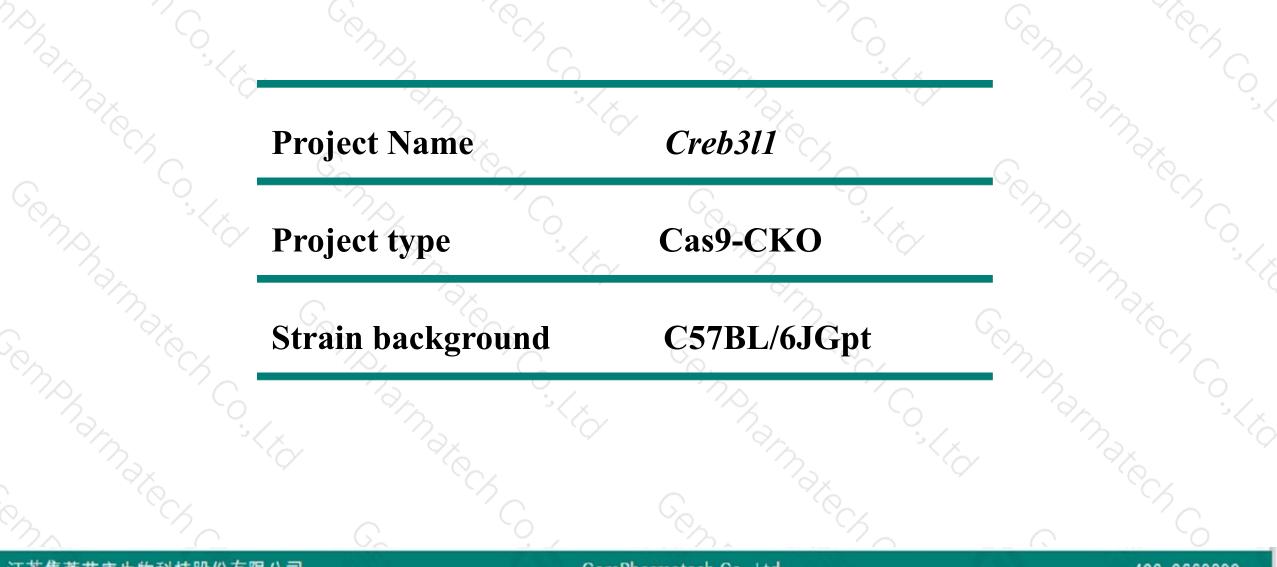


# Creb3l1 Cas9-CKO Strategy

Designer:Xueting Zhang Reviewer:Yanhua Shen Date:2019-12-13

## **Project Overview**





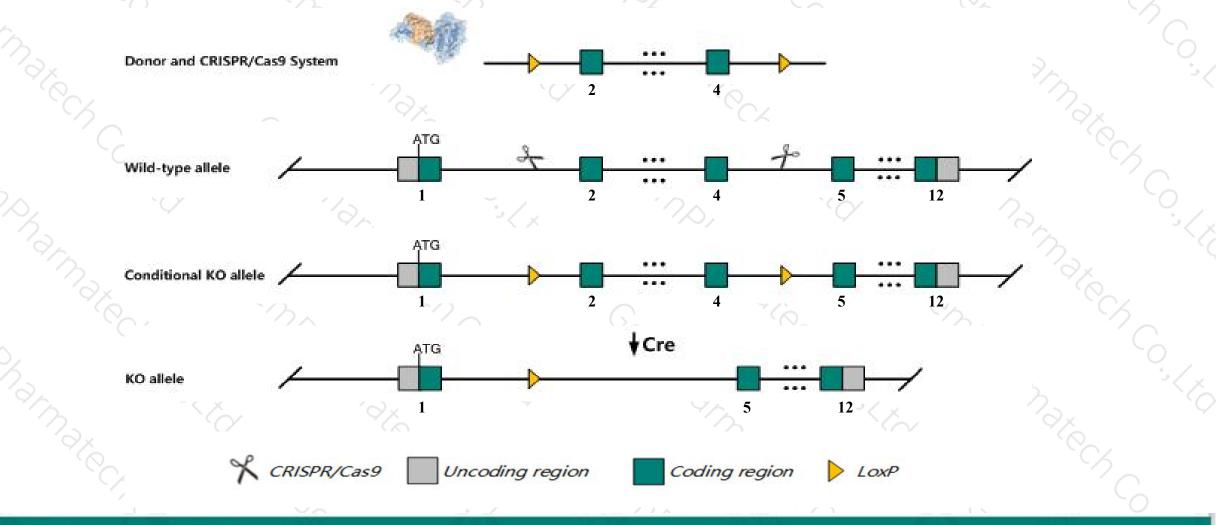
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### **Conditional Knockout strategy**



This model will use CRISPR/Cas9 technology to edit the Creb311 gene. The schematic diagram is as follows:



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The Creb311 gene has 1 transcript. According to the structure of Creb311 gene, exon2-exon4 of Creb311-201 (ENSMUST00000028663.4) transcript is recommended as the knockout region. The region contains 493bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Creb3l1* 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.

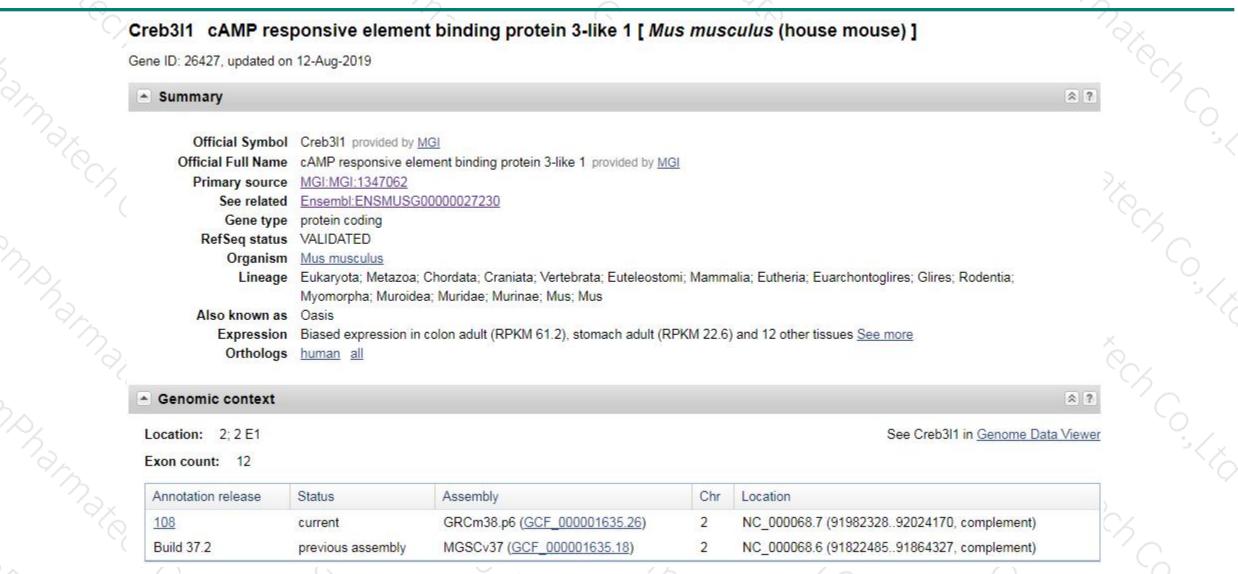
The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.



- According to the existing MGI data, Mice homozygous for a knock-out allele exhibit postnatal growth retardation, fragile skeleton, and decreased bone density, cortical and trabecular thickness, and osteoblast maturation.
- The Creb311 gene is located on the Chr2. 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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The gene has 1 transcript, and the transcript is shown below:

| 1                                      |                                    |                        |   |   | and a second sec |  |         | · / Dec.                      |     |
|--|------------------------------------|------------------------|---|---|--|--|---------|-------------------------------|-----|
| Name                                   | Transcript ID ENSMUST00000028663.4 |                        | bp         Protein           2603         520aa |   | Biotype  | CCDS                                   | UniProt | Flag                          | S   |
| Creb3l1-201                            |                                    |                        |   |   | Protein coding   | CCDS38181 A0A0R4J082                   |         | TSL:1 GENCODE basic APPRIS P1 |     |
| ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~ |                                    |                        |   | o <sub>x</sub>  |  |  |         |                               | 12  |
|  | C                                  | G                      |   |   |  |  |         |                               |     |
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|  |                                    |                        |   |   |  | ~~/                                    |         |                               |     |
| 6                                      |                                    | C.                     |   |   |  | -                                      |         |                               | Co. |
|  | °°CK                               | $\gamma_{\mathcal{O}}$ |   |   |  |  | C.      | Solo Solo                     | -20 |
|  |                                    |                        |   |   |  |  |         |                               |     |

The strategy is based on the design of Creb311-201 transcript, The transcription is shown below

< Creb3l1-201 protein coding

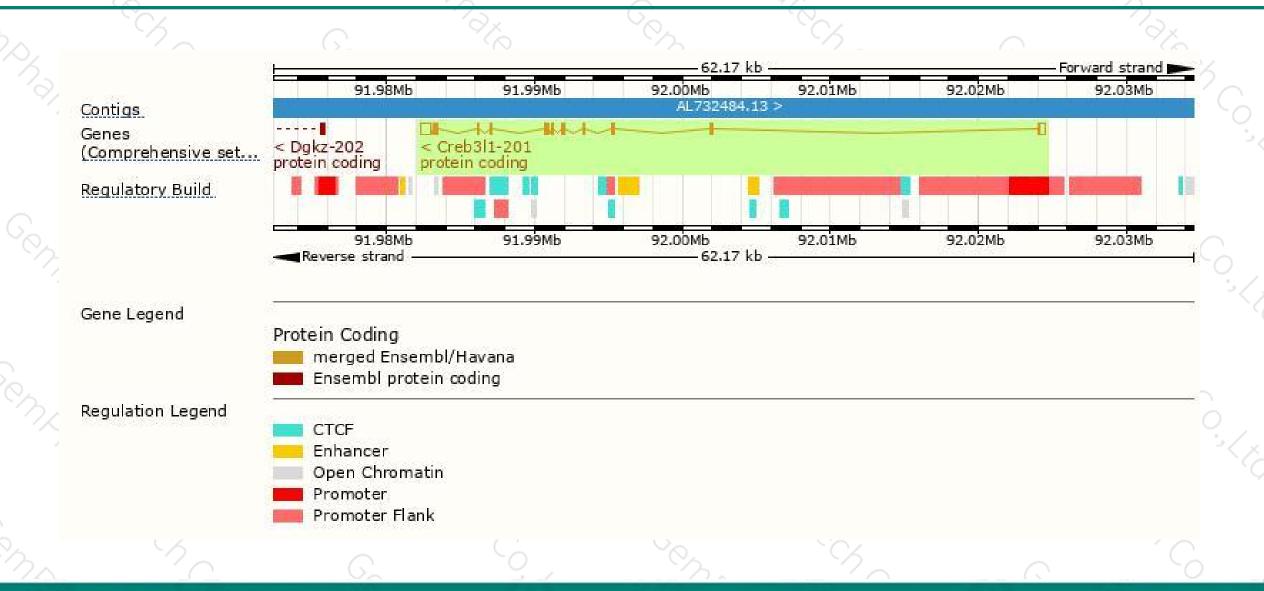
Reverse strand -

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42.17 kb

### **Genomic location distribution**



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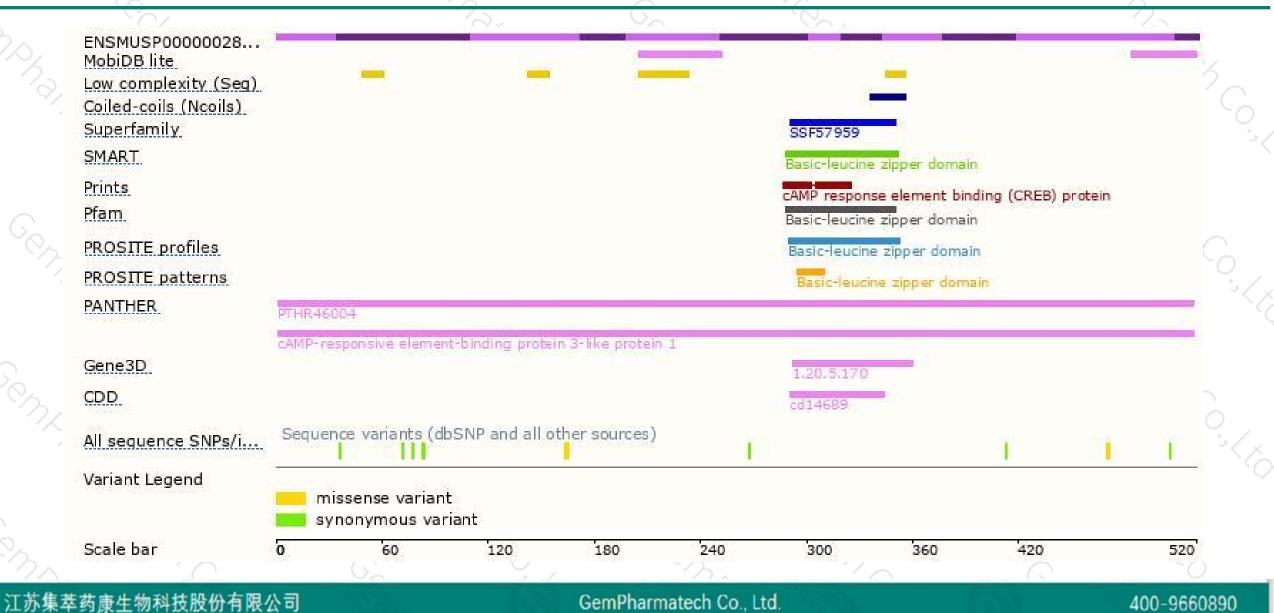
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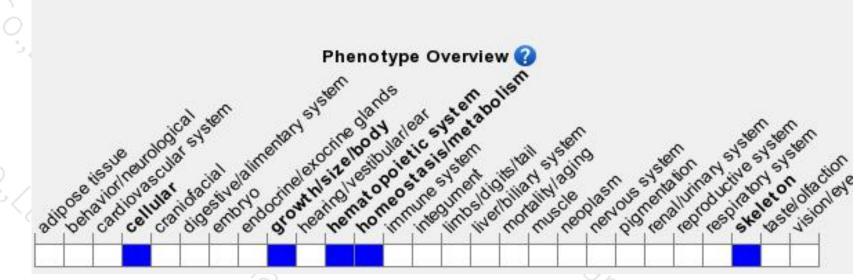
### **Protein domain**





### 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 knock-out allele exhibit postnatal growth retardation, fragile skeleton, and decreased bone density, cortical and trabecular thickness, and osteoblast maturation.



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



