

# Acot6 Cas9-KO Strategy

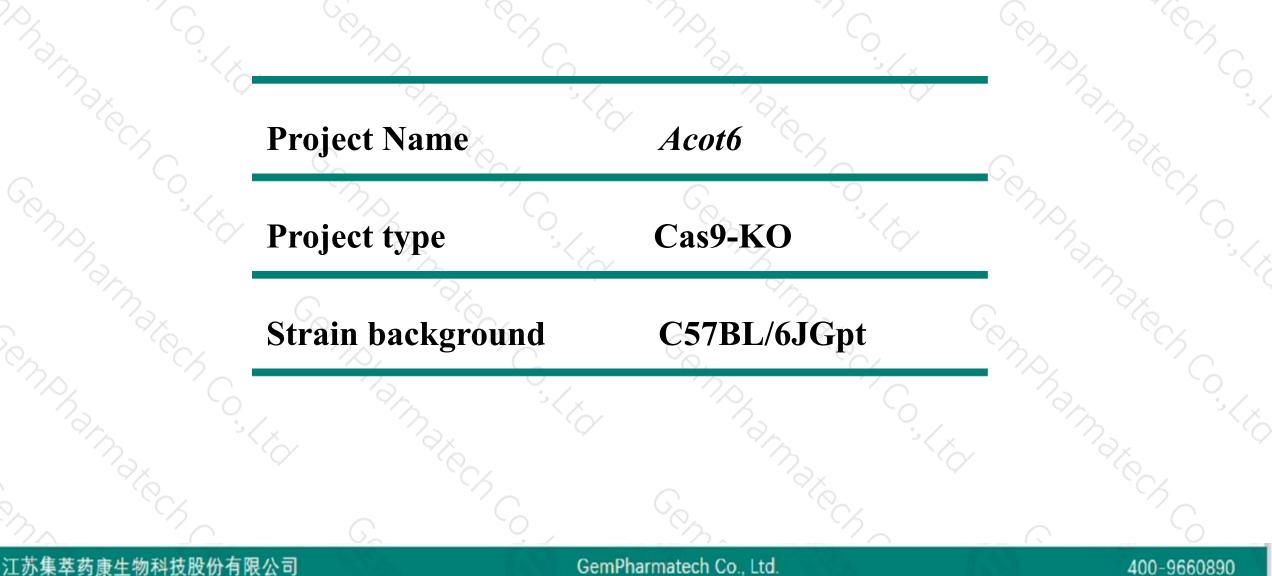
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Reviewer: Daohua Xu

Design Date: 2020-5-8

### **Project Overview**





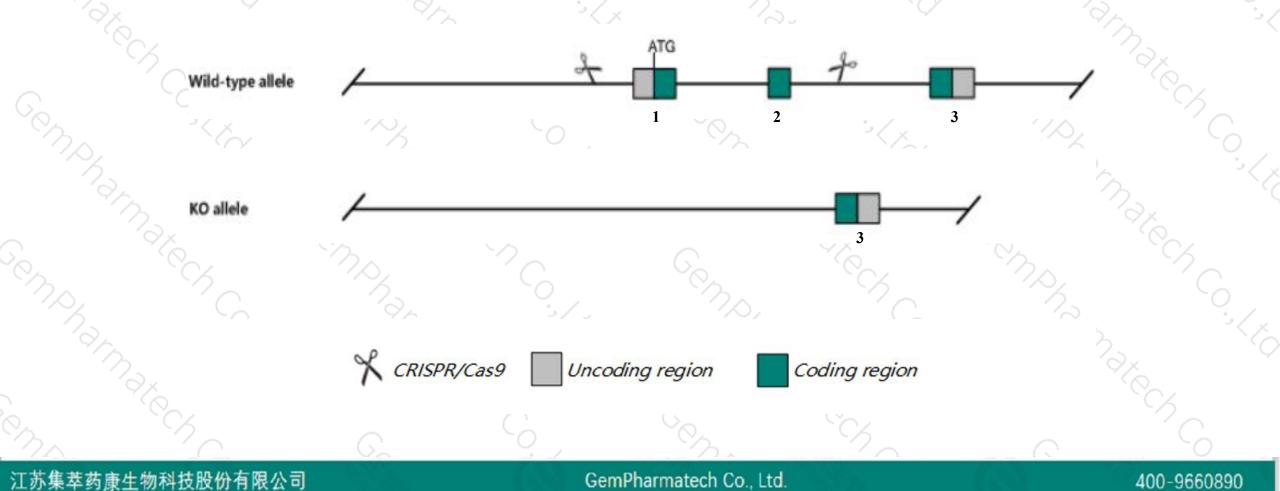
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### **Knockout** strategy



This model will use CRISPR/Cas9 technology to edit the Acot6 gene. The schematic diagram is as follows:





The Acot6 gene has 2 transcripts. According to the structure of Acot6 gene, exon1-exon2 of Acot6-201 (ENSMUST00000056822.3) 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 Acot6 gene. The brief process is as follows: CRISPR/Cas9 system



- According to the existing MGI data, mice homozygous for a knock-out allele exhibit no detectable phenotypic abnormalities.
- The knockout region is near to the N-terminal of *Dnall* gene, this strategy may influence the regulatory function of the N-terminal of *Dnall* gene.
- The Acot6 gene is located on the Chr12. 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.

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### **Gene information (NCBI)**



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#### Acot6 acyl-CoA thioesterase 6 [Mus musculus (house mouse)]

Gene ID: 217700, updated on 13-Mar-2020

#### Summary

Official SymbolAcot6 provided by MGIOfficial Full Nameacyl-CoA thioesterase 6 provided by MGIPrimary soureMGI:MGI:1921287See relateEnsembl:ENSMUSG0000043487Gene typeprotein codingprotein codingPROVISIONALOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;<br/>Muroidea; Murinae; Mus; MusAlso knownas4632408A20Rik, A330054B07, BE688602ExpressionUbiquitous expression in ovary adult (RPKM 11.9), kidney adult (RPKM 8.5) and 27 other tissuesSee more<br/>human all

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## **Transcript information (Ensembl)**



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

| - 1 m           |                      | Sugar      |                |                |        | <u></u>                          |  |  |  |  |  |  |  |
|-----------------|----------------------|------------|----------------|----------------|--------|----------------------------------|--|--|--|--|--|--|--|
| Name            | Transcript ID        | bp Protein | Protein        | Protein coding | CCDS   | UniProt   B2RTE4 Q32Q92   Q32Q92 | Flags   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  |  |  |  |  |  |  |
| Acot6-201       | ENSMUST00000056822.3 |            |                |                |        |                                  |  |  |  |  |  |  |  |
| Acot6-202       | ENSMUST00000222921.1 | 3622       | <u>176aa</u> P |                |        |                                  | TSL:NA GENCODE basic   |  |  |  |  |  |  |
|                 | Ye K C               |            |                |                | 6      |                                  |  |  |  |  |  |  |  |
|                 |                      | ~<br>~     |                |                |        | nder and                         | South Constant Andra Constant Start Star |  |  |  |  |  |  |
|                 | Max.                 | 3          |                |                |        | 2                                | are constant are   |  |  |  |  |  |  |
| The             | strategy is h        | ase        | don            | the de         | esign  | of Acott                         | 6-201 transcript, the transcription is shown below:  |  |  |  |  |  |  |
| 1 He            | indiegy is e         | aber       | u On           |                | 651511 | orneon                           | 5 201 transeription is shown below.  |  |  |  |  |  |  |
| 1               |                      |            |                |                |        |                                  | 10.70 kb — Forward strand  |  |  |  |  |  |  |
| Acot6<br>protei | 201 ><br>n coding    |            |                |                |        |                                  |  |  |  |  |  |  |  |

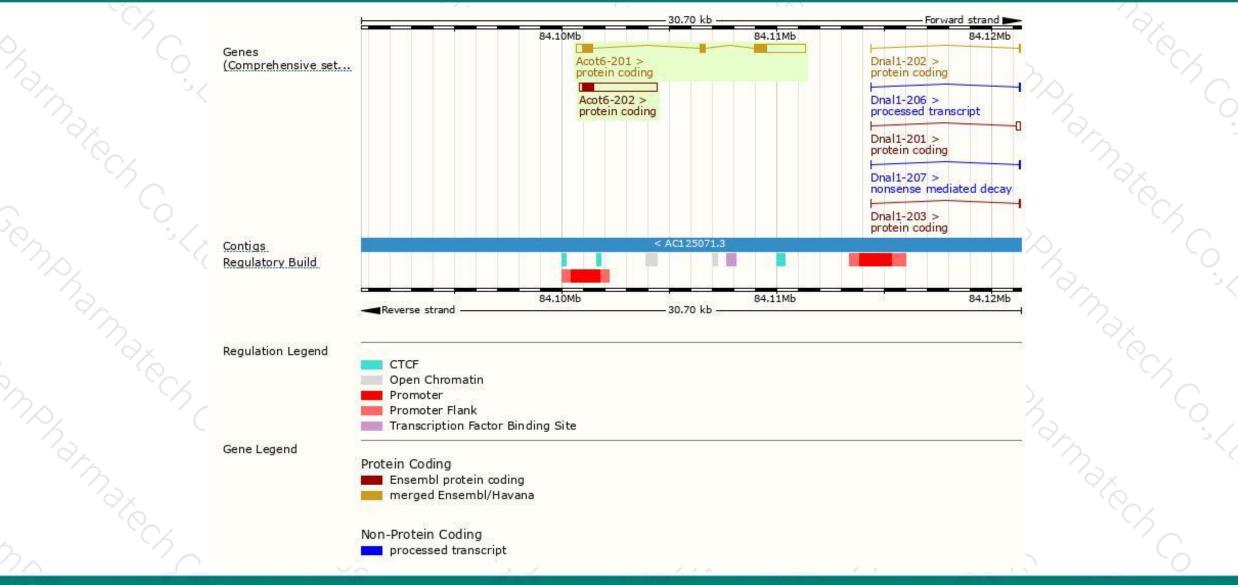
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### **Genomic location distribution**



400-9660890



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### **Protein domain**



|    | 10                                      | G <sub>N</sub>                         |                 | ~,                 | $\sim$         | $\sim$     |                |                |              |      | Ye,            |
|----|---|--|-----------------|--------------------|----------------|------------|----------------|----------------|--------------|------|----------------|
| 3  | ENSMUSP00000056<br>Low complexity (Seg) |  |                 | -                  |                |            |                |                |              |      | 20             |
|    | Superfamily                             |  | Alpha/Beta hy   | drolase fold       |                |            |                |                |              |      |                |
|    | Pfam.                                   |  |                 |                    |                | BAAT,      | /Acyl-CoA thic | oester hydrola | se C-termin: | al   |                |
|    |   | Acyl-CoA thioe                         | ster hydrolase/ | bile acid-Co/      | A amino acid I | N-acetylti | ansferase      |                |              |      |                |
|    | PIRSF                                   | Acyl-CoA thioestera                    | ise, long chain |                    |                |            |                |                |              |      |                |
|    | PANTHER                                 | PTHR10824;SF17                         |                 |                    |                |            |                |                |              |      | 2              |
| 1  | Gene3D                                  | PTHR 10824                             |                 | 1                  |                |            |                |                |              |      |                |
|    |   |  |                 |                    | /Beta hydrola  | se told    |                |                |              |      |                |
| 5, | All sequence SNPs/i                     | Acyl-CoA thioester<br>Sequence variant |                 |                    |                | 1          | ų              | E.             | i)           | 1.01 | 1              |
|    | Variant Legend                          | missense va                            |                 |                    |                |            |                |                |              |      | ~<             |
|    | Scale bar                               | 0 40                                   | 80              | 120                | 160            | 200        | 240            | 280            | 320          | 360  | 419            |
|    | °C/                                     |  |                 | $\hat{\mathbf{O}}$ | Go)            | 5.         |                |                |              |      | <sup>2</sup> C |

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



