

Col4a3 Cas9-CKO Strategy

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Reviewer:

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Project Overview

Project Name

Col4a3

Project type

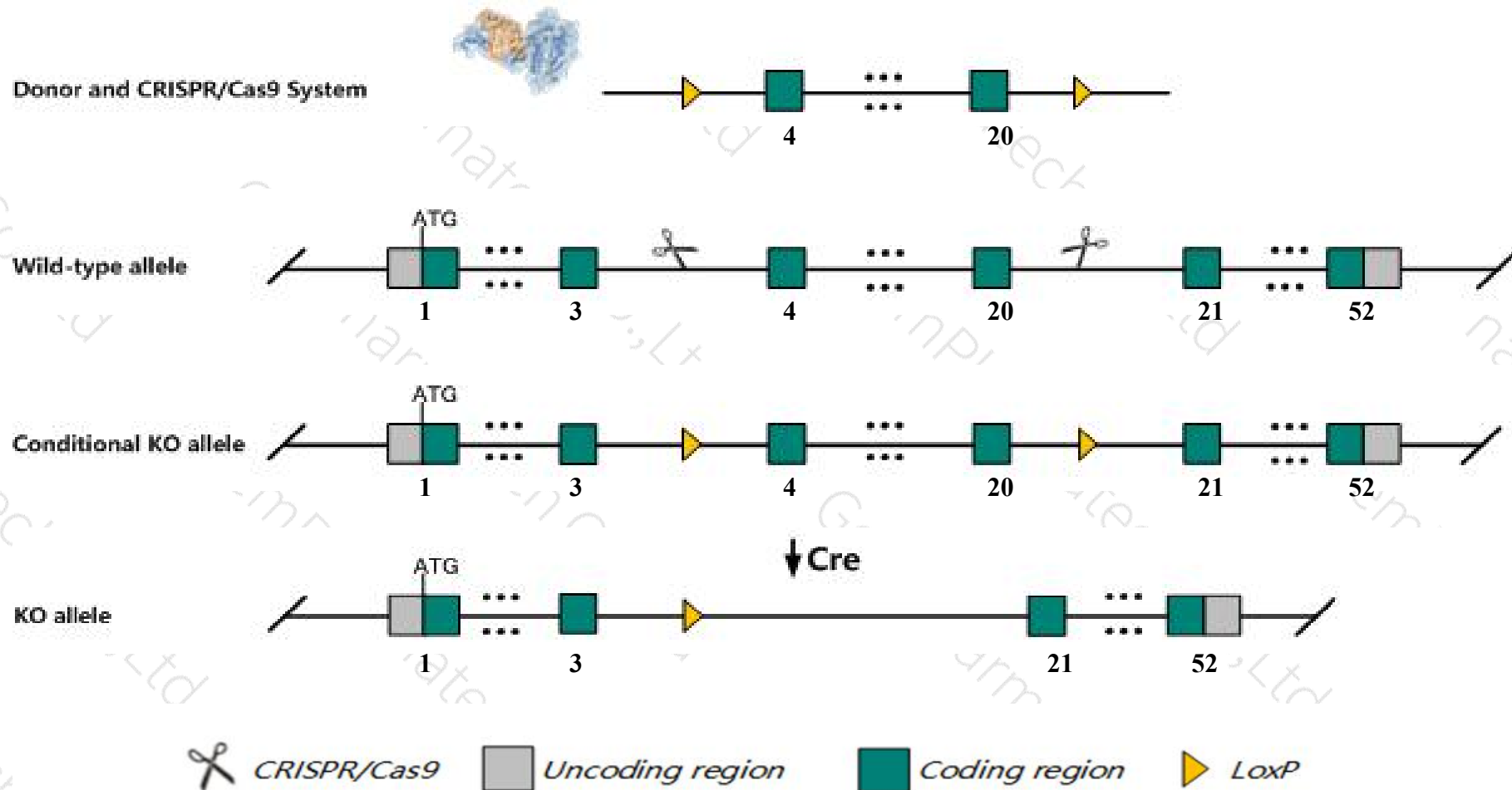
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Col4a3* gene. The schematic diagram is as follows:



Technical routes

- The *Col4a3* gene has 4 transcripts. According to the structure of *Col4a3* gene, exon4-exon20 of *Col4a3-201* (ENSMUST00000113457.8) transcript is recommended as the knockout region. The region contains 916bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Col4a3* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed. Cas9, gRNA 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, Homozygotes for targeted null mutations exhibit renal pathology including reduced glomerular filtration, impaired glomerular integrity, and glomerulonephrosis, resulting in uremia, proteinuria, and high mortality in young adults. Auditory thresholds are mildly increased across all test frequencies.
- The Transcript *Col4a3-204* is incomplete, so the effect on it is unknown.
- The *Col4a3* 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)

Col4a3 collagen, type IV, alpha 3 [*Mus musculus* (house mouse)]

Gene ID: 12828, updated on 27-Aug-2019

Summary

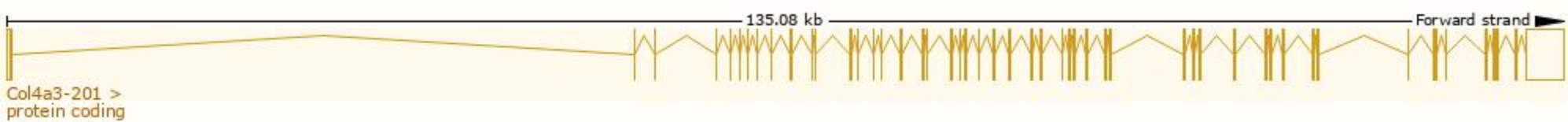
Official Symbol	Col4a3 provided by MGI
Official Full Name	collagen, type IV, alpha 3 provided by MGI
Primary source	MGI:MGI:104688
See related	Ensembl:ENSMUSG00000079465
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	[a]3(IV); alpha3(IV)
Expression	Biased expression in kidney adult (RPKM 16.1), lung adult (RPKM 12.8) and 2 other tissues See more

Transcript information (Ensembl)

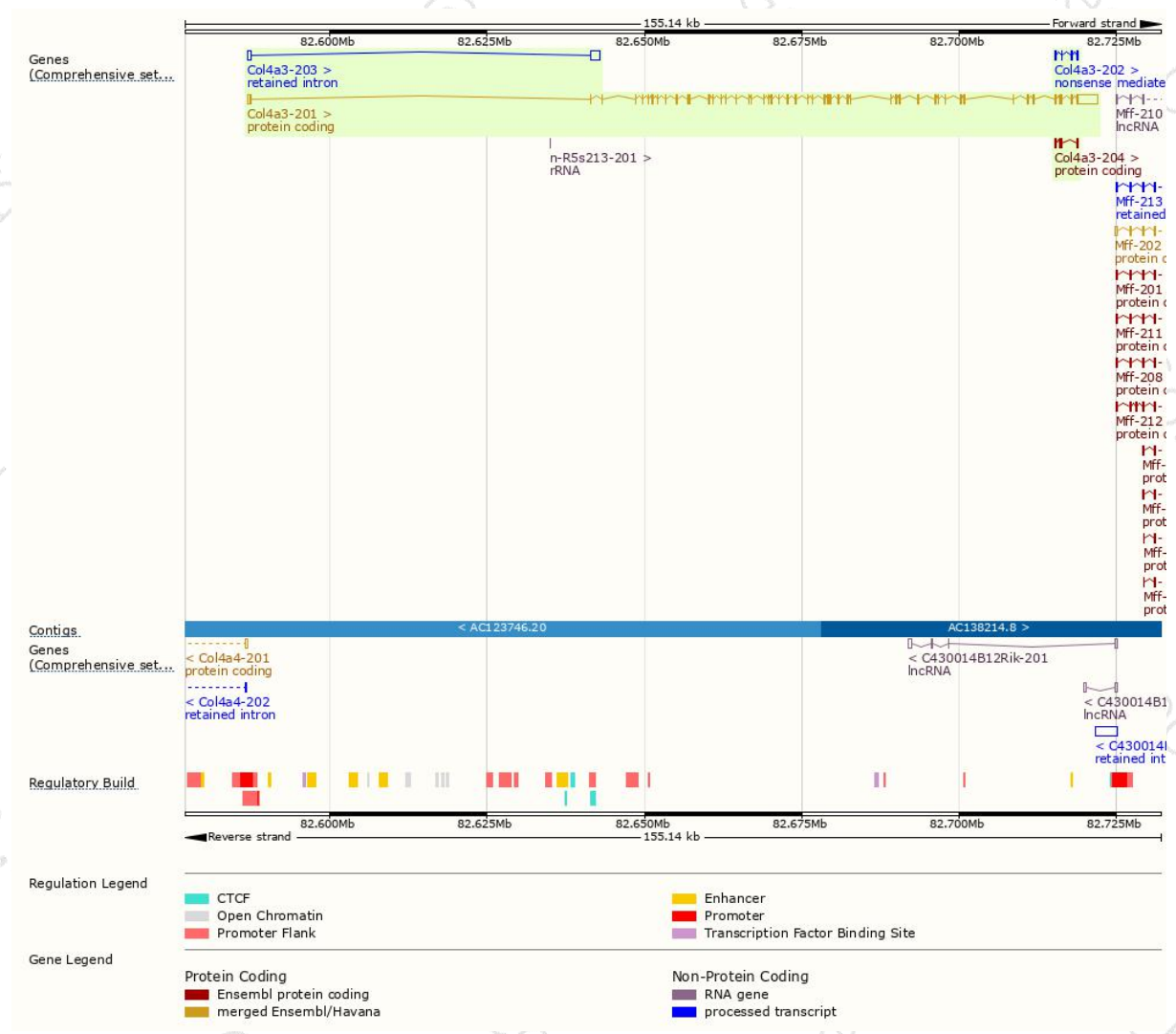
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col4a3-201	ENSMUST00000113457.8	8554	1669aa	Protein coding	CCDS35631	Q9QZS0	TSL:1 GENCODE basic APPRIS P1
Col4a3-204	ENSMUST00000152664.1	627	197aa	Protein coding	-	F6RIS8	CDS 5' incomplete TSL:5
Col4a3-202	ENSMUST00000125563.7	524	61aa	Nonsense mediated decay	-	M0QWQ2	CDS 5' incomplete TSL:5
Col4a3-203	ENSMUST00000141994.1	1926	No protein	Retained intron	-	-	TSL:2

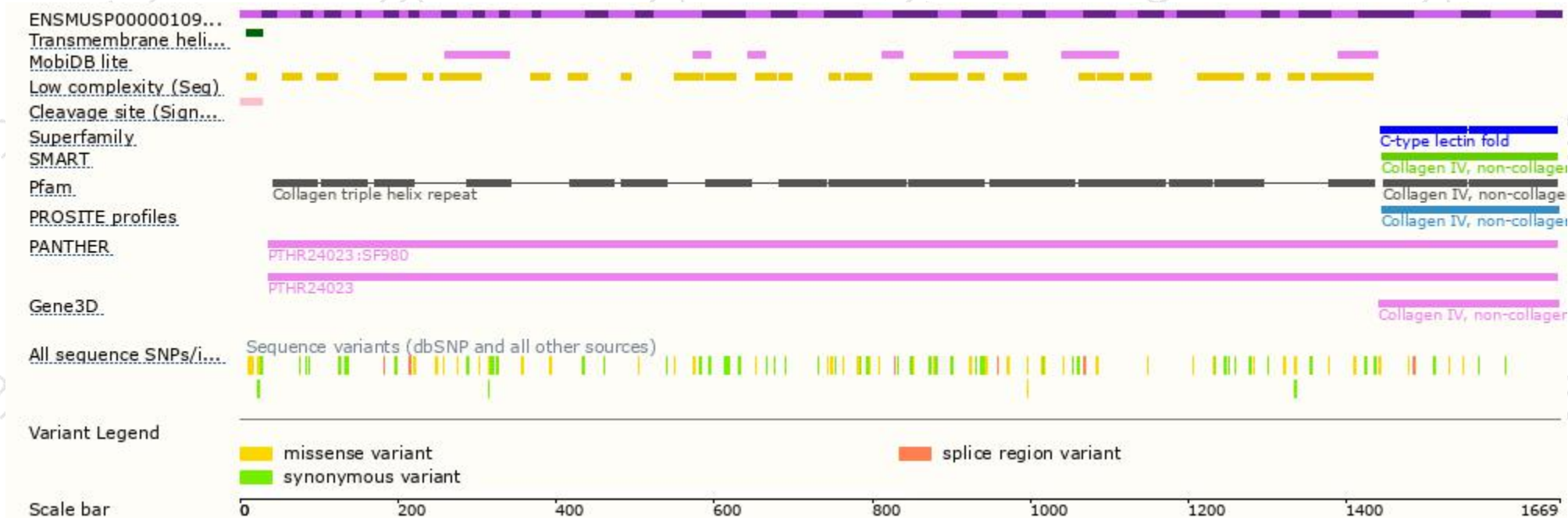
The strategy is based on the design of *Col4a3-201* transcript,The transcription is shown below



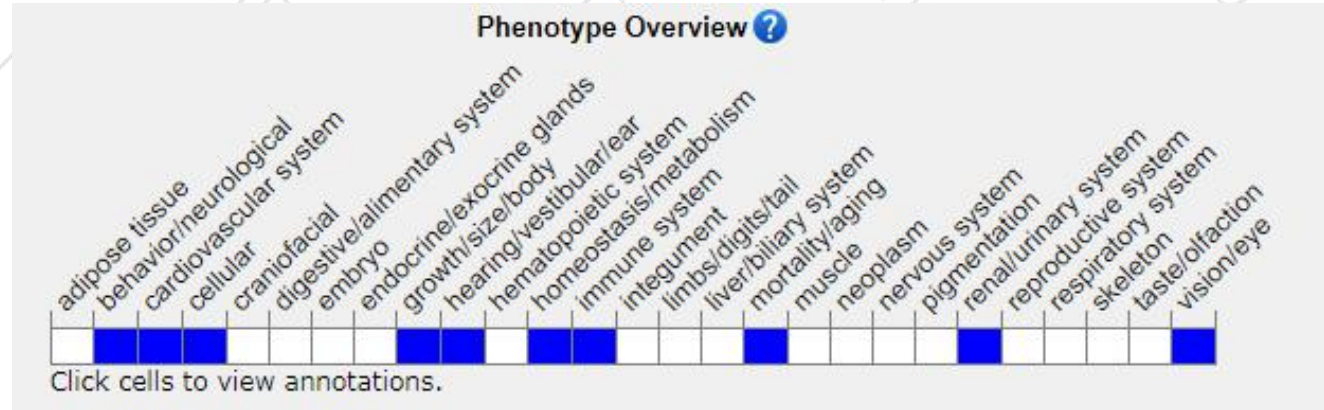
Genomic location distribution



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, Homozygotes for targeted null mutations exhibit renal pathology including reduced glomerular filtration, impaired glomerular integrity, and glomerulonephrosis, resulting in uremia, proteinuria, and high mortality in young adults. Auditory thresholds are mildly increased across all test frequencies.

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

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