Nphs1 Cas9-KO Strategy

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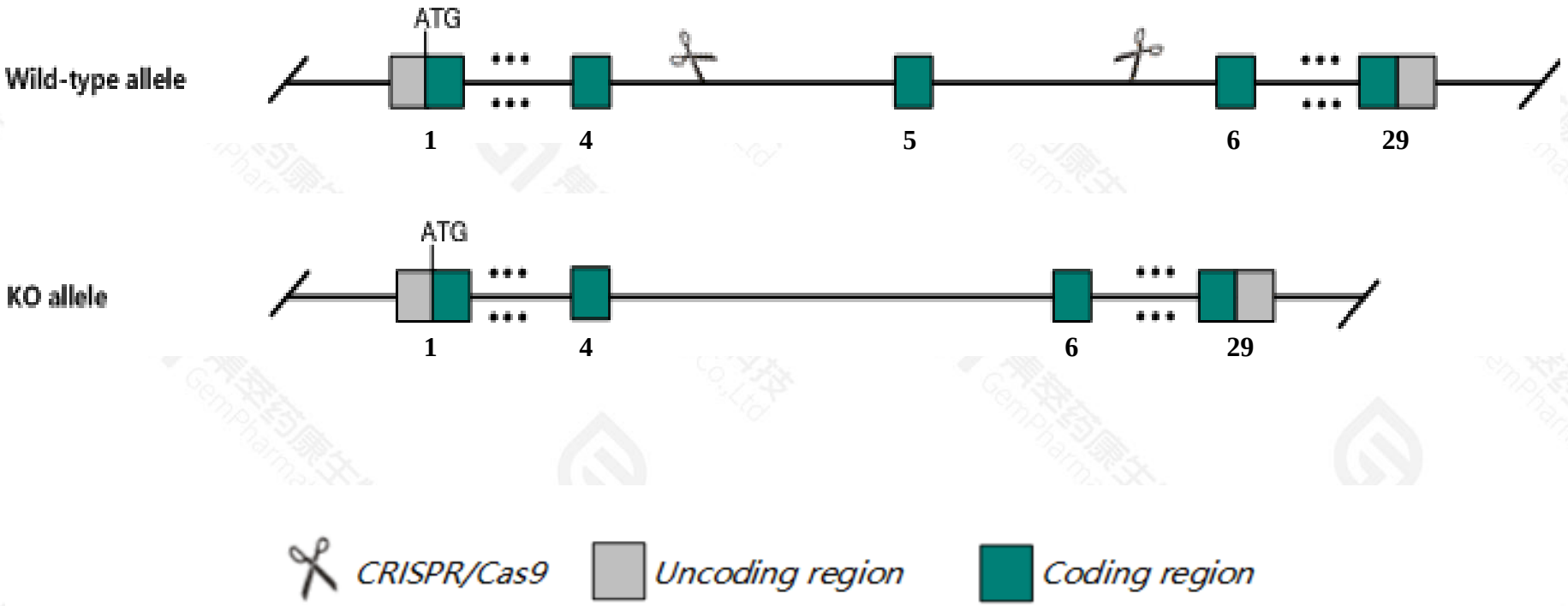
Project Overview

Project Name	***Nphs1***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Nphs1* gene. The schematic diagram is as follows:



Technical routes

➢ The *Nphs1* gene has 5 transcripts. According to the structure of *Nphs1* gene, exon5 of *Nphs1-201*(ENSMUST00000006825.9) transcript is recommended as the knockout region.The region contains 82bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Nphs1* 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 a targeted null mutation exhibit severe proteinuria associated with kidney defects and die soon after birth. Heterozygotes exhibit fusion of one-third of glomerular foot processes.

➢ The Intron4 and Intron5 are only 483bp and 376bp,loxp insertion may affect mRNA splicing.

➢ The *Nphs1* gene is located on the Chr7. 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）

Nphs1 nephrosis 1, nephrin [Mus musculus (house mouse)]

Gene ID: 54631, updated on 29-Dec-2020

Summary

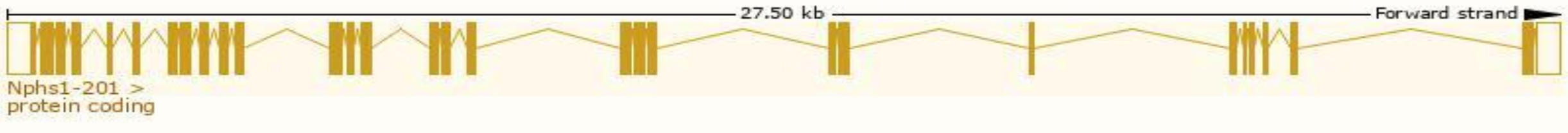
Official Symbol	Nphs1 provided by MGI
Official Full Name	nephrosis 1, nephrin provided byMGI
Primary source	MGI:MGI:1859637
See related	Ensembl:ENSMUSG00000006649
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	NephrinB, ne, nephrin
Expression	Biased expression in kidney adult (RPKM 9.9), small intestine adult (RPKM 0.8) and 2 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

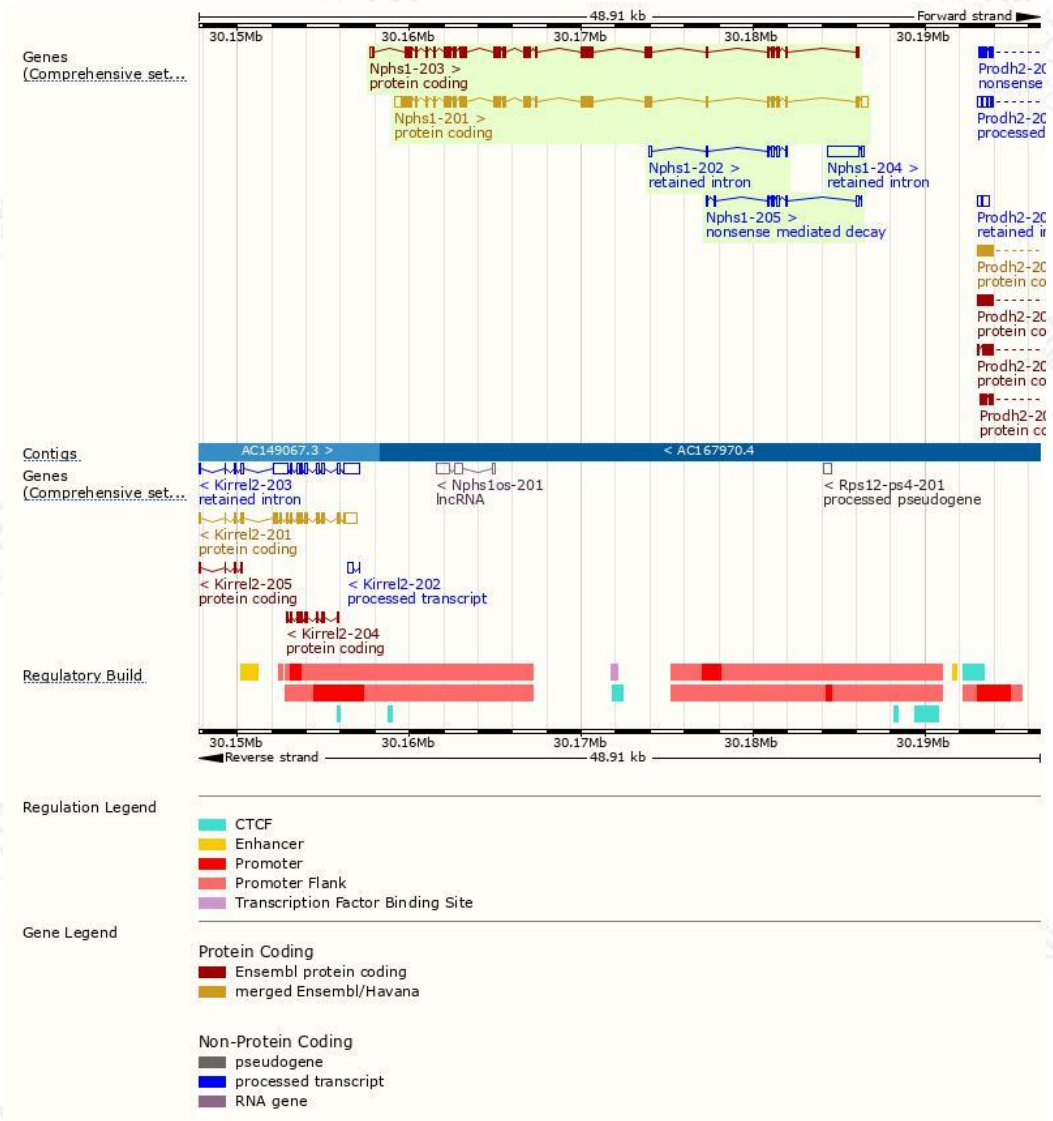
The gene has 5 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nphs1-201	ENSMUST00000006825.9	4608	1256aa	Protein coding	CCDS39884		TSL:1 , GENCODE basic , APPRIS P2 ,
Nphs1-203	ENSMUST00000126297.9	3877	1242aa	Protein coding	-		TSL:1 , GENCODE basic , APPRIS ALT2 ,
Nphs1-205	ENSMUST00000149086.2	752	72aa	Nonsense mediated decay	-		CDS 5' incomplete , TSL:5 ,
Nphs1-204	ENSMUST00000131004.3	1987	No protein	Retained intron	-		TSL:2 ,
Nphs1-202	ENSMUST00000123880.8	664	No protein	Retained intron	-		TSL:5 ,

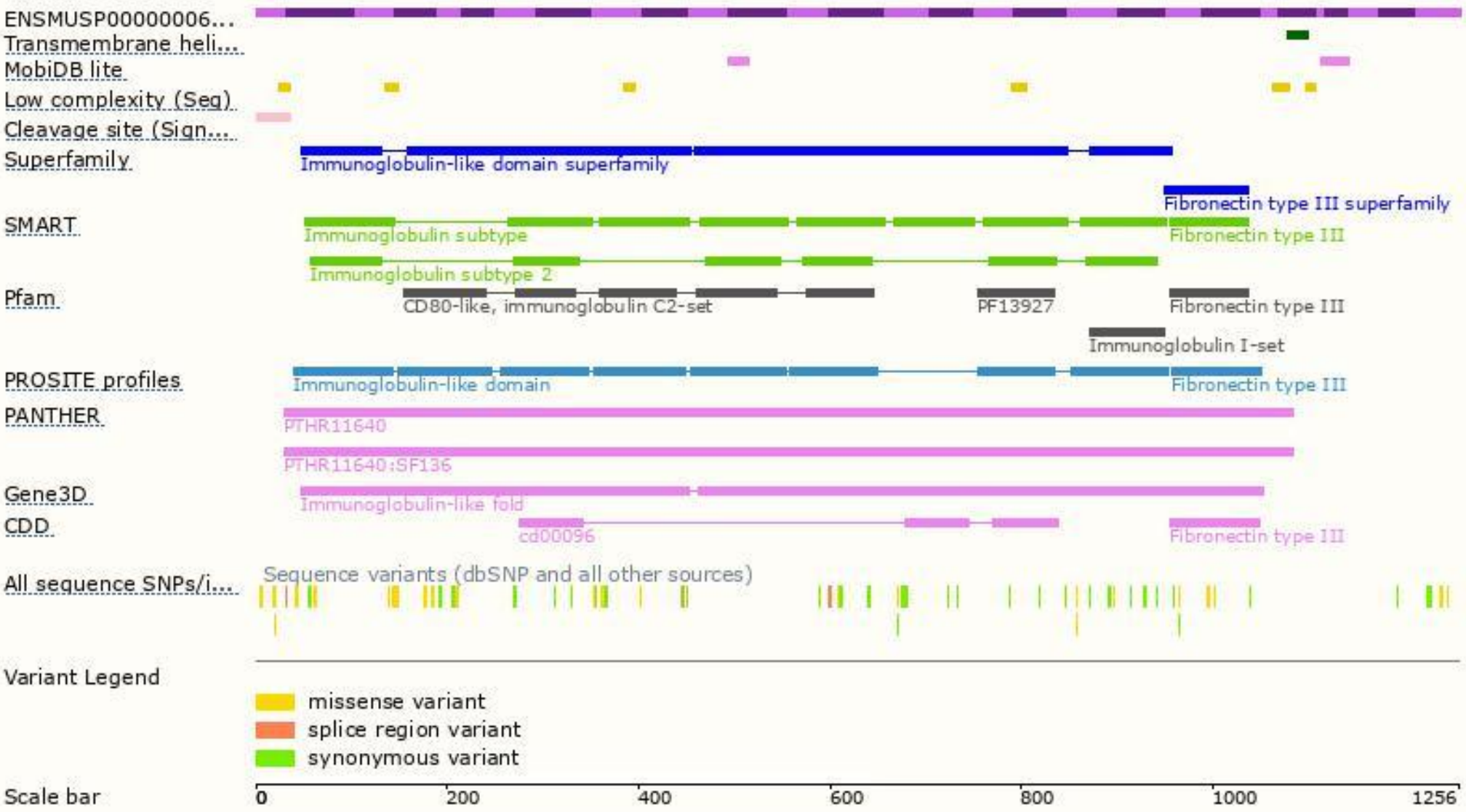
The strategy is based on the design of *Nphs1-201* transcript,the transcription is shown below:



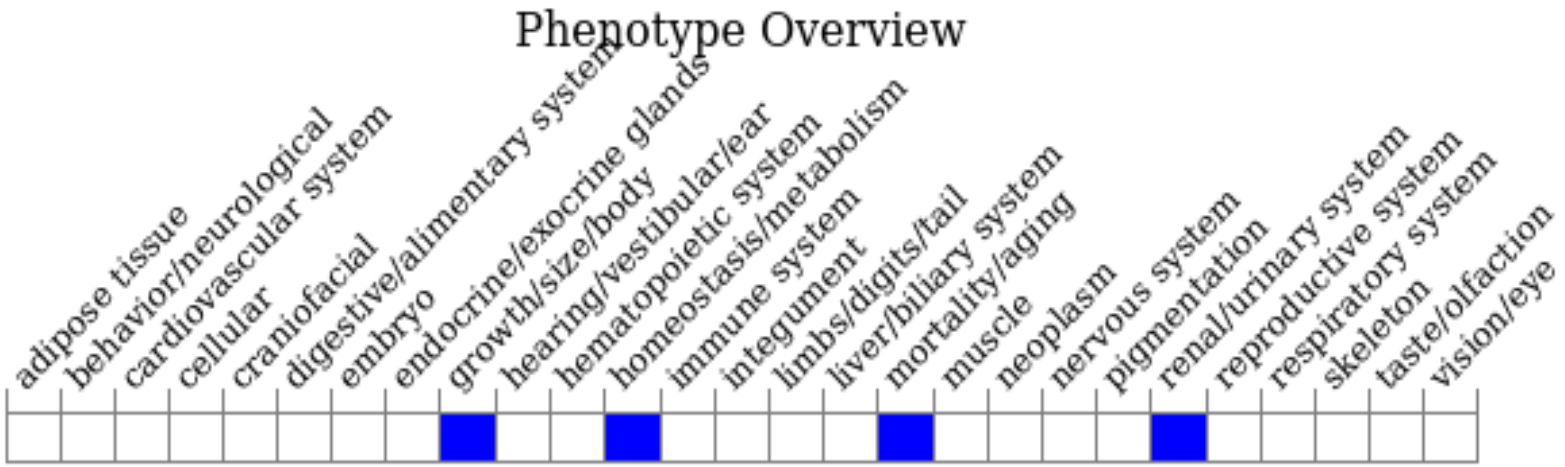
Genomic location distribution



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/).

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

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