

# Nprl2 Cas9-CKO Strategy

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# **Project Overview**

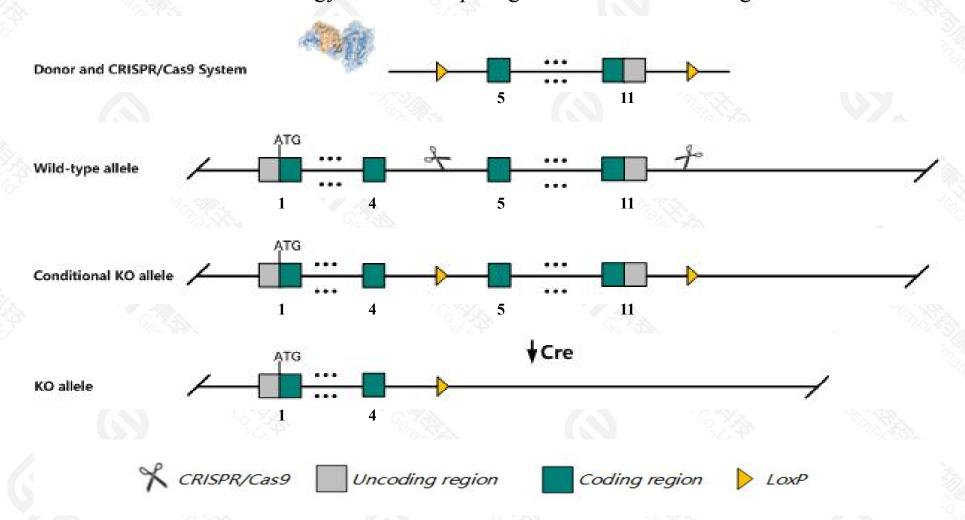


Project Name	Nprl2
Project type	Cas9-CKO
Strain background	C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Nprl2* gene. The schematic diagram is as follows:



### **Technical routes**



- ➤ The *Nprl2* gene has 5 transcripts. According to the structure of *Nprl2* gene, exon5-exon11 of *Nprl2*201(ENSMUST00000010201.9) transcript is recommended as the knockout region. The region contains 695bp coding sequence.

  Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Nprl2* 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.

### **Notice**



- > According to the existing MGI data,mice homozygous for a knock-out allele exhibit reduced embryo size, microphthalmia, occaional anophthalmia, pale liver, reduced fetal liver hematopoiesis, impaired erythropoiesis and reduced methionine synthesis.
- The flox region is about 1.6kb away from the 5th end of the *Cyb561d2,Zmynd10* gene separately, which may affect the regulation of their gene.
- ➤ The Intron4 is only 535bp,loxp insertion may affect mRNA splicing.
- > The *Nprl2* gene is located on the Chr9. 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)



#### Nprl2 NPR2 like, GATOR1 complex subunit [Mus musculus (house mouse)]

Gene ID: 56032, updated on 17-Feb-2021

#### Summary

☆ ?

Official Symbol Nprl2 provided by MGI

Official Full Name NPR2 like, GATOR1 complex subunit provided by MGI

Primary source MGI:MGI:1914482

See related Ensembl: ENSMUSG00000010057

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 2810446G01Rik, G21, N, NPR2L, Tusc, Tusc4

Expression Ubiquitous expression in CNS E18 (RPKM 22.0), whole brain E14.5 (RPKM 21.6) and 28 other tissuesSee more

Orthologs <u>human all</u>

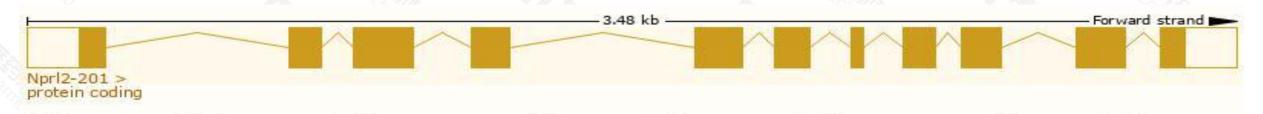
# Transcript information (Ensembl)



The gene has 5 transcripts, all transcripts are shown below:

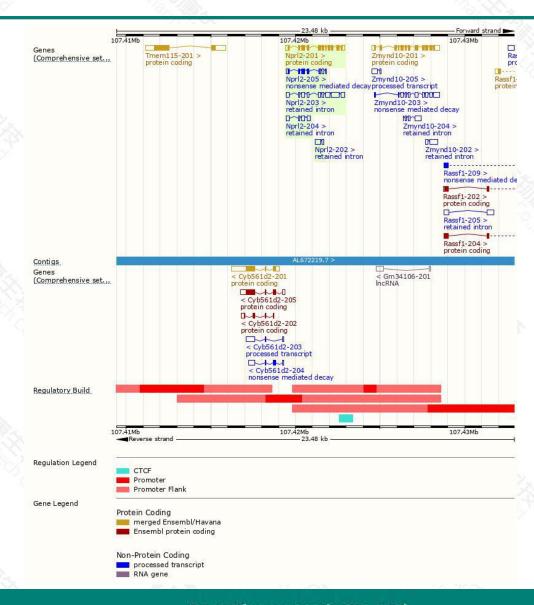
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nprl2-201	ENSMUST00000010201.9	1441	380aa	Protein coding	CCDS23492		TSL:1 , GENCODE basic , APPRIS P1
Nprl2-205	ENSMUST00000195370.6	829	<u>163aa</u>	Nonsense mediated decay	3-		TSL:3,
Nprl2-203	ENSMUST00000193628.6	1736	No protein	Retained intron	12		TSL:2,
Nprl2-204	ENSMUST00000194848.2	637	No protein	Retained intron	-		TSL:2,
Nprl2-202	ENSMUST00000192951.2	391	No protein	Retained intron	32		TSL:3,

The strategy is based on the design of *Nprl2-201* transcript, the transcription is shown below:



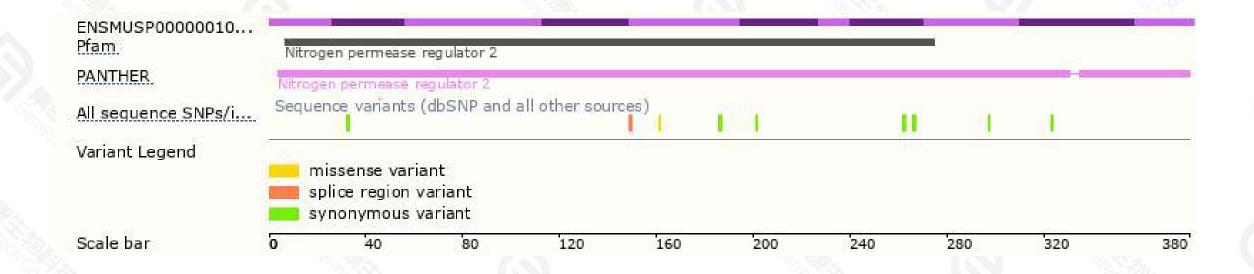
### Genomic location distribution





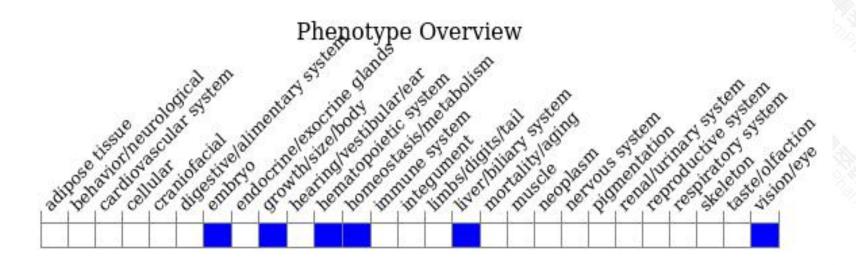
### Protein domain





# 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-out allele exhibit reduced embryo size, microphthalmia, occaional anophthalmia, pale liver, reduced fetal liver hematopoiesis, impaired erythropoiesis and reduced methionine synthesis.



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

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