# Wtap Cas9-CKO Strategy

Designer: Daohua Xu

**Design Date:** 2019-7-18

# **Project Overview**



**Project Name** 

Wtap

**Project type** 

Cas9-CKO

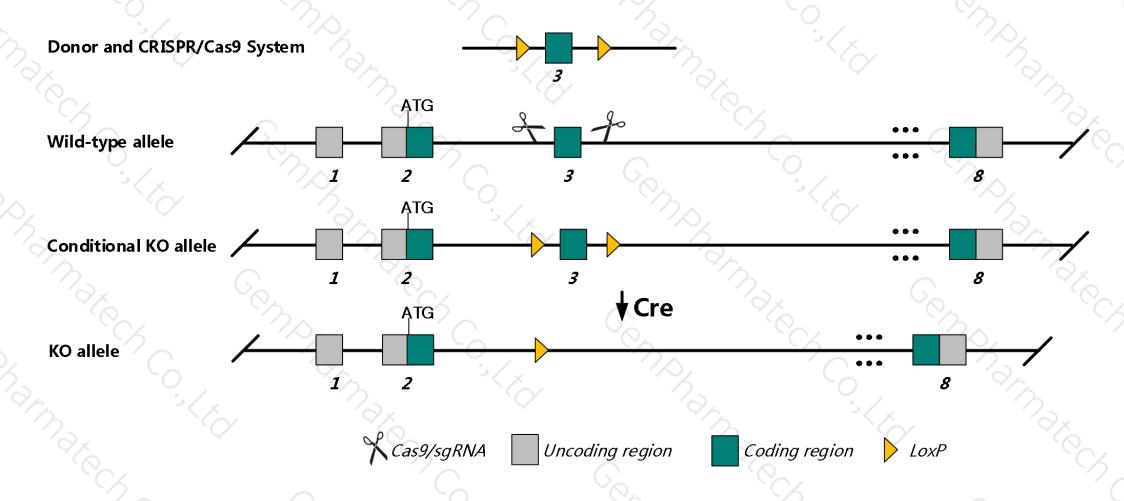
Strain background

C57BL/6JGpt

### Conditional Knockout strategy



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



### **Technical routes**



- The *Wtap* gene has 9 transcripts. According to the structure of *Wtap* gene, exon3 of *Wtap*-201 (ENSMUST0000007007.13) transcript is recommended as the knockout region. The region contains 56bp coding sequence. Knock out the region will result in disruption of protein function.
- In this project we use CRISPR/Cas9 technology to modify *Wtap* 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 mutation display lethality during embryogenesis with abnormalities appearing during gastrulation.
- The *Wtap* 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 the loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

# Gene information (NCBI)



#### Wtap Wilms tumour 1-associating protein [ Mus musculus (house mouse) ]

Gene ID: 60532, updated on 5-Aug-2018

#### Summary

Official Symbol Wtap provided by MGI

Official Full Name Wilms tumour 1-associating protein provided by MGI

Primary source MGI:MGI:1926395

See related Ensembl:ENSMUSG00000060475 Vega:OTTMUSG00000034274

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 2810408K05Rik; 9430038B09Rik

Expression Ubiquitous expression in CNS E11.5 (RPKM 10.9), CNS E18 (RPKM 10.6) and 28 other tissues See more

Orthologs human all

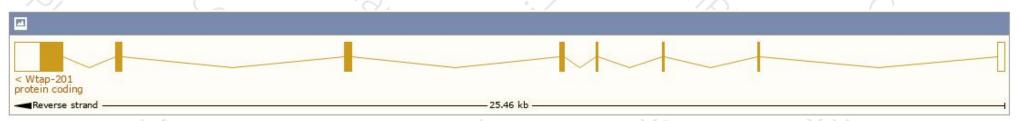
# Transcript information (Ensembl)



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

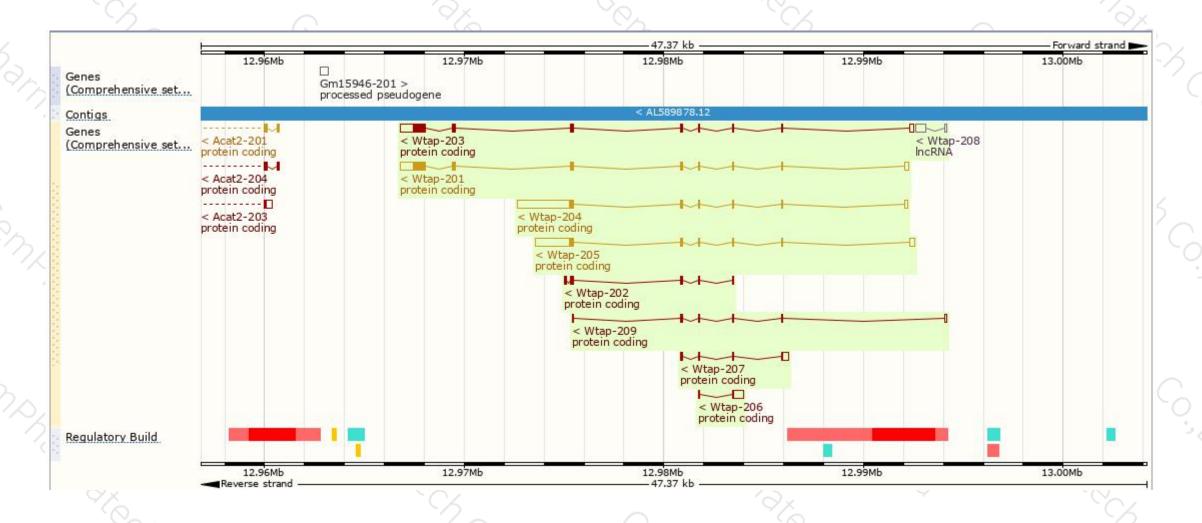
Name 🍦	Transcript ID	bp 🍦	Protein	Biotype	CCDS 🍦	UniProt	RefSeq	Flags
Wtap-204	ENSMUST00000159986.7	3187	<u>151aa</u>	Protein coding	CCDS49949 ₽	Q9ER69₽	NM_001113532년 NP_001107004년	TSL:1 GENCODE basic
Ntap-205	ENSMUST00000160781.7	2442	<u>151aa</u>	Protein coding	CCDS49949₽	Q9ER69 ₽	NM_175394 & NP_780603 &	TSL:1 GENCODE basic
Ntap-203	ENSMUST00000159551.7	2060	396aa	Protein coding	CCDS49950 ₽	E0CYH0₽	g. T	TSL:5   GENCODE basic   APPRIS P1
Wtap-201	ENSMUST00000007007.13	2051	396aa	Protein coding	CCDS49950 ₽	E0CYH0₽	NM_001113533년 NP_001107005년	TSL:1 GENCODE basic APPRIS P1
Ntap-206	ENSMUST00000161901.1	574	<u>28aa</u>	Protein coding	2	2	2	CDS 3' incomplete TSL:3
Ntap-207	ENSMUST00000162395.1	536	80aa	Protein coding	¥	E0CZF5 ₽	[ 4	CDS 3' incomplete TSL:3
Ntap-202	ENSMUST00000159104.7	495	139aa	Protein coding	ā	F7CCI4 ₺	G.	CDS 5" incomplete TSL:5
Ntap-209	ENSMUST00000233867.1	446	<u>110aa</u>	Protein coding	5	15	-	CDS 3' incomplete
Wtap-208	ENSMUST00000233467.1	593	No protein	Processed transcript				

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



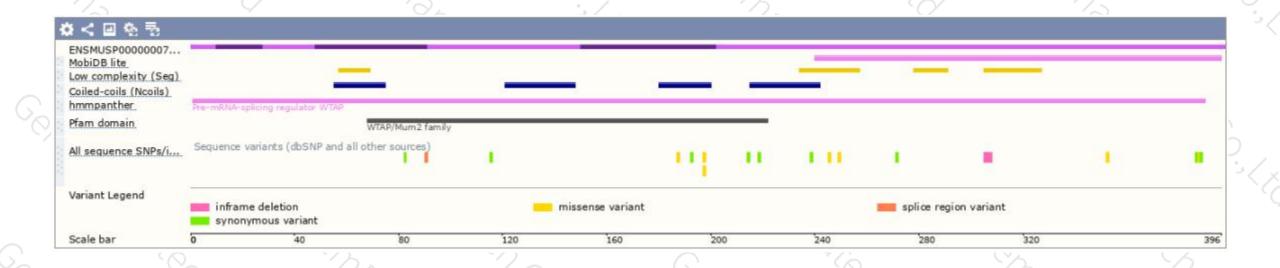
### Genomic location distribution





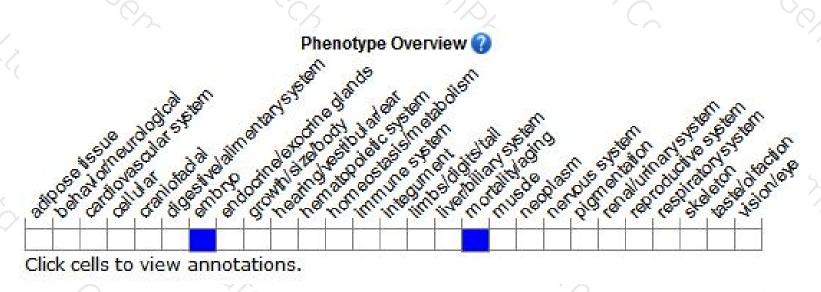
### Protein domain





## 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, Mice homozygous for a mutation display lethality during embryogenesis with abnormalities appearing during gastrulation.

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





