

Col2a1 Cas9-CKO Strategy

Designer: Xiaojing Li
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Reviewer: Jia Yu

Project Overview

Project Name

Col2a1

Project type

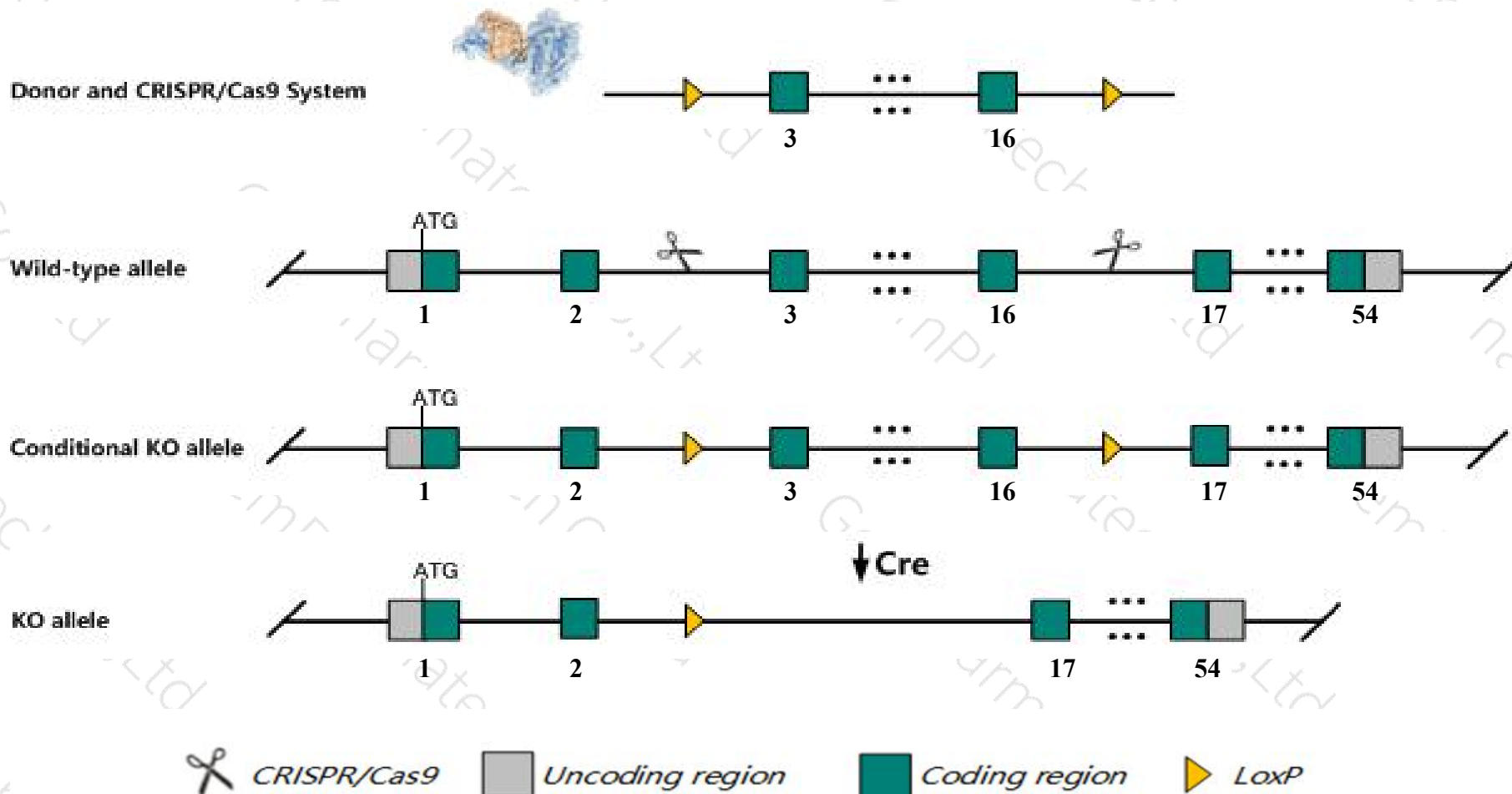
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Col2a1* gene. The schematic diagram is as follows:



- The *Col2a1* gene has 10 transcripts. According to the structure of *Col2a1* gene, exon3-exon16 of *Col2a1-201* (ENSMUST00000023123.14) transcript is recommended as the knockout region. The region contains 734bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Col2a1* 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, Mutations in this locus affect cartilage development. Homozygotes die perinatally with anomalies such as shortened limbs without epiphiseal growth plates, cleft palate and persistence of notochord. Heterozygotes are dwarfed with reduced cartilage matrix.
- The *Col2a1* gene is located on the Chr15. 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)

Col2a1 collagen, type II, alpha 1 [Mus musculus (house mouse)]

Gene ID: 12824, updated on 9-Apr-2019

Summary



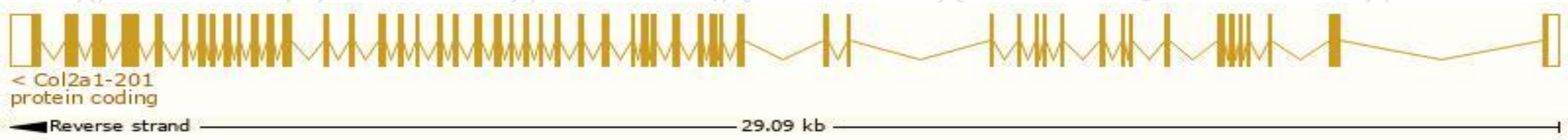
Official Symbol	Col2a1 provided by MGI
Official Full Name	collagen, type II, alpha 1 provided by MGI
Primary source	MGI:MGI:88452
See related	Ensembl:ENSMUSG000000022483
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Col2, Col2a, Col2a-1, Del1, Dmm, Lpk, M100413, Rgsc413, Rgsc856
Summary	This gene encodes the alpha-1 subunit of the fibril-forming type II collagen, the major component of cartilage and the vitreous humor of the eye. The encoded preproprotein forms homotrimeric, triple helical procollagen that undergoes proteolytic processing during fibril formation. Mice harboring certain mutations in this gene exhibit severe chondrodysplasia characterized by short limbs and trunk, craniofacial deformities and cleft palate. A complete lack of the encoded protein in mice results in postnatal lethality. Alternative splicing results in multiple transcript variants encoding different isoforms that may undergo similar proteolytic processing. [provided by RefSeq, Dec 2015]
Expression	Biased expression in limb E14.5 (RPKM 504.7), CNS E14 (RPKM 67.6) and 1 other tissue See more
Orthologs	human all

Transcript information (Ensembl)

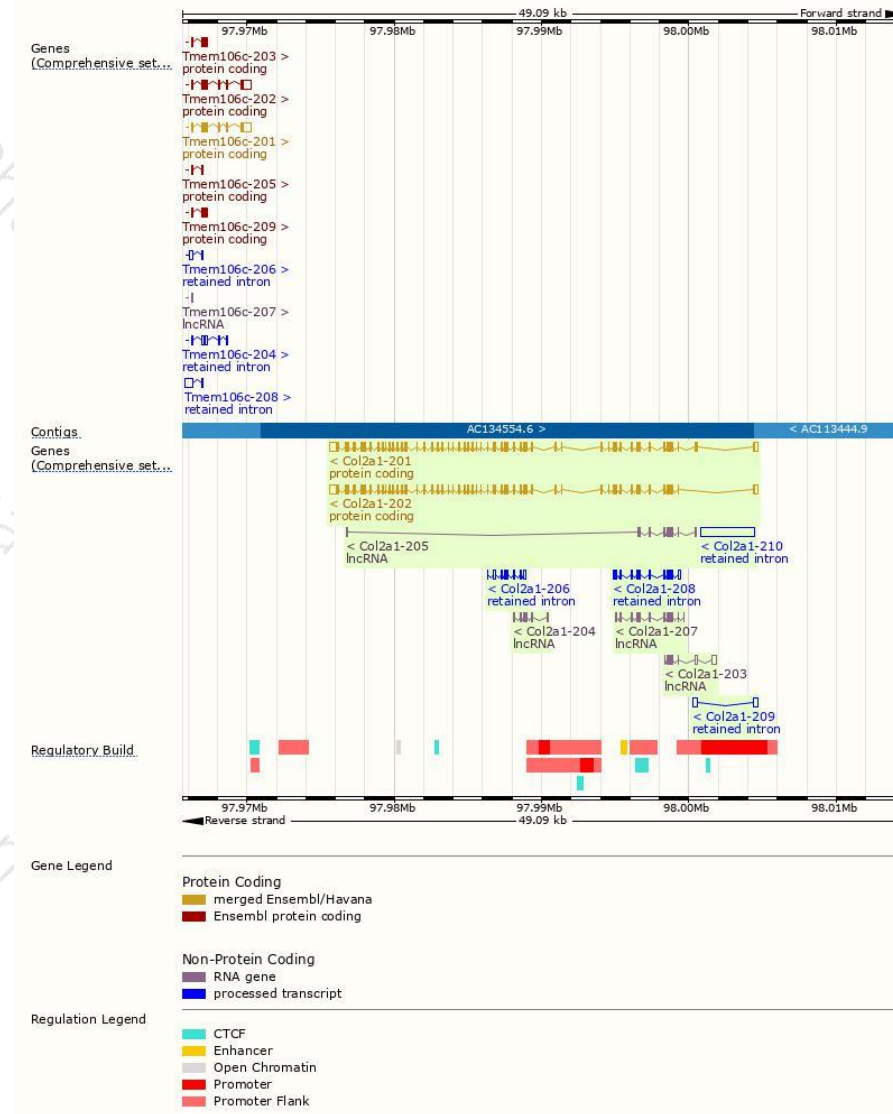
The gene has 10 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col2a1-201	ENSMUST00000023123.14	5104	1487aa	Protein coding	CCDS37189	P28481	TSL:1 GENCODE basic APPRIS P3
Col2a1-202	ENSMUST00000088355.11	4862	1419aa	Protein coding	CCDS49716	P28481	TSL:1 GENCODE basic APPRIS ALT 1
Col2a1-210	ENSMUST00000230148.1	3666	No protein	Retained intron	-	-	
Col2a1-208	ENSMUST00000139246.7	809	No protein	Retained intron	-	-	TSL:5
Col2a1-206	ENSMUST00000131910.7	654	No protein	Retained intron	-	-	TSL:5
Col2a1-209	ENSMUST00000140064.1	613	No protein	Retained intron	-	-	TSL:2
Col2a1-203	ENSMUST00000127879.1	715	No protein	lncRNA	-	-	TSL:3
Col2a1-207	ENSMUST00000133488.7	626	No protein	lncRNA	-	-	TSL:5
Col2a1-205	ENSMUST00000131560.2	575	No protein	lncRNA	-	-	TSL:3
Col2a1-204	ENSMUST00000128547.1	491	No protein	lncRNA	-	-	TSL:3

The strategy is based on the design of *Col2a1-201* transcript,The transcription is shown below



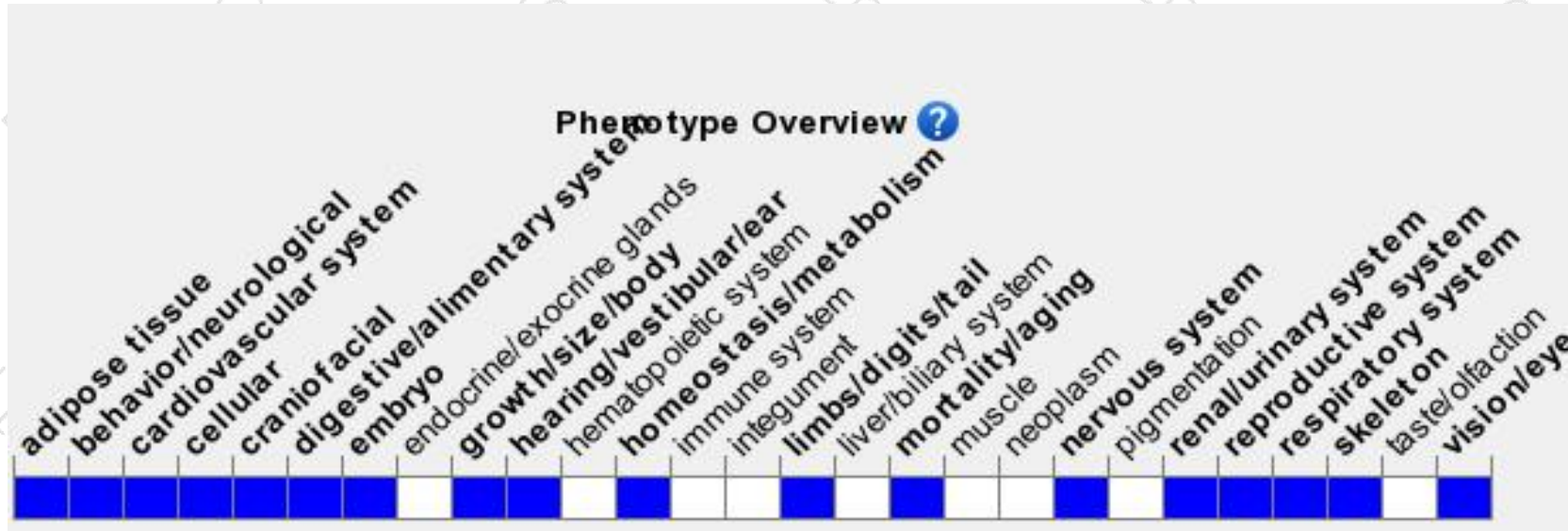
Genomic location distribution



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/>).

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

Tel: 400-9660890

