

Col5a3 Cas9-CKO Strategy

Designer:	Huan Fan
Reviewer	Huan Wang
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Project Overview

Project Name

Col5a3

Project type

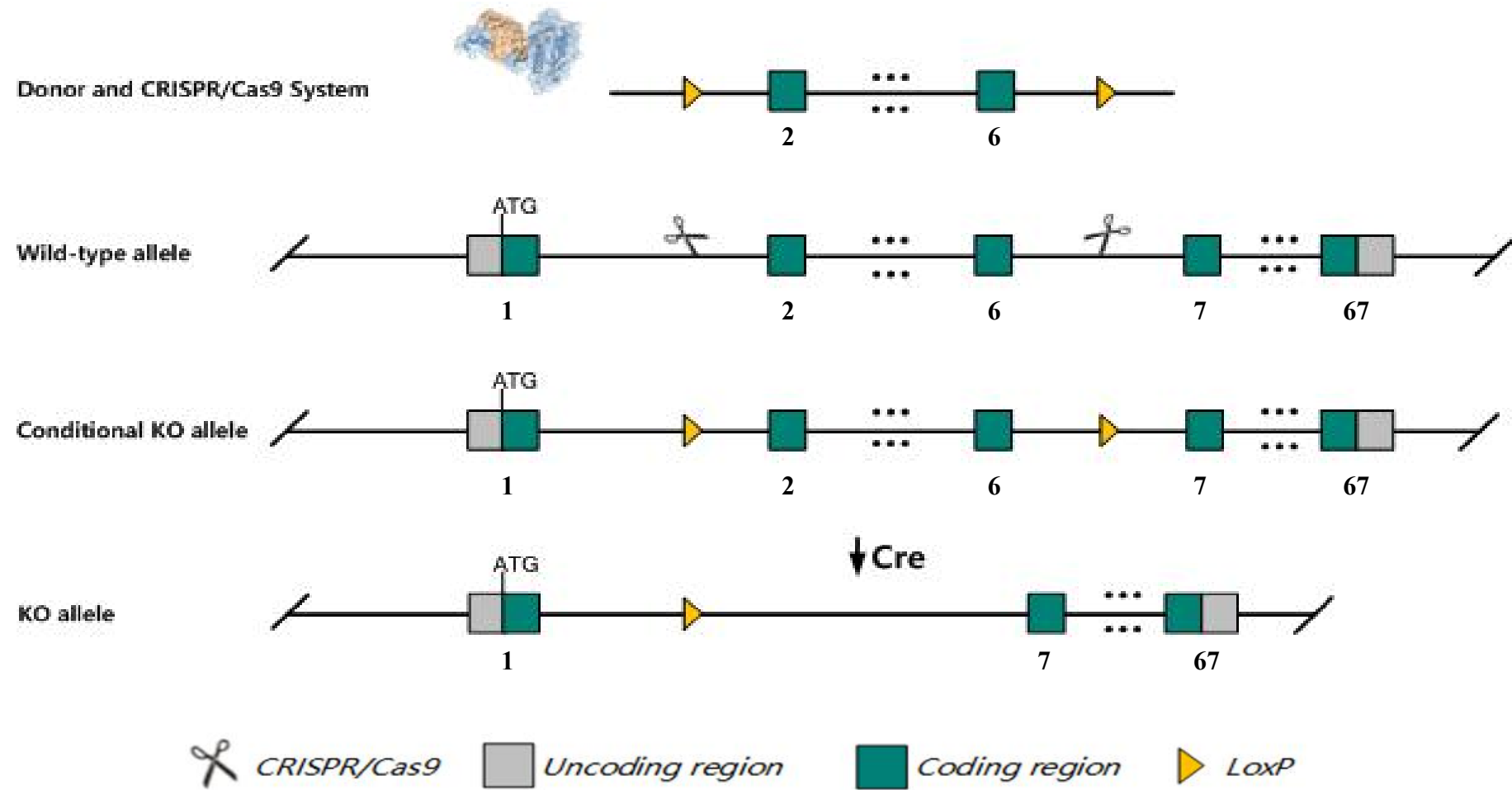
Cas9-CKO

Strain background

C57BL/6JGpt

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Col5a3* gene. The schematic diagram is as follows:



The *Col5a3* gene has 2 transcripts. According to the structure of *Col5a3* gene, exon2-exon6 of *Col5a3-201* (ENSMUST00000004201.7) transcript is recommended as the knockout region. The region contains 764bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Col5a3* 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 show decreased pancreatic beta cell mass, hyperglycemia, hypoinsulinemia, impaired glucose tolerance, insulin resistance and impaired glucose uptake. homozygous females show decreased susceptibility to diet-induced obesity and a thin hypodermal fat layer. *Gm26274* will be deleted.

The *Col5a3* gene is located on the Chr9. 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.

Col5a3 collagen, type V, alpha 3 [Mus musculus (house mouse)]

Gene ID: 53867, updated on 13-Mar-2020

Summary

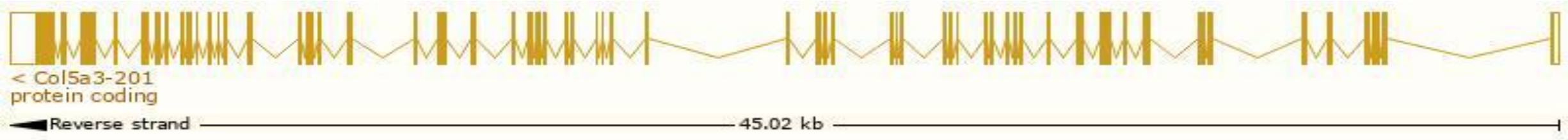
Official Symbol	Col5a3 provided by MGI
Official Full Name	collagen, type V, alpha 3 provided by MGI
Primary source	MGI:MGI:1858212
See related	Ensembl:ENSMUSG000000004098
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Biased expression in subcutaneous fat pad adult (RPKM 60.2), adrenal adult (RPKM 48.3) and 7 other tissues See more
Orthologs	human all

Transcript information Ensembl

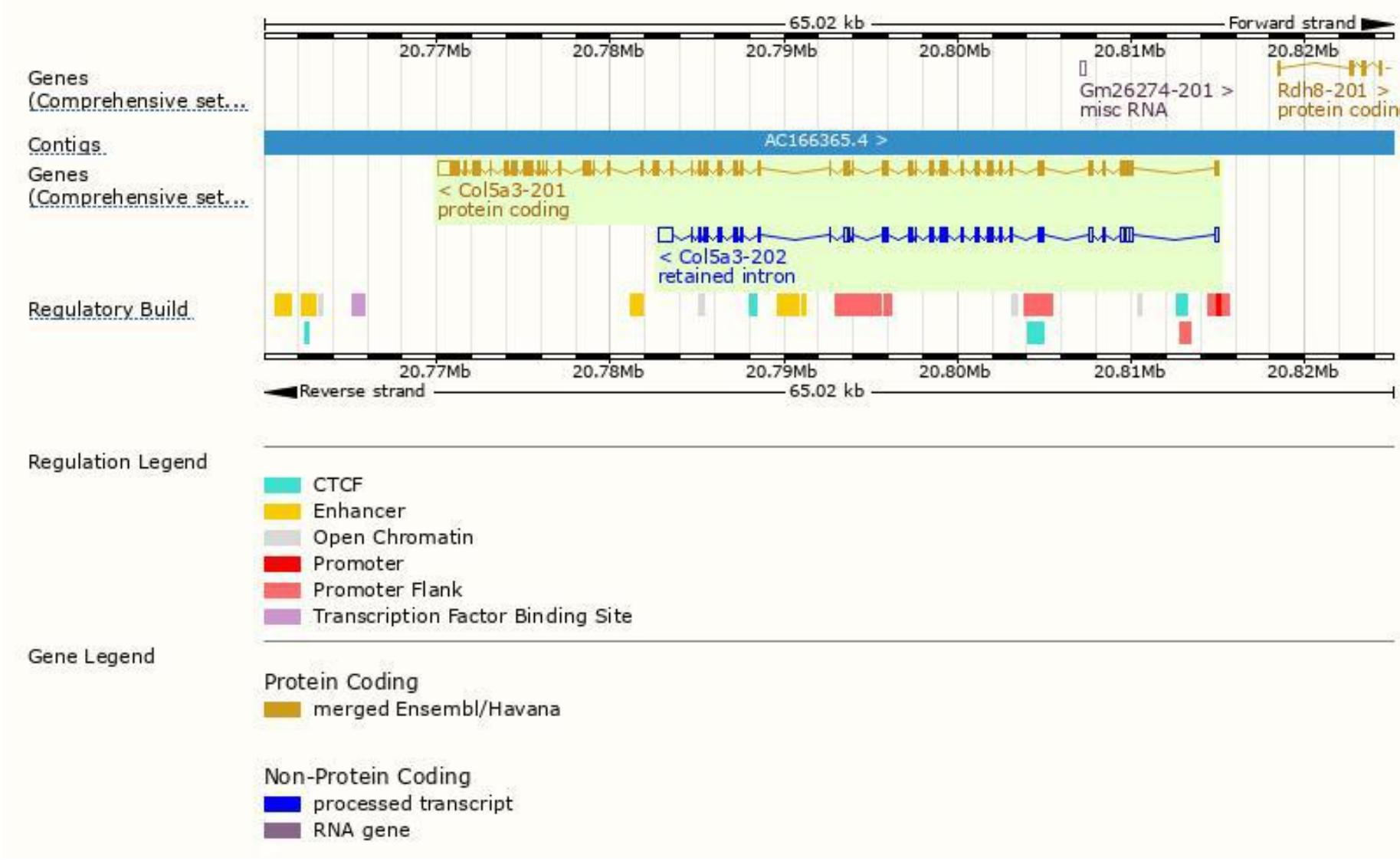
The gene has 2 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col5a3-201	ENSMUST00000004201.7	6119	1739aa	Protein coding	CCDS22883	Q9JLI2	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
Col5a3-202	ENSMUST00000145974.1	3927	No protein	Retained intron	-	-	TSL:2

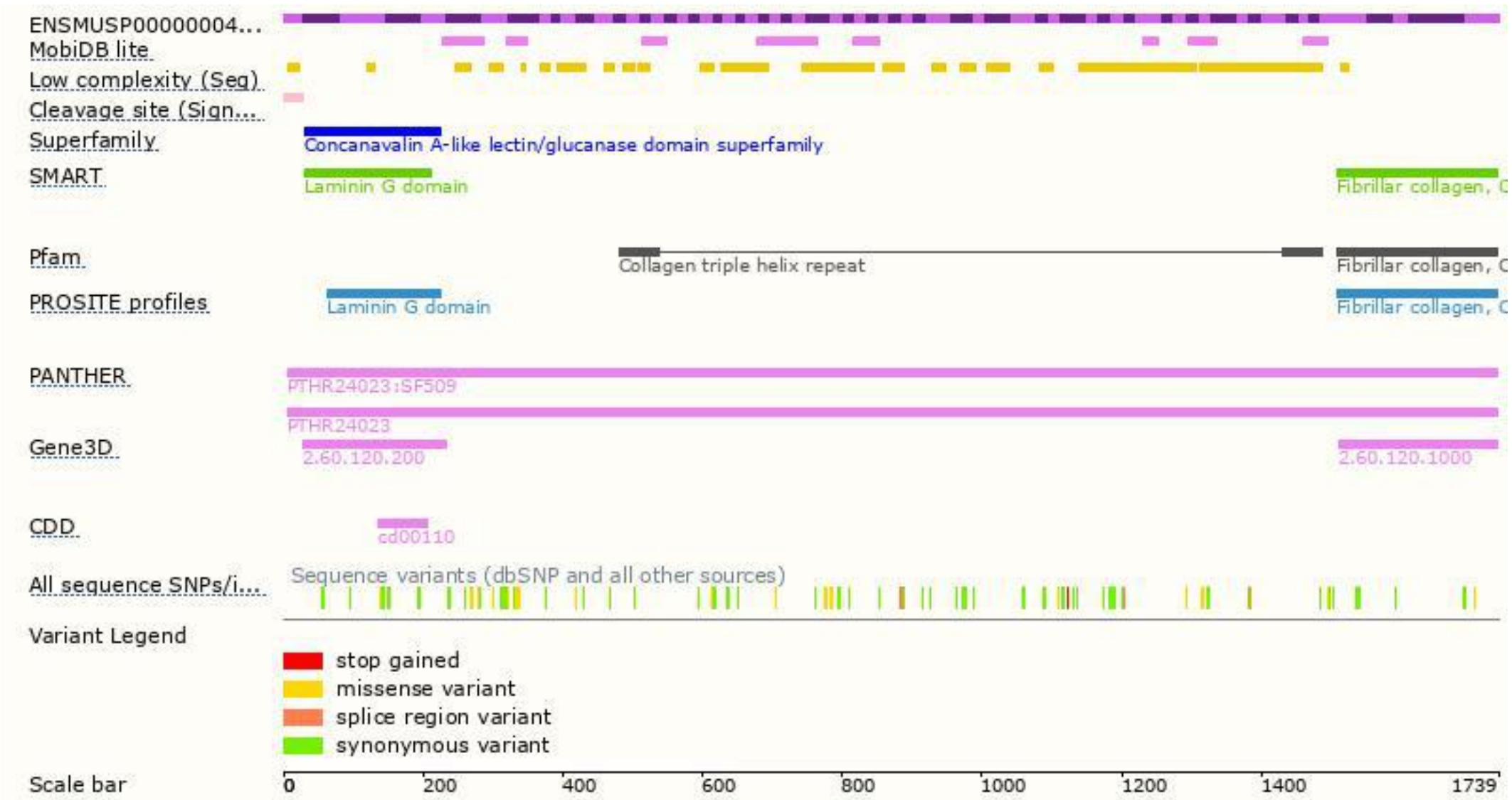
The strategy is based on the design of *Col5a3-201* transcript,the transcription is shown below



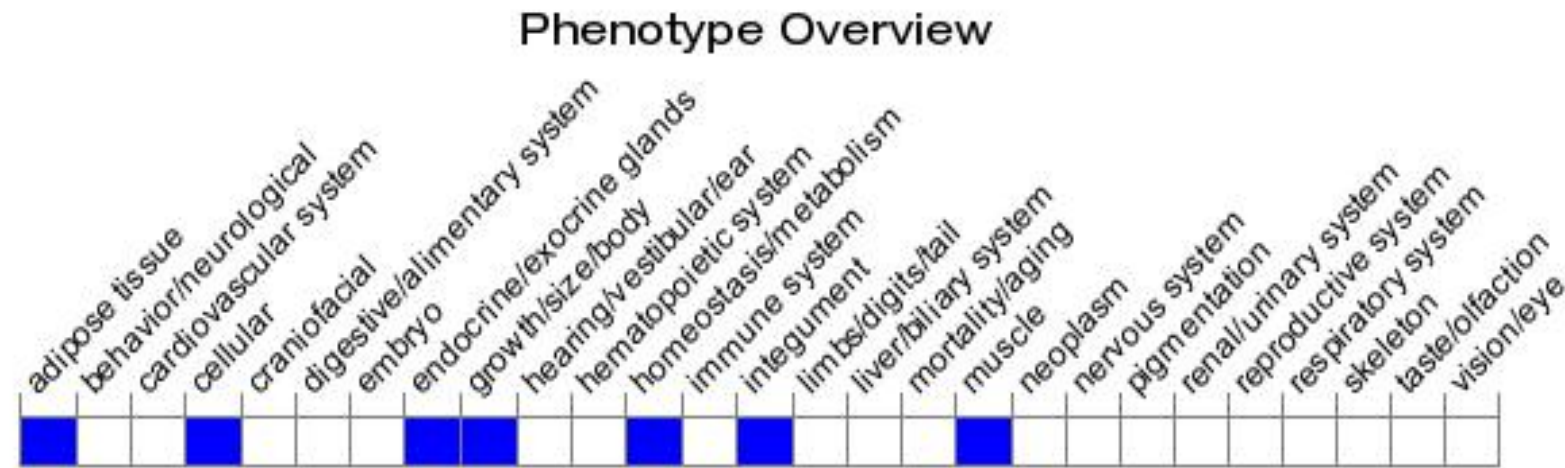
Genomic location distribution



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, mice homozygous for a null mutation show decreased pancreatic beta cell mass, hyperglycemia, hypoinsulinemia, impaired glucose tolerance, insulin resistance and impaired glucose uptake. Homozygous females show decreased susceptibility to diet-induced obesity and a thin hypodermal fat layer.

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

