

Col4a4 Cas9-CKO Strategy

Designer:

Huimin Su

Reviewer:

Ruirui Zhang

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Project Overview

Project Name

Col4a4

Project type

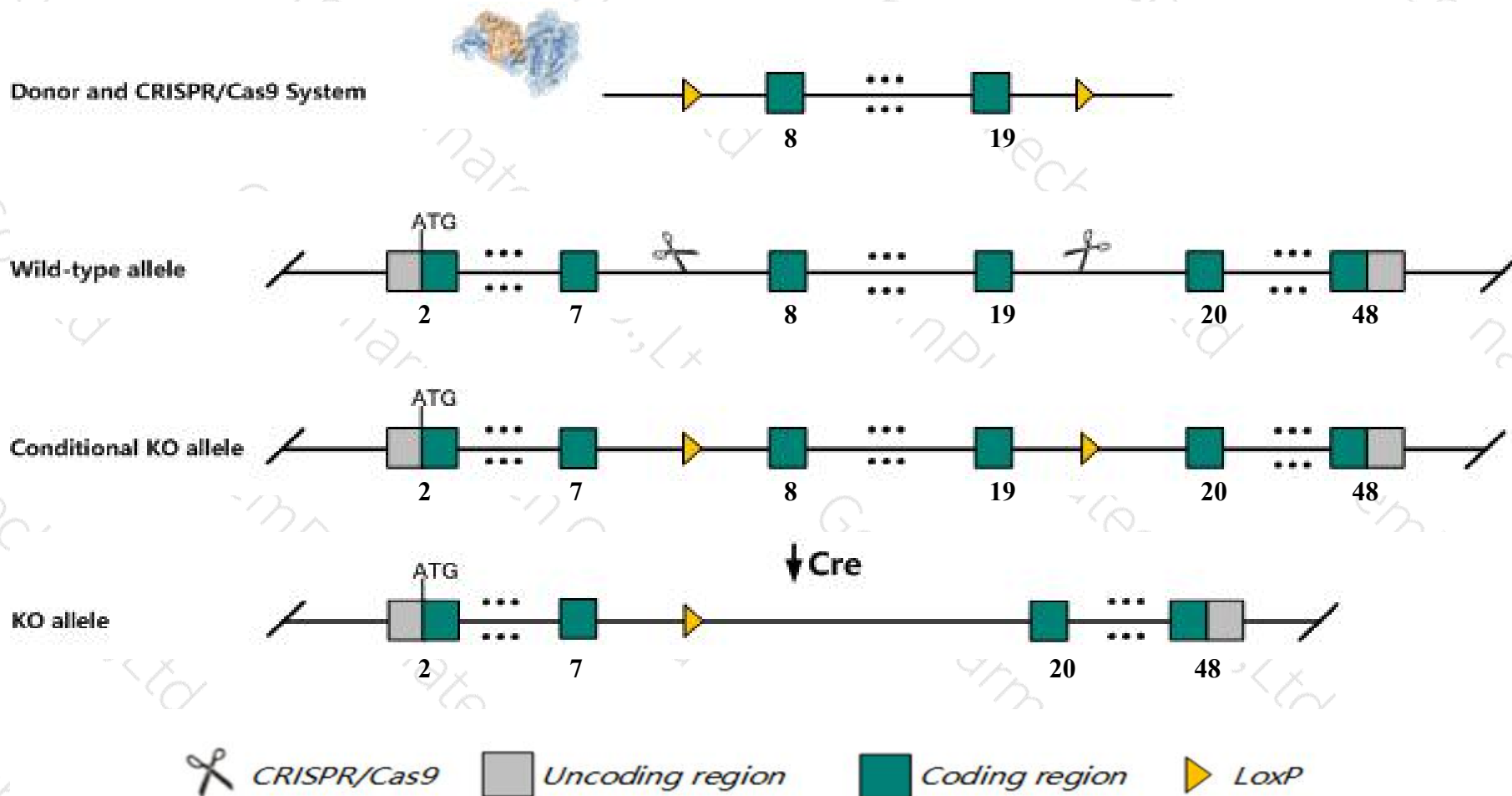
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Col4a4* gene. The schematic diagram is as follows:



Technical routes

- The *Col4a4* gene has 2 transcripts. According to the structure of *Col4a4* gene, exon8-exon19 of *Col4a4-201* (ENSMUST00000087050.6) transcript is recommended as the knockout region. The region contains 715bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Col4a4* 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.

- According to the existing MGI data, Mice homozygous for an ENU-induced mutation develop an early nephritic syndrome associated with uremia, proteinuria, hematuria, leukocyturia, and focal segmental glomerulosclerosis, and die prematurely of kidney failure. Some homozygotes exhibit moderate sensorineural hearing loss.
- The *Col4a4* gene is located on the Chr1. 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)

Col4a4 collagen, type IV, alpha 4 [*Mus musculus* (house mouse)]

Gene ID: 12829, updated on 12-Aug-2019

Summary

Official Symbol	Col4a4 provided by MGI
Official Full Name	collagen, type IV, alpha 4 provided by MGI
Primary source	MGI:MGI:104687
See related	Ensembl:ENSMUSG000000067158
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	[a]4(IV); E130010M05Rik
Expression	Biased expression in kidney adult (RPKM 12.0), lung adult (RPKM 10.4) and 6 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

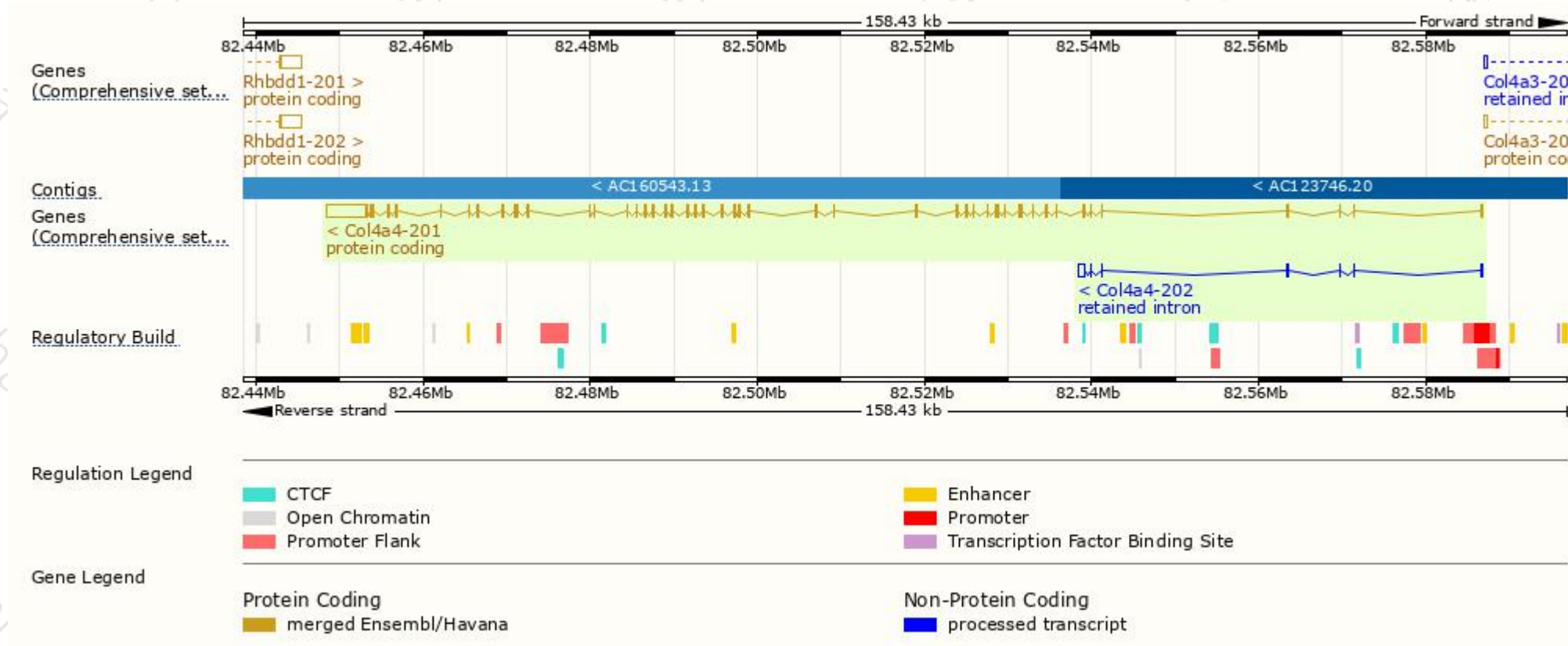
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col4a4-201	ENSMUST00000087050.6	10108	1682aa	Protein coding	CCDS35630	Q9QZR9	TSL:1 GENCODE basic APPRIS P1
Col4a4-202	ENSMUST00000135271.1	1263	No protein	Retained intron	-	-	TSL:5

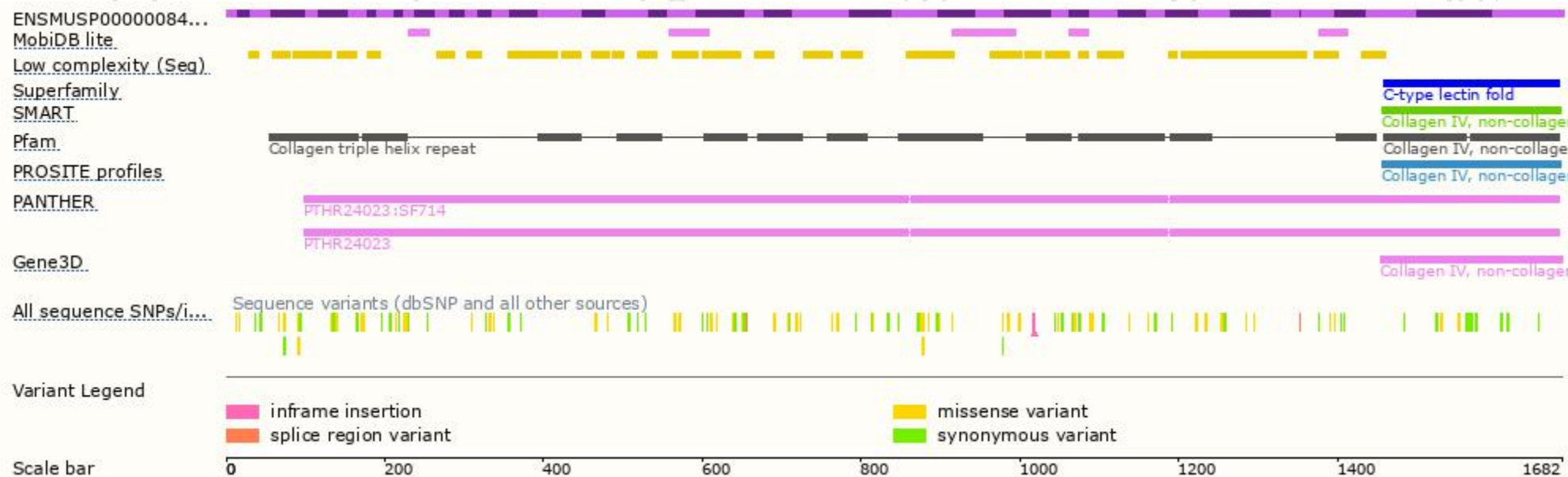
The strategy is based on the design of *Col4a4-201* transcript,The transcription is shown below



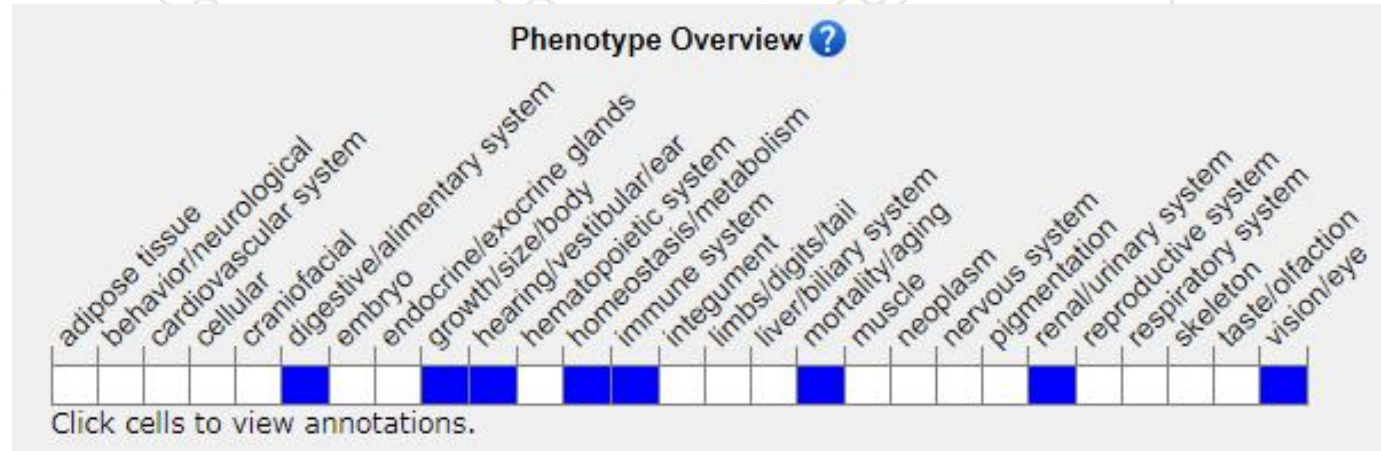
Genomic location distribution



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 an ENU-induced mutation develop an early nephritic syndrome associated with uremia, proteinuria, hematuria, leukocyturia, and focal segmental glomerulosclerosis, and die prematurely of kidney failure. Some homozygotes exhibit moderate sensorineural hearing loss.

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

Tel: 400-9660890

