

Col24a1 Cas9-CKO Strategy

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Reviewer: Xiaojing Li

Design Date: 2020-2-28

Project Overview



Project Name

Col24a1

Project type

Cas9-CKO

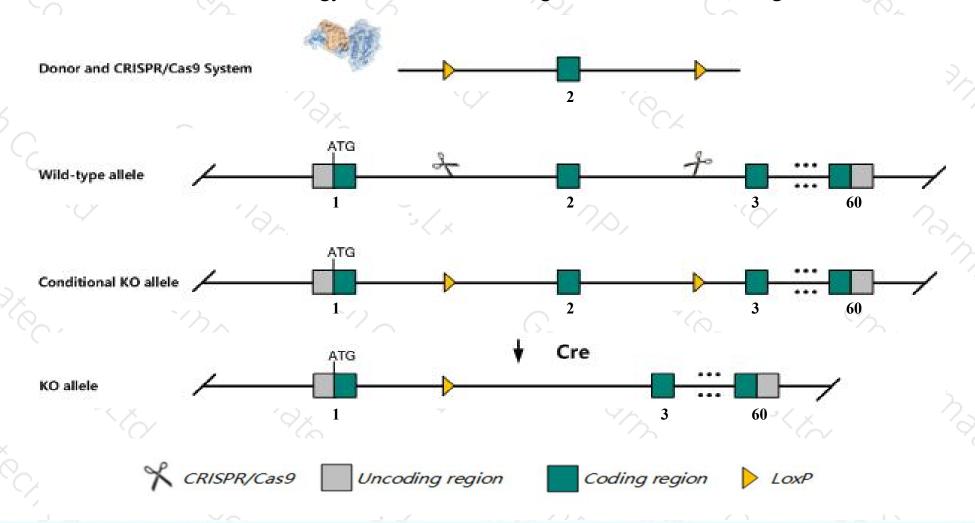
Strain background

C57BL/6JGpt

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Col24a1 gene. The schematic diagram is as follows:



Technical routes



- The Col24a1 gene has 4 transcripts. According to the structure of Col24a1 gene, exon2 of Col24a1-201 (ENSMUST00000029848.4) transcript is recommended as the knockout region. The region contains 65bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Col24a1* 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.

Notice



- ➤ The *Col24a1* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)



Col24a1 collagen, type XXIV, alpha 1 [Mus musculus (house mouse)]

Gene ID: 71355, updated on 3-Feb-2019

Summary

↑ ?

Official Symbol Col24a1 provided by MGI

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

Primary source MGI:MGI:1918605

See related Ensembl:ENSMUSG00000028197

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 5430404K19Rik

Summary This gene encodes the alpha-1 subunit of type XXIV collagen, one of the low abundance fibril-forming collagens found in cartilage. The

encoded protein has structural features of invertebrate fibrillar collagens and is expressed predominantly in bone tissue. Alternate splicing of

this gene results in multiple transcript variants encoding different isoforms. [provided by RefSeq, Dec 2015]

Expression Biased expression in limb E14.5 (RPKM 3.8), frontal lobe adult (RPKM 3.5) and 7 other tissuesSee more

Orthologs <u>human</u> all

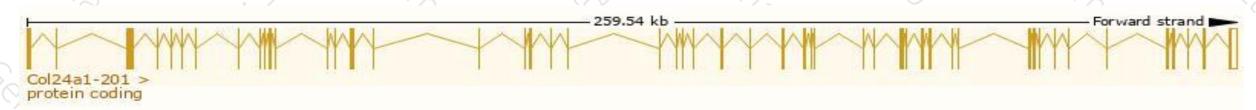
Transcript information (Ensembl)



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

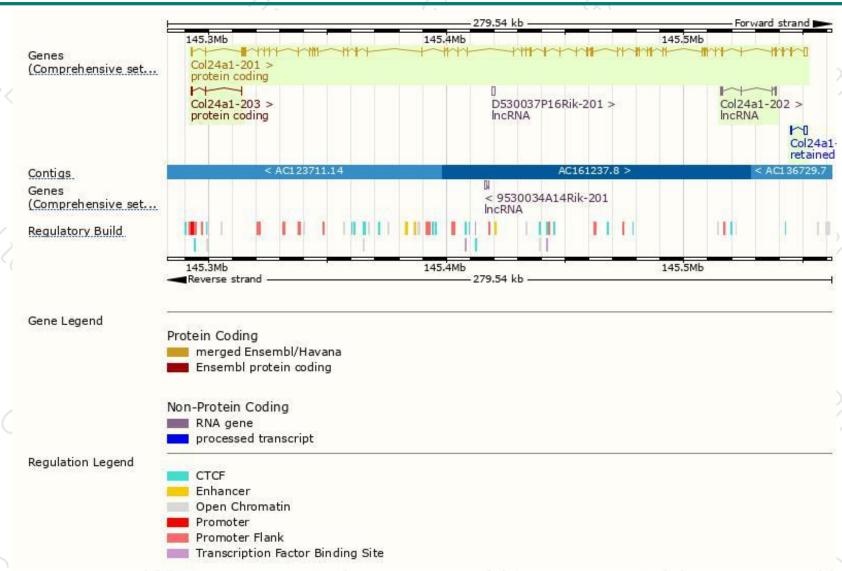
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col24a1-201	ENSMUST00000029848.4	7143	<u>1733aa</u>	Protein coding	CCDS17892	Q30D77	TSL:1 GENCODE basic APPRIS P1
Col24a1-203	ENSMUST00000139001.1	330	<u>62aa</u>	Protein coding		D3Z1L7	CDS 3' incomplete TSL:3
Col24a1-204	ENSMUST00000142441.1	1777	No protein	Retained intron	2	9 1	TSL:1
Col24a1-202	ENSMUST00000127379.1	620	No protein	IncRNA	<u>.</u>	12	TSL:3

The strategy is based on the design of Col24a1-201 transcript, The transcription is shown below



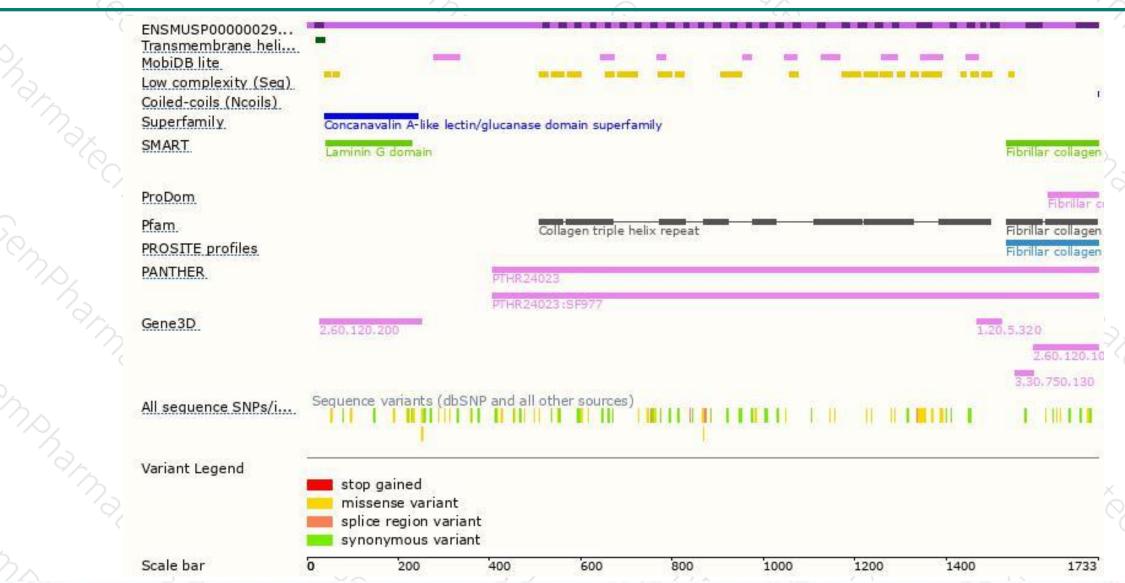
Genomic location distribution





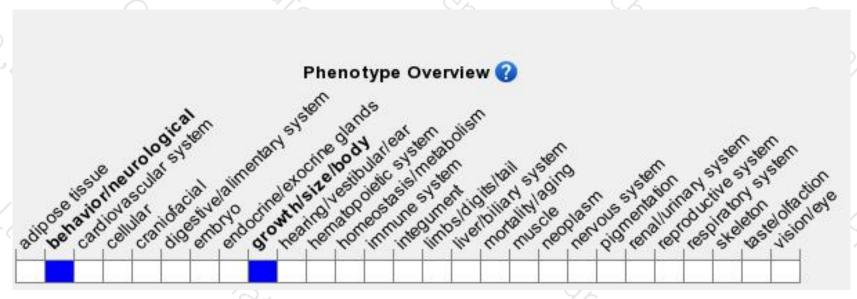
Protein domain





Mouse phenotype description(MGI)





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



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





