

Tap1 Cas9-CKO Strategy

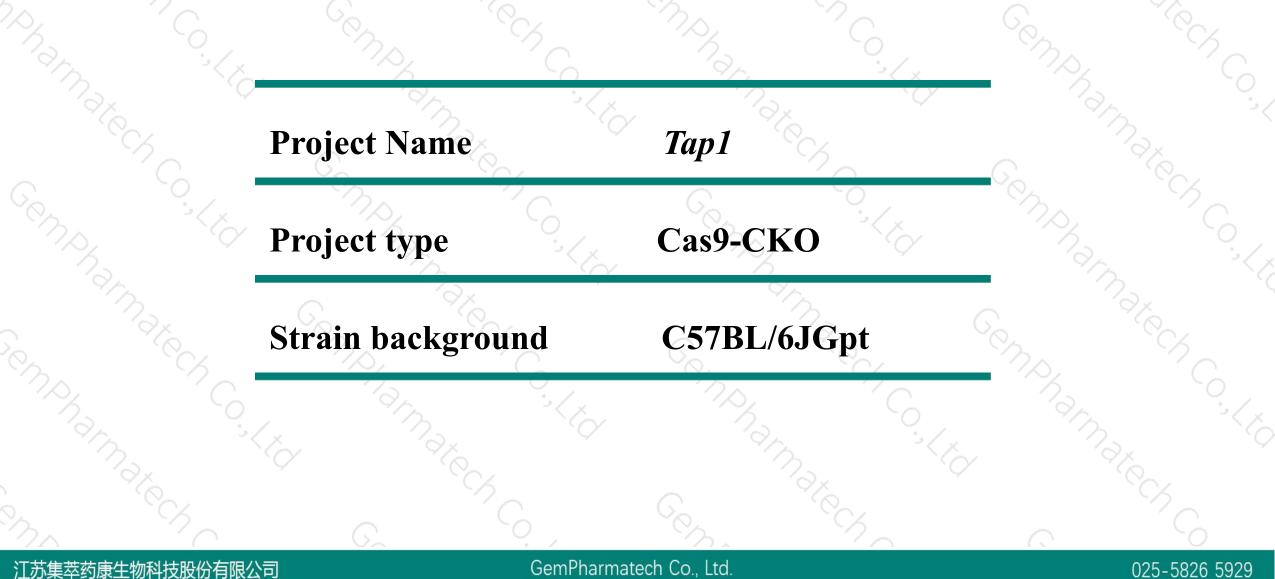
Designer: Jinling Wang

Reviewer: Huan Wang

Design Date: 2022-4-7

Project Overview





江苏集萃药康生物科技股份有限公司

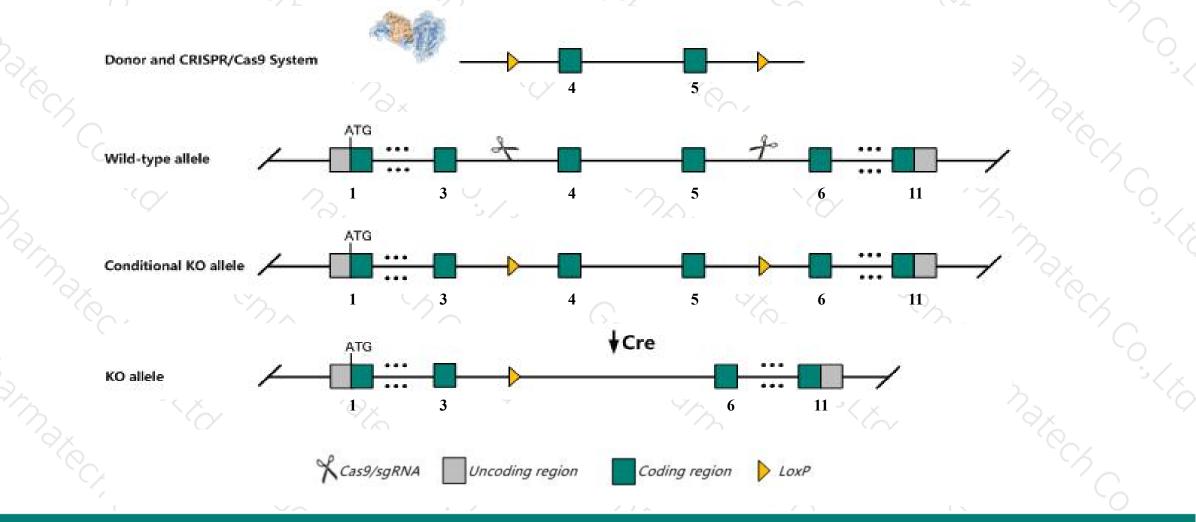
GemPharmatech Co., Ltd.

Conditional Knockout strategy



025-5826 5929

This model will use CRISPR/Cas9 technology to edit the *Tap1* 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 *Tap1* gene has 7 transcripts. According to the structure of *Tap1* gene, exon4-exon5 of *Tap1-* 206(ENSMUST00000170086.7) transcript is recommended as the knockout region. The region contains 404bp coding sequence.
Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Tap1* 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.



025-5826 5929

- > According to the existing MGI data, mice homozygous for targeted mutations that inactivate the gene are deficient in antigen presentation, surface class I antigens, and CD4-8+ T cells.
- > The flox region is near the gene *Psmb9*, this strategy may destroy the function of gene *Psmb9*.
- ➤ This strategy directly destroy the transcript 202, 204 and 205.
- > The *Tap1* 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.
- > 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)



2

025-5826 5929

Tap1 transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) [Mus musculus (house mouse)]

Gene ID: 21354, updated on 13-Mar-2020

Summary

Official Symbol	Tap1 provided by MGI							
Official Full Name	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP) provided by MGI							
Primary source	MGI:MGI:98483							
See related	Ensembl:ENSMUSG0000037321							
Gene type	protein coding							
RefSeq status	REVIEWED							
Organism	Mus musculus							
Lineage	ge Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomor							
	Muroidea; Muridae; Murinae; Mus; Mus							
Also known as	ABC17, APT1, Abcb, Abcb2, Ham, Ham-, Ham-1, Ham1, MTP, MTP1, PSF, PSF1, RI, RING4, T, TAP, Tap-1, Y3							
Summary	The membrane-associated protein encoded by this gene is a member of the superfamily of ATP-binding cassette (ABC) transporters.							
	ABC proteins transport various molecules across extra- and intra-cellular membranes. ABC genes are divided into seven distinct							
	subfamilies (ABC1, MDR/TAP, MRP, ALD, OABP, GCN20, White). This protein is a member of the MDR/TAP subfamily. Members of the							
	MDR/TAP subfamily are involved in multidrug resistance. This protein forms a heterodimer with Tap2 that transports short peptides from							
	the cytosol into the endoplasmic reticulum lumen. Mutations in the human gene may be associated with ankylosing spondylitis, insulin-							
	dependent diabetes mellitus, and celiac disease. Two transcript variants encoding different isoforms have been found for this gene.							
	[provided by RefSeq, Jun 2009]							
Expression	Broad expression in spleen adult (RPKM 66.8), thymus adult (RPKM 59.4) and 17 other tissues See more							
Orthologs	human all							

GemPharmatech Co., Ltd.

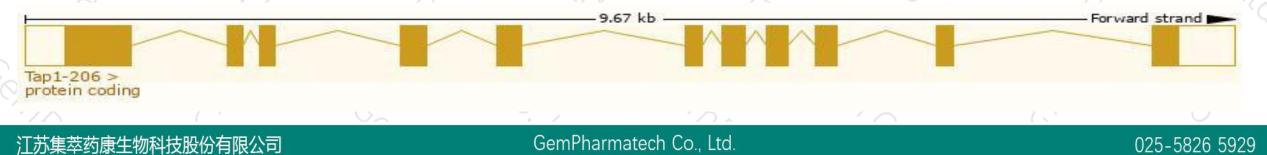
Transcript information (Ensembl)



The gene has 7 transcripts, all transcripts are shown below:

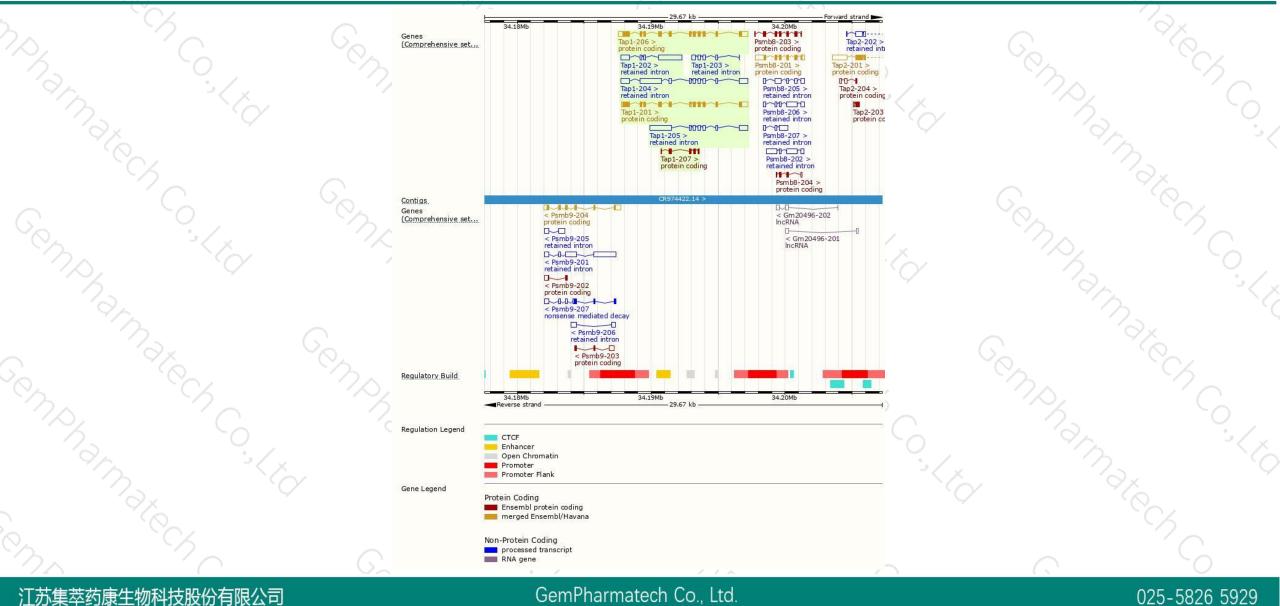
		1		100000			- I have I have a second secon
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tap1-206	ENSMUST00000170086.7	2953	<u>724aa</u>	Protein coding	CCDS28643	P21958	TSL:1 GENCODE basic APPRIS P3
Tap1-201	ENSMUST00000041633.14	2614	<u>696aa</u>	Protein coding	CCDS50072	Q3TBA3	TSL:1 GENCODE basic APPRIS ALT2
Tap1-207	ENSMUST00000171148.1	587	<u>196aa</u>	Protein coding	114	F6QHF1	CDS 5' and 3' incomplete TSL:5
Tap1-204	ENSMUST00000166853.7	3911	No protein	Retained intron	1923	-	TSL:1
Tap1-205	ENSMUST00000168351.7	3081	No protein	Retained intron	150		TSL:1
Tap1-202	ENSMUST00000166287.1	2638	No protein	Retained intron	(- 21	-	TSL:1
Tap1-203	ENSMUST00000166582.1	757	No protein	Retained intron	10400	2	TSL:3

The strategy is based on the design of *Tap1-206* 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

Protein domain





江苏集萃药康生物科技股份有限公司

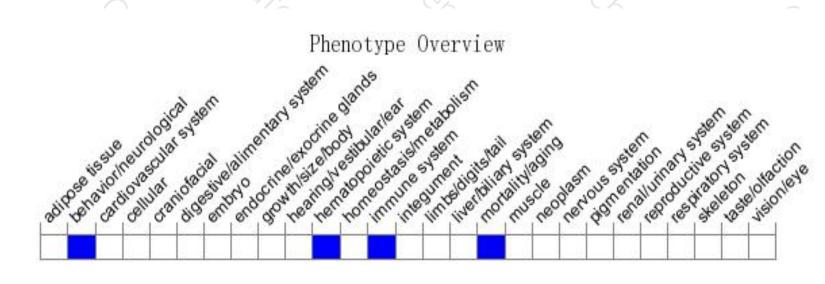
GemPharmatech Co., Ltd.

025-5826 5929

Mouse phenotype description(MGI)



025-5826



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 targeted mutations that inactivate the gene are deficient in antigen presentation, surface class I antigens, and CD4-8+ T cells.

GemPharmatech Co., Ltd,



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



