

***H11-Nphs1-iCre-ployA* Cas9-KI Strategy**

Designer:

Jia Yu

Design Date:

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Reviewer

Xueting Zhang

Project Overview

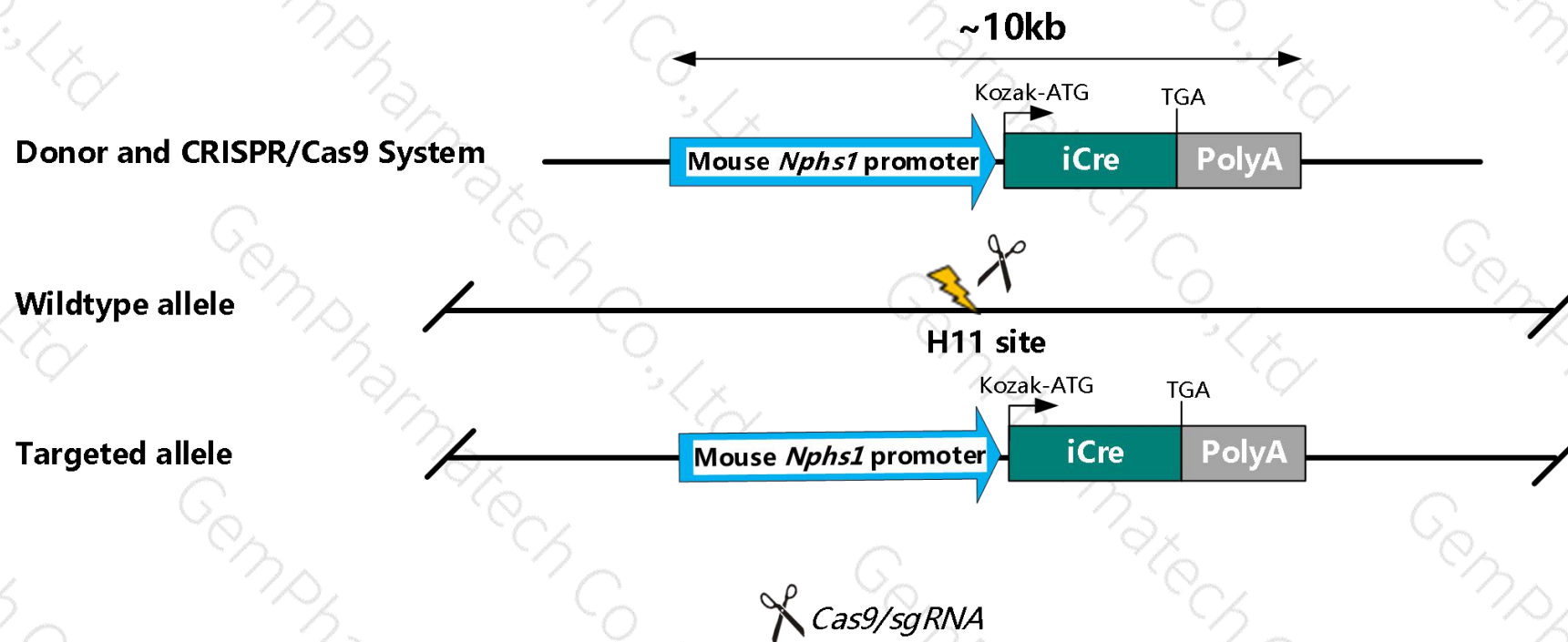
Project Name	<i>H11-Nphs1-iCre-ployA</i>
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Project type	Cas9-KI
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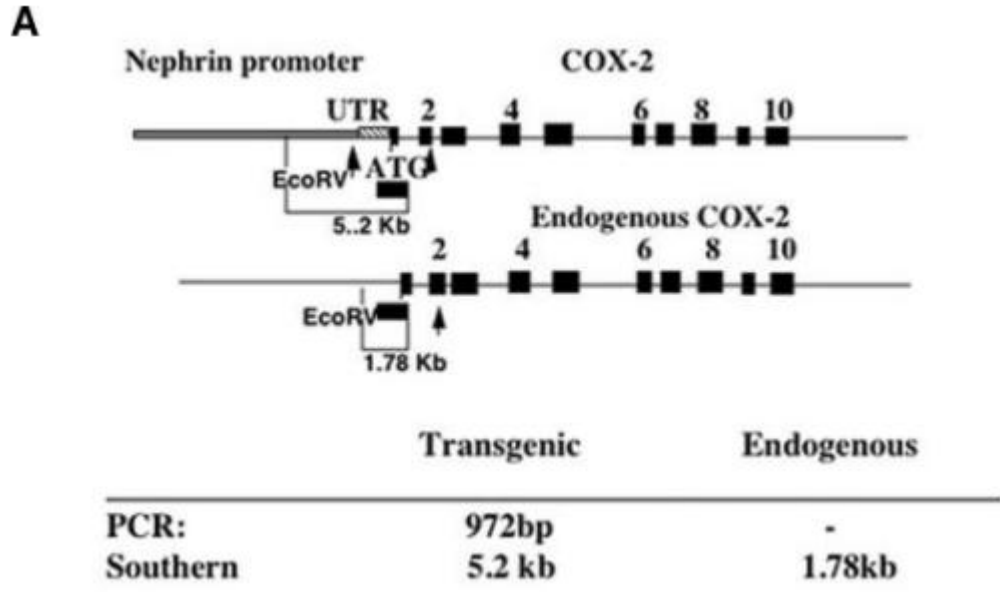
Strain background	C57BL/6J
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Knockin strategy

The *Nphs1-iCre-loyA* fragment was inserted into H11 site of mice and the schematic diagram is as follows:



Summary of mouse *Nphs1* promoter^[1]



Animal Model and Genotype

All animal study protocols were reviewed and approved by the Vanderbilt University Institutional Animal Care and Use Committee, and all experiments were conducted according to National Institutes of Health guidelines. An 8.5-kb fragment of the murine 5' nephrin promoter was ligated to the murine COX-2 gene in pBluescript SK(-),

Figure 1. Genotyping of nephrin promoter-driven cyclooxygenase-2 (COX-2) transgenic mice. (A) Schematic illustration of genotyping strategy: An 8.5-kb fragment of the murine 5' nephrin promoter was ligated to the murine COX-2 gene, beginning at the ATG in exon 1. (B) Southern hybridization: Lanes 1, 2, and 4 from transgenic mice F10, F14, and F16, respectively, which exhibited an expected 5.2-kb band in EcoRV-digested DNA, whereas only a 1.78-kb band from endogenous COX-2 was detected in wild-type mice (lane 2). (C) Southern hybridization: Lanes 1, 3, and 4 are from transgenic mice F16; lanes 2 and 5 are from wild-type mice. Lanes 1 and 4 represented homogeneous transgenic mice, and lane 3 was from a mouse that was heterozygous for the transgene, which was confirmed further by genotyping of their offspring. (C) A single PCR product of 972 bp was detected for the respective transgenic mouse lines (lanes 1, 3, and 4) but not in wild-type (lane 2).

1、Cheng H; Wang S; Jo YI; Hao CM; Zhang M; Fan X; Kennedy C; Breyer MD; Moeckel GW; Harris RC. 2007. Overexpression of cyclooxygenase-2 predisposes to podocyte injury. J Am Soc Nephrol 18(2):551-9.

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- The *Nphs1* gene has 5 transcripts. According to the structure of *Nphs1* gene, *Nphs1-201* (ENSMUST00000006825.8) is selected for presentation of the recommended strategy.
- *Nphs1-201* gene has 30 exons, with the ATG start codon in exon1 and TGA stop codon in exon29.
- The *Nphs1* promoter is from article, the length is about 8.5kb.
- H11, located on mouse chromosome 11, is a safe site for foreign gene insertion. The foreign gene integrated into this site can be expressed stably and efficiently without destroying the function of endogenous gene.
- In this study, the *Nphs1-iCre-plotA* gene fragment was inserted into H11 site of mice by CRISPR/Cas9 technology. The brief process is as follows: the donor vector and sgRNA were constructed in vitro, Cas9, donor and sgRNA were microinjected into the fertilized eggs of C57BL/6J mice, and F0 generation mice were obtained. The F0 positive mice were mated with C57BL/6J mice by PCR, sequencing, and southern blot, then the stable inheritance of F1 positive mice model was obtained.

- H11 is located on Chr11. Please take the loci in consideration when breeding the Knock-in mice with other gene modified (e.g., iCre) strains, if the other gene is also on Chr11, it may be extremely hard to get double gene positive homozygotes.
- The scheme is designed according to the genetic information in the existing database. Due to the complex process of gene transcription and translation, it cannot be predicted completely at the present technology level.

Gene information (NCBI)

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

Gene ID: 54631, updated on 12-Aug-2019

 Summary  

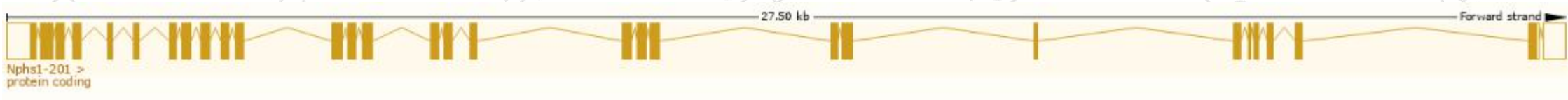
Official Symbol	Nphs1 provided by MGI
Official Full Name	nephrosis 1, nephrin provided by MGI
Primary source	MGI:MGI:1859637
See related	Ensembl:ENSMUSG00000006649
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;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

Transcript information (Ensembl)

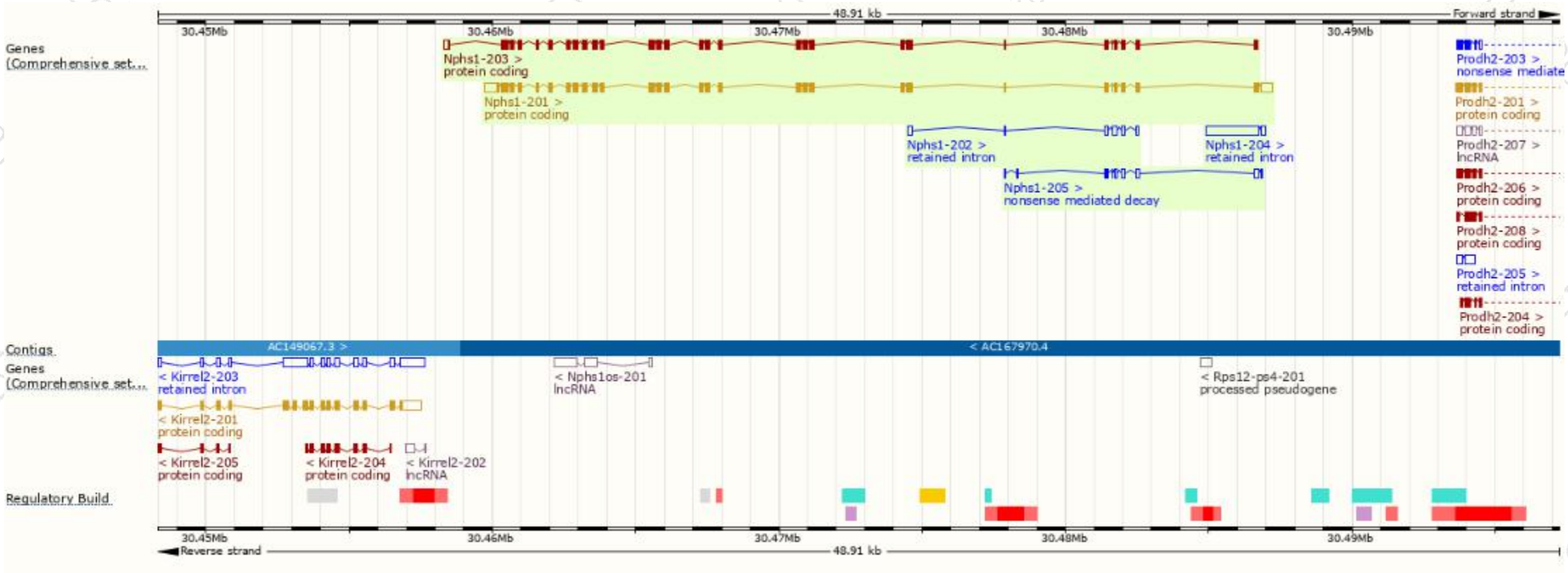
The gene has 5 transcript, and the transcript is shown below :

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nphs1-201	ENSMUST00000006825.8	4608	1256aa	Protein coding	CCDS39884	Q9QZS7	TSL:1 GENCODE basic APPRIS P2
Nphs1-203	ENSMUST000000126297.8	3877	1242aa	Protein coding	-	Q9QZS7	TSL:1 GENCODE basic APPRIS ALT2
Nphs1-205	ENSMUST000000149086.1	752	72aa	Nonsense mediated decay	-	F6RCI4	CDS 5' incomplete TSL:5
Nphs1-204	ENSMUST000000131004.2	1987	No protein	Retained intron	-	-	TSL:2
Nphs1-202	ENSMUST000000123880.7	664	No protein	Retained intron	-	-	TSL:5

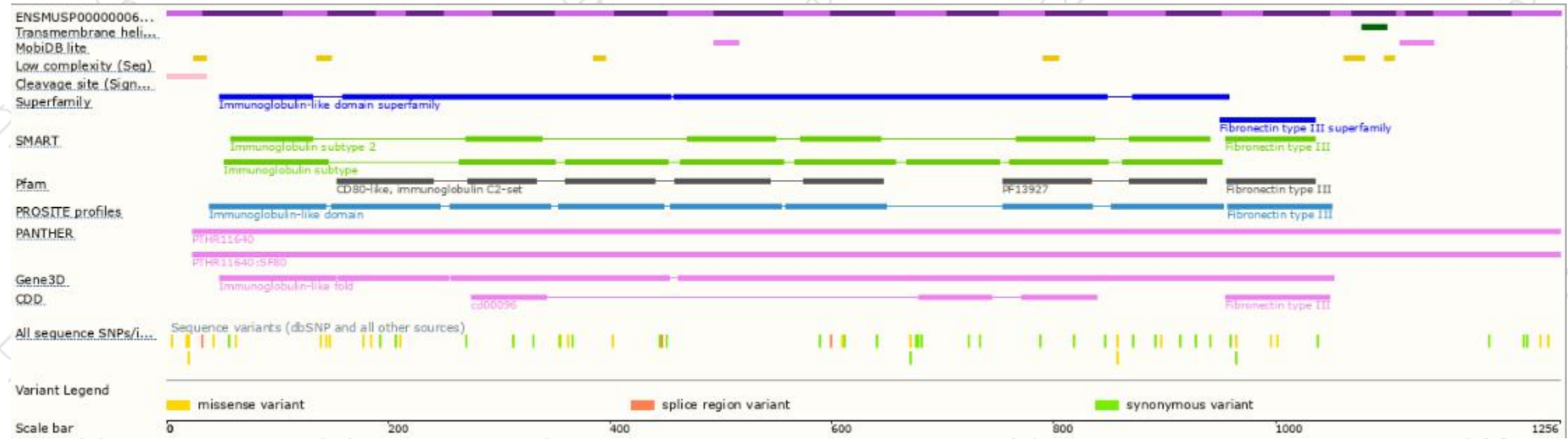
The strategy is based on the design of *Nphs1-201* transcript, The transcription is shown below



Genomic location distribution



Protein domain



If you have any questions, you are welcome to inquire.
Tel: 025-5864 1534



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