Galc Cas9-CKO Strategy

Designer: Xiaojing Li

Reviewer: JiaYu

Design Date: 2020-7-24

Project Overview

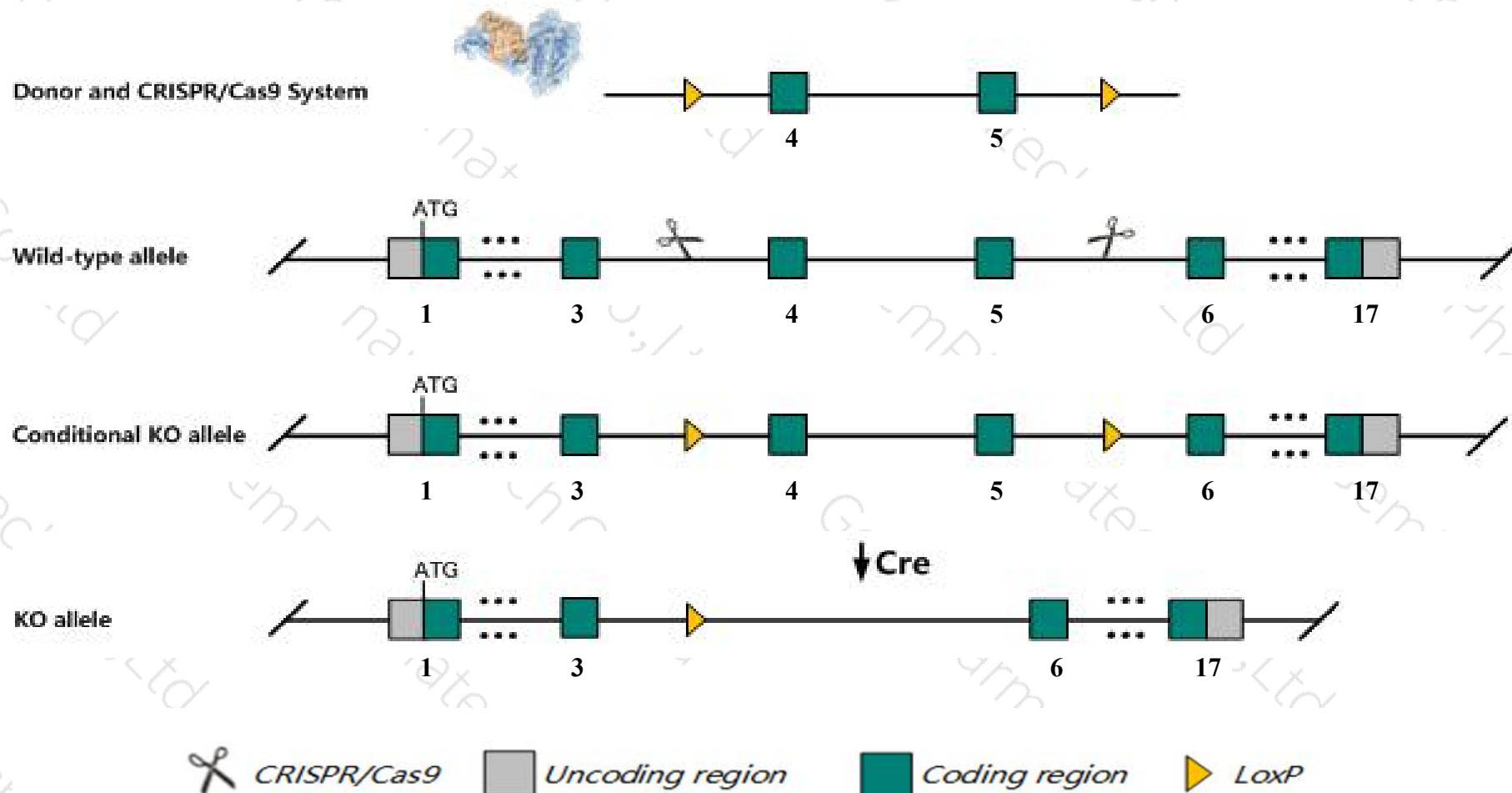|	
Project Name	*Galc*
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

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

Technical routes

➤ The *Galc* gene has 7 transcripts. According to the structure of *Galc* gene, exon4-exon5 of *Galc-201*(ENSMUST00000021390.8) transcript is recommended as the knockout region.The region contains Ens 254bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Galc* 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,homozygotes for spontaneous and targeted mutations exhibit tremors, progressive weakness, wasting, both central and peripheral demyelination, massive accumulation of galactosylceramide, abnormal macrophages, and death by 4 months of age.

➢ The *Galc* gene is located on the Chr12. 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）

Galc galactosylceramidase [Mus musculus (house mouse)]

Gene ID: 14420, updated on 13-Mar-2020

▲ Summary

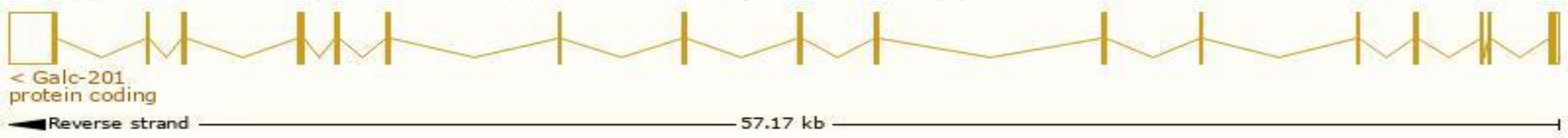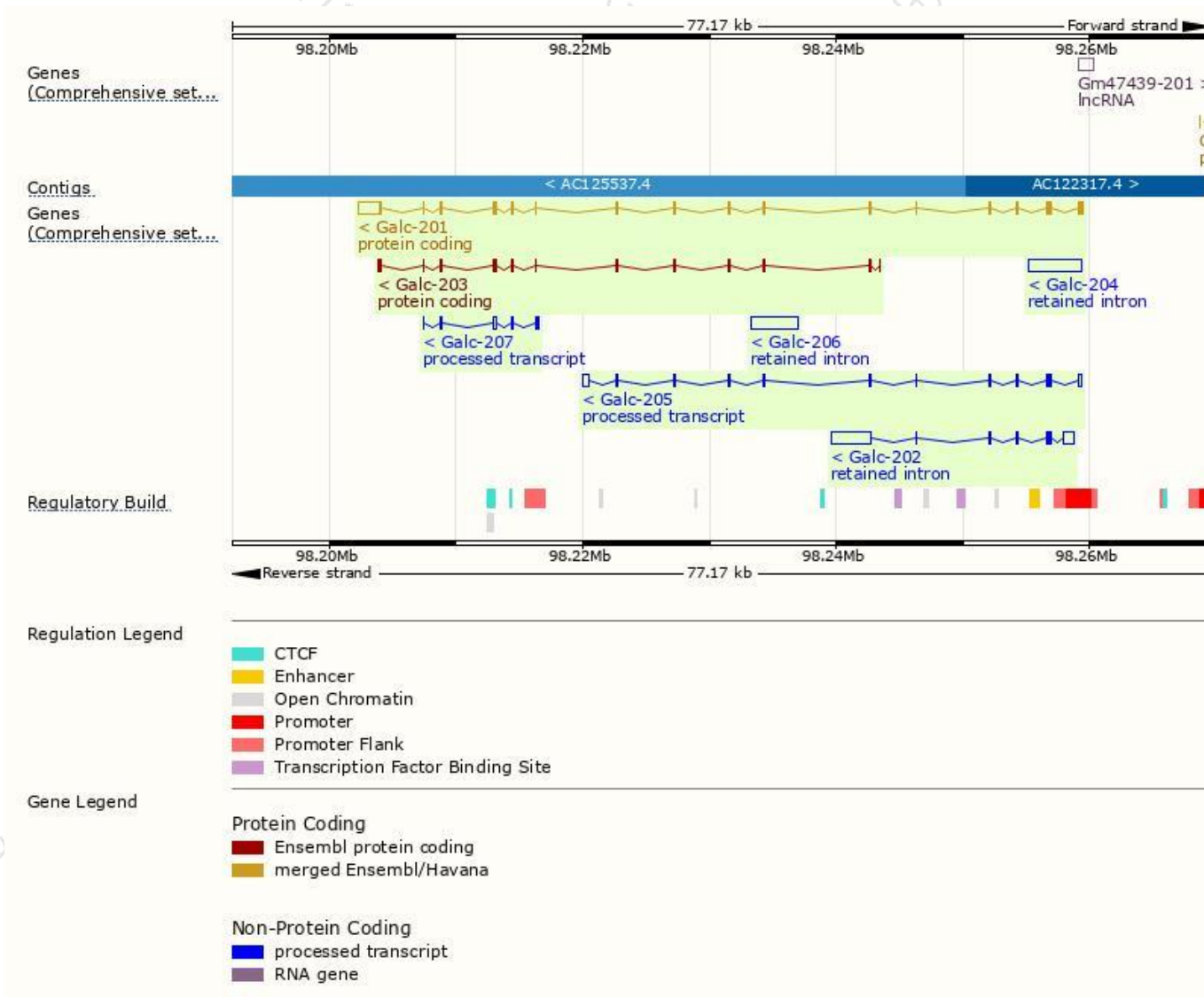|	
Official Symbol	Galc provided by MGI
Official Full Name	galactosylceramidase provided byMGI
Primary source	MGI:MGI:95636
See related	Ensembl:ENSMUSG00000021003
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2310068B06Rik, A930008M05Rik, AW212969, AW413532, Gacy, twi, twitcher
Summary	This gene encodes galactosylceramidase, the lysosomal hydryolase involved in the catabolism of galactosylceramide. Mutations in this gene result in slow growth, tremors and hind leg weakness, collectively termed as the 'twitcher' phenotype. In humans, deficiency of this gene product causes a lysosomal storage disorder known as Krabbe disease. [provided by RefSeq, Dec 2014]
Expression	Ubiquitous expression in kidney adult (RPKM 11.0), testis adult (RPKM 4.6) and 25 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Galc-201	ENSMUST00000021390.8	3832	684aa	Protein coding	CCDS36517	P54818	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Galc-203	ENSMUST00000221063.1	1619	491aa	Protein coding	-	A0A1Y7VM63	CDS 5' incomplete TSL:1
Galc-205	ENSMUST00000222042.1	1646	No protein	Processed transcript	-	-	TSL:1
Galc-207	ENSMUST00000222703.1	685	No protein	Processed transcript	-	-	TSL:5
Galc-202	ENSMUST00000220501.1	4439	No protein	Retained intron	-	-	TSL:1
Galc-204	ENSMUST00000221322.1	4153	No protein	Retained intron	-	-	TSL:NA
Galc-206	ENSMUST00000222379.1	3771	No protein	Retained intron	-	-	TSL:NA

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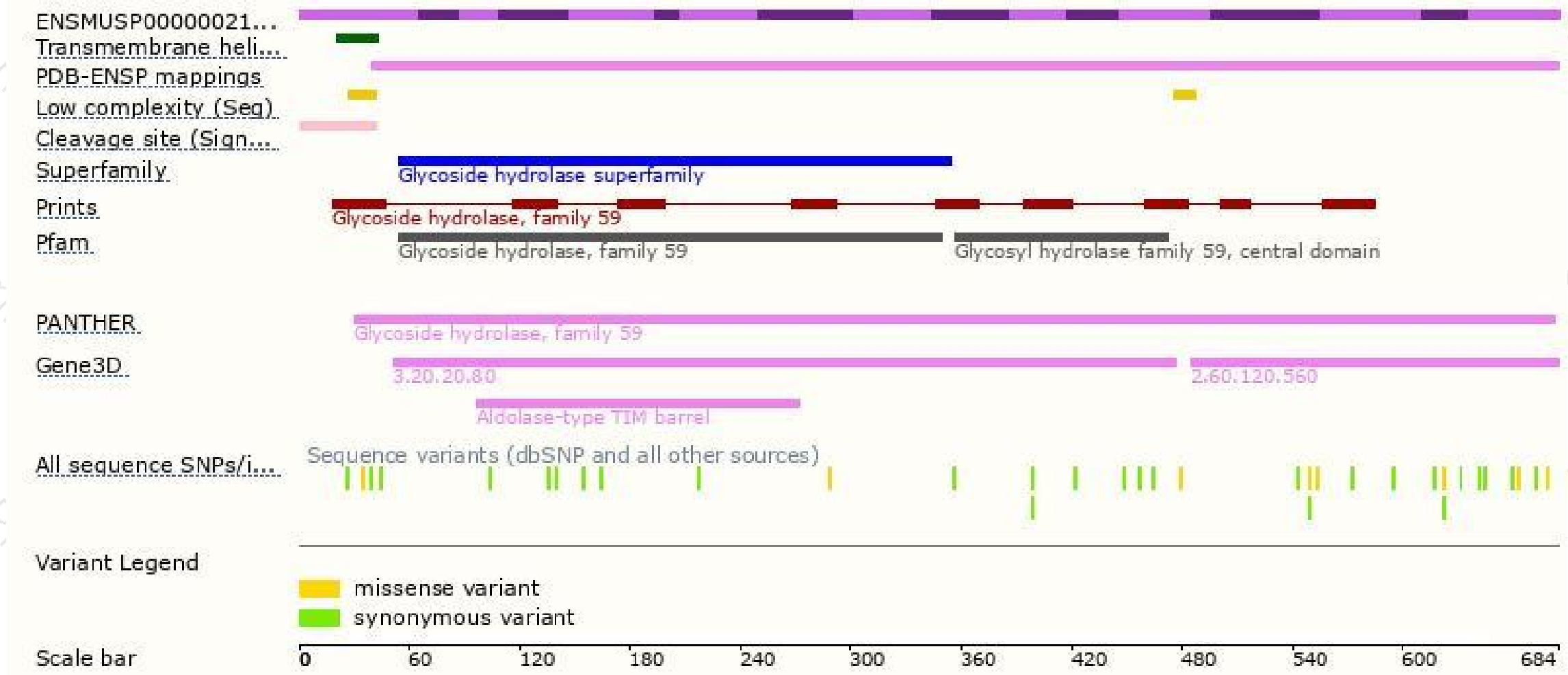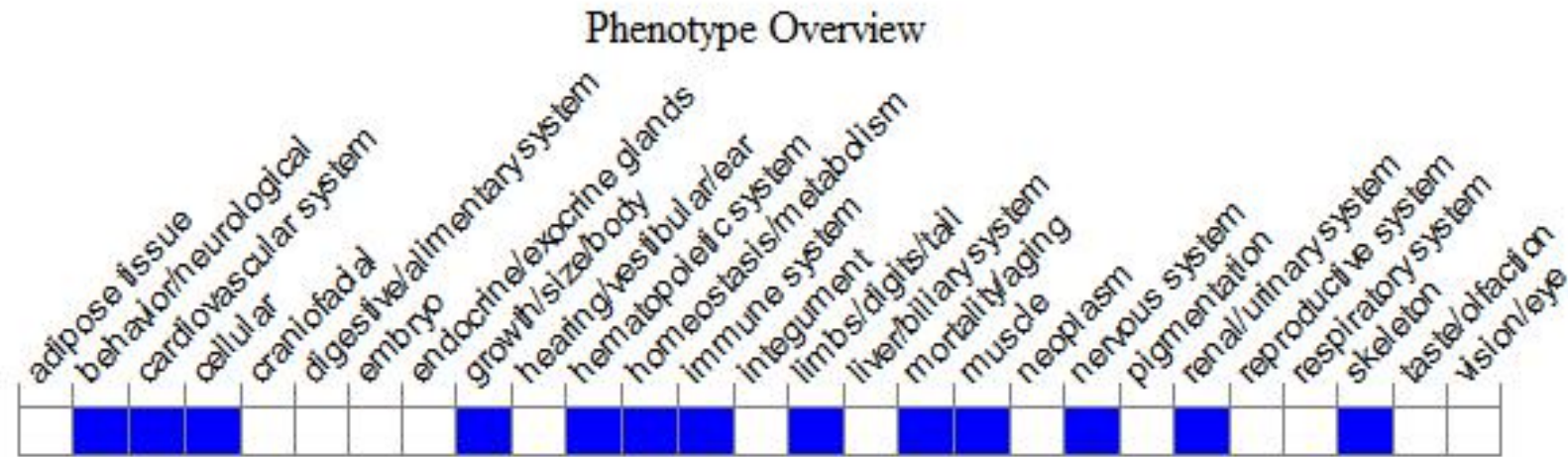

< Galc-201
protein coding

◄ Reverse strand

57.17 kb

Genomic location distribution

Protein domain

ENSMUSP00000021...	
Transmembrane heli...	
PDB-ENSP mappings	
Low complexity (Seg)	
Cleavage site (Sign...	
Superfamily	Glycoside hydrolase superfamily
Prints	Glycoside hydrolase, family 59
Pfam	Glycoside hydrolase, family 59 — Glycosyl hydrolase family 59, central domain
PANTHER	Glycoside hydrolase, family 59
Gene3D	3.20.20.80 — 2.60.120.560
	Aldolase-type TIM barrel
All sequence SNPs/i...	Sequence variants (dbSNP and all other sources)
Variant Legend	▮ missense variant ▮ synonymous variant
Scale bar	0 60 120 180 240 300 360 420 480 540 600 684

Mouse phenotype description(MGI)

Phenotype Overview

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,homozygotes for spontaneous and targeted mutations exhibit tremors, progressive weakness, wasting, both central and peripheral demyelination, massive accumulation of galactosylceramide, abnormal macrophages, and death by 4 months of age.

If you have any questions, you are welcome to inquire.
Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd