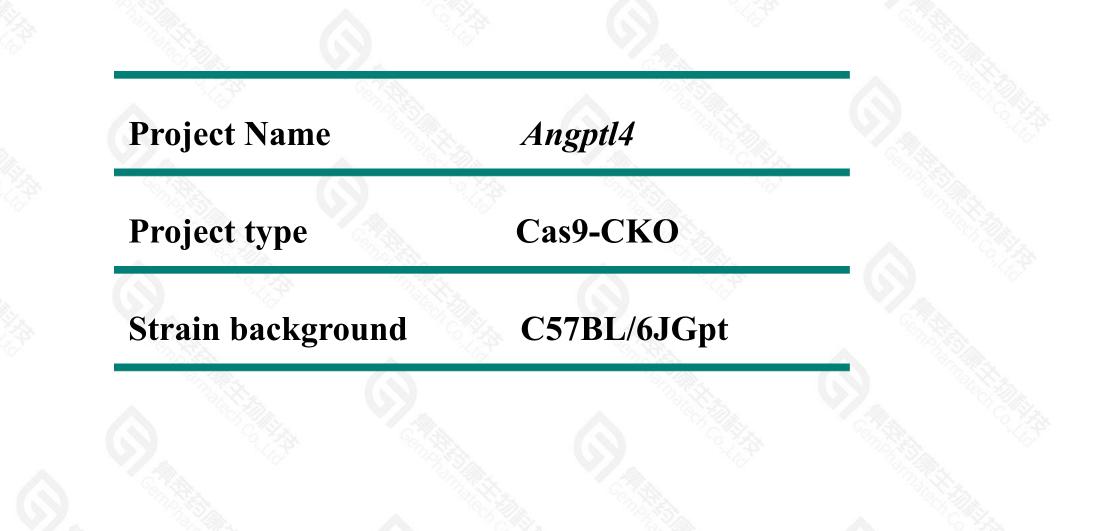


# Angptl4 Cas9-CKO Strategy

**Designer: Qiong Zhou** 

# **Project Overview**



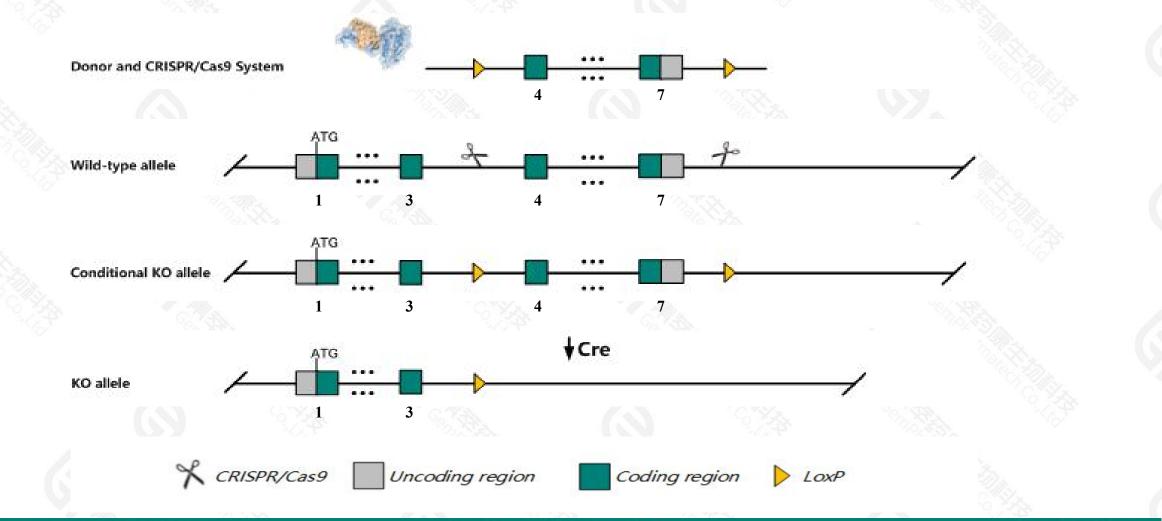


# **Conditional Knockout strategy**

D

生物料药制

This model will use CRISPR/Cas9 technology to edit the Angptl4 gene. The schematic diagram is as follows:



GemPharmatech Co., Ltd.

# **Technical routes**



➤ The Angptl4 gene has 4 transcripts. According to the structure of Angptl4 gene, exon4-exon7 of Angptl4-201(ENSMUST0000002360.17) transcript is recommended as the knockout region. The region contains 674bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Angptl4* 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.

 $\succ$  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 disruptions in this gene display decreased levels of triglycerides and cholesterol and a lower increase in body fat after exposure to gut microbiota.
- ➤ The KO region contains functional region of the Angptl4 gene.Knockout the region may affect the function of LOC118568328 gene.
- > The *Angptl4* 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.

# Gene information (NCBI)

### Angptl4 angiopoietin-like 4 [Mus musculus (house mouse)]

Gene ID: 57875, updated on 20-Dec-2020

### Summary

Official SymbolAngptl4 provided by MGIOfficial Full Nameangiopoietin-like 4 provided by MGIPrimary sourceMGI:MGI:1888999See relatedEnsembl:ENSMUSG0000002289Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;<br/>Myomorpha; Muroidea; Muridae; Mus; MusAlso known asArp4, Bk89, FI, Fiaf, HF, Hfarp, NG2, Ng27, Pgarg, Pp1158ExpressionBiased expression in subcutaneous fat pad adult (RPKM 130.4), genital fat pad adult (RPKM 109.0) and 14 other tissuesSeeMoreMuran all



### GemPharmatech Co., Ltd.





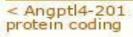
2

# **Transcript information (Ensembl)**

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

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags	
Angptl4-201	ENSMUST0000002360.17	3308	<u>410aa</u>	Protein coding	CCDS28629		TSL:1, GENCODE basic, APPRIS P1,	
Angptl4-202	ENSMUST00000173869.8	1859	<u>154aa</u>	Nonsense mediated decay	( <del>-</del> )		TSL:1,	
Angptl4-204	ENSMUST00000174872.2	575	No protein	Processed transcript	123		TSL:3 ,	
Angptl4-203	ENSMUST00000174858.2	539	No protein	Retained intron	670		TSL:3,	

The strategy is based on the design of Angptl4-201 transcript, the transcription is shown below:



Reverse strand

- 8.08 kb

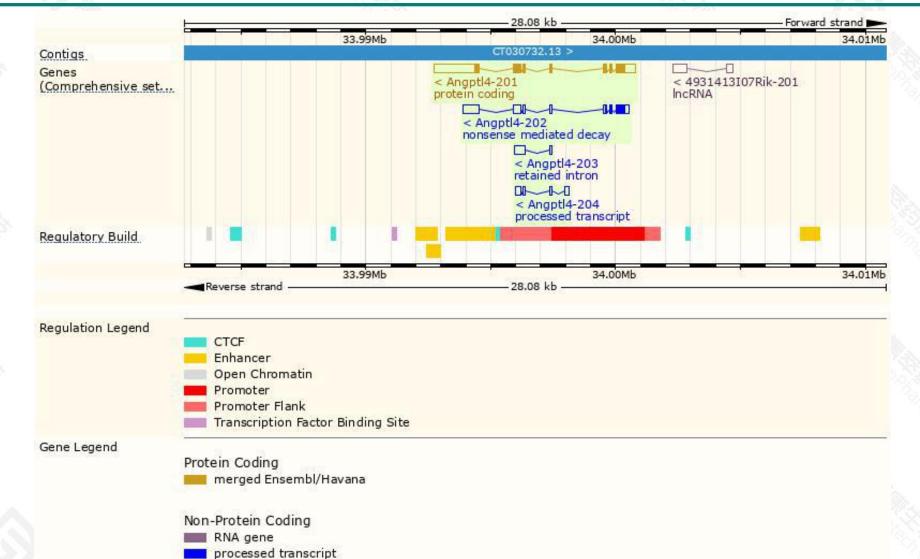
### 江苏集萃药康生物科技股份有限公司

### GemPharmatech Co., Ltd.

### 400-9660890

# **Genomic location distribution**





江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

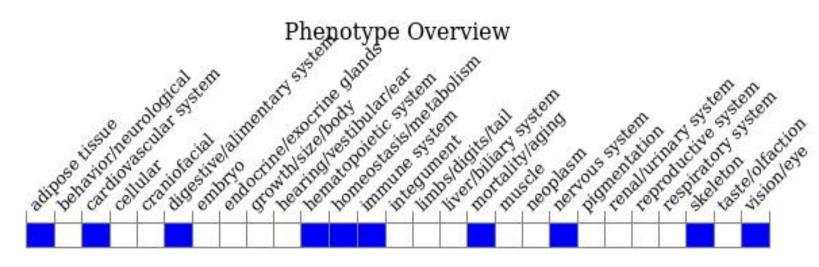
400-9660890

# **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 disruptions in this gene display decreased levels of triglycerides and cholesterol and a lower increase in body fat after exposure to gut microbiota.

### 江苏集萃药康生物科技股份有限公司

### GemPharmatech Co., Ltd.

#### 400-9660890



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



