

A white mouse is shown inside a green nitrile glove, which is being held by a hand. The mouse is looking forward and appears to be holding a small object in its mouth. The background is a soft-focus blue with a faint DNA double helix structure on the left side.

Models to
Accelerate Innovation



***Col2a1-P2A-CreERT2* Mouse Model Strategy**

CRISPR-Cas9 Technology

Designer

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Reviewer

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Date

2024-04-08

Project Overview



Project Name

Col2a1-P2A-CreERT2

Project Type

Cas9-KI

Background

N000013 C57BL/6JGpt

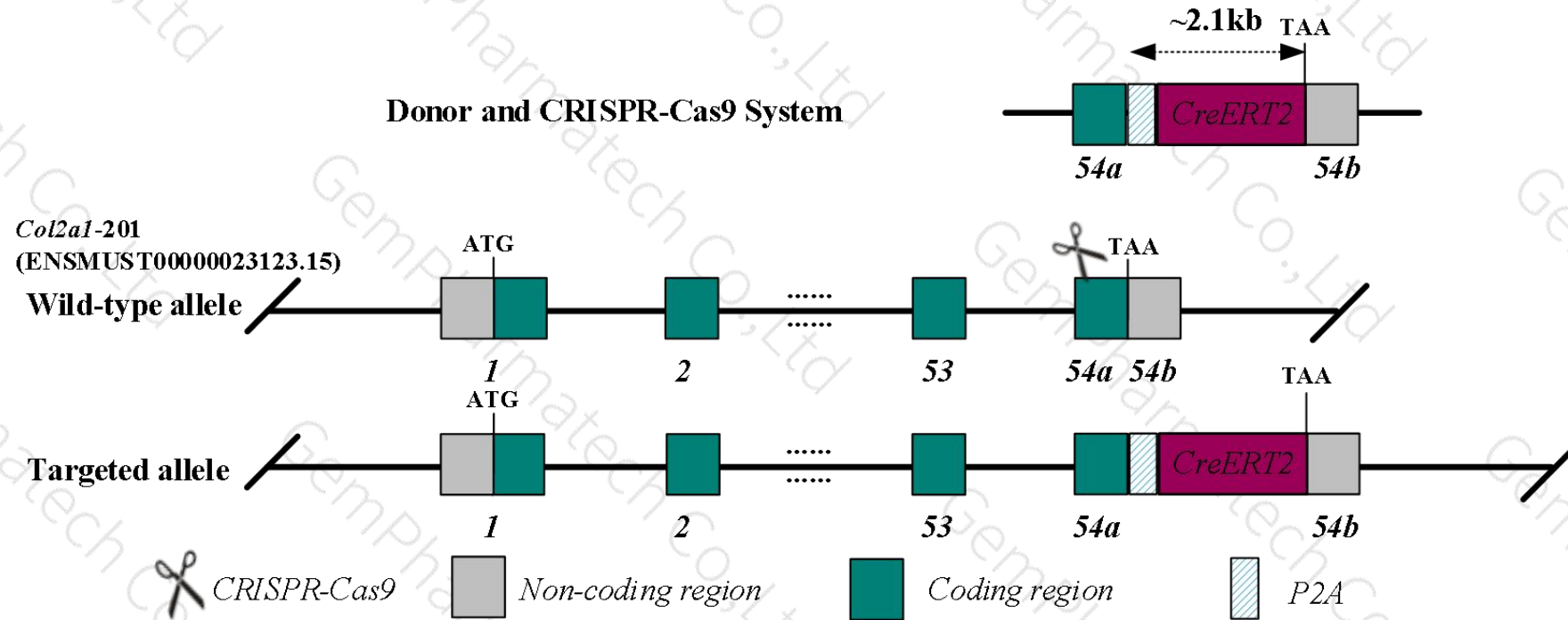
Timeline

5-8 Months



Strategy

This model will use CRISPR-Cas9 technology to edit the mouse *Col2a1* gene and the schematic diagram is as follows:





Technical Description

- The Mouse *Col2a1* gene has 10 transcripts. The transcript *Col2a1*-201 (ENSMUST00000023123.15) is selected for this strategy. It contains 54 exons and codes 1487 aa. The ATG is located in exon 1, and the TAA is located in exon 54.
- According to the structure of *Col2a1* gene, before inserting *P2A-CreERT2* into the translation stop codon of *Col2a1*-201 (ENSMUST00000023123.15), the length of the inserted fragment is about 2.1 kb.
- The project will use CRISPR-Cas9 technology to modify mouse *Col2a1* gene. Briefly, the donor vector and gRNA will be constructed in vitro. Cas9, donor vector and gRNA will be microinjected into fertilized eggs of C57BL/6JGpt mice to obtain positive F0 generation mice. The F0 positive mice will be bred with C57BL/6JGpt mice to obtain positive F1 mice. Pups from both F0 and F1 generations will be genotyped by PCR, followed by on-target sequencing analysis.

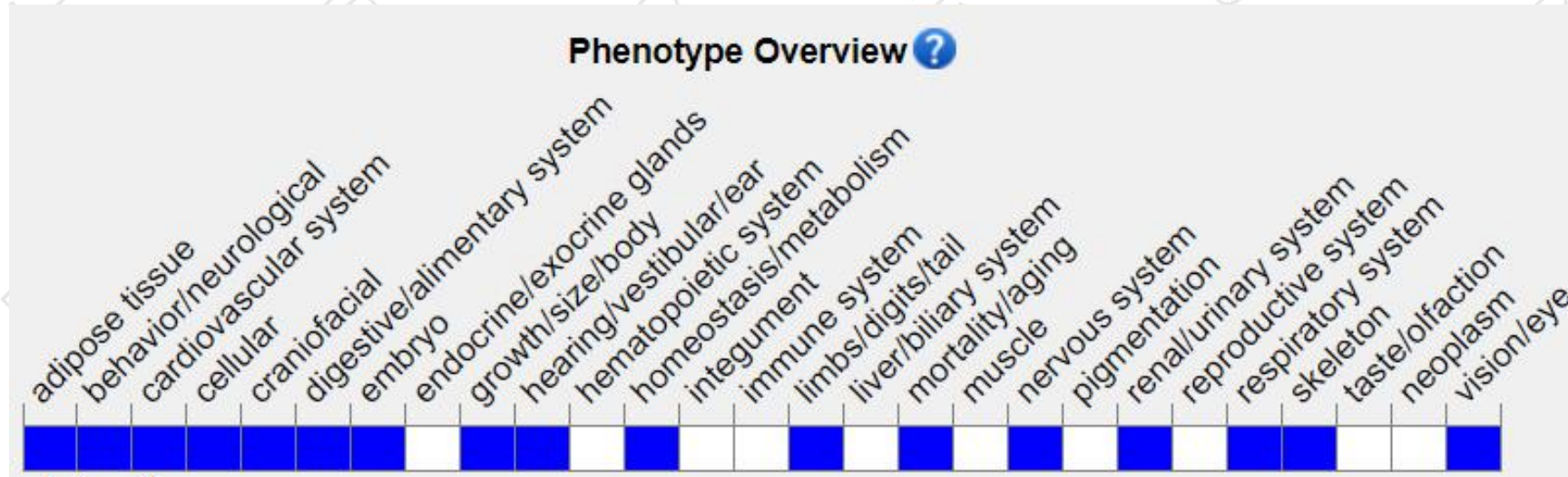


Note

- According to 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.
- There may be 1 to 2 mutations of amino acid in exon 54 of *Col2a1* gene in this strategy.
- Mouse *Col2a1* gene is located on Chr 15. Please take the loci in consideration when breeding this mutation mice with other gene modified strains, if the other gene is also on Chr 15, it may be extremely hard to get double gene positive homozygotes.
- The *CreERT2* and *Col2a1* linked by P2A will expressed under the regulation of endogenous *Col2a1*, and protein products will bedivided into two protein molecules. The anterior protein will carry the peptide translated from the P2A sequence.
- The scheme is designed according to the genetic information in the existing database. Inserting a foreign gene between the 3'UTR and the gene coding region may affect the expression of endogenous and foreign genes. Due to the complexityofbiological processes, it cannot be predicted completely at the present technology level.



Phenotype Information (MGI)



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.

<https://www.informatics.jax.org/marker/MGI:88452>



Basic Information of Mouse *Col2a1* Gene

Gene name	Mouse <i>Col2a1</i>
Gene ID (NCBI)	12824
Gene link (NCBI)	https://www.ncbi.nlm.nih.gov/gene/12824
Gene link (Ensembl)	https://asia.ensembl.org/Mus_musculus/Gene/Summary?g=ENSMUSG00000022483;r=15:97873483-97902576
Chromosome location	Chr 15

Mouse *Col2a1* Gene Information (NCBI)



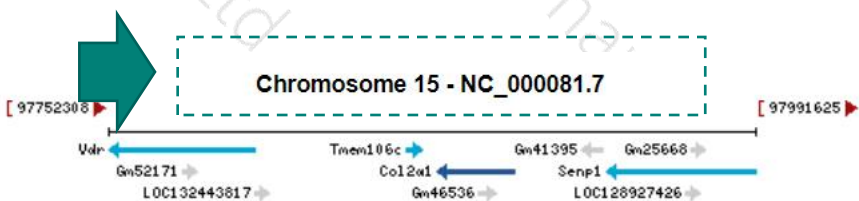
Col2a1 collagen, type II, alpha 1 [*Mus musculus* (house mouse)]

Gene ID: 12824, updated on 26-Mar-2024

[Download Datasets](#)

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:ENSMUSG00000022483 AllianceGenome:MGI:88452
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	Dmm; Lpk; Col2; Del1; Col2a; Col2a-1; 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



Mouse *Col2a1* Transcript Information (Ensembl)



The gene has 10 transcripts. All transcripts are shown below:

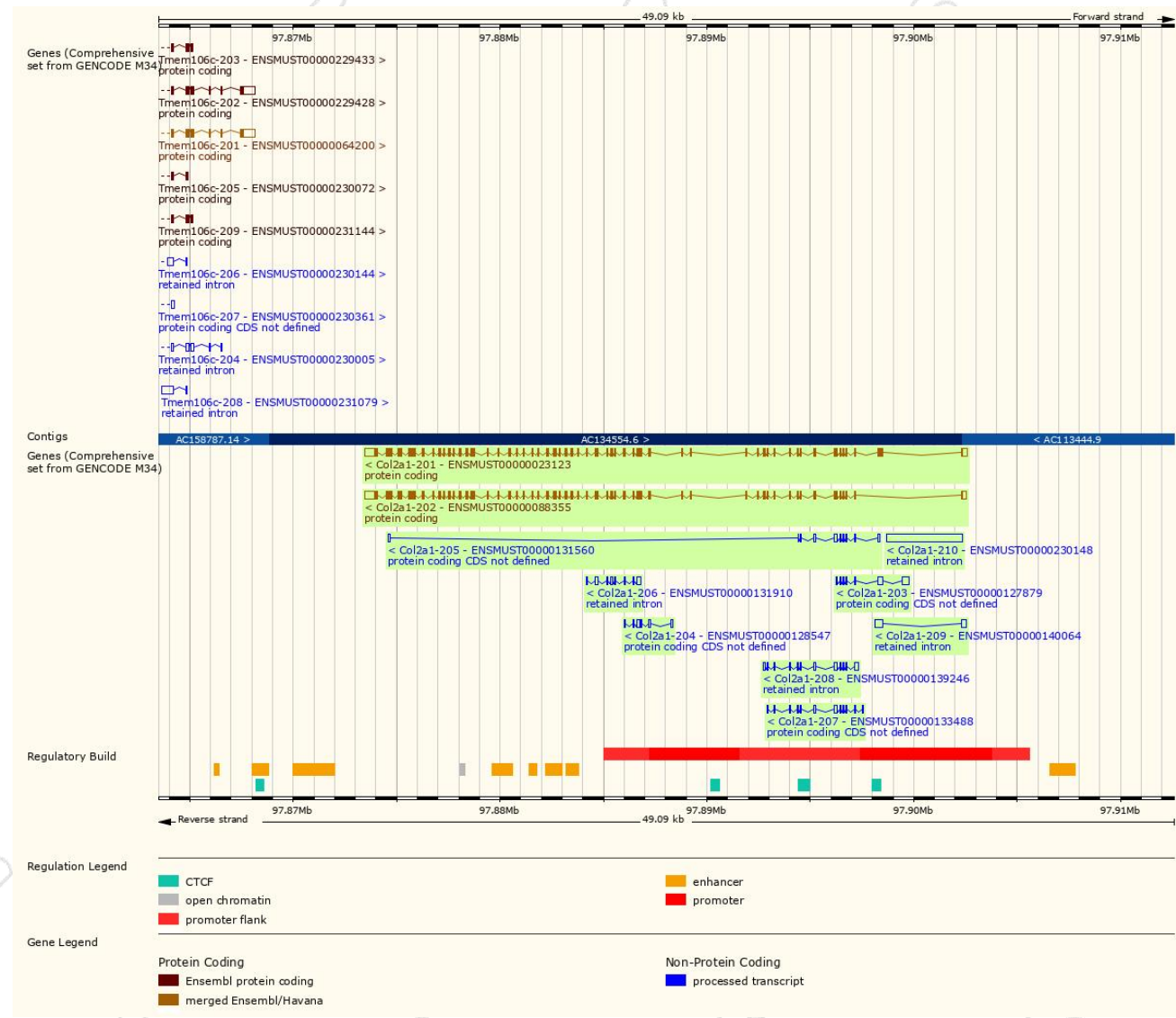
Show/hide columns (1 hidden)					Filter		
Transcript ID	Name	bp	Protein	Biotype	CCDS	UniProt Match	Flags
ENSMUST00000023123.15	Col2a1-201	5104	1487aa	Protein coding	CCDS37189	P28481	Ensembl Canonical Gencode basic APPRIS P1 TSL:1
ENSMUST00000088355.12	Col2a1-202	4862	1419aa	Protein coding	CCDS49716	P28481-7	Gencode basic TSL:1
ENSMUST00000127879.2	Col2a1-203	715	No protein	Protein coding CDS not defined		-	TSL:3
ENSMUST00000133488.8	Col2a1-207	626	No protein	Protein coding CDS not defined		-	TSL:5
ENSMUST00000131560.3	Col2a1-205	575	No protein	Protein coding CDS not defined		-	TSL:3
ENSMUST00000128547.2	Col2a1-204	491	No protein	Protein coding CDS not defined		-	TSL:3
ENSMUST00000230148.2	Col2a1-210	3666	No protein	Retained intron		-	-
ENSMUST00000139246.8	Col2a1-208	809	No protein	Retained intron		-	TSL:5
ENSMUST00000131910.8	Col2a1-206	654	No protein	Retained intron		-	TSL:5
ENSMUST00000140064.2	Col2a1-209	613	No protein	Retained intron		-	TSL:2

The strategy is based on *Col2a1*-201(ENSMUST00000023123.15), which contains 54 exons, is 5104 bps long, and encodes 1487 amino acids.

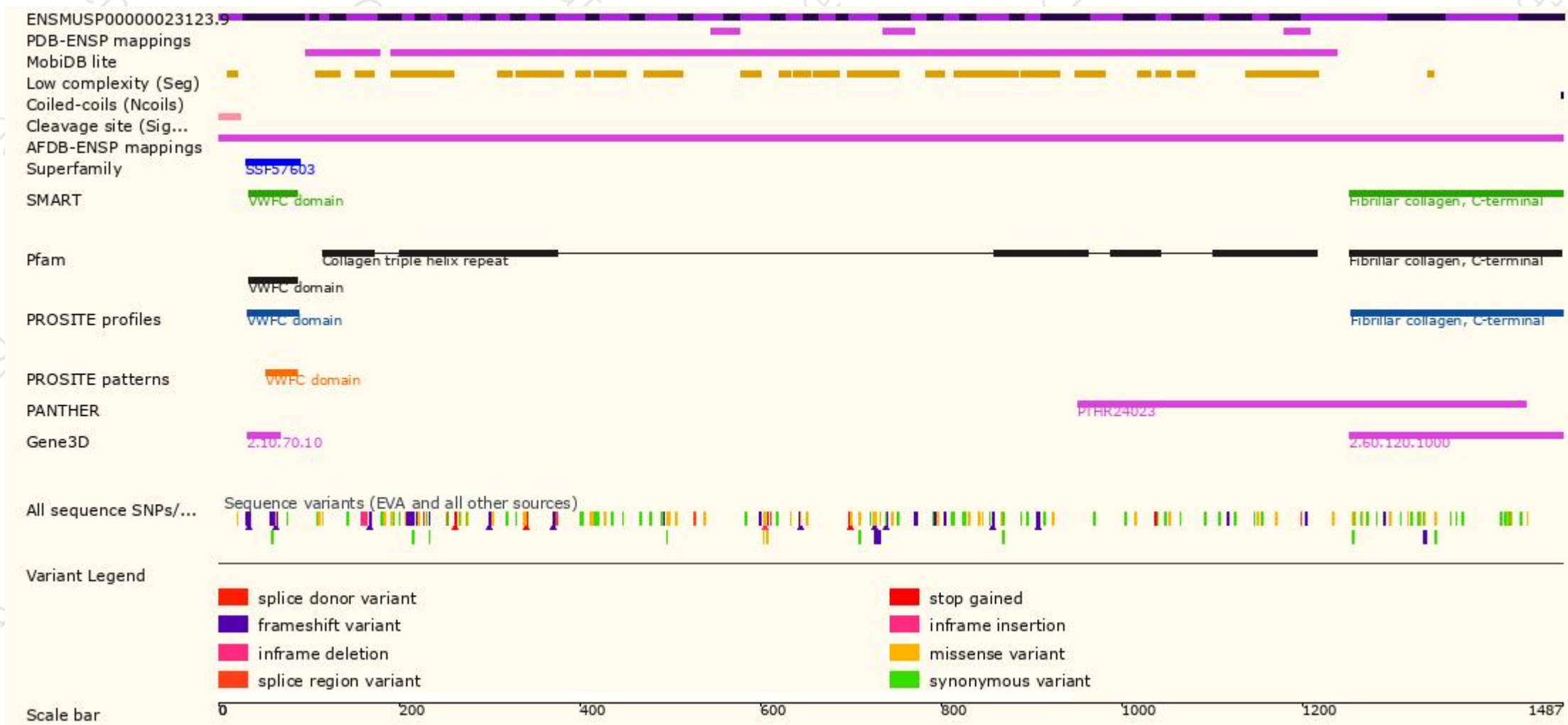




Mouse *Col2a1* Genomic Information



Mouse *Col2a1* Protein Information





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