

Col5a1 Cas9-CKO Strategy

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Reviewer	Huan Wang
Design Date:	2020-4-16

Project Overview

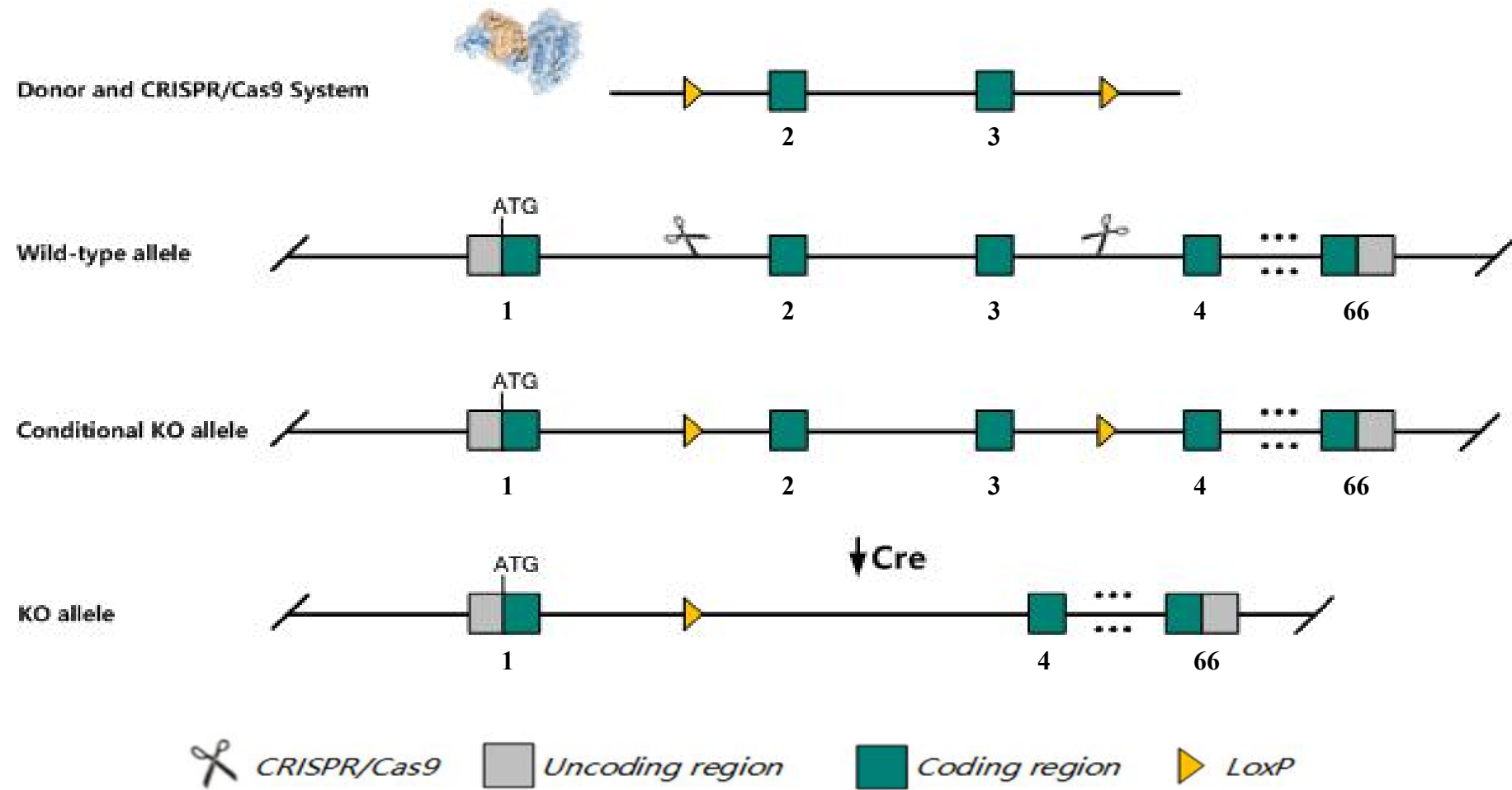
Project Name	<i>Col5a1</i>
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Project type	Cas9-CKO
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Strain background	C57BL/6JGpt
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Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Col5a1* gene. The schematic diagram is as follows:



The *Col5a1* gene has 3 transcripts. According to the structure of *Col5a1* gene, exon2-exon3 of *Col5a1-201* (ENSMUST00000028280.13) transcript is recommended as the knockout region. The region contains 382bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Col5a1* 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 lethality around e10-11 due to cardiovascular insufficiency and lack of collagen fibril formation. heterozygotes exhibit poorly organized and less dense fibers in the dermis and reduced skin tensile strength and are a model for ehlers-danlos syndrome.

Transcript *Col5a1-202,203* may not be affected.

The *Col5a1* gene is located on the Chr2. 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.

Col5a1 collagen, type V, alpha 1 [Mus musculus (house mouse)]

Gene ID: 12831, updated on 13-Mar-2020

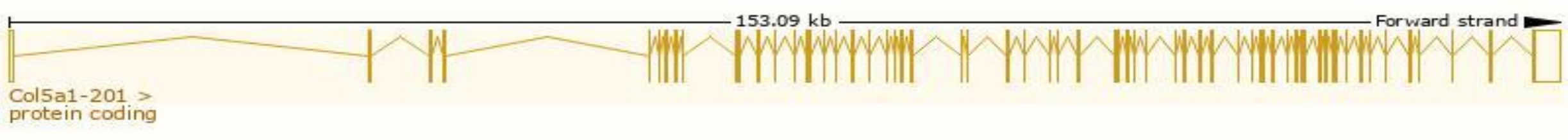
Summary**Official Symbol** Col5a1 provided by [MGI](#)**Official Full Name** collagen, type V, alpha 1 provided by [MGI](#)**Primary source** [MGI:MGI:88457](#)**See related** [Ensembl:ENSMUSG00000026837](#)**Gene type** protein coding**RefSeq status** VALIDATED**Organism** [Mus musculus](#)**Lineage** Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus**Also known as** AI413331**Expression** Broad expression in limb E14.5 (RPKM 82.9), bladder adult (RPKM 28.4) and 15 other tissues [See more](#)**Orthologs** [human](#) [all](#)

Transcript information Ensembl

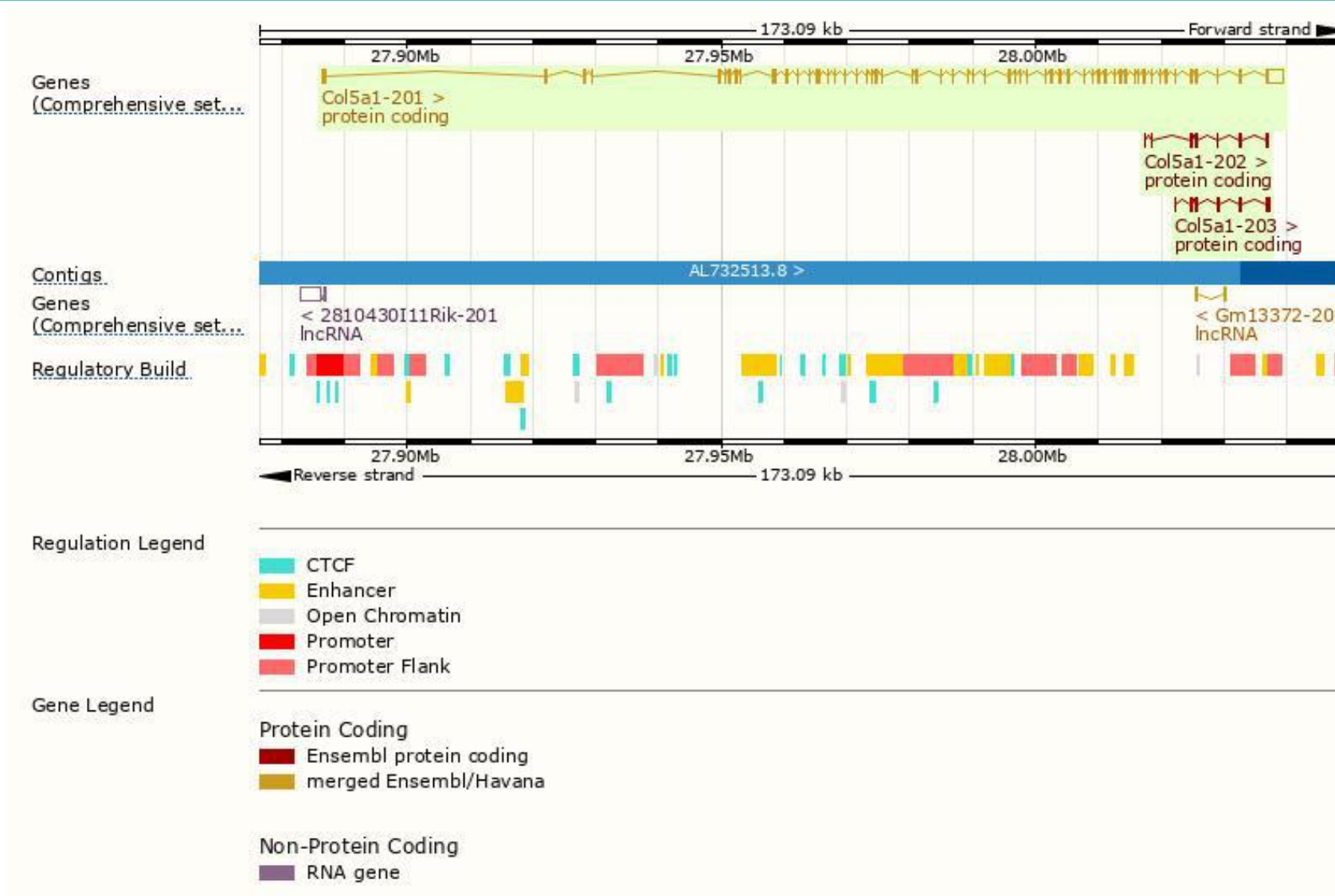
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col5a1-201	ENSMUST00000028280.13	8406	1838aa	Protein coding	CCDS15831	B1AWB9 O88207	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Col5a1-203	ENSMUST00000023885.1	1281	289aa	Protein coding	-	-	CDS 5' incomplete
Col5a1-202	ENSMUST000000145423.1	1049	349aa	Protein coding	-	A3KGE7	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:5

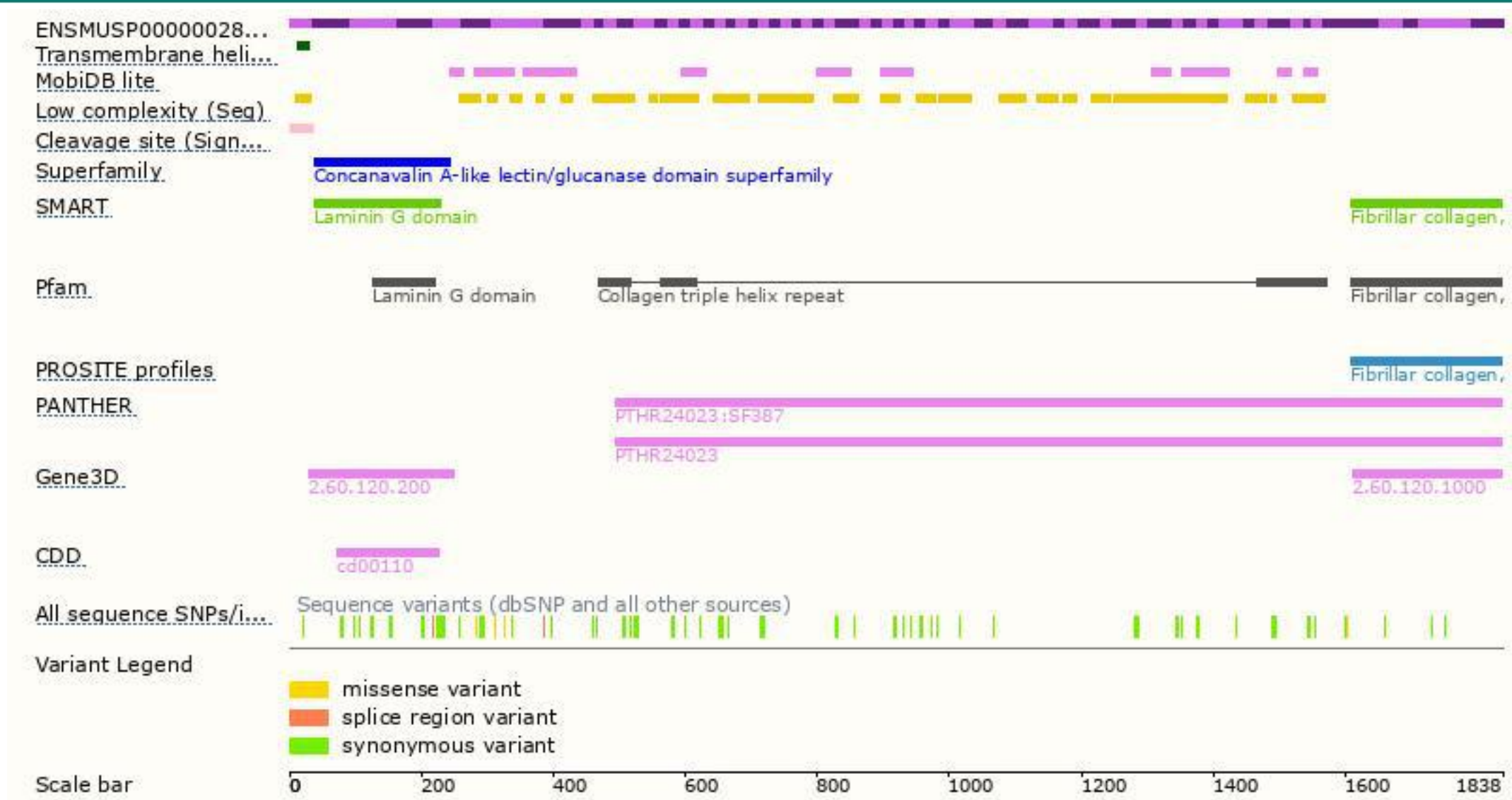
The strategy is based on the design of *Col5a1-201* transcript,the transcription is shown below



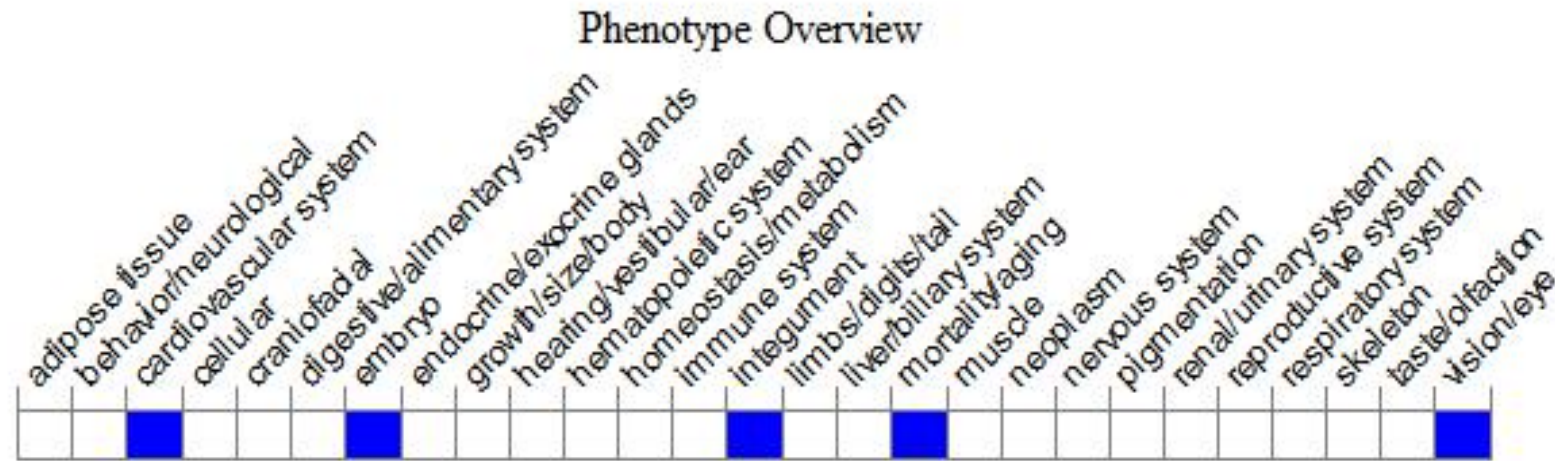
Genomic location distribution



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 lethality around E10-11 due to cardiovascular insufficiency and lack of collagen fibril formation. Heterozygotes exhibit poorly organized and less dense fibers in the dermis and reduced skin tensile strength and are a model for Ehlers-Danlos Syndrome.

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