

Chrnbl Cas9-CKO Strategy

Designer: Qiong Zhou

Project Overview

Project Name

Chrnbl

Project type

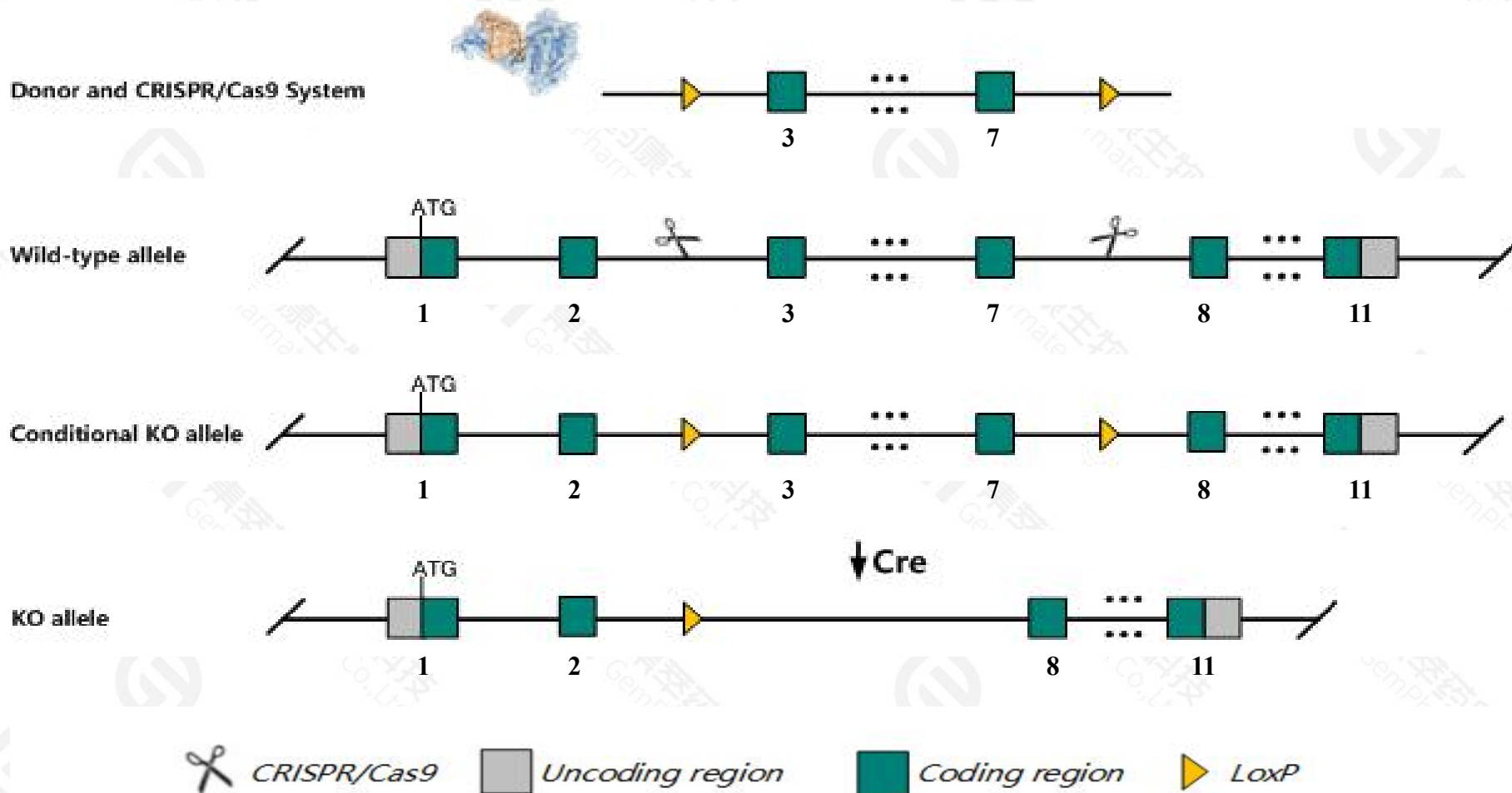
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

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



- The *Chrnbl* gene has 4 transcripts. According to the structure of *Chrnbl* gene, exon3-exon7 of *Chrnbl*-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 *Chrnbl* gene. The brief process is as follows: CRISPR/Cas9 system and Donor 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.
- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

- 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 *Chrnbl* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)

Chrn1 cholinergic receptor, nicotinic, beta polypeptide 1 (muscle) [Mus musculus (house mouse)]

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

Summary

Official Symbol Chrn1 provided by [MGI](#)

Official Full Name cholinergic receptor, nicotinic, beta polypeptide 1 (muscle) provided by [MGI](#)

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 tissues [See more](#)

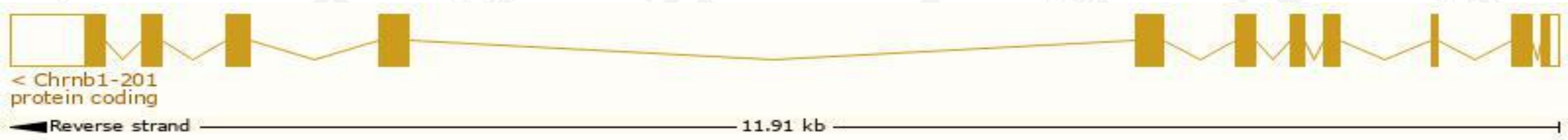
Orthologs [human](#) [all](#)

Transcript information (Ensembl)

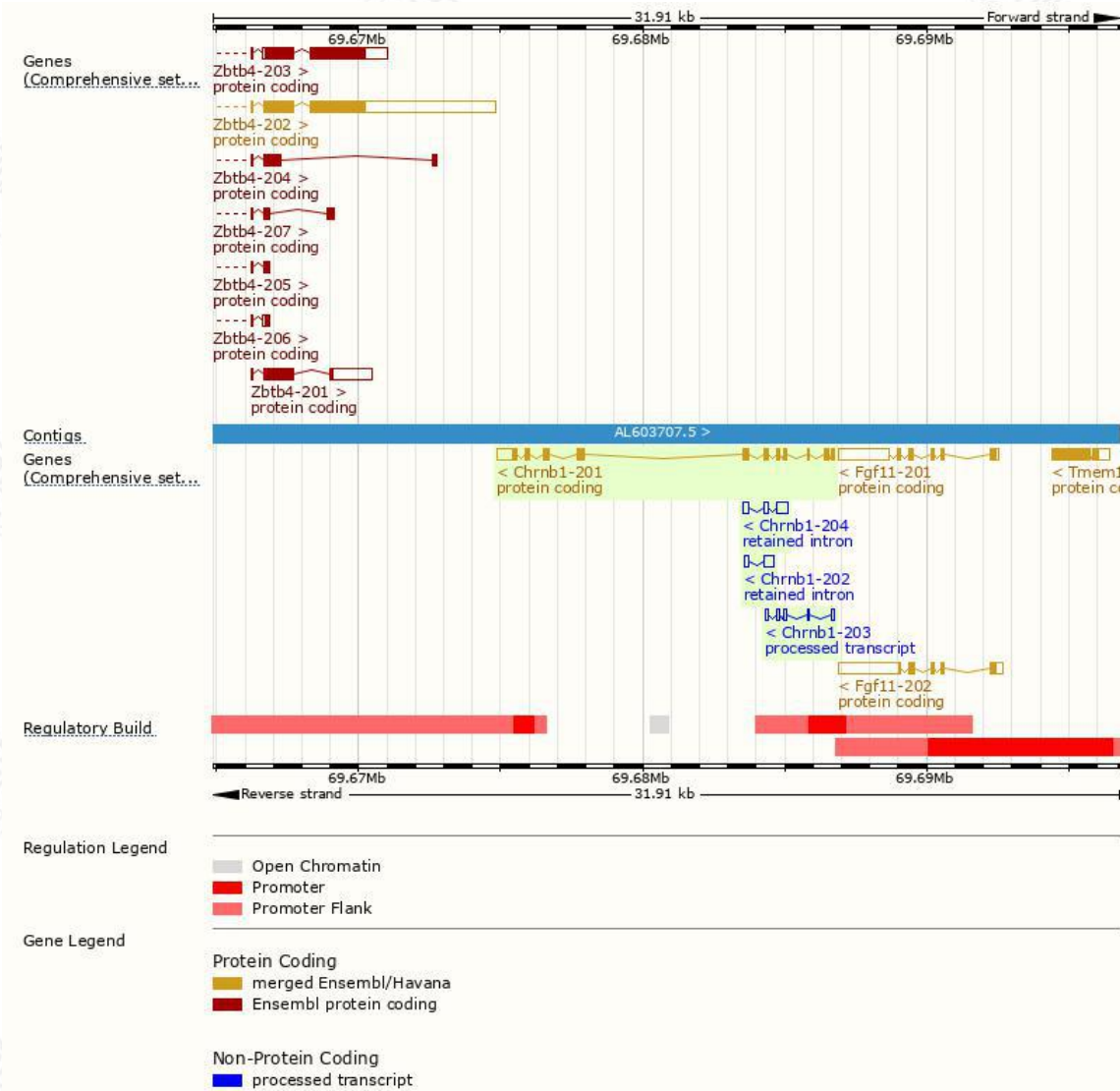
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Chrn1-201	ENSMUST0000045971.8	2155	501aa	Protein coding	CCDS24910	P09690	TSL:1 GENCODE basic APPRIS P1
Chrn1-203	ENSMUST00000147791.1	441	No protein	Processed transcript	-	-	TSL:3
Chrn1-204	ENSMUST00000154816.1	740	No protein	Retained intron	-	-	TSL:3
Chrn1-202	ENSMUST00000129381.1	486	No protein	Retained intron	-	-	TSL:3

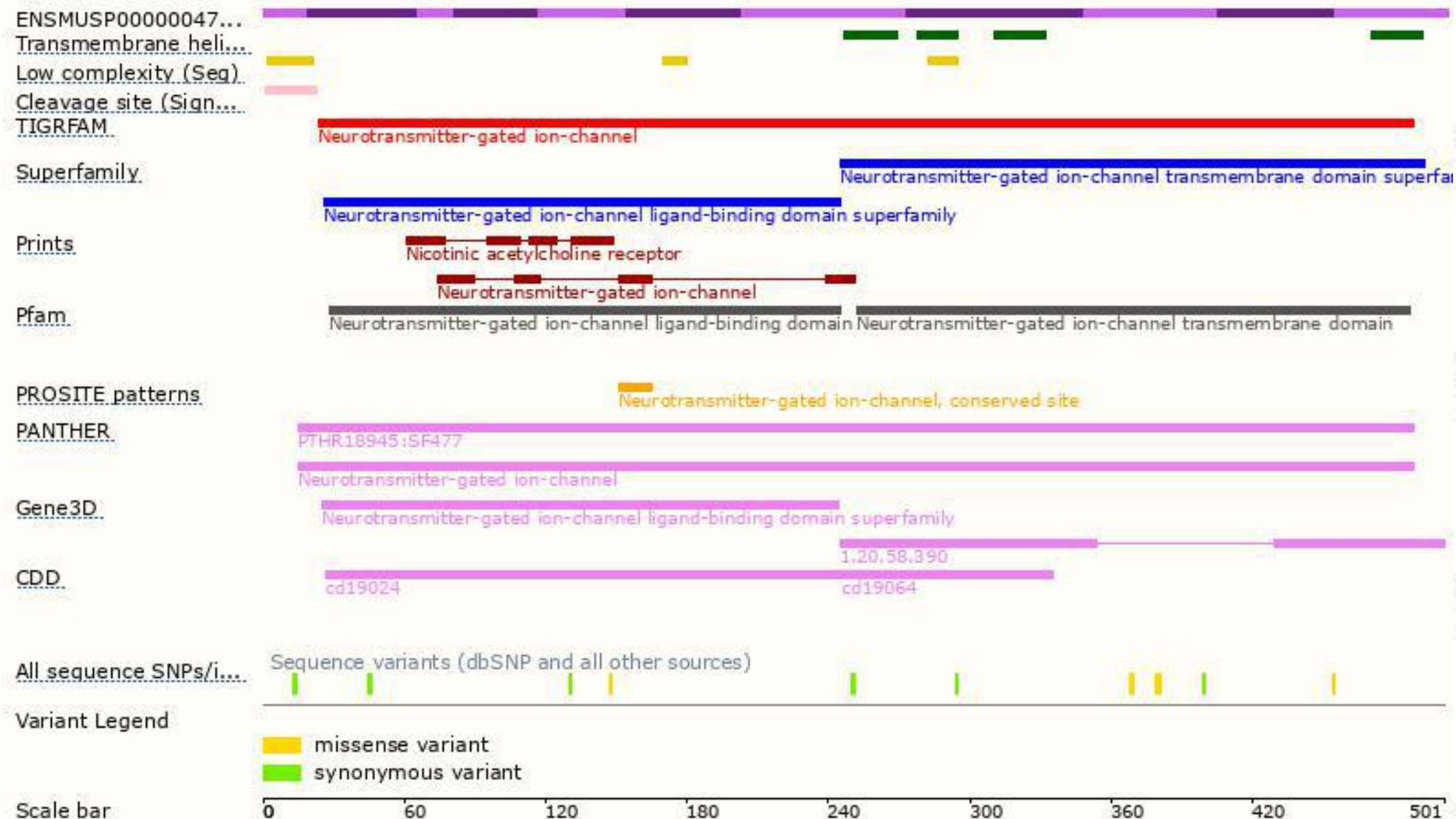
The strategy is based on the design of *Chrn1-201* transcript,the transcription is shown below:



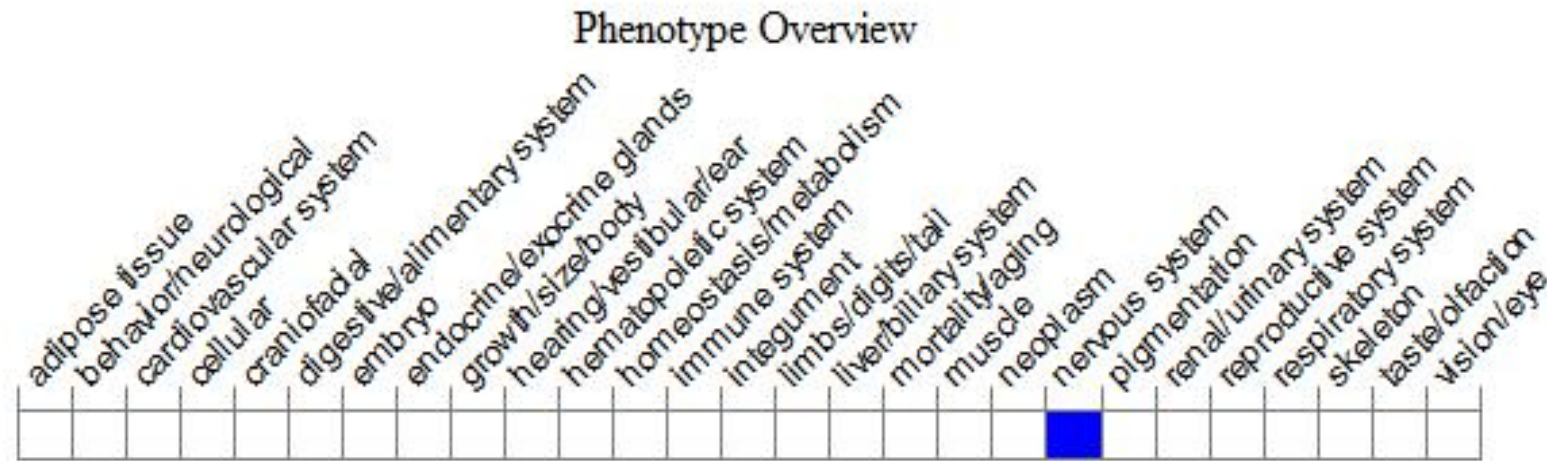
Genomic location distribution



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 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.

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