Cnr2 Cas9-KO *Strategy*

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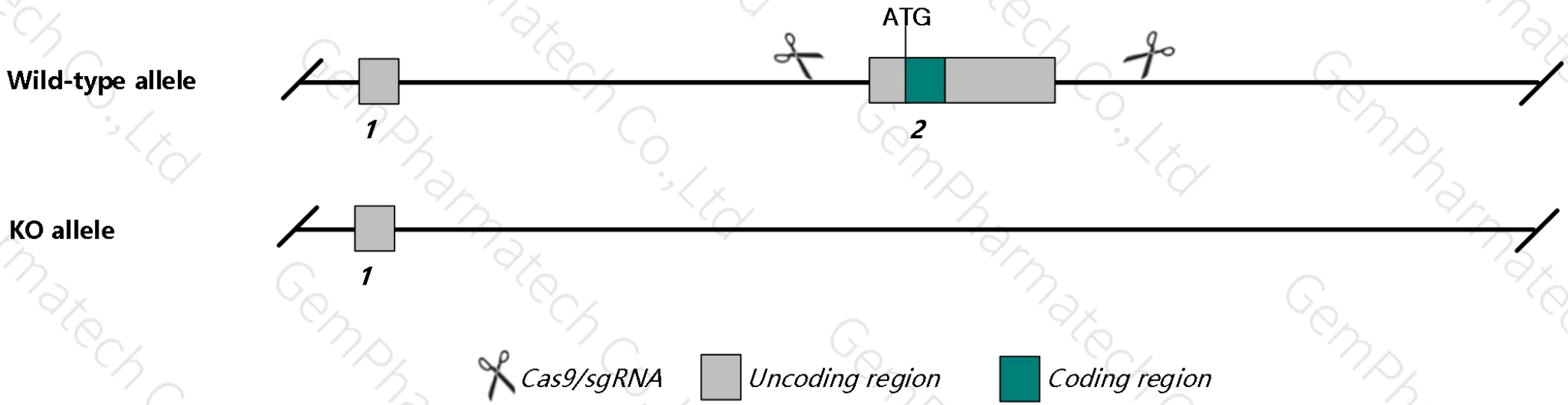
Project Overview

Project Name	***Cnr2***
Project type	**Cas9-KO**
Strain background	**C57BL/6J**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Cnr2* gene. The schematic diagram is as follows:



Technical routes

- The *Cnr2* gene has 2 transcripts.According to the structure of *Cnr2* gene, exon2 of *Cnr2*-201 (ENSMUST00000068830.3) transcript is recommended as the knockout region.The region contains all 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 *Cnr2* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA were microinjected into the fertilized eggs of C57BL/6J 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/6J mice.

Notice

- According to the existing MGI data，Macrophages from homozygous mutant animals are resistant to the inhibitory effects of delta9-Tetrahydrocannabinol. Alopecia is seen in some but not all homozygotes.
- The *Cnr2* gene is 527bp away from *Fuca1* gene. Knockout the region may affect the function of *Fuca1* gene.
- The *Cnr2* gene is located on the Chr4. 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)

集萃药康 GemPharmatech

Cnr2 cannabinoid receptor 2 (macrophage) [*Mus musculus* (house mouse)]

Gene ID: 12802, updated on 21-Oct-2019

Summary

Official Symbol	Cnr2 provided by MGI
Official Full Name	cannabinoid receptor 2 (macrophage) provided by MGI
Primary source	MGI:MGI:104650
See related	Ensembl:ENSMUSG00000062585
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	CB2; CB-2; CB2-R
Expression	Biased expression in spleen adult (RPKM 19.7), mammary gland adult (RPKM 6.0) and 6 other tissues See more
Orthologs	human all

Transcript information （Ensembl）

The gene has 2 transcripts, and all transcripts are shown below:

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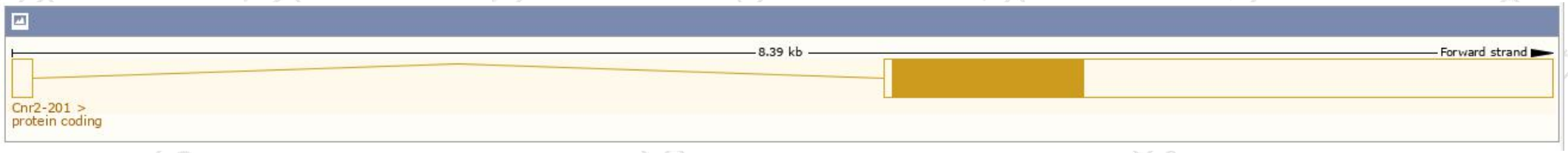
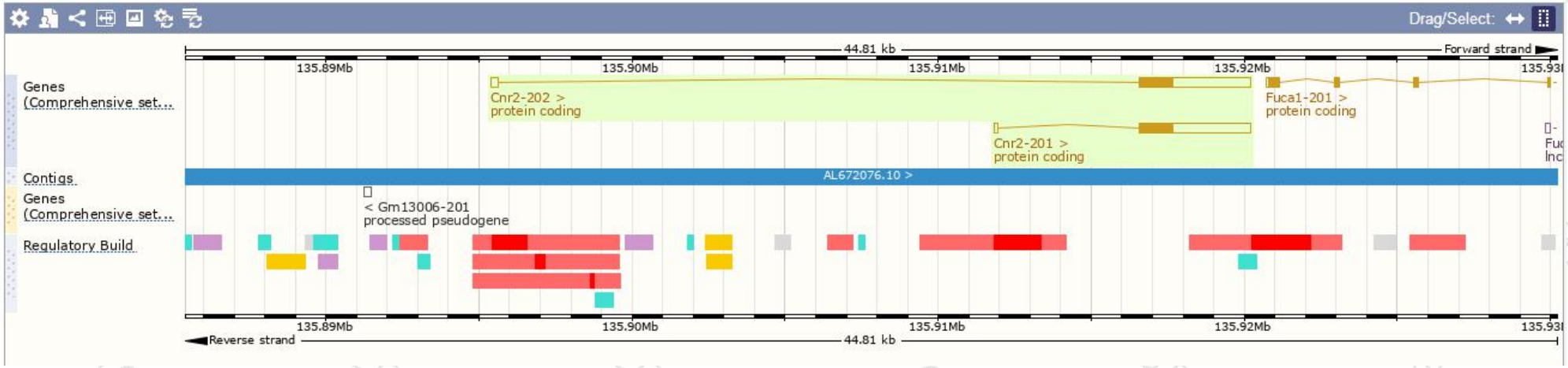
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cnr2-202	ENSMUST00000097843.8	3867	347aa	Protein coding	CCDS18793	P47936	TSL:1 GENCODE basic APPRIS P1
Cnr2-201	ENSMUST00000068830.3	3756	347aa	Protein coding	CCDS18793	P47936	TSL:1 GENCODE basic APPRIS P1

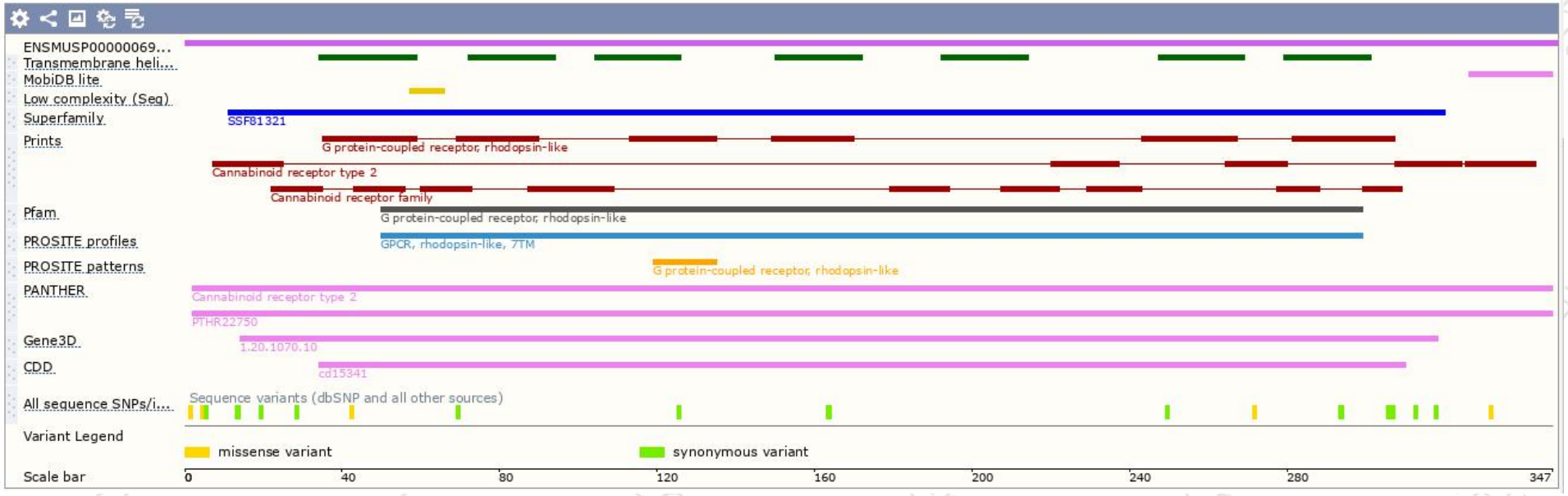
The strategy is based on the design of *Cnr2*-201 transcript,The transcription is shown below



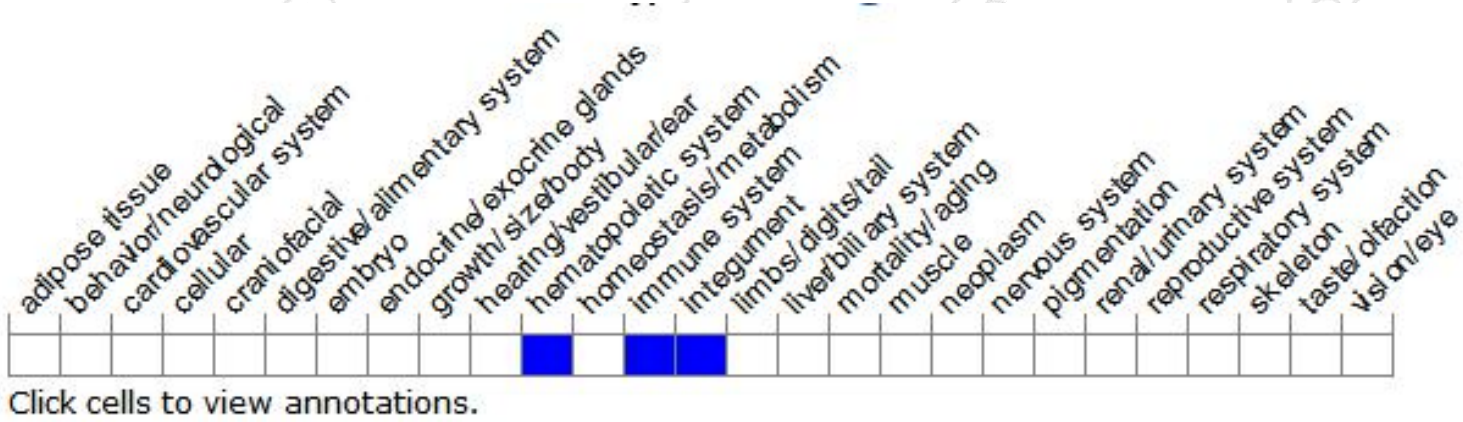
Genomic location distribution



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,Mutations in this locus affect cell-cycle regulation and apoptos is. Null homozygotes show high, early-onset tumor incidence; some have persistent hyaloid vasculature and cataracts. Truncated or temperature-sensitive alleles cause early aging phenotypes.

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

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