

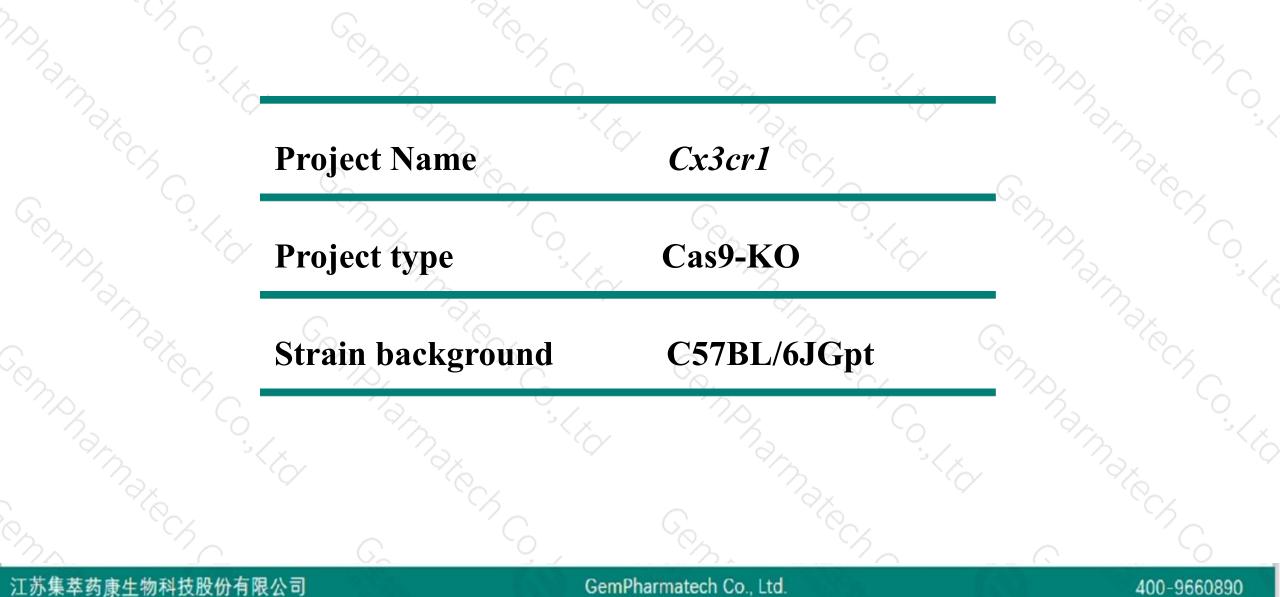
# Cx3cr1 Cas9-KO Strategy

Designer: Xueting Zhang Design Date: 2019-8-5

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# **Project Overview**

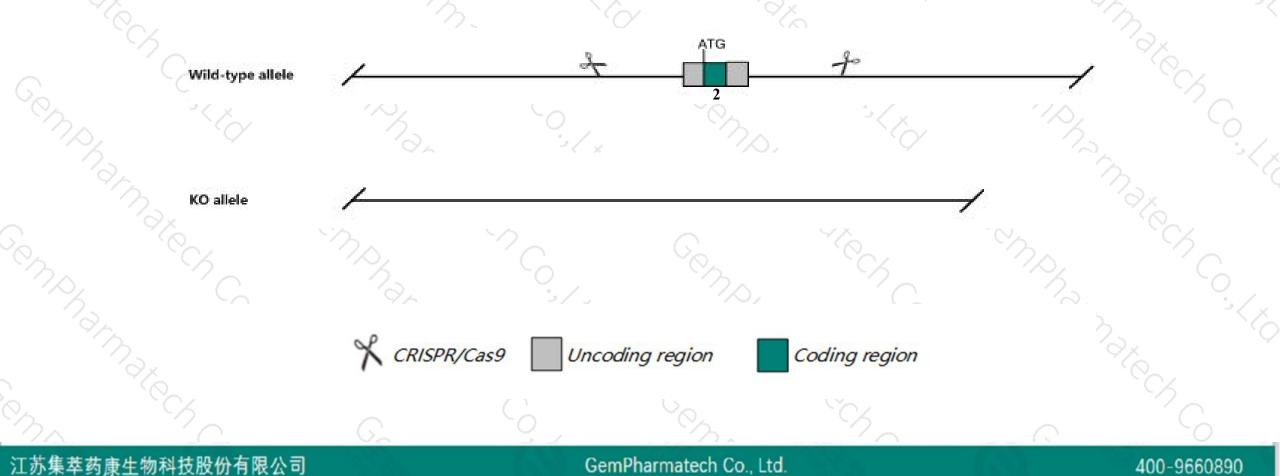




# **Knockout** strategy



This model will use CRISPR/Cas9 technology to edit the *Cx3cr1* gene. The schematic diagram is as follows:





- The Cx3cr1 gene has 3 transcripts. According to the structure of Cx3cr1 gene, exon2 of Cx3cr1-201 (NSMUST00000064165.4) transcript is recommended as the knockout region. The region contains all of the coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Cx3cr1* gene. The brief process is as follows: gRNA was transcribed in vitro.Cas9 and gRNA 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, Age related retinal degeneration with abnormal subretinal microglial cell accumulation in one homozygous null mice. Other null mice shows impaired monocyte recruitment after vascular injury, kidney ischemia and reperfusion, and bacterial infection of the instestine.
- The Cx3cr1 gene is located on the Chr9. 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.

# **Gene information (NCBI)**



## Cx3cr1 chemokine (C-X3-C motif) receptor 1 [Mus musculus (house mouse)]

Gene ID: 13051, updated on 9-Apr-2019

### Summary

Official Symbol	Cx3cr1 provided by MGI
Official Full Name	chemokine (C-X3-C motif) receptor 1 provided by MGI
Primary source	MGI:MGI:1333815
See related	Ensembl:ENSMUSG0000052336
Gene type	protein coding
<b>RefSeq status</b>	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;
	Muroidea; Muridae; Murinae; Mus; Mus
Expression	Ubiquitous expression in cortex adult (RPKM 8.3), frontal lobe adult (RPKM 7.0) and 27 other tissues See more
Orthologs	human all

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# **Transcript information (Ensembl)**



# The gene has 3 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Cx3cr1-203	ENSMUST00000215016.1	4493	<u>354aa</u>	Protein coding	CCDS40806	Q543X3 Q9Z0D9	TSL:5 GENCODE basic APPRIS P1
Cx3cr1-201	ENSMUST0000064165.4	3753	<u>354aa</u>	Protein coding	CCDS40806	Q543X3 Q9Z0D9	TSL:1 GENCODE basic APPRIS P1
Cx3cr1-202	ENSMUST00000177637.1	3156	<u>354aa</u>	Protein coding	CCDS40806	Q543X3 Q9Z0D9	TSL:5 GENCODE basic APPRIS P1

The strategy is based on the design of *Cx3cr1-201* transcript, The transcription is shown below

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< Cx3cr1-201 protein coding ■Reverse strand —		19.61 kb -		i	ľ S
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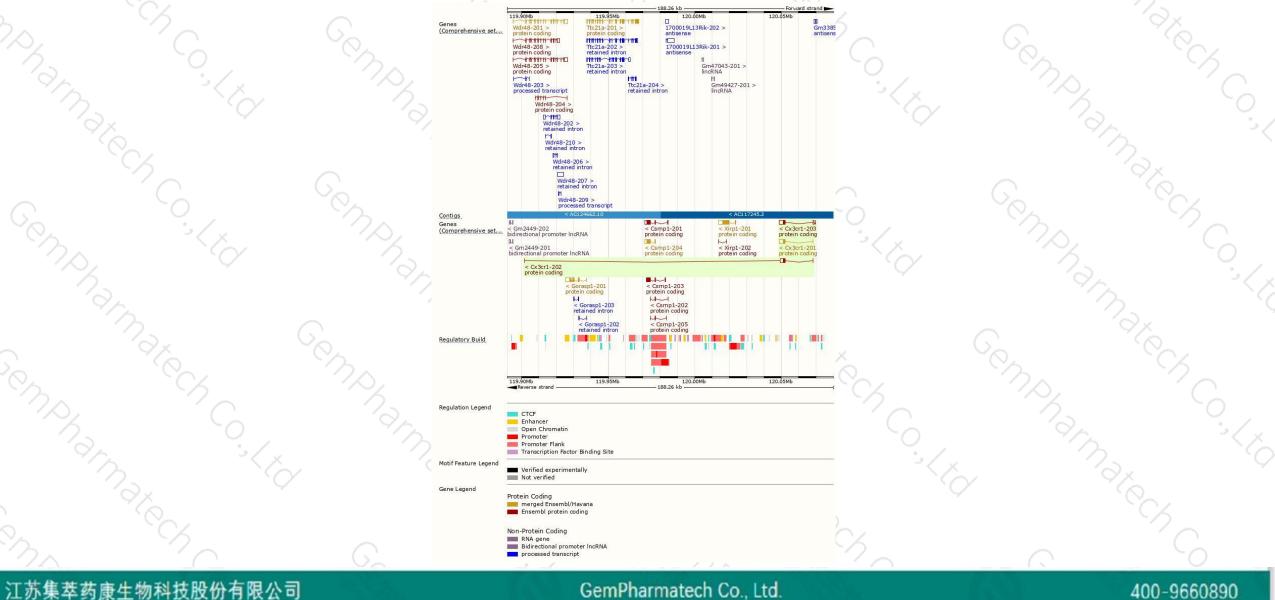
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# **Genomic location distribution**





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# **Protein domain**



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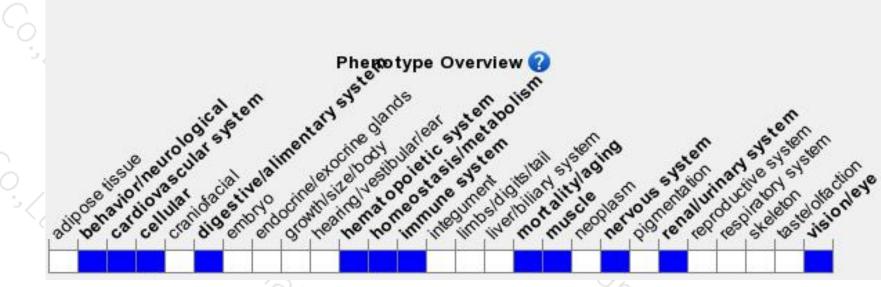
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# Mouse phenotype description(MGI)





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, Age related retinal degeneration with abnormal subretinal microglial cell accumulation in one homozygous null mice. Other null mice shows impaired monocyte recruitment after vascular injury, kidn ischemia and reperfusion, and bacterial infection of the instestine.

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



