

Cxcr5 Cas9-KO Strategy

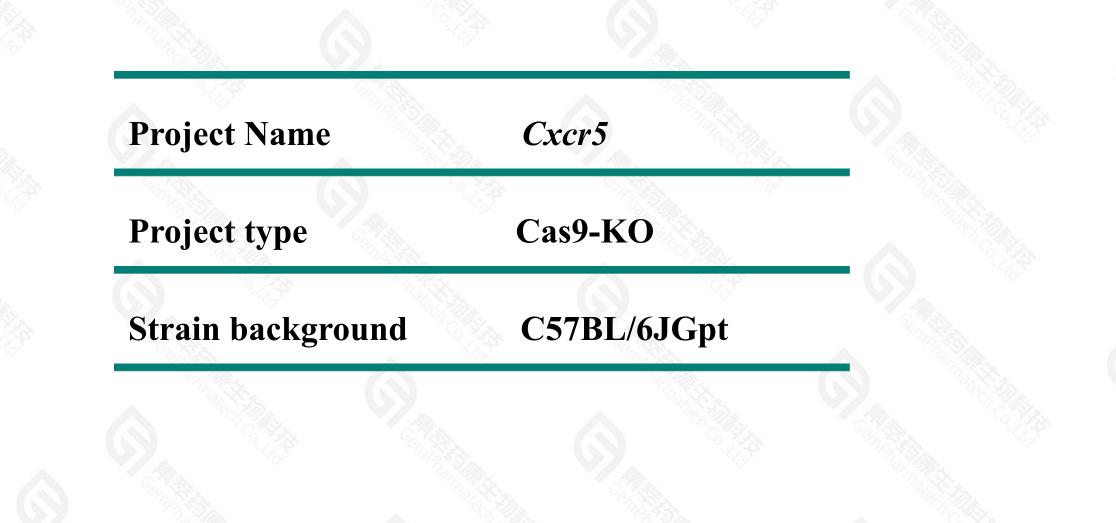
Designer: Daohua Xu

Reviewer: Xueting Zhang

Design Date: 2021-11-19

Project Overview





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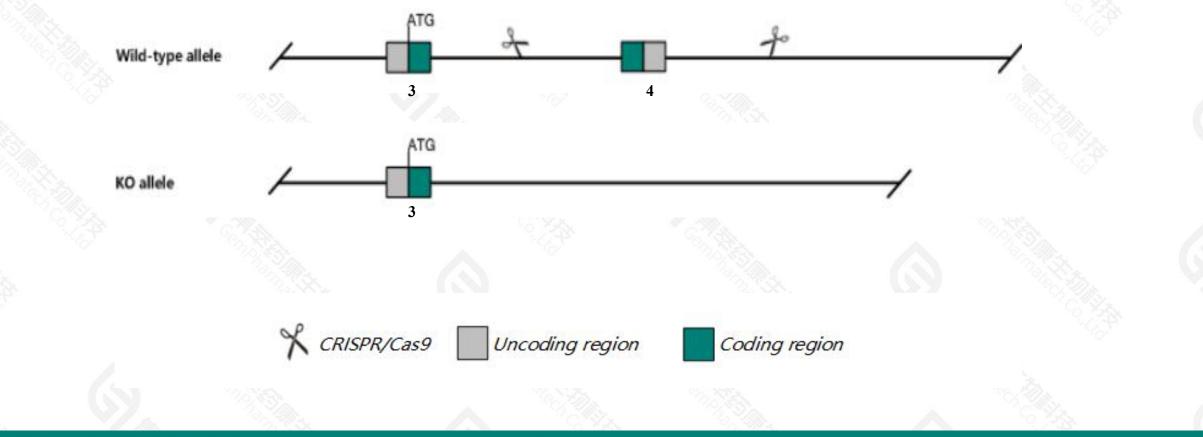
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Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Cxcr5 gene. The schematic diagram is as follows:



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> The *Cxcr5* gene has 5 transcripts. According to the structure of *Cxcr5* gene, exon4 of *Cxcr5*-204(ENSMUST00000215293.2) transcript is recommended as the knockout region. The region contains most 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 *Cxcr5* gene. The brief process is as follows: CRISPR/Cas9 system 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, homozygous null mutants lack inguinal lymph nodes, have a few abnormal or no Peyer's patches, morphologically altered primary lymphoid follicles and no functional germinal centers in their spleen.
The KO region contains functional region of the *Bcl9l* gene. Knockout the region may affect the function of *Bcl9l* gene.
The *Cxcr5* 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.

Cxcr5 chemokine (C-X-C motif) receptor 5 [Mus musculus (house mouse)]

Gene ID: 12145, updated on 13-Dec-2020

Summary

Official Symbol	Cxcr5 provided by MGI
Official Full Name	chemokine (C-X-C motif) receptor 5 provided by MGI
Primary source	MGI:MGI:103567
See related	Ensembl:ENSMUSG00000047880
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	Bl, Blr1, CXC-R5, CXCR-, CXCR-5, Gpc, Gpcr6, MDR15
Expression	Biased expression in spleen adult (RPKM 61.9), mammary gland adult (RPKM 12.5) and 1 other tissueSee more
Orthologs	human all

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Gene information (NCBI)

Transcript information (Ensembl)



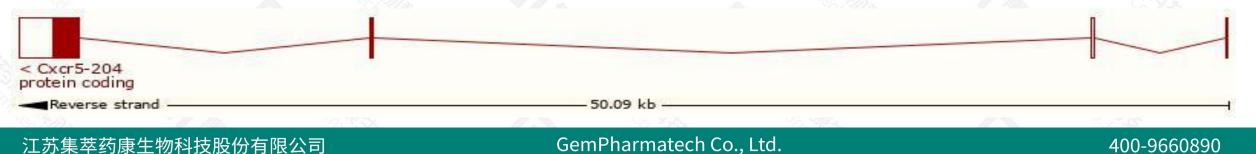
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The gene has 5 transcripts, all transcripts are shown below:

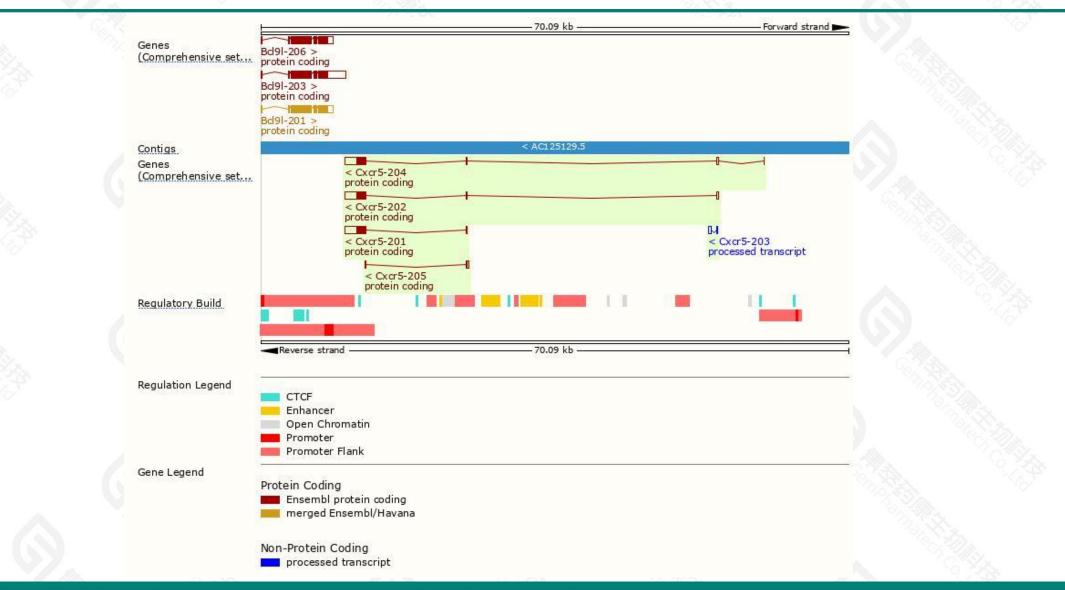
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Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
ENSMUST00000215293.2	2846	<u>374aa</u>	Protein coding	CCDS23115		TSL:1, GENCODE basic, APPRIS P1,
ENSMUST00000179828.8	2824	<u>374aa</u>	Protein coding	CCDS23115		TSL:5, GENCODE basic, APPRIS P1,
ENSMUST0000062215.8	2614	<u>374aa</u>	Protein coding	CCDS23115		TSL:1, GENCODE basic, APPRIS P1,
ENSMUST00000215661.2	358	<u>51aa</u>	Protein coding	-		CDS 3' incomplete , TSL:1 ,
ENSMUST00000213357.2	377	No protein	Processed transcript	20		TSL:3,
	ENSMUST00000215293.2 ENSMUST00000179828.8 ENSMUST00000062215.8 ENSMUST00000215661.2	ENSMUST00000215293.2 2846 ENSMUST00000179828.8 2824 ENSMUST0000062215.8 2614 ENSMUST00000215661.2 358	ENSMUST00000215293.2 2846 374aa ENSMUST00000179828.8 2824 374aa ENSMUST0000062215.8 2614 374aa ENSMUST00000215661.2 358 51aa	ENSMUST00000215293.22846374aaProtein codingENSMUST00000179828.82824374aaProtein codingENSMUST0000062215.82614374aaProtein codingENSMUST00000215661.235851aaProtein coding	ENSMUST00000215293.22846374aaProtein codingCCDS23115ENSMUST00000179828.82824374aaProtein codingCCDS23115ENSMUST0000062215.82614374aaProtein codingCCDS23115ENSMUST00000215661.235851aaProtein coding-	ENSMUST0000215293.22846374aaProtein codingCCDS23115ENSMUST00000179828.82824374aaProtein codingCCDS23115ENSMUST0000062215.82614374aaProtein codingCCDS23115ENSMUST00000215661.235851aaProtein coding-

The strategy is based on the design of *Cxcr5-204* transcript, the transcription is shown below:



Genomic location distribution



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Protein domain



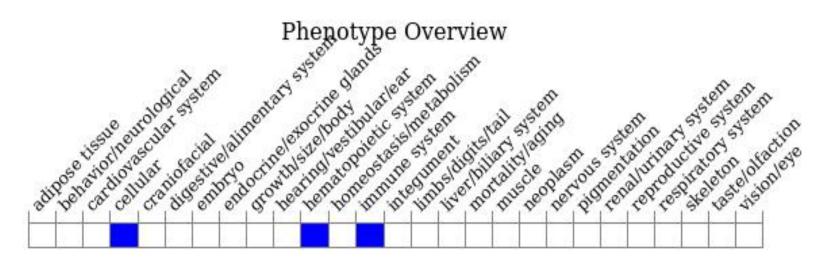
ENSMUSP00000149 Transmembrane heli						-		
Low complexity (Seg)								
Superfamily	SSF81321							
Prints	Gpro	tein-coupled recep	tor, rhodopsin-like		-			
	CXC chemokine rec				-		N	_
Pfam		manufacture and a second se	receptor, rhodopsin-lik	e				
PROSITE profiles		GPCR, rhodopsin-li	ke, 7TM					
PROSITE patterns			G protein-coupler	receptor, rho	lopsin-like			
PANTHER	PTHR104891SF618							
	PTHR 10489							
Gene3D	1.20,10	070.10						
<u>CDD</u>	cd15:						-	
All sequence SNPs/i	Sequence variants (dl		r sources)			a	-	
An sequence Shrs/I			Weberg Strategy					
Variant Legend	splice region var	iant						
	synonymous var							
Scale bar	0 40	80	160	200	240	280	320	374
				1423/				

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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,homozygous null mutants lack inguinal lymph nodes, have a few abnormal or no Peyer's patches, morphologically altered primary lymphoid follicles and no functional germinal centers in their spleen.



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



