

# Cfb Cas9-KO Strategy

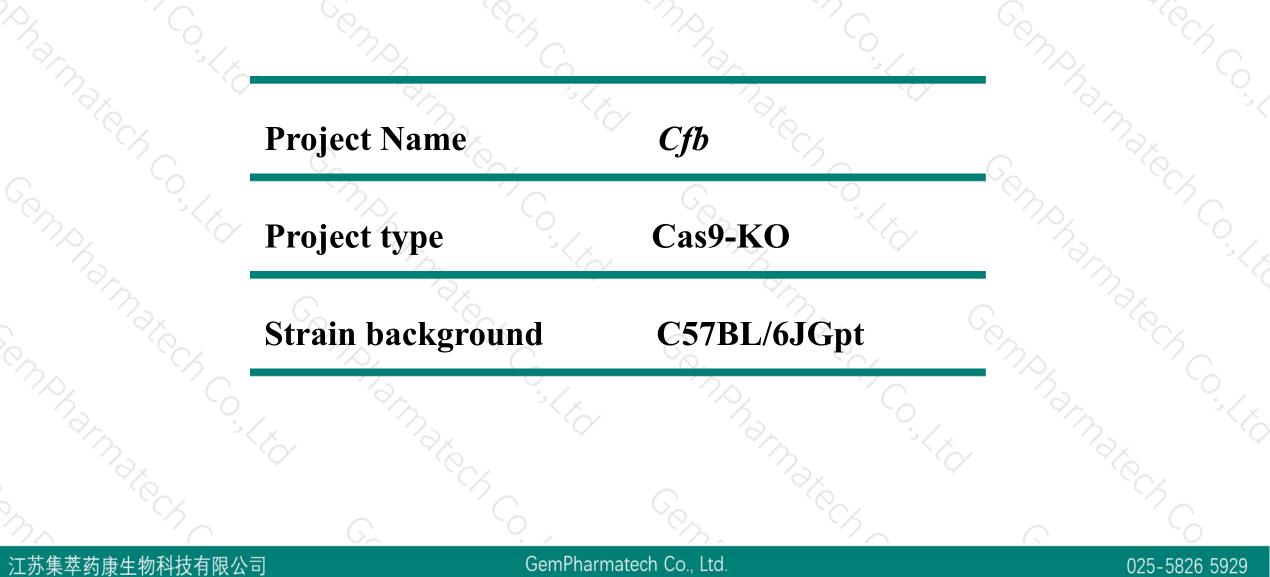
Designer: Daohua Xu

**Reviewer: Xueting Zhang** 

Design Date: 2020-10-20

# **Project Overview**





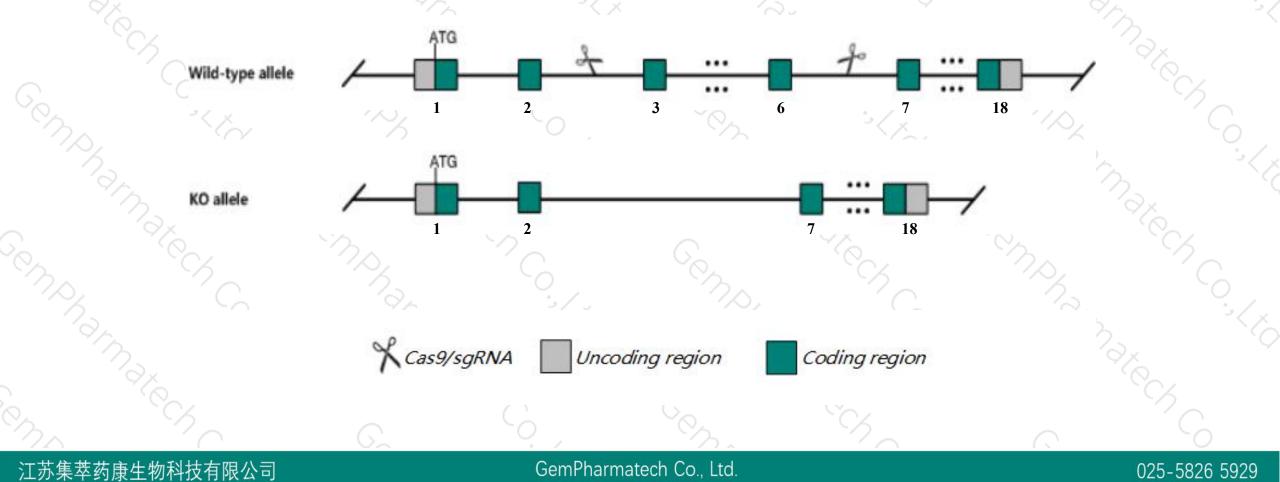
江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd.

# **Knockout** strategy



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





> The *Cfb* gene has 8 transcripts. According to the structure of *Cfb* gene, exon3-exon6 of *Cfb*-201(ENSMUST00000025229.10) transcript is recommended as the knockout region. The region contains 599bp coding sequence. Knock out the region will result in disruption of protein function.

> In this project we use CRISPR/Cas9 technology to modify *Cfb* gene. The brief process is as follows: sgRNA was transcribed in vitro.Cas9 and sgRNA 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.

- > According to the existing MGI data, homozygotes for targeted null mutations lack the alternative complement pathway, and have reduced overall complement activity.
- > The KO region contains functional region of the Gm20547 gene.Knockout the region may affect the function of Gm20547 gene.
- ➤ Transcript *Cfb-204*, *Cfb-205* and *Cfb-208* may not be affected.
- > The *Cfb* 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 the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Notice

# **Gene information (NCBI)**



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025-5826 5929

### Cfb complement factor B [Mus musculus (house mouse)]

Gene ID: 14962, updated on 13-Mar-2020

#### Summary

Official SymbolCfb provided by MGIOfficial Full Namecomplement factor B provided byMGIPrimary sourceMGI:MGI:105975See relatedEnsembl:ENSMUSG0000090231Gene typeprotein codingRefSeq statusVALIDATEDOrganismMus musculusLineageEukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;<br/>Myomorpha; Muroidea; Murinae; Mus; MusAlso knownasAl195813, Al255840, B, Bf, C2, Fb, H2-BfExpressionBiased expression in liver E18 (RPKM 279.0), liver adult (RPKM 221.3) and 9 other tissuesSee moreOrthologhuman all

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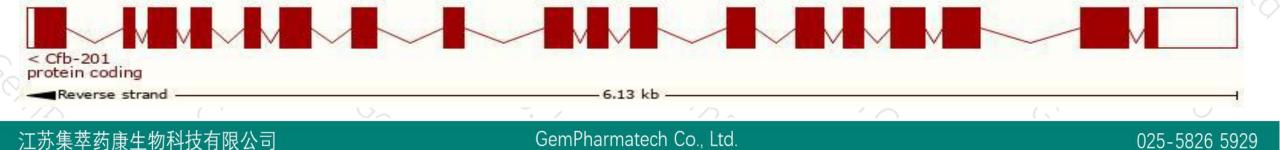
# **Transcript information (Ensembl)**



### The gene has 8 transcripts, all transcripts are shown below:

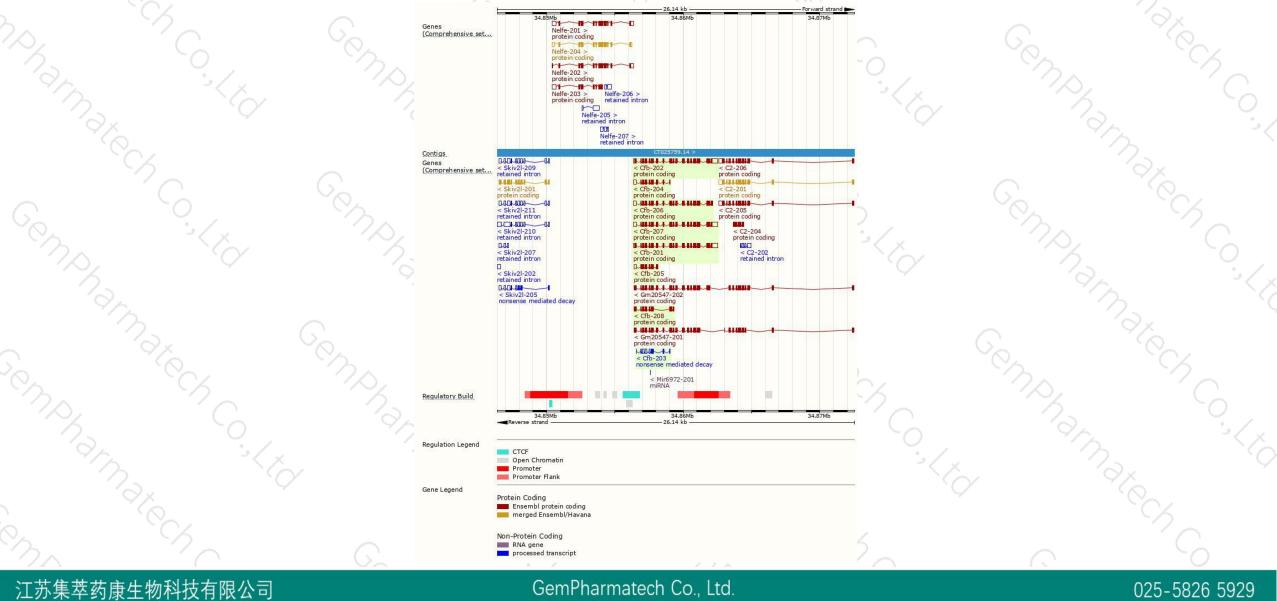
Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
ENSMUST0000025229.10	2737	<u>763aa</u>	Protein coding	CCD528663	B8JJM5	TSL:1 GENCODE basic APPRIS P2
ENSMUST00000176203.8	2733	<u>713aa</u>	Protein coding	CCDS50079	<u>B8JJM6</u>	TSL:1 GENCODE basic
ENSMUST00000128767.7	2751	<u>761aa</u>	Protein coding	8 <u>2</u> 8	P04186 Q3UEG8	TSL:1 GENCODE basic APPRIS ALT2
ENSMUST00000154526.7	2397	<u>711aa</u>	Protein coding		<u>F6XQ00</u>	TSL:1 GENCODE basic
ENSMUST00000141295.7	973	<u>259aa</u>	Protein coding	-	<u>F6W2T4</u>	CDS 5' incomplete TSL:5
ENSMUST00000176332.1	905	<u>296aa</u>	Protein coding	1070	<u>H3BK95</u>	CDS 5' incomplete TSL:5
ENSMUST00000153400.7	887	<u>234aa</u>	Protein coding	19 <b>-</b> 1	<u>B8JJM3</u>	CDS 5' incomplete TSL:5
ENSMUST00000133127.1	655	<u>81aa</u>	Nonsense mediated decay		F6WZA2	CDS 5' incomplete TSL:5
	ENSMUST0000025229.10 ENSMUST00000176203.8 ENSMUST00000128767.7 ENSMUST00000154526.7 ENSMUST00000141295.7 ENSMUST00000176332.1	ENSMUST0000025229.10   2737     ENSMUST00000176203.8   2733     ENSMUST00000128767.7   2751     ENSMUST00000154526.7   2397     ENSMUST00000141295.7   973     ENSMUST00000176332.1   905     ENSMUST00000153400.7   887	ENSMUST0000025229.10 2737 763aa   ENSMUST00000176203.8 2733 713aa   ENSMUST0000128767.7 2751 761aa   ENSMUST0000154526.7 2397 711aa   ENSMUST0000176332.1 973 259aa   ENSMUST0000153400.7 887 234aa	ENSMUST0000025229.102737763aaProtein codingENSMUST0000176203.82733713aaProtein codingENSMUST0000128767.72751761aaProtein codingENSMUST0000154526.72397711aaProtein codingENSMUST0000141295.7973259aaProtein codingENSMUST0000176332.1905296aaProtein codingENSMUST0000153400.7887234aaProtein coding	ENSMUST0000025229.102737763aaProtein codingCCDS28663ENSMUST0000176203.82733713aaProtein codingCCDS50079ENSMUST0000128767.72751761aaProtein coding-ENSMUST0000154526.72397711aaProtein coding-ENSMUST0000141295.7973259aaProtein coding-ENSMUST0000176332.1905296aaProtein coding-ENSMUST0000153400.7887234aaProtein coding-	ENSMUST0000025229.102737763aaProtein codingCCDS28663B8JJM5ENSMUST00000176203.82733713aaProtein codingCCDS50079B8JJM6ENSMUST00000128767.72751761aaProtein coding-P04186 Q3UEG8ENSMUST00000154526.72397711aaProtein coding-F6XQ00ENSMUST00000141295.7973259aaProtein coding-F6W2T4ENSMUST0000015632.1905296aaProtein coding-H3BK95ENSMUST00000153400.7887234aaProtein coding-B8JJM3

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



## **Genomic location distribution**



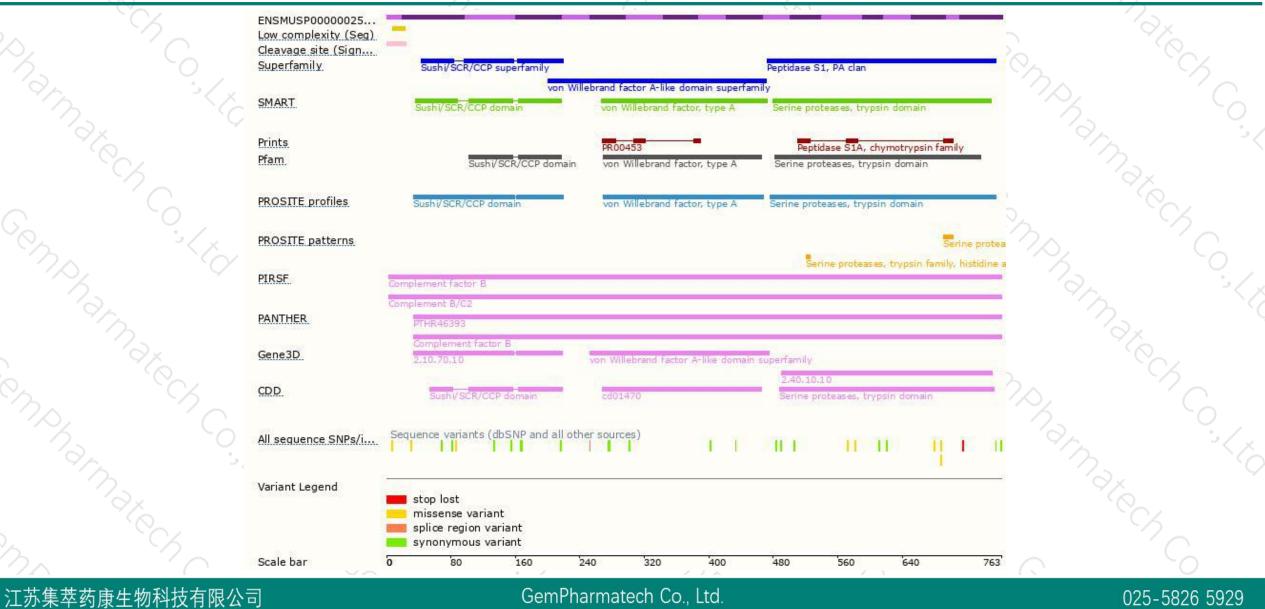


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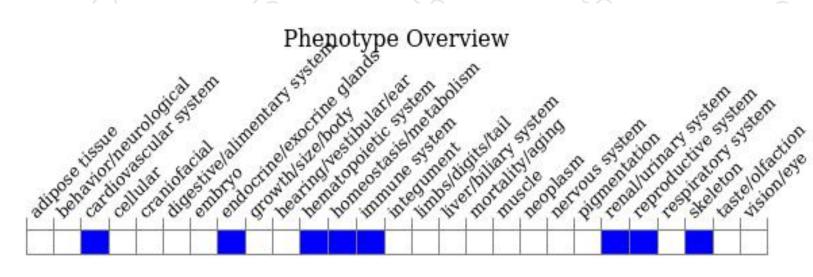
### **Protein domain**





### 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, homozygotes for targeted null mutations lack the alternative complement pathway, and have reduced overall complement activity.

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



