

Tmx1 Cas9-CKO Strategy

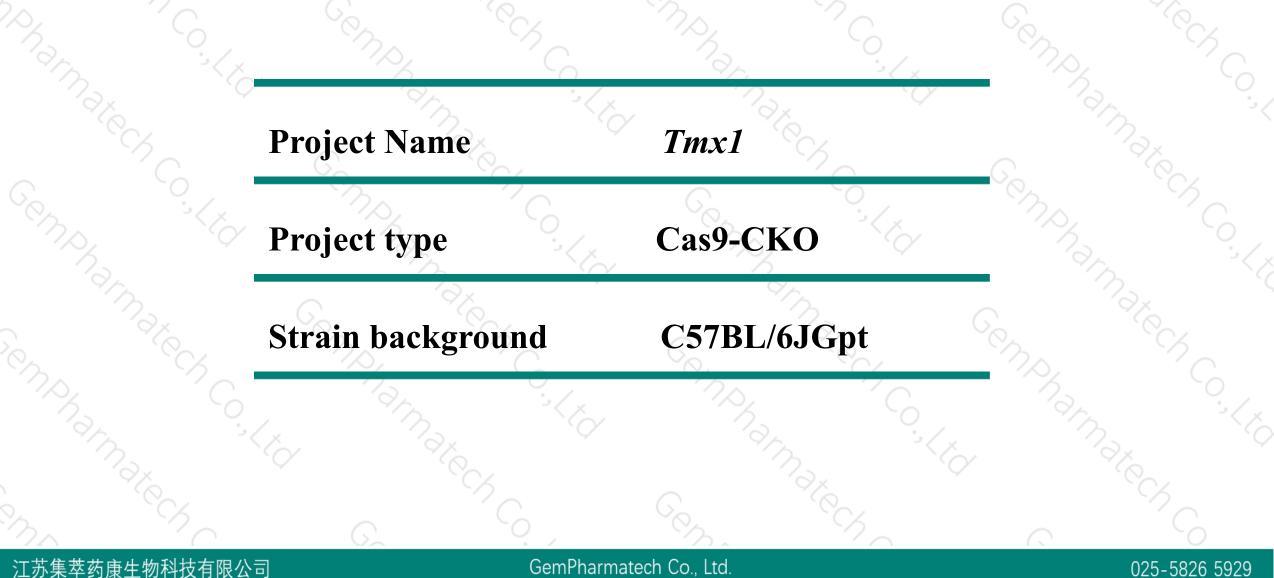
Designer: Jia Yu

Reviewer: Xiaojing Li

Design Date: 2020-10-12

Project Overview





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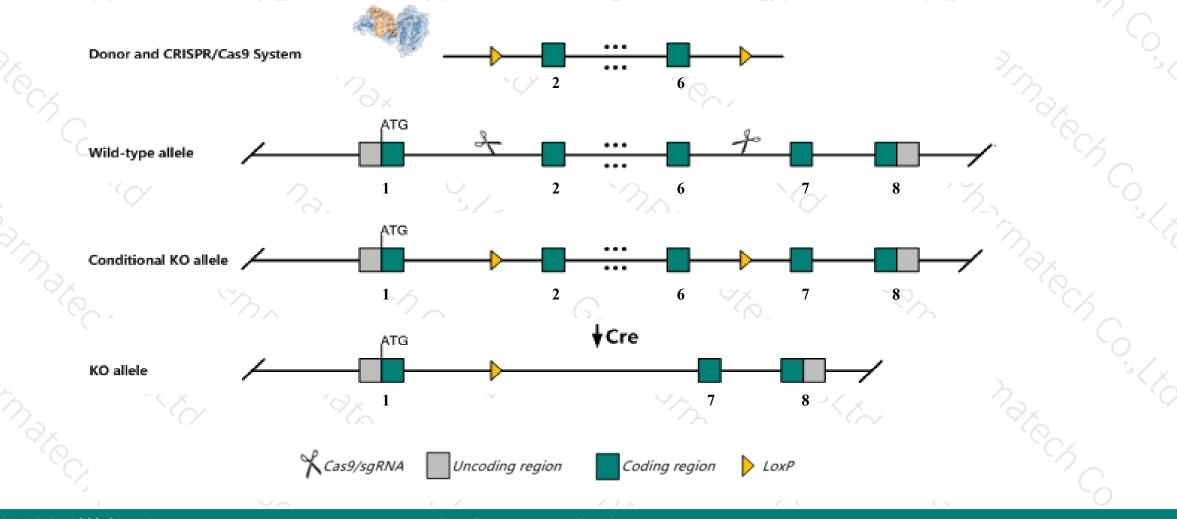
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Conditional Knockout strategy



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This model will use CRISPR/Cas9 technology to edit the *Tmx1* gene. The schematic diagram is as follows:



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> The *Tmx1* gene has 3 transcripts. According to the structure of *Tmx1* gene, exon2-exon6 of *Tmx1*-201(ENSMUST0000021471.12) transcript is recommended as the knockout region. The region contains 439bp coding sequence. Knock out the region will result in disruption of protein function.

In this project we use CRISPR/Cas9 technology to modify *Tmx1* 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.



> According to the existing MGI data, no notable phenotype was detected in a high-throughput screen of homozygous mice.

The *Tmx1* gene is located on the Chr12. 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)



☆ ?

Tmx1 thioredoxin-related transmembrane protein 1 [Mus musculus (house mouse)]

Gene ID: 72736, updated on 13-Mar-2020

Summary

Official Symbol	Tmx1 provided by MGI
Official Full Name	thioredoxin-related transmembrane protein 1 provided by <u>MGI</u>
Primary source	MGI:MGI:1919986
See related	Ensembl:ENSMUSG0000021072
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	<u>Mus musculus</u>
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
	Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2810425A04Rik, Txndc1
Expression	Broad expression in liver E14 (RPKM 28.3), placenta adult (RPKM 26.2) and 25 other tissues <u>See more</u>
Orthologs	human all

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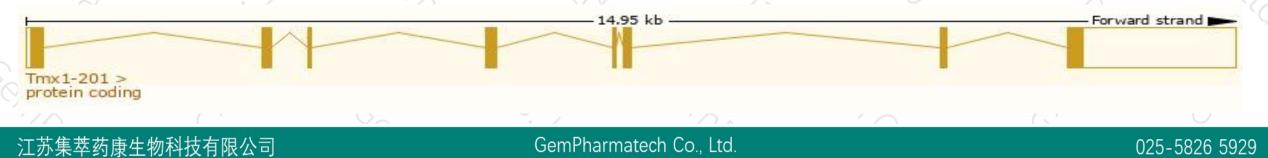
Transcript information (Ensembl)



The gene has 3 transcripts, all transcripts are shown below:

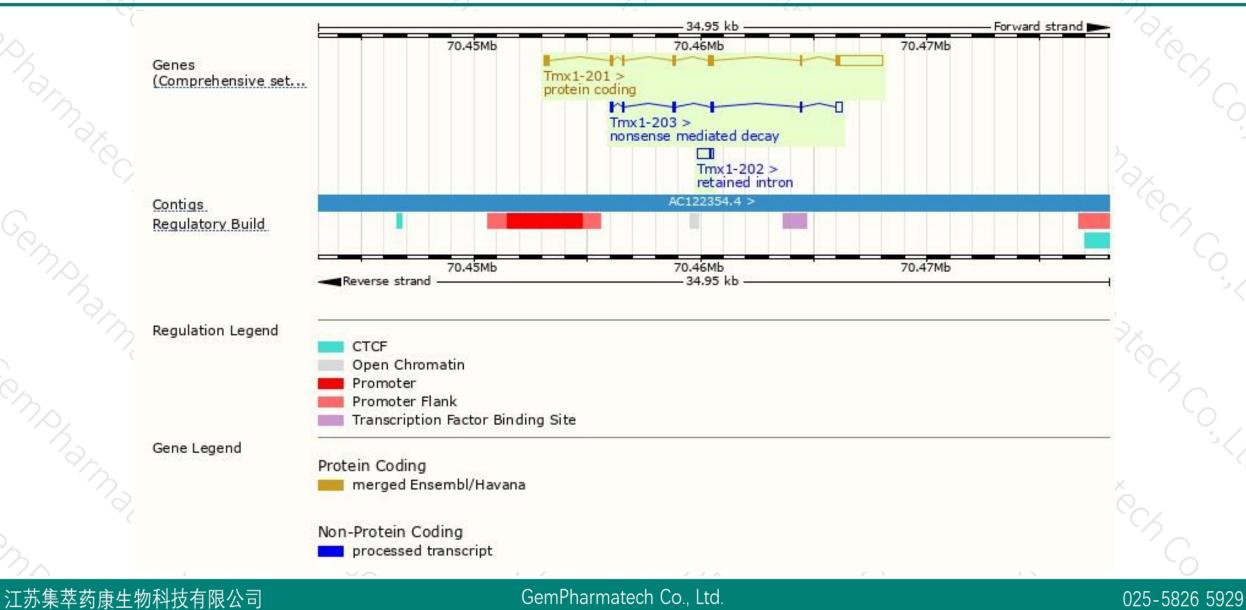
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Tmx1-201	ENSMUST0000021471.12	2795	<u>278aa</u>	Protein coding	CCDS25958	Q8VBT0	TSL:1 GENCODE basic APPRIS P1
Tmx1-203	ENSMUST00000162277.1	710	<u>124aa</u>	Nonsense mediated decay	-	<u>F6V084</u>	CDS 5' incomplete TSL:3
Tmx1-202	ENSMUST00000160865.1	648	No protein	Retained intron	-	-	TSL:2

The strategy is based on the design of *Tmx1-201* transcript, the transcription is shown below:



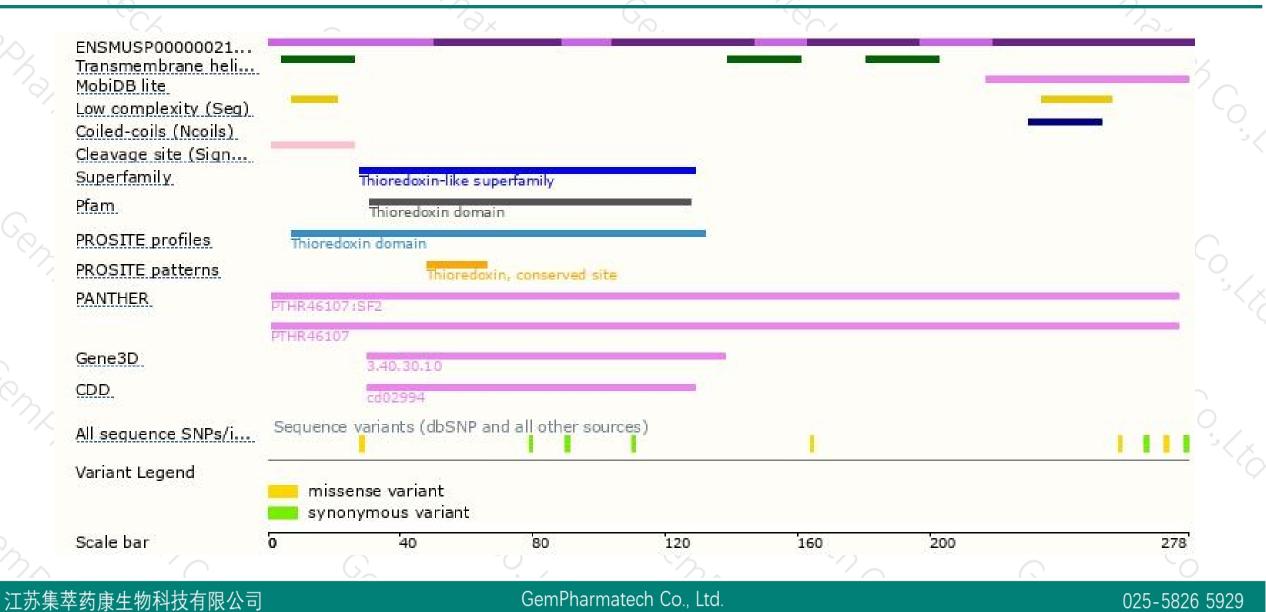
Genomic location distribution





Protein domain







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



