

Lrrc8e Cas9-CKO Strategy

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Overview

Target Gene Name

- Lrrc8e

Project Type

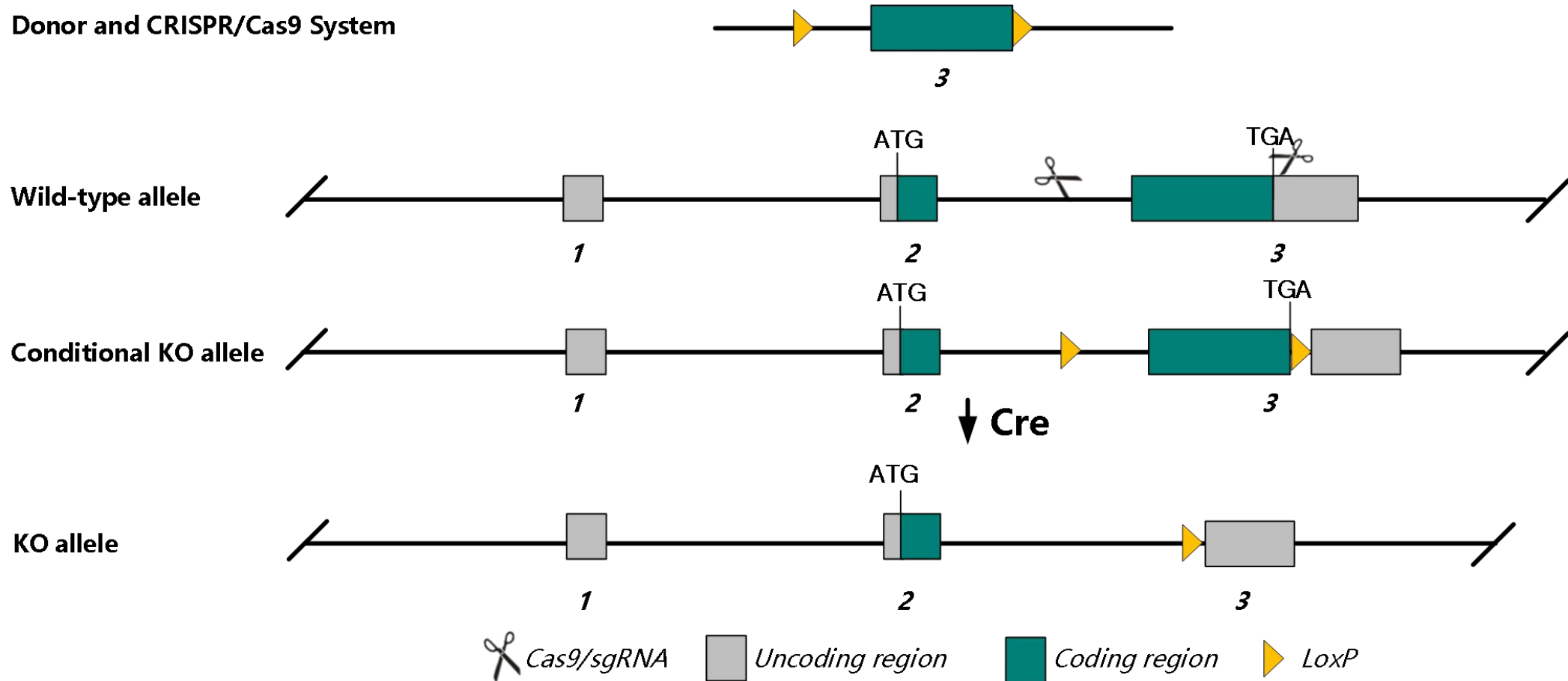
- Cas9-CKO

Genetic Background

- C57BL/6JGpt

Strain Strategy

Donor and CRISPR/Cas9 System



Schematic representation of CRISPR-Cas9 engineering used to edit the *Lrrc8e* gene.

Technical Information

- The *Lrrc8e* gene has 2 transcripts. According to the structure of *Lrrc8e* gene, exon3 of *Lrrc8e*-201 (ENSMUST00000053035.7) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knocking out the region will result in disruption of protein function.
- In this project we use CRISPR-Cas9 technology to modify *Lrrc8e* gene. The brief process is as follows: CRISPR-Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice. Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and on-target amplicon sequencing. A stable F1-generation mouse strain was obtained by mating positive F0-generation mice with C57BL/6JGpt mice and confirmation of the desired mutant allele was carried out by PCR and on-target amplicon sequencing.
- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Gene Information

Lrrc8e leucine rich repeat containing 8 family, member E [*Mus musculus* (house mouse)]

Gene ID: 72267, updated on 25-Jan-2024

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Summary

Official Symbol	Lrrc8e provided by MGI
Official Full Name	leucine rich repeat containing 8 family, member E provided by MGI
Primary source	MGI:MGI:1919517
See related	Ensembl:ENSMUSG00000046589 AllianceGenome:MGI:1919517
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1810049O03Rik
Summary	Predicted to contribute to volume-sensitive anion channel activity. Involved in cyclic-GMP-AMP transmembrane import across plasma membrane. Predicted to be located in cytoplasm and plasma membrane. Predicted to be integral component of endoplasmic reticulum membrane; integral component of lysosomal membrane; and integral component of plasma membrane. Predicted to be part of ion channel complex. Orthologous to human LRRC8E (leucine rich repeat containing 8 VRAC subunit E). [provided by Alliance of Genome Resources, Apr 2022]
Expression	Biased expression in adrenal adult (RPKM 4.6), bladder adult (RPKM 3.6) and 8 other tissues See more
Orthologs	human all
NEW	Try the new Gene table Try the new Transcript table

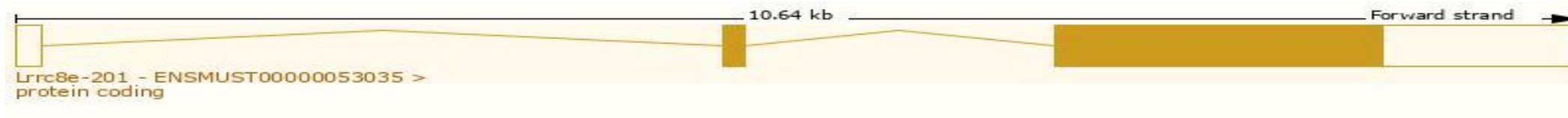
Source: <https://www.ncbi.nlm.nih.gov/>

Transcript Information

The gene has 2 transcripts, all transcripts are shown below:

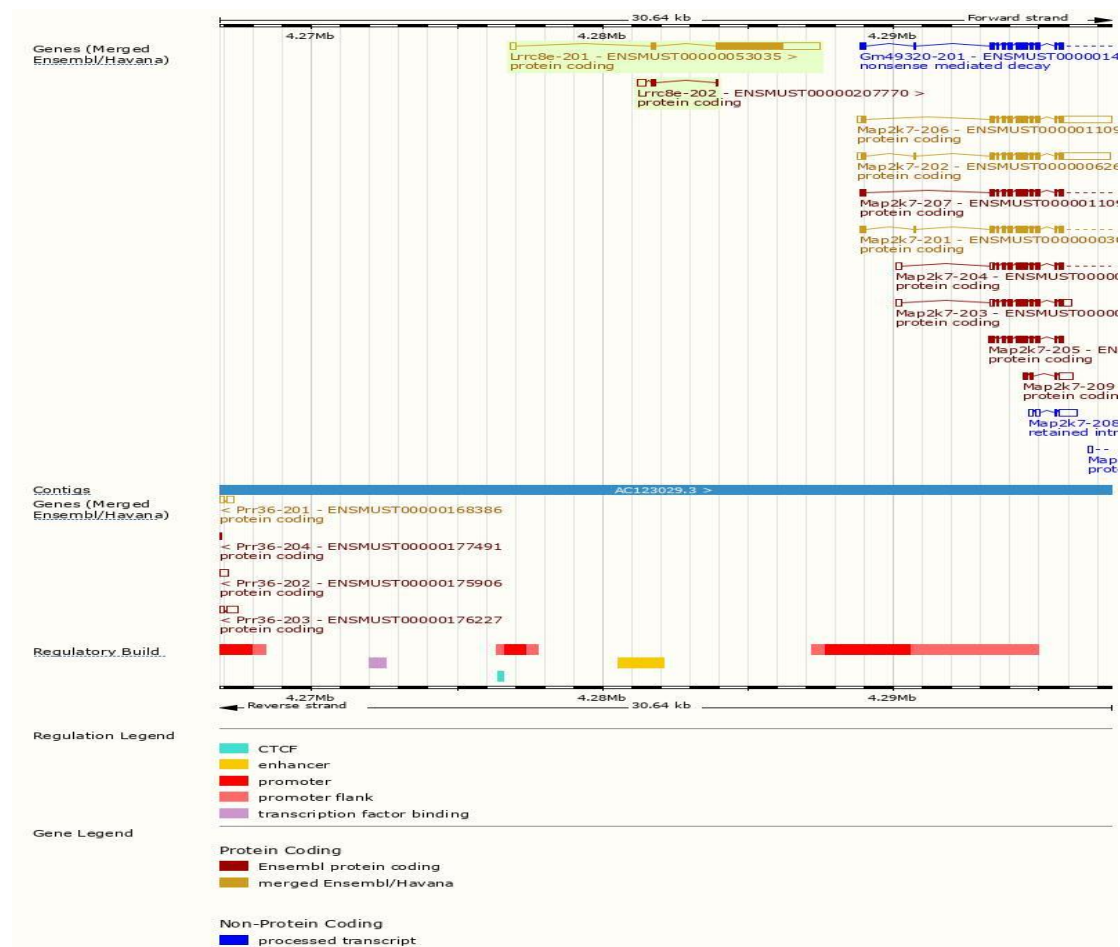
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000053035.7	Lrrc8e-201	3878	795aa	Protein coding	CCDS22079	Q66JT1	Ensembl Canonical Gencode basic APPRIS P1 TSL:1
ENSMUST00000207770.2	Lrrc8e-202	455	64aa	Protein coding		A0A140LI19	TSL:3 CDS 3' incomplete

The strategy is based on the design of *Lrrc8e*-201 transcript, the transcription is shown below:

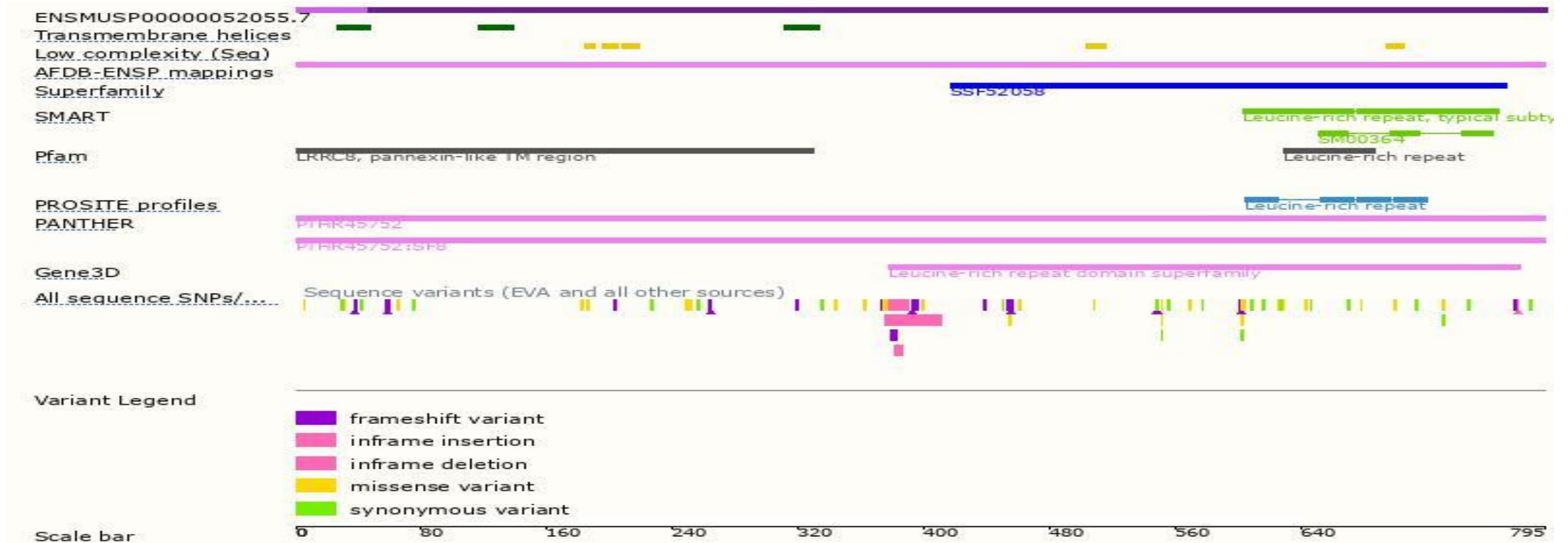


Source: <https://www.ensembl.org>

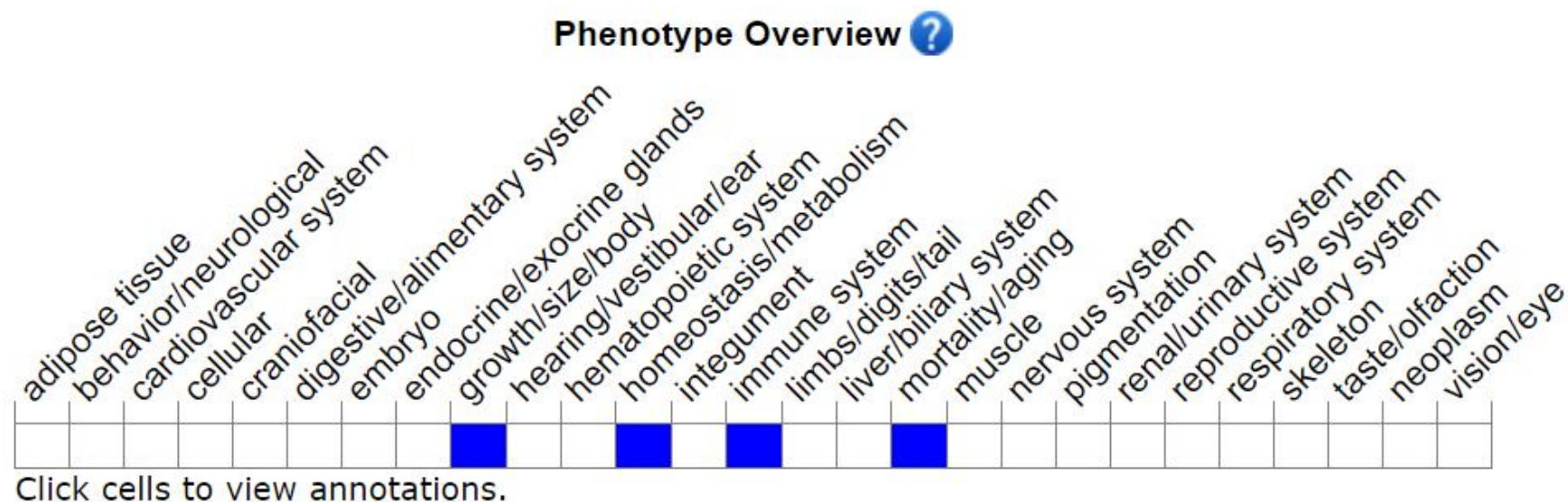
Genomic Information



Protein Information



Mouse Phenotype Information (MGI)



- Mice homozygous for a null allele exhibit impaired response to HSV-1 infection.

Important Information

- According to MGI information, mice homozygous for a null allele exhibit impaired response to HSV-1 infection.
- The second loxP is inserted at the stop codon, it may affect the regulatory function of *Lrrc8e* gene.
- The floxed region is not frame shift, but it contains most of the coding sequence.
- The floxed region is about 2.5kb away from the N-terminal of *Map2k7* and *Gm49320* genes, this strategy may influence the regulatory function of these genes.
- *Lrrc8e* is located on Chr8. If the knockout mice are crossed with other mouse strains to obtain double homozygous mutant offspring, please avoid the situation that the second gene is on the same chromosome.
- This Strategy is designed based on genetic information in existing databases. Due to the complexity of biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

References

<https://www.informatics.jax.org/allele/MGI:4436449>

Mutation description	Allele Type:	Targeted (Conditional ready, Null/knockout, Reporter)
	Mutation:	Insertion Vector: L1L2_Bact_P
	▼ Mutation details: The L1L2_Bact_P cassette was inserted at position 4283407 of Chromosome 8 upstream of the critical exon(s) (Build GRCm39). The cassette is composed of an FRT site followed by lacZ sequence and a loxP site. This first loxP site is followed by a neomycin resistance gene under the control of the human beta-actin promoter, SV40 polyA, a second FRT site and a second loxP site. A third loxP site is inserted downstream of the targeted exon(s) at position 4287140. The critical exon(s) is/are thus flanked by loxP sites. A "conditional ready" (floxed) allele can be created by flp recombinase expression in mice carrying this allele. Subsequent cre expression results in a knockout mouse. If cre expression occurs without flp expression, a reporter knockout mouse will be created. Further information on targeting strategies used for this and other IKMC alleles can be found at http://www.informatics.jax.org/mgihome/nomen/IKMC_schematics.shtml (J:157065)	

<https://www.mousephenotype.org/data/genes/MGI:1919517>

