Col3a1 Cas9-CKO Strategy

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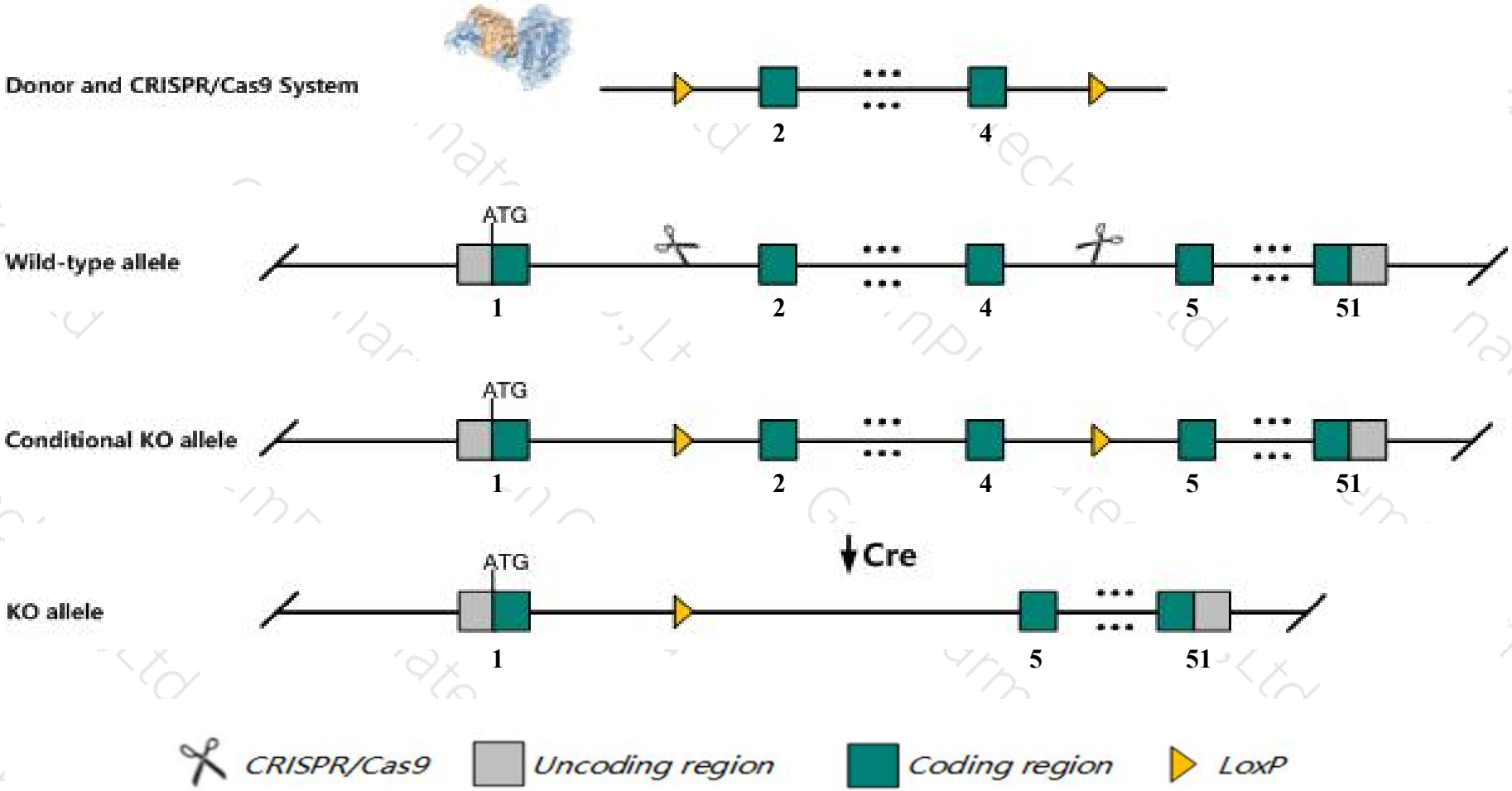
Project Overview

Project Name	***Col3a1***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Col3a1* gene. The schematic diagram is as follows:



Technical routes

- The *Col3a1* gene has 7 transcripts. According to the structure of *Col3a1* gene, exon2-exon4 of *Col3a1-201* (ENSMUST00000087883.12) transcript is recommended as the knockout region.The region contains 365bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Col3a1* 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

- According to the existing MGI data,Most homozygous mutants die within 48 hours after birth. Surviving mutants have reduced body size, skin lesions, enlarged intestines, and die by 6 months of age from ruptured blood vessels. Occasionally intestinal rupture also results in early death. Heterozygotes exhibit tight skin.

- The *Col3a1* 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）

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Col3a1 collagen, type III, alpha 1 [Mus musculus (house mouse)]

Gene ID: 12825, updated on 2-Apr-2019

Summary

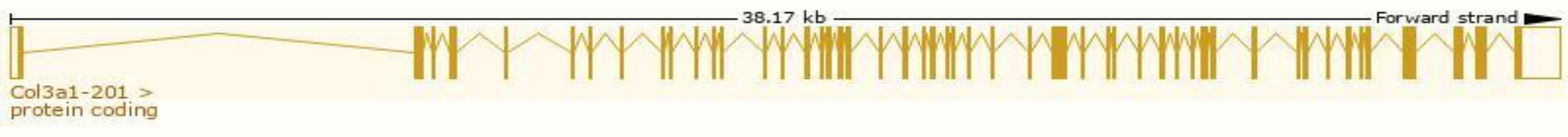
Official Symbol	Col3a1 provided by MGI
Official Full Name	collagen, type III, alpha 1 provided by MGI
Primary source	MGI:MGI:88453
See related	Ensembl:ENSMUSG00000026043
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW550625, Col3a-1, Tsk-2, Tsk2
Summary	This gene encodes the alpha-1 subunit of the fibril-forming type III collagen found in bone, cartilage, dentin, tendon, bone marrow stroma and other connective tissue. The encoded protein forms homotrimeric type III procollagen that undergoes proteolytic processing during fibril formation. A majority of mice lacking the encoded protein die within two days of birth but about 5% of the animals survive to adulthood. The surviving mice exhibit severe cortical malformation and experience significantly shorter lifespan. The mutant mouse named "tight skin 2" exhibiting systemic sclerosis phenotype was found to harbor a missense point mutation in this gene. A pseudogene of this gene has been defined on chromosome 8. [provided by RefSeq, Nov 2015]
Expression	Biased expression in bladder adult (RPKM 483.4), limb E14.5 (RPKM 351.2) and 13 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

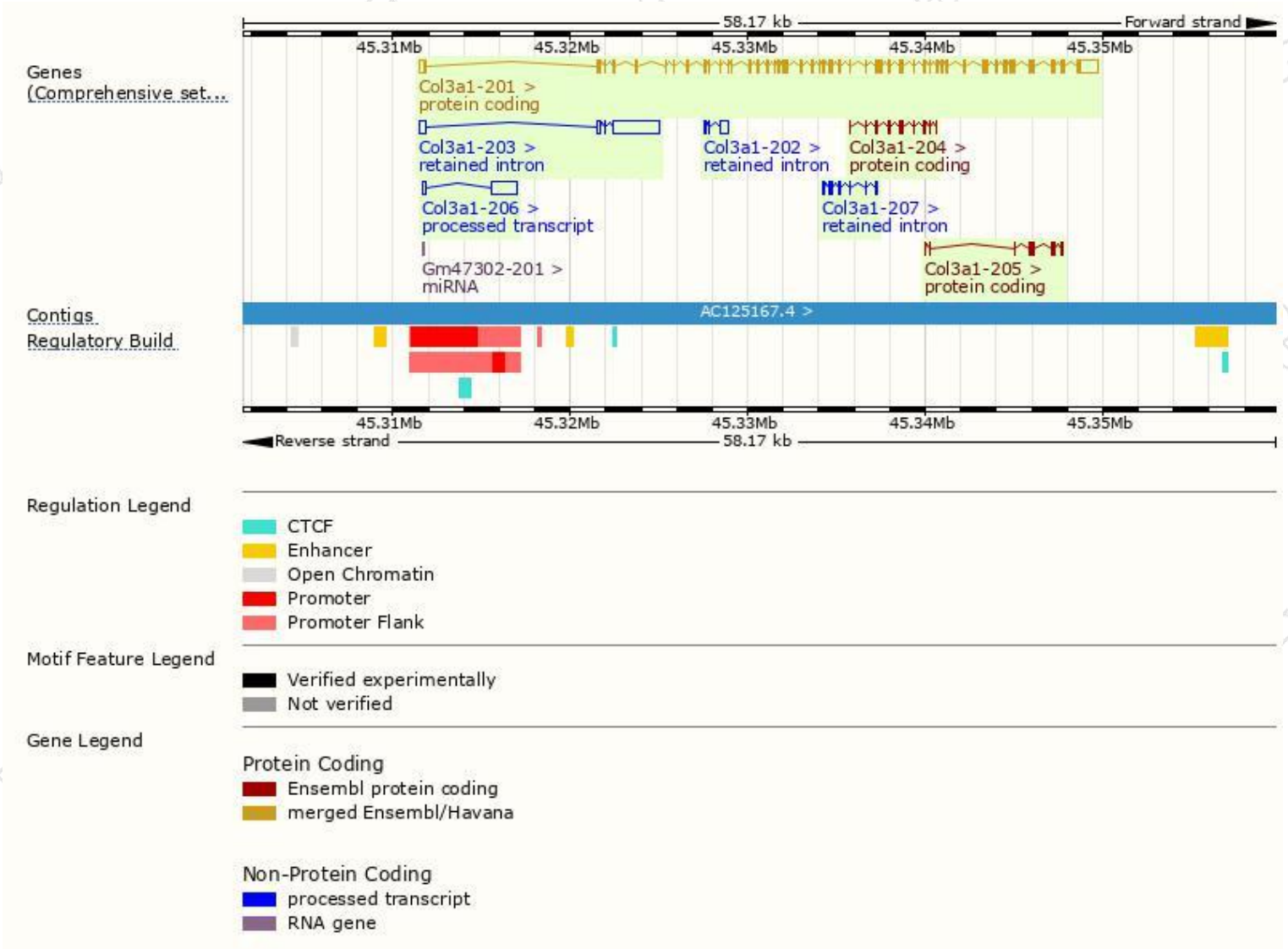
The gene has 7 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col3a1-201	ENSMUST00000087883.12	5564	1464aa	Protein coding	CCDS35554	P08121 Q3TVI5	TSL:1 GENCODE basic APPRIS P1
Col3a1-205	ENSMUST00000186021.1	737	245aa	Protein coding	-	A0A087WPJ5	5' and 3' truncations in transcript evidence prevent annotation of the start and the end of the CDS. CDS 5' and 3' incomplete TSL:3
Col3a1-204	ENSMUST00000185687.1	583	195aa	Protein coding	-	A0A087WPS3	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
Col3a1-206	ENSMUST00000189818.1	1610	No protein	Processed transcript	-	-	TSL:3
Col3a1-203	ENSMUST00000129611.1	3159	No protein	Retained intron	-	-	TSL:2
Col3a1-202	ENSMUST00000123204.1	570	No protein	Retained intron	-	-	TSL:3
Col3a1-207	ENSMUST00000190943.1	431	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Col3a1-201* transcript,The transcription is shown below

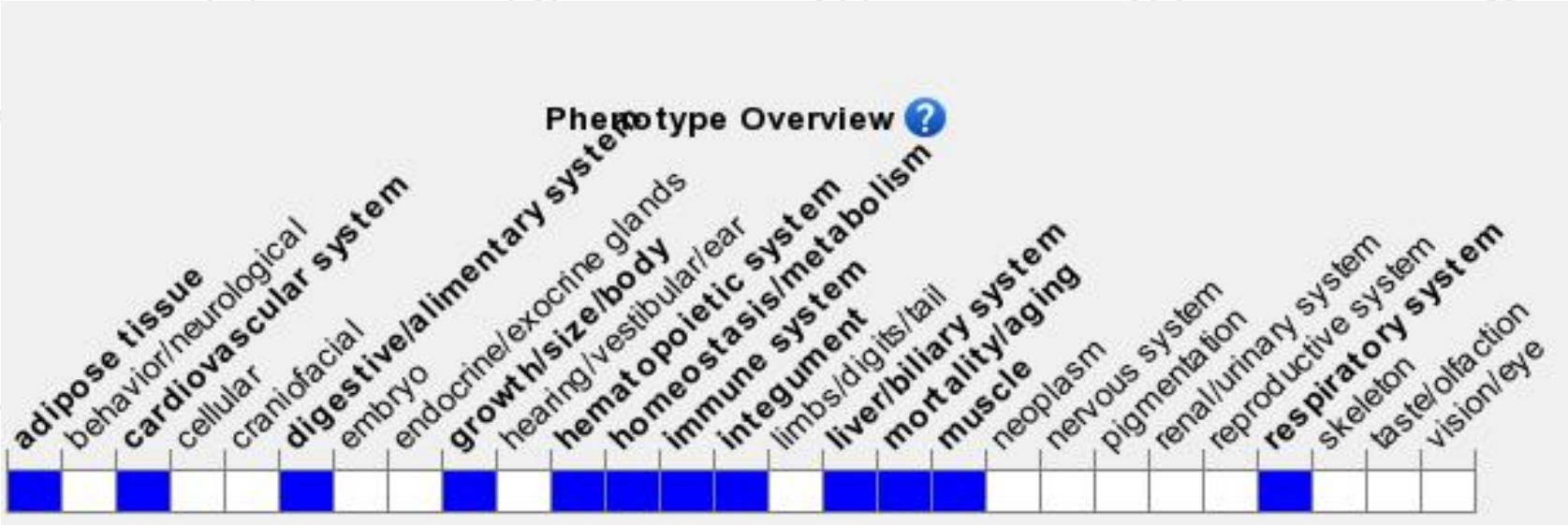


Genomic location distribution



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,Most homozygous mutants die within 48 hours after birth. Surviving mutants have reduced body size, skin lesions, enlarged intestines, and die by 6 months of age from ruptured blood vessels. Occasionally intestinal rupture also results in early death. Heterozygotes exhibit tight skin.

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

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