

Abcc3 Cas9-CKO Strategy

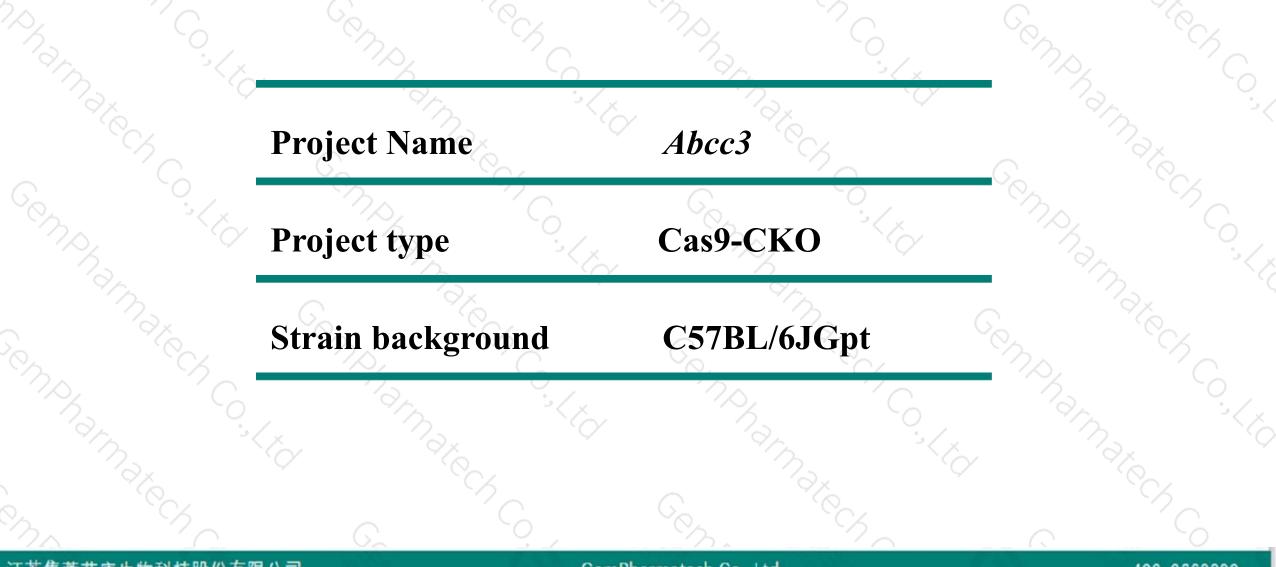
Designer: Design Date:

0.

Bingxuan Li 2019-10-17

Project Overview





江苏集萃药康生物科技股份有限公司

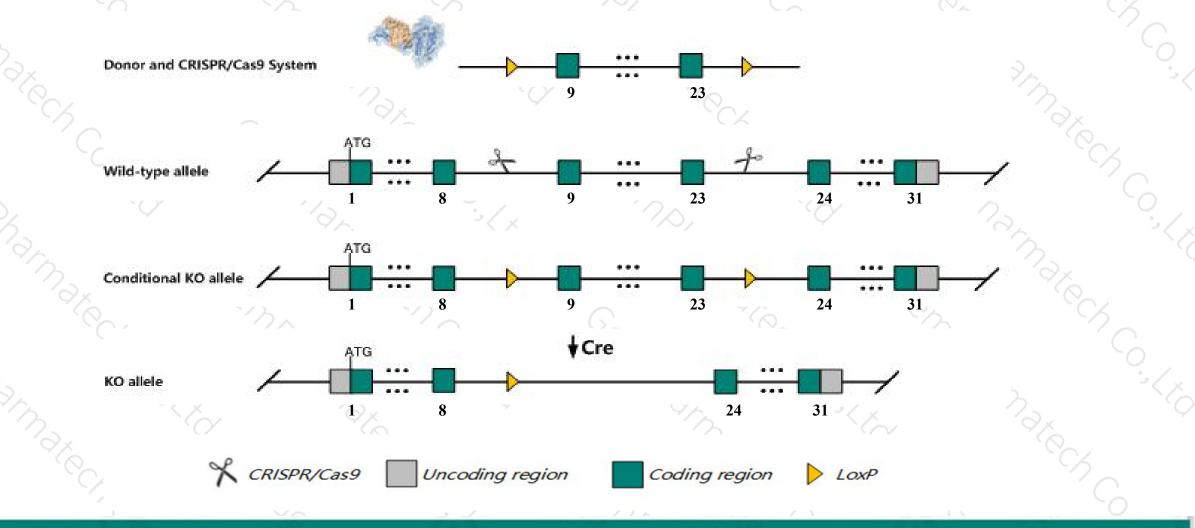
GemPharmatech Co., Ltd.

400-9660890

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Abcc3* 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.

400-9660890



The Abcc3 gene has 6 transcripts. According to the structure of Abcc3 gene, exon9-exon23 of Abcc3-201 (ENSMUST00000021231.7) transcript is recommended as the knockout region. The region contains 2368bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Abcc3* gene. The brief process is as follows:gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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 disruptions in this gene exhibit increased liver bile acid levels after bile duct ligation
- The *Abcc3* gene is located on the Chr11. 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)



\$?

Abcc3 ATP-binding cassette, sub-family C (CFTR/MRP), member 3 [Mus musculus (house mouse)]

Gene ID: 76408, updated on 10-Oct-2019

Summary

| Official Symbol | Abcc3 provided by MGI | | | | | | | |
|---------------------------|--|--|--|--|--|--|--|--|
| Official Full Name | ATP-binding cassette, sub-family C (CFTR/MRP), member 3 provided by MGI | | | | | | | |
| Primary source | MGI:MGI:1923658 | | | | | | | |
| See related | Ensembl:ENSMUSG0000020865 | | | | | | | |
| Gene type | protein coding | | | | | | | |
| RefSeq status | VALIDATED | | | | | | | |
| Organism | Mus musculus | | | | | | | |
| Lineage | Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; | | | | | | | |
| | Muroidea; Murinae; Mus; Mus | | | | | | | |
| Also known as | MLP2; MRP3; ABC31; MOAT-D; 1700019L09Rik | | | | | | | |
| Expression | Biased expression in liver adult (RPKM 63.1), colon adult (RPKM 54.5) and 14 other tissues See more | | | | | | | |
| Orthologs | human all | | | | | | | |
| | | | | | | | | |

Transcript information (Ensembl)



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

限公司

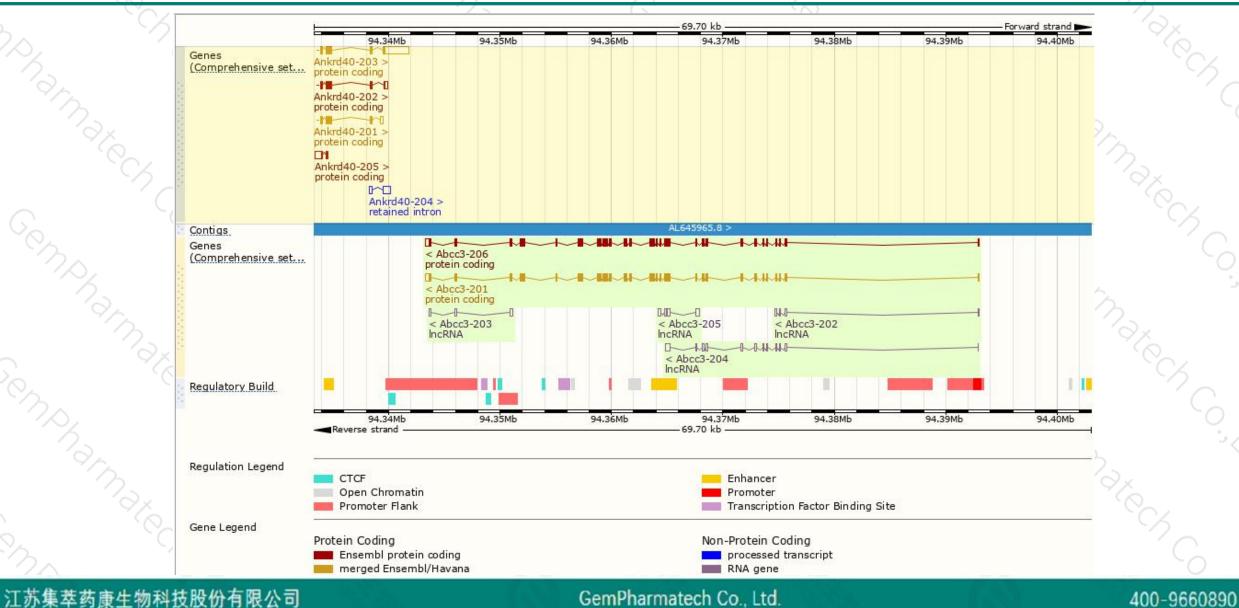
汀苏集萃药

| Show/hid | le columns (1 hidden) | | | | | | Filter |
|-----------|-----------------------|------|---------------|----------------|-----------|---------------------|---------------------------------|
| Name 🖕 | Transcript ID 💧 | bp 👙 | Protein 🖕 | Biotype | CCDS 🖕 | UniProt 💧 | Flags |
| Abcc3-201 | ENSMUST0000021231.7 | 4999 | <u>1522aa</u> | Protein coding | CCDS25254 | <u>A0A0R4J015</u> & | TSL:1 GENCODE basic APPRIS P2 |
| Abcc3-206 | ENSMUST00000178136.7 | 5005 | <u>1523aa</u> | Protein coding | 5 | <u>J3QML2</u> & | TSL:1 GENCODE basic APPRIS ALT: |
| Abcc3-204 | ENSMUST00000151124.7 | 1808 | No protein | IncRNA | 5 | 5 | TSL:5 |
| Abcc3-205 | ENSMUST00000155253.1 | 915 | No protein | IncRNA | | . | TSL:3 |
| Abcc3-202 | ENSMUST00000126566.1 | 667 | No protein | IncRNA | × | | TSL:3 |
| Abcc3-203 | ENSMUST00000140985.1 | 567 | No protein | IncRNA | * | - | TSL:3 |

The strategy is based on the design of Abcc3-201 transcript, The transcription is shown below

Abcc3-206 protein coding
49.68 kb

Genomic location distribution



GemPharmatech Co., Ltd.

集萃

集卒药康 GemPharmatech

Protein domain

限公司

技股份有

江苏集萃药

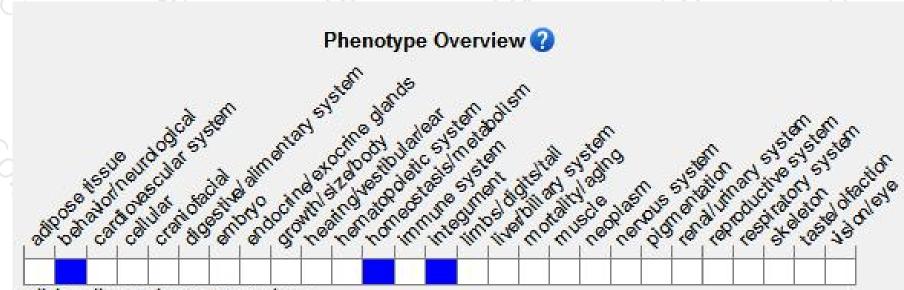


| | <u> </u> | | | | | | | 1/2 | | |
|----------|--|----------------------------------|--|------------------------------|----------------------------|---------------------------------------|--------------------------|---------|--|--|
| Dohan an | ENSMUSP00000136 Transmembrane heli MobiDB lite Coiled-coils (Ncoils) TIGRFAM | | | | | | | | | |
| 1 | | Multi drug resistance-associated | d protein | | | | | , | | |
| 1 | Superfamily | | | P-loop | containing nucleoside trip | osphate hydrolase | | | | |
| | | | ABC transporter type 1, trans | smembrane dor | main superfamily | | () | | | |
| | SMART | | | AAA | + ATPase domain | | | | | |
| | Pfam | | | ABC | transporter-like | | | | | |
| \cap | | | ABC transporter type 1, tra | nsmembrane d | omain | | | | | |
| | PROSITE profiles | | ABC transporter type 1, tra | and shares an arrival | | 4 | | | | |
| 1 | | | | and the second second second | ansporter-like | | | | | |
| | PROSITE patterns | | | nbo ci | ABC transporter, o | onserved site | | | | |
| | PANTHER | PTHR24223 | | | | | | | | |
| | | PTHR24223:SF241 | | | | | | | | |
| | Gene3D | | | | | | | | | |
| | | | ABC transporter type 1, transr | A REAL PROPERTY OF | 2 THE PROPERTY IN THE REAL | | | | | |
| | CDD | | | 3,40.5 | | 85 | | | | |
| 2 | | | cd18595 | cd0325 | 50 | cd18603 | cd03244 | | | |
| | 1 | | | | | | | | | |
| | All sequence SNPs/i | Sequence variants (dbSNP a | nd all other sources) | | 100 Mar 100 M | 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 | The second second second | 1 N N | | |
| 1 | | Let Letter ett | n <mark>in an an</mark> | 1 HII | 11.11.11.11.1 | C IS HER | enneme arbh ran | . D. D. | | |
| | | | 1. | | | | | | | |
| | Variant Legend | 20 20 20 10 | | | | e es es | | | | |
| | | missense variant | | | | | | | | |
| | | synonymous variant | 1 | 1 | E and a | Laborer | 1.50.507 | 0 | | |
| 5. I | Scale bar | 0 200 | 400 | 600 | 800 | 1000 | 1200 | 1523 | | |
| | () | | nat d | | | | () | | | |

GemPharmatech Co., Ltd.

Mouse phenotype description(MGI)





Click cells to view annotations.

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 exhibit increased liver bile acid levels after bile duct ligation

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



