

***Grb2* Cas9-KO Strategy**

Designer: Jinling Wang

Reviewer: Lingyan Wu

Design Date: 2018-9-8

Project Overview

Project Name

Grb2

Project type

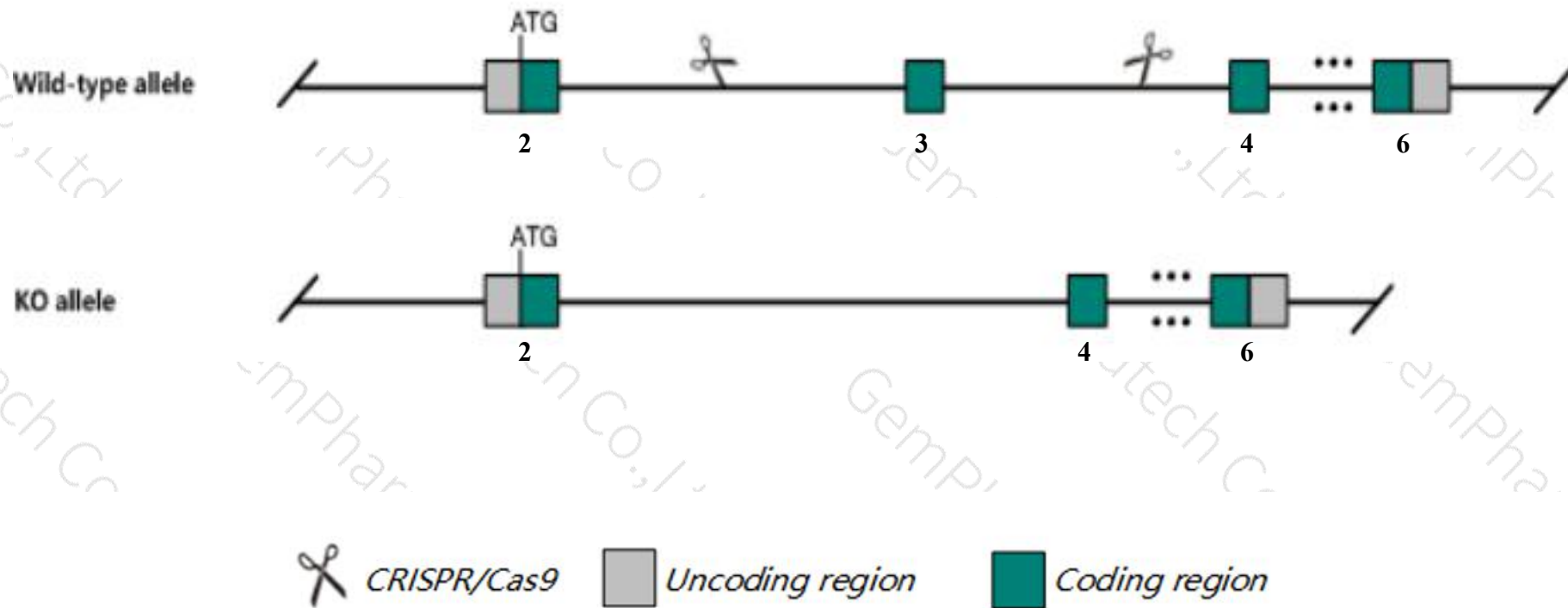
Cas9-KO

Strain background

C57BL/6JGpt

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Grb2* gene. The schematic diagram is as follows:



Technical routes

- The *Grb2* gene has 5 transcripts. According to the structure of *Grb2* gene, exon3 of *Grb2-201*(ENSMUST00000021090.13) transcript is recommended as the knockout region. The region contains 98bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Grb2* gene. The brief process is as follows: CRISPR/Cas9 system 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, embryos homozygous for a targeted null mutation lack expanded inner cell masses, show only a few endodermal cells, and die by embryonic day 7.5. Heterozygotes have defective T cell signaling and lack the cardiac hypertrophy response to pressure overload.
- The *Grb2* gene is located on the Chr11. 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)

Grb2 growth factor receptor bound protein 2 [Mus musculus (house mouse)]

Gene ID: 14784, updated on 13-Mar-2020

Summary

Official Symbol Grb2 provided by [MGI](#)

Official Full Name growth factor receptor bound protein 2 provided by [MGI](#)

Primary source [MGI:MGI:95805](#)

See related [Ensembl:ENSMUSG00000059923](#)

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 AA408164, Ash

Summary The protein encoded by this gene binds the epidermal growth factor receptor and contains one SH2 domain and two SH3 domains. Its two SH3 domains direct complex formation with proline-rich regions of other proteins, and its SH2 domain binds tyrosine phosphorylated sequences. This gene is similar to the Sem5 gene of *C.elegans*, which is involved in the signal transduction pathway. Three alternatively spliced transcript variants encoding the same protein have been found for this gene. [provided by RefSeq, Sep 2015]

Expression Ubiquitous expression in CNS E18 (RPKM 40.7), frontal lobe adult (RPKM 37.9) and 28 other tissues [See more](#)

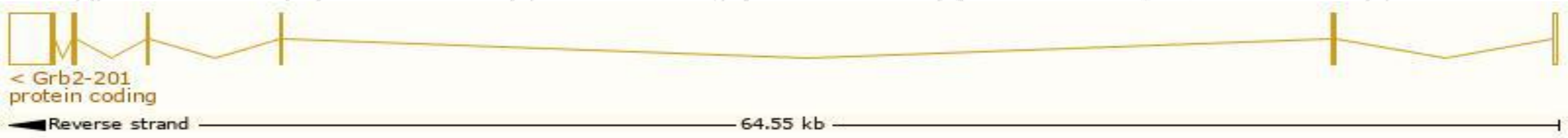
Orthologs [human](#) [all](#)

Transcript information（Ensembl）

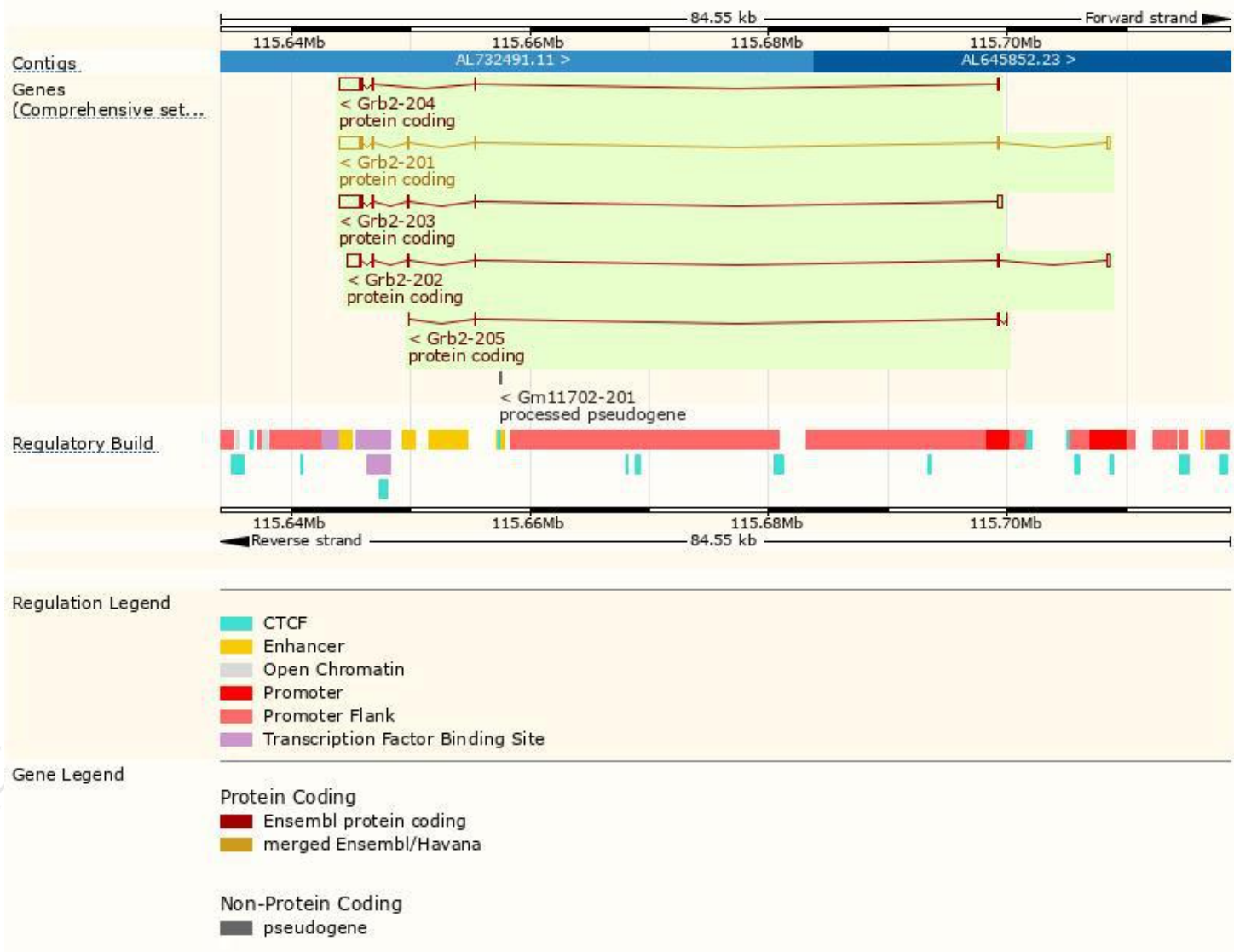
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Grb2-201	ENSMUST00000021090.13	2709	217aa	Protein coding	CCDS25645	Q3U5I5 Q60631	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
Grb2-203	ENSMUST00000106497.7	2683	217aa	Protein coding	CCDS25645	Q3U5I5 Q60631	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
Grb2-204	ENSMUST00000106499.7	2333	176aa	Protein coding	-	Q60631	TSL:1 GENCODE basic
Grb2-202	ENSMUST00000106495.7	2041	203aa	Protein coding	-	B1AT92	TSL:5 GENCODE basic
Grb2-205	ENSMUST00000135065.1	369	71aa	Protein coding	-	B1AT95	CDS 3' incomplete TSL:5

The strategy is based on the design of *Grb2-201* transcript,the transcription is shown below:



Genomic location distribution



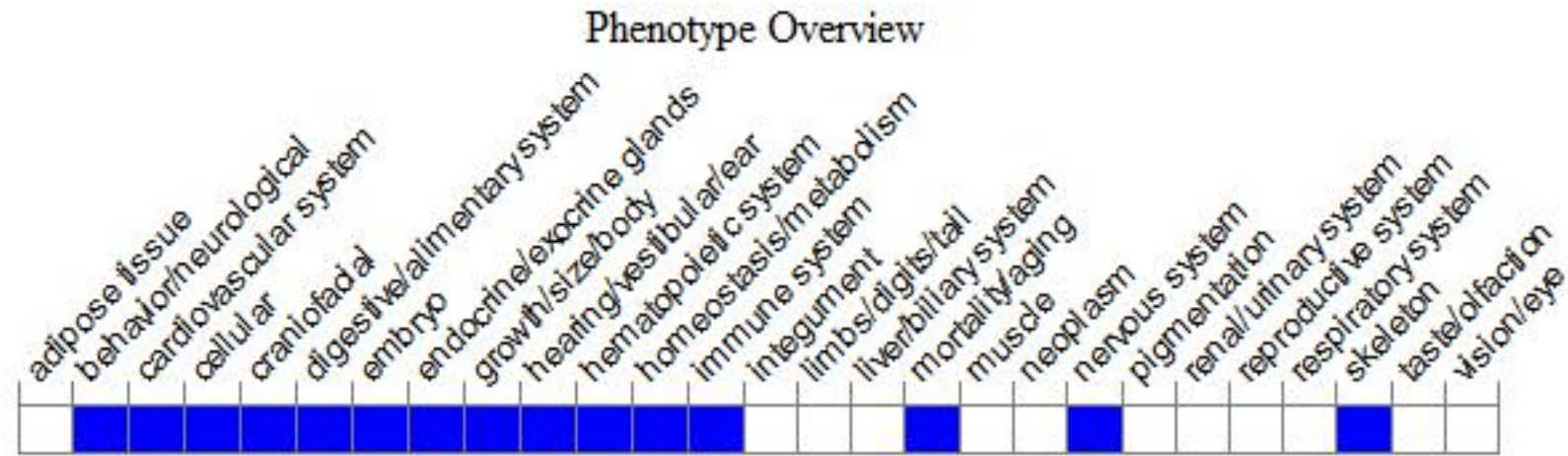
Protein domain



集萃药康
GemPharmatech



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, embryos homozygous for a targeted null mutation lack expanded inner cell masses, show only a few endodermal cells, and die by embryonic day 7.5. Heterozygotes have defective T cell signaling and lack the cardiac hypertrophy response to pressure overload.

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

Tel: 400-9660890

