Gbel Cas9-KO Strategy

Designer: Yupeng Yang

Design Date: 2019-9-30

Project Overview



Project Name

Gbe1

Project type

Cas9-KO

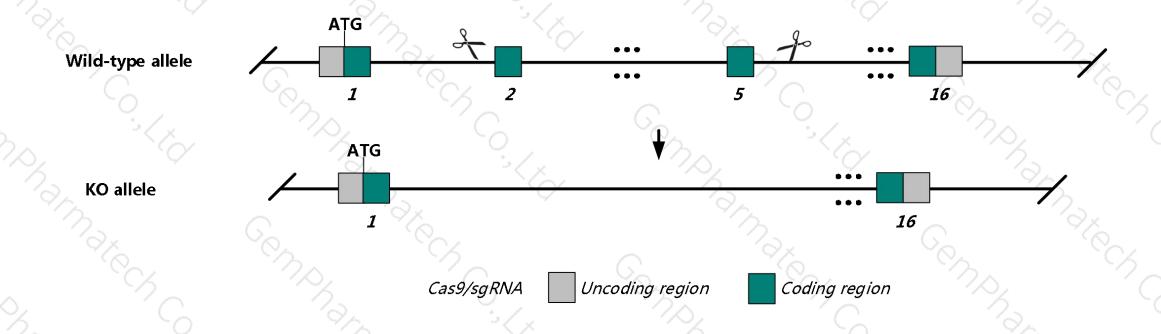
Strain background

C57BL/6JGpt

Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Gbe1* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Gbe1* gene has 7 transcripts. According to the structure of *Gbe1* gene, exon2-5 of *Gbe1*-203 (
 ENSMUST00000163832.7)transcript is recommended as the knockout region. The region contains 548bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Gbe1* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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, Mice homozygous for an ENU-induced allele exhibit mid-to-late gestation lethality, decreased heart rate, glycogen storage defects, and ventricles that were small, hypertrabeculated, and noncompacted.
- ➤ The *Gbe1* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information (NCBI)



Gbe1 glucan (1,4-alpha-), branching enzyme 1 [Mus musculus (house mouse)]

Gene ID: 74185, updated on 12-Aug-2019

Summary

Official Symbol Gbe1 provided by MGI

Official Full Name glucan (1,4-alpha-), branching enzyme 1 provided by MGI

Primary source MGI:MGI:1921435

See related Ensembl: ENSMUSG00000022707

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 D16Ertd536e; 2310045H19Rik; 2810426P10Rik

Expression Broad expression in liver E18 (RPKM 14.7), adrenal adult (RPKM 14.5) and 27 other tissues See more

Orthologs human all

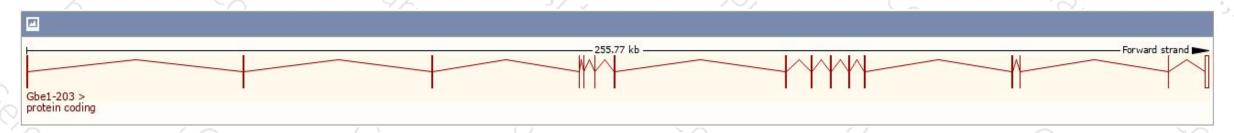
Transcript information (Ensembl)



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

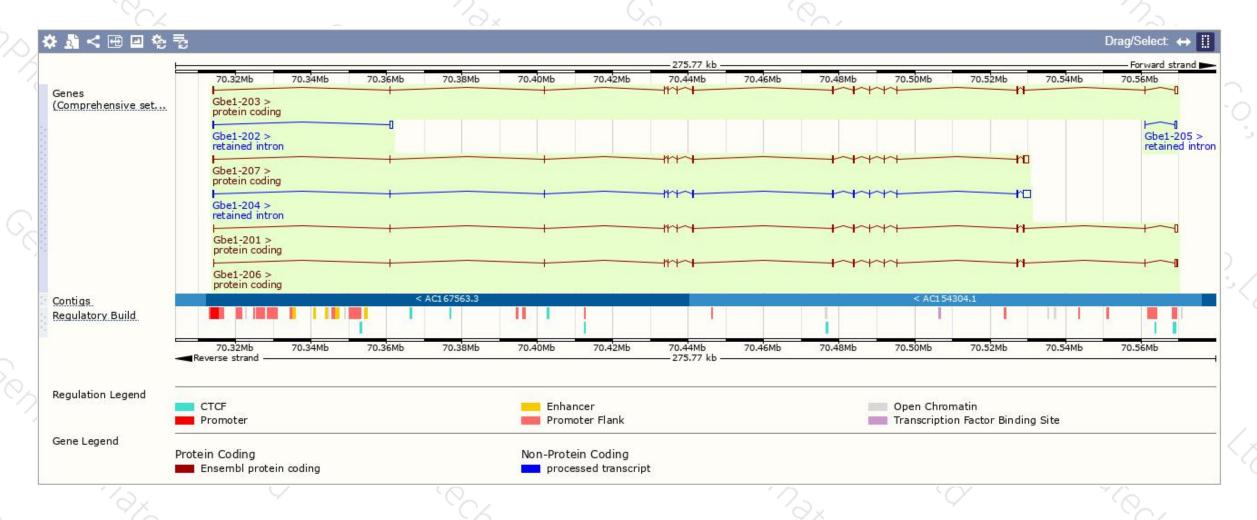
| Name A | Transcript ID | bp 🛔 | Protein A | Biotype | CCDS & | UniProt 4 | Flags |
|----------|-----------------------|------|--------------|-----------------|--------|-----------------|---------------------------------|
| Gbe1-207 | ENSMUST00000171132.7 | 3303 | <u>660aa</u> | Protein coding | - | <u>G3UW30</u> ₽ | TSL:1 GENCODE basic |
| 6be1-203 | ENSMUST00000163832.7 | 2984 | 702aa | Protein coding | 82 | Q9D6Y9₽ | TSL:5 GENCODE basic APPRIS ALT2 |
| Sbe1-201 | ENSMUST00000023393.14 | 2846 | 702aa | Protein coding | 525 | F6ZHD8₽ | TSL:5 GENCODE basic APPRIS P5 |
| Sbe1-206 | ENSMUST00000170464.2 | 2736 | 702aa | Protein coding | 82 | F6ZHD8₽ | TSL:5 GENCODE basic APPRIS ALT2 |
| Sbe1-204 | ENSMUST00000164300.1 | 3734 | No protein | Retained intron | 184 | - | TSL:5 |
| 6be1-202 | ENSMUST00000099628.4 | 1026 | No protein | Retained intron | 82 | - | TSL:1 |
| Gbe1-205 | ENSMUST00000169432.1 | 658 | No protein | Retained intron | 1 828 | 2 | TSL:1 |

The strategy is based on the design of *Gbe1-203* transcript, The transcription is shown below



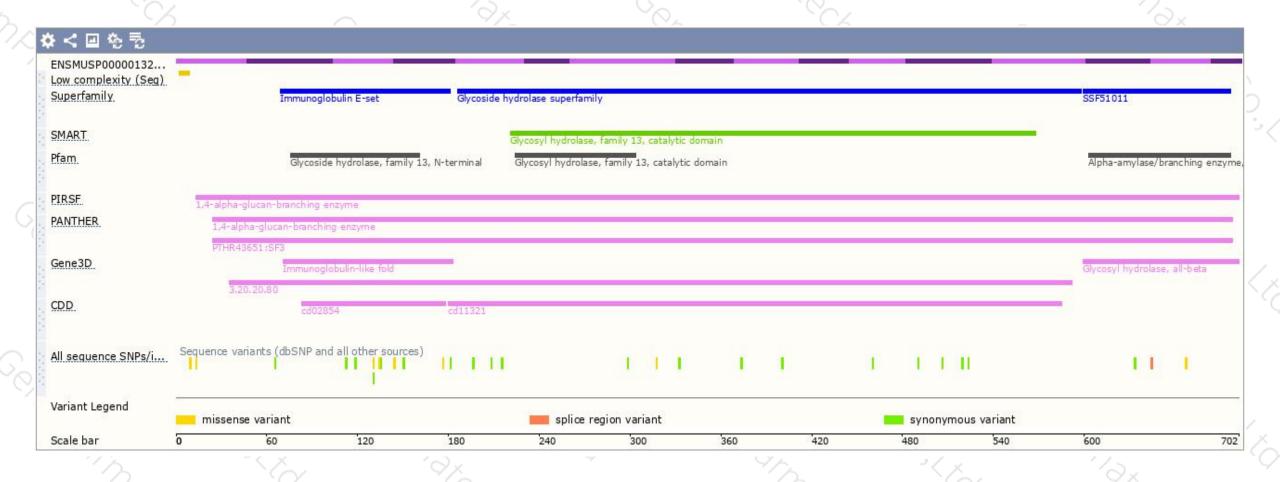
Genomic location (Ensembl)





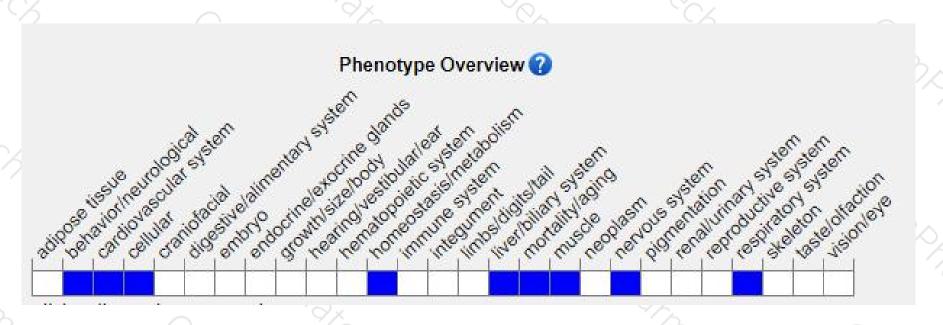
Protein domain (Ensembl)





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 an ENU-induced allele exhibit mid-to-late gestation lethality, decreased heart rate, glycogen storage defects, and ventricles that were small, hypertrabeculated, and noncompacted.

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





