

Igsf6 Cas9-CKO Strategy

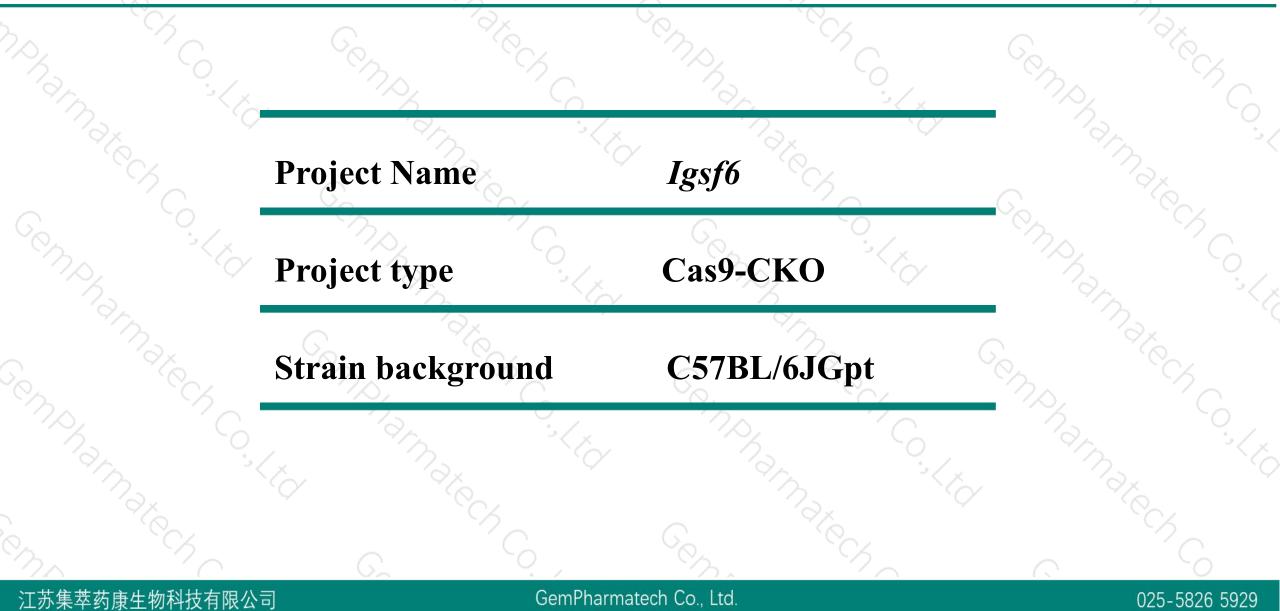
Designer: Xueting Zhang

Reviewer: Daohua Xu

Design Date: 2020-9-29

Project Overview



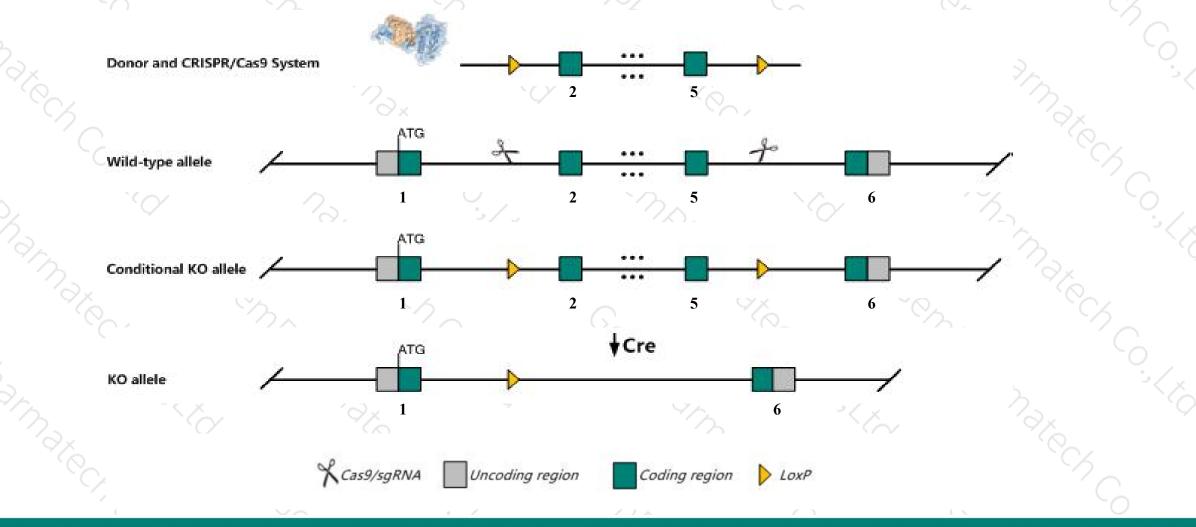


Conditional Knockout strategy



025-5826 5929

This model will use CRISPR/Cas9 technology to edit the *Igsf6* gene. The schematic diagram is as follows:



江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd.



The Igsf6 gene has 1 transcript. According to the structure of Igsf6 gene, exon2-exon5 of Igsf6-201(ENSMUST00000047194.3) transcript is recommended as the knockout region. The region contains 590bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Igsf6* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor vector was constructed.Cas9, sgRNA 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 was 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.



- > The floxed region is near to the N-terminal of 2210406H18Rik gene, this strategy may influence the regulatory function of the N-terminal of 2210406H18Rik gene.
- > The partial intron of *Mettl9* gene will be deleted together after Cre recombination in this strategy.
- > The *Igsf6* gene is located on the Chr7. 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)



☆ ?

Igsf6 immunoglobulin superfamily, member 6 [Mus musculus (house mouse)]

Gene ID: 80719, updated on 13-Mar-2020

Summary

Official Symbol	Igsf6 provided by MGI
Official Full Name	immunoglobulin superfamily, member 6 provided by <u>MGI</u>
Primary source	MGI:MGI:1891393
See related	Ensembl:ENSMUSG0000035004
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
	Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Expression	Broad expression in liver E18 (RPKM 6.0), liver E14 (RPKM 3.2) and 19 other tissuesSee more
Orthologs	human all

江苏集萃药康生物科技有限公司

GemPharmatech Co., Ltd.

025-5826 5929



The gene has 1 transcript, and the transcript is shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
gsf6-201	ENSMUST00000047194.3	00047194.3 2175		Protein coding	coding <u>CCDS21801</u> <u>P0C6B7</u>		TSL:1 GENCODE basic APPRIS P1
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	5		27	$\sim$	<u></u>	<	
			°°G -				
		2					$\gamma_{\mathcal{S}_{\mathcal{L}}}$
		912			CAN .		and the
					1 drps		

The strategy is based on the design of *Igsf6-201* transcript, the transcription is shown below:

#### < Igsf6-201 protein coding

Reverse strand

江苏集萃药康生物科技有限公司

#### GemPharmatech Co., Ltd

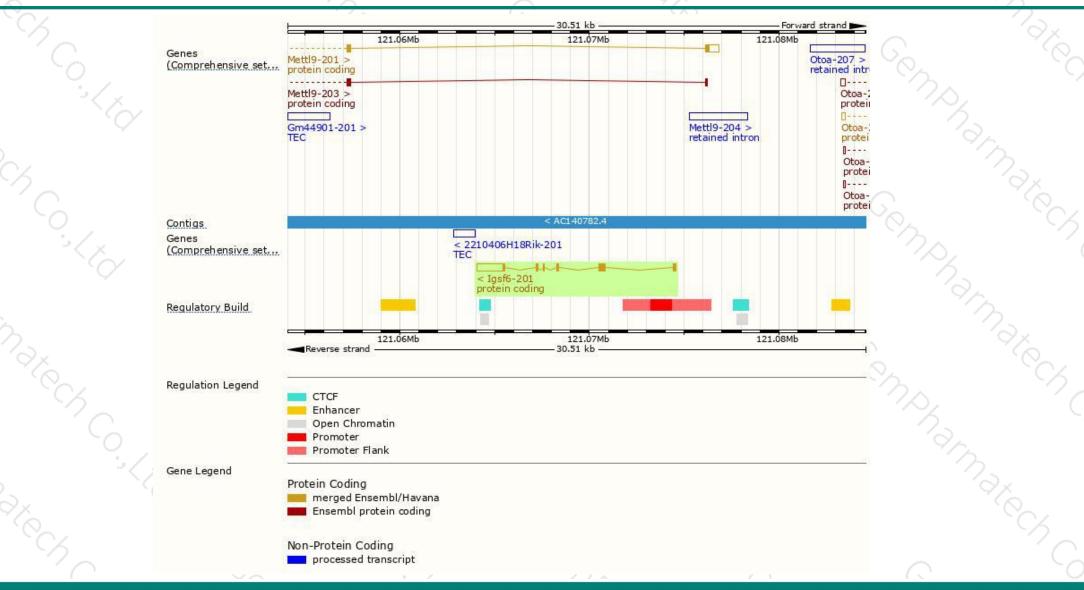
10.51 kb



### **Genomic location distribution**



025-5826 5929



#### <u>江苏集萃药康生物科技有限公司</u>

GemPharmatech Co., Ltd.

## **Protein domain**



			°070		[°] [°] [°]		100			)	G	ions,	^C C	
	ENSMUSP00000039 Transmembrane heli MobiDB lite Superfamily			mmunogla	bulin-like d	omain sup	erfamily	l pr	÷.		-			-0
Go.	PROSITE profiles	-	mmunoglob	oulin-like d	lomain									
~??;	PANTHER	PS5125	7. Iglobulin su	perfamily	member 6	_								0.
	Gene3D			inoglobulir	the state of the state				))					
	All sequence SNPs/i	Sequer	nce variant	ts (dbSNF	P and all o	ther sourc	ces)		11					
'Ony	Variant Legend		issense va nonymou		[									0
	Scale bar	0	20	40	60	80	100	120	140	160	180	200	237	- 6
	nate ch	<i>~</i>						1					are C	
江苏集	萃药康生物科技有限公司				Ger	nPharma	tech Co., Lto	∽ d.		$\sim$		(.)	025-5826	6 5929 ·



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



