

Tead3 Cas9-CKO Strategy

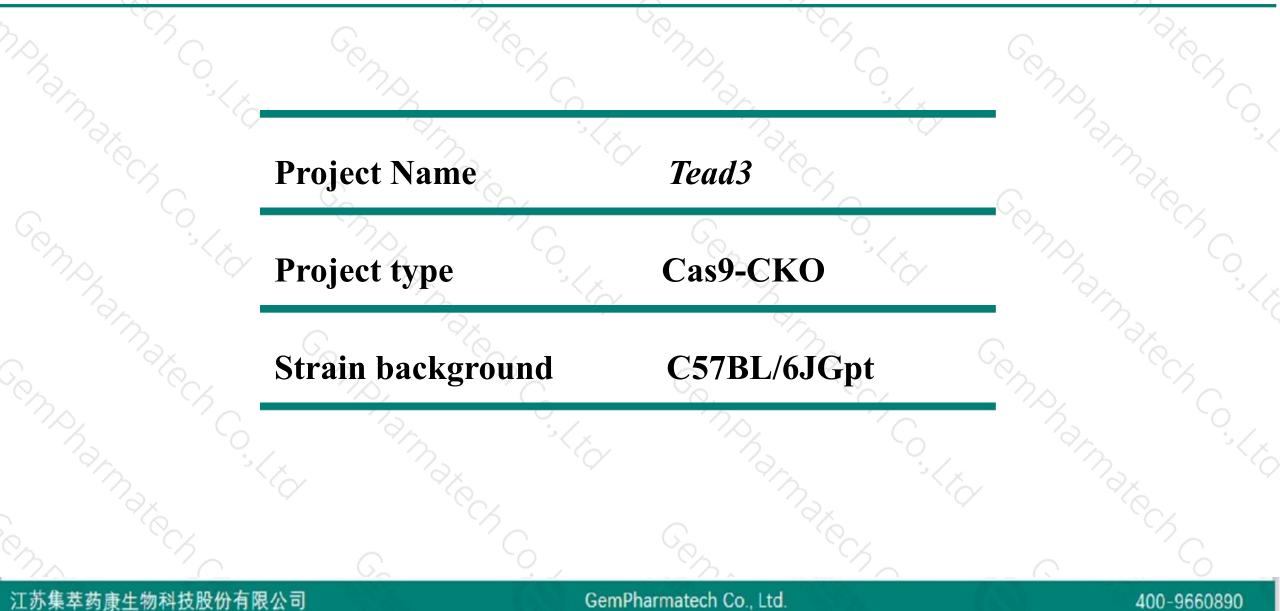
Designer: Huan Fan

Reviewer: Lingyan Wu

Design Date: 2018-9-12

Project Overview



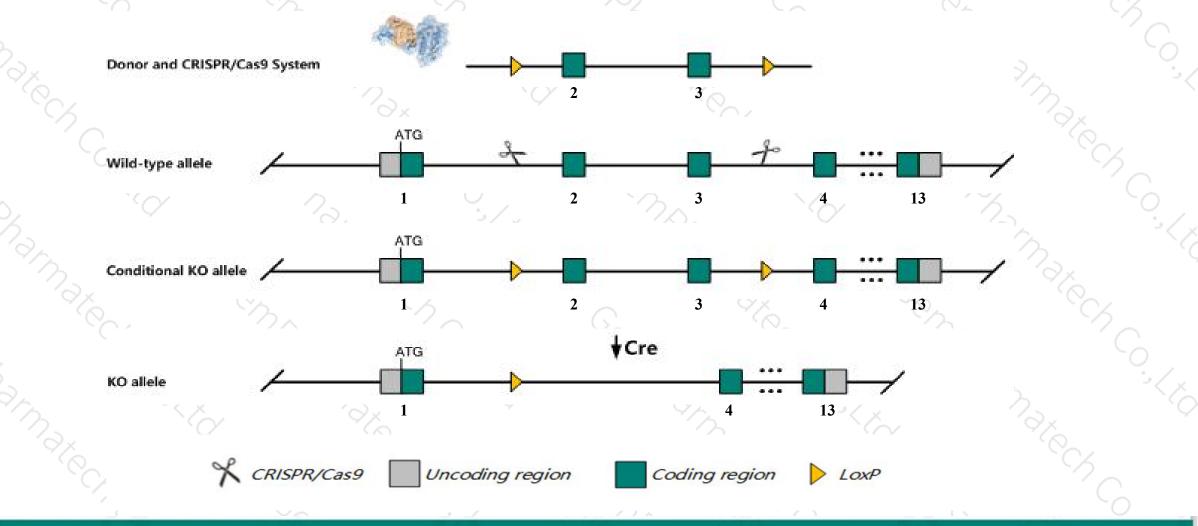


Conditional Knockout strategy



400-9660890

This model will use CRISPR/Cas9 technology to edit the *Tead3* 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 *Tead3* gene has 9 transcripts. According to the structure of *Tead3* gene, exon2-exon3 of *Tead3*-201(ENSMUST00000114799.7) transcript is recommended as the knockout region. The region contains 317bp coding sequence. Knock out the region will result in disruption of protein function.

➤ In this project we use CRISPR/Cas9 technology to modify *Tead3* 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.



- > The *Tead3* gene is located on the Chr17. 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.
- ➤ Transcript *Tead3-203, 204, 208* may not be affected.
- > 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)



~ 1

400-9660890

Tead3 TEA domain family member 3 [Mus musculus (house mouse)]

Gene ID: 21678, updated on 13-Mar-2020

Summary

Official Symbol Tead3 provided by MGI Official Full Name TEA domain family member 3 provided by MGI Primary source MGI:MGI:109241 See related Ensembl:ENSMUSG0000002249 Gene type protein coding RefSeg status REVIEWED Organism Mus musculus Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus Also known as DTEF-1, ETFR-1, TEAD-3, TEF-5, Tcf13r2 Summary This gene product is a member of the transcriptional enhancer factor (TEF) family of transcription factors, which contain the TEA/ATTS DNAbinding domain. It is predominantly expressed in the placenta and thought to play a role in placental gene regulation and development. Alternative splicing, and alternate use of an upstream AUG translation initiation codon, and an in-frame downstream non-AUG (AUA) codon, results in 2 isoforms. [provided by RefSeq, Jul 2008] Broad expression in limb E14.5 (RPKM 22.9), bladder adult (RPKM 15.0) and 22 other tissuesSee more Expression Orthologs human all

江苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

Transcript information (Ensembl)



The gene has 9 transcripts, all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tead3-201	ENSMUST00000114799.7	2448	<u>465aa</u>	Protein coding	CCDS50046	F8VPU0	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P
Tead3-205	ENSMUST00000154873.8	2775	<u>439aa</u>	Protein coding		F6TTJ9	TSL:1 GENCODE basic
Tead3-206	ENSMUST00000156862.2	2585	<u>439aa</u>	Protein coding		F6TTJ9	TSL:1 GENCODE basic
Tead3-207	ENSMUST00000219703.2	1579	<u>439aa</u>	Protein coding	120	A0A3F2YNR7	TSL:1 GENCODE basic
Tead3-208	ENSMUST00000226172.1	11	No protein	Processed transcript	-	æ	
Tead3-202	ENSMUST00000127212.8	3443	No protein	Retained intron			TSL:2
Tead3-209	ENSMUST00000233330.1	2870	No protein	Retained intron		12	
Tead3-203	ENSMUST00000146668.8	944	No protein	Retained intron	1.020	14	TSL:3
Tead3-204	ENSMUST00000151557.1	347	No protein	Retained intron		-	TSL:5

The strategy is based on the design of *Tead3-201* transcript, the transcription is shown below:

< Tead3-201 protein coding

Reverse strand -

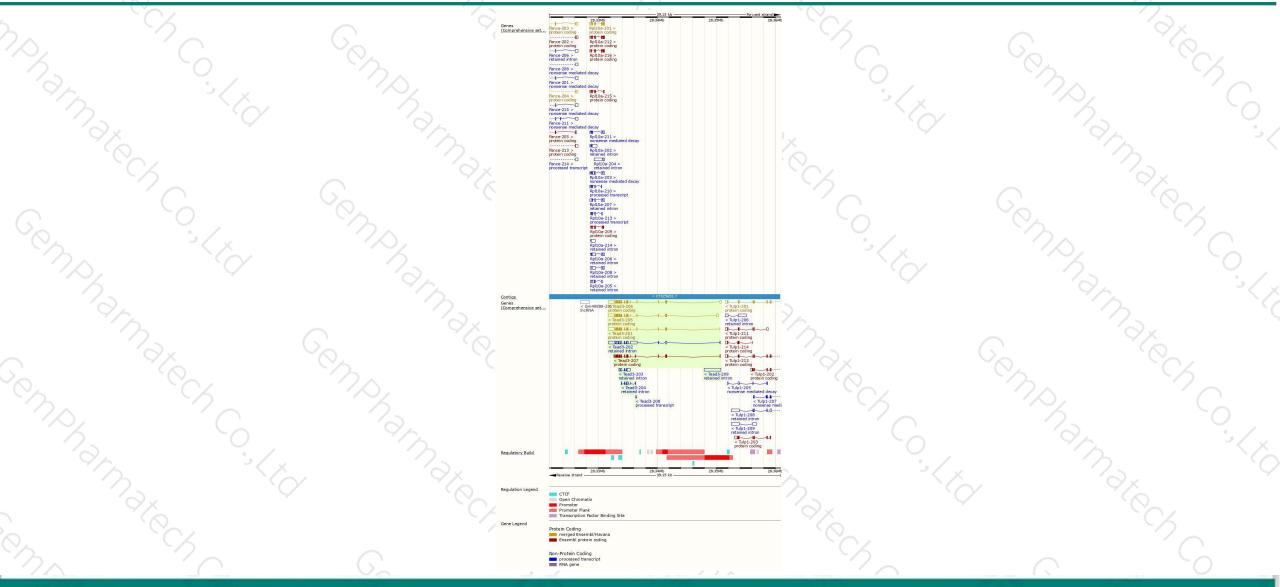
汀苏集萃药康生物科技股份有限公司

GemPharmatech Co., Ltd.

18.93 kb

Genomic location distribution





<u>江苏集萃药康生物科技股份有限公司</u>

GemPharmatech Co., Ltd.

400-9660890

Protein domain

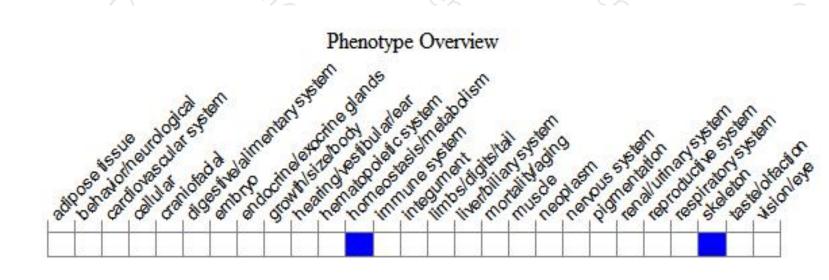
江苏



ENSMUSP0000110 MobiDB lite. Low complexity. (Seq) SMARI. Prints Prints ProSITE profiles PROSITE patterns PIRSE. PANTHER. PA	主共	药康生物科技股份有限少	2				GemPha	armatech (Co Itd					400-966	0890
MobiDB lite. Low complexity. (Seq). SMART. Fints. Prints. TEA/ATTS domain Pfam. TEA/ATTS domain PROSITE profiles. TEA/ATTS domain PROSITE patterns. TEA/ATTS domain PROSITE patterns. TEA/ATTS domain PROSITE patterns. Teanscriptional enhancer factor, metazoa PANTHER. PTHRI1834 PTHRI1834:FF4 Gene3D. Sequence SNPs/i Sequence variants (dbSNP and all other sources) Variant Legend missense variant	5	Scale bar	0	40	80	120	160	200	240	280	320	360	400	465	5
MobiDB lite Low complexity (Seg) SMART. Prints Prints Pfam. PROSITE profiles PROSITE patterns PROSITE patterns PIRSE. Transcriptional enhancer factor, metazoa Transcriptional enhancer factor, metazoa PANTHER. PTHR11834 PTHR11834 PTHR11834:SF4 Sene3D. All sequence SNPs/i		Variant Legend													
MobiDB lite Low. complexity. (Seg). SMART. Prints. Pfam. TEA/ATTS domain PROSITE profiles PROSITE patterns PIRSF. Transcriptional enhancer factor. metazoa Transcriptional enhancer factor. TEF-S (TEAD3) PANTHER. PTHR11834+ISF4 Gene3D. TEA/ATTS domain superfamily	~	85 - <u>8</u> 9	Sequen	ce variant	s (dbSNF	² and all of	ther source	s)	Û.	1	i.	11		1	
MobiDB lite Low complexity (Seg) SMART. Prints Pfam. TEA/ATTS domain PROSITE profiles PROSITE patterns PIRSE Transcriptional enhancer factor, metazoa Transcriptional enhancer factor, TEF-S (TEAD3) PANTHER.		Gene3D		1.	22012	ain superfa	mily		2.70	.50.80					3
MobiDB lite. Low complexity (Seg). SMART. Prints. Pfam. PROSITE profiles. PROSITE patterns. PIRSE. Transcriptional enhancer factor, metazoa		PANTHER	F	THR11834		Sentactor (1	EH-S (TEADS								
MobiDB lite Low complexity (Seg) SMART. Prints TEA/ATTS domain	5,	PROSITE patterns		scriptional	TE/	A/ATT <mark>S don</mark> factor, met	azoa								; G.
MobiDB lite Low complexity (Seg)				TE	A/ATTS do	omain			YAP	binding do	main				0.
	2	MobiDB lite Low complexity (Seg)	-	TEA	ATTS dom	ain	-		-		-				3

Mouse phenotype description(MGI)





Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).



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



