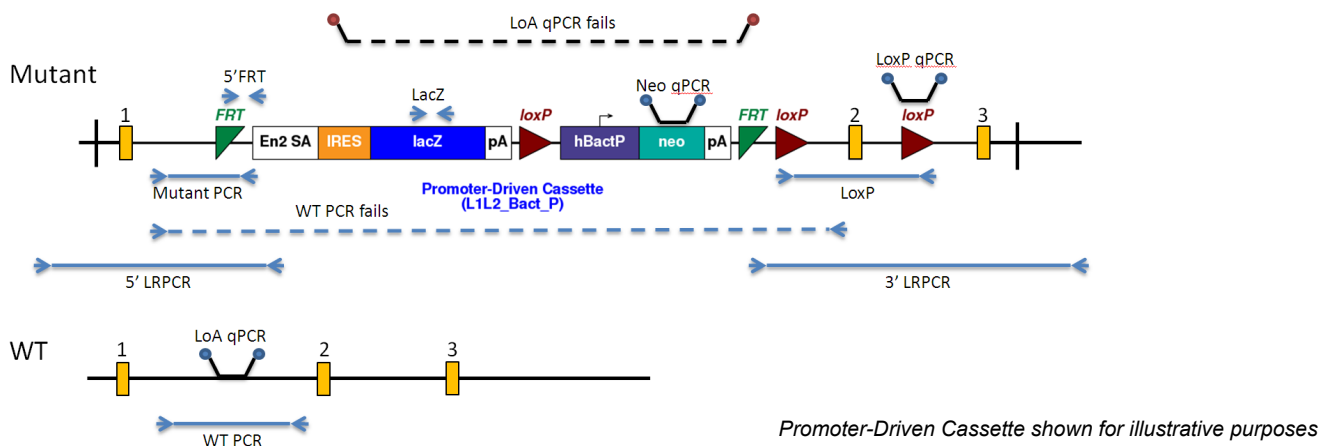
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<b>LacZ SR-PCR</b>	pass	<b>5' Cassette Integrity</b>	pass	<b>Neo SR-PCR</b>	na
<b>Mutant Specific SR-PCR</b>	pass	<b>LoxP Confirmation</b>	pass	<b>3' LR-PCR</b>	na
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