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GemPharmatech

# *Col4a6* Cas9-CKO Strategy

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# Project Overview

| | |
|---|---|
| **Project Name** | ***Col4a6*** |
| **Project type** | **Cas9-CKO** |
| **Strain background** | **C57BL/6JGpt** |

# Conditional Knockout strategy

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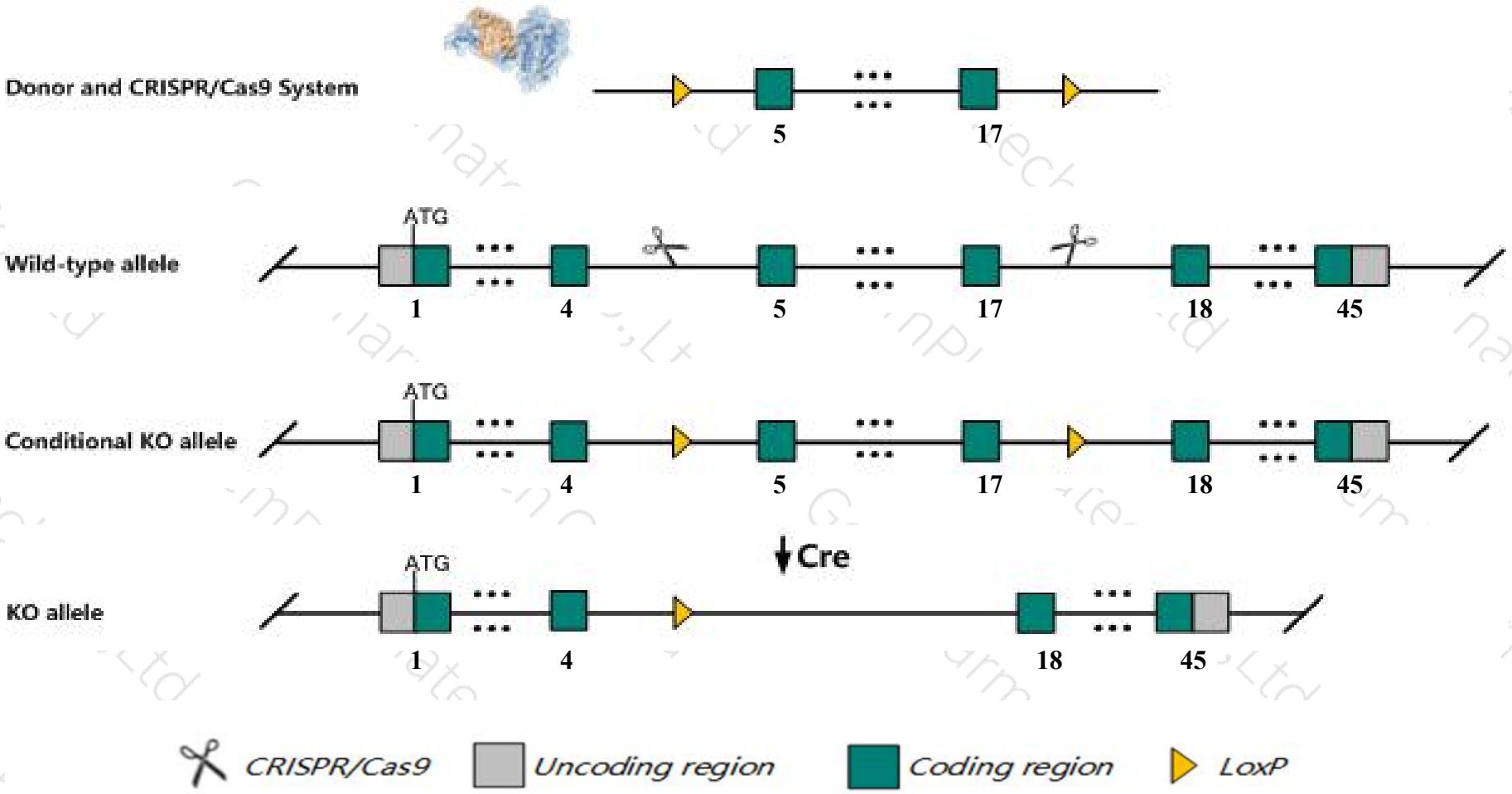
This model will use CRISPR/Cas9 technology to edit the *Col4a6* gene. The schematic diagram is as follows:



# Technical routes

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- The *Col4a6* gene has 3 transcripts. According to the structure of *Col4a6* gene, exon5-exon17 of *Col4a6-201* (ENSMUST00000101205.2) transcript is recommended as the knockout region.The region contains 784bp coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Col4a6* 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,male mice hemizygous for a knock-out allele are viable, fertile and healthy with no apparent defects in the topology of neuromuscular junctions in the diaphragm muscle.

- The *Col4a6* gene is located on the ChrX. 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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**Col4a6 collagen, type IV, alpha 6 [ *Mus musculus* (house mouse) ]**

Gene ID: 94216, updated on 24-Aug-2019

**Summary**

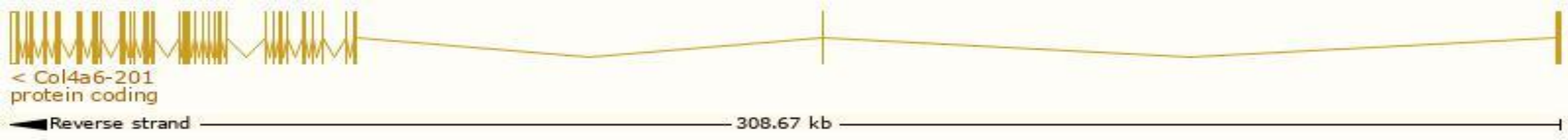
| | |
|---|---|
| **Official Symbol** | Col4a6 provided by MGI |
| **Official Full Name** | collagen, type IV, alpha 6 provided by MGI |
| **Primary source** | MGI:MGI:2152695 |
| **See related** | Ensembl:ENSMUSG00000031273 |
| **Gene type** | protein coding |
| **RefSeq status** | VALIDATED |
| **Organism** | Mus musculus |
| **Lineage** | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus |
| **Also known as** | BB116301 |
| **Expression** | Broad expression in bladder adult (RPKM 12.0), ovary adult (RPKM 3.7) and 15 other tissues See more |
| **Orthologs** | human all |

# Transcript information（Ensembl）

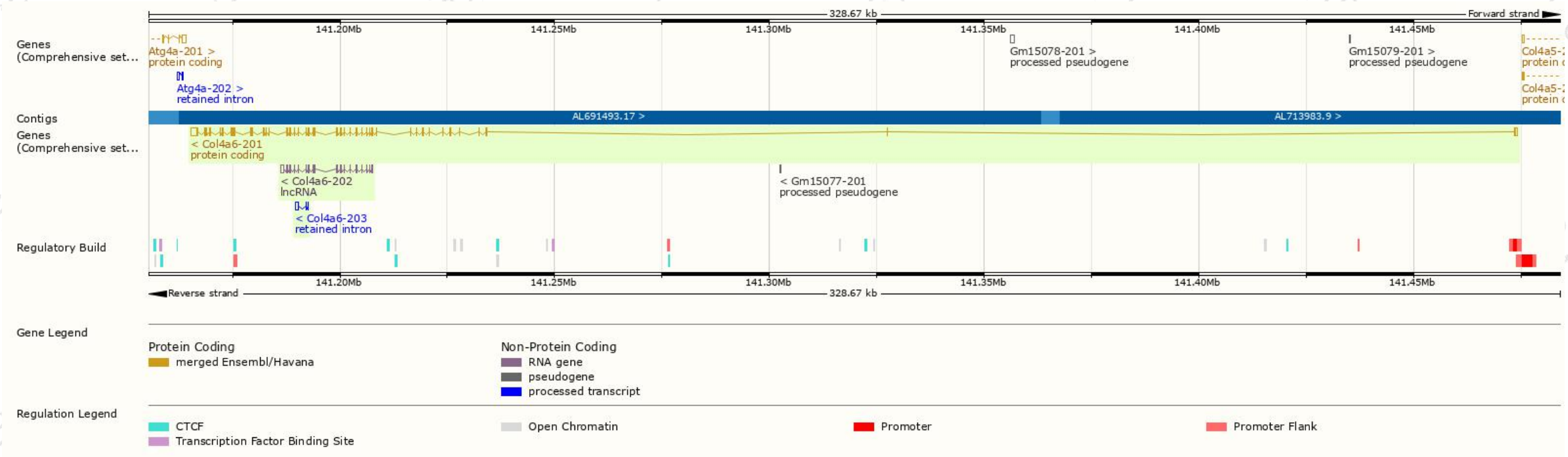
The gene has 3 transcripts,all transcripts are shown below:

| Name | Transcript ID | bp | Protein | Biotype | CCDS | UniProt | Flags |
|---|---|---|---|---|---|---|---|
| Col4a6-201 | ENSMUST00000101205.2 | 6648 | 1691aa | Protein coding | CCDS41153 | B1AVK5 | TSL:1 GENCODE basic APPRIS P1 |
| Col4a6-203 | ENSMUST00000151459.1 | 845 | No protein | Retained intron | - | - | TSL:3 |
| Col4a6-202 | ENSMUST00000137051.7 | 3092 | No protein | lncRNA | - | - | TSL:1 |

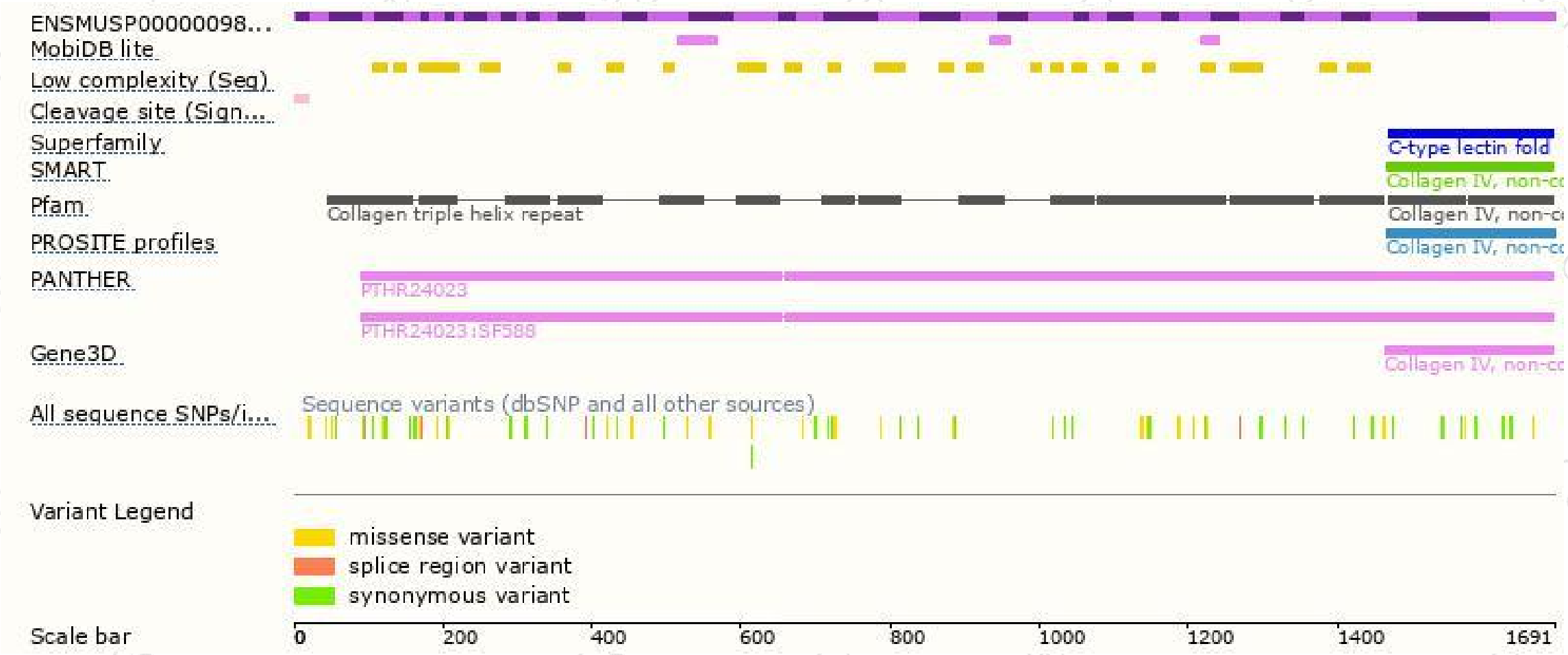
The strategy is based on the design of *Col4a6-201* transcript,The transcription is shown below



# Genomic location distribution



# Protein domain



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