Chrnb1 Cas9-KO Strategy

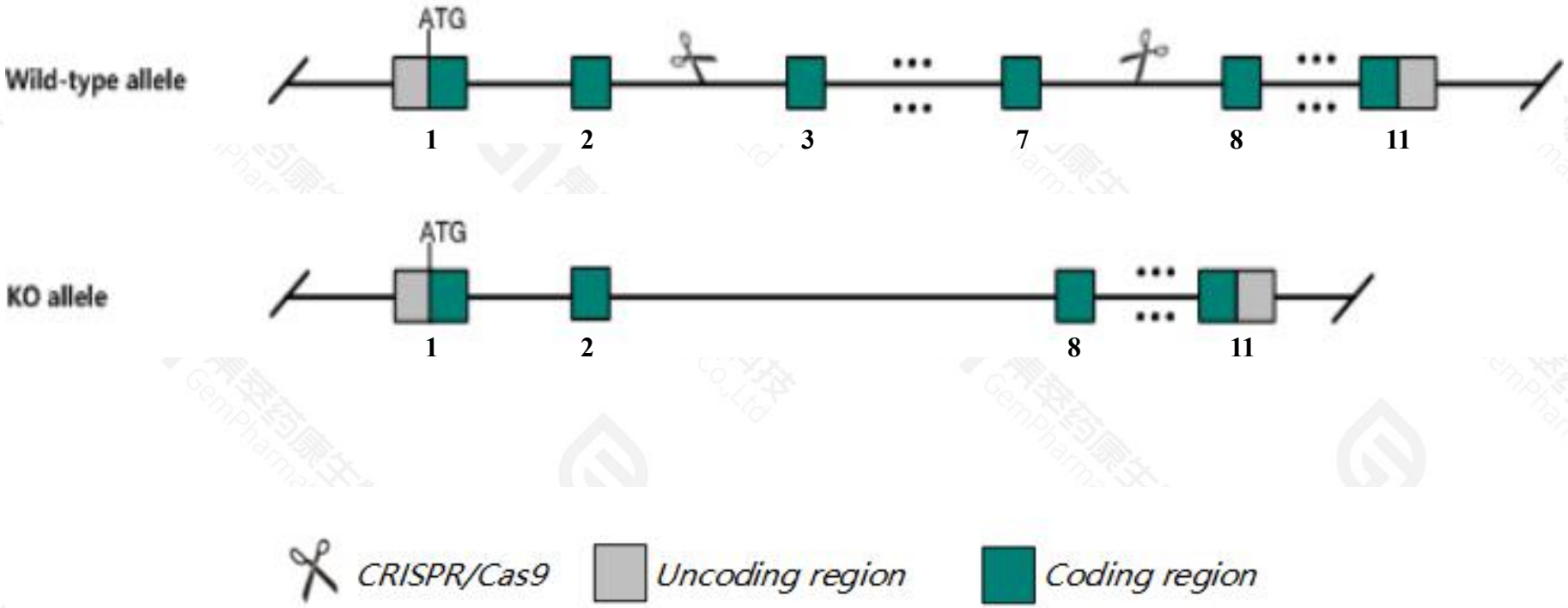
Designer: Qiong Zhou

Project Overview

Project Name	***Chrnb1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Chrnb1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Chrnb1* gene has 4 transcripts. According to the structure of *Chrnb1* gene, exon3-exon7 of *Chrnb1-201*(ENSMUST00000045971.9) transcript is recommended as the knockout region.The region contains 622bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Chrnb1* 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.

Notice

➢ According to the existing MGI data,mice homozygous for a knock-in allele lack all tyrosine residues in the beta subunit intracellular loop, display reduced and simplified neuromuscular junctions, and show defective acetylcholine receptor clustering and anchoring at synapses.

➢ The Intron2 and Intron7 are only 581bp and 5594bp,loxp insertion may affect mRNA splicing.

➢ The *Chrnb1* 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）

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Chrnb1 cholinergic receptor, nicotinic, beta polypeptide 1 (muscle) [Mus musculus (house mouse)]

Gene ID: 11443, updated on 13-Mar-2020

Summary

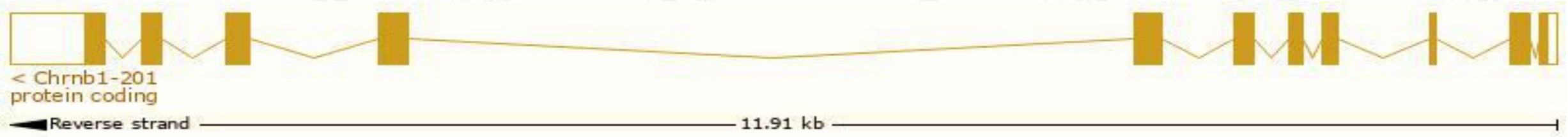
Official Symbol	Chrnb1 provided by MGI
Official Full Name	cholinergic receptor, nicotinic, beta polypeptide 1 (muscle) provided byMGI
Primary source	MGI:MGI:87890
See related	Ensembl:ENSMUSG00000041189
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Achr-2, Acrb
Expression	Broad expression in limb E14.5 (RPKM 18.2), lung adult (RPKM 13.4) and 21 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

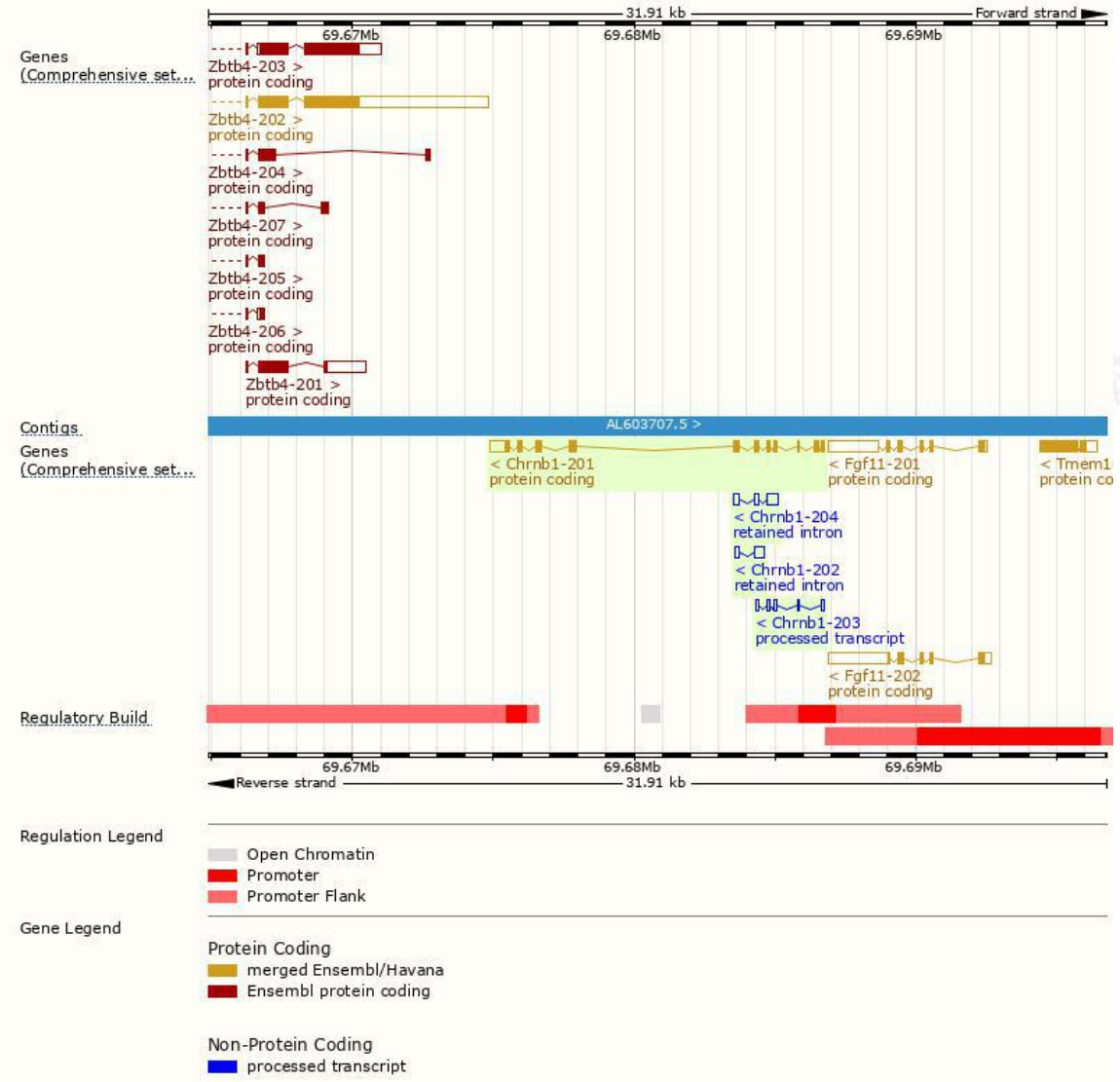
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Chrnb1-201	ENSMUST00000045971.8	2155	501aa	Protein coding	CCDS24910	P09690	TSL:1 GENCODE basic APPRIS P1
Chrnb1-203	ENSMUST00000147791.1	441	No protein	Processed transcript	-	-	TSL:3
Chrnb1-204	ENSMUST00000154816.1	740	No protein	Retained intron	-	-	TSL:3
Chrnb1-202	ENSMUST00000129381.1	486	No protein	Retained intron	-	-	TSL:3

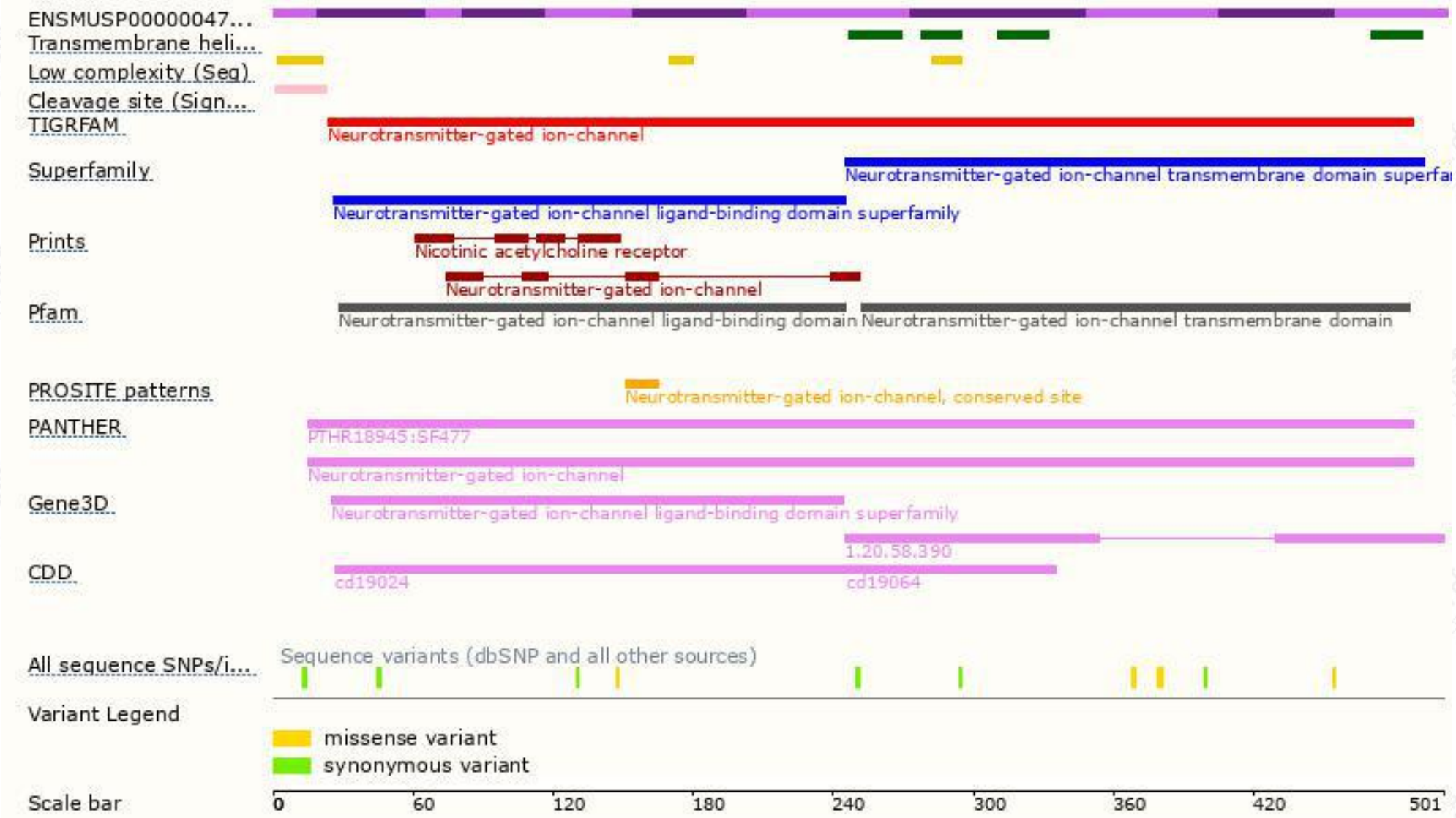
The strategy is based on the design of *Chrnb1-201* transcript,the transcription is shown below:



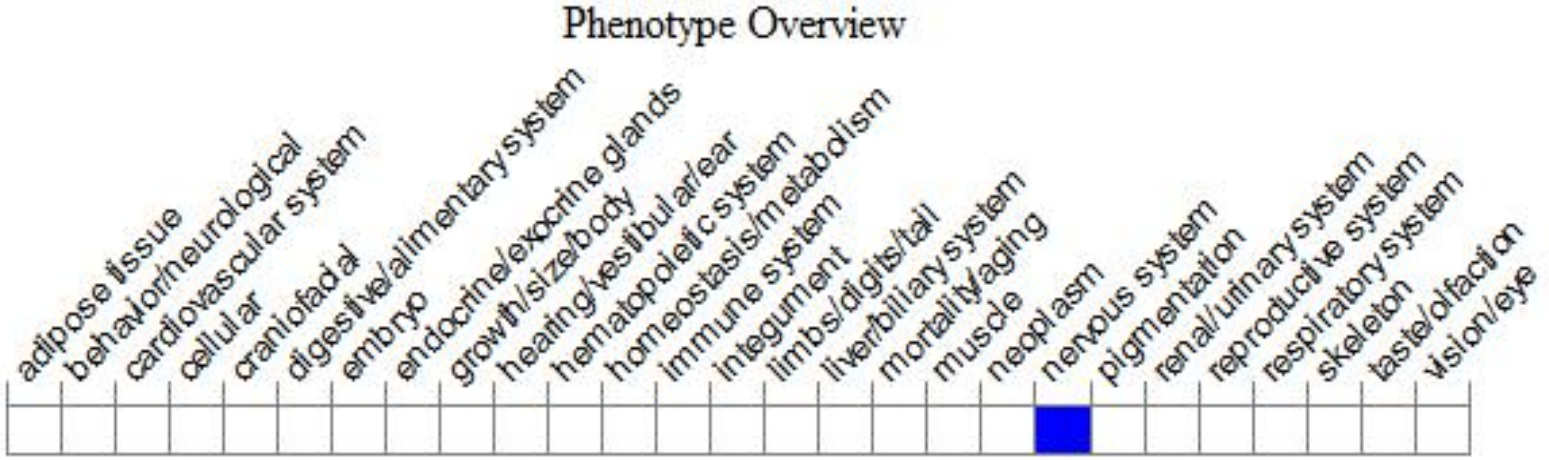
Genomic location distribution



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/).

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