Chrne Cas9-KO Strategy

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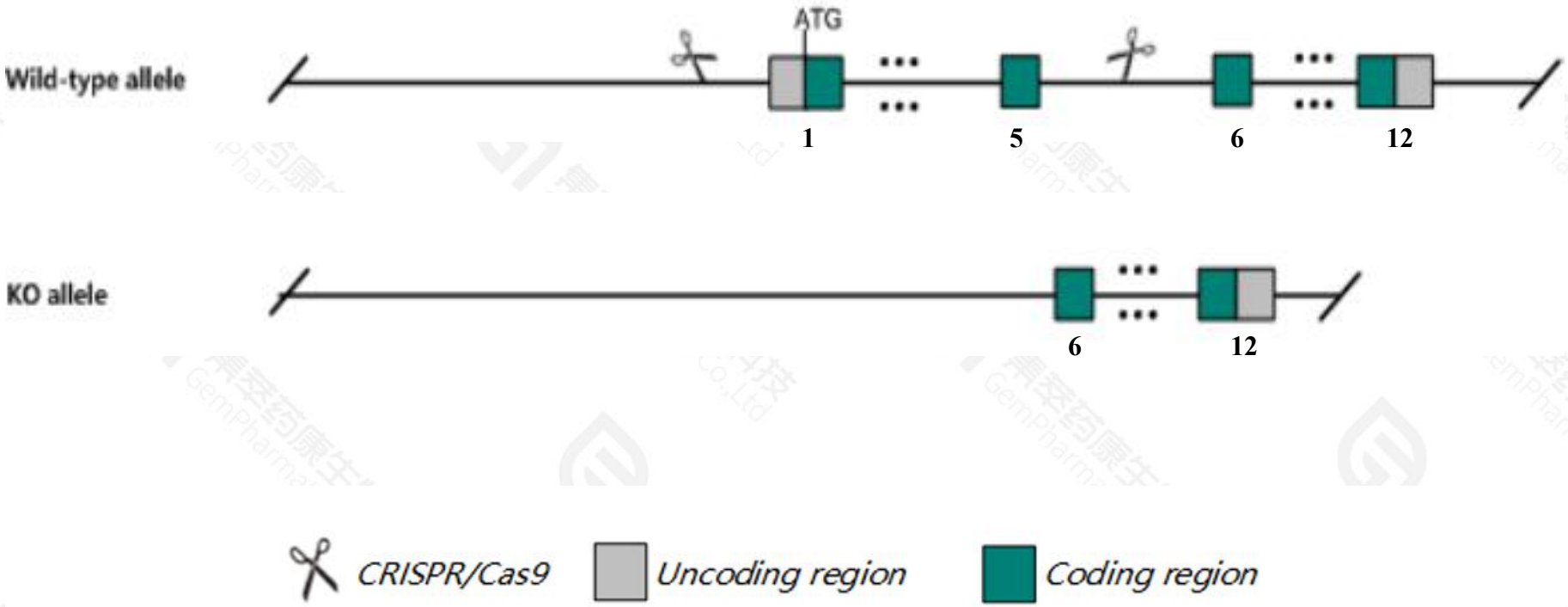
Design Date: 2021-3-3

Project Overview

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

Knockout strategy

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



Technical routes

➢ The *Chrne* gene has 4 transcripts. According to the structure of *Chrne* gene, exon1-exon5 of *Chrne-202*(ENSMUST00000102556.9) transcript is recommended as the knockout region.The region contains start codon ATG.Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Chrne* 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,homozygotes for targeted null mutations exhibit reduced AChR receptor density at neuromuscular synapses, impaired neuromuscular transmission, progressive muscular weakness and atrophy, and lethality at 2-3 months of age.

➢ The Intron5 is only 449bp,loxp insertion may affect mRNA splicing.

➢ The *Chrne* 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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Chrne cholinergic receptor, nicotinic, epsilon polypeptide [Mus musculus (house mouse)]

Gene ID: 11448, updated on 13-Mar-2020

Summary

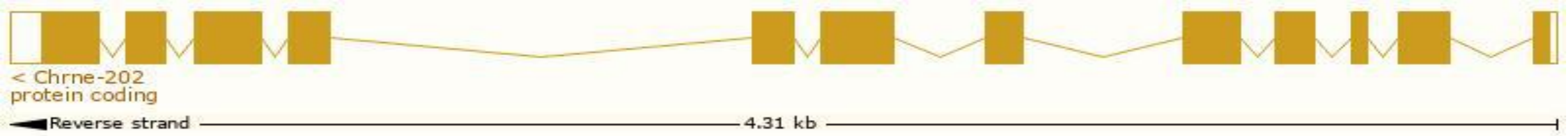
Official Symbol	Chrne provided by MGI
Official Full Name	cholinergic receptor, nicotinic, epsilon polypeptide provided byMGI
Primary source	MGI:MGI:87894
See related	Ensembl:ENSMUSG00000014609
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AChrepsilon, Acre, nAChRE
Summary	This gene encodes the epsilon subunit of the muscle-derived nicotinic acetylcholine receptor, a pentameric neurotransmitter receptor and member of the ligand-gated ion channel superfamily. The acetylcholine receptor changes subunit composition shortly after birth when the epsilon subunit replaces the gamma subunit seen in embryonic receptors. In mice, deficiency of this gene can lead to a decline in the number of nicotinic acetylcholine receptors at neuromuscular junctions and causes progressive muscle weakness, atrophy and premature death. Mutations in this gene serve as a pathophysiological model for human congenital myasthenia. Several alternatively spliced transcript variants of this gene have been described, but their full-length nature is not known. [provided by RefSeq, Nov 2012]
Expression	Restricted expression toward testis adult (RPKM 26.9)See more
Orthologs	human all

Transcript information（Ensembl）

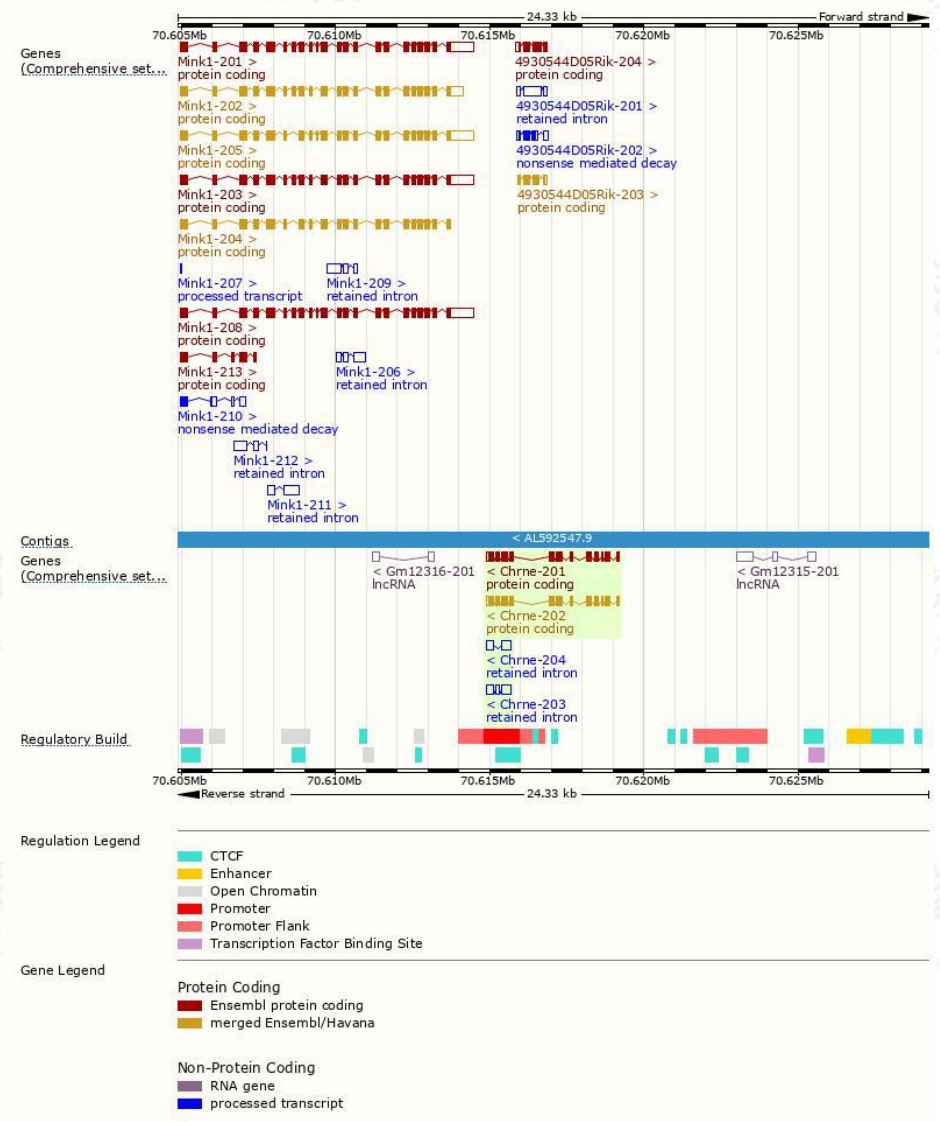
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Chrne-202	ENSMUST00000102556.9	1591	493aa	Protein coding	CCDS24956	P20782	TSL:1 GENCODE basic APPRIS P2
Chrne-201	ENSMUST00000014753.8	1616	494aa	Protein coding	-	Q5SXG9	TSL:5 GENCODE basic APPRIS ALT2
Chrne-203	ENSMUST00000134836.1	641	No protein	Retained intron	-	-	TSL:1
Chrne-204	ENSMUST00000135920.1	518	No protein	Retained intron	-	-	TSL:2

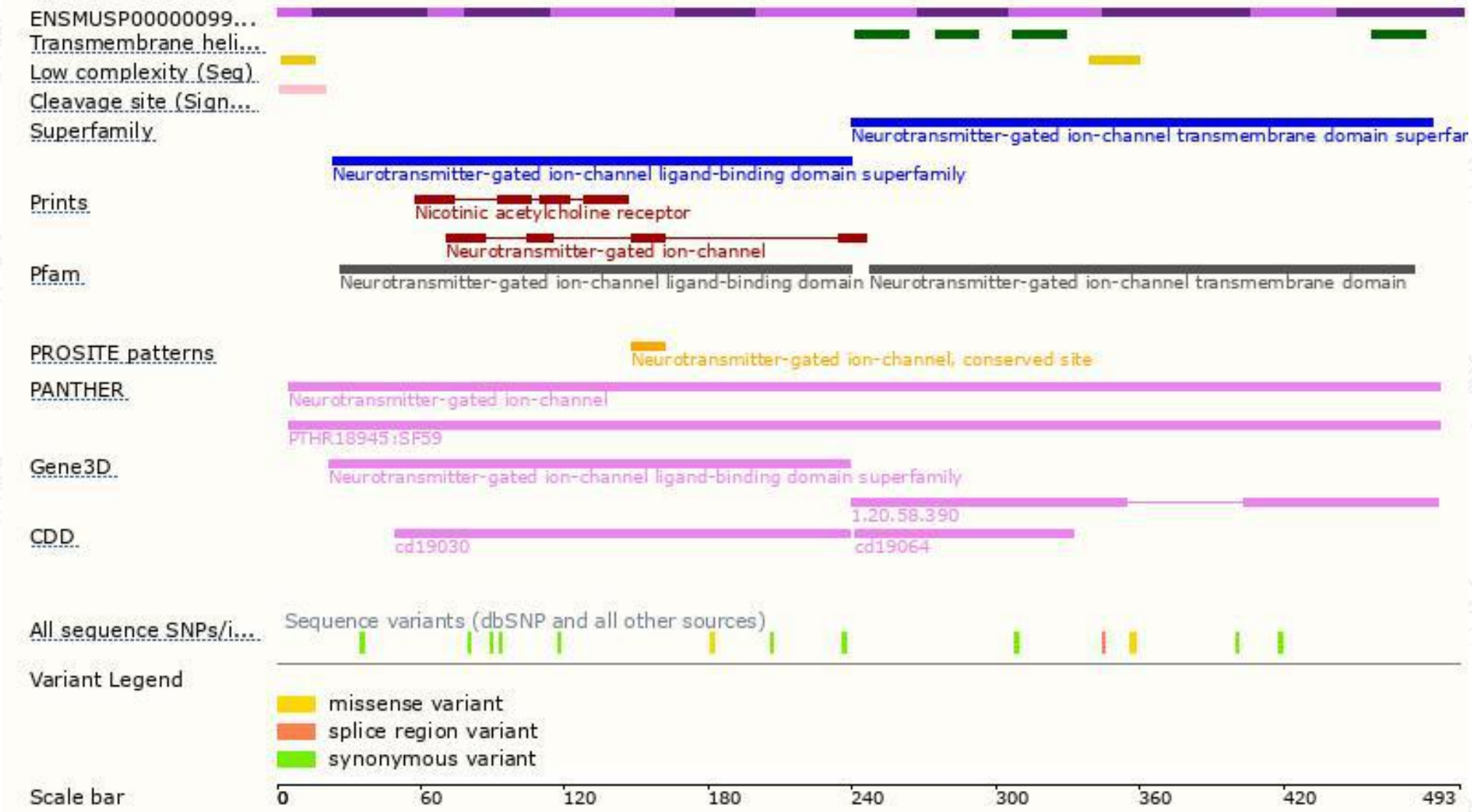
The strategy is based on the design of *Chrne-202* transcript,the transcription is shown below:



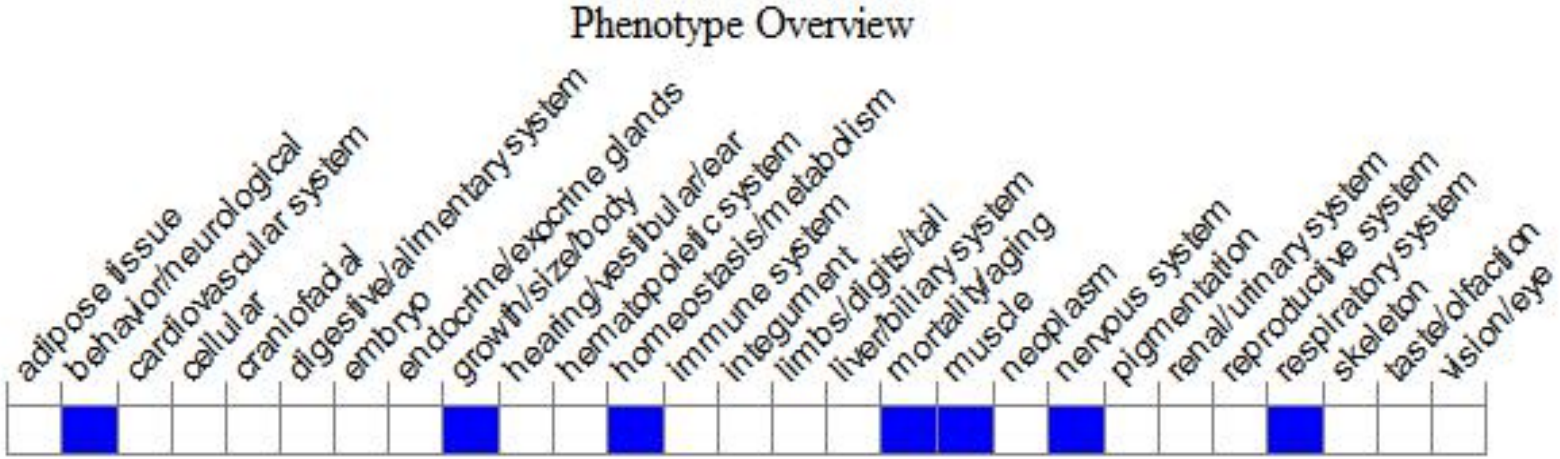
Genomic location distribution



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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