

***Col5a2* Cas9-CKO Strategy**

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Design Date:2020-3-6

Project Overview

Project Name

Col5a2

Project type

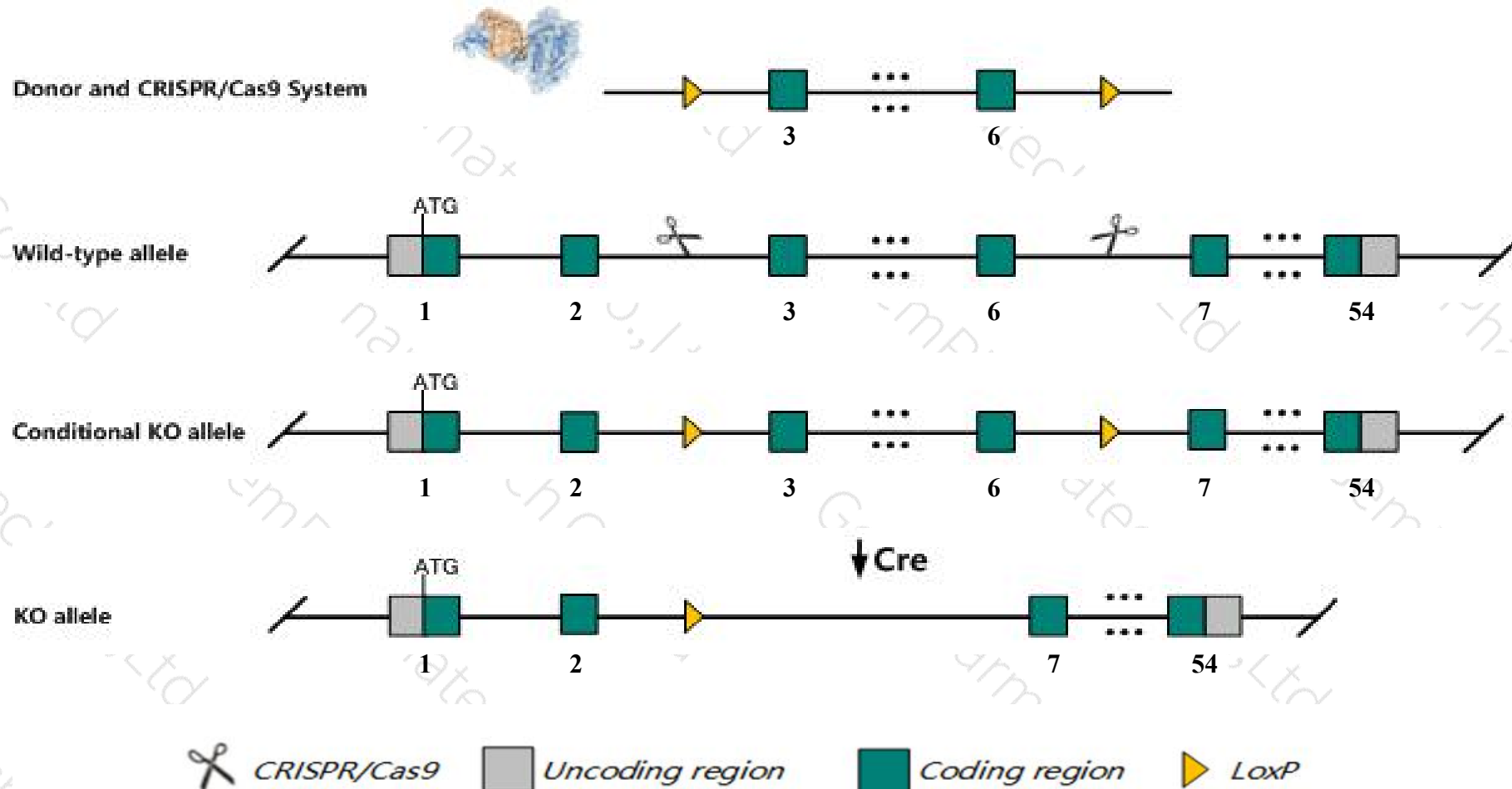
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Col5a2* gene. The schematic diagram is as follows:



Technical routes

- The *Col5a2* gene has 4 transcripts. According to the structure of *Col5a2* gene, exon3-exon6 of *Col5a2-201* (ENSMUST00000086430.4) transcript is recommended as the knockout region. The region contains 134bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Col5a2* 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, Homozygous mutation of this gene results in perinatal lethality. Mutant animals exhibit reduced body weight, reduced bone growth rate, thin, fragile skin, variable degrees of lordosis and kyphosis, abnormal localization of hair follicles in the dermis, and thinned stroma of the cornea.
- The *Col5a2* 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)

Col5a2 collagen, type V, alpha 2 [*Mus musculus* (house mouse)]

Gene ID: 12832, updated on 10-Oct-2019

Summary

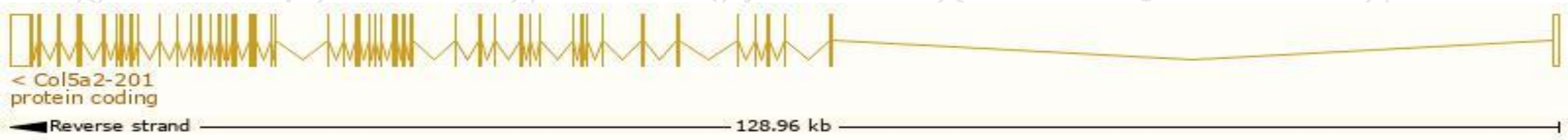
Official Symbol	Col5a2 provided by MGI
Official Full Name	collagen, type V, alpha 2 provided by MGI
Primary source	MGI:MGI:88458
See related	Ensembl:ENSMUSG00000026042
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	1110014L14Rik
Summary	This gene encodes the alpha-2 subunit of type V collagen, one of the low abundance fibrillar collagens that gets incorporated into growing fibrils with type I collagen. The encoded protein, in association with alpha-1 and/or alpha-3 subunits, forms homo- or heterotrimeric type V procollagen that undergoes proteolytic processing. Mice lacking the encoded protein die in utero. Transgenic mice that produce a structurally abnormal form of the encoded protein survive poorly and exhibit skin fragility, skeletal abnormalities and alterations in the collagen fiber organization. [provided by RefSeq, Dec 2015]
Expression	Biased expression in limb E14.5 (RPKM 56.0), bladder adult (RPKM 29.0) and 14 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

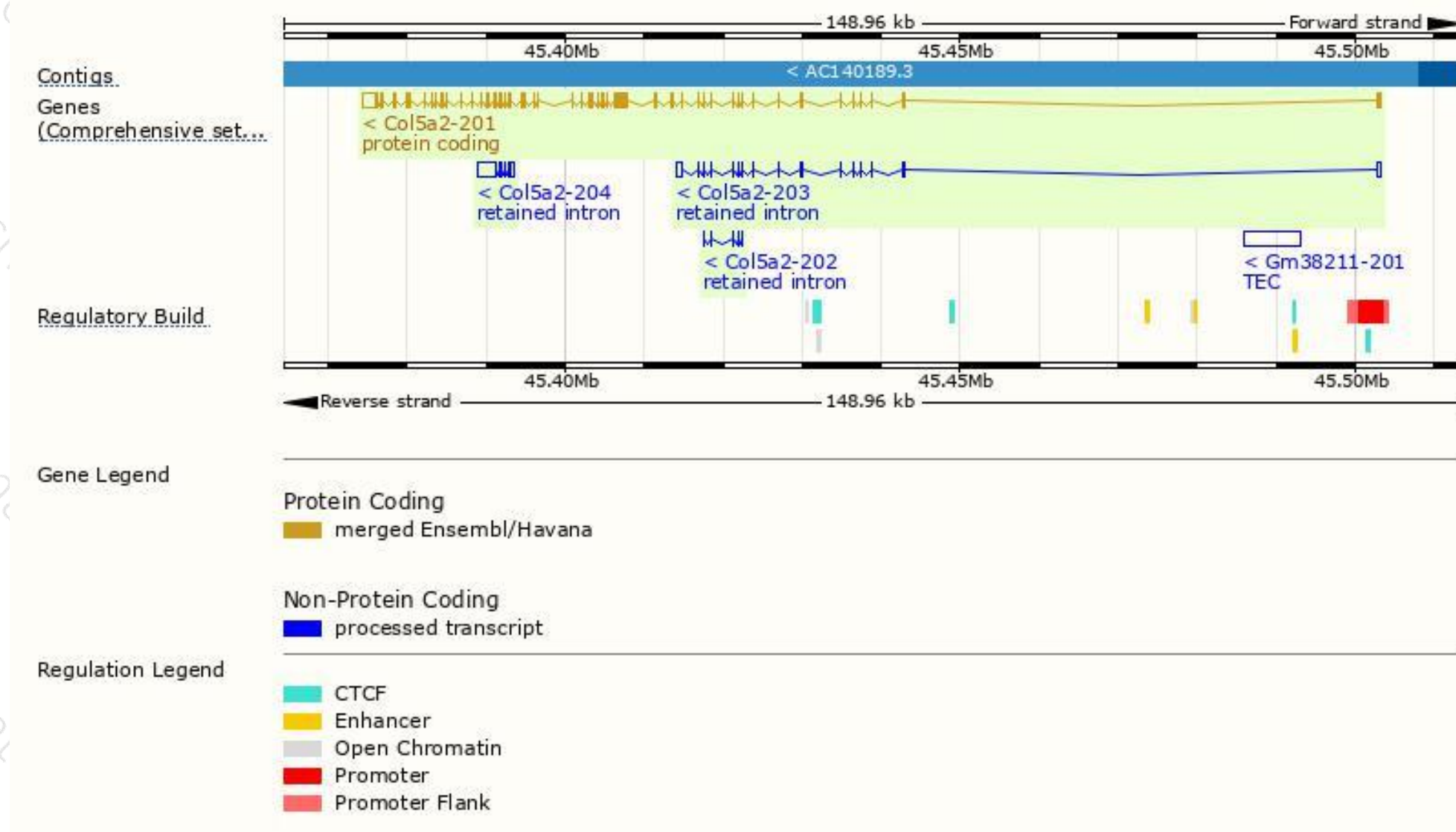
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col5a2-201	ENSMUST00000086430.4	6625	1497aa	Protein coding	CCDS35555	Q3U962	TSL:1 GENCODE basic APPRIS P1
Col5a2-204	ENSMUST00000150143.1	2951	No protein	Retained intron	-	-	TSL:2
Col5a2-203	ENSMUST00000134681.7	1970	No protein	Retained intron	-	-	TSL:2
Col5a2-202	ENSMUST00000131535.1	356	No protein	Retained intron	-	-	TSL:3

The strategy is based on the design of *Col5a2-201* transcript,The transcription is shown below



Genomic location distribution

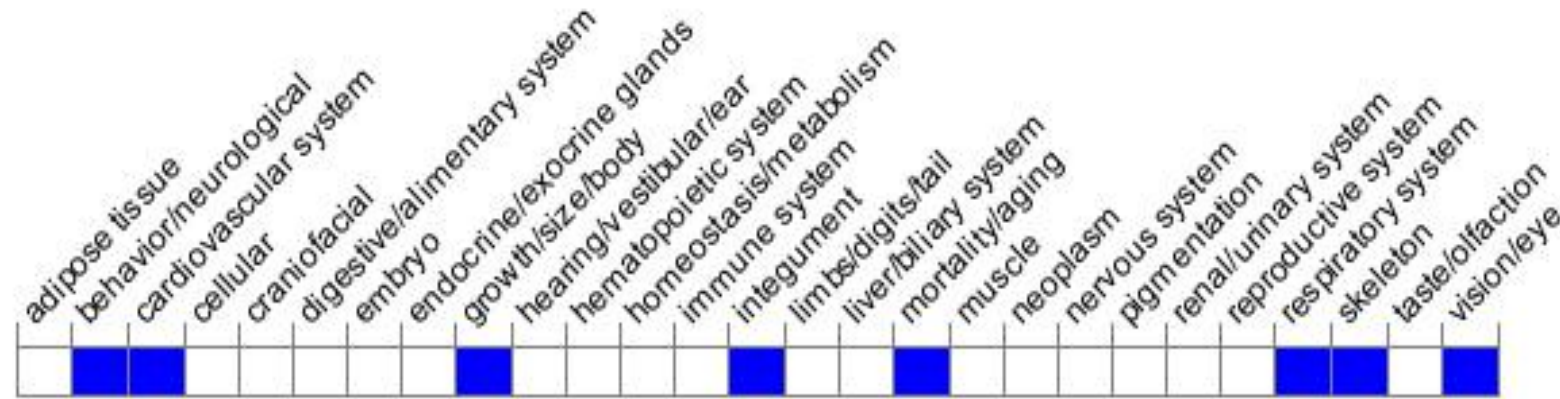


Protein domain



Mouse phenotype description(MGI)

Phenotype Overview



Phenotypes affected by the gene are marked in blue. Data quoted from MGI database(<http://www.informatics.jax.org/>).

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

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