

Thumpd2 Cas9-CKO Strategy

Designer: Lingyan Wu

Reviewer: Miaomiao Cui

Design Date: 2021-6-11

Project Overview

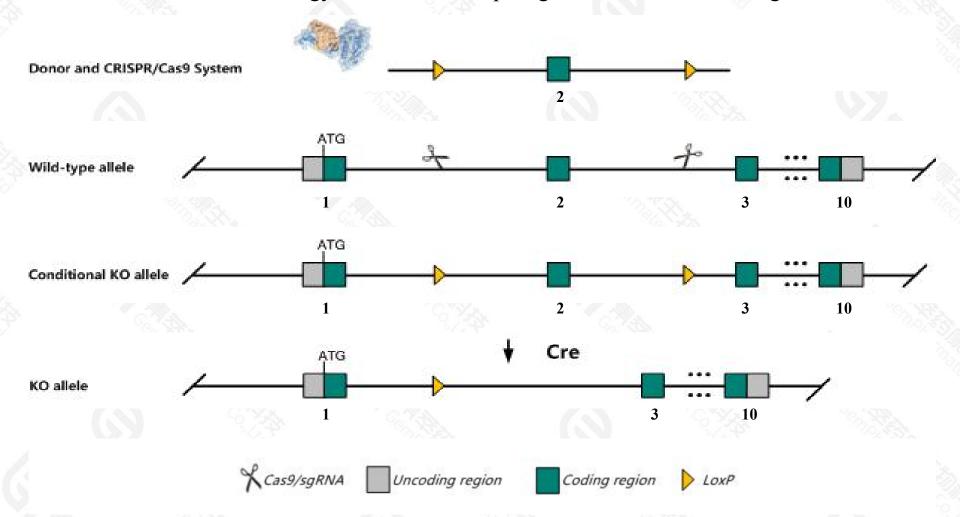


Project Name	Thumpd2			
Project type	Cas9-CKO			
Strain background	C57BL/6JGpt			

Conditional Knockout strategy



This model will use CRISPR/Cas9 technology to edit the *Thumpd2* gene. The schematic diagram is as follows:



Technical routes



- ➤ The *Thumpd2* gene has 2 transcripts. According to the structure of *Thumpd2* gene, exon2 of *Thumpd2*-201(ENSMUST00000025093.6) transcript is recommended as the knockout region. The region contains 136bp coding sequence. Knock out the region will result in disruption of protein function.
- ➤ In this project we use CRISPR/Cas9 technology to modify *Thumpd2* gene. The brief process is as follows:sgRNA was transcribed in vitro, donor 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.

Notice



- > The *Thumpd2* 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)



Thumpd2 THUMP domain containing 2 [Mus musculus (house mouse)]

Gene ID: 72167, updated on 17-Dec-2020

Summary

☆ ?

Official Symbol Thumpd2 provided by MGI

Official Full Name THUMP domain containing 2 provided by MGI

Primary source MGI:MGI:1919417

See related Ensembl: ENSMUSG00000024246

Gene type protein coding
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 2810025A12Rik

Expression Low expression observed in reference datasetSee more

Orthologs <u>human all</u>

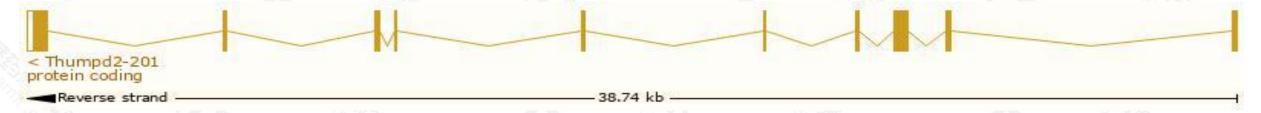
Transcript information (Ensembl)



The gene has 2 transcripts, all transcripts are shown below:

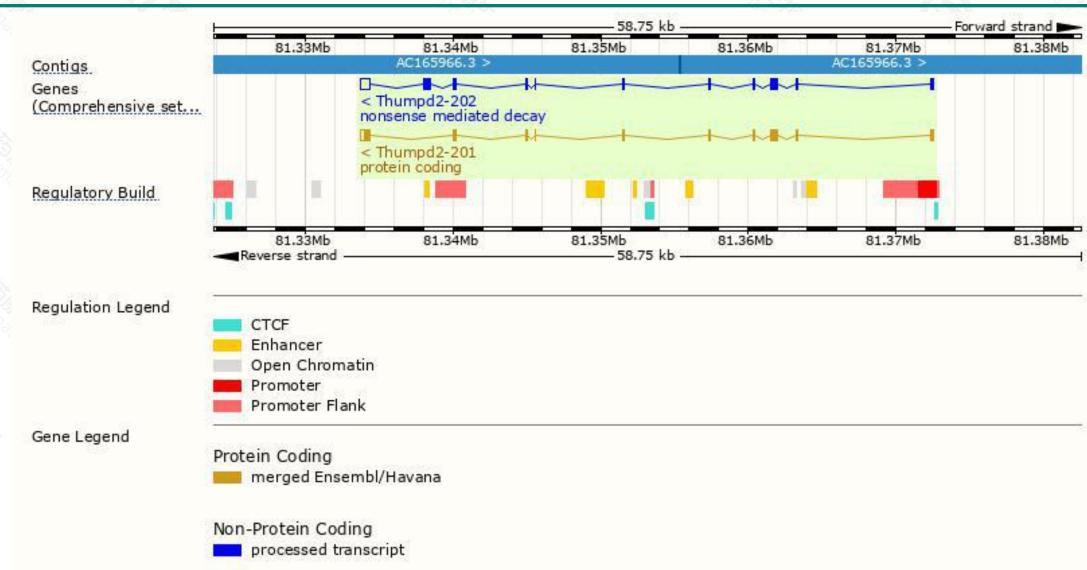
Name		Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Thumpd2	-201	ENSMUST00000025093.6	1833	528aa	Protein coding	CCDS50190		TSL:1 , GENCODE basic , APPRIS P1 ,
Thumpd2	-202	ENSMUST00000234801.2	2239	<u>506aa</u>	Nonsense mediated decay			

The strategy is based on the design of *Thumpd2-201* transcript, the transcription is shown below:



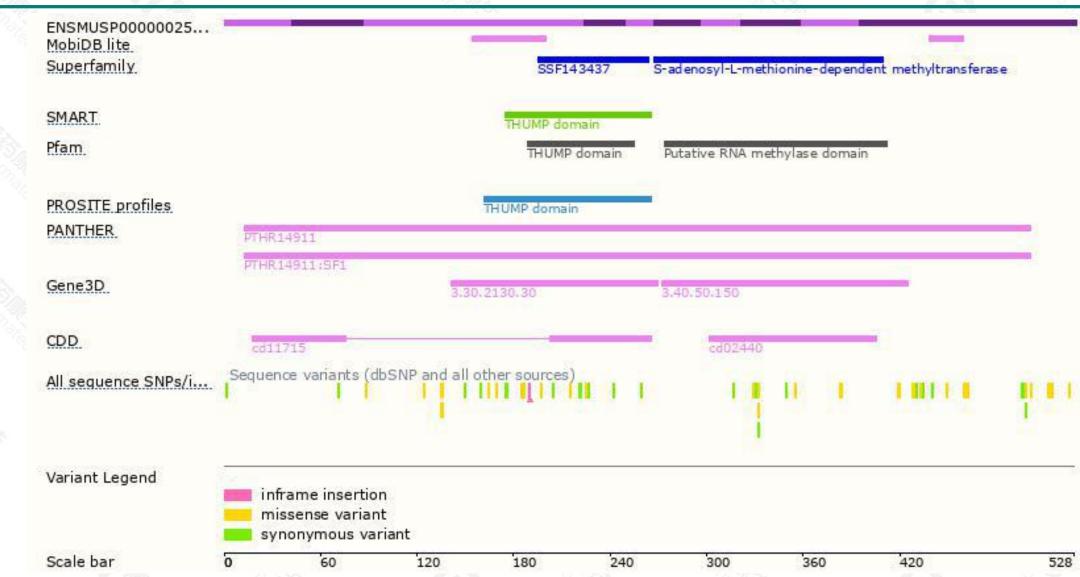
Genomic location distribution





Protein domain







If you have any questions, you are welcome to inquire.

Tel: 025-5864 1534





