Col8a1 Cas9-CKO *Strategy*

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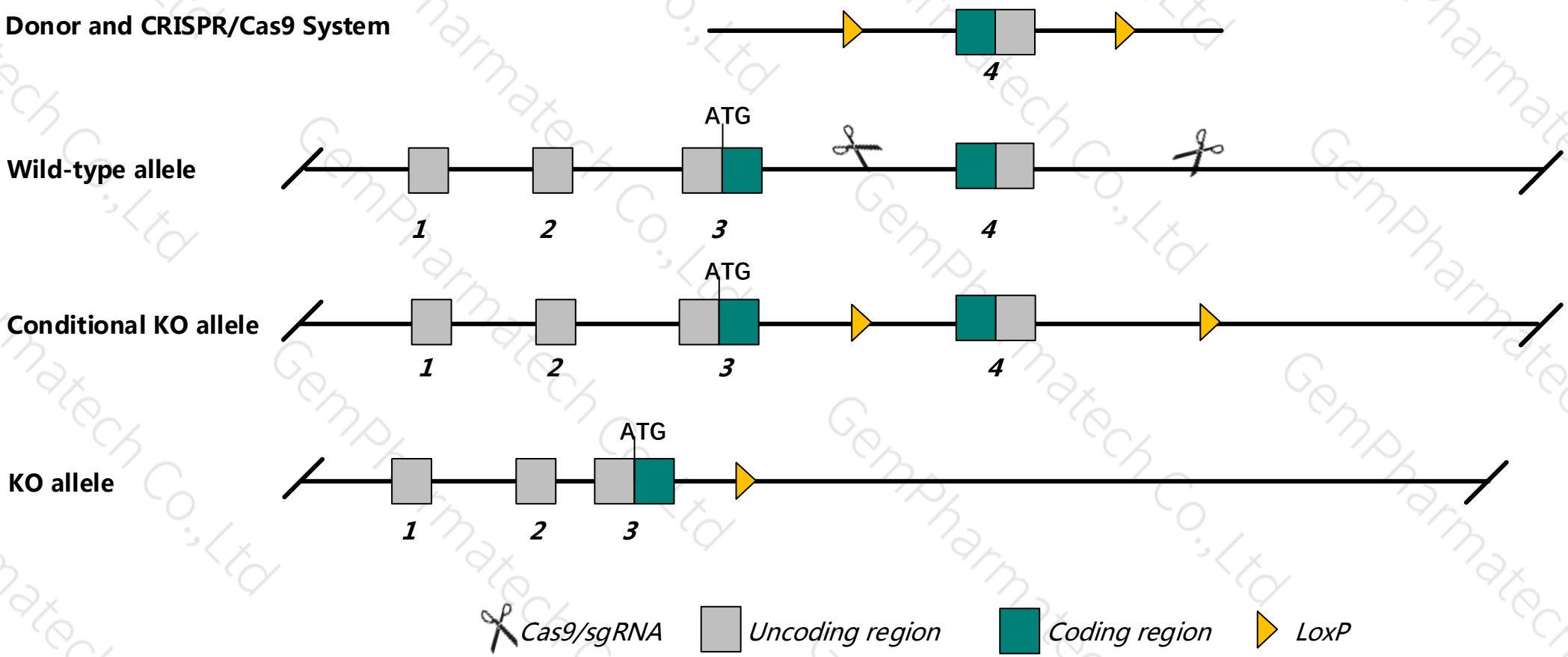
Project Overview

Project Name	***Col8a1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Col8a1* gene. The schematic diagram is as follows:



Technical routes

- The *Col8a1* gene has 2 transcripts.According to the structure of *Col8a1* gene, exon4 of *Col8a1*-201 (ENSMUST00000089332.4) transcript is recommended as the knockout region.The region contains most 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 *Col8a1* gene. The brief process is as follows: sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues or cell types.

Notice

- According to the existing MGI data，Mutation of this gene causes cornea abnormalities that include increased depth of the anterior chamber and a thinner corneal stroma and Descemet's membrane.
- The *Col8a1* gene is located on the Chr16. 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Col8a1 collagen, type VIII, alpha 1 [*Mus musculus* (house mouse)]

Gene ID: 12837, updated on 12-Aug-2019

Summary

Official Symbol	Col8a1 provided by MGI
Official Full Name	collagen, type VIII, alpha 1 provided by MGI
Primary source	MGI:MGI:88463
See related	Ensembl:ENSMUSG00000068196
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	Col8a-1
Expression	Biased expression in bladder adult (RPKM 10.3), limb E14.5 (RPKM 3.6) and 13 other tissues See more
Orthologs	human all

Genomic context

Location: 16; 16 C1.1

See Col8a1 in Genome Data Viewer

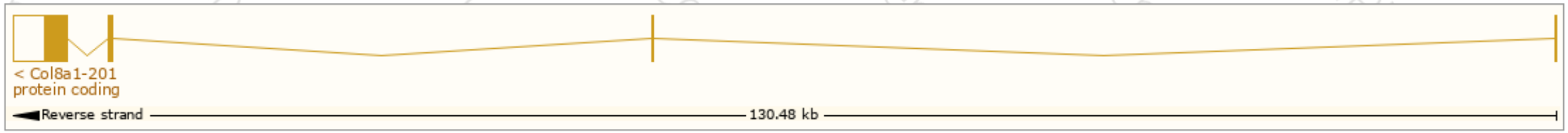
Exon count: 4

Transcript information（Ensembl）

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

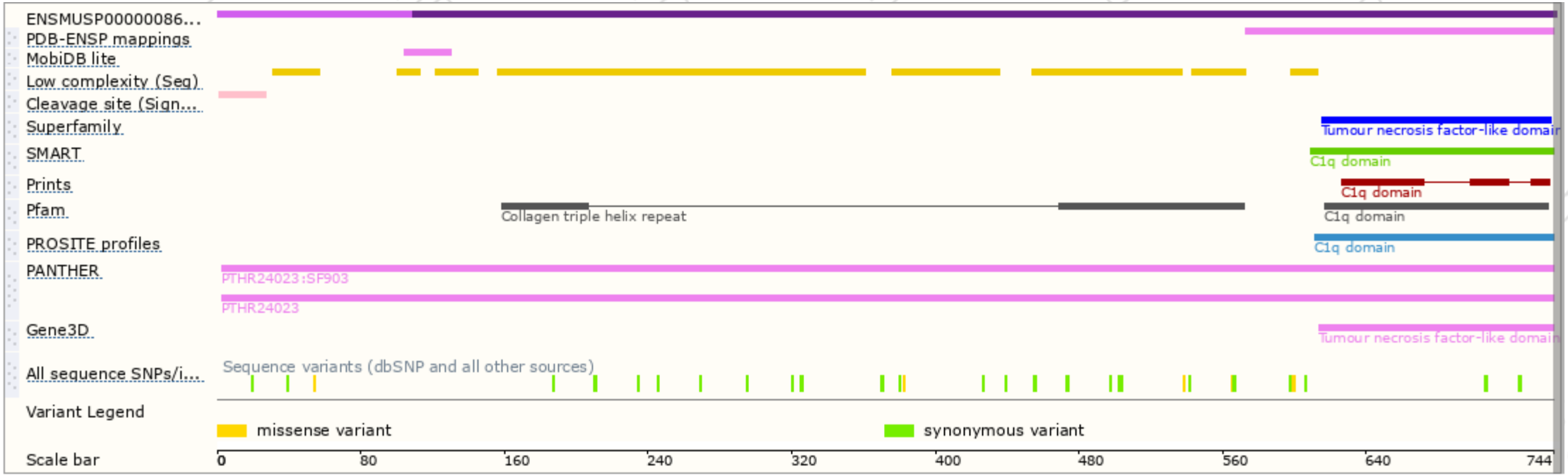
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col8a1-201	ENSMUST00000089332.4	5108	744aa	Protein coding	CCDS37367	Q00780	TSL:1 GENCODE basic APPRIS P1
Col8a1-202	ENSMUST00000232382.1	2801	No protein	Retained intron	-	-	-

The strategy is based on the design of *Col8a1*-201 transcript,The transcription is shown below

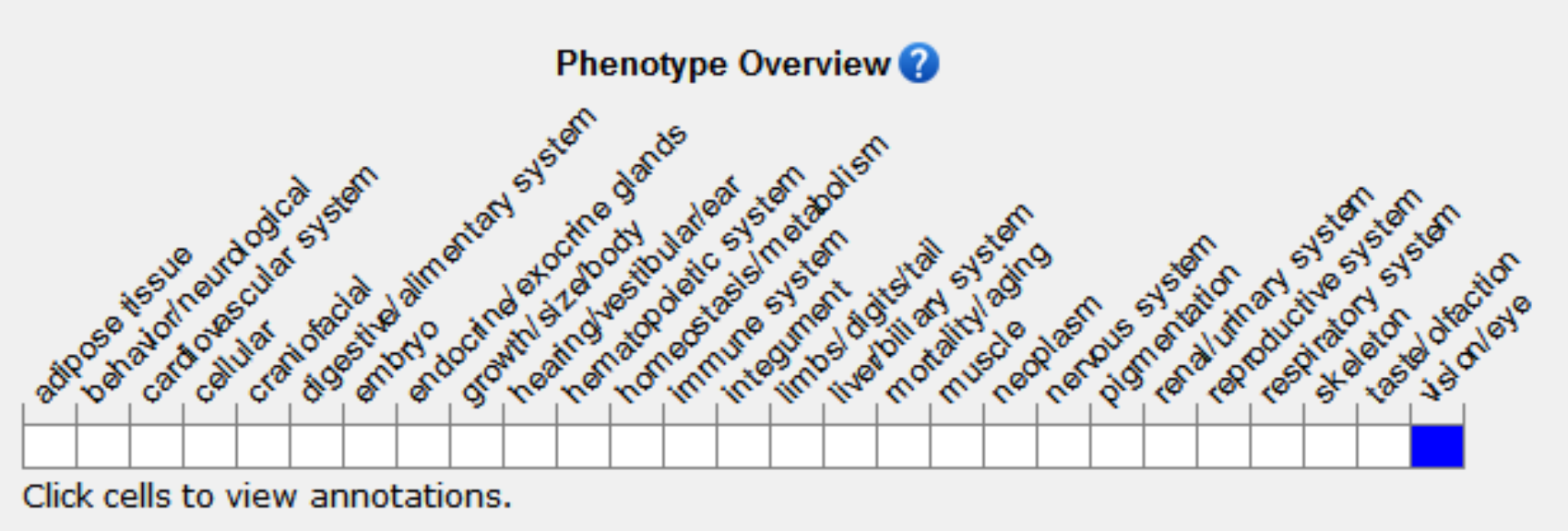


Genomic location distribution

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,Mutation of this gene causes cornea abnormalities that include increased depth of the anterior chamber and a thinner corneal stroma and Descemet's membrane.

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

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