

# Coll1a1 Cas9-KO Strategy

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Reviewer: Miaomiao Cui

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## **Project Overview**



**Project Name** 

Col11a1

**Project type** 

Cas9-KO

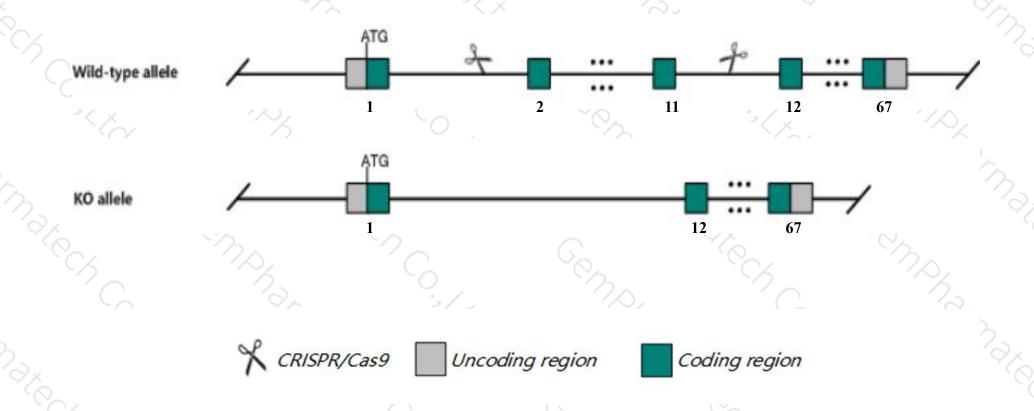
Strain background

C57BL/6JGpt

## **Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the Coll1a1 gene. The schematic diagram is as follows:



### **Technical routes**



- The *Coll1a1* gene has 7 transcripts. According to the structure of *Coll1a1* gene, exon2-exon11 of *Coll1a1-201*(ENSMUST00000092155.11) transcript is recommended as the knockout region. The region contains 1304bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Coll1a1* gene. The brief process is as follows: CRISPR/Cas9 system 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.

### **Notice**



- > According to the existing MGI data, homozygous mutation of this gene results in perinatal lethality by asphyxia. Mutants animals display weak tracheal cartilage, short snout, short mandible, cleft palate, short limbs, and externally rotated distal portion of the hindlimbs.
- The *Coll1a1* gene is located on the Chr3. 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)



#### Coll1a1 collagen, type XI, alpha 1 [Mus musculus (house mouse)]

Gene ID: 12814, updated on 13-Mar-2020

#### Summary

↑ ?

Official Symbol Coll1a1 provided by MGI

Official Full Name collagen, type XI, alpha 1 provided by MGI

Primary source MGI:MGI:88446

See related Ensembl: ENSMUSG00000027966

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 C530001D20Rik, cho

Summary This gene encodes the alpha-1 subunit of type XI collagen, one of the low abundance fibrillar collagens that is essential for

normal embryonic skeletal development and the cohesive properties of cartilage. The encoded protein, in association with the

alpha-1 subunit of type II collagen, forms a heterotrimeric type XI procollagen that undergoes proteolytic processing. Mice

lacking the encoded protein develop severe chondrodysplasia and die at birth. [provided by RefSeq, Dec 2015]

Expression Biased expression in limb E14.5 (RPKM 65.0), CNS E14 (RPKM 14.0) and 3 other tissuesSee more

Orthologs <u>human all</u>

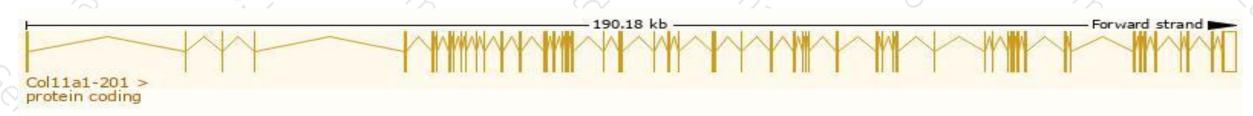
## Transcript information (Ensembl)



The gene has 7 transcripts, all transcripts are shown below:

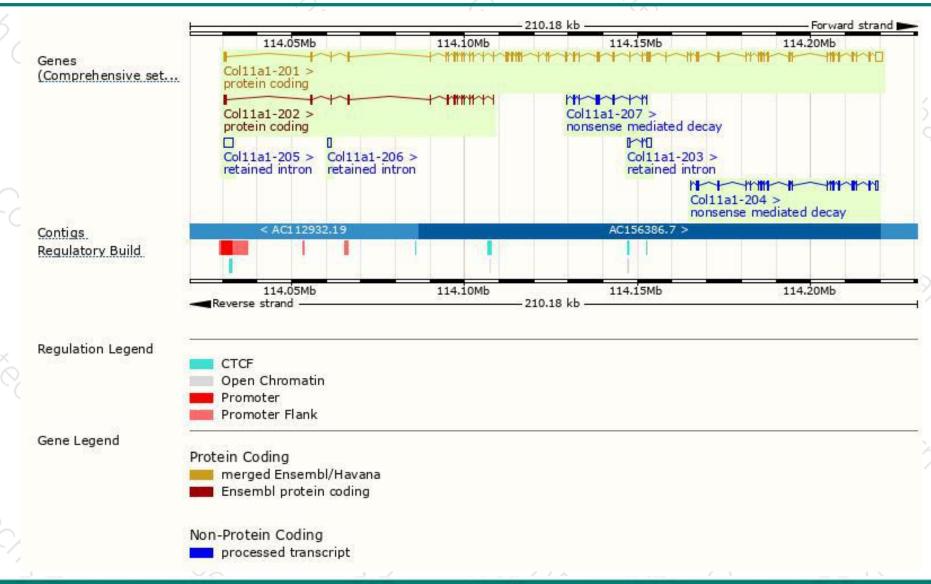
| Name        | Transcript ID         | bp   | Protein                                 | Biotype                 | CCDS      | UniProt    | Flags                        |
|-------------|-----------------------|------|---|-------------------------|-----------|------------|------------------------------|
| Col11a1-201 | ENSMUST00000092155.11 | 7667 | 1804aa                                  | Protein coding          | CCDS17778 | Q61245     | TSL:1 GENCODE basic APPRIS P |
| Col11a1-202 | ENSMUST00000123619.7  | 2064 | <u>567aa</u>                            | Protein coding          | -:        | E9PVT8     | CDS 3' incomplete TSL:5      |
| Col11a1-204 | ENSMUST00000184978.1  | 2570 | 536aa                                   | Nonsense mediated decay | 20        | V9GWX8     | CDS 5' incomplete TSL:1      |
| Col11a1-207 | ENSMUST00000211802.1  | 675  | 128aa                                   | Nonsense mediated decay | -         | A0A1D5RMJ0 | CDS 5' incomplete TSL:3      |
| Col11a1-205 | ENSMUST00000196345.1  | 2519 | No protein                              | Retained intron         | 20        | <u> 12</u> | TSL:NA                       |
| Col11a1-203 | ENSMUST00000138680.1  | 2112 | No protein                              | Retained intron         | -         | 95         | TSL:1                        |
| Col11a1-206 | ENSMUST00000196654.1  | 969  | No protein                              | Retained intron         |           | -          | TSL:NA                       |
|             |                       | 1    | • |                         |           |            | 1 1 1/10                     |

The strategy is based on the design of *Coll1a1-201* transcript, the transcription is shown below:



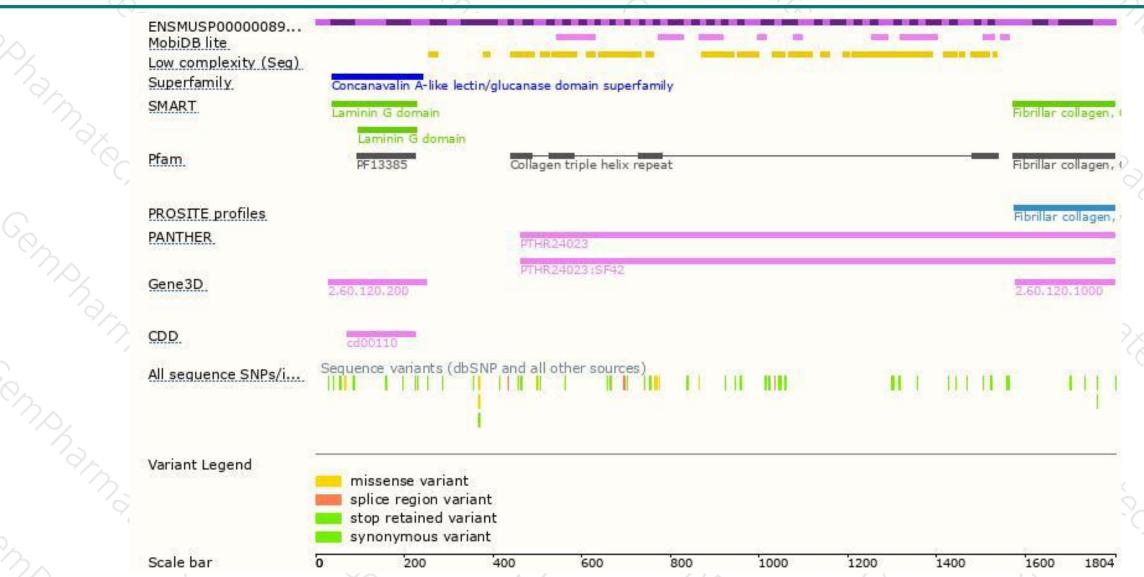
### Genomic location distribution





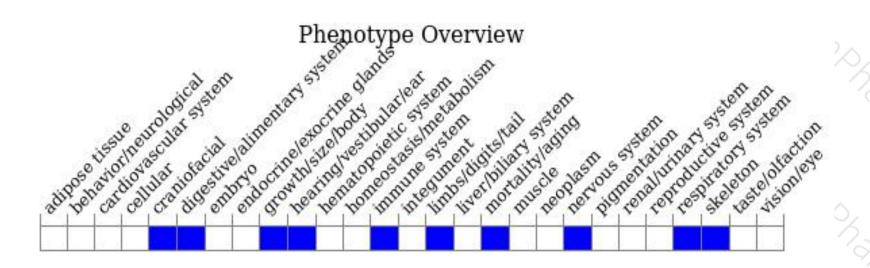
### Protein domain





## 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, homozygous mutation of this gene results in perinatal lethality by asphyxia.

Mutants animals display weak tracheal cartilage, short snout, short mandible, cleft palate, short limbs, and externally rotated distal portion of the hindlimbs.



If you have any questions, you are welcome to inquire. Tel: 400-9660890





