

Jam3 Cas9-CKO Strategy

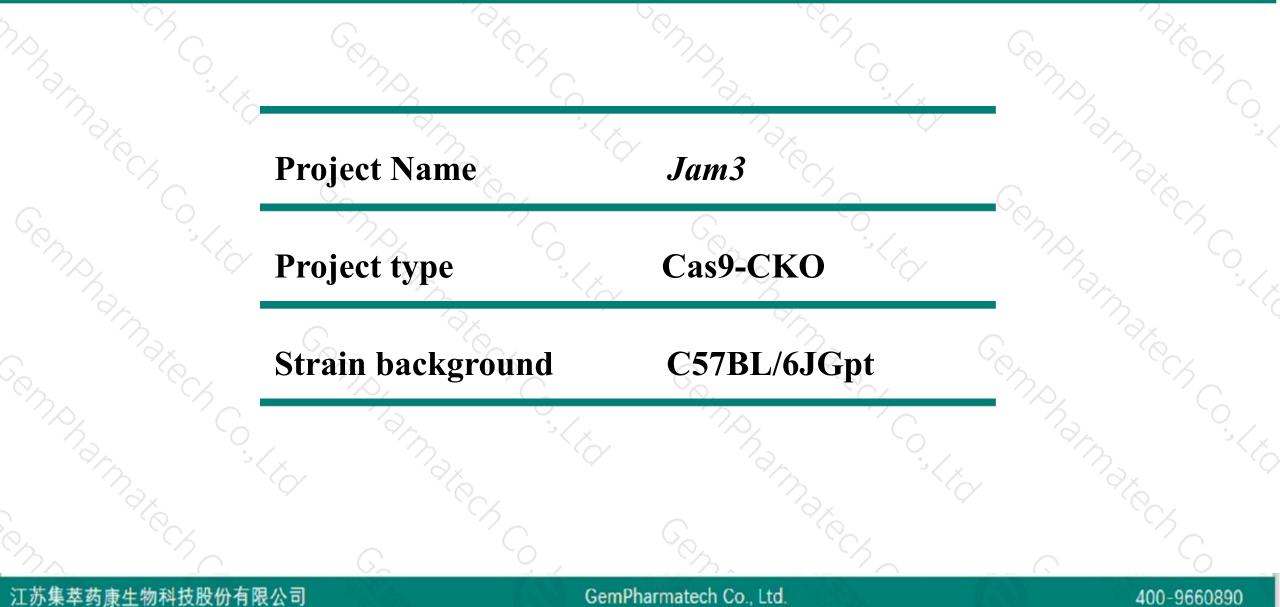
Designer: Reviewer:

Design Date:

Daohua Xu Huimin Su 2020-1-20

Project Overview

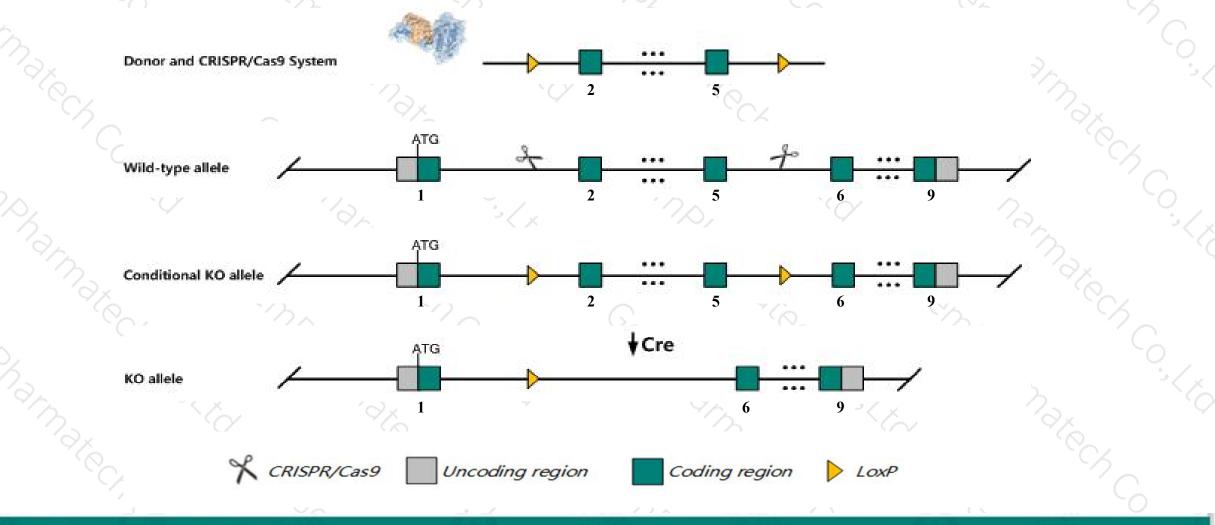




Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the Jam3 gene. The schematic diagram is as follows:



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The Jam3 gene has 5 transcripts. According to the structure of Jam3 gene, exon2-exon5 of Jam3-201 (ENSMUST00000034472.15) transcript is recommended as the knockout region. The region contains 536bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify Jam3 gene. The brief process is as follows:CRISPR/Cas9 system 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 will be 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.

Notice



- According to the existing MGI data, Approximately 60% of mice homozygous for a targeted mutation exhibit postnatal lethality. Males are infertile and display small testes and arrested differentiation of round spermatids into spermatozoa, as shown by the absence of acrosomes, elongated nuclei, and morphological signs of polarization.
- The KO region contains functional region of the Gm48796 gene.Knockout the region may affect the function of Gm48796 gene.
- The *Jam3* 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 loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information (NCBI)



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Jam3 junction adhesion molecule 3 [Mus musculus (house mouse)]

Gene ID: 83964, updated on 31-Jan-2019

Summary

Official Symbol	Jam3 provided by <u>MGI</u>
Official Full Name	junction adhesion molecule 3 provided by MGI
Primary source	MGI:MGI:1933825
See related	Ensembl:ENSMUSG00000031990
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	1110002N23Rik, JAM-3, JAM-C, Jcam3
Expression	Broad expression in CNS E11.5 (RPKM 37.0), limb E14.5 (RPKM 30.4) and 21 other tissues See more
Orthologs	human all

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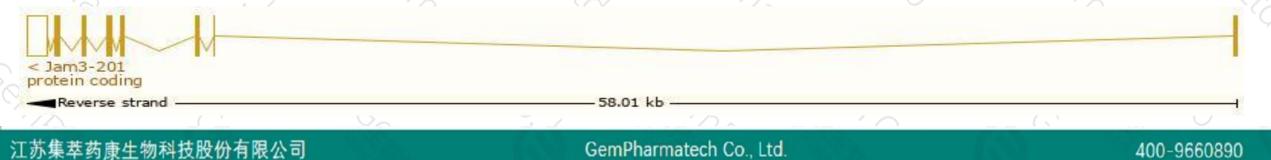
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The gene has 5 transcripts, all transcripts are shown below:

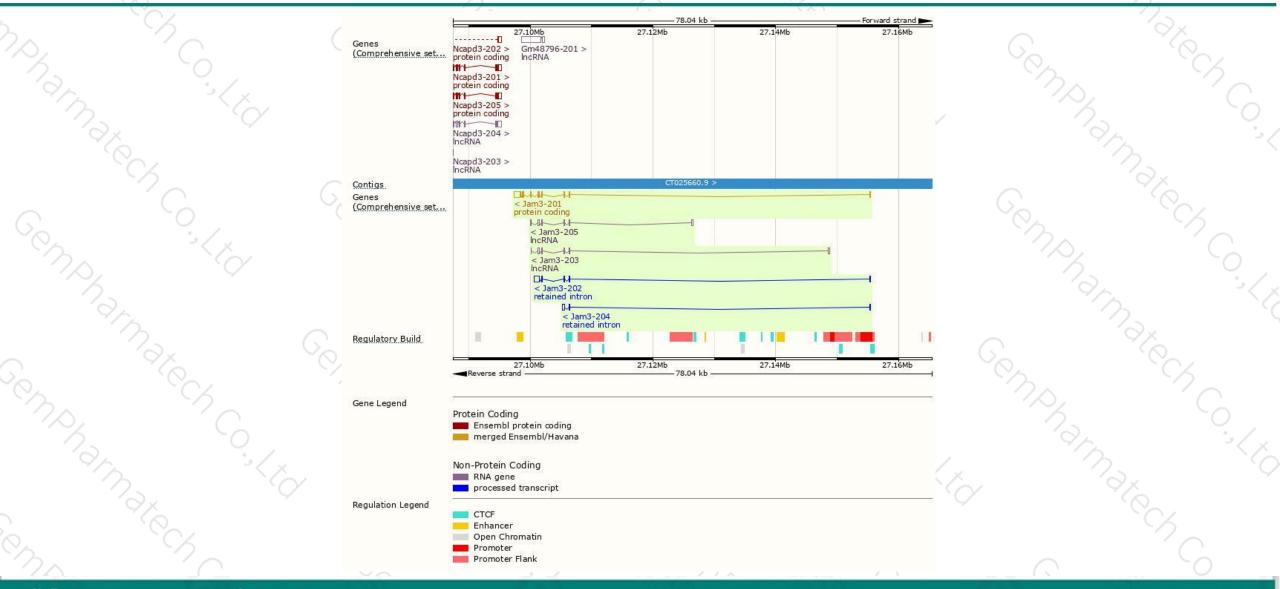
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Jam3-201	ENSMUST0000034472.15	1942	<u>310aa</u>	Protein coding	CCDS22939	<u>Q9D8B7</u>	TSL:1 GENCODE basic APPRIS P1
Jam3-202	ENSMUST00000167074.1	1234	No protein	Retained intron	-	-	TSL:1
Jam3-204	ENSMUST00000213682.1	597	No protein	Retained intron		-	TSL:2
Jam3-205	ENSMUST00000215446.1	863	No protein	IncRNA	12	22	TSL:3
Jam3-203	ENSMUST00000213170.1	731	No protein	IncRNA	70	-	TSL:3

The strategy is based on the design of Jam3-201 transcript, The transcription is shown below



Genomic location distribution





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Protein domain

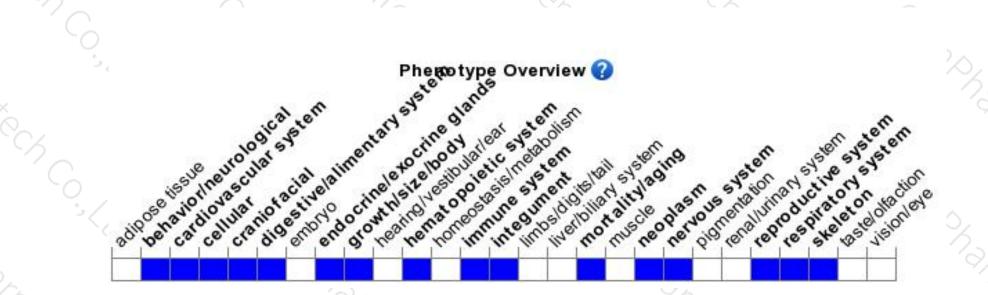
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Low complexity (Seg) Cleavage site (Sign Superfamily SMART Immunoglobulin-like domain superfamily Immunoglobulin subtype 2	
ENSMUSP00000034 Transmembrane heli SIFTS import	and Co

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, Approximately 60% of mice homozygous for a targeted mutation exhibit postnatal lethality. Males are infertile and display small testes and arrested differentiation of round spermatids into spermatozoa, as shown by the absence of acrosomes, elongated nuclei, and morphological signs of polarization.

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



