Slc32a1-IRES-EGFP Mouse Model Strategy

Designer: Lingyan Wu

Reviewer: Yun Li

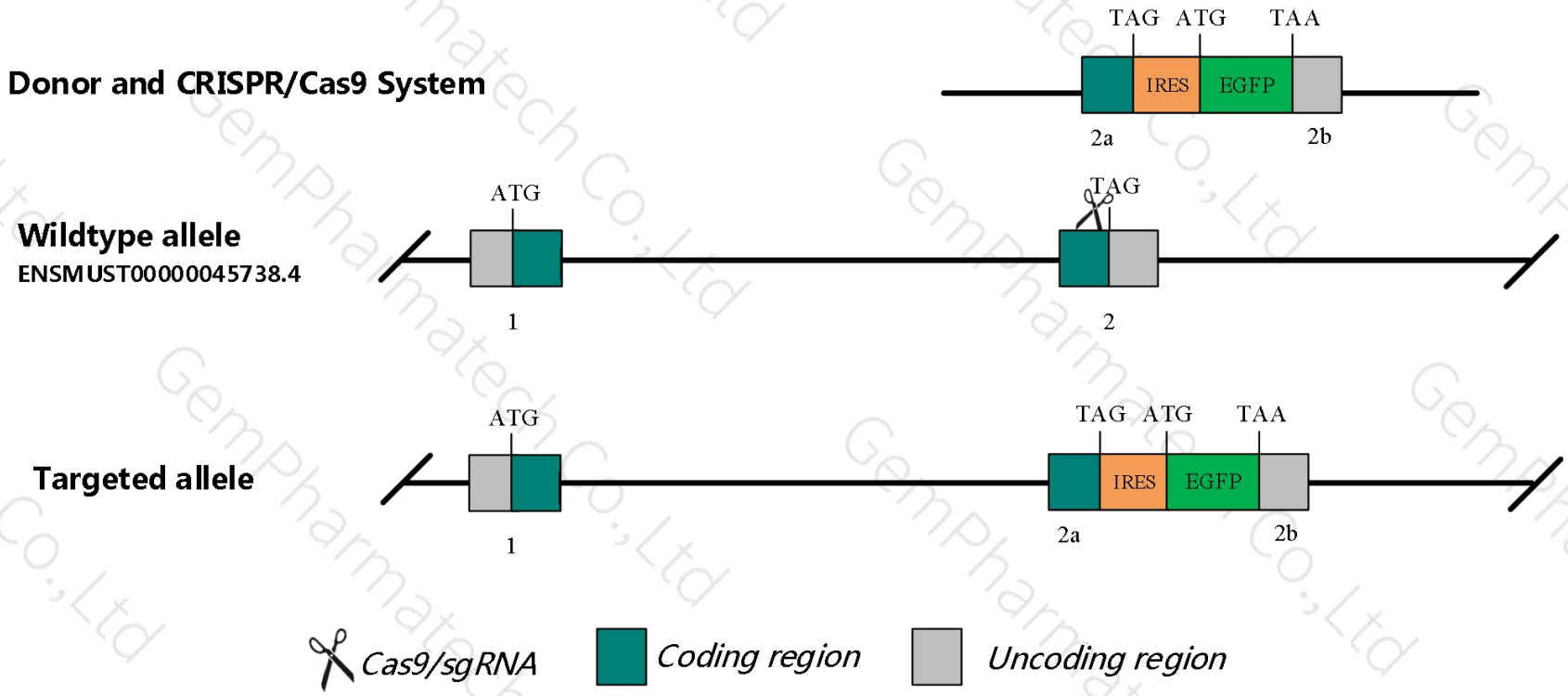
Design Date: 2020-11-5

Project Overview

Project Name	***Slc32a1*-IRES-EGFP**
Project type	**Cas9-KI**
Strain background	**C57BL/6JGpt**

Strategy

This model will use CRISPR/Cas9 technology to edit the *Slc32a1* gene. The schematic diagram is as follows:



Technical Description

➢ The mouse *Slc32a1* gene has 1 transcript.

➢ According to the structure of *Slc32a1* gene, the element IRES-EGFP will be inserted at the translation stop codon of *Slc32a1*-201(ENSMUST00000045738.4), the length of inserted fragment is about 1.3kb.

➢ In this project we use CRISPR/Cas9 technology to modify *Slc32a1* 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.

Notice

➢ According to the existing MGI data,homozygous null mice been independently reported to die perinatally exhibiting a hunched posture, respiratory failure, cleft secondary palate due to failure of palate shelf elevation, umbilical hernia or omphalocele, and loss of neurotransmitter release in both GABAergic and glycinergic neurons.

➢ It is necessary to introduce 1-2 synonymous mutation in exon2.

➢ The IERS-linked genes will be tarnscripted together and then be translated two protein separately, but the downstream protein is lower than the upstream protein.

➢ The insert site is about 3.1kb away from the N-terminal of *Gm14204* and *Gm14205* gene, this strategy may influence the regulatory function of the N-terminal of *Gm14204* gene and *Gm14205* gene.

➢ The *Slc32a1* gene is located on the Chr2. Please take the loci in consideration when breeding this knockin mice with other gene modified (e.g., Tg, iCre) strains, if the other gene is also on Chr2, it may be extremely hard to get double gene positive homozygotes.

➢ The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene between the 3'UTR and the gene coding region may affect the expression of endogenous and foreign genes. Due to the complexity of biological processes, it cannot be predicted completely at the present technology level.

Gene information （NCBI）

集萃药康 GemPharmatech

Slc32a1 solute carrier family 32 (GABA vesicular transporter), member 1 [Mus musculus (house mouse)]

Gene ID: 22348, updated on 13-Mar-2020

Summary

Official Symbol	Slc32a1 provided by MGI
Official Full Name	solute carrier family 32 (GABA vesicular transporter), member 1 provided by MGI
Primary source	MGI:MGI:1194488
See related	Ensembl:ENSMUSG00000037771
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	R75019, VGAT, Viaat
Expression	Biased expression in cerebellum adult (RPKM 56.5), frontal lobe adult (RPKM 42.0) and 5 other tissues See more
Orthologs	human all

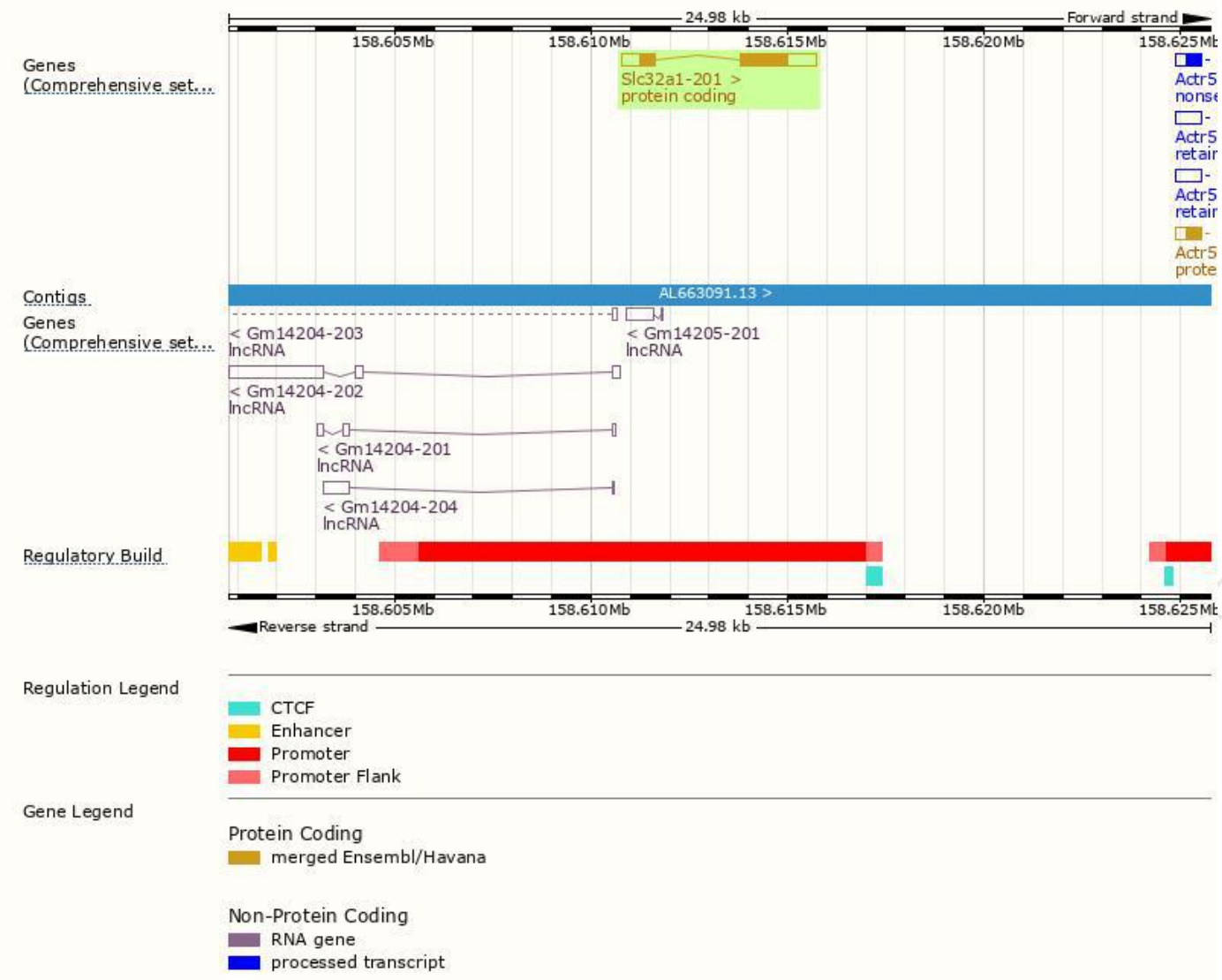
Transcript information（Ensembl）

The gene has 1 transcript,and the transcript is shown below:

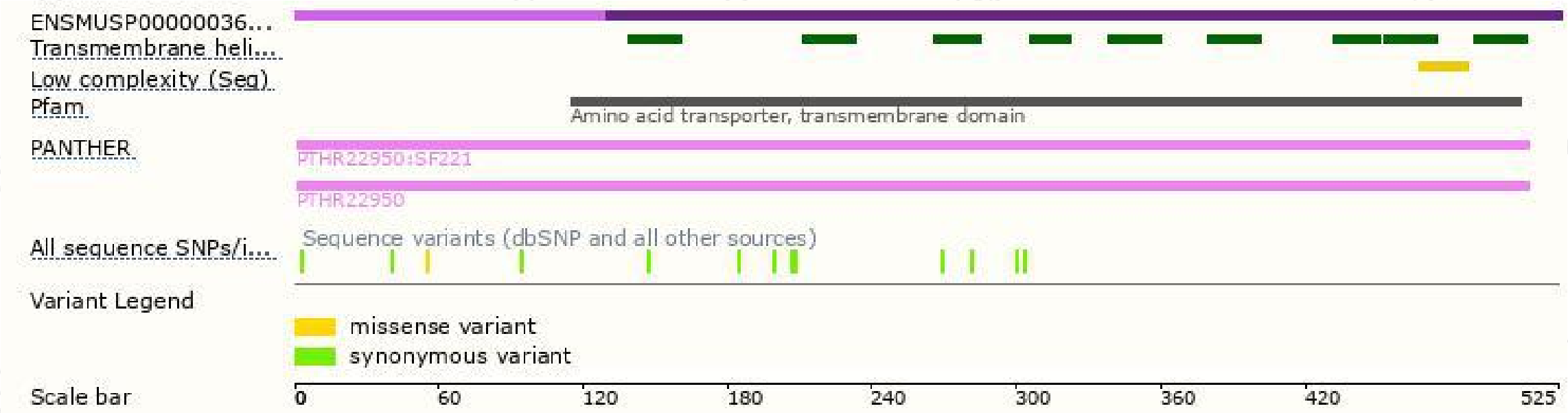
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Slc32a1-201	ENSMUST00000045738.4	2797	525aa	Protein coding	CCDS38309	O35633 Q49S98	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1

The strategy is based on the design of *Slc32a1-201* transcript,the transcription is shown below:

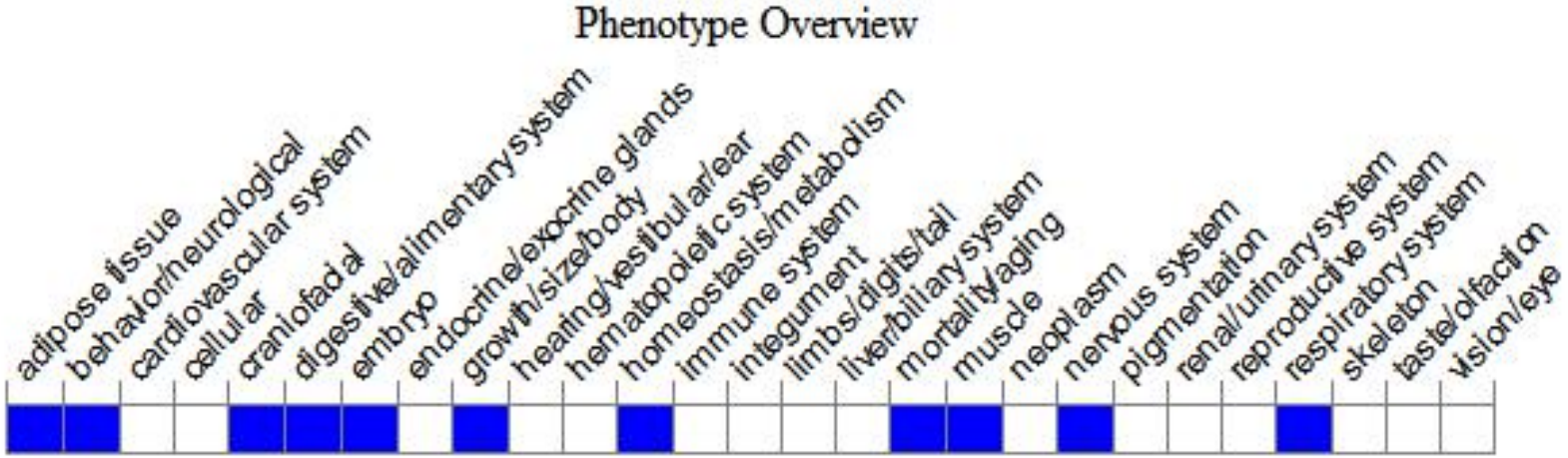
Genomic location distribution



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,homozygous null mice been independently reported to die perinatally exhibiting a hunched posture, respiratory failure, cleft secondary palate due to failure of palate shelf elevation, umbilical hernia or omphalocele, and loss of neurotransmitter release in both GABAergic and glycinergic neurons.

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

Tel: 400-9660890

集萃药康生物科技
GemPharmatech Co.,Ltd