

# Atp9b Cas9-CKO Strategy

**Designer:** 

**Reviewer:** 

**Design Date:** 

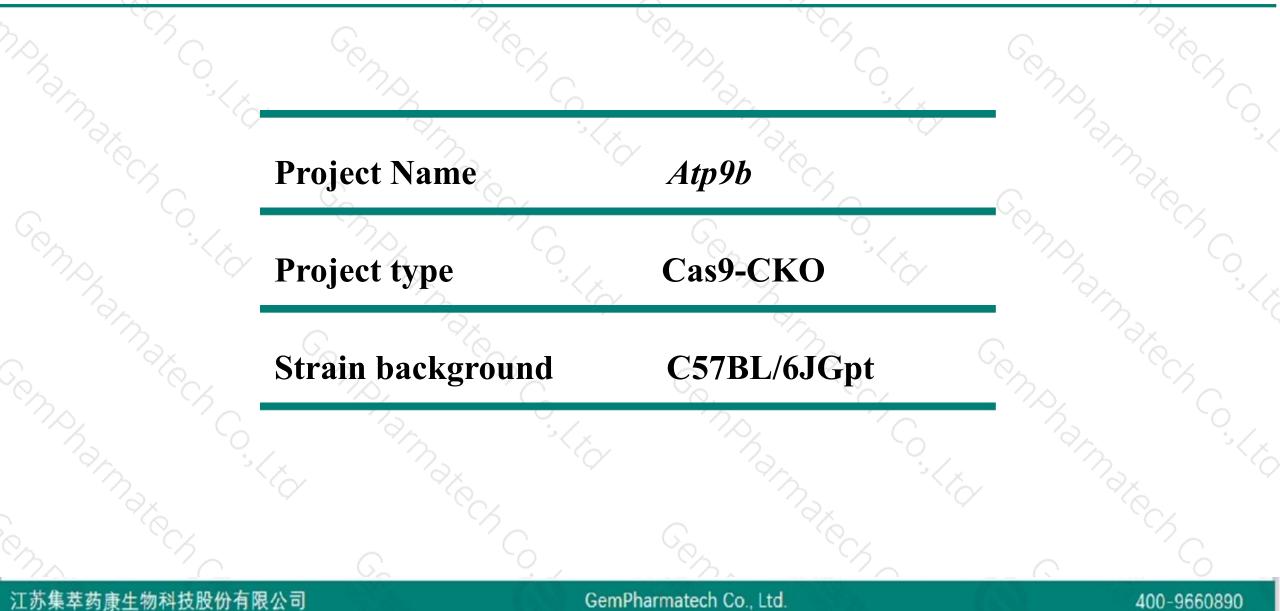
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## **Project Overview**

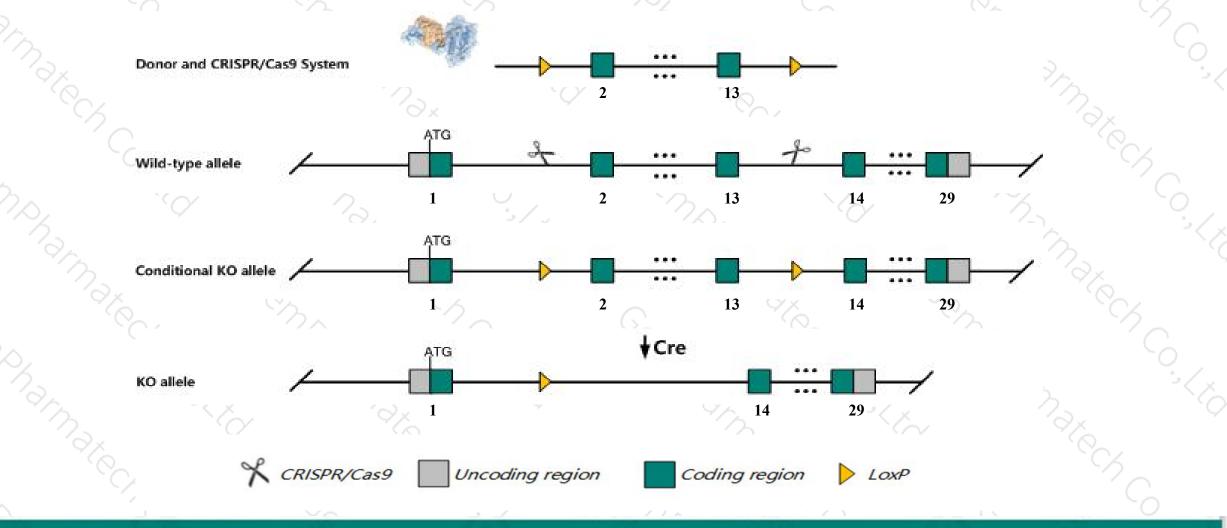




## **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the *Atp9b* gene. The schematic diagram is as follows:



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The Atp9b gene has 15 transcripts. According to the structure of Atp9b gene, exon2-exon13 of Atp9b-212 (ENSMUST00000225980.1) transcript is recommended as the knockout region. The region contains 1292bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Atp9b* 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



- The Atp9b gene is located on the Chr18. 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)**



\$ ?

400-9660890

## Atp9b ATPase, class II, type 9B [Mus musculus (house mouse)]

Gene ID: 50771, updated on 13-Mar-2020

### Summary

| Official Symbol      | Atp9b provided by MGI  |
|----------------------|--|
| •                    | ATPase, class II, type 9B provided byMGI   |
|                      | MGI:MGI:1354757  |
| 250                  | Ensembl:ENSMUSG0000024566  |
| Gene type            | protein coding   |
| <b>RefSeq status</b> | 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        | AA934181, Atpc2b, Ilb, MMR   |
| Expression           | Ubiquitous expression in testis adult (RPKM 29.2), thymus adult (RPKM 16.9) and 28 other tissuesSee more                             |
| Orthologs            | human all  |

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## **Transcript information (Ensembl)**



## The gene has 15 transcripts, all transcripts are shown below:

| Name      | Transcript ID        | bp   | Protein       | Biotype                 | CCDS              | UniProt    | Flags                   |
|-----------|----------------------|------|---------------|-------------------------|-------------------|------------|-------------------------|
| Atp9b-212 | ENSMUST00000225980.1 | 5150 | <u>1135aa</u> | Protein coding          | CCDS29370         | A0A286YCV0 | GENCODE basic APPRIS P1 |
| Atp9b-201 | ENSMUST0000091790.4  | 5357 | <u>1146aa</u> | Protein coding          | 674               | D3YV00     | TSL:5 GENCODE basic     |
| Atp9b-213 | ENSMUST00000226064.1 | 815  | <u>235aa</u>  | Protein coding          | 1420              | A0A286YDR0 | CDS 3' incomplete       |
| Atp9b-209 | ENSMUST00000225235.1 | 4326 | <u>380aa</u>  | Nonsense mediated decay | 100               | A0A286YCT2 |                         |
| Atp9b-207 | ENSMUST00000225205.1 | 3038 | <u>380aa</u>  | Nonsense mediated decay | 1781              | A0A286YCT2 |                         |
| Atp9b-204 | ENSMUST00000224709.1 | 770  | <u>78aa</u>   | Nonsense mediated decay |                   | A0A286YDF8 | CDS 5' incomplete       |
| Atp9b-202 | ENSMUST00000223926.1 | 732  | <u>163aa</u>  | Nonsense mediated decay | (12)              | A0A286YDZ3 | CDS 5' incomplete       |
| Atp9b-208 | ENSMUST00000225218.1 | 456  | No protein    | Processed transcript    | 1023              | 1020       |                         |
| Atp9b-205 | ENSMUST00000225075.1 | 454  | No protein    | Processed transcript    | 1793              | (2)        |                         |
| Atp9b-215 | ENSMUST00000237845.1 | 2920 | No protein    | Retained intron         | ( <del>1</del> 7) | -          |                         |
| Atp9b-214 | ENSMUST00000235385.1 | 2533 | No protein    | Retained intron         | 1440              | (a)        |                         |
| Atp9b-203 | ENSMUST00000224283.1 | 794  | No protein    | Retained intron         | 1023              | 1021       |                         |
| Atp9b-206 | ENSMUST00000225092.1 | 748  | No protein    | Retained intron         | 1753              | 151        |                         |
| Atp9b-211 | ENSMUST00000225692.1 | 721  | No protein    | Retained intron         | 6 <del>.</del> %  | -          |                         |
| Atp9b-210 | ENSMUST00000225345.1 | 533  | No protein    | Retained intron         | (2)               | (12)       |                         |

The strategy is based on the design of *Atp9b-212* transcript, the transcription is shown below:

#### < Atp9b-212 protein coding

Reverse strand -

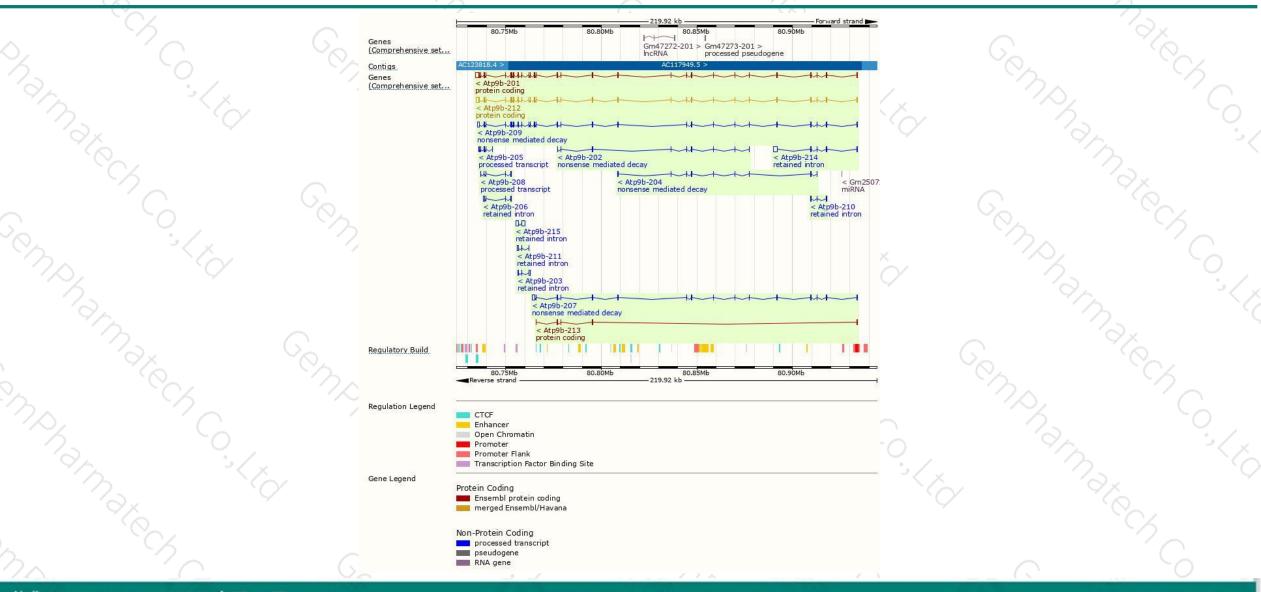
– 199.67 kb –

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## **Genomic location distribution**



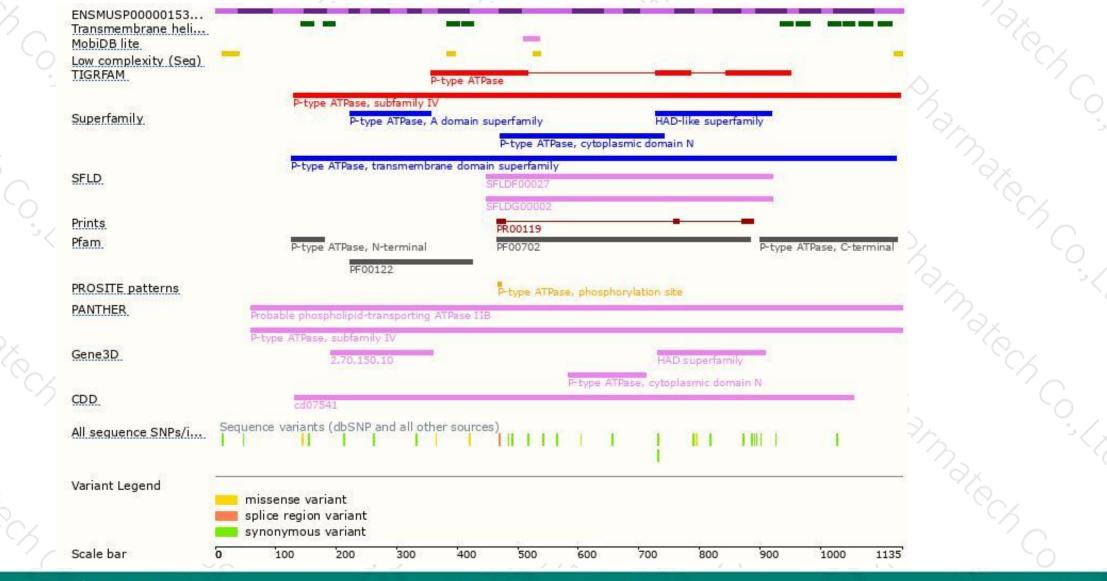


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## **Protein domain**





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



