Col4a2 Cas9-KO Strategy

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Design Date: 2020-1-4

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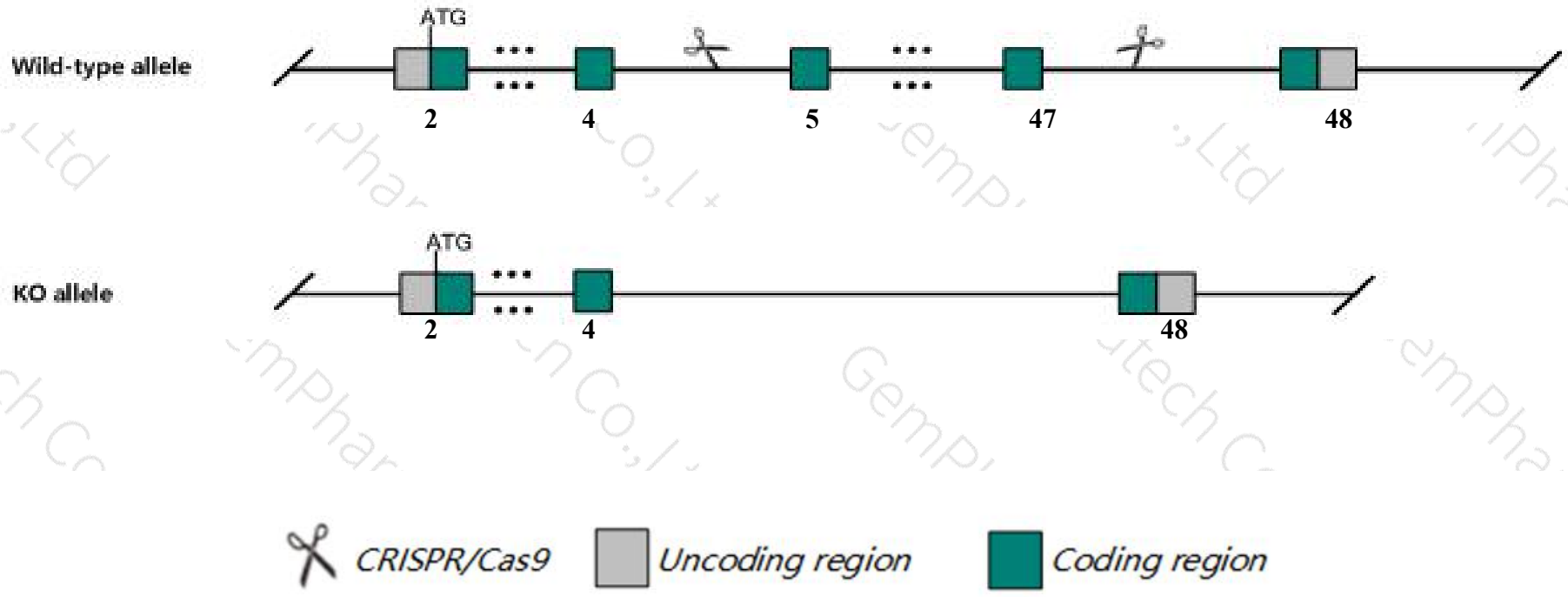
Project Overview

Project Name	***Col4a2***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Col4a2* gene. The schematic diagram is as follows:



Technical routes

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- The *Col4a2* gene has 3 transcripts. According to the structure of *Col4a2* gene, exon5-exon47 of *Col4a2-201* (ENSMUST00000033899.13) 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 *Col4a2* gene. The brief process is as follows: CRISPR/Cas9 syster

Notice

- According to the existing MGI data,ENU-induced missense mutations of this gene result in a variable phenotype affecting the eye, brain and vascular stability in heterozygotes, and fetal or postnatal survival in homozygotes.
- The KO region contains the *Gm15419* gene. Knockout the region may affect the function of *Gm15419* gene.
- The *Col4a2* gene is located on the Chr8. 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 gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information （NCBI）

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Col4a2 collagen, type IV, alpha 2 [Mus musculus (house mouse)]

Gene ID: 12827, updated on 3-Feb-2019

Summary

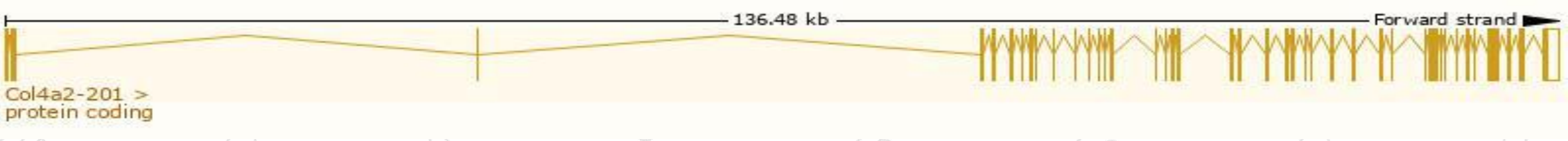
Official Symbol	Col4a2 provided by MGI
Official Full Name	collagen, type IV, alpha 2 provided byMGI
Primary source	MGI:MGI:88455
See related	Ensembl:ENSMUSG00000031503
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Col4a-2
Summary	This gene encodes the alpha-2 subunit of the type IV collagens, an essential component of basement membranes. The encoded protein forms a triple helical heterotrimer comprised of alpha-1 and alpha-2 subunits that assembles into a type IV collagen network. Canstatin, a peptide derived fom the C-terminus of the collagen chain, is a matrikine that has been shown to inhibit angiogenesis. Homozygous knockout mice for this gene exhibit impaired basement membrane integrity and embryonic lethality. This gene shares a bi-directional promoter with a related gene on chromosome 8. [provided by RefSeq, Nov 2015]
Expression	Broad expression in subcutaneous fat pad adult (RPKM 132.8), lung adult (RPKM 102.4) and 22 other tissuesSee more
Orthologs	human all

Transcript information（Ensembl）

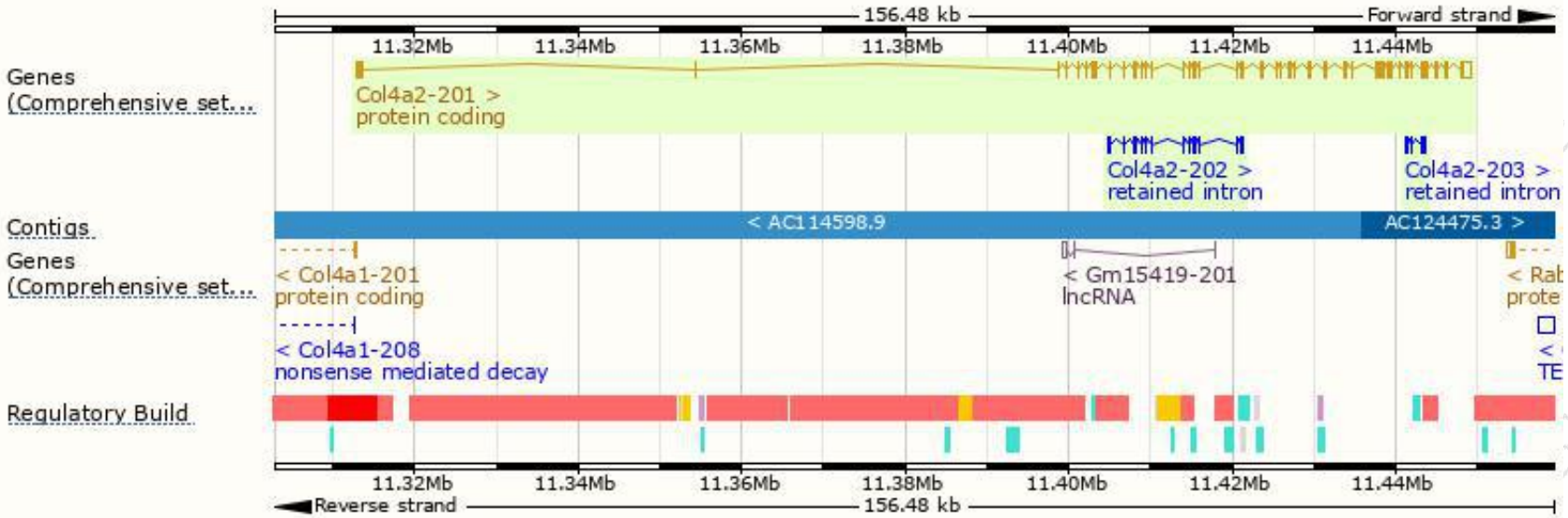
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col4a2-201	ENSMUST00000033899.13	6450	1707aa	Protein coding	CCDS40220	B2RQQ8 P08122	TSL:1 GENCODE basic APPRIS P1
Col4a2-202	ENSMUST00000145295.1	921	No protein	Retained intron	-	-	TSL:5
Col4a2-203	ENSMUST00000146219.1	764	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Col4a2-201* transcript,The transcription is shown below



Genomic location distribution



Gene Legend

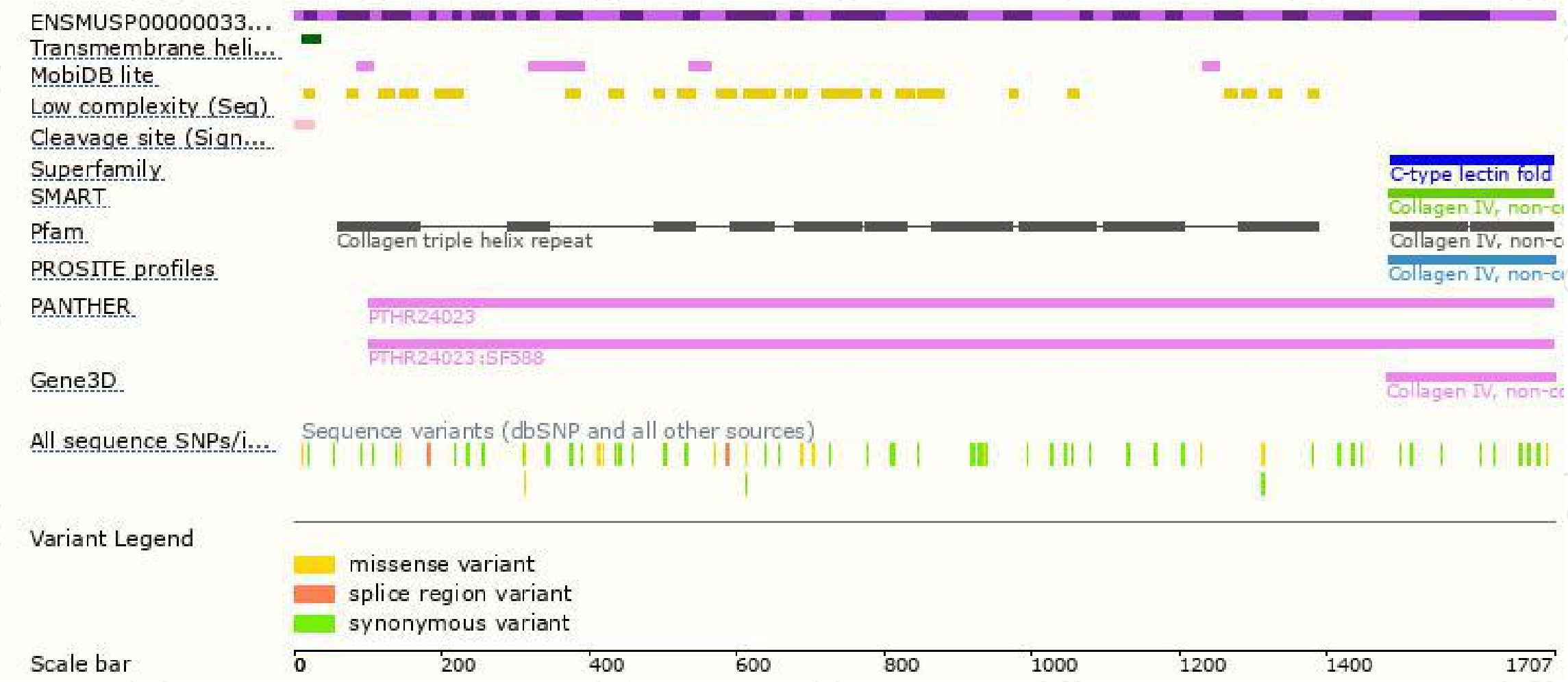
Protein Coding
- merged Ensembl/Havana

Non-Protein Coding
- RNA gene
- processed transcript

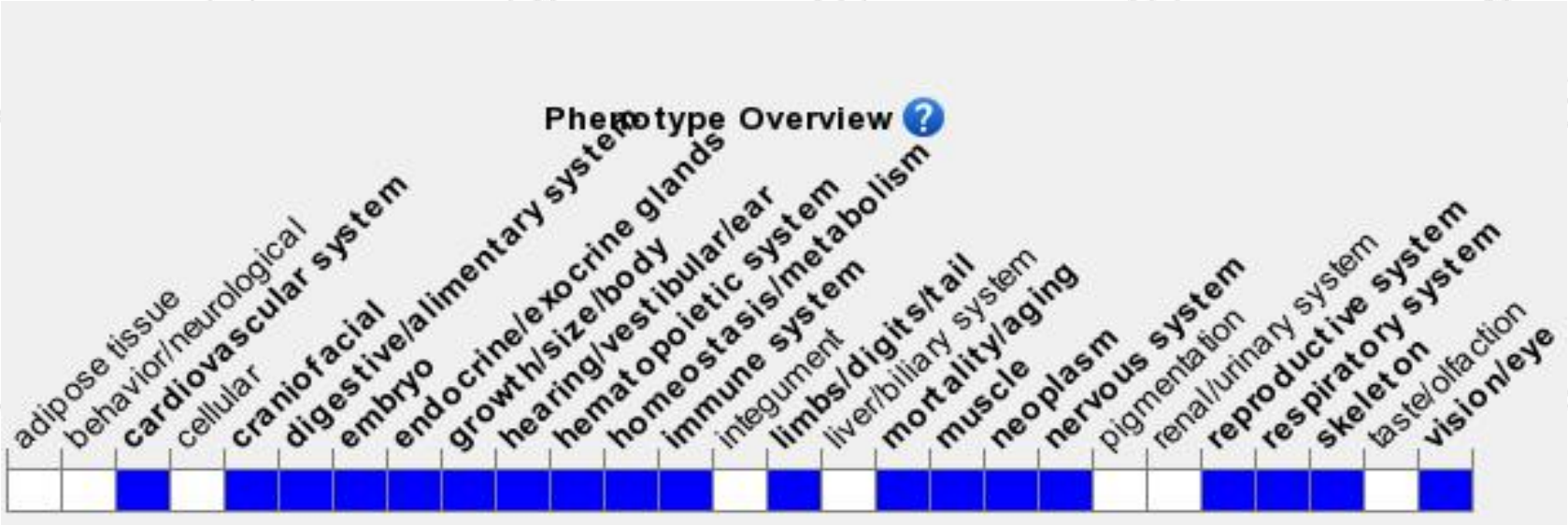
Regulation Legend

- CTCF
- Enhancer
- Open Chromatin
- Promoter
- Promoter Flank
- Transcription Factor Binding Site

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,ENU-induced missense mutations of this gene result in a variable phenotype affecting the eye, brain and vascular stability in heterozygotes, and fetal or postnatal survival in homozygotes.

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

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