Rtn4r Cas9-CKO Strategy

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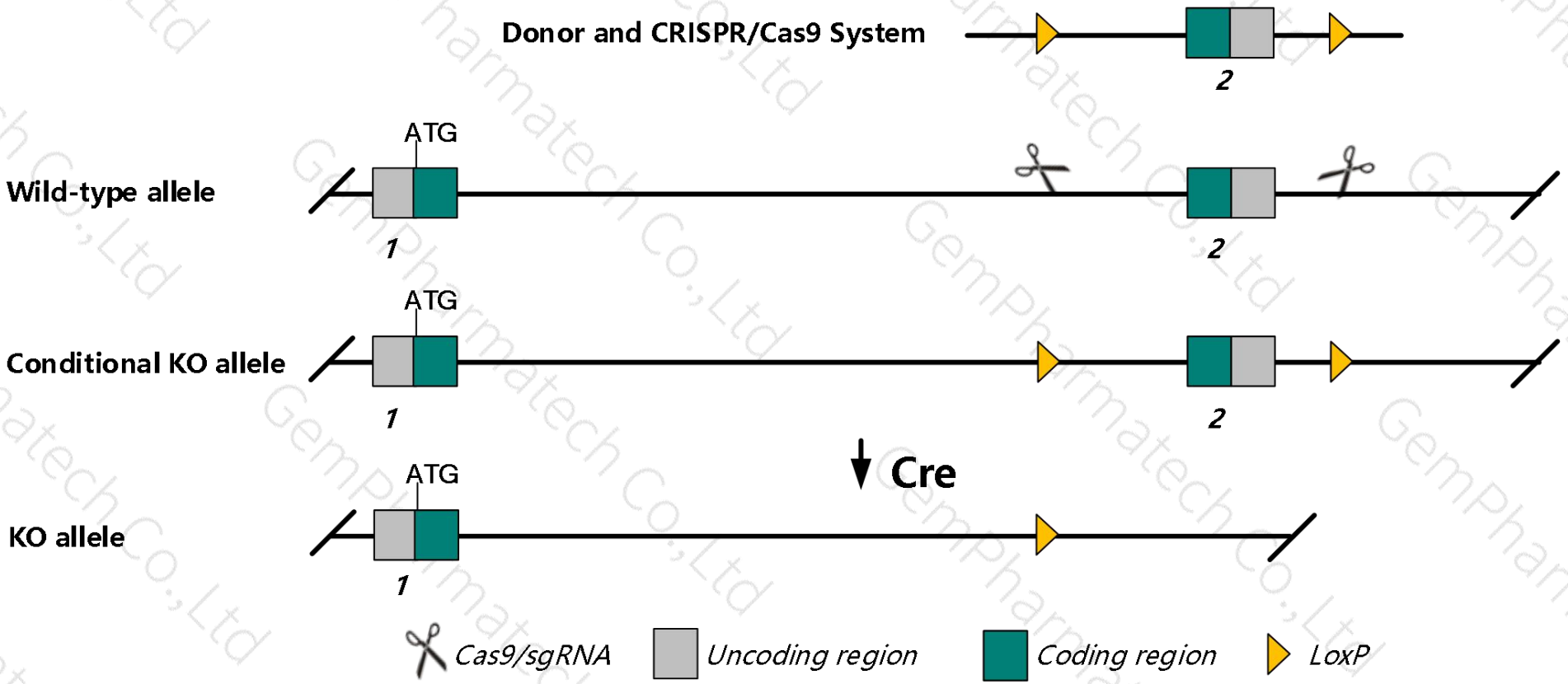
Project Overview

Project Name	***Rtn4r***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Rtn4r* gene. The schematic diagram is as follows:



Technical routes

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- The *Rtn4r* gene has 1 transcript. According to the structure of *Rtn4r* gene, exon2 of *Rtn4r-201* (ENSMUST00000059589.5) transcript is recommended as the knockout region.The region contains most of coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Rtn4r* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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.

Notice

➢ According to the existing MGI data, Homozygous null mice display decreased exploration in new environment, impaired coordination, and improved recovery and rubrospinal axon regeneration following spinal cord injury.

➢ The *Rtn4r* gene is located on the Chr16. 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）

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Rtn4r reticulon 4 receptor [*Mus musculus* (house mouse)]

Gene ID: 65079, updated on 5-Nov-2019

Summary

Official Symbol	Rtn4r provided by MGI
Official Full Name	reticulon 4 receptor provided by MGI
Primary source	MGI:MGI:2136886
See related	Ensembl:ENSMUSG00000043811
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	NgR; NgR1; NOGOR
Expression	Broad expression in cortex adult (RPKM 55.4), frontal lobe adult (RPKM 33.2) and 15 other tissues See more
Orthologs	human all

Genomic context

Location: 16; 16 A3

See Rtn4r in Genome Data Viewer

Exon count: 2

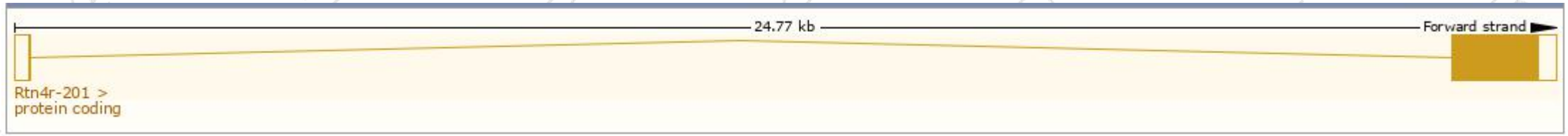
Annotation release	Status	Assembly	Chr	Location
108	current	GRCm38.p6 (GCF_000001635.26)	16	NC_000082.6 (18127642..18152408)
Build 37.2	previous assembly	MGSCv37 (GCF_000001635.18)	16	NC_000082.5 (18127799..18152501)

Transcript information（Ensembl）

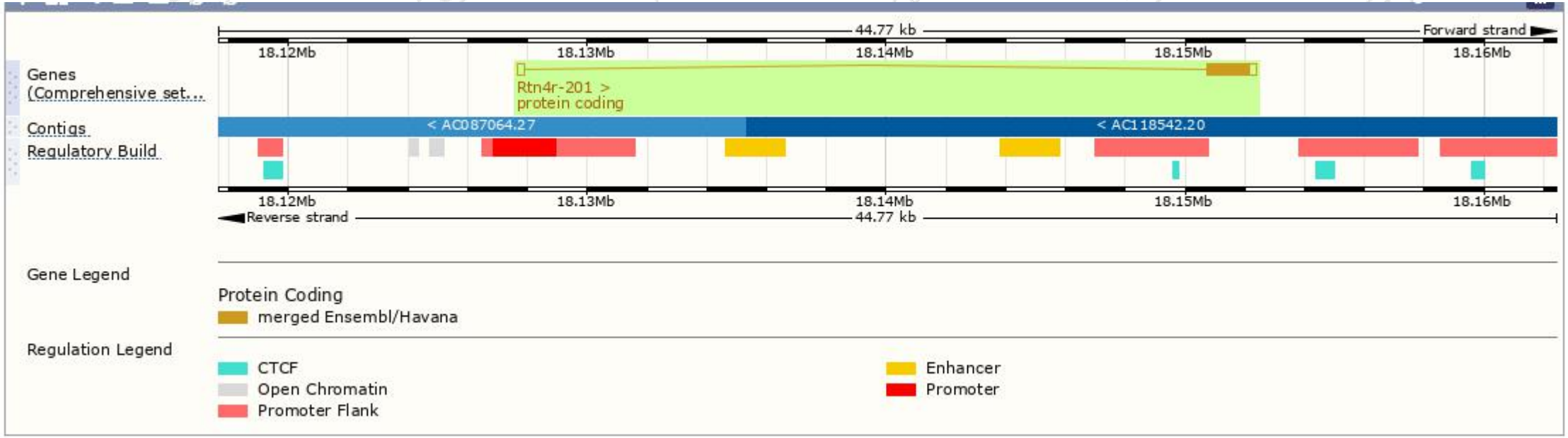
The gene has 1 transcript,and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Rtn4r-201	ENSMUST00000059589.5	1938	473aa	Protein coding	CCDS37279	Q99PI8	TSL:1 GENCODE basic APPRIS P1

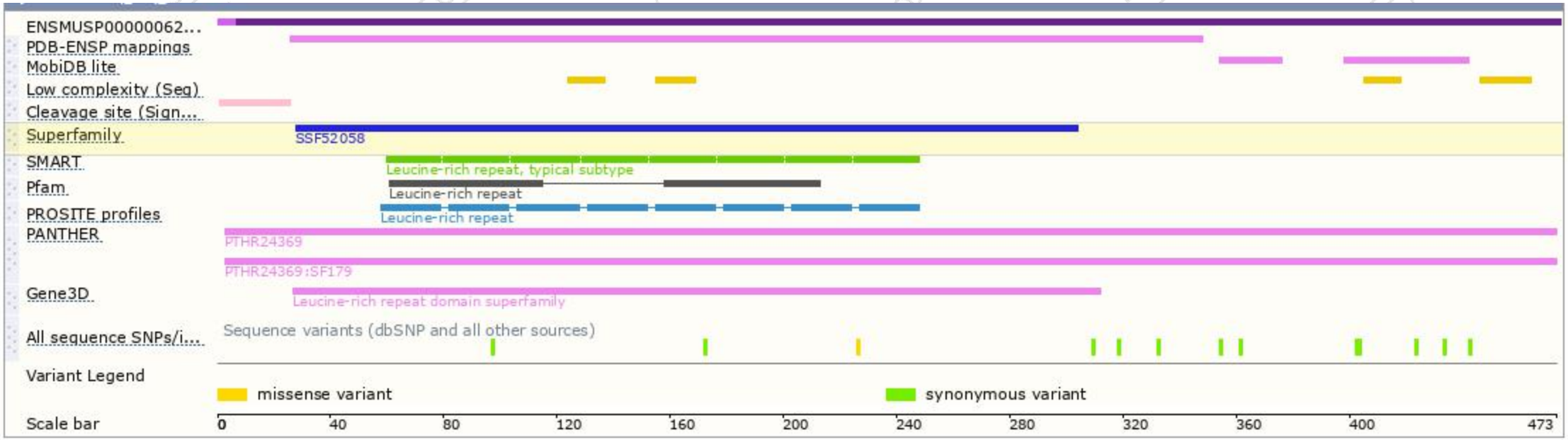
The strategy is based on the design of *Rtn4r-201* transcript,The transcription is shown below



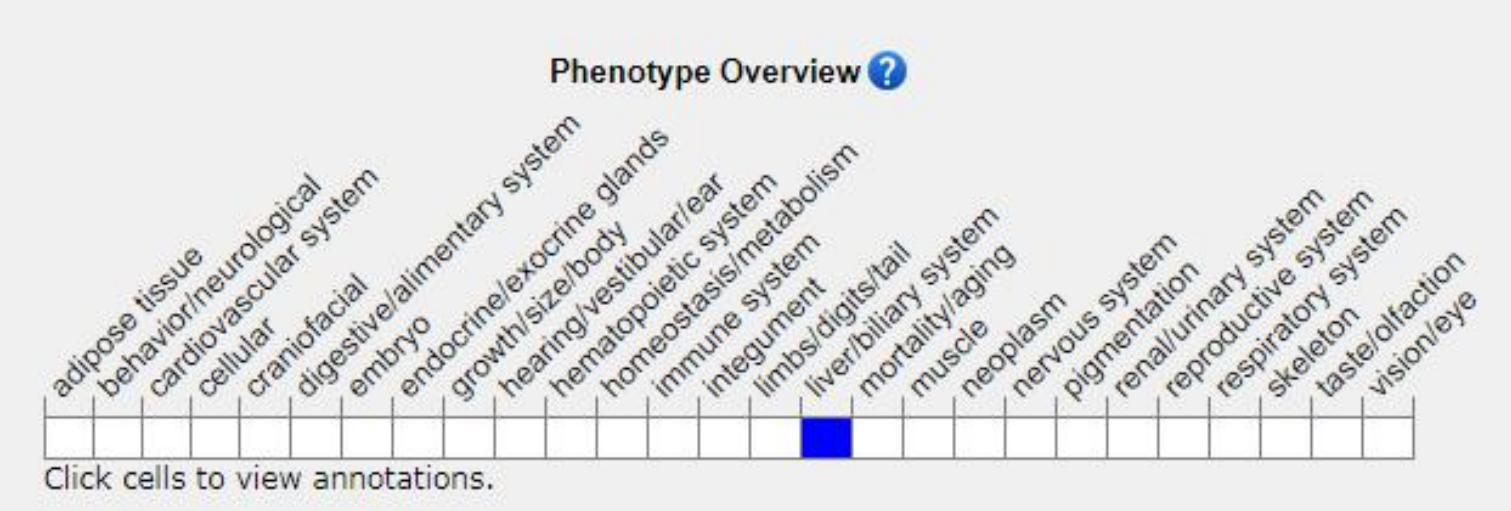
Genomic location distribution



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, Homozygous null mice display decreased exploration in new environment, impaired coordination, and improved recovery and rubrospinal axon regeneration following spinal cord injury.

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

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