

Icall Cas9-CKO Strategy

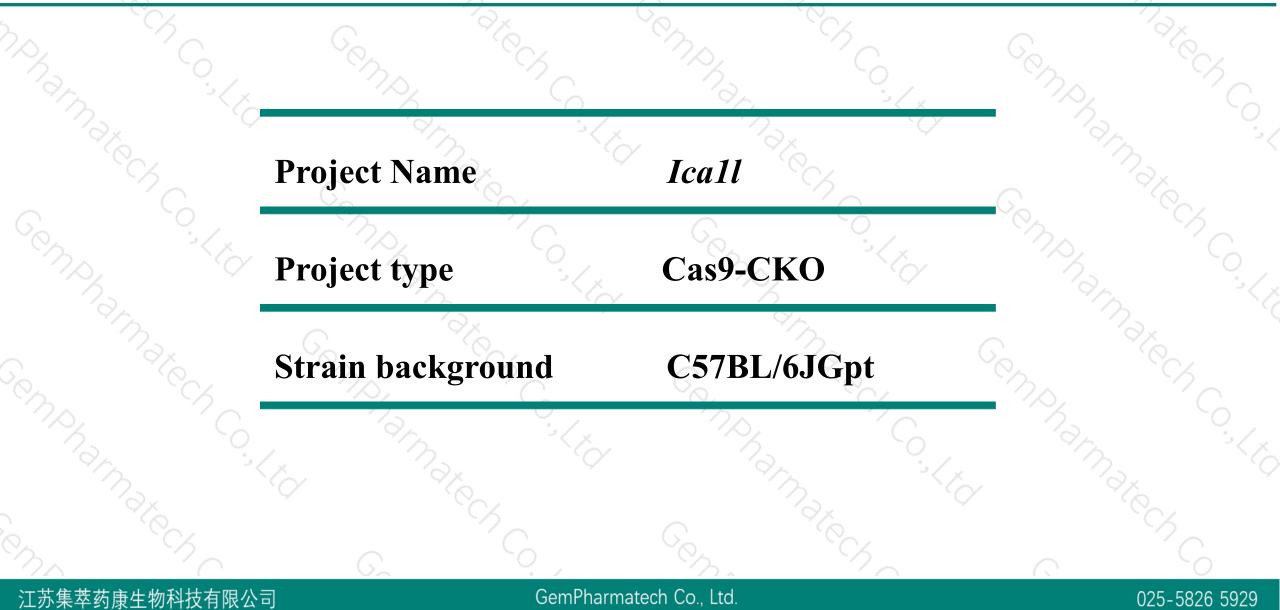
Designer: Jia Yu

Reviewer:Xiaojing Li

Design Date: 2020-9-25

Project Overview



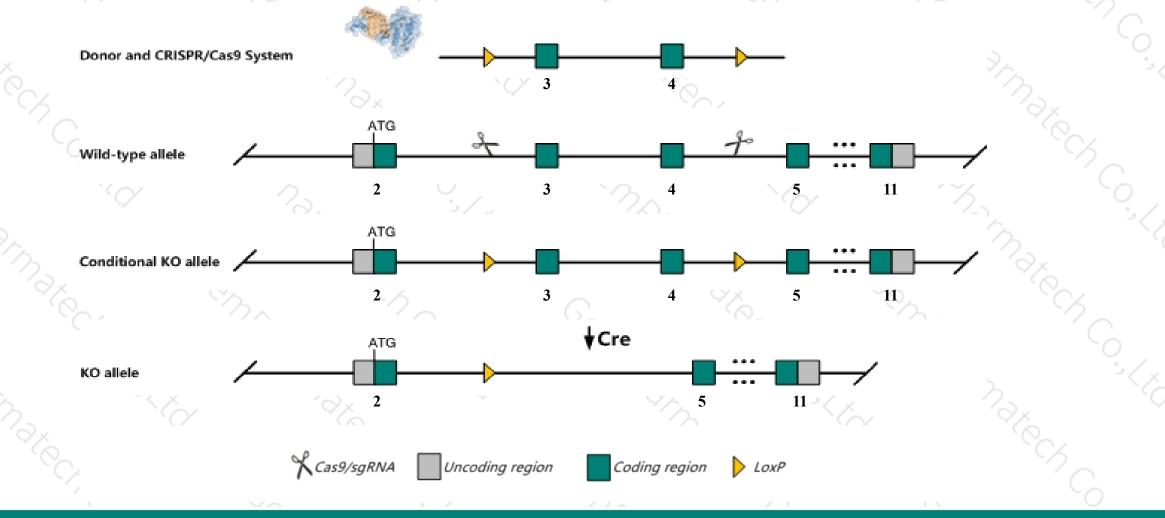


Conditional Knockout strategy



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This model will use CRISPR/Cas9 technology to edit the *Ica11* gene. The schematic diagram is as follows:



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The *Icall* gene has 6 transcripts. According to the structure of *Icall* gene, exon3-exon4 of *Icall-201*(ENSMUST0000027172.12) transcript is recommended as the knockout region. The region contains 197bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Ica11* 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.



According to the existing MGI data,mice homozygous for a hypomorphic allele exhibit reduced male fertility with oligospermia, globospermia, and abnormal spermiogenesis, sperm nucleus and mitochondrial sheath morphology.
 The *Ica11* gene is located on the Chr1. 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)



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Ica1l islet cell autoantigen 1-like [Mus musculus (house mouse)]

Gene ID: 70375, updated on 20-Mar-2020

Summary

Official Symbol	Icall provided by MGI							
Official Full Name	islet cell autoantigen 1-like provided by <u>MGI</u>							
Primary source	MGI:MGI:1917625							
See related	Ensembl:ENSMUSG0000026018							
Gene type	protein coding							
RefSeq status	VALIDATED							
Organism	Mus musculus							
Lineage	e Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;							
	Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus							
Also known as	1700030B17Rik, Als2cr15, b2b3465Clo							
Expression	Biased expression in testis adult (RPKM 8.3), CNS E18 (RPKM 6.5) and 6 other tissuesSee more							
Orthologs	human all							

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Transcript information (Ensembl)



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

Transcript ID NSMUST00000027172.12	bp 3650	Protein 431aa	Biotype	CCDS	UniProt	Flags
	3650	43122				
		45100	Protein coding	CCDS14987	<u>Q3TY65</u>	TSL:1 GENCODE basic APPRIS P2
NSMUST00000191251.6	3415	<u>431aa</u>	Protein coding	CCDS14987	<u>Q3TY65</u>	TSL:1 GENCODE basic APPRIS P2
NSMUST00000189776.1	1320	<u>439aa</u>	Protein coding	-	A0A087WSM1	TSL:5 GENCODE basic APPRIS ALT2
NSMUST00000186970.1	951	No protein	Processed transcript	-	-	TSL:1
NSMUST00000185891.1	516	No protein	Processed transcript	-	-	TSL:3
NSMUST00000187364.1	619	No protein	Retained intron	-	-	TSL:3
EN	ISMUST00000186970.1 ISMUST00000185891.1	ISMUST00000186970.1 951 ISMUST00000185891.1 516	ISMUST00000186970.1 951 No protein ISMUST00000185891.1 516 No protein	ISMUST00000186970.1 951 No protein Processed transcript ISMUST00000185891.1 516 No protein Processed transcript	ISMUST00000186970.1 951 No protein Processed transcript - ISMUST00000185891.1 516 No protein Processed transcript -	ISMUST0000186970.1 951 No protein Processed transcript - - ISMUST00000185891.1 516 No protein Processed transcript - -

The strategy is based on the design of *Ica11-201* transcript, the transcription is shown below:

< Ica11-201 protein coding

Reverse strand

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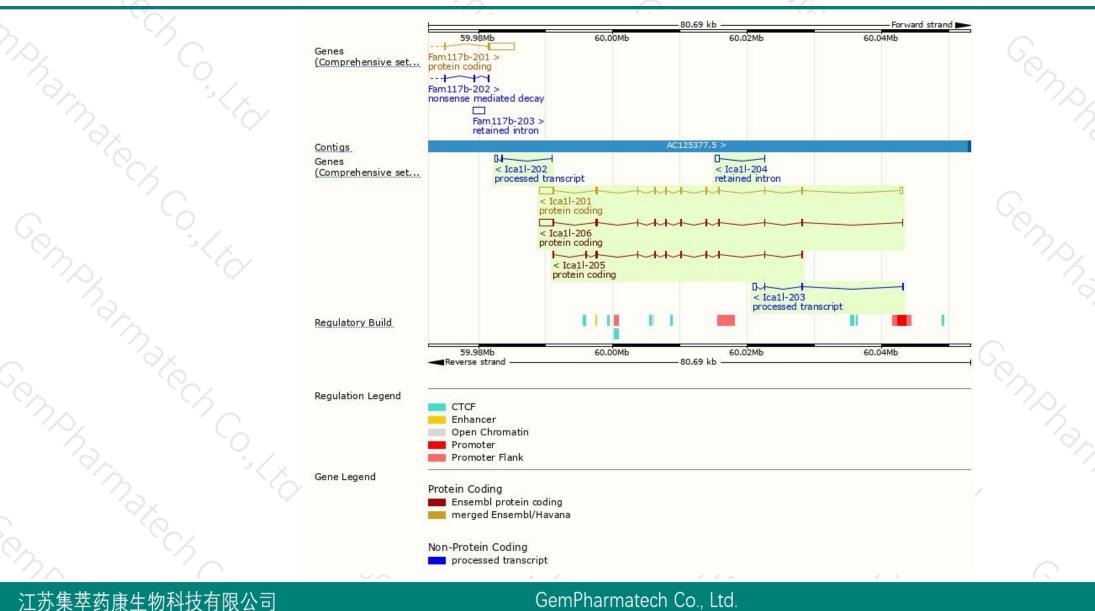
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54.02 kb

Genomic location distribution



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Protein domain

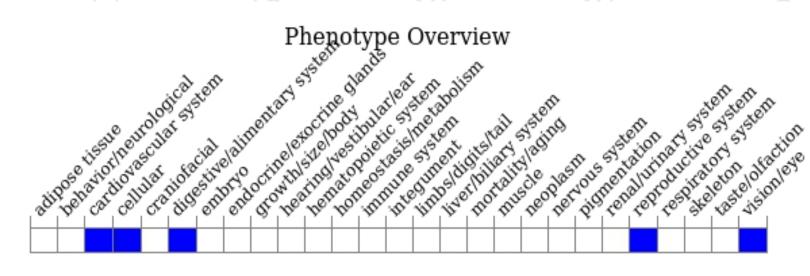


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	ENSMUSP00000027 MobiDB lite Low complexity (Seg)						=	-	20
	Superfamily	AH/BAR d	lomain superfamily						ي د .
	SMART	Arfaptin homology	(AH) domain			Islet c	ell autoantiger	n Ica1, C-terminal	
	<u>Pfam</u>	Artaptin homology	(AH) domain			_	Islet (cell autoantigen Ica1	, C-terminal
	PROSITE profiles	Arfaptin	homology (AH) domain						
	PANTHER	PTHR10164:SF5							
	Gene3D	Islet cell autoantigen : AH/BAR	L/Ica1-like domain superfamily						
	All sequence SNPs/i	Sequence variants (dbSNP and all other	sources)	- 11	4	1	1.1	
	Variant Legend	missense varia							
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Mouse phenotype description(MGI)



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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 a hypomorphic allele exhibit reduced male fertility with oligospermia, globospermia, and abnormal spermiogenesis, sperm nucleus and mitochondrial sheath morphology.



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



