Pik3r2 Cas9-CKO Strategy

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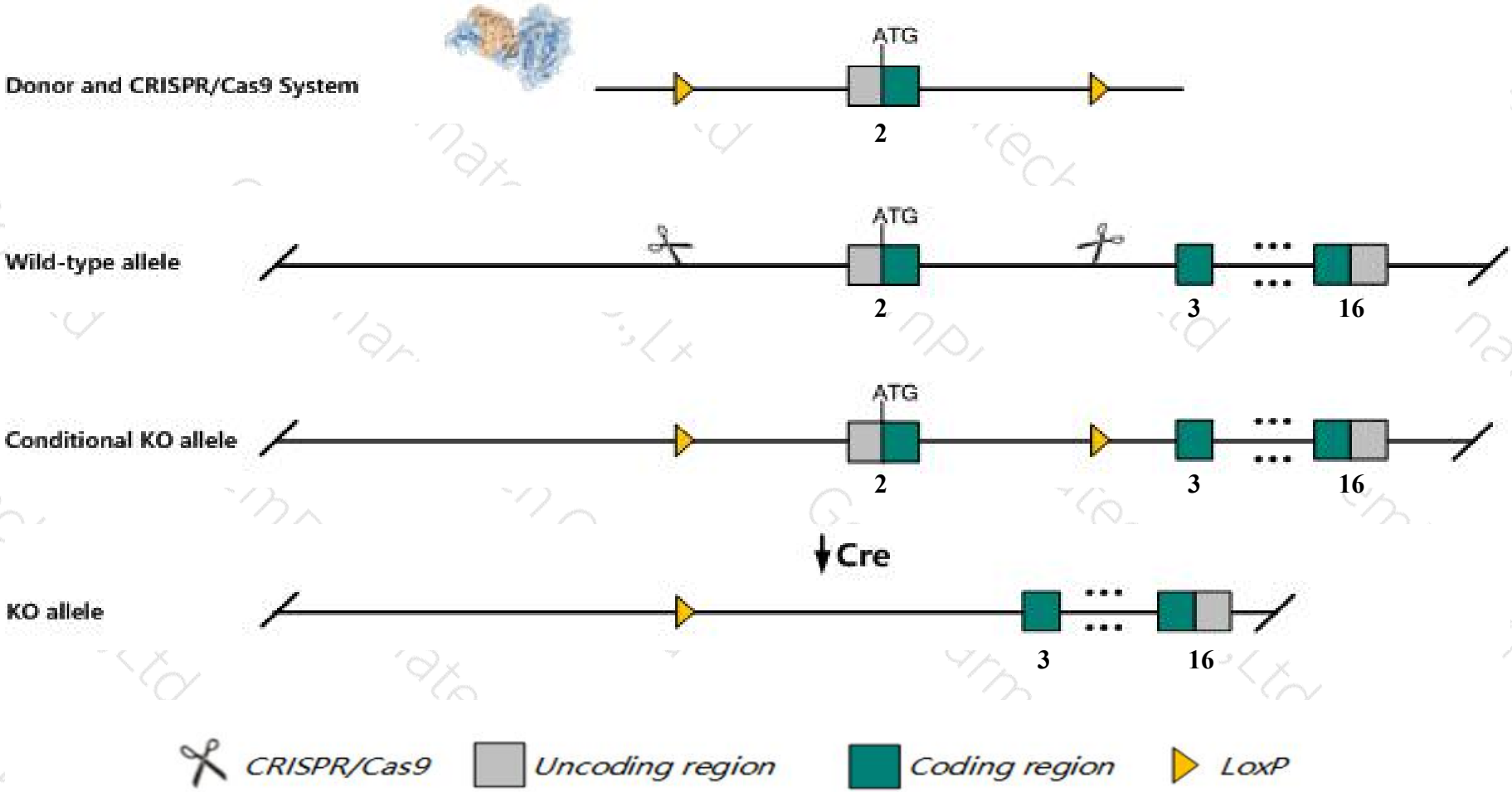
Project Overview

Project Name	***Pik3r2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Pik3r2* gene. The schematic diagram is as follows:



Technical routes

➢ The *Pik3r2* gene has 6 transcripts. According to the structure of *Pik3r2* gene, exon2 of *Pik3r2-201* (ENSMUST00000034296.14) transcript is recommended as the knockout region.The region contains start codon ATG. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Pik3r2* 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 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 disruptions in this gene have lower blood glucose levels both when fed and after fasting. Insulin sensitivity is improved as well.
- There is a risk of recognizing a new ATG to form an unknown protein after knocking out ATG.
- Transcripts 203, 204, 205 may not be affected.
- The flox region is about 1.3 kb away from the 5th end of the 2010320M18Rik gene, which may affect the 5-terminal regulation of the gene.
- The *Pik3r2* gene is located on the Chr8. 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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Pik3r2 phosphoinositide-3-kinase regulatory subunit 2 [*Mus musculus* (house mouse)]

Gene ID: 18709, updated on 12-Aug-2019

Summary

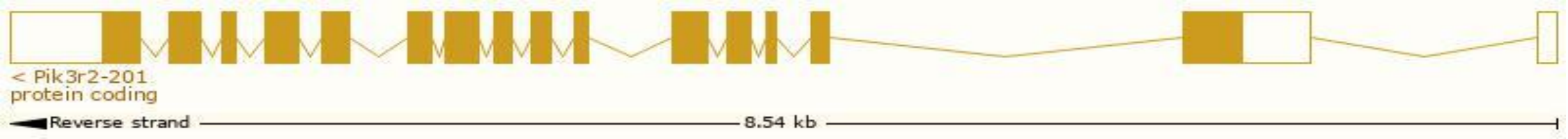
Official Symbol	Pik3r2 provided by MGI
Official Full Name	phosphoinositide-3-kinase regulatory subunit 2 provided by MGI
Primary source	MGI:MGI:1098772
See related	Ensembl:ENSMUSG00000031834
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	p85beta
Expression	Ubiquitous expression in ovary adult (RPKM 65.5), duodenum adult (RPKM 57.1) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

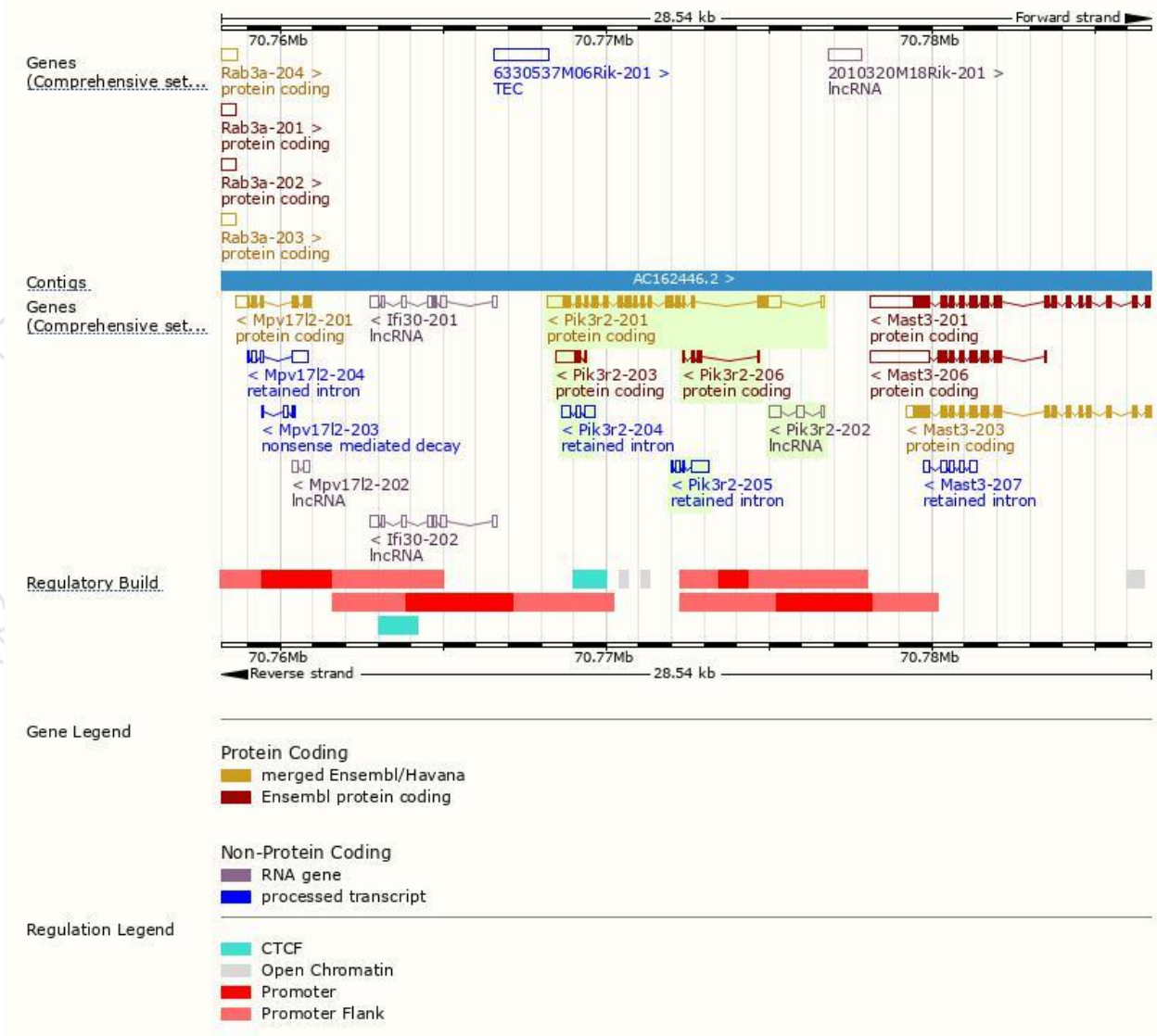
The gene has 6 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Pik3r2-201	ENSMUST00000034296.14	3165	722aa	Protein coding	CCDS22381	O08908	TSL:1 GENCODE basic APPRIS P1
Pik3r2-203	ENSMUST00000143785.1	813	71aa	Protein coding	-	F7CB91	CDS 5' incomplete TSL:3
Pik3r2-206	ENSMUST00000154685.1	250	84aa	Protein coding	-	F6R2V5	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:1
Pik3r2-202	ENSMUST00000142370.1	626	No protein	Processed transcript	-	-	TSL:3
Pik3r2-205	ENSMUST00000152545.1	783	No protein	Retained intron	-	-	TSL:5
Pik3r2-204	ENSMUST00000146707.1	746	No protein	Retained intron	-	-	TSL:2

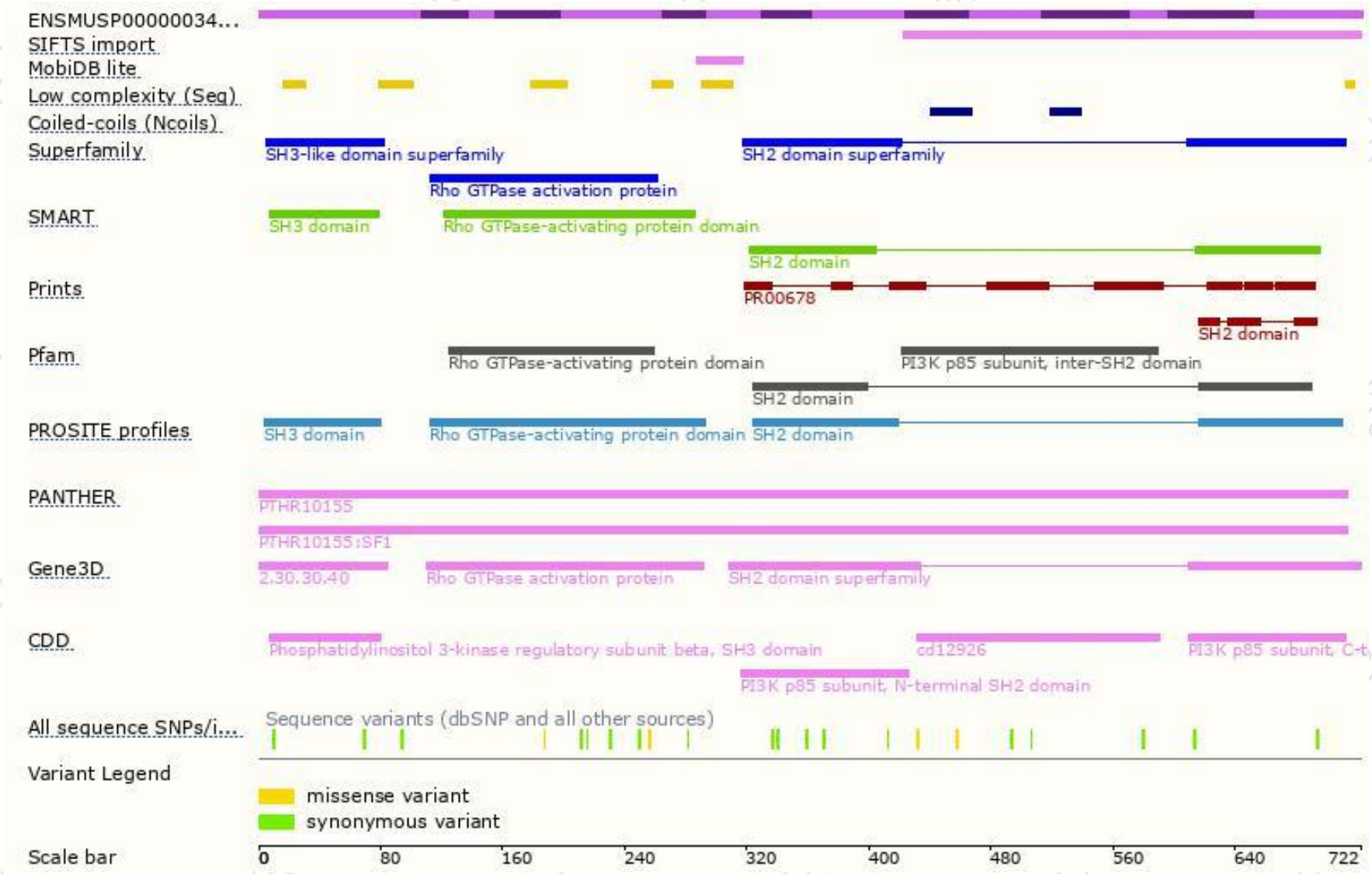
The strategy is based on the design of *Pik3r2-201* transcript,The transcription is shown below



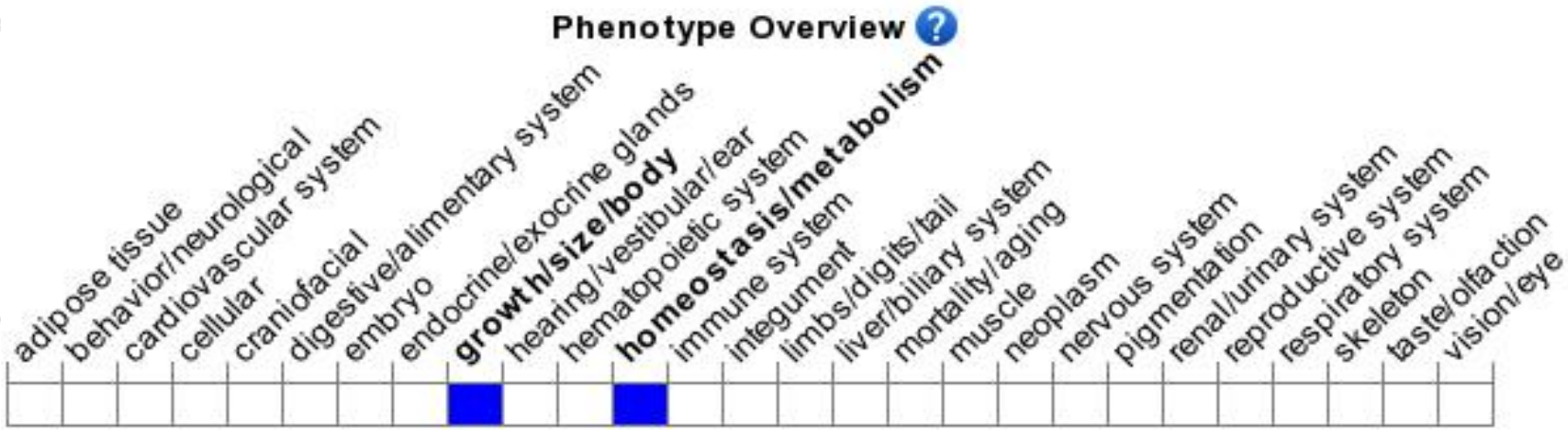
Genomic location distribution



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/).

According to the existing MGI data,Mice homozygous for disruptions in this gene have lower blood glucose levels both when fed and after fasting. Insulin sensitivity is improved as well.

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

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