

Kcnb1 Cas9-KO Strategy

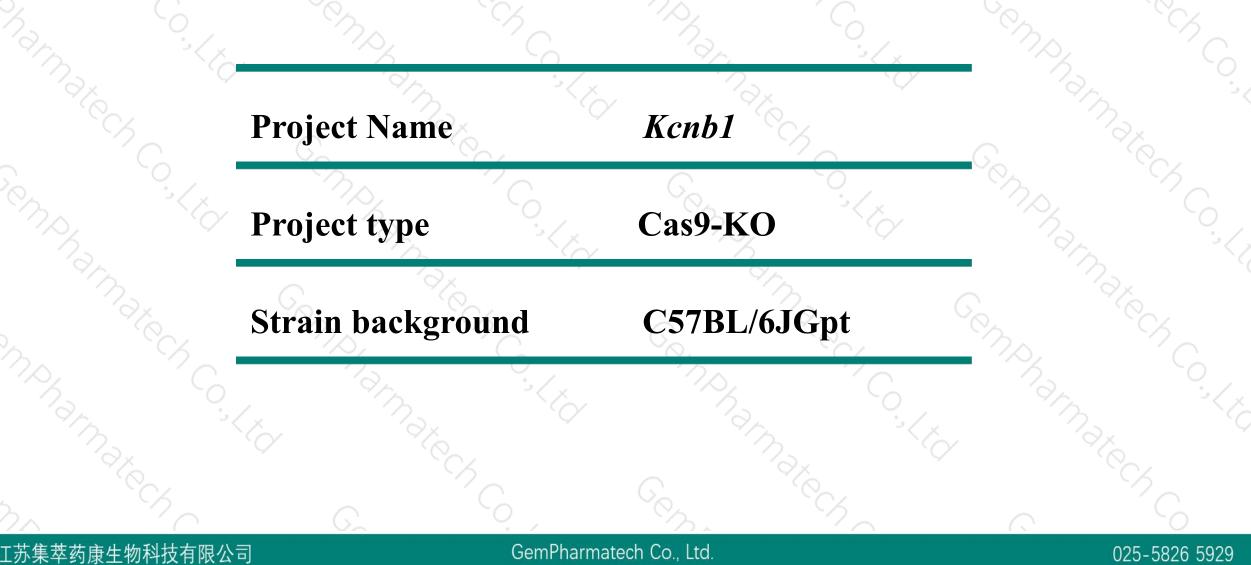
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Reviewer: Ruiuri Zhang

Design Date: 2020-7-2

Project Overview



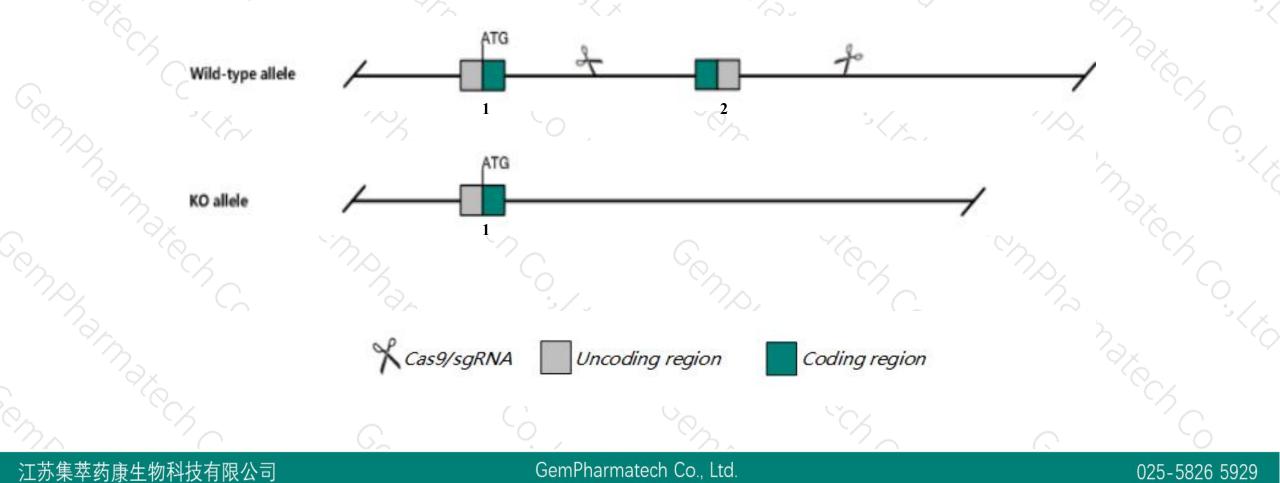


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Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Kcnb1 gene. The schematic diagram is as follows:





The Kcnb1 gene has 4 transcripts. According to the structure of Kcnb1 gene, exon2 of Kcnb1-204(ENSMUST00000207917.1) transcript is recommended as the knockout region. The region contains most of the coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Kcnb1* 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.

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> According to the existing MGI data,mice homozygous for a null allele show reduced fasting glucose levels, hyperinsulinemia, improved glucose tolerance, and enhanced insulin secretion. Mice homozygous for another null allele exhibit loss of photoreceptors with increased apoptosis.

> The *Kcnb1* gene is located on the Chr2. 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.

Notice

Gene information (NCBI)



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Kcnb1 potassium voltage gated channel, Shab-related subfamily, member 1 [Mus musculus (house mouse)]

Gene ID: 16500, updated on 26-Jun-2020

Summary

 Official Symbol
 Kcnb1 provided by MGI

 Official Full Name
 potassium voltage gated channel, Shab-related subfamily, member 1 provided by MGI

 Primary source
 MGI:MGI:96666

 See related
 Ensembl:ENSMUSG00000050556

 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; Musi Mus

 Also known as
 Shab; Kv2.1; Kcr1-1

 Expression
 Broad expression in cortex adult (RPKM 12.1), frontal lobe adult (RPKM 11.6) and 19 other tissues See more

 Orthologs
 human all

Transcript information (Ensembl)



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The gene has 4 transcripts, all transcripts are shown below:

| Name 🖕 | Transcript ID | bp 🛔 | Protein 🔺 | Biotype 🖕 | CCDS 🖕 | UniProt 🖕 | Flags |
|-----------|----------------------|--------------------|--------------|----------------------|--------------------|-----------------|-------------------------------|
| Kcnb1-204 | ENSMUST00000207917.1 | 11153 | <u>857aa</u> | Protein coding | <u>CCDS17096</u> 교 | <u>Q03717</u> & | TSL:1 GENCODE basic APPRIS P1 |
| Kcnb1-201 | ENSMUST0000059826.9 | 4015 | <u>857aa</u> | Protein coding | <u>CCDS17096</u> 교 | <u>Q03717</u> & | TSL:1 GENCODE basic APPRIS P1 |
| Kcnb1-202 | ENSMUST00000144351.1 | <mark>189</mark> 4 | No protein | Processed transcript | 5 | 8 7 8 | TSL:1 |
| Kcnb1-203 | ENSMUST00000148226.2 | 1119 | No protein | Processed transcript | - | 1.71 | TSL:3 |

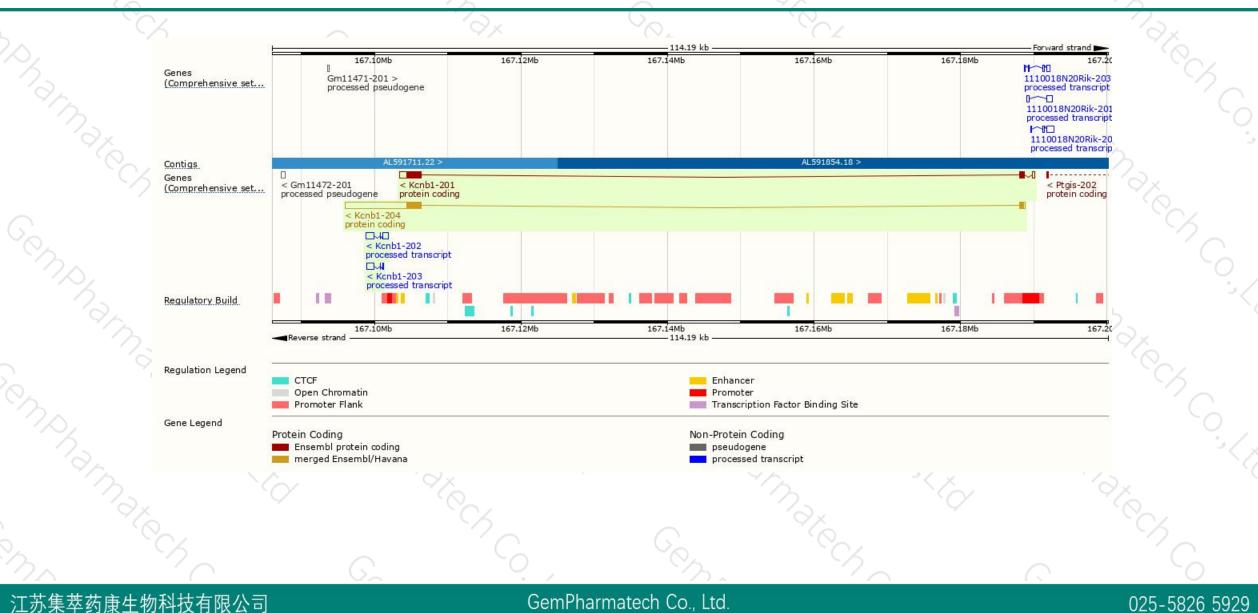
The strategy is based on the design of *Kcnb1-204* transcript, the transcription is shown below:

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< Kcnb1-204

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Genomic location distribution



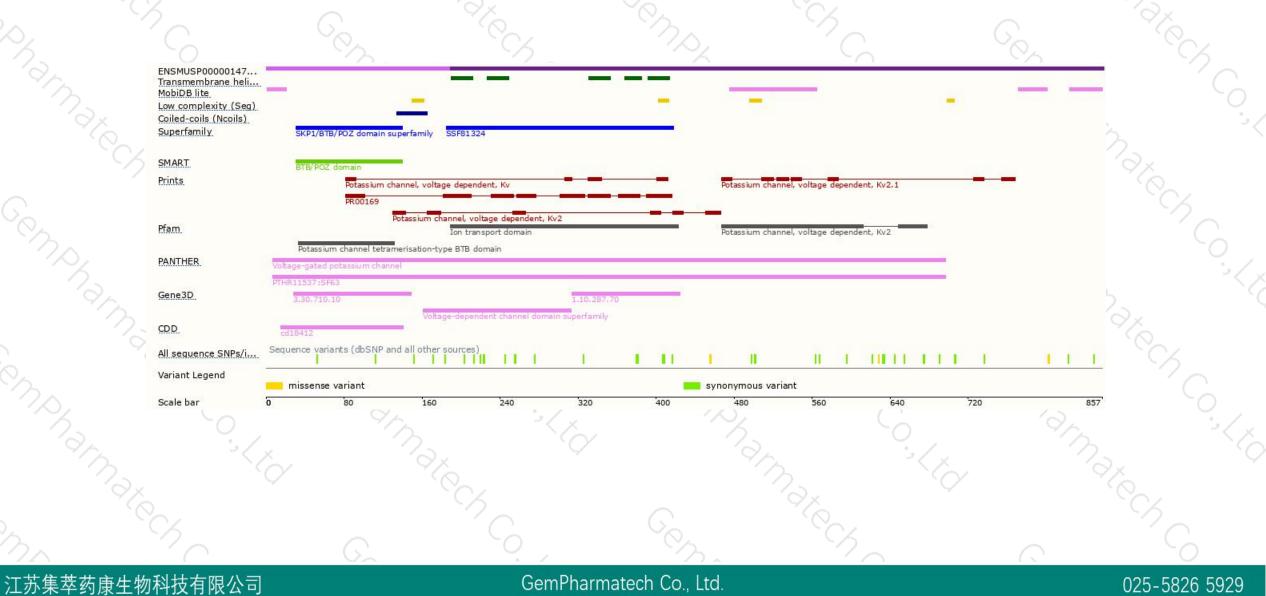
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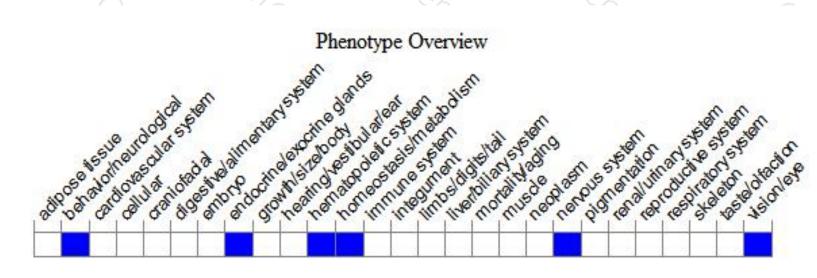
Protein domain





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 show reduced fasting glucose levels, hyperinsulinemia, improved glucose tolerance, and enhanced insulin secretion. Mice homozygous for another null allele exhibit loss of photoreceptors with increased apoptosis.

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If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



