# Tead4 Cas9-CKO Strategy

Designer: Huan Fan

**Design Date:** 2019-7-25

# **Project Overview**



**Project Name** 

Tead4

**Project type** 

Cas9-CKO

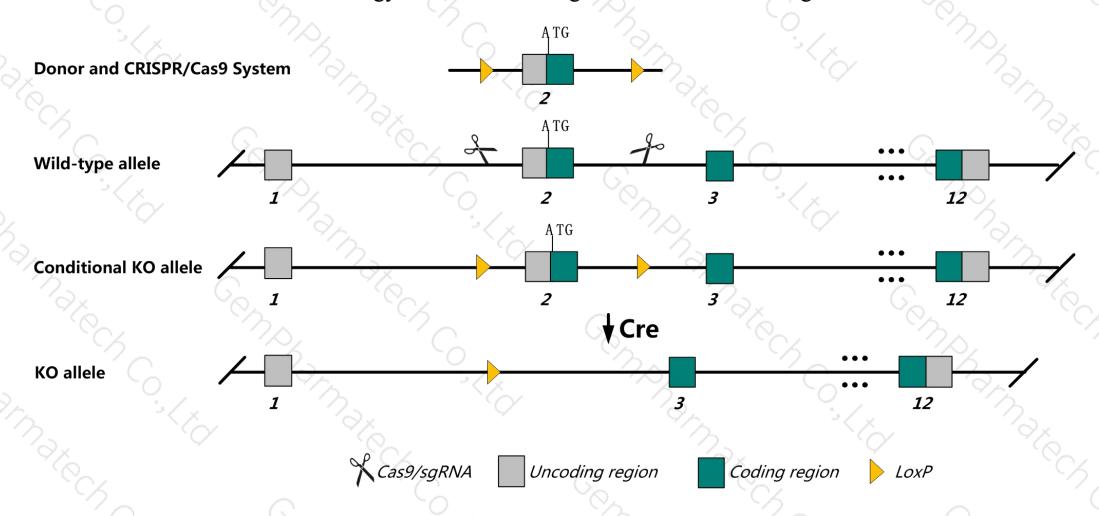
Strain background

C57BL/6JGpt

## Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Tead4* gene. The schematic diagram is as follows:



### **Technical routes**



- ➤ The *Tead4* gene has 5 transcripts. According to the structure of *Tead4* gene, exon2 of *Tead4*-201 transcript is recommended as the knockout region. The region contains start codon ATG coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Tead4* gene. The brief process is as follows: gRNA was transcribed in vitro, donor was constructed.Cas9, gRNA 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 or cell types.

### **Notice**



- According to the existing MGI data, Mice homozygous for a null allele die prior to somitogenesis, lack trophoblast stem cells and develop abnormally.
- ➤ The *Tead4* gene is located on the Chr6. 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

# Gene information (NCBI)



#### Tead4 TEA domain family member 4 [ Mus musculus (house mouse) ]

Gene ID: 21679, updated on 2-Jul-2019

Summary

↑ ?

Official Symbol Tead4 provided by MGI

Official Full Name TEA domain family member 4 provided by MGI

Primary source MGI:MGI:106907

See related Ensembl: ENSMUSG00000030353

Gene type protein coding
RefSeq 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 Tef3; Tefr; Etfr2; FR-19; Rtef1; TEF-3; Tefr1; ETFR-2; TEAD-4; Tefr1a; Tcf13r1

Summary This gene product is a member of the transcriptional enhancer factor (TEF) family of transcription factors, which contain the TEA/ATTS DNA-binding domain. It is

preferentially expressed in the skeletal muscle, and binds to the M-CAT regulatory element found in promoters of muscle-specific genes to direct their gene expression. This factor may play a role in the embryonic development of skeletal muscle. Alternatively spliced transcripts encoding distinct isoforms, which are

translated through the use of a non-AUG (AUU) initiation codon, have been described for this gene. [provided by RefSeq, Jul 2008]

Expression Broad expression in placenta adult (RPKM 4.9), lung adult (RPKM 4.8) and 15 other tissues See more

Orthologs human all

# Transcript information (Ensembl)



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

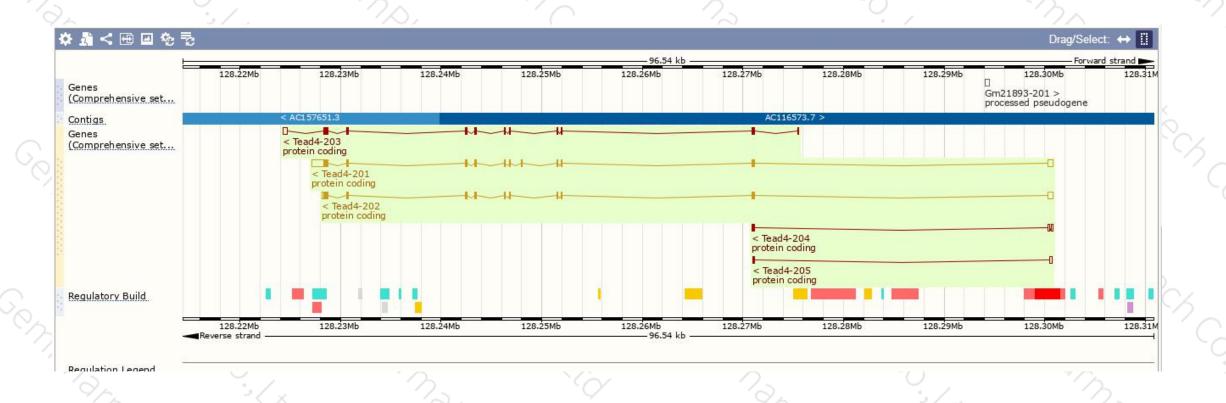
Show/hide columns (1 hidden)							Filter	
Name	Transcript ID	bp 🍦	Protein 🍦	Biotype	CCDS	UniProt	Flags	
Tead4-201	ENSMUST00000006311.12	3076	<u>427aa</u>	Protein coding	CCDS39646 ₽	A0A0R4IZY5₽	TSL:1 GENO	CODE basic
Tead4-202	ENSMUST00000112157.3	1909	384aa	Protein coding	CCDS39645 ₽	<u>A0A0R4J1U4</u> ₽	TSL:1 GENO	CODE basic
Tead4-203	ENSMUST00000130454.7	1718	384aa	Protein coding	CCDS39645@	<u>A0A0R4J1U4</u> ₽	TSL:5 GENO	CODE basic
Tead4-204	ENSMUST00000133118.1	520	<u>54aa</u>	Protein coding	-	F6UKF4₽	CDS 3' incomplete	TSL:2 APPRIS P5
Tead4-205	ENSMUST00000143004.1	368	<u>38aa</u>	Protein coding	-	F6ULG6®	CDS 3" incomplete TS	SL:3 APPRIS ALT2

The strategy is based on the design of *Tead4-201* transcript, The transcription is shown below



### Genomic location distribution





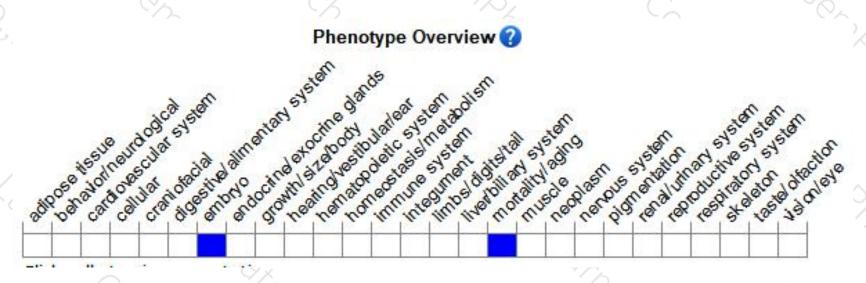
### Protein domain





### 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, Mutations in this locus affect cell-cycle regulation and apoptos is. Null homozygotes show high, early-onset tumor incidence; some have persistent hyaloid vasculature and cataracts. Truncated or temperature-sensitive alleles cause early aging phenotypes.

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





