

Tac2 Cas9-CKO Strategy

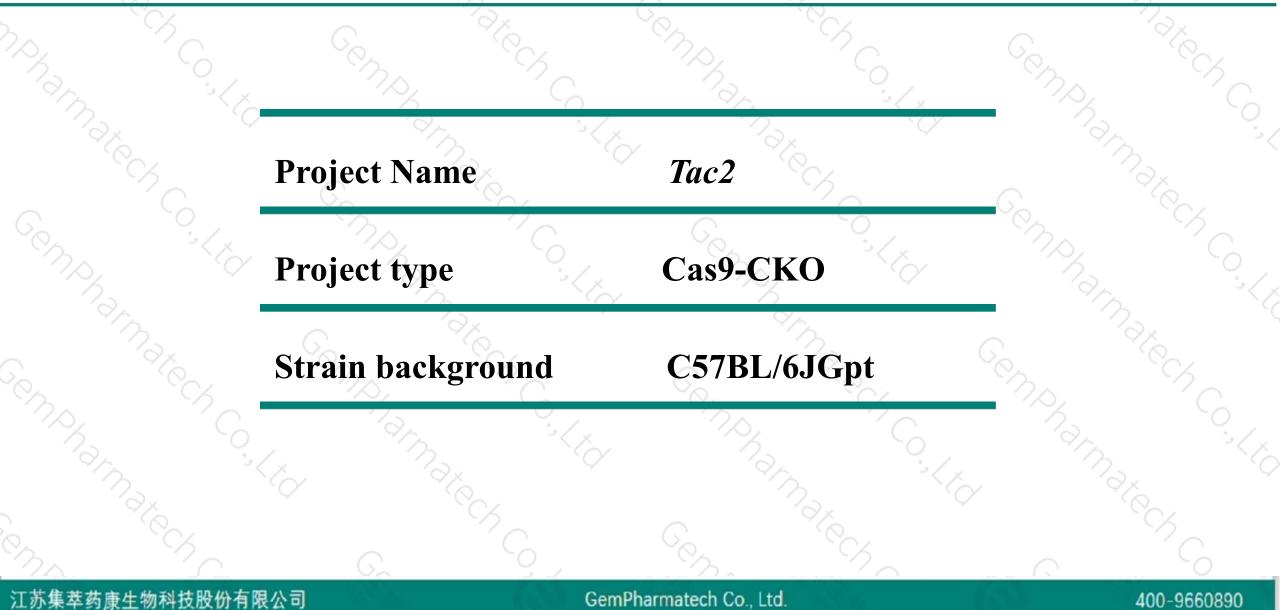
Designer: Reviewer:

Design Date:

Daohua Xu Huimin Su 2020-2-14

Project Overview



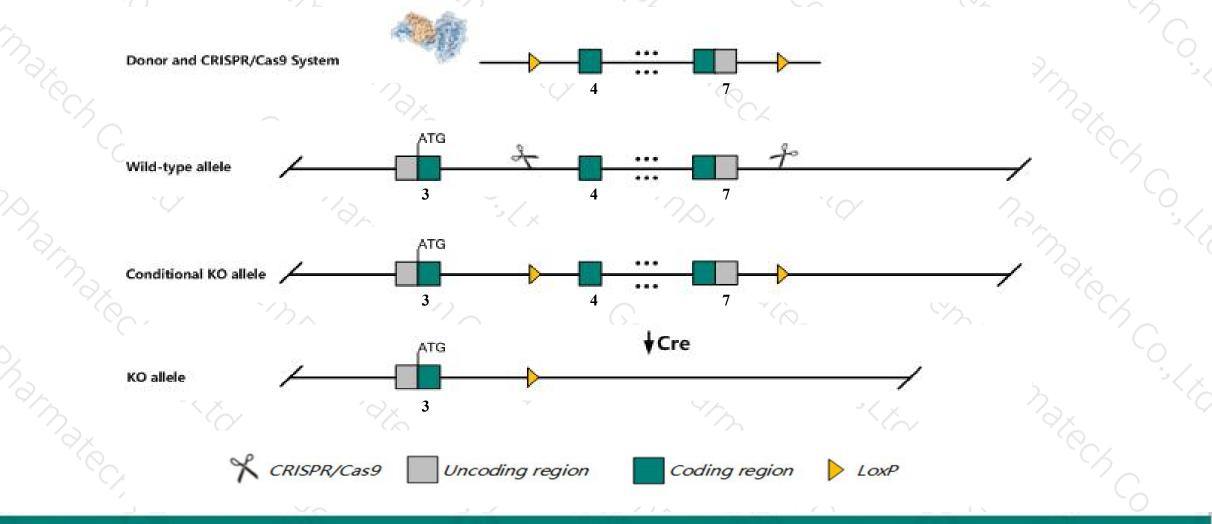


Conditional Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the Tac2 gene. The schematic diagram is as follows:



江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.



The *Tac2* gene has 4 transcripts. According to the structure of *Tac2* gene, exon4-exon7 of *Tac2-202* (ENSMUST00000179960.7) transcript is recommended as the knockout region. The region contains 247bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Tac2* 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, Mice homozygous for a knock-out allele exhibit delayed female sexual maturation and fertility.
- The *Tac2* gene is located on the Chr10. 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)

Muroidea; Muridae; Murinae; Mus; Mus



2

Tac2 tachykinin 2 [Mus musculus (house mouse)]

Gene ID: 21334, updated on 31-Jan-2019

Summary Official Symbol Tac2 provided by MGI Official Full Name tachykinin 2 provided byMGI Primary source MGI:MGI:98476 See related Ensembl:ENSMUSG00000025400 Gene type protein coding RefSeq status REVIEWED Organism Mus musculus Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha;

Also known as PPT-B, Tac3

Summary This gene encodes a member of the tachykinin family of signaling peptides that is widely expressed in the central nervous system and plays a role in diverse processes such as water homeostasis, pulmonary inflammation, cognition, fear memory consolidation and preeclampsia. The encoded protein is enzymatically processed to generate the mature neuropeptide. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Jul 2015]

Expression Biased expression in liver E14.5 (RPKM 9.9), liver E14 (RPKM 8.0) and 9 other tissues See more

Orthologs human all

江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

400-9660890



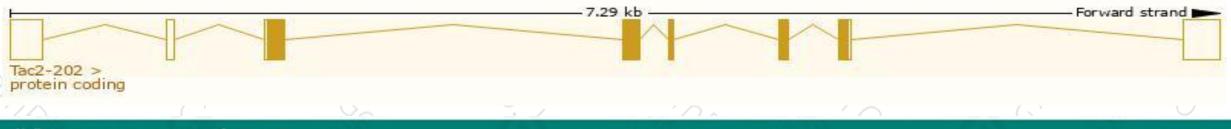
400-9660890

The gene has 4 transcripts, all transcripts are shown below:

江苏集萃药康生物科技股份有限公司

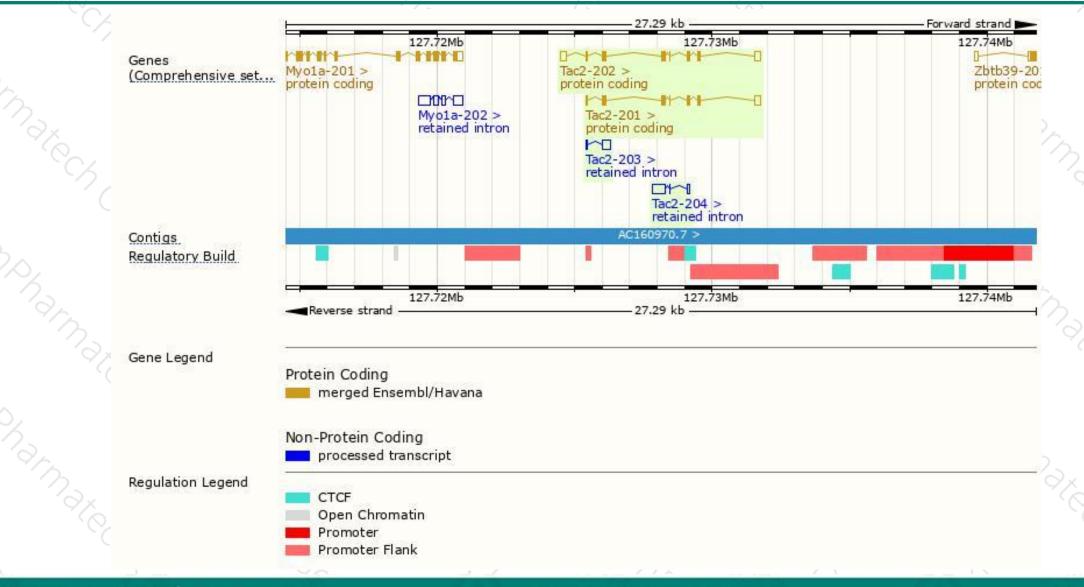
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tac2-202	ENSMUST00000179960.7	852	<u>116aa</u>	Protein coding	CCDS24248	P55099	TSL:1 GENCODE basic APPRIS P1
Tac2-201	ENSMUST00000026466.4	678	<u>116aa</u>	Protein coding	CCDS24248	P55099	TSL:1 GENCODE basic APPRIS P1
Tac2-204	ENSMUST00000218048.1	514	No protein	Retained intron	<u>.</u>	-	TSL:5
Tac2-203	ENSMUST00000217729.1	358	No protein	Retained intron	<u></u>	-	TSL:3

The strategy is based on the design of *Tac2-202* transcript, The transcription is shown below



Genomic location distribution





江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

400-9660890

Protein domain

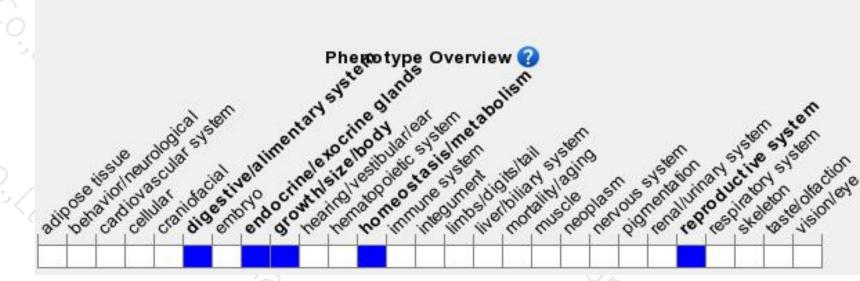
江苏集萃药康生物科技股份有限公司



2/1			No.		20	0	The second) An an	0		8	3 De		
	ENSMUSP00000136 MobiDB lite									1				<
	Cleavage site (Sign Prints		Neur	okinin-B/I	achykinin-	3			1				-	
	<u>Pfam</u>	Neurok	inin-B/Tachy	DN:										
	PROSITE patterns										Tachykinin	/Neurokinin-li	ike, conser	
	PIRSF	Neurok	inin-B/Tachy	kinin-3										~./ x
	PANTHER	Neurok	inin-B/Tachy	kinin-3										
	All sequence SNPs/i	Seque	nce variants	s (dbSNP	and all ot	her sourc	es)							
2	Variant Legend		issense vai nonymous										2	
	Scale bar	0	10	20	30	40	50	60	70	80	90	100	116	- 6
	Mate Ch	Ŷ								Ģ			in the second se	
集萃	药康生物科技股份有限	公司				GemPh	armatech C	o., Ltd.			163		400-96608	90

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, Mice homozygous for a knock-out allele exhibit delayed female sexual maturation and fertility.



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



