

Gabre Cas9-KO Strategy

Designer: Xiaojing Li

Reviewer: Jia Yu

Design Date: 2020-8-24

Project Overview

Project Name

Gabre

Project type

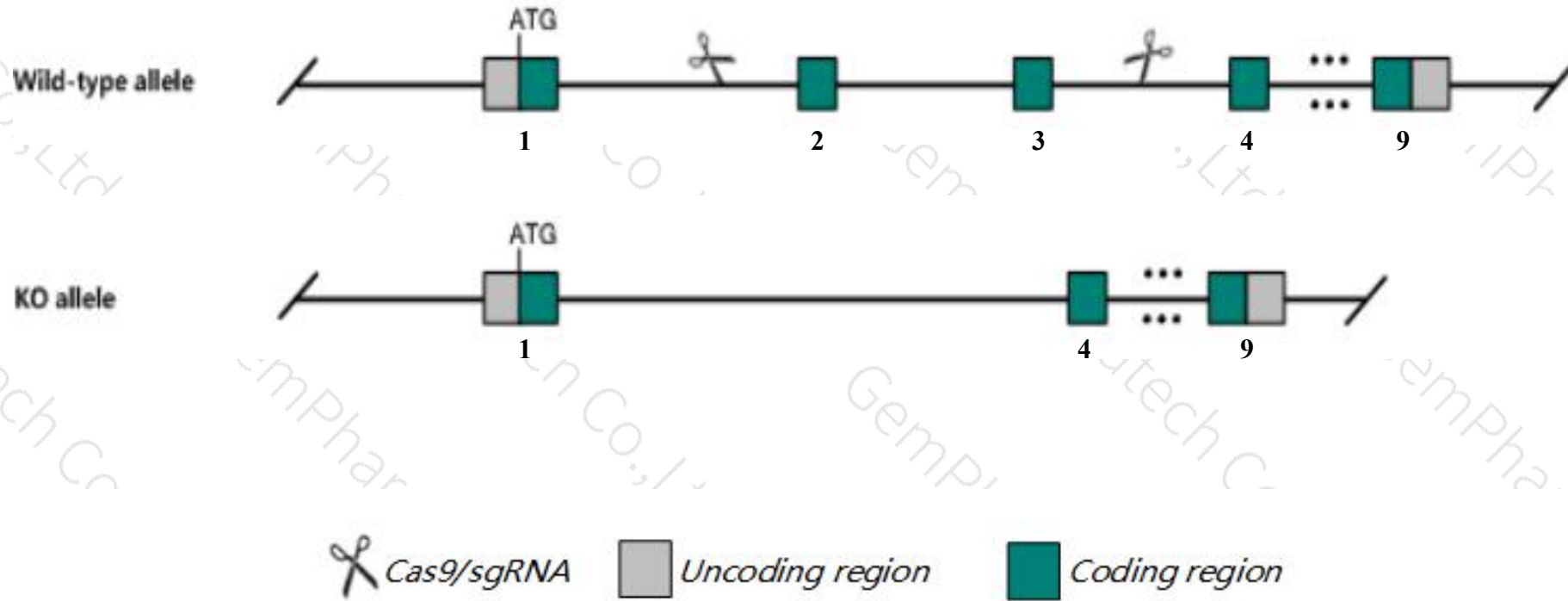
Cas9-KO

Strain background

C57BL/6JGpt

Knockout strategy

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



- The *Gabre* gene has 3 transcripts. According to the structure of *Gabre* gene, exon2-exon3 of *Gabre-201*(ENSMUST00000064780.3) transcript is recommended as the knockout region. The region contains 1519bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Gabre* gene. The brief process is as follows: sgRNA was transcribed in vitro. Cas9 and sgRNA 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.

- According to the existing MGI data, mice homozygous for a null allele exhibit impaired fertility and impaired response to repeated sevoflurane inhalation.
- The *Gabre* gene is located on the ChrX. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)

Gabre gamma-aminobutyric acid (GABA) A receptor, subunit epsilon [Mus musculus (house mouse)]

Gene ID: 14404, updated on 20-Mar-2020

Summary

Official Symbol Gabre provided by [MGI](#)

Official Full Name gamma-aminobutyric acid (GABA) A receptor, subunit epsilon provided by [MGI](#)

Primary source [MGI:MGI:1330235](#)

See related [Ensembl:ENSMUSG000000031340](#)

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

Expression Broad expression in limb E14.5 (RPKM 5.8), bladder adult (RPKM 1.5) and 18 other tissues [See more](#)

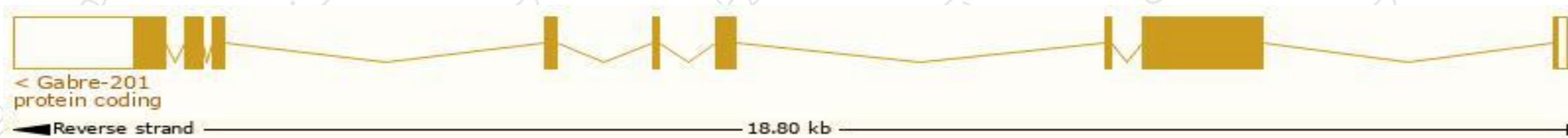
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

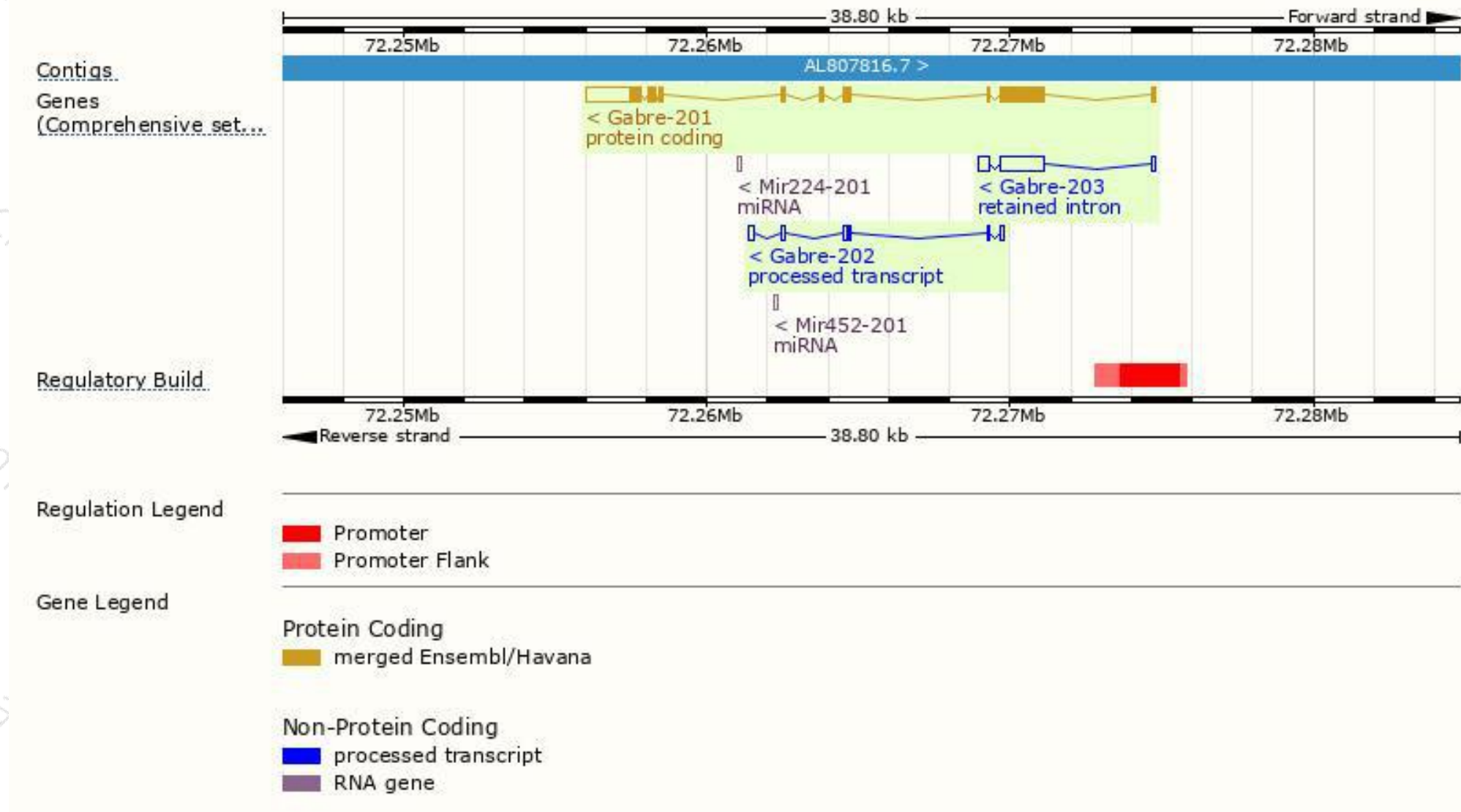
The gene has 3 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Gabre-201	ENSMUST00000064780.3	4306	916aa	Protein coding	CCDS30186	A2AMW3	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
Gabre-202	ENSMUST00000148499.1	579	No protein	Processed transcript	-	-	TSL:3
Gabre-203	ENSMUST00000153740.1	1938	No protein	Retained intron	-	-	TSL:1

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



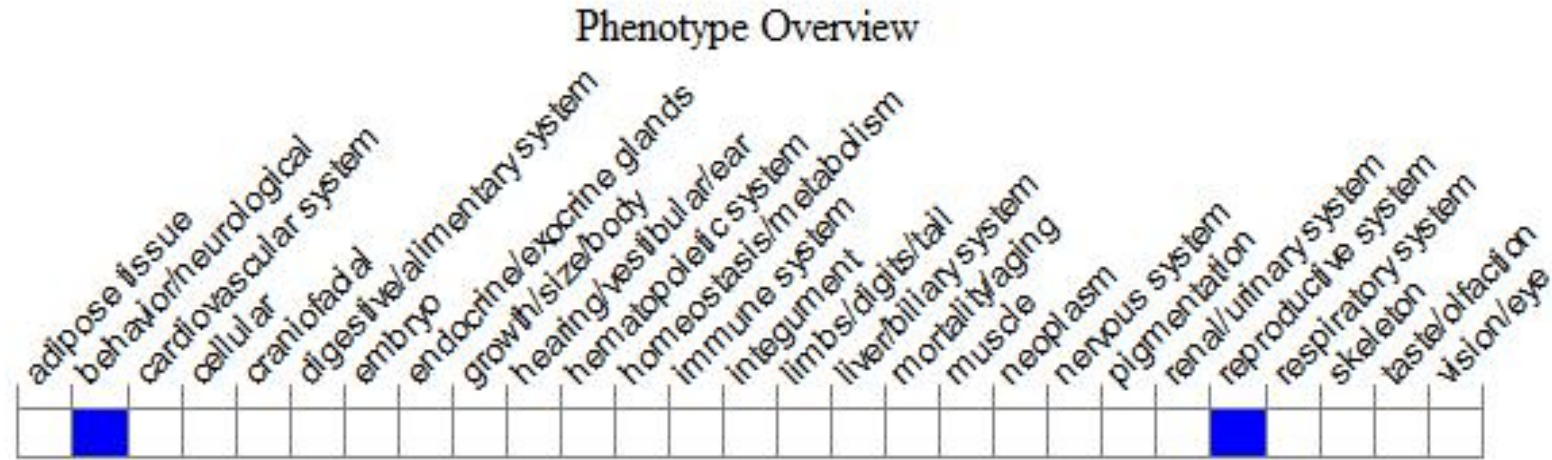
Genomic location distribution



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 a null allele exhibit impaired fertility and impaired response to repeated sevoflurane inhalation.