

# Col28a1 Cas9-KO Strategy

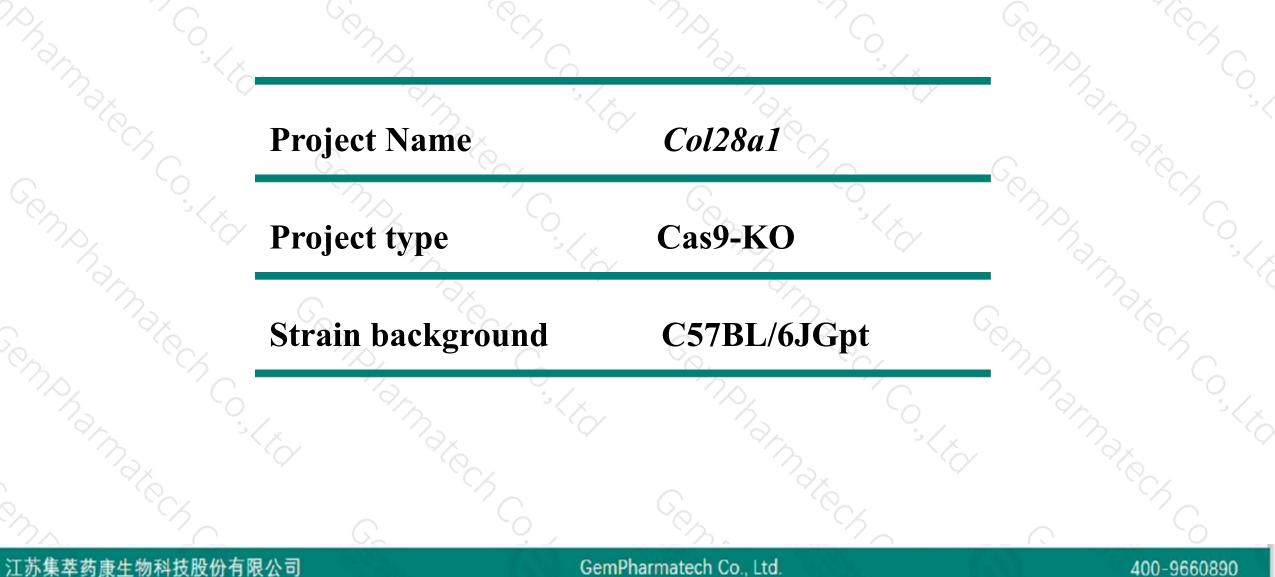
Designer: Huan Wang

**Reviewer: Wenjing Li** 

Design Date: 2020-8-5

## **Project Overview**





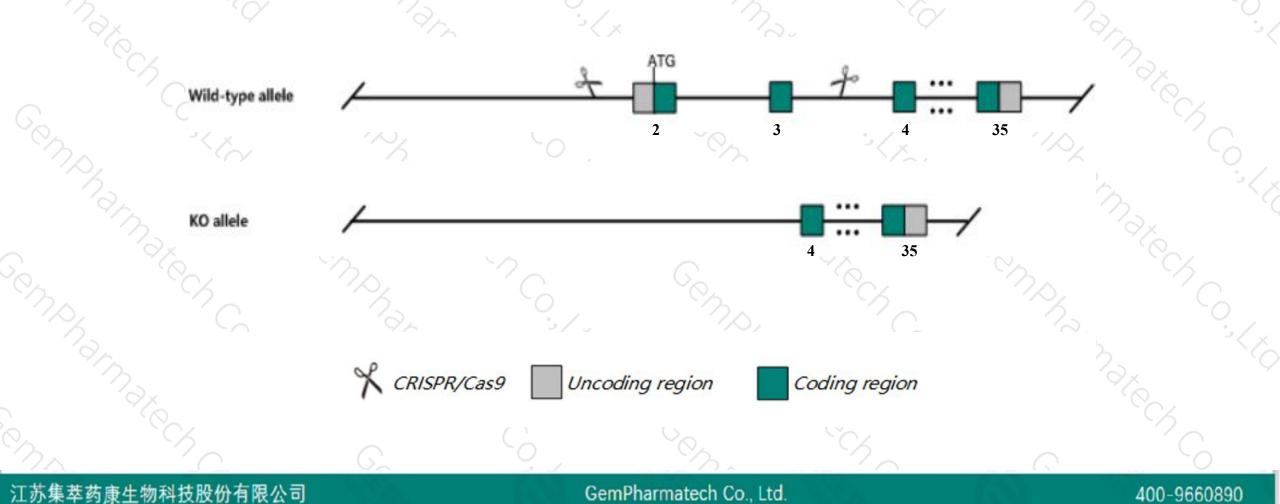
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# **Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the Col28a1 gene. The schematic diagram is as follows:





➤ The Col28a1 gene has 4 transcripts. According to the structure of Col28a1 gene, exon2-exon3 of Col28a1-201(ENSMUST00000115537.1) transcript is recommended as the knockout region. The region contains start codon ATG.Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Col28a1* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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 *Col28a1* gene is located on the Chr6. 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Notice

# **Gene information (NCBI)**



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#### Col28a1 collagen, type XXVIII, alpha 1 [Mus musculus (house mouse)]

Gene ID: 213945, updated on 13-Mar-2020

#### Summary

Official Symbol	Col28a1 provided by MGI
<b>Official Full Name</b>	collagen, type XXVIII, alpha 1 provided by <u>MGI</u>
Primary source	MGI:MGI:2685312
See related	Ensembl:ENSMUSG0000068794
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
	Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	Col28, Gm466
Expression	Biased expression in bladder adult (RPKM 1.7), mammary gland adult (RPKM 0.6) and 14 other tissuesSee more
Orthologs	human all

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## **Transcript information (Ensembl)**



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

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Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Col28a1-201	ENSMUST00000115537.1	4230	<u>1141aa</u>	Protein coding	CCD539424	<u>Q2UY11</u>	TSL:1 GENCODE basic APPRIS P1
Col28a1-204	ENSMUST00000213284.1	749	<u>234aa</u>	Protein coding	3 <b>-</b> 0	A0A1L1SSY2	CDS 3' incomplete TSL:5
Col28a1-203	ENSMUST00000134731.1	214	No protein	Processed transcript	-	2	TSL:3
Col28a1-202	ENSMUST00000123163.1	708	No protein	Retained intron	-		TSL:1

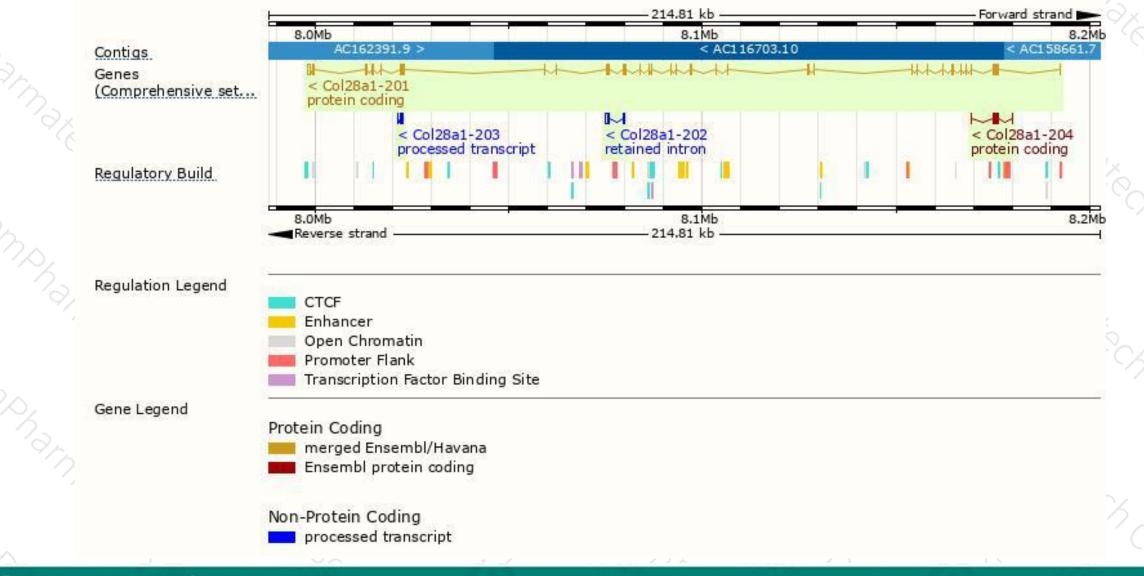
The strategy is based on the design of *Col28a1-201* transcript, the transcription is shown below:

< Col28a1-201 protein coding Reverse strand

- 194.81 kb -

### **Genomic location distribution**





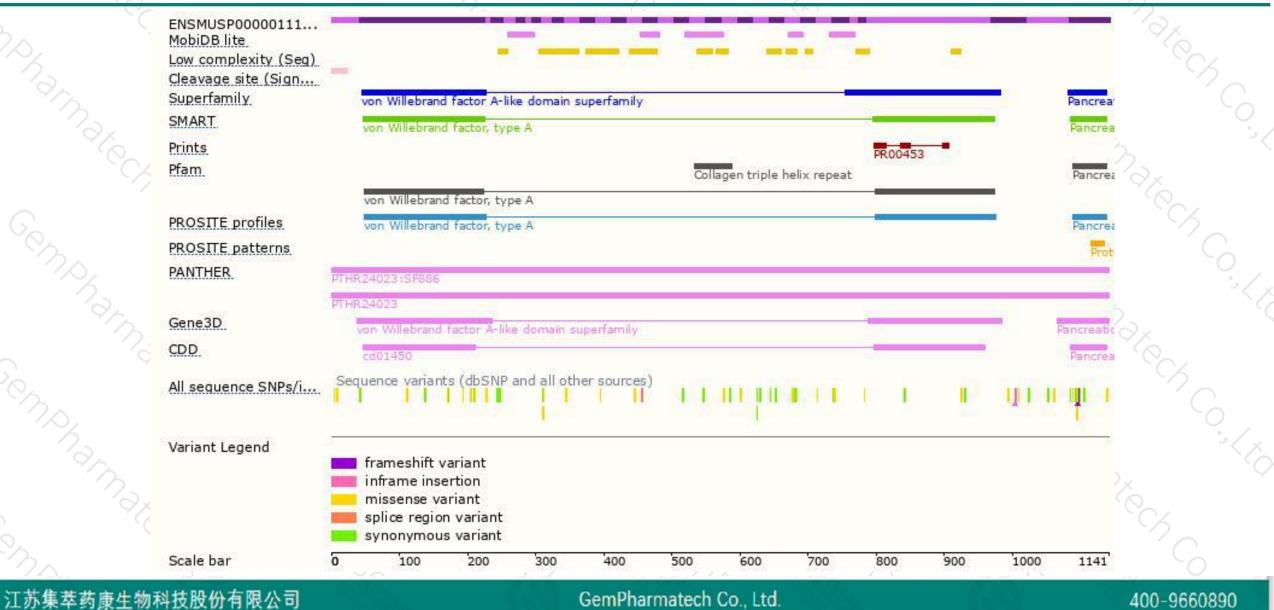
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### **Protein domain**







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



