Prph Cas9-CKO Strategy

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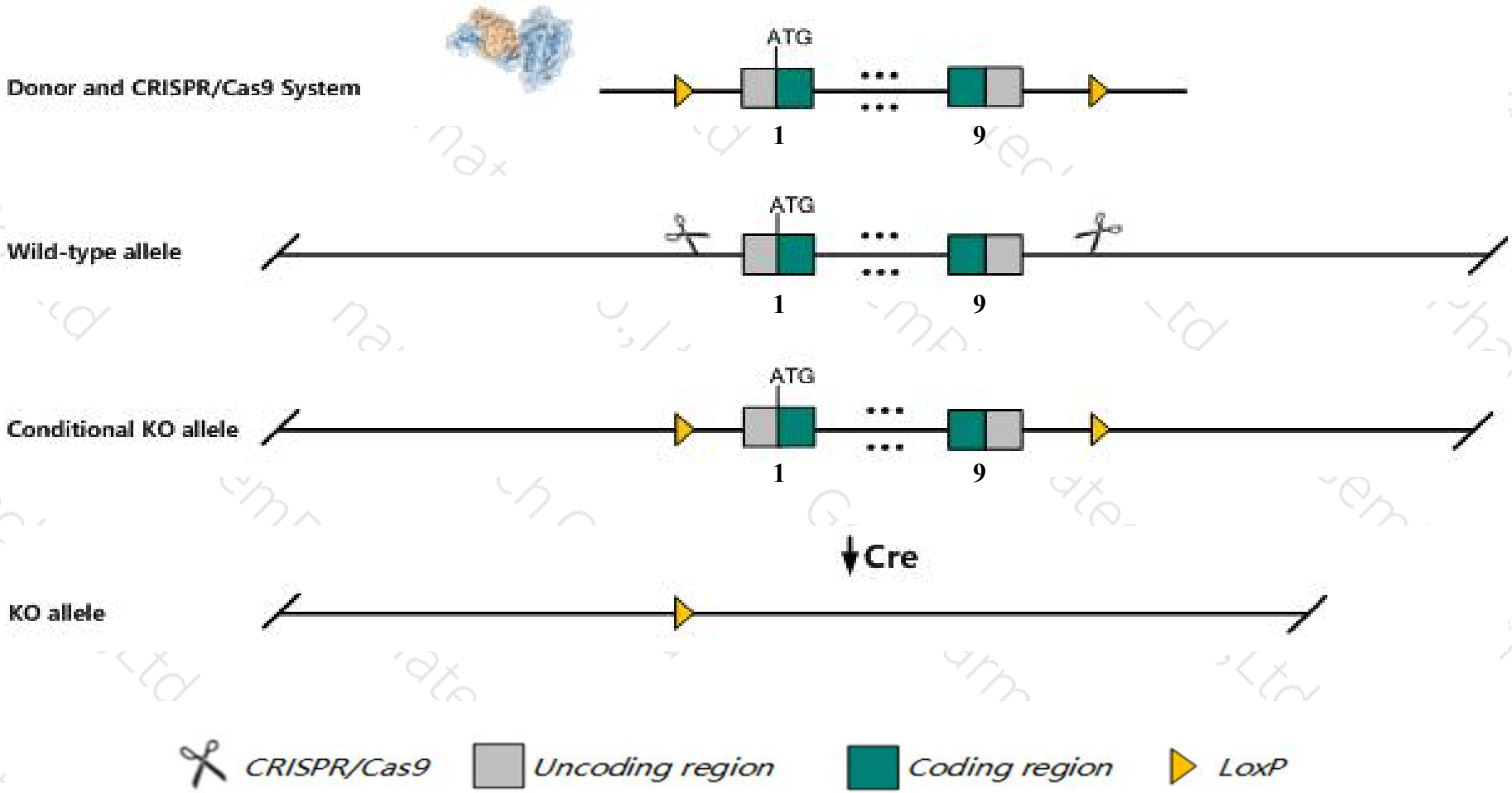
Design Date:2019/3/7

Project Overview

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

Conditional Knockout strategy

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



Technical routes

➢ The *Prph* gene has 4 transcripts. According to the structure of *Prph* gene, exon1-exon9 of *Prph-201*(ENSMUST00000024249.4) 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 *Prph* 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,homozygous null mice showed no overt phenotype up to 14 months of age. While overall structure, number, and caliber of large myelinated axons was normal, mice had reduced numbers of a small subset of unmelinated sensory axons.

➢ The *Prph* gene is located on the Chr15. 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）

集萃药康 GemPharmatech

Prph peripherin [Mus musculus (house mouse)]

Gene ID: 19132, updated on 13-Mar-2020

Summary

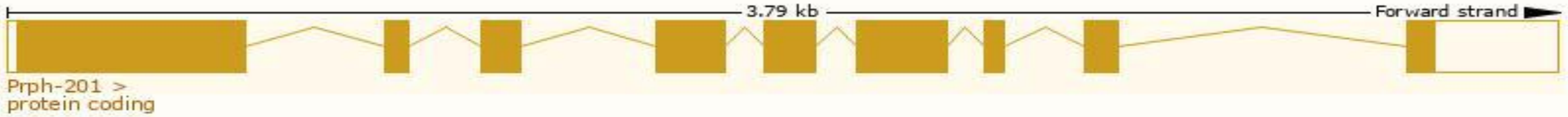
Official Symbol	Prph provided by MGI
Official Full Name	peripherin provided byMGI
Primary source	MGI:MGI:97774
See related	Ensembl:ENSMUSG00000023484
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Prph1
Expression	Biased expression in CNS E14 (RPKM 15.6), CNS E18 (RPKM 8.0) and 13 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

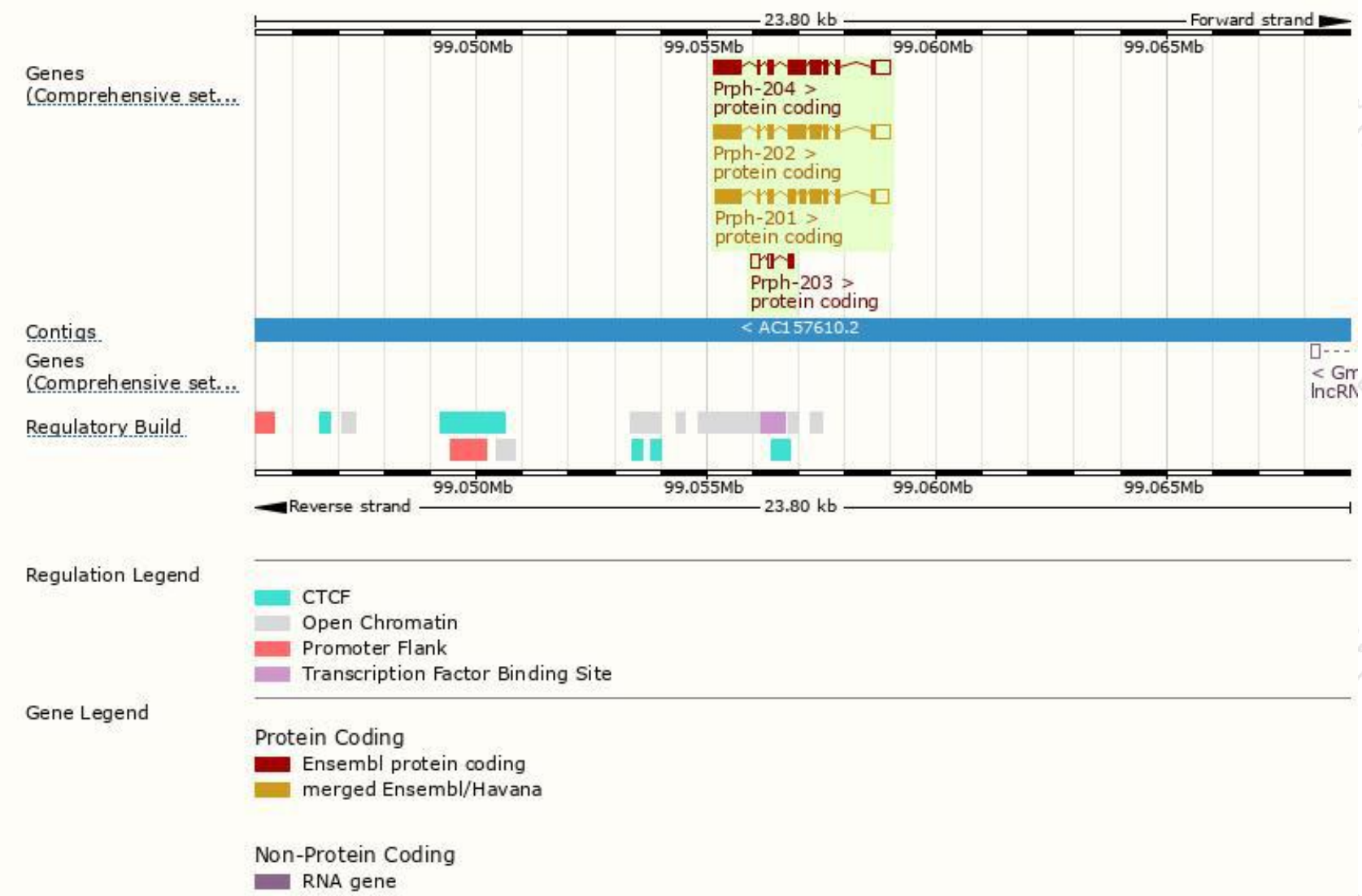
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Prph-202	ENSMUST00000047104.14	1863	507aa	Protein coding	CCDS27813	G3X981	TSL:1 GENCODE basic APPRIS P3
Prph-201	ENSMUST00000024249.4	1757	475aa	Protein coding	CCDS49727	G5E846	TSL:1 GENCODE basic APPRIS ALT2
Prph-204	ENSMUST00000230021.1	1862	506aa	Protein coding	-	A0A2R8W6R6	GENCODE basic APPRIS ALT2
Prph-203	ENSMUST00000229268.1	397	48aa	Protein coding	-	A0A2R8VKR9	CDS 3' incomplete

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

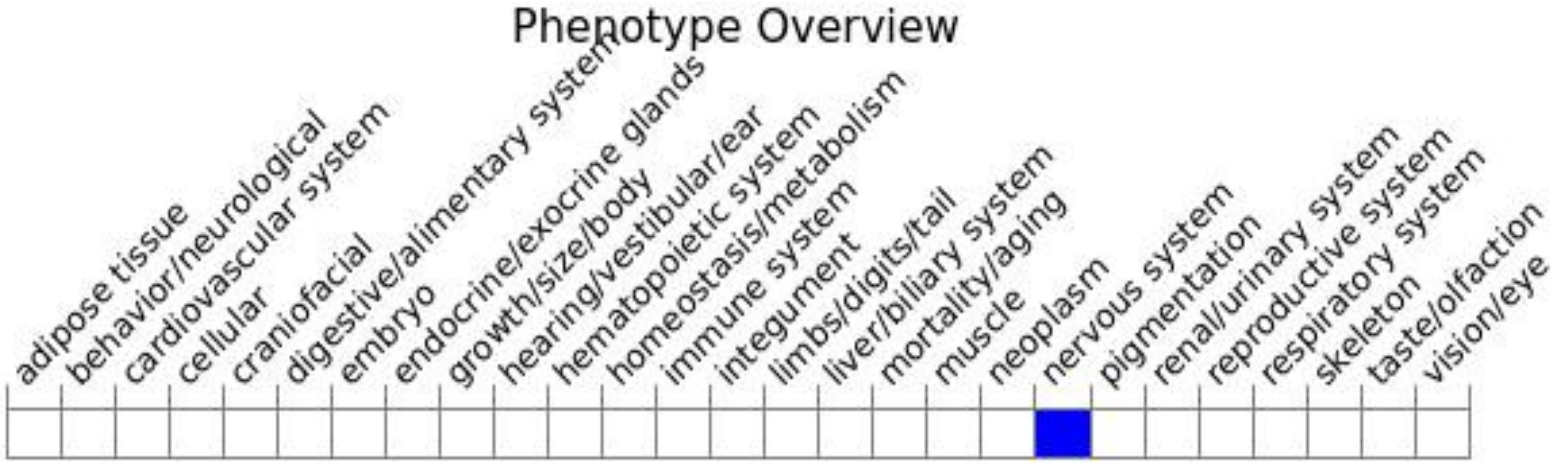


Genomic location distribution



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 showed no overt phenotype up to 14 months of age. While overall structure, number, and caliber of large myelinated axons was normal, mice had reduced numbers of a small subset of unmelinated sensory axons.

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

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