

# L3mbtl4 Cas9-CKO Strategy

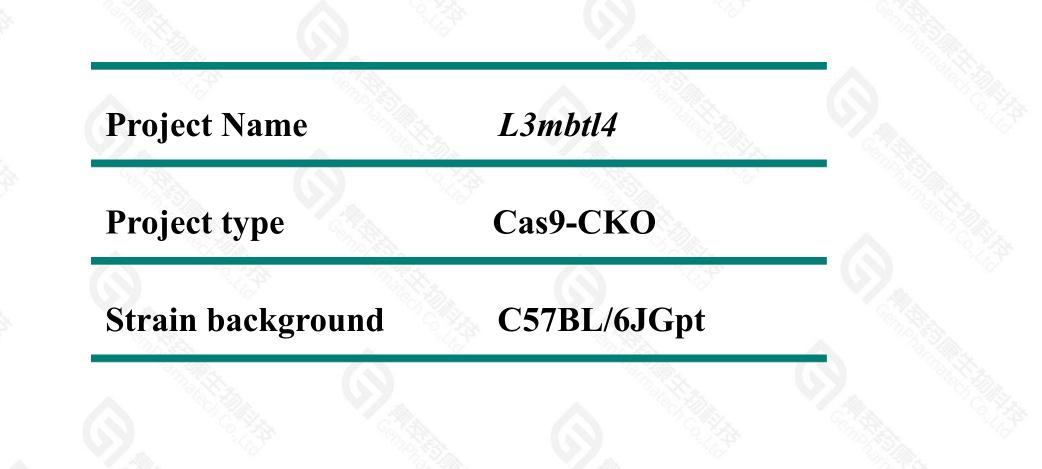
**Designer: Daohua Xu** 

**Reviewer: Xueting Zhang** 

**Design Date: 2021-2-8** 

# **Project Overview**



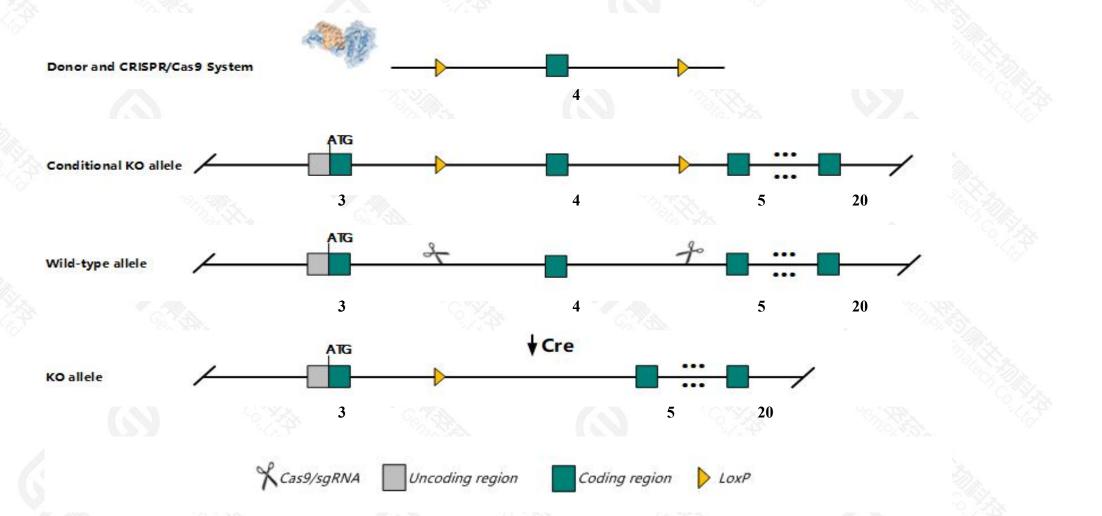


江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

# **Conditional Knockout strategy**

This model will use CRISPR/Cas9 technology to edit the L3mbtl4 gene. The schematic diagram is as follows:



江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

# **Technical routes**



The L3mbtl4 gene has 6 transcripts. According to the structure of L3mbtl4 gene, exon4 of L3mbtl4 201(ENSMUST0000093007.5) transcript is recommended as the knockout region. The region contains 55bp coding sequence.
Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify L3mbtl4 gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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.



- > Transcript L3mbtl4-205 may not be affected.
- > The L3mbtl4 gene is located on the Chr17. 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.

Notice

# Gene information (NCBI)

## L3mbtl4 L3MBTL4 histone methyl-lysine binding protein [Mus musculus (house mouse)]

Gene ID: 320858, updated on 13-Mar-2020

## Summary

| Official Symbol           | L3mbtl4 provided by MGI   |
|---------------------------|---|
| <b>Official Full Name</b> | L3MBTL4 histone methyl-lysine binding protein provided by <u>MGI</u>  |
| <b>Primary source</b>     | MGI:MGI:2444889   |
| See related               | Ensembl:ENSMUSG0000041565   |
| 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             | A730037L19Rik, D930040M24Rik  |
| Expression                | Low expression observed in reference datasetSee more  |
| Orthologs                 | human all   |

## 江苏集萃药康生物科技股份有限公司

## GemPharmatech Co., Ltd.

☆ ?

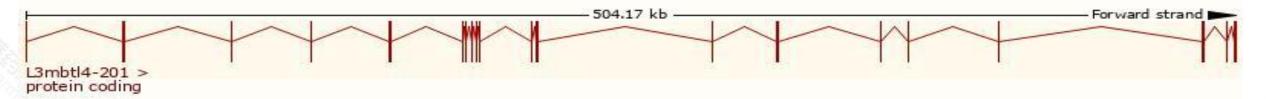
**う**泉 atech

# **Transcript information (Ensembl)**

## The gene has 6 transcripts, all transcripts are shown below:

#### Transcript ID Name bp Protein Biotype CCDS UniProt Flags L3mbtl4-201 ENSMUST0000093007.5 Protein coding TSL:5 GENCODE basic APPRIS P1 2445 621aa D3YW39 -Protein coding L3mbtl4-205 ENSMUST00000233625.1 A0A3B2WCI8 CDS 5' incomplete 2216 131aa -L3mbtl4-202 ENSMUST00000124543.7 2023 Protein coding CDS 3' incomplete TSL:5 481aa A0A0A0MQK6 -Nonsense mediated decay L3mbtl4-203 ENSMUST00000139383.7 2308 292aa D6REI4 TSL:1 -Retained intron L3mbtl4-206 ENSMUST00000233696.1 2790 No protein -1564 No protein Retained intron TSL:1 L3mbtl4-204 ENSMUST00000150573.7 -

The strategy is based on the design of L3mbtl4-201 transcript, the transcription is shown below:

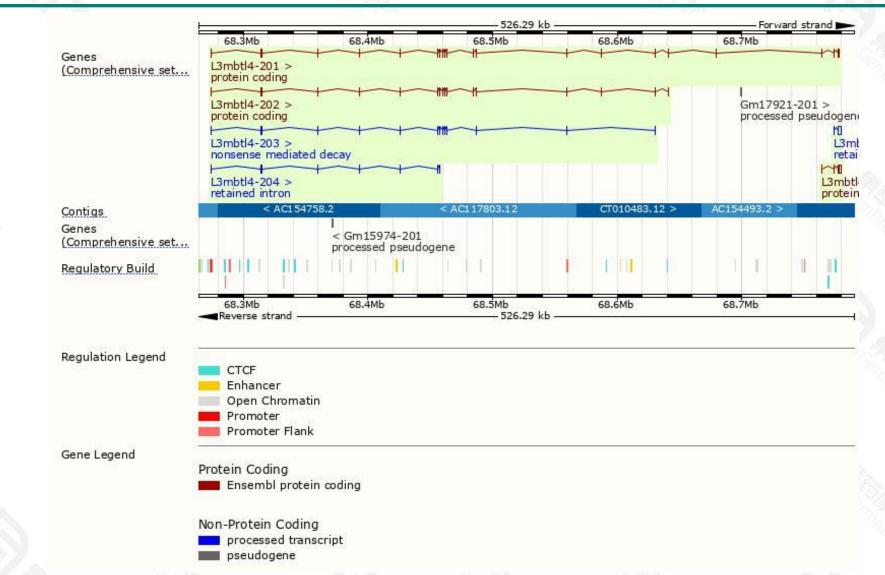


## 江苏集萃药康生物科技股份有限公司

## GemPharmatech Co., Ltd.

# **Genomic location distribution**





江苏集萃药康生物科技股份有限公司

## GemPharmatech Co., Ltd.

# **Protein domain**





## 江苏集萃药康生物科技股份有限公司

## GemPharmatech Co., Ltd.



If you have any questions, you are welcome to inquire. Tel: 025-5864 1534



