

Col14a1 Cas9-CKO Strategy

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Reviewer:

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Design Date:

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Project Overview

Project Name

Col14a1

Project type

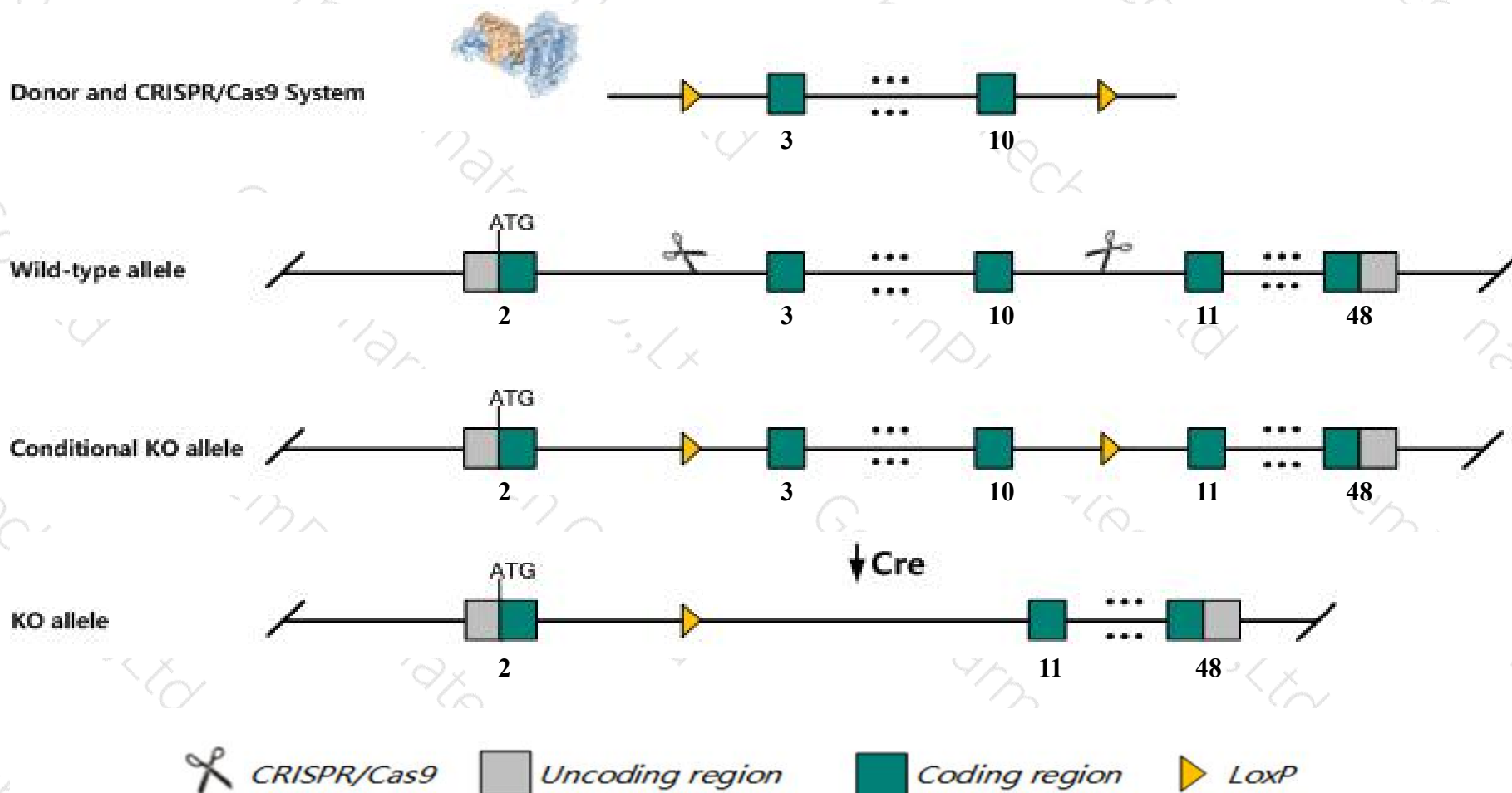
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Coll4a1* gene. The schematic diagram is as follows:



Technical routes

- The *Coll4a1* gene has 4 transcripts. According to the structure of *Coll4a1* gene, exon3-exon10 of *Coll4a1*-203 (ENSMUST00000110221.9) transcript is recommended as the knockout region. The region contains 1106bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Coll4a1* 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, Mice homozygous for a null mutation display abnormal tendon morphology and abnormal biomechanical properties of the skin and tendons.
- Transcript 204 CDS 5' incomplete the influences is unknown.
- The *Coll4a1* 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)

Col14a1 collagen, type XIV, alpha 1 [*Mus musculus* (house mouse)]

Gene ID: 12818, updated on 13-Mar-2020

Summary

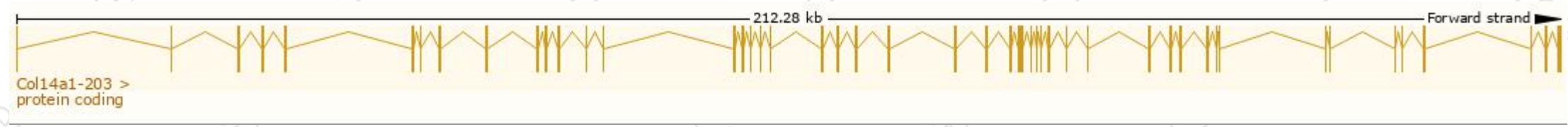
Official Symbol	Col14a1 provided by MGI
Official Full Name	collagen, type XIV, alpha 1 provided by MGI
Primary source	MGI:MGI:1341272
See related	Ensembl:ENSMUSG00000022371
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	AW108078; 5730412L22Rik
Expression	Broad expression in bladder adult (RPKM 45.3), limb E14.5 (RPKM 16.4) and 15 other tissues See more
Orthologs	human all

Transcript information (Ensembl)

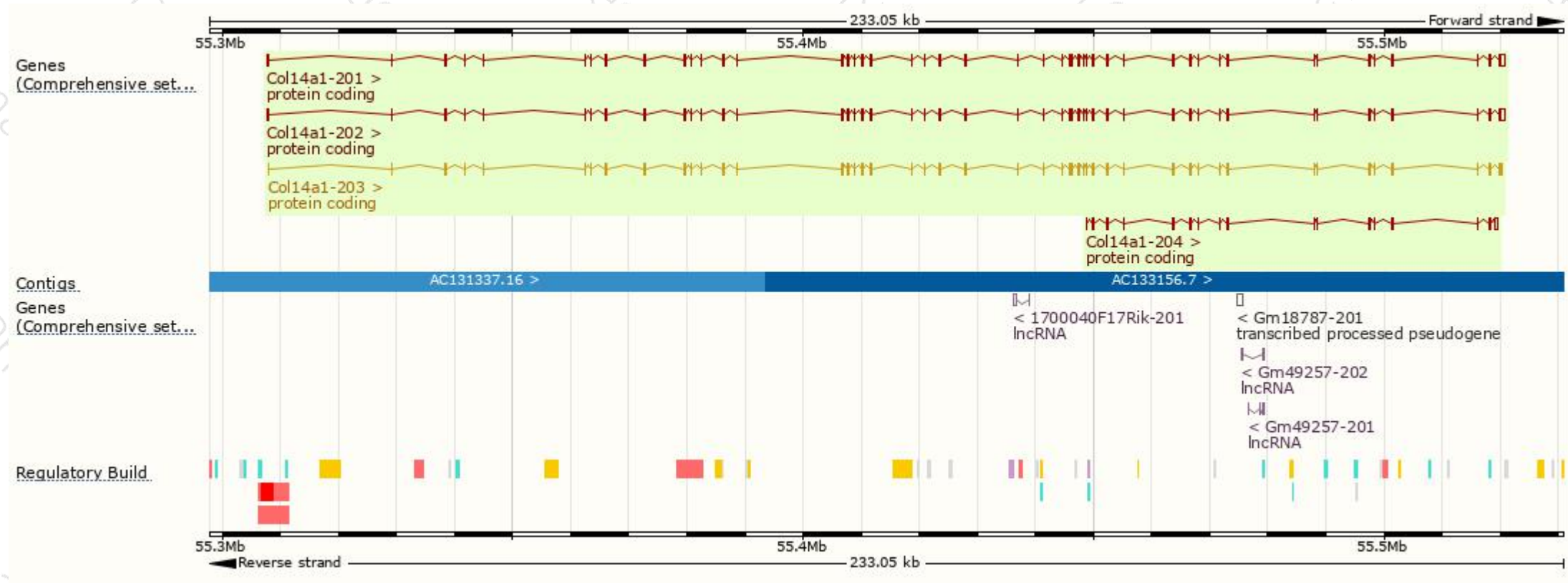
The gene has 4 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col14a1-203	ENSMUST00000110221.9	5679	1794aa	Protein coding	CCDS37076	B7ZNH7	TSL:1 Gencode basic APPRIS P2
Col14a1-201	ENSMUST00000023053.11	6457	1797aa	Protein coding	-	Q80X19	TSL:5 Gencode basic APPRIS ALT1
Col14a1-202	ENSMUST00000110217.9	6457	1798aa	Protein coding	-	K3W4R4	TSL:5 Gencode basic APPRIS ALT1
Col14a1-204	ENSMUST00000125416.1	2085	521aa	Protein coding	-	F7D5Y4	CDS 5' incomplete TSL:1

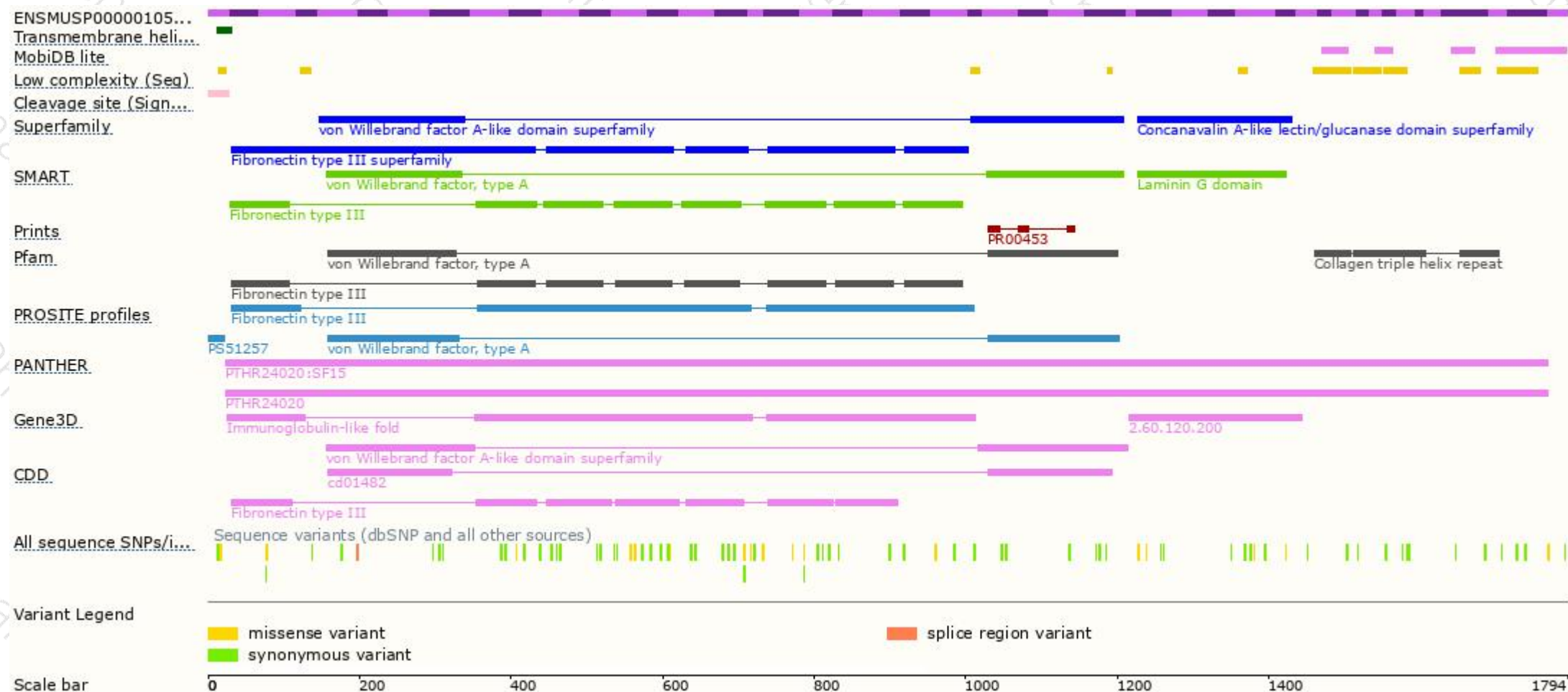
The strategy is based on the design of *Col14a1-203* transcript,The transcription is shown below



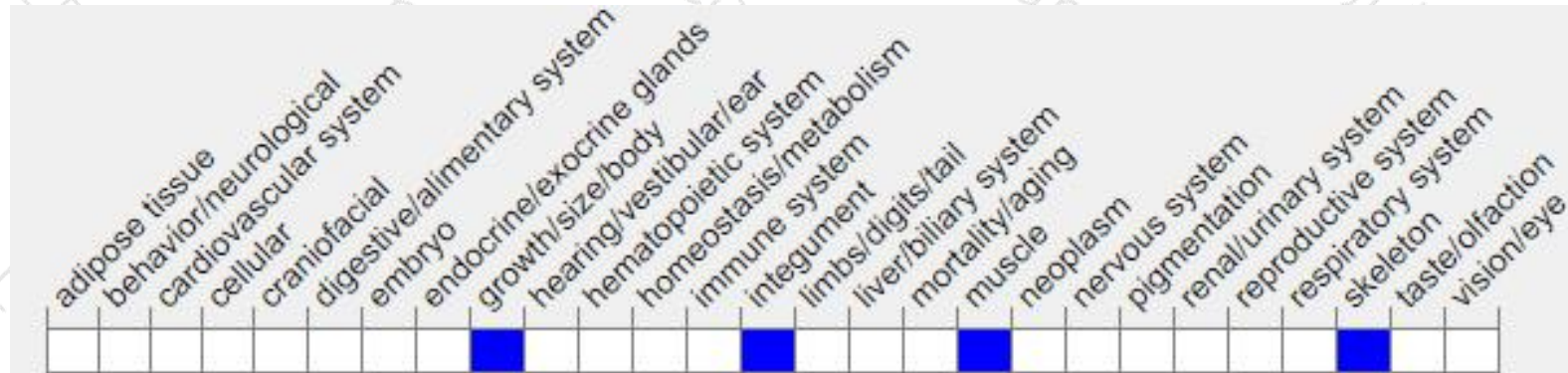
Genomic location distribution



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, Mice homozygous for a null mutation display abnormal tendon morphology and abnormal biomechanical properties of the skin and tendons.

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

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