Kcne2 Cas9-CKO Strategy

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Design Date: 2019-9-11
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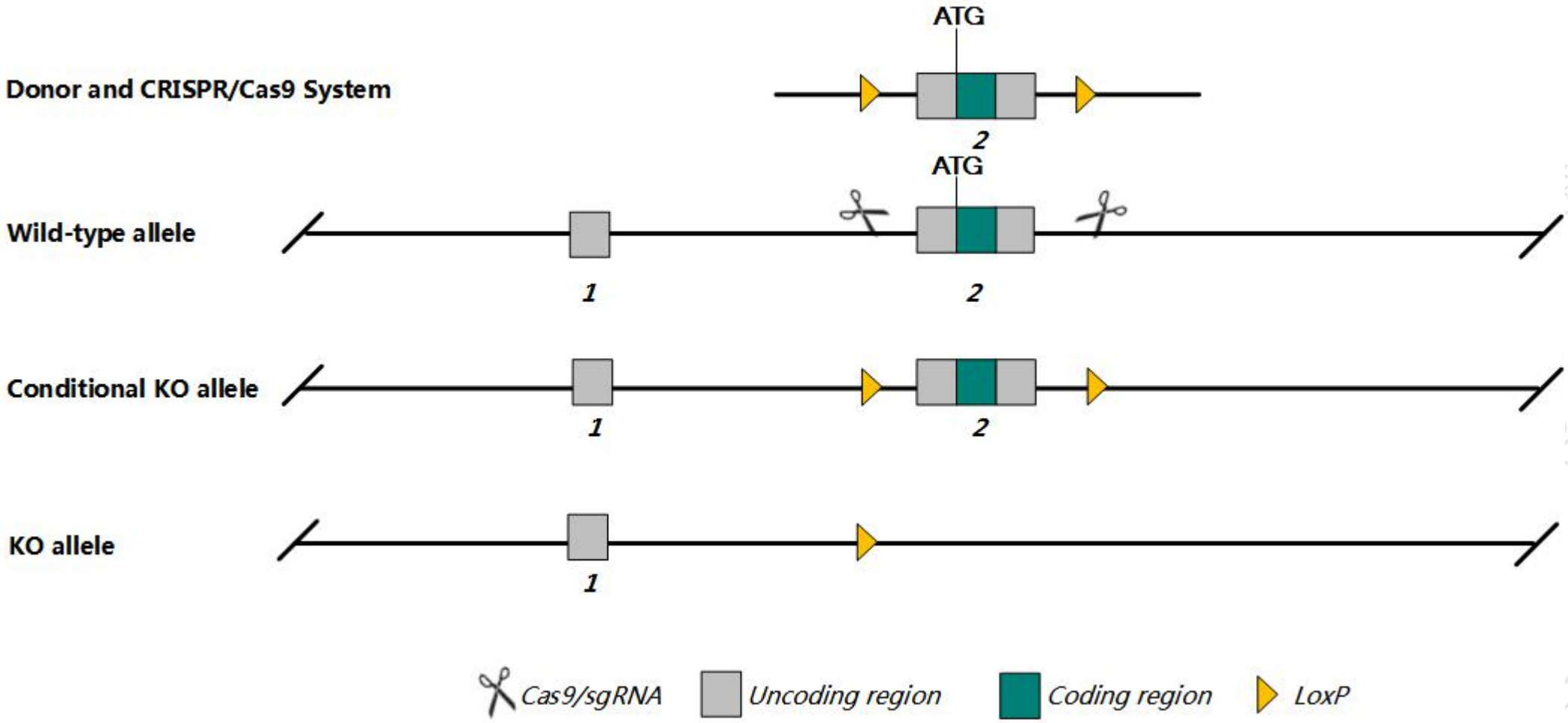
Project Overview

Project Name	***Kcne2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Kcne2* gene. The schematic diagram is as follows:



Technical routes

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- The *Kcne2* gene has 2 transcripts. According to the structure of *Kcne2* gene, exon2 of *Kcne2-201* (ENSMUST00000047383.9) transcript is recommended as the knockout region.The region contains all coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Kcne2* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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 sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

- 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.

Notice

➢ According to the existing MGI data, Mice homozygous for a knock-out allele show enlarged stomachs, reduced parietal cell proton secretion, altered parietal cell morphology, achlorhydria, hypergastrinemia, gastric hyperplasia, and increased gastric pH. Males homozygous for a different knock-out allele develop iron-deficient anemia.

➢ The knockout area is about 3.1 kb from the 5-terminal of Smim11, which may affect the 5-terminal regulation of Smim11

➢ The *Kcne2* gene is located on the Chr16. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes 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 existing technological level.

Gene information （NCBI）

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Kcne2 potassium voltage-gated channel, Isk-related subfamily, gene 2 [*Mus musculus* (house mouse)]

Gene ID: 246133, updated on 14-Aug-2019

Summary

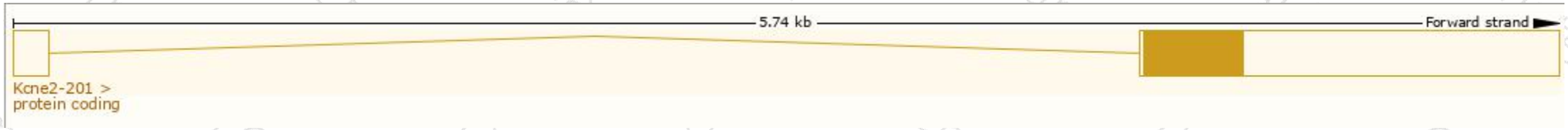
Official Symbol	Kcne2 provided by MGI
Official Full Name	potassium voltage-gated channel, Isk-related subfamily, gene 2 provided by MGI
Primary source	MGI:MGI:1891123
See related	Ensembl:ENSMUSG00000039672
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	MiRP1; AW048273; 2200002I16Rik
Expression	Biased expression in lung adult (RPKM 4.7), stomach adult (RPKM 4.4) and 7 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

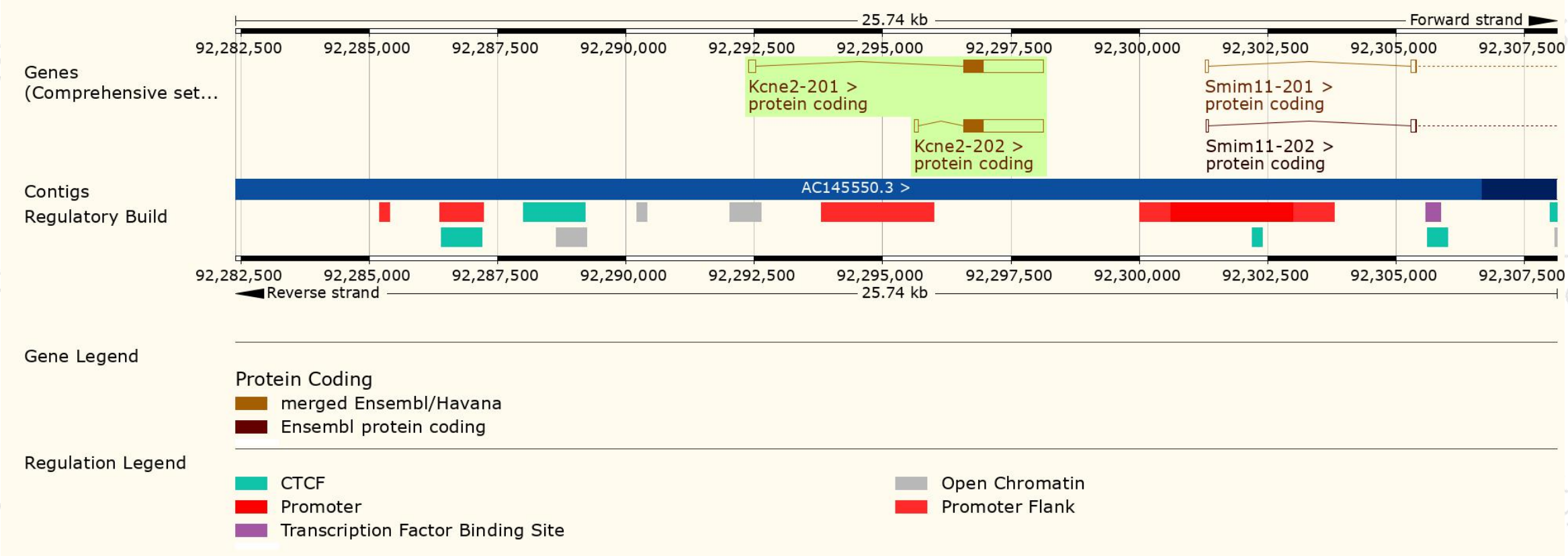
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Kcne2-201	ENSMUST00000047383.9	1690	123aa	Protein coding	CCDS28333	A0A0R4J1J2	TSL:1 GENCODE basic APPRIS P1
Kcne2-202	ENSMUST00000113971.1	1632	123aa	Protein coding	CCDS28333	A0A0R4J1J2	TSL:1 GENCODE basic APPRIS P1

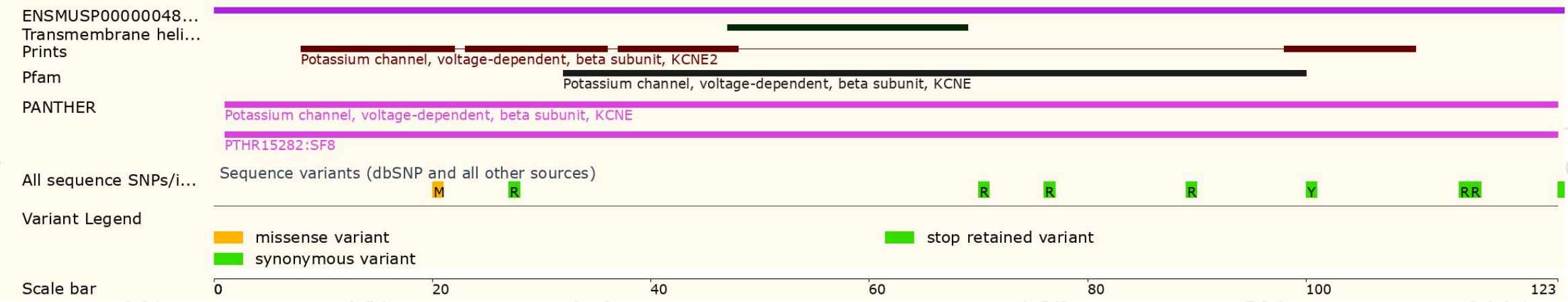
The strategy is based on the design of *Kcne2-201* transcript,The transcription is shown below



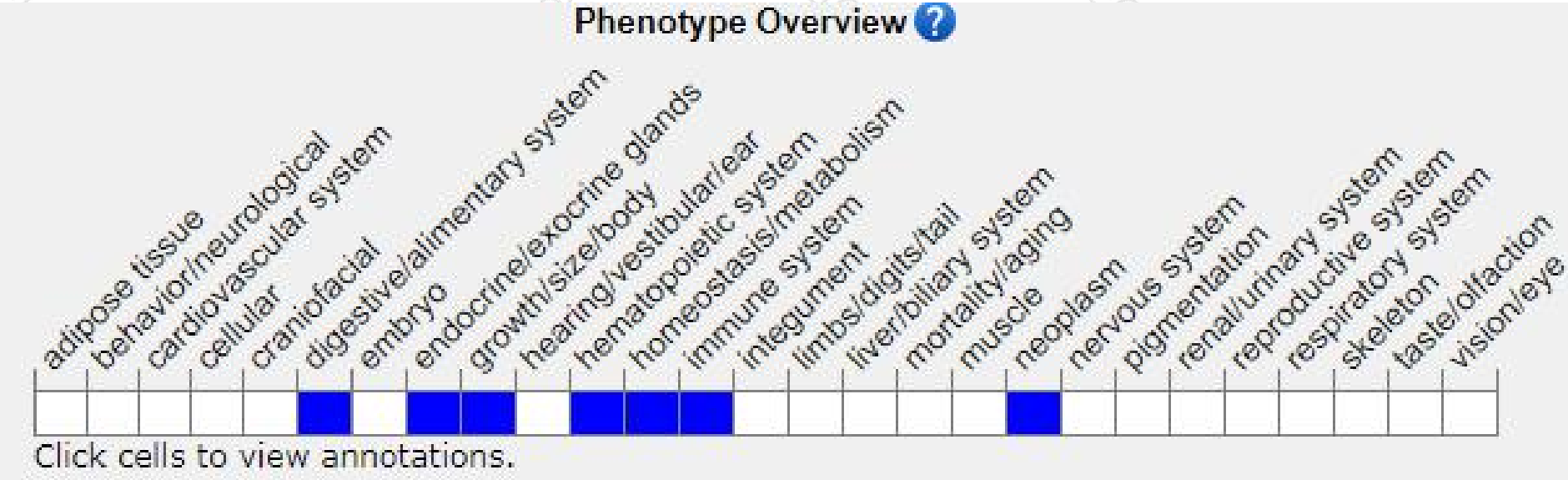
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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If you have any questions, you are welcome to inquire.

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